University of Groningen

# Diversification dynamics in Caribbean rain frogs (Eleutherodactylus) are uncoupled from the anuran community and consistent with adaptive radiation

Jiménez-Ortega, Dante; Valente, Luis; Dugo-Cota, Álvaro; Rabosky, Daniel L.; Vilà, Carles; Gonzalez-Voyer, Alejandro

*Published in:*
Proceedings of the Royal Society B: Biological Sciences

*DOI:*
10.1098/rspb.2022.2171

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*
Publisher's PDF, also known as Version of record

*Publication date:*
2023

Link to publication in University of Groningen/UMCG research database

*Citation for published version (APA):*
Jiménez-Ortega, D., Valente, L., Dugo-Cota, Á., Rabosky, D. L., Vilà, C., & Gonzalez-Voyer, A. (2023). Diversification dynamics in Caribbean rain frogs (*Eleutherodactylus*) are uncoupled from the anuran community and consistent with adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences*, *290*(1990), Article 20222171. https://doi.org/10.1098/rspb.2022.2171

**Copyright**
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

**Take-down policy**
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

**Cite this article:** Jiménez-Ortega D, Valente L, Dugo-Cota Á, Rabosky DL, Vilà C, Gonzalez-Voyer A. 2023 Diversification dynamics in Caribbean rain frogs (*Eleutherodactylus*) are uncoupled from the anuran community and consistent with adaptive radiation. *Proc. R. Soc. B* **290**: 20222171.
https://doi.org/10.1098/rspb.2022.2171

Received: 28 October 2022
Accepted: 8 December 2022

**Subject Category:**
Evolution

**Subject Areas:**
evolution, ecology

**Keywords:**
adaptive radiation, diversification, equilibrium dynamics, anurans, Caribbean

**Author for correspondence:**
Alejandro Gonzalez-Voyer
e-mail: alejandro.gonzalez@iecologia.unam.mx

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6350490.

THE ROYAL SOCIETY
PUBLISHING

# Diversification dynamics in Caribbean rain frogs (*Eleutherodactylus*) are uncoupled from the anuran community and consistent with adaptive radiation

Dante Jiménez-Ortega[1,2], Luis Valente[3,4], Álvaro Dugo-Cota[5], Daniel L. Rabosky[6], Carles Vilà[5] and Alejandro Gonzalez-Voyer[1]

[1]Instituto de Ecología and [2]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad de México 04510, México
[3]Naturalis Biodiversity Center, Leiden, The Netherlands
[4]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands
[5]Conservation and Evolutionary Genetics Group, Estación Biológica de Doñana (EBD-CSIC), Seville 41092, Spain
[6]Museum of Zoology and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA

DJ-O, 0000-0003-0110-1244; LV, 0000-0003-4247-8785; ÁD-C, 0000-0001-8474-9163; DLR, 0000-0002-7499-8251; CV, 0000-0002-4206-5246; AD-V, 0000-0002-5072-1688

Adaptive radiation is proposed to play a key role in generating differences in species richness among lineages and geographical regions. Due to the importance of ecological divergence in adaptive radiation, species richness is predicted to be influenced by equilibrium diversity dynamics, although the concept continues to generate much debate. An additional important question is whether radiating clades have intrinsic biological characteristics that make them particularly prone to diversify. We tackle these questions by analysing (i) the temporal patterns of diversification of Caribbean *Eleutherodactylus* frogs, and (ii) assembly of the complete native anuran community of the Caribbean archipelago (197 species), testing for the presence of equilibrium dynamics and whether diversification patterns of *Eleutherodactylus* differ from those of the rest of the Caribbean anurans. Diversification rates follow the predicted pattern of rapid diversification early in the radiation which gradually decreases towards the present. *Eleutherodactylus* diversification is significantly faster than that of the Caribbean anuran community, and although equilibrium dynamics influence richness of all Caribbean anurans, *Eleutherodactylus* shows higher carrying capacity. Our results indicate that ecological opportunity *per se* is not sufficient for adaptive radiation and that diverse lineages present intrinsic characteristics that enable them to make the most of available opportunity.

## 1. Introduction

Identifying the causes of the heterogeneous distribution of species richness across the branches of the tree of life, and across geographical regions, remains one of the key unresolved questions in evolutionary biology [1]. One process proposed to play a key role in generating differences in species richness is adaptive radiation, a particular case of speciation by ecological divergence, in which lineages exhibit rapid adaptive diversification to exploit distinct ecological niches [2–4]. Due to the tight link between ecological divergence and speciation, adaptive radiation has been proposed to be driven by access to ecological opportunity, that is niches that are available to be filled, either because of lineages having invaded a habitat with under-exploited resources or as a result of being able to exploit resources that were hitherto inaccessible [5]. Thus, at the onset of adaptive radiation, diversification is predicted to be rapid, as species invade available ecological niches. As the number of diverging species increases, the number of accessible niches decreases and diversification rates are expected to slow down [4,5].

Most well-studied cases of adaptive radiation come from geographically restricted habitats, such as lakes or oceanic islands [6,7], where two geographical attributes are proposed to play a key role. The first is their isolation, which functions as a filter for the mainland biota and allows early colonizers to evolve free from the constraints of competition or predation [6]. Second, the clear boundaries of these regions, which result in colonization-diversification dynamics that tend toward equilibria in species richness, likely determined to a certain extent by the number of distinct ecological niches [6,8]. Indeed, during adaptive radiation species richness is proposed to be determined by the number of available niches, and thus ecological limits on diversity are expected to influence diversity dynamics [4].

Although the concept of equilibrium dynamics in species richness continues to generate much debate [9,10], studies of radiations in islands or lakes have provided the strongest evidence in its favour [11–14]. In fact, equilibrium dynamics are a central part of the island biogeography theory of McArthur and Wilson [8,15], which suggests that island biotas are a result of the interplay between colonization and extinction, and reach a dynamic equilibrium influenced by the distance to the mainland and island size, where larger or less isolated islands are expected to support greater species richness. Overall, the consistent and strong scaling of species richness with island area [16] is difficult to reconcile with non-equilibrium models for island community assembly [17]. Recent evidence from island biota suggests that a dynamic equilibrium can be achieved and maintained over evolutionary timescales, despite the unpredictable nature of geological changes to which an island is exposed [13,14]. At the same time, even within the same insular system, both equilibrium and non-equilibrium dynamics can be at play [12].

Despite the importance of ecological opportunity in fueling adaptive radiation, another unresolved question is whether the presence of such opportunity is sufficient to trigger adaptive radiation or whether radiating lineages are unusual in their ability to make the most of the particular ecological scenario [18]. Lakes or islands where adaptive radiations have been documented were colonized by different lineages, only one of which radiated and filled available niches. For example, recent evidence suggests that intrinsic attributes of *Anolis* lizards, and not the ecological or biogeographic context, played a prominent role in enabling their radiation in the Caribbean islands [18,19]. Similarly, although the Galapagos islands were colonized eight times by different bird lineages, only Darwin's finches present high *in-situ* radiation, and their diversification patterns are uncoupled from those of the avian community of the archipelago [12].

Amphibians are particularly underrepresented within the study of adaptive radiations, which is surprising given the group's broad diversity of life histories, ecological specializations, and variation in species richness among clades, offering interesting opportunities to study the importance of adaptive radiation in generating diversity [20]. Here, we focus on the frog genus *Eleutherodactylus*, one of the largest extant island radiations of vertebrates, which diversified mainly in the Caribbean archipelago, where approximately 167 species have been described [21]. *Eleutherodactylus* belongs to the superfamily Brachycephaloidea, a Neotropical group with direct development (i.e. froglets emerge from eggs and thus avoid the tadpole stage [22]), which includes over 1100 described species [21] and represents roughly 28% of the amphibian diversity of the Neotropics [22]. Direct development is suggested to be a key innovation enabling the invasion of diverse terrestrial environments, as reproduction is no longer limited by access to water bodies [22]. The diversity of *Eleutherodactylus* in the Caribbean archipelago was recently proposed to be the result from parallel adaptive radiations due to evidence of phenotypic ecological specialization, and morphological evolutionary convergence, resulting from independent colonization of the same micro-habitats across the different islands [23]. Interestingly, *Eleutherodactylus* frogs diversified in the same biogeographic scenario as the parallel radiation of *Anolis* lizards [24], where both groups most probably arrived by overwater dispersal [24–26]. The biogeographic configuration of the Caribbean archipelago provides multiple opportunities for allopatric speciation to occur [24], highlighting the Caribbean region as a remarkable scenario for the study of evolution and community assembly.

We analysed the temporal diversification patterns of Caribbean *Eleutherodactylus* as well as community assembly of the entire anuran fauna of the archipelago to test whether these follow the patterns expected for an adaptive radiation [4,7]. First, we tested for differences in diversification rates among clades and whether rates varied through time. Because island size is one potential predictor for speciation and extinction rates [17,27], we expected to observe variation in diversification rates across clades on different islands given the large differences in island size in the Caribbean archipelago. We also expected temporal variation in diversification, with high rates early in the radiation followed by a progressive decline, as predicted by theory [4]. Finally, we analysed whether macroevolutionary dynamics of Caribbean *Eleutherodactylus* are uncoupled from the dynamics of the anuran community where they evolved and whether species richness in *Eleutherodactylus* reached a dynamic equilibrium, as predicted by the island biogeography theory, but also expected during adaptive radiation [12,15]. These analyses allowed us to address the question of whether *Eleutherodactylus*'s high species richness is the result of its particular ability to exploit ecological opportunity in the Caribbean archipelago compared to other amphibians.

## 2. Methods

### (a) Temporal patterns of diversification in *Eleutherodactylus*

We first focused on Caribbean *Eleutherodactylus* to test hypotheses of diversification patterns through time. We used the phylogeny reconstructed by Dugo-Cota *et al.* [23], which is the most complete regarding taxon sampling and genetic information. The phylogeny includes 148 species (89%) out of the 167 Caribbean *Eleutherodactylus* and was constructed with four mitochondrial and three nuclear genes, and time-calibrated with a combination of fossil and geological calibration points. First, we explored whether diversification rates differed among *Eleutherodactylus* clades. We expected to find higher rates of diversification in clades that colonized larger islands such as Cuba or Hispaniola. We used BAMM version 2.5.0 (Bayesian Analysis of Macroevolutionary Mixtures [28]), which can identify heterogeneity in diversification rates along the branches of a phylogeny produced by a mixture of processes, such as diversity dependence, constant and time-varying diversification [28]. BAMM uses reversible jump Markov chain Monte Carlo (rjMCMC) [28] to explore a universe of alternative models that differ in the number of macroevolutionary regimes. We used the R package BAMMtools [29] to estimate suitable priors and to visualize and manage the results. To account for missing species, we used the global option implemented in BAMM, which corrects for unsampled species across the phylogeny. We ran a total of six chains with 10 million generations, sampled every 1000 generations and discarded 10% of the chains as burn-in. Convergence was based on an effective sampling fraction for the log likelihood and a number of shifts greater than 200. Finally, we computed Bayes factors (BFs) to compare between alternative models with different number of rate-shift configurations.

We also analysed the temporal patterns of diversification in *Eleutherodactylus* using the RPANDA package [30] in R. We compared the fit of six birth–death and pure birth models, including models with constant and time-varying speciation ($\lambda$) and extinction ($\mu$) rates [30] (see electronic supplementary material, methods ST1). To account for phylogenetic uncertainty, we repeated the analyses with BAMM and RPANDA on a sample of 20 trees from the posterior distribution.

## (b) Assembly dynamics and carrying capacity of the Caribbean anuran biota

### (i) Data collection and phylogenies

We restricted our study area to islands regarded as the native range of Caribbean *Eleutherodactylus*, which is the Greater and Lesser Antilles, and the Bahamas. We considered all islands as a single unit since previous studies have demonstrated the importance of archipelagos as biogeographic units in studies of community assembly [31] (see electronic supplementary material, methods ST2). We searched the literature to compile a checklist of all anuran species in the Caribbean, including whether they were endemic to the Caribbean, to a certain island in the Caribbean, or non-endemic (electronic supplementary material, table S1 and figure 1*a*). For each clade or single species (figure 1*a*), we obtained an estimated colonization age of the Caribbean islands (we used of the divergence age from the continental sister group), and branching times (i.e. cladogenetic events occurring within the archipelago). For *Eleutherodactylus*, colonization and branching times were based on the phylogeny by Dugo-Cota *et al*. [23]. For most of the remaining Caribbean anuran fauna, we used the phylogeny of Pyron [34], which was reconstructed using nine nuclear and three mitochondrial genes and time-calibrated with a set of fossil constraints using a maximum-likelihood approach. For *Allobates chalcopis*, the only species not included in the phylogeny of Pyron [34], we used the divergence age estimated by Fouquet *et al*. [35], who reconstructed the phylogeny using four mitochondrial and one nuclear gene, including four calibration points from a combination of fossil and biogeographic information. Finally, to test the robustness of our results to phylogenetic uncertainty due mainly to differences in estimated divergence times, we repeated our analyses using the phylogeny of Jetz & Pyron [33]. This phylogeny includes all the genera and practically all species considered in this study, but many are placed on the phylogeny based on taxonomic information (16%).

### (ii) DAISIE analyses

We were interested in testing whether *Eleutherodactylus*'s diversity is the result of diversification patterns that are uncoupled from the rates of the remaining anuran biota within the Caribbean archipelago. At the same time, we explored whether species richness in anurans, but particularly in eleutherodactylids, shows evidence of equilibrium dynamics, derived from diversity-dependent processes. We applied DAISIE (Dynamic Assembly of Islands through Speciation, Immigration and Extinction [12]), a likelihood-based phylogenetic approach that combines the theory of island biogeography [15] with phylogenetic birth–death models [32] within a dynamic stochastic biogeography model [12]. For a given insular community consisting of multiple colonizing lineages, DAISIE estimates rates of cladogenesis, anagenesis, colonization, extinction, as well as the presence of a limit to species richness in each insular clade. In DAISIE, all estimated parameters may depend on the number of species on the island (diversity dependence), but that dependence is currently only explicitly considered in the case of cladogenesis and colonization [12] (i.e. under diversity dependence rates of cladogenesis and colonization decrease as the number of species increases). We used three different status for clades, following categories in DAISIE: 'Endemic' for lineages with one or more species that only occur in the archipelago; 'Non-Endemic' for lineages that have populations on both mainland and the islands; and 'Endemic_MaxAge' for endemic clades whose stem nodes are older than the age of the islands (*Peltophryne* and *Pristimantis*) and in the case of *A. chalcopis*, because the divergence time for this species was estimated independently based on Fouquet *et al*. [35]. The 'Endemic_MaxAge' option in DAISIE integrates over the possible colonization times between the specified upper bound and the stem age of the clades.

Since island age may influence colonization dynamics, we compared results using two estimates for the age of the Caribbean islands based on the paleogeographic reconstructions of Iturralde-Vinent [36], who estimates a minimum of 40 Myr and a maximum of 65 Myr for the presence of continuously emerged land for the Greater Antilles. More recent studies of geomorphology of the region exist, but they discuss processes that postdate the formation of the archipelago (e.g. [37]). Colonization rate estimates in DAISIE are also influenced by the mainland species pool, thus we repeated analyses using two different mainland pool sizes. The first estimate was of 2715 species, consisting in an approximate number of anurans distributed from North Amazonia through Central America to Southeast North America [21]. For the second estimate we restricted the area to Central America and northern South America, where there are around 2195 anuran species [21].

We contrasted 17 alternative models (electronic supplementary material, table S3) in DAISIE, for which we obtained maximum-likelihood estimates for cladogenesis ($\lambda^c$), anagenesis ($\lambda^a$), extinction ($\mu$), colonization ($\gamma$, here an estimate of colonization) and carrying capacity ($K$, which is infinite when richness is unbounded). Models with diversity dependence and a finite $K$ are equilibrium models with an upper bound to diversity. In four equal-rates DAISIE models, we assumed that the entire Caribbean anuran fauna shares the same macroevolutionary dynamics (M1–M4; electronic supplementary material, table S3). In the remaining 13 models, we assume that *Eleutherodactylus*'s (type 2 species) macroevolutionary dynamics are completely or partially uncoupled from the dynamics of the other Caribbean anurans (type 1 species; heterogeneous models, M5–M17; electronic supplementary material, table S3). For the heterogeneous-rates models, the number of *Eleutherodactylus* species in the mainland pool was based on the proportion of species from the mainland pool that are part of the Brachycephaloidea superfamily, which are 0.366 for a mainland pool size of 2715, and 0.215 for a mainland pool size of 2195. As a test of the goodness of the fit of the preferred model to the empirical data, we conducted 1000 island simulations based on the estimated ML parameters. We plotted the resulting summary statistics to assess the accuracy of the model to reproduce the observed data (electronic supplementary material, figure S1). To obtain confidence intervals on parameters estimates, we performed a parametric bootstrap analysis by fitting DAISIE to these 1000 datasets and calculating percentiles from the distribution of estimated parameters.

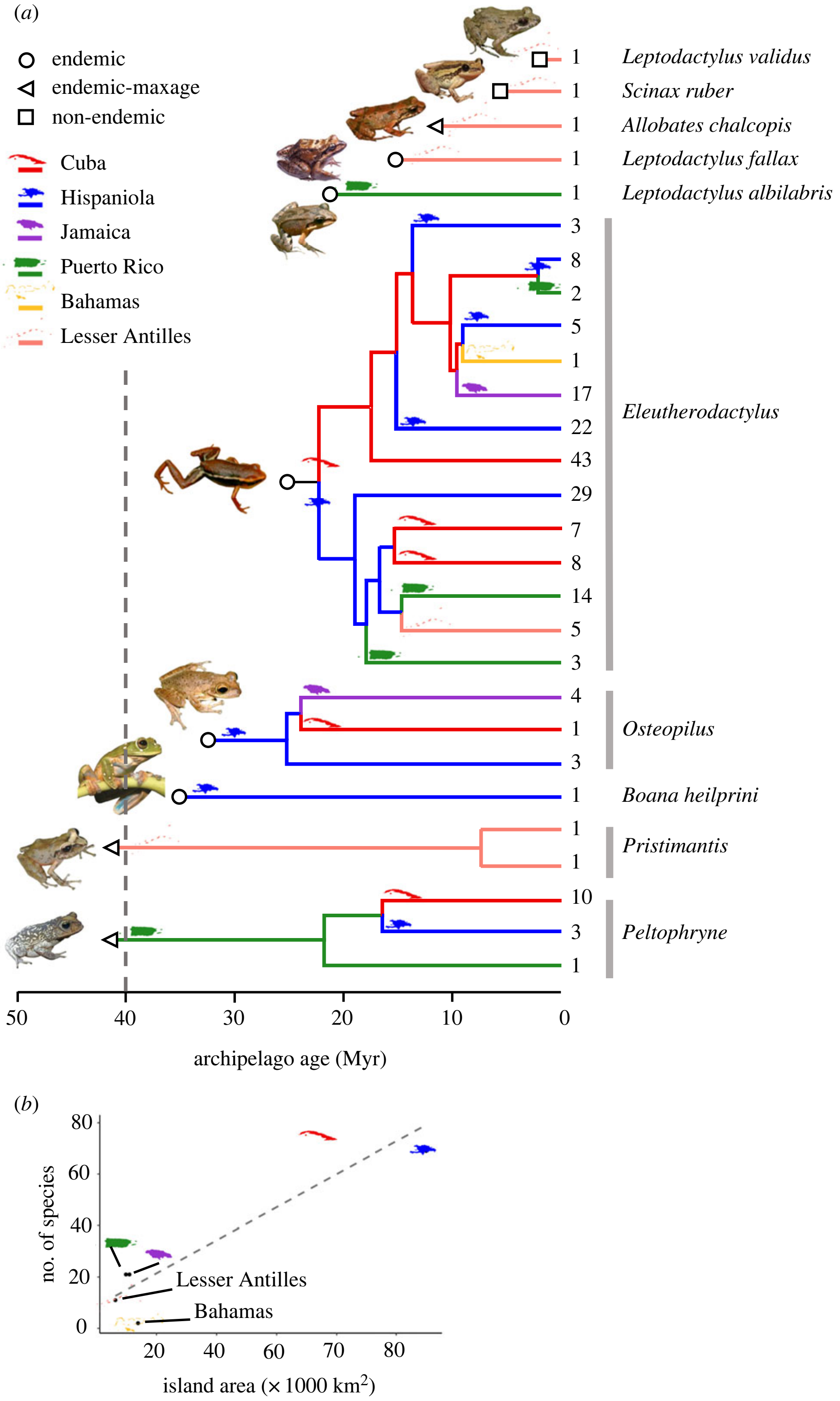

**Figure 1.** Colonization times, representative branching events and species–area relationship in Caribbean anuran fauna. (*a*) Schematic representation of the colonization times of the Caribbean archipelago and the major branching times associated with between-island colonization events. Numbers at the tips of each edge represent the species richness represented by that branch. The branching events within islands are not shown. Edge colour and silhouettes represent the Caribbean Island where the different clades diversified. Dashed vertical line indicates the estimated minimum age for the archipelago. Myr: million years. Divergence times are based on the phylogenies of Dugo-Cota *et al.* [23] for *Eleutherodactylus*, Fouquet *et al.* [32] for *A. chalcopis* and Pyron [33] for the remaining anurans. A single species, *O. septentrionalis,* colonized both Cuba and the Bahamas, here only the former colonization is represented. (*b*) Species–area relationship of the entire anuran fauna within the islands of the Caribbean archipelago, dashed line represents the OLS (ordinary least squares) regression line. Picture credits: *Leptodactylus validus*: F. J. M. Rojas-Runjaic; *Scinax ruber*: Todd Pierson; *Allobates chalcopis*: Maël Dewynter; *Leptodactylus fallax*: Jake M. Hutton; *Leptodactylus albilabris*: Luis J. Villanueva-Rivera; *Eleutherodactylus limbatus*: Alejandro Gonzalez-Voyer; *Osteopilus septentrionalis*: Will Flaxington; *Boana heilprini*: Harry W. Greene; *Pristimantis shrevei*: Robert Powell; and *Peltophryne taladai* Thomas Brown.

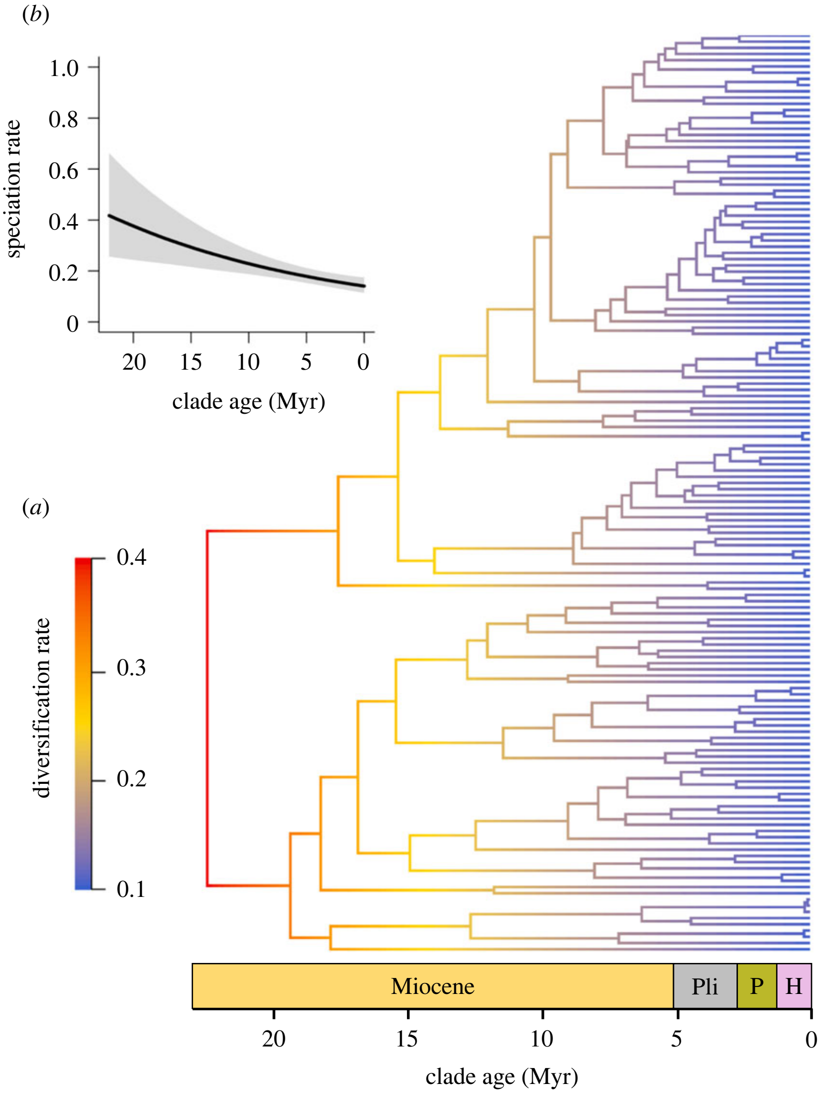

**Figure 2.** Speciation rate through time estimated in BAMM for Caribbean *Eleutherodactylus*. (*a*) Phylorate plot showing speciation rate across the *Eleutherodactylus* phylogeny based on the maximum credibility set from the analyses with BAMM. Warmer colours indicate higher diversification rates, the scale bar represents speciation rate per Myr. (*b*) Speciation rate through time for *Eleutherodactylus*, the shaded area represents the 95% confidence intervals of the estimated rate. Pli = Pliocene, P = Pleistocene, H = Holocene.

# 3. Results

## (a) Temporal patterns of diversification in *Eleutherodactylus*

The analyses with BAMM indicated that the diversity of Caribbean *Eleutherodactylus* accumulated with homogeneous rates across coeval branches of the phylogeny, as we found no evidence for diversification rate shifts. A scenario with zero rate shifts was the best supported model with a posterior probability of 85% (BF = 1 indicating evidence in favour of the model with zero rate shifts, compared with models with one or more rate shifts, BF < 0.3). Effective sample size for both the log-likelihood and the number of rate shifts exceeded 5700. Nonetheless, the analyses showed temporal variation in the diversification rate, as the mean of the marginal posterior density for speciation along the phylogeny indicated higher rates in the past (i.e. toward the root) and lower rates toward the present (figure 2*a*,*b*). Given that we find no evidence for heterogeneity in diversification rates across the *Eleutherodactylus* phylogeny, we focus on the Caribbean radiation of the genus as a whole for the remaining analyses.

The temporal variation in diversification rates of *Eleutherodactylus* was further confirmed by the results of the pure birth and birth–death models. The model that best fit the data corresponded to a pure birth model with an exponential variation in speciation rate with time (ΔAIC > 3, relative to the constant speciation rate models; electronic supplementary material, table S2), i.e. higher speciation towards the root of the tree and a decrease toward the tips. By contrast, birth–death models as well as those with constant rates of speciation had a poor fit to the data (electronic supplementary material, table S2). The results are the same when repeating the analyses (with BAMM and RPANDA) on a sample of 20 phylogenies from the posterior distribution (see electronic supplementary material, results ST3, figures S2 and S3, and table S8 for a summary of the results).

## (b) Diversification dynamics of the Caribbean anuran community assembly

In addition to the single colonization event by *Eleutherodactylus* (which generated 167 species), the Antilles have been

**Table 1.** Maximum-likelihood parameters estimated in the macroevolutionary diversification analysis for the anuran community excluding *Eleutherodactylus* (background) and specific for *Eleutherodactylus* under the best-supported model (M5; see complete set of results in electronic supplementary material, table S4), using an island age of 40 Myr and a mainland pool size of 2715 species. $\lambda^c$ and $\lambda^a$: speciation by cladogenesis and anagenesis, respectively; $\mu$ extinction rate; $K$, carrying capacity; $\gamma$ colonization rate; LogLik: log-likelihood; BIC: Bayesian information criterium; BICw: BIC weight of the competing models. Parameter units are in events per lineage per million years, except $K$, which is in number of species. Confidence intervals are the 2.5 and 97.5 percentiles from the bootstrap analysis.

| parameter | background | *Eleutherodactylus* | LogLik | BIC | BICw |
|---|---|---|---|---|---|
| $\lambda^c$ | 0.057 (0.04–0.17) | 0.455 (0.39–1.05) | −537.44 | 1172.34 | 0.965 |
| $\mu$ | 0.007 (0–0.08) | 0.007 (0–0.43) | | | |
| $K$ | 41 ($3-6\times10^4$) | 214 (4.98–217) | | | |
| $\gamma$ | 0.0001 (0.0001–0.0003) | $3.48\times10^{-5}$ (0.00002–0.0001) | | | |
| $\lambda^a$ | 0.165 (0–1.95) | 75.909 ($3.8\times10^{-7}$ –5.03) | | | |

colonized at least nine times by members of seven other anuran genera, leading to 30 native non-*Eleutherodactylus* extant species (figure 1*a*). These genera have been present in the Caribbean during a period similar or even prior to the estimated colonization by *Eleutherodactylus*, indicating that other anuran lineages evolved in the same biogeographic context and probably had access to comparable ecological opportunity. The results of the analyses with DAISIE indicate that diversification dynamics of *Eleutherodactylus* are distinct from those of the rest of the Caribbean anuran community, since the best-fitting model is one where *Eleutherodactylus* presents parameters that differ from those of the other anuran lineages (model M5; table 1, including confidence intervals, and electronic supplementary material, table S4 for the complete set of tested models). *Eleutherodactylus* presented higher rates of cladogenesis and anagenesis ($\lambda^c = 0.455$; $\lambda^a = 75.909$) as well as a higher carrying capacity ($K = 214$ species), compared to the rest of the anuran community ($\lambda^c = 0.057$; $\lambda^a = 0.165$; and $K = 41$ species). At the same time, in line with the expected equilibrium dynamics predicted for island biota under island biogeography theory, both *Eleutherodactylus* and the rest of the anuran community are governed by equilibrium dynamics (electronic supplementary material, figure S4), although with different limits to species richness (table 1, electronic supplementary material, figure S4). In the preferred model, the equilibrium number of species is determined by diversity dependence in rates of cladogenesis and colonization. In other words, diversity tends toward an equilibrium because the more species there are on the island the lower the rates of cladogenesis and colonization, and those rates will approach zero when the carrying capacity (or diversity 'limit') is reached. Although both groups are driven by diversity dependence, their diversities are still below the estimated equilibrium diversity limits. On the other hand, the estimated extinction and colonization rates are very low (but non-zero) for both groups ($\mu = 0.007$, and $\gamma = 3.48 \times 10^{-5}$ for *Eleutherodactylus*; $\mu = 0.007$, and $\gamma = 0.0001$ for the rest of the Caribbean anurans). These results were consistent when we repeated analyses varying the island age (electronic supplementary material, table S5), mainland pool size (electronic supplementary material, table S6), as well as colonization and branching times (electronic supplementary material, table S7). Finally, the simulated island set indicates that model M5 provides a good fit to the empirical data (electronic supplementary material, figure S1).

## 4. Discussion

The results of our analyses of temporal patterns of diversification of Caribbean *Eleutherodactylus* showed high rates of diversification early in the radiation with a gradual decrease towards the present, as predicted for adaptive radiation [4,7,38]. Interestingly, although we found temporal variation in diversification rates of Caribbean *Eleutherodactylus*, said rates were relatively homogeneous across the different lineages, which is surprising given that the group colonized islands that differ notably in size. Consistent with the described slow-down in diversification, we also found support for the presence of equilibrium dynamics influencing anuran richness, as proposed by the island biogeography theory [4,15]. Finally, our results indicate that the elevated species richness of *Eleutherodactylus* in the Caribbean is likely the result of clade-specific processes and intrinsic characteristics, as the genus shows higher rates of speciation and higher carrying capacity than other anurans that also colonized the archipelago at comparable times and thus shared the same ecological setting.

### (a) Diversification patterns in *Eleutherodactylus*

A key requirement for diversification through adaptive radiation is the physical and evolutionary access to ecological opportunity, that is, the availability of empty or underexploited niches, and the evolutionary flexibility to explore new adaptive zones [4,7]. Ecological opportunity is predicted to dictate the temporal variation in diversification rates during adaptive radiation, that is, high speciation rates when niches are abundant and subsequent decrease as species accumulate and niches become saturated [5,7]. This temporal sequence of events is expected to leave signatures in the evolutionary history of the radiating lineage, reflected by temporal changes in diversification rates [38]. In line with this prediction, we found temporal variation in the rate of diversification in *Eleutherodactylus* with higher rates of diversification early in the radiation followed by a gradual slow-down toward the present.

Previous studies have found a general trend for diversification rates which slowdown with time [39–41]. Such temporal declines in diversification rates are sometimes interpreted as providing evidence for niche-filling, adaptive radiations [37] or presence of ecological limits to diversity [40,42,43]. Alternative explanations have been offered for such patterns [43], one of which is a decrease in global temperatures from the late Cretaceous to the present [40]. In a study, spanning a broad range of taxa, Condamine *et al*. [41] analysed the temporal patterns of diversification of Eleutherodactylidae, the family to which *Eleutherodactylus* belongs, and found strong support for a model with time-varying rates of diversification, consistent with our results. This is not surprising since most of the diversity in the Eleutherodactylidae (total 234 species [21]) is represented by *Eleutherodactylus* (204 species [21]). A decrease in the temporal pattern of diversification should not *per se* be interpreted as signature of adaptive radiation, without evidence of ecological diversification and adaptation [44]. However, for *Eleutherodactylus* evidence for adaptive diversification comes from previous work [23] which shows significant associations between phenotypes and the different micro-habitats where species are found, and significant morphological convergence among species having independently colonized similar micro-habitats on the different islands.

Recently, Louca & Pennell [45] showed that a given pattern of branching through time in a molecular phylogeny is equally probable under a vast set of models, i.e. the parameters describing the rates may be non-identifiable [46]. Nonetheless, we agree with others that—despite these concerns—the wholesale abandonment of phylogenetic diversification studies is unwarranted at the present time, and further, that some proposed solutions—such as the use of 'pulled' diversification rates—does not necessarily add clarity to our understanding of macroevolutionary dynamics [46,47]. Furthermore, the issues raised by Louca & Pennell [45] do not undermine the hypothesis-driven model selection procedure widely applied in the field [47], and by employing different approaches, which make different assumptions about the data, greater confidence on the results can be obtained when these converge on the same conclusion (as they do in our case). We acknowledge that the field is undergoing rapid changes and the development of novel methods could lead to changes in our results but remain confident the main conclusions will be unchanged.

We hypothesized that diversification rates would differ among islands due to the large differences in geographical area, in line with the island biogeography theory [15]. The species richness of Caribbean *Eleutherodyactylus* scales with island size, where larger islands, such as Cuba and Hispaniola, harbour higher species richness (58 and 67 species, respectively), while smaller islands such as Puerto Rico or Jamaica, have lower richness (19 and 17 species, respectively; figure 1*b*). The diversity of Jamaica emerged from a single colonization event as also occurred in *Anolis* lizards [11]. Interestingly however, we did not find evidence for differences in diversification rates between *Eleutherodactylus* clades occurring on different islands. This contrasts with results for *Anolis* lizards [11], which show differences in the temporal patterns of species accumulation across the four major islands of the Greater Antilles. Although we cannot rule out that our analyses lacked the power to detect shifts in the rate of diversification along the phylogeny, we think it is an unlikely explanation for the results, as the *Eleutherodactylus* phylogeny includes a higher number of species (148 compared with 117 for *Anolis* [11]), and because we were able to detect temporal shifts in the rate of diversification from the root to the tips of the phylogeny, a result that is corroborated by two different analytical approaches. Instead, we propose that the reason we do not detect heterogeneous diversification rates for Caribbean *Eleutherodactylus* is the variation in colonization times for the different islands, with smaller islands having been colonized more recently, in conjunction with the temporal decrease in diversification rates. While the large islands of Cuba and Hispaniola were first colonized before 20 Ma, the smaller islands of Puerto Rico and Jamaica were first colonized around 15 Ma and 7 Ma, respectively [23]. We therefore suggest that a combination of time-for-speciation resulting from different colonization times of the different islands, coupled with dispersal events among major islands from the Greater Antilles might explain our finding of homogeneous diversification rates across the *Eleutherodactylus* phylogeny.

### (b) *Eleutherodactylus*'s diversification patterns are uncoupled from those of the remaining Caribbean anuran biota

Our analysis of the macroevolutionary dynamics of the assembly of the anuran community indicates that the best-supported model is one where Caribbean *Eleutherodactylus* present diversification patterns that are decoupled from those of the rest of the Caribbean anurans. The rates of speciation for Caribbean *Eleutherodactylus* are nearly one order of magnitude higher than those of the other Caribbean anurans, with nearly identically low rates of extinction (table 1). At the same time, our results indicate that both groups tend towards a dynamic equilibrium, as predicted by island biogeography theory [15], although with notable differences in the estimated limits on diversity. The estimated carrying capacity for *Eleutherodactylus* is more than five times higher than that for the other Caribbean anurans. Together, these findings suggest that intrinsic attributes of *Eleutherodactylus* enabled them to make the most of the available ecological opportunity in the Caribbean, as suggested for *Anolis* lizards [18]. In addition to *Eleutherodactylus* two genera presented *in situ* diversifications in the Caribbean, *Peltophryne* and *Osteopilus*. However, the resulting species richness of these two clades (14 and 8 species, respectively) is comparably low. The remarkable discrepancies in species richness and carrying capacity between *Eleutherodactylus* and the other Caribbean anurans, suggest that the limits to diversity are not only determined by the availability of ecological opportunity, but also by the intrinsic capacity of species to exploit it [18]. Key innovations are one such intrinsic characteristic which has been proposed to facilitate access to ecological opportunity in adaptive radiation [48]. Direct development, by reducing dependency on water bodies for reproduction, may enable species to invade a greater diversity of niches [22,23]. Interestingly, *Eleutherodactylus* is not the only genus with direct development to have invaded the Caribbean archipelago. *Pristimantis*, another member of the Brachicephalidae (the superfamily to which *Eleutherodactylus* belongs), whose diversity in south America amounts to more than 500 species, is also found in the Caribbean but represented by only two species (*P. sherevei* and *P. eupronides*). A possible explanation is that *Pristimantis* did not have the physical access to the same array of ecological opportunity as *Eleutherodactylus*. The former only reached the Lesser Antilles, whereas the latter first reached Cuba and Hispaniola, and then colonized the rest of the archipelago.

We are aware that using a contemporary diversity estimate as the mainland pool in our analysis can be problematic. However, it is currently not possible to obtain an accurate estimate of the number of species of neotropical amphibians in the mainland pool over the last 40–60 Myr. We thus favour using the contemporary diversity as the mainland pool, as we found that using a different smaller mainland pool did not affect our main conclusions (electronic supplementary material, table S6). Colonization rates in Caribbean anuran fauna are low compared with other island colonist taxa such as birds [12,14], which can be directly explained by the fact that anurans are not a particularly dispersive group. Evidence suggests that insular anuran fauna have emerged mainly through oceanic overwater dispersal [25,26,34]. Such is probably also the case for Caribbean *Eleutherodactylus*, as a South American origin and overseas dispersal, probably on flotsam, has been proposed based on divergence estimates from time-calibrated molecular phylogenies [23,25] (figure 1*a*). The low dispersal estimates from the DAISIE analyses, coupled by the fact that *Eleutherodactylus* colonized the Caribbean archipelago only once, indicate that colonization events from the mainland were rare, although inter-island colonization has occurred multiple times. Since most continental species are absent from the Antilles, island colonists had access to very diverse resources [6,49]. In a similar example, the anuran genus *Limnonectes* is widespread in South and Southeast Asia, but in the island of Sulawesi in central Indonesia, where the anuran community is not as diverse as in the neighbouring islands of Borneo and the Philippines, the genus displays higher levels of species richness and morphological variation [49]. It is possible that low competition at the onset of the radiation contributed to the ecological release of *Eleutherodactylus* in the Caribbean.

In conclusion, our results using both a lineage-specific and a community-wide approach highlight the remarkable ability of Caribbean *Eleutherodactylus* to exploit available ecological opportunity, as other anurans colonized the archipelago at comparable times and thus probably had access to comparable diversifying opportunity. The remarkable differences in estimated carrying capacity between *Eleutherodactylus* and the rest of the Caribbean anurans suggest that the limits to diversity are not only determined by the availability of ecological opportunity, but also by the intrinsic capacity of species to exploit it. This is supported by the fact that *Eleutherodactylus* is the only anuran lineage in the Caribbean which has been found to invade a large number of different micro-habitats [23]. Finally, this work also shows the relevance of the Caribbean region for studying macroevolutionary processes, given the fact that parallel evolutionary radiations have occurred more than once (e.g. [50]), which points to potentially fruitful avenues to further explore the geologic and biogeographic attributes of the Antilles that contribute to heterogeneous patterns of spatial and phylogenetic biodiversity. Our work thus highlights how the historical biogeographic scenario interacts with a lineage's evolutionary lability to spur the emergence of heterogeneity in richness across space and throughout the branches of the tree of life.

Data accessibility. All data, code and phylogenies are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.34tmpg4pq [51].

Additional data are provided in electronic supplementary material [52].

Authors' contributions. D.J.-O.: data curation, formal analysis, investigation, writing—original draft, writing—review and editing; L.V.: conceptualization, formal analysis, methodology, software, supervision, validation, writing—review and editing; A.D.-C.: data curation, validation; D.L.R.: formal analysis, software, supervision, validation, writing—review and editing; C.V.: data curation, funding acquisition, validation, writing—review and editing; A.G.-V.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was funded by UNAM PAPIIT project (grant no. IA201716) to A.G.-V. C.V. thanks the Spanish Government for funding project CGL2016-75227-P. L.V. was funded by a NWO Vidi grant (grant no. NWO 016.Vidi.189.006). This manuscript is part of the requirements for graduation of D.J.-O. in the Posgrado en Ciencias Biológicas PhD program at Universidad Nacional Autónoma de México. D.J.-O. was funded by a PhD scholarship from CONACyT (grant no. CVU-704115).

Acknowledgements. We thank Edgar Ávila Luna for logistic support.

## References

1. Nürk N *et al.* 2020 Diversification in evolutionary arenas—assessment and synthesis. *Ecol. Evol.* **10**, 6163–6182. (doi:10.1002/ece3.6313)
2. Schluter D. 2000 *The ecology of adaptive radiation*. Oxford, UK: Oxford University Press.
3. Simpson GG. 1953 *The major features of evolution*. New York: NY: Columbia University Press.
4. Gavrilets S, Losos JB. 2009 Adaptive radiation: contrasting theory with data. *Science* **323**, 732–737. (doi:10.1126/science.1157966)
5. Stroud JT, Losos JB. 2016 Ecological opportunity and adaptive radiation. *Annu. Rev. Ecol. Evol. Sys.* **47**, 507–532. (doi:10.1146/annurev-ecolsys-121415-032254)
6. Losos JB, Ricklefs RE. 2009 Adaptation and diversification on islands. *Nature* **457**, 830–836. (doi:10.1038/nature07893)
7. Gillespie RG *et al.* 2020 Comparing adaptive radiations across space, time, and taxa. *J. Hered.* **111**, 1–20. (doi:10.1093/jhered/esz064)
8. MacArthur RH, Wilson EO. 1963 An equilibrium theory of insular zoogeography. *Evolution* **17**, 373–387.
9. Harmon LJ, Harrison S. 2015 Species diversity is dynamic and unbounded at local and continental scales. *Am. Nat.* **185**, 584–593. (doi:10.1086/680859)
10. Rabosky DL, Hurlbert AH. 2015 Species richness at continental scales is dominated by ecological limits. *Am. Nat.* **185**, 572–583. (doi:10.1086/680850)
11. Rabosky DL, Glor RE. 2010 Equilibrium speciation dynamics in a model adaptive radiation of island lizards. *Proc. Natl. Acad. Sci. USA* **107**, 22 178–22 183. (doi:10.1073/pnas.1007606107)
12. Valente LM, Phillimore AB, Etienne RS. 2015 Equilibrium and non-equilibrium dynamics simultaneously operate in the Galápagos islands. *Ecol. Lett.* **18**, 844–852. (doi:10.1111/ele.12461)
13. Lim JY, Marshall CR. 2017 The true tempo of evolutionary radiation and decline revealed on the Hawaiian archipelago. *Nature* **543**, 710–713. (doi:10.1038/nature21675)
14. Valente L, Illera JC, Havenstein K, Pallien T, Etienne RS, Tiedemann R. 2017 Equilibrium bird species diversity in Atlantic islands. *Curr. Biol.* **27**, 1660–1666. (doi:10.1016/j.cub.2017.04.053)
15. MacArthur RH, Wilson EO. 1967 *The theory of island biogeography*. Princeton, NJ: Princeton University Press.
16. Triantis KA, Economo EP, Guilhaumon F, Ricklefs RE. 2015 Diversity regulation at macro-scales: species richness on oceanic archipelagos. *Glob. Ecol. Biogeogr.* **24**, 594–605. (doi:10.1111/geb.12301)

17. Rosenzweig ML. 1995 *Species diversity in space and time*. Cambridge, UK: Cambridge University Press.
18. Burress ED, Muñoz MM. 2022 Ecological opportunity from innovation, not islands, drove the anole lizard adaptive radiation. *Syst. Biol.* **71**, 93–104. (doi:10.1093/sysbio/syab031)
19. Patton AH, Harmon LJ, del Rosario Castañeda M, Frank HK, Donihue CM, Herrel A, Losos JB. 2021 When adaptive radiations collide: different evolutionary trajectories between and within island and mainland lizard clades. *Proc. Natl. Acad. Sci. USA* **118**, e2024451118. (doi:10.1073/pnas.2024451118)
20. Blackburn DC, Siler CD, Diesmos AC, McGuire JA, Cannatella DC, Brown RM. 2013 An adaptive radiation of frogs in a Southeast Asian island archipelago. *Evolution* **67**, 2631–2646. (doi:10.1111/evo.12145)
21. Frost DR. 2021 *Amphibian species of the world: an online reference. Version 6.1 (April 23 of 2021)*. New York, NY: American Museum of Natural History. See https://amphibiansoftheworld.amnh.org/index.php
22. Hedges SB, Duellman WE, Heinicke MP. 2008 New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* **1737**, 1–182. (doi:10.11646/zootaxa.1737.1.1)
23. Dugo-Cota Á, Vilà C, Rodríguez A, Gonzalez-Voyer A. 2019 Ecomorphological convergence in *Eleutherodactylus* frogs: a case of replicate radiations in the Caribbean. *Ecol. Lett.* **22**, 884–893. (doi:10.1111/ele.13246)
24. Losos JB. 2009 *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. Berkeley, CA: University of California Press.
25. Heinicke MP, Duellman WE, Hedges SB. 2007 Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proc. Natl. Acad. Sci. USA* **104**, 10 092–10 097. (doi:10.1073/pnas.0611051104)
26. Vences M, Vieites DR, Glaw F, Brinkmann H, Kosuch J, Veith M, Meyer A. 2003 Multiple overseas dispersal in amphibians. *Proc. R. Soc. B* **270**, 2435–2442. (doi:10.1098/rspb.2003.2516)
27. Losos JB, Schluter D. 2000 Analysis of an evolutionary species–area relationship. *Nature* **408**, 847–850. (doi:10.1038/35048558)
28. Rabosky DL. 2014 Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. *PLoS ONE* **9**, e89543. (doi:10.1371/journal.pone.0089543)
29. Rabosky DL, Grundler M, Anderson C, Title P, Shi JJ, Brown JW, Huateng H, Larson JG. 2014 BAMM tools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. *Methods Ecol. Evol.* **5**, 701–707. (doi:10.1111/2041-210X.12199)
30. Morlon H, Lewitus E, Condamine FL, Manceau M, Clavel J, Drury J. 2016 RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. *Methods Ecol. Evol.* **7**, 589–597. (doi:10.1111/2041-210X.12526)
31. Triantis KA, Whittaker RJ, Fernández-Palacios JM, Geist DJ. 2016 Oceanic archipelagos: a perspective on the geodynamics and biogeography of the World's smallest biotic provinces. *Front. Biogeogr.* **8**, e29605. (doi:10.21425/F5FBG29605)
32. Nee S, Mooers AO, Harvey PH. 1992 Tempo and mode of evolution revealed from molecular phylogenies. *Proc. Natl Acad. Sci. USA* **89**, 8322–8326. (doi:10.1073/pnas.89.17.8322)
33. Jetz W, Pyron RA. 2018 The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nat. Ecol. Evol.* **2**, 850–858. (doi:10.1038/s41559-018-0515-5)
34. Pyron RA. 2014 Biogeographic analysis reveals ancient continental vicariance and recent oceanic dispersal in amphibians. *Syst. Biol.* **63**, 779–797. (doi:10.1093/sysbio/syu042)
35. Fouquet A, Pineau K, Rodrigues MT, Mailles J, Schneider JB, Ernst R, Dewynter M. 2013 Endemic or exotic: the phylogenetic position of the Martinique volcano frog *Allobates chalcopis* (Anura: Dendrobatidae) sheds light on its origin and challenges current conservation strategies. *Syst. Biodivers.* **11**, 87–101. (doi:10.1080/14772000.2013.764944)
36. Iturralde-Vinent MA. 2006 Meso-Cenozoic Caribbean paleogeography: implications for the historical biogeography of the region. *Int. Geol. Rev.* **48**, 791–827. (doi:10.2747/0020-6814.48.9.791)
37. Cornée JJ *et al.* 2020 Eocene intra-plate shortening responsible for the rise of a faunal pathway in the northeastern Caribbean realm. *PLoS ONE* **15**, e0241000. (doi:10.1371/journal.pone.0241000)
38. Glor RE. 2010 Phylogenetic insights on adaptive radiation. *Annu. Rev. Ecol. Evol. Syst.* **41**, 251–270. (doi:10.1146/annurev.ecolsys.39.110707.173447)
39. McPeek MA. 2008 The ecological dynamics of clade diversification and community assembly. *Am. Nat.* **172**, E270–E284. (doi:10.1086/593137)
40. Phillimore AB, Price TD. 2008 Density-dependent cladogenesis in birds. *PLoS Biol.* **6**, e71. (doi:10.1371/journal.pbio.0060071)
41. Condamine FL, Rolland J, Morlon H. 2019 Assessing the causes of diversification slowdowns: temperature-dependent and diversity-dependent models receive equivalent support. *Ecol. Lett.* **22**, 1900–1912. (doi:10.1111/ele.13382)
42. Rabosky DL, Lovette IJ. 2008 Density-dependent diversification in North American wood warblers. *Proc. R. Soc. B* **275**, 2363–2371. (doi:10.1098/rspb.2008.0630)
43. Etienne RS, Haegeman B. 2012 A conceptual and statistical framework for adaptive radiations with a key role for diversity dependence. *Am. Nat.* **180**, E75–E89. (doi:10.1086/667574)
44. Moen D, Morlon H. 2014 Why does diversification slow down? *Trends Ecol. Evol.* **29**, 190–197. (doi:10.1016/j.tree.2014.01.010)
45. Louca S, Pennell MW. 2020 Extant timetrees are consistent with a myriad of diversification histories. *Nature* **580**, 502–505. (doi:10.1038/s41586-020-2176-1)
46. Helmstetter AJ *et al.* 2021 Pulled diversification rates, lineage-through-time plots and modern macroevolutionary modelling. *Syst. Biol.* **71**, 758–773. (doi:10.1093/sysbio/syab083)
47. Morlon H, Robin S, Hartig F. 2022 Studying speciation and extinction dynamics from phylogenies: addressing identifiability issues. *Trends Ecol. Evol.* **37**, 497–506. (doi:10.1016/j.tree.2022.02.004))
48. Rabosky DL. 2013 Diversity-dependence, ecological speciation, and the role of competition in macroevolution. *Ann. Rev. Ecol. Evol. Syst.* **44**, 481–502.
49. Setiadi MI, McGuire JA, Brown RM, Zubairi M, Iskandar DT, Andayani N, Supriatna J, Evans BJ. 2011 Adaptive radiation and ecological opportunity in Sulawesi and Philippine fanged frog (*Limnonectes*) communities. *Am. Nat.* **178**, 221–240. (doi:10.1086/660830)
50. Rodriguez-Silva R, Schlupp I. 2021 Biogeography of the West Indies: a complex scenario for species radiations in terrestrial and aquatic habitats. *Ecol. Evol.* **11**, 2416–2430. (doi:10.1002/ece3.7236)
51. Jiménez-Ortega D, Valente L, Dugo-Cota Á, Rabosky DL, Vilà C, Gonzalez-Voyer A. 2023 Data from: Diversification dynamics in Caribbean rain frogs (*Eleutherodactylus*) are uncoupled from the anuran community and consistent with adaptive radiation. Dryad Digital Repository. (doi:10.5061/dryad.34tmpg4pq)
52. Jiménez-Ortega D, Valente L, Dugo-Cota Á, Rabosky DL, Vilà C, Gonzalez-Voyer A. 2023 Diversification dynamics in Caribbean rain frogs (*Eleutherodactylus*) are uncoupled from the anuran community and consistent with adaptive radiation. Figshare. (doi:10.6084/m9.figshare.c.6350490)