Population divergence associated with spatial asynchrony in precipitation in Neotropical frogs

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Abstract
Aim: The role that geography plays in promoting speciation by spatially isolating populations has been studied extensively, but less attention has been devoted to assessing the role of isolation by time mediated by spatial variation in climate as a mechanism promoting population divergence. Gene flow between populations differing in their reproductive schedules might be reduced relative to that between populations with overlapping breeding seasons, potentially leading to genetic divergence. Assuming that precipitation influences reproductive schedules, we explored the effect of asynchronous precipitation regimes on intraspecific genetic divergence. In addition, we examined whether microevolutionary processes are reflected in macroevolutionary patterns by assessing whether regions with more asynchronous precipitation across space have higher frog species richness and have experienced greater speciation rates than regions with more spatially synchronous precipitation regimes.

Location: Central and South America.
Taxon: Anurans: across 38 species of Neotropical frogs.
Methods: For microevolutionary analyses, we assessed the relationship between spatial asynchrony in precipitation and mitochondrial DNA genetic divergence accounting for ecological connectivity using multiple regression and phylogenetic meta-analyses. For macroevolutionary analyses, we assessed the relationship between species richness, speciation rates estimated from a comprehensive molecular phylogeny, and the degree of spatial asynchrony in precipitation using generalized linear models.

Results: We found that spatial asynchrony in precipitation is positively associated with genetic differentiation in 39% of the species tested, resulting in a weak cross-species effect. However, the effect of asynchrony in precipitation on population divergence seems not to scale to macroevolutionary patterns because spatial asynchrony in precipitation was not associated with geographical patterns of species richness nor speciation rates.

Main Conclusions: Our results indicate that asynchronous breeding may promote genetic divergence even in the absence of geographical barriers in species where breeding is associated with water availability, but such effects may not be stable enough through time to influence macroevolutionary patterns.

Keywords
allochronic, allochrony, genetic distance, phenology, seasonality, speciation
Understanding the mechanisms underlying spatial variation in biodiversity is a major goal of biogeography and evolutionary biology. The tropics harbour much of the world’s biodiversity, but a thorough understanding of the historical and evolutionary processes underlying such patterns is still lacking (Mittelbach et al., 2007). A variety of hypotheses about potential drivers of latitudinal patterns in species richness focus on mechanisms that enhance tropical rates of speciation, leading to the accumulation of biodiversity over time (Fine, 2015; Fischer, 1960). While the fundamental role that geographical barriers play in promoting speciation by spatially isolating populations has been studied extensively in the tropics and elsewhere, less attention has been devoted to assessing the role of temporal isolation as a mechanism promoting population divergence (Alexander & Bigelow, 1960; Hendry & Day, 2005; Walter et al., 2017). Namely, if neighboring populations differ in their reproductive schedules, then gene flow among them is expected to be lower than among populations with overlapping breeding seasons, which may eventually lead to the formation of new species (Martin et al., 2009; Taylor & Friesen, 2017; Wadgymar & Weis, 2017). Temporal isolation as a mechanism promoting population divergence (hereafter allochronic divergence) and subsequent speciation may often occur between separate populations (i.e. in allopatry), but may also arise within a single population (i.e. in sympatry) in cases in which disruptive selection favours distinct breeding seasons in a given area (e.g. in association with pulses in resources; Friesen et al., 2007). Although there is evidence supporting allochronic speciation across various groups of animals including insects (Santos et al., 2007; Yamamoto & Sota, 2009) and birds (Taylor & Friesen, 2017), the extent and generality of allochronic speciation remains largely unknown, and the role that evolutionary divergence due to asynchronous reproduction may play in establishing spatial patterns of diversity has seldom been considered (Martin et al., 2009).

The ‘asynchrony of seasons’ hypothesis (Martin et al., 2009) posits that spatial asynchrony in breeding seasons may promote genetic divergence among populations, potentially scaling up to influence speciation rates and thereby regional spatial patterns of species richness. In species that time their reproductive phenology tracking annual fluctuations in resource availability, populations from regions varying in patterns of climatic seasonality across space should experience spatial asynchrony in breeding phenology. This, in turn, would result in reduced temporal overlap in reproductive seasons among populations, potentially restricting gene flow, thereby promoting divergence and speciation. Thus, areas in which climate is more spatially variable should promote greater spatial asynchrony among populations in breeding schedules, which should result in greater opportunities for allopatric speciation and the accumulation of species richness (Martin et al., 2009).

The prevalence of allochronic divergence resulting from spatial variation in climatic seasonality is predicted to vary with latitude (reviewed by Martin et al., 2009). In temperate zones, organisms typically time their breeding periods in association with seasonal changes in factors such as temperature and solar radiation; because such changes are synchronous over broad spatial extents, populations at high latitudes exhibit low spatial variation in breeding phenologies, with limited potential for allochronic divergence. By contrast, tropical ecosystems exhibit only minor seasonal variations in temperature, and thus breeding seasonality depends more on resource pulses associated with temporal variation in precipitation. Because seasonality in precipitation regimes may vary substantially over space even over short distances owing to factors such as topography and airflow, the potential for asynchronous breeding and allochronic divergence is therefore greater in the tropics (Martin et al., 2009). In particular, the Neotropical region of Central and South America exhibits substantial geographical variation in precipitation regimes (Knoben et al., 2019) due to a combination of a variety of high- and low-elevation jet streams (Poveda & Mesa, 2000), complex topography (Junquas et al., 2016), the oscillation of the Intertropical Convergence Zone (ITCZ; Sierra et al., 2015) and vegetation feedbacks (Pöhler et al., 2012). Hence, the Neotropics is an ideal setting in which to test the hypothesis that spatial variation in seasonality promotes speciation via the asynchrony of breeding phenologies.

Despite the potential for geographical variation in climatic seasonality to drive population structure in the Neotropics through its effect on breeding seasons, phylogenetic and phylogeographical analyses in the region typically interpret spatial patterns in genetic variation in the context of topographic barriers such as mountains, valleys or rivers, or of historical changes in habitat connectivity influencing gene flow (Baker et al., 2020; Silveira et al., 2020; Smith et al., 2014). Exceptions are a study employing data for several species of birds, which found that genetic distance among individuals increased with the degree of precipitation asynchrony among sampling localities after controlling for ecological connectivity (Quintero et al., 2014), and recent work on a species of frog showing that population structure appears to be better accounted for by spatial variation in climatic regimes than by geographical barriers, ecological gradients or historical refugia (Thomé et al., 2021). Although spatial shifts in climatic regimes have been shown to potentially underlie phylogeographical and phylogenetic turnover of lineages in other systems (e.g. rainforest trees in Central Africa; Helmstetter et al., 2020), we are unaware of comparative studies examining the role of spatially shifting climate as a driver of population divergence within regions of the Neotropics lacking overt barriers to gene flow but experiencing distinct climates (e.g. areas North and South of the Equator where seasonality in precipitation is influenced by the ITCZ). The only study attempting to link spatial variation in breeding seasons with population divergence in the Neotropics explicitly that we are aware of documented that plant species from Neotropical cloud forests showing greater asynchrony in flowering between localities (presumably driven by climate) exhibited greater genetic structure than plants with more synchronous phenologies (Gamba-Moreno, 2020).
Given the paucity of data on population-level breeding cycles across different regions, putative evidence for population divergence presumably linked to asynchronous breeding relies on the assumption that temporal variation in precipitation reflects reproductive activity due to its effect on food availability. However, precipitation may not be the only (nor the most important) driver of breeding schedules in the tropics and any effect of precipitation on reproduction of some organisms (e.g. birds) would be indirect, via its influence on food resources (Quintero et al., 2014). Consequently, further assessments of the hypothesis that climatic asynchrony drives population divergence are needed, particularly by studying organisms in which climate and reproductive schedules are tightly linked.

Because most frogs are expected to closely match their breeding phenology to track temporal variation in precipitation patterns given their strict dependence on water availability for reproduction, they are an appropriate group to test for effects of spatial asynchrony in precipitation on population divergence. The hydric physiological restrictions of frogs, which derive mainly from their semipermeable skin and non-amniotic eggs (Lillywhite, 2006), constrain most species to tightly match their reproductive activities with rainy seasons (Kaefer et al., 2012; Saenz et al., 2006; Schalk & Saenz, 2016). Consequently, we hypothesize that frogs from populations with asynchronous precipitation regimes should show limited overlap in their breeding seasons, which may restrict gene flow, thereby promoting speciation. However, not all frog species are expected to be impacted equally by climatic variation because species vary extensively with respect to reproductive mode and on whether they may exhibit plasticity in the onset of breeding seasons triggered by environmental cues (Brooke et al., 2000). Intuitively, one might expect to find stronger effects of precipitation asynchrony on genetic differentiation in species with explosive reproduction because their breeding is temporally restricted and, therefore, temporal isolation among regions is more probable. However, if rainy periods are unpredictable, then frog species may be selected for plasticity and breed opportunistically whenever it starts raining, as in birds inhabiting unpredictable environments (Hau et al., 2004). Furthermore, one might expect that frogs laying eggs on microhabitats requiring more water would show stronger effects of precipitation asynchrony on genetic differentiation. For example, species laying eggs on vegetation need moisture for clutch development and high water levels for larvae to drop safely into ponds or streams (Gottsberger & Gruber, 2004), whereas direct-development species lay eggs in leaf litter where they are more buffered from fluctuations in moisture driven by precipitation. Therefore, analyses testing for genetic divergence linked to climatic asynchrony on a diversity of frog species occupying various habitats and exhibiting different reproductive modes have the potential to reveal the generality of this mechanism of evolutionary divergence and how it relates to the natural history of species.

We used mitochondrial DNA (mtDNA) sequences and remotely sensed climatic data to assess whether spatial asynchrony in precipitation seasonality is associated with genetic differentiation in Neotropical frogs with a variety of reproductive modes. In keeping with previous work, we tested the population-level prediction that precipitation asynchrony should have an effect on genetic distance among individuals of the same species, after controlling for the influence of barriers to dispersal (Quintero et al., 2014). In addition, we tested the macroevolutionary hypothesis that if allochronic speciation driven by climate is recurrent, then lineages inhabiting areas with higher levels of spatial asynchrony in climate should speciate more often, contributing to increased accumulation of species in such areas. All else being equal, this hypothesis predicts that the degree of spatial asynchrony in precipitation within regions should be positively related to regional speciation rates and species richness.

2 | MATERIALS AND METHODS

2.1 | Genetic divergence data

We performed a thorough search of phylogeographical studies of Neotropical frogs and retrieved information on DNA sequences with associated geographical coordinates from GenBank (see Section 3 for the number of species/sequences included). We considered species with few localities only when these were well-spaced across the known distribution range of species. We inspected intraspecific scatterplots of genetic versus geographical distances to make sure there were no highly divergent DNA sequences which may reflect identification errors (Guarnizo & Cannatella, 2013). For each species, sequences corresponded to either mtDNA regions (cytochrome oxidase I, cytochrome b, the control region, NADH dehydrogenase subunit 2) or the 16S ribosomal RNA gene. We included only one marker by species and analysed data for each species separately. To control for possible effects of variation in evolutionary rates among genes, we included the gene as a covariate in a meta-analysis combining data of multiple species (see below). We aligned sequences using the MUSCLE algorithm (Edgar, 2004) and then calculated pairwise genetic distances between all individuals under the best-fit model of nucleotide substitution for each species selected using JModelTest (Posada, 2008) based on the Akaike information criterion in the program MEGA 6.0 (Tamura et al., 2013).

2.2 | Asynchrony in precipitation

Asynchrony in precipitation refers to the time lag between peaks in precipitation at two given sites, irrespective of the magnitude of differences in total precipitation between sites. For example, a locality with a precipitation peak occurring in June has greater asynchrony relative to a locality with a precipitation peak in December than relative to a locality where precipitation peaks in April (Figure 1). Maximum asynchrony occurs between sites with shifted precipitation regimes such that when one site experiences its peak precipitation, the other one experiences its nadir in precipitation (Quintero et al., 2014).
To estimate asynchrony in precipitation between sites, we followed the approach used by Quintero et al. (2014). We used remotely sensed cloud-cover data to characterize precipitation seasonality based on a public database integrating 15 years of twice-daily remote-sensing cloud observations (MODIS satellite) at 1-km² resolution (Wilson & Jetz, 2016). We acknowledge that this is a short-term database and that the processes we are investigating are more likely to occur along longer time spans. However, to our knowledge, there are no alternative databases to evaluate historical precipitation asynchrony.

Using Fourier transformations, we determined whether each locality experienced a discernible annual (one-peaked) or biannual (two-peaked) rainfall pattern by contrasting the fit of sinusoidal curves with these periodicities to that of a null model. We discarded localities lacking a significant periodic component (i.e., localities with uniform precipitation throughout the year). Next, we estimated the lag between the peaks of the sinusoidal curves that best described seasonality among all localities. The maximum lag is 6 months for annual curves and 3 months for biannual curves. The estimated lag in peaks was our measure of precipitation asynchrony between localities.

### 2.3 | Accounting for ecological connectivity

We estimated the effect of precipitation asynchrony on genetic divergence while accounting for other factors known to affect population differentiation, namely geographical distance and barriers to dispersal. We used species distribution models to estimate likely dispersal paths given species-specific niche preferences between georeferenced localities, which we then accounted for when assessing the relationship between precipitation asynchrony and genetic differentiation (Quintero et al., 2014). We first obtained geographical coordinates for collecting localities of museum specimens of each species from the Global Biodiversity Information Facility (https://www.gbif.org) and VertNet (http://vertnet.org). We vetted localities by removing coordinates outside range maps defined by experts and the known elevational limits for each species (http://www.iucnredlist.org/). Because range maps constructed based on expert knowledge are reliable at resolutions equal or higher than ca. 1° longitude/latitude (Hurlbert & Jetz, 2007), we incorporated a buffer of 0.5° longitude/latitude around map polygons and discarded georeferenced records located outside. We complemented the clean localities with the coordinates associated with DNA data and used to estimate the asynchrony in precipitation. To build species distribution models, we used bioclimatic variables from WorldClim (Hijmans et al., 2005), which describe different aspects of annual temperature and precipitation. Bioclimatic layers that were highly correlated (>70%) were excluded from model estimations using a variance inflation factor analysis in the package ‘USDM’ in R (Naimi, 2015). Subsequently, we estimated a species distribution model for each species using MaxEnt v3.3.3k (Phillips & Dudík, 2008) with 10,000 background points and evaluating model performance with cross-validation using 20% of the data. In addition to the coordinates from VertNet, we also included coordinates from the genetically verified locality records to build the models (see Table S1 for details on model inference). Finally, we used the inverse of environmental suitability for each species inferred by the models as an ecological resistance matrix from which we estimated the ecological connectivity among localities (i.e., least-cost path distances) using the ‘gdistance’ package for R (van Etten, 2017). We also calculated linear geographical distances (taking into account Earth’s curvature) using the program Geographic Distance Matrix Generator (Ersts, 2011).

### 2.4 | Data analyses at intraspecific level

To assess the effect of asynchrony in precipitation, geographical distance and ecological connectivity on genetic divergence, we used multiple-matrix regression with randomization (MMRR), an...
FIGURE 2. Top: Beta regression coefficients describing the relationship between asynchrony in precipitation and genetic distances, after controlling for the effect of ecological connectivity employing paths of least resistance in 38 species of Neotropical frogs. Black dots indicate a significant association between asynchrony in precipitation and genetic distances \((p < 0.05)\) and white dots indicate non-significant associations between these two variables. Bars indicate 95% confidence intervals. The mean effect of precipitation asynchrony across species using a meta-analysis (horizontal black line) indicates a positive relationship between asynchrony in precipitation and genetic distances. The grey thick horizontal line corresponds to the effects estimated across all of the 100 trees and their confidence intervals (see text). The red reference line corresponds to zero. Bottom: Beta coefficients of the multiple regression (MMRR) between ecological connectivity (least-cost path distances) and genetic distances within species, after controlling for the effect of precipitation asynchrony. Black dots indicate a significant association between asynchrony in precipitation and genetic distances \((p < 0.05)\) and white dots indicate non-significant associations between these two variables. Bars indicate 95% confidence intervals. The horizontal red line corresponds to zero and the black line to the mean beta coefficient for ecological connectivity.
approach tailored to deal with the non-independence of pairwise distance estimators (Wang, 2013). All distances were standardized (mean = 0, variance = 1) to allow comparison among regression coefficients (Figure 2). We performed MMRR separately on each species using the R script provided by Wang (2013) with 10,000 permutations. Because Dendropsophus minutus is a species complex, we did not analyse it as a single species, but separately based on the highly divergent mtDNA lineages recovered by Gehara et al. (2014).

We evaluated an overall, cross-species effect of asynchrony in precipitation on genetic divergence using a phylogenetic meta-analysis (Adams, 2008), where the effect estimated separately for each species was inversely weighted by its associated standard error (i.e. less weight was given to estimates with higher uncertainty) while accounting for phylogenetic covariance structure. We sampled 100 amphibian phylogenetic trees from the posterior distribution of Jetz and Pyron (2018), a species-complete phylogeny built using sequence data for 15 genes (5 mitochondrial and 10 nuclear) for 4061 amphibians and with the rest of the species imputed using taxonomic constraints. For the analysis, we pruned all species not considered in our study. For each tree, we estimated this cross-species effect on genetic divergence using Phylogenetic Generalized Least Squares with simultaneous optimization of Pagel’s Lambda under restricted maximum-likelihood using the gls() function of the ‘nlme’ package (Pinheiro et al., 2014) for R (R Core Team, 2020). We included the genetic marker as a covariable in the meta-analysis to accommodate that variation among markers in substitution rate may affect the detection of effects of asynchrony on divergence. Also, we included two variables describing the area encompassed by localities because precipitation asynchrony may affect more those species occupying large areas over which climate may be more variable (Quintero et al., 2014). We calculated the perimeter occupied by the localities used for the asynchrony estimation for each species and the maximum linear distance among these using functions of the ‘geosphere’ (Hijmans, 2019) and ‘raster’ (Hijmans et al., 2015) packages. We also included the topographic heterogeneity within species ranges as a covariable, considering that species inhabiting more complex topographic areas might experience greater potential for divergence owing to precipitation asynchrony because of the effect of topography on climate. In the next section, we explain how we generated the topographic heterogeneity data.

2.5 Species richness and speciation rates

Because the asynchrony of seasons hypothesis posits that seasonal asynchrony in precipitation promotes population differentiation that might scale up to the origin of new species and accumulation of species in space, we predicted regions with higher spatial asynchrony in precipitation to have higher speciation rates and, all else being equal, a greater number of species than areas with more spatially uniform precipitation seasonality. We tested these predictions in the Neotropical region (xmin = -118.1333, xmax = -34.7999, ymin = -54.39167, ymax = 33.10834), dividing the region in quadrats of 5 x 5 decimal degrees (grid size of −550 x 550 km), a grain size large enough to enable within-quadrat speciation (Kisiel & Barraclough, 2010). We estimated the asynchrony in precipitation, species richness and speciation rates within each of these quadrats. In the species richness model, we also included productivity and topographic heterogeneity as covariates because these are key factors explaining spatial richness patterns in frogs (Buckley & Jetz, 2007).

We estimated species richness as the sum of all overlapped expert-based maps for anuran species (IUCN, 2018) within each 5 x 5 decimal-degree quadrat using the ‘raster’ (Hijmans et al., 2015) and ‘maptools’ (Bivand & Lewin-Koh, 2019) R packages. We measured the degree of spatial asynchrony in precipitation within quadrats based on variation in the MODCF seasonality theta parameter (Wilson & Jetz, 2016), which estimates the timing of peak cloudiness in a grid cell as a circular variable ranging from 0 (peak cloudiness on January 1st) to 360 (peak cloudiness on December 31st). Because day of year is a circular variable, we estimated the circular variance of theta values across all the 1-km2 cells contained within each 5 x 5 decimal-degree quadrat as our measure of within-region spatial asynchrony in precipitation. The circular variance measures the variation in the angles about the mean direction, ranging from 0 when all data are concentrated at one point (the precipitation peak is at the same time across grid cells within the quadrat) to 1 when the data have broad spread (precipitation peaks are distributed throughout the year within the quadrat). We estimated the circular variance using package ‘circular’ (Agostinelli & Lund, 2017).

To assess frog speciation rates within regions, we used the DR metric (Jetz et al., 2012), which approximates speciation rates under a pure-birth model of diversification and is best interpreted as an estimate of recent speciation rates (Title & Rabosky, 2019). We calculated the DR metric for each species as the median DR across a sample of 100 posterior phylogenetic trees published by Jetz and Pyron (2018). We mapped speciation rates on geography using the harmonic mean value of DR for all species present on each quadrat.

Net primary productivity was obtained from the productivity layer at 0.25° resolution generated by SEDAC (Imhoff et al., 2004) (http://sedac.ciesin.columbia.edu/data/set/hanpp-net-primary-productivity), and we calculated mean values for each of the quadrats in our 5 x 5 decimal degree grid (Figure S1). This variable is measured in grams of carbon per year, obtained by applying the Carnegie-Ames-Stanford Approach terrestrial carbon model to the 17-year average of maximum monthly Normalized Difference Vegetation Index and other climatological data (Imhoff & Bounoua, 2006). Topographic heterogeneity was estimated as the variance in elevation across 1-km2 grid cells within each quadrat using the data from STRM (Figure S2; Farr et al., 2007).

2.6 Data analyses at interspecific level

We ran generalized linear models (GLM) with Gaussian-distributed errors to examine the relationship between asynchrony in
precipitation and the logarithm of speciation rates, and with Poisson-distributed errors to examine the relationship between asynchrony in precipitation and species richness, using net primary productivity and topographic heterogeneity as covariates. We performed GLM using the `glm()` function in R. We used a Lambert-Azimuthal equal-area projection centered in South America’s centroid (lat = −16.1912568, lon = −68.90625) to give the same weight to values representing areas with different size. To account for spatial autocorrelation, we used the residual autocovariates (RAC) GLM following Crase et al. (2012), which have shown unbiased coefficients using simulated data. The RAC specifies the relationship between the value of the residuals at a given location and those at neighbouring locations. We used the residuals of each GLM model to calculate the RAC as the focal mean value on the first-order neighbourhood, and then included it in the model as covariate. The focal mean value was calculated with `focal()` function of ‘raster’ package. We tested the presence of spatial autocorrelation in our data using Moran’s I with the `lm.morantest()` function in package ‘spdep’ (Bivand et al. 2013) for all models.

3 | RESULTS

We evaluated information in ca. 90 papers on Neotropical phylogeography and phylogenetics. After removing species lacking information on geographical sampling or species occupying landscapes lacking significant periodicity in precipitation patterns, we considered 38 species in 13 genera of Neotropical frogs in our study (Table S1). Of these 38 species, 13 exhibit aquatic breeding, 18 terrestrial breeding and 7 are direct developers (Table S5).

In more than 90% of the species, geographical distance and ecological connectivity were highly correlated (Pearson correlation > 0.8). Because MMRR and other multiple regression approaches cannot discriminate separate effects due to collinearity (Wang, 2013), we chose to use ecological connectivity (i.e. least-cost distance) to describe the effect of geography on genetic distances. Ecological connectivity explained most of the variation in genetic distances within species (Figure 2), with significant effects in 73% of species (68% after Bonferroni correction). Nonetheless, precipitation asynchrony was positively associated with genetic distances in 39% of species (29% after Bonferroni correction; Figure 2; Figure S3; Table S2), and negatively associated in 10% of the species (5% after Bonferroni correction).

The phylogenetic meta-analysis revealed a significant albeit weak cross-species effect of precipitation asynchrony on genetic distances across species (phylogenetic inverse-variance-weighted standardized average effect across 100 phylogenetic trees = 0.067 and range = [0.055–0.086], average standard error [SE] = 0.005, SE range = [0.001–0.023]). We did not find an effect of any of the covariates included in the models: the genetic marker used to measure divergence, the area encompassed by localities and topographic heterogeneity in the range of species did not influence the relationship between genetic divergence and asynchrony in precipitation (Table 1; Figure S4).

To examine the relationship among precipitation asynchrony, speciation rates and species richness at the regional level, we collected geographical and phylogenetic data for 2438 frog species occurring in our study area (Table S3). We found no relationship between spatial asynchrony in precipitation and mean speciation rates (Figure 3; glm: slope = 0.008, SE = 0.046, p = 0.86; Moran’s I: 0.006, p = 0.22), nor between spatial asynchrony in precipitation and species richness after accounting for net primary productivity and topographic heterogeneity (Figure 3; glm: slope = 0.039, SE = 0.054, p = 0.466, complete model in Table S4; Moran’s I: 0.022, p = 0.1517). In this latter model, the covariates (primary productivity and topographic heterogeneity) showed a significantly positive, albeit weak, relationship with species richness (Table S4).

**Table 1** Medians of parameters estimated for the variables included in the meta-analysis performed across 100 trees.

| Response | Variable | Value | SE | p value | Lambda |
|----------|----------|-------|----|---------|---------|
| Effect of precipitation asynchrony on genetic divergence | Cross-species average | 0.0667 | 0.0048 | 1.82E-15 | −0.4234 |
| | Effect of haplotype diversity | 0.0144 | 0.037 | 0.7018 | −0.393 |
| | Effect of nucleotide diversity | 0.0396 | 0.0508 | 0.4166 | −0.4191 |
| | Effect of maximum distance | 0.0374 | 0.0271 | 0.1774 | −0.42 |
| | Effect of perimeter | 0.0522 | 0.0301 | 0.0944 | −0.3636 |
| | Effect of topographic heterogeneity | −0.0142 | 0.0226 | 0.5344 | −0.4071 |
| Effects of gene type | 16S | 0.011 | 0.0508 | 0.8315 | −0.4009 |
| | CO1 | −0.0143 | 0.0911 | 0.8241 | −0.4009 |
| | CR | 0.0193 | 0.192 | 0.9162 | −0.4009 |
| | Cytb | 0.0812 | 0.3398 | 0.8135 | −0.4009 |
| | ND2 | −0.2354 | 0.2659 | 0.386 | −0.4009 |

Note: The meta-analysis aims to explain variation in the effects of precipitation asynchrony on genetic distance across 38 species by assessing the relationship with species traits hypothesized to have an effect, while accounting for phylogenetic relationships and species-level uncertainty.
4 | DISCUSSION

4.1 | Overview

Although aspects of breeding biology are largely unknown for many Neotropical species, seasonal variation in precipitation generally correlates with reproductive activity in frogs, including some of the species we studied (Donnelly & Guyer, 1994; Kaefer et al., 2012; Moreira & Lima, 1991). Assuming that precipitation is a proximate surrogate for breeding activity in frogs, our study advances the understanding on the importance of reproductive timing as a factor promoting lineage divergence at two different evolutionary scales: intraspecific genetic divergence and speciation. The positive association between spatial asynchrony in precipitation and genetic distance found across 15 species (Figure S4) is consistent with the hypothesis that, if spatial variation in water availability causes spatial asynchrony in breeding activity, then regions with asynchronous precipitation regimes should experience reduced gene flow among populations. This pattern led to a statistically significant— albeit weak— cross-species effect of asynchrony in precipitation on genetic distance using a phylogenetic meta-analysis that considered 38 species. However, our results suggest that population divergence potentially resulting from asynchronous phenologies linked to climate does not seem to scale up to drive macroevolutionary patterns nor the accumulation of regional diversity because spatial variation in recent rates of species origination and species richness were not associated with spatial variation in precipitation asynchrony.

4.2 | Natural history of species and the role of climatic asynchrony as a driver of divergence

For the asynchrony of seasons hypothesis to hold, a given species should exhibit discontinuous breeding throughout the year, the onset of its breeding periods should depend on climate (e.g. on precipitation) and climatic regimes within its range should be spatially asynchronous. Therefore, a close look at the natural history of organisms and at the environments they inhabit may help identify which species are most expected to experience population divergence in association with asynchrony in precipitation and which are not. For example, frog species from temperate regions that use photoperiod instead of precipitation as a cue to initiate breeding (Canavero & Arim, 2009) are not expected to experience sufficient temporal isolation between populations to exhibit genetic divergence derived from asynchronous breeding across space (Martin et al., 2009). Below we discuss some of our results in light of the natural history of our study species and the areas where they occur.

For some of the species in which we found a strong effect of precipitation asynchrony on genetic distances, a potential link between asynchronous breeding phenology and population structure may be reasonably expected given their life histories and geographical ranges. For example, Leptodactylus mystaceus is a seasonal breeder (Caldwell & Lopez, 1989) that uses precipitation as a cue to time its breeding period (Ulloa et al., 2019) and lives in a region with highly heterogeneous precipitation regimes but no major geographical barriers (Knoben et al., 2019). Therefore, asynchronous precipitation peaks may serve to temporally isolate genetically distinct populations of L. mystaceus from the Guianas— where precipitation is bimodal with peaks in December and June— from those of northern Amazonian Brazil, where precipitation is unimodal, with a peak in March and a dry period with a peak in August (Knoben et al., 2019) (Figure S4). In other species inhabiting the same region, and thus experiencing similar asynchrony patterns, such as Ameerega trivittata, Hypsiboa semilineatus and Adenomera hylaedactyla, we also found a significant effect of precipitation asynchrony on genetic distance. Nonetheless, our observation of greater genetic distances associated with more asynchronous precipitation does not imply a causal relationship because these species range over a broad area (much of Amazonia), and disentangling the effects of precipitation asynchrony from those of topography or large rivers on genetic divergence is challenging. Another noteworthy case is that of Craugastor podociferus, which inhabits
a region where precipitation regimes appear less heterogeneous across space (Knoben et al., 2019) than in the ranges of other species, yet shows a moderate significant association between precipitation asynchrony and genetic distances.

Another factor which may explain variation across species with respect to whether precipitation asynchrony is associated with genetic divergence is the tremendous diversity of reproductive modes existing among tropical frogs (Haddad & Prado, 2005). For example, explosive breeders are usually found in dry habitats where water is scarce during most of the year; after heavy rains, individuals congregate and mate in recently formed water bodies during one or a few nights. In such species, one might expect particularly strong effects of climatic asynchrony in population divergence. Accordingly, in the explosive breeder Rhinella granulosa, genetic structure appears to be better explained by asynchrony in breeding seasons between southern and northern regions of Brazil than by geographical barriers or by historical habitat stability (Thomé et al., 2021). By contrast, prolonged breeders mate throughout the year and usually inhabit sites maintaining humidity through time (Touchon & Warkentin, 2008; Wells & Schwartz, 2006). For instance, populations of three species of Pristimantis (not included in our study) from the Colombian Andes, despite living in areas with heterogeneous climate, mate throughout the year and show no geographical variation in breeding seasons (Granados-Pérez & Ramirez-Pinilla, 2020).

Although we expected that reproductive mode and microhabitat would mediate the association between precipitation and breeding time, species in which we encountered a positive and significant relationship between asynchrony in precipitation and genetic distances belonged to a variety of frog families characterized by different reproductive modes (Bufonidae, Leptodactylidae, Craugastoridae Dendrobatidae, Hylidae and Aromobatidae). For instance, among the 15 species showing a positive association between asynchrony in precipitation and genetic distance were frogs with direct development (C. podociferus), aquatic eggs (R. margaritifera, R. ornata, Dendropsophus minutus), eggs in water tanks on epiphytic plants (Anomaloglossus baebiatus) and foam nests built on the ground (L. mystaceus, Ad. thomei, Ad. heyeri, Ad. hylaedactyla, Engystomops petersi). Therefore, populations of species with particular reproductive modes (Table S5) do not appear more likely to diverge owing to spatial asynchrony in precipitation. We also found in 5% of the species a negative and significant relationship between precipitation asynchrony and genetic divergence (see also Quintero et al., 2014 for similar examples in birds). This result is unexpected, and we cannot explain why precipitation synchronicity would promote divergence. Perhaps unconsidered factors, such as other sources of local adaptation, are promoting divergence in these few species.

In sum, we found that spatial asynchrony in precipitation is linked to genetic distance across populations in some Neotropical frog species, but there is much to be learned about whether and how such variables may be casually linked via the influence of precipitation on breeding seasonality. For instance, we can only measure present-day precipitation asynchrony, but genetic differentiation results from population isolation over larger temporal scales. Furthermore, we used mtDNA to measure genetic divergence and this marker does not necessarily correspond to that of the nuclear genome and may not reflect divergence history among populations. Examples like those mentioned above serve to illustrate that work aiming to test additional predictions of the hypothesis that asynchronous climates promote population divergence owing to asynchronous breeding are required to unravel the mechanisms involved in the patterns we uncovered at the organismal level.

4.3 | Mechanisms of evolutionary divergence and the origins of tropical diversity

Our multiple regression models indicated that the effect of ecological connectivity on within-species genetic distances was, on average, three times larger than the effect of asynchrony in precipitation. This result confirms the substantial role that spatial isolation (particularly in topographically heterogeneous regions) has on the genetic differentiation of Neotropical amphibians. Nonetheless, we found that in some species spatial asynchrony in precipitation had a substantial, and possibly causal, association with genetic distance. Therefore, the influence of geographical variation in climate and its role as a driver of population divergence via its influence on breeding seasons should be considered in future work assessing the build-up of Neotropical diversity.

A salient conclusion of our work is that support for the asynchrony of seasons hypothesis in Neotropical frogs seems to be observable only at a microevolutionary scale because asynchrony in precipitation was associated positively with genetic distances, but not with speciation rates nor with species richness. This may reflect temporal instability of spatial patterns in precipitation driven by factors such as orbital precession (Merlis et al., 2013), climatic change associated with glacial cycles (Whitney et al., 2011) or climate modulation caused by the uplift of the Andes (Insel et al., 2010). Such historical events could impede speciation processes by destabilizing geographical patterns in precipitation asynchrony which promote intraspecific differentiation across specific Neotropical frog populations. A similar explanation involving climatic instability has been advanced to account for the lack of evidence of parapatric speciation across habitat ecotones despite numerous cases of adaptive divergence of populations in such ecological settings (Smith et al., 2005). Unfortunately, we cannot assess the effect of such climatic dynamics because historical data on fine-grained spatiotemporal variation of precipitation regimes are unavailable.

More broadly, our results show that spatial asynchrony in precipitation is associated with divergence at the population level but not with increased recent speciation rates or species richness of Neotropical frogs, which is consistent with studies showing no association between characteristics of species thought to promote speciation and macroevolutionary rates. For example, recent speciation rates in various groups estimated using phylogenetic methods are unrelated to factors such as the rates at which current populations...
evolve premating barriers (Freeman et al., 2022), postzygotic reproductive isolation (Rabosky & Matute, 2013) or genetic isolation (Singhal et al., 2018). However, the observed decoupling between factors involved in speciation and present-day patterns resulting from macroevolutionary processes might also suggest that other factors are at play, such as species extinction rates or the ability of populations to establish persistent populations in dynamic environments (Singhal et al., 2022). Understanding the links between microevolutionary processes and macroevolutionary patterns is therefore an important area for speciation research.

Historically, topography and temperature gradients have been the main variables considered when studying patterns of genetic variation and diversification of frogs. Our results suggest that genetic divergence can be promoted in the absence of geographical barriers or temperature gradients in species where breeding pathology is associated with water availability. As information on reproductive modes, population-level breeding seasons and current and past precipitation patterns becomes available through remote sensing and public databases, our ability to measure phenological shifts and their dependence on environmental cues will considerably advance our understanding on how precipitation asynchrony promotes genetic divergence and diversification in the Neotropics.

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CONFLICT OF INTEREST

The authors declare they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

DNA sequence alignments, geographical coordinates and scripts for analyses are deposited in the Zenodo Digital Repository (https://doi.org/10.5281/zenodo.6399145).

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BIOSKETCH

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**Author contributions:** Carlos E. Guarnizo, Paola Montoya, Ignacio Quintero and Carlos Daniel Cadena conceived the ideas, designed the methodology and analysed the data; Carlos E. Guarnizo and Paola Montoya collected the data; Carlos E. Guarnizo and Paola Montoya led writing the manuscript. All authors contributed critically to the manuscript and gave final approval for publication.

**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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