**LETTER**

## Using historical biogeography to test for community saturation

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### Abstract

Saturation is the idea that a community is effectively filled with species, such that no more can be added without extinctions. This concept has important implications for many areas of ecology, such as species richness, community assembly, invasive species and climate change. Here, we illustrate how biogeography can be used to test for community saturation, when combined with data on local species richness, phylogeny and climate. We focus on a clade of frogs (Terrarana) and the impact of the Great American Biotic Interchange on patterns of local richness in Lower Middle America and adjacent regions. We analyse data on species richness at 83 sites and a time-calibrated phylogeny for 363 species. We find no evidence for saturation, and show instead that biotic interchange dramatically increased local richness in the region. We suggest that historical biogeography offers thousands of similar long-term natural experiments that can be used to test for saturation.

### Keywords

Amphibians, biogeography, community assembly, dispersal, Great American Biotic Interchange, local richness, phylogeny, saturation, Terrarana.

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## INTRODUCTION

Saturation is a fundamental concept in ecology (e.g. Elton 1950; MacArthur & Wilson 1963; Terborgh & Faarborg 1980), with far-reaching and urgent implications. Saturation occurs when a community reaches a limit on the number of species that it can sustain of a given type (i.e. of a given clade or ecological guild), such that no more can be added without others going locally extinct. Saturation is critically important to many areas of ecology. For example, saturation is relevant to understanding many species richness patterns, including local and regional richness (e.g. Cornell & Lawton 1992; Caley & Schluter 1997; Harrison & Cornell 2008) and richness of clades (e.g. constant richness in clades over time due to limited resources; Rabosky 2009). Saturation is also essential to community assembly, and to the potential invasion of natural communities by exotics (e.g. MacDougall *et al.* 2009). Finally, saturation may be relevant to species persistence under climate change, given that many species are shifting their ranges (e.g. Chen *et al.* 2011) and may be forced to invade new communities to avoid extinction (e.g. HilleRisLambers *et al.* 2013). Thus, saturation might determine whether such species will persist under changed climatic conditions.

Community saturation can be tested in many ways. First, experimental studies can add new species to a community and test whether new and incumbent species can co-exist (e.g. Myers & Harms 2009). However, this approach is necessarily limited to relatively short time scales, much shorter than the scales at which many species richness patterns are generated, and may be problematic for natural communities. Second, studies of invasive species have documented that invasives rarely drive native species to extinction through competition alone, and instead tend to increase regional richness (e.g. Sax & Gaines 2008). Human-mediated invasions may also increase local richness (e.g. Smith *et al.* 2004). Third, many studies have tested the relationship between local and regional richness across several regions (e.g. Harrison & Cornell 2008). If local richness plateaus as regional richness increases, this is traditionally interpreted as indicating that communities in those high-richness regions are saturated, despite the large regional species pool from which more species could be added (e.g. Cornell & Lawton 1992; Caley & Schluter 1997). In contrast, a strong relationship between local and regional richness would potentially reject saturation, and this latter pattern is often supported (e.g. Caley & Schluter 1997; Harrison & Cornell 2008). However, many factors might influence this relationship besides saturation (e.g. Loreau 2000; Mouquet *et al.* 2003; Stephens & Wiens 2003). A fourth, similar approach tests if regions inhabited longer by the group have higher local richness, using biogeographic inferences from time-calibrated phylogenies. A positive relationship between time and local richness implies that communities are not saturated (e.g. Wiens *et al.* 2011; Kozak & Wiens 2012; Hutter *et al.* 2013). A fifth approach involves examining the impact of biotic interchanges on regional species pools using fossil data (e.g. Vermeij 1991; Patzkowski & Holland 2007; Tilman 2011). These paleontological studies suggest that such exchanges rarely cause extinctions and instead tend to increase regional richness in invaded areas.

Here, we illustrate a new approach to testing for saturation, combining historical biogeography with data on local species richness. We use phylogeny-based inferences of biogeography (i.e. historical biogeography) to identify lineages that have dispersed between regions. We then analyse patterns of local species richness to determine whether invasion of a region by lineages from another region significantly increased local richness in invaded communities (a pattern rejecting saturation; Fig. 1). Our approach differs from experimental approaches and analyses of human-mediated invasions in addressing invasions in natural systems over long time scales (i.e. millions of

years). Our approach differs from paleontological studies of biotic interchange in that we address local species richness. Importantly, without considering local richness, invading species may have limited co-occurrence with incumbent species, and so community saturation is not tested directly. Furthermore, by using data on extant communities, other confounding factors can be tested (e.g. variation in climatic conditions, differing ages of communities).

We illustrate our approach by analysing local richness and historical biogeography in terraranan frogs, in association with the Great American Biotic Interchange (GABI) between North and South American landmasses. Terrarana is a species-rich Neotropical clade (~ 980 species, ~ 15% of all frog species; AmphibiaWeb 2013) that includes the species formerly placed in *Eleutherodactylus* (until recently among the largest vertebrate genera). Terraranan species are now divided among ~29 genera and the families Brachycephalidae, Ceuthomantidae, Craugastoridae (including Strabomantidae) and Eleutherodactylidae (Pyron & Wiens 2011; AmphibiaWeb 2013). Terraranans share direct development and have major



**Figure 1** Figure illustrating the hypothetical impact of biotic interchange or biogeographic colonisation on local richness. When communities are saturated, clades from the two regions co-occur in some communities, but local richness is not higher in these mixed communities. When communities are not saturated, local richness is significantly higher in those communities in which species from the clades from the two regions co-occur. Note that local richness is similar between regions in this hypothetical example, but need not be in empirical analyses. In cases of asymmetry, saturation is rejected if richness is higher in communities where clades from the two regions co-occur, relative to local richness in unmixed sites from both the species-poor and species-rich regions (and after controlling for climate and other factors). Saturation would potentially be supported if local richness in mixed communities was similar to local richness in unmixed communities of the more species-rich region.

centres of diversification in South America, Middle America (Mexico to Panama) and the West Indies (Hedges *et al.* 2008). On average, they make up > 40% of all frog species at local sites where they occur (see Results).

The GABI is an important model system for biogeographic analyses (e.g. Weir *et al.* 2009; Cody *et al.* 2010; Smith & Klicka 2010; Pinto-Sánchez *et al.* 2012; Leigh *et al.* 2014; earlier literature reviewed by Marshall *et al.* 1982). However, previous studies have not used this system to test for local community saturation (although Marshall *et al.* (1982) analysed continent-scale saturation using mammalian families). It is often thought that the North and South American landmasses were separated during most of the Cenozoic, but reconnected in the last ~ 3 million years (e.g. Coates & Obando 1996). However, some geological and molecular evidence suggests an earlier reconnection (~ 15 million years; Montes *et al.* 2012), and biotic interchange > 3 million years ago (e.g. Cody *et al.* 2010). Regardless, there were ancient radiations of terraranans in both Middle and South America (e.g. Crawford & Smith 2005; Heinike *et al.* 2007), which moved between regions more recently as part of the GABI (e.g. Pinto-Sánchez *et al.* 2012).

Here, we use terraranan frogs as a case study to test for community saturation where lineages from South and Middle America co-occur. We combine data on local richness and climate with biogeographic inferences from a new large-scale, time-calibrated phylogeny (363 species). First, we test whether local richness patterns are explained by climate or by the timing of colonisation of different regions across the range of terraranans. We then focus on species richness and composition at local sites in Middle and South America. Using our biogeographic inferences, we assign species in each community as being from radiations in Middle vs. South America. We then test whether local richness is significantly higher in communities where species are derived from both regions, vs. those containing species from only one region, a pattern that would reject saturation of local communities (Fig. 1). In contrast, saturation would be supported if species richness were similar between these two community types (Fig. 1), or at least not significantly increased (richness need not be symmetrical between regions before invasion). We also test whether species richness of Middle and South American clades is significantly lower in communities where they occur together, a potential signature of competition on local richness patterns that is less extreme than community saturation.
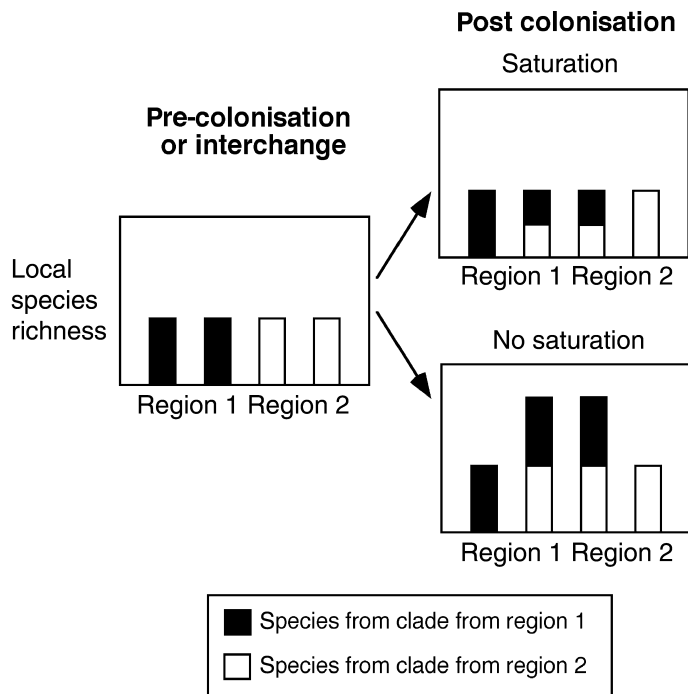
## MATERIALS AND METHODS

### Species richness of local sites

We obtained data on local species composition and richness for 83 sites across the range of Terrarana (Tables S1, S2). To search for sites, we began with the 123 sites analysed by Wiens *et al.* (2011) for hylid frogs, which targeted sites a few km$^2$ in size or less that were the subject of multi-year amphibian surveys (but included many sites outside the range of terraranans). We used 17 of these sites, and searched the literature for additional sites, especially in Middle and South America. Species lists for 78 sites were obtained from published accounts, and data for five sites were from unpublished

species lists from extensive, multi-year surveys (Table S1). To ensure that local sites included potentially co-occurring species, we used only localities that represented a single major habitat type (e.g. lowland rainforest, montane cloud forest), and an elevational range of < 500 m.

Sites included multiple microhabitats, but individuals may move between these microhabitats daily (e.g. ground by day, vegetation at night; Miyamoto 1982), suggesting that finer subdivision of sites would be problematic. Nevertheless, some species within a given site might not interact (e.g. due to microhabitat differences), but our study focuses on comparisons between sites, not microhabitats within sites.

Local richness data were ln-transformed for all analyses to improve normality. Normality tests and alternative analyses using the raw data are presented in Appendix S1.

Some sites differed in area, and some lacked explicit information on the area surveyed. Given that area might influence richness (e.g. MacArthur & Wilson 1963), we regressed richness and area for those 39 sites with relevant data (Table S1). We initially eliminated two sites of exceptional size (107 800–860 000 ha) but limited richness to avoid biasing this analysis against a size-richness relationship. Other sites ranged from 0.058 to 48 600 ha. Richness was not significantly related to area (richness and area ln-transformed: $r^2 = 0.005$, $F = 0.178$, $P = 0.676$; including all 39 sites: $r^2 = 0.000$, $F = 0.016$, $P = 0.900$). Therefore, we did not account for area in subsequent analyses, and no sites were excluded. Unless otherwise noted, statistical analyses utilised JMP version 9.0.1 (Sall et al. 2005).

Similarly, richness might be related to sampling effort. However, analysing the 39 sites with relevant data (Table S1) showed no relationship between sampling effort and richness (ln-transformed variables: $r^2 = 0.006$, $F = 0.229$, $P = 0.635$).

### Climatic data for local sites

We first tested the hypothesis that local richness is correlated with climate. We extracted data for 19 climatic variables from all 83 georeferenced sites (Table S3) using ArcView GIS 3.3 and the WorldClim database with $\sim 1\ km^2$ resolution (Hijmans et al. 2005). We extracted climatic data from the site's same $\sim 1\ km^2$ pixel or the pixel closest to the site's geographic centre.

To summarise the 19 variables, we conducted a principal components analysis (PCA) using a correlation matrix. We used broken stick analysis (Jackson 1993) to select PCs to retain (PCs 1–4 explained more variation than expected by chance). PCA results, variable loadings and PC1–4 scores for each locality are given in Tables S4–S6. We also performed analyses using only annual mean temperature (Bio1) and annual precipitation (Bio12), two obvious descriptors of climate. We then used linear regression to test relationships between local richness and Bio1, Bio12 and PC1–PC4.

### Phylogeny and divergence times

Testing hypotheses about historical biogeography required a time-calibrated phylogeny. We generated a new phylogeny based on published sequence data in Pyron & Wiens (2011)

and Pinto-Sánchez et al. (2012); for details see Appendix S2. The final matrix contained 363 ingroup species and 12 712 aligned, concatenated base pairs from three mitochondrial and nine nuclear genes, although many species lacked data for some genes (Table S7). Sampling included all terraranan families and 13 genera, with dense species sampling in Lower Middle America. The primary goal of our phylogenetic sampling was to infer the biogeographic origin of the five genera involved in GABI. Therefore, including all terraranan species and genera was not necessary. Relationships and divergence times were estimated using the Bayesian uncorrelated lognormal approach (Drummond et al. 2006) in BEAST version 1.7.5 (Drummond & Rambaut 2007). See Table S8 for calibration points and Fig. S1 for the tree.

### Ancestral areas and the time-richness relationship

We used ancestral area reconstruction to estimate the region of origin of clades involved in GABI (see below), and to test if local richness is higher in regions colonised earlier (both a test for saturation and potential confounding factor). We also estimated the number, direction and timing of dispersal events between South and Middle America (i.e. GABI). Biogeographic shifts among eight regions (see below) were estimated using maximum likelihood with LAGRANGE version 20130526 (Ree & Smith 2008). For details see Appendix S3.

We assigned all 83 sites (Table S1) and all 363 species in the tree to eight major biogeographic regions (Table S9). For South America, we used five regions of high amphibian endemism from Duellman (1999): (1) Amazonia-Guiana, (2) Andes, (3) Chocó, (4) Caribbean Coastal Forest and (5) Atlantic Forest. Outside South America we used: (6) West Indies, (7) Upper Middle America (Mexico to Nicaragua) and (8) Lower Middle America (Costa Rica, Panama). We then created a geographic adjacency matrix among regions (Table S10).

To determine the relationship between local richness and time, we estimated the timing of first colonisation of terraranans in each region based on the age of the oldest clade unambiguously reconstructed as occurring in that region, using area reconstructions on our time-calibrated phylogeny. For each clade, we used both the minimum age (crown-group) and maximum age (stem-group). Furthermore, we tested the relationship between colonisation time and maximum local richness among sites in each region and mean local richness (mean richness might be impacted by non-random sampling of localities within regions, whereas maximum richness might reflect outliers). These relationships were tested using linear regression.

Our approach assumed that the age of the first colonisation of each region was the most important for explaining richness patterns, even if there were subsequent colonisations. Previous studies suggest that using only the first (oldest) colonisation time gives similar results to using all colonisation times summed (e.g. Stephens & Wiens 2003; Hutter et al. 2013) and simulations suggest that use of the first colonisation age is more accurate for estimating time-richness relationships (Rabosky 2012).

We also tested for a linear relationship between ln-transformed regional and ln-transformed local richness (Table 1)

as another test of saturation (Appendix S4). Finally, we compared regional richness and time (Appendix S4).

## Testing for saturation using historical biogeography

Given saturation, local richness should be similar in sites where species from Middle and South American clades co-occur, relative to sites with species from only one continent. In contrast, saturation would be rejected if richness were higher in communities where clades from these two regions co-occur. Many sites in Lower Middle America have species from both regions (as do some in Upper Middle America and north-western South America), whereas many in Middle and South America have species from only that region. Our sampling included 14 sites in Upper Middle America, 16 in Lower Middle America and 45 in South America (Amazonia-Guiana: 18; Andes: 18; Atlantic Forest: 4; Caribbean Coastal Forest: 1; Chocó: 4). We assigned all 75 sites to one of three categories (Table S11): (1) those containing species from Middle American clades only (MA; $n = 9$), (2) South American clades only (SA; $n = 42$) and (3) clades from both regions (MASA; $n = 24$). Analyses of these 75 sites (Table S11) were conducted using ANOVA.

We also accounted for the potential impact of non-terraranan frog species on patterns of terraranan richness (e.g. if lower richness of other frog clades might explain higher terraranan richness in MASA sites). Many sites ($n = 66$) had data on overall anuran richness (Table S12).

Analyses of climatic variables showed that annual precipitation was related to richness (see Results). To address whether higher richness in MASA localities is potentially explained by climate rather than GABI, we conducted ANCOVA in R version 3.0.2. We tested the impact of community type (MA, MASA, SA), precipitation, and their interaction (community type × precipitation) on local richness for the 75 relevant sites. We then compared the fit of these models using Akaike weights to account for different numbers of parameters among models.

As an additional test of the potential imprint of competition on local richness, we tested whether local richness of each clade involved in the GABI is higher in its native region relative to regions where it co-occurs with species from other regions (data in Table S13). This is not a strict test of saturation, but rather addresses whether there is any potential signature of clade co-occurrence on local richness (although such patterns might have other explanations unrelated to competition, making this a liberal test).

All terraranan species involved in the GABI belong to five genera. Biogeographic reconstructions (Fig. S1) showed that *Pristimantis* and *Strabomantis* (Craugastoridae: Strabomantinae) originated in South America and *Craugastor* (Craugastoridae: Craugastorinae) and mainland *Eleutherodactylus* (Eleutherodactylidae) originated in Upper Middle America (see also Crawford & Smith 2005; Heinike *et al.* 2007; Pinto-Sánchez *et al.* 2012). *Diasporus* (Eleutherodactylidae) is species-poor and under-represented in our phylogeny, but unpublished analyses suggest a Middle American origin (L. Barrientos, pers. comm.). However, an alternative assignment would have little impact on our conclusions, because *Diasporus* only occurs in sampled communities with *Craugastor* and *Pristimantis* (i.e. MASA communities, regardless of where *Diasporus* originated). We treated the two genera of Eleutherodactylidae (*Diasporus, Eleutherodactylus*) as a single clade. Therefore, as an example, we tested whether richness of *Pristimantis* in MASA communities (co-occurring with Middle American clades) is different from that in SA communities (co-occurring only with other South American clades).

A potential criticism of this analysis is that each clade typically co-occurs with other terraranan clades. However, the two clades of Middle American origin typically co-occur with each other, as do the two South American clades (Table S2). In Middle America, only these four clades are present. In South America, most terraranan communities included here are dominated by *Pristimantis*, but some contain *Strabomantis*, and/or a few species from genera not involved in GABI. Atlantic Forest communities do not include *Pristimantis* or *Strabomantis*, and so are not included here. Overall, if there are strong competitive effects of clades on local richness of other clades, the two Middle American clades should have higher richness where they occur only with each other and without South American clades, and the two focal South American genera should have higher richness where they occur without Middle American genera.

Table 1 Summary data on the eight biogeographic regions studied here, including mean and maximum local richness, regional species richness and the estimated timing of the first colonisation of terraranans in each region (in millions of years before present), the latter based on both the crown and stem-group ages for the oldest endemic clade in each region
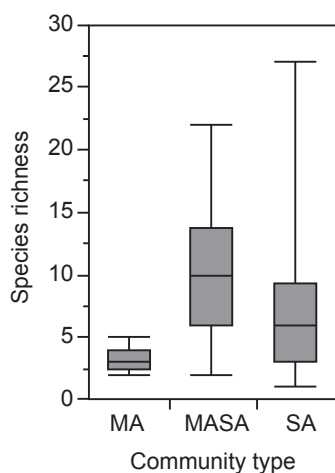
| Region | Mean local richness | Maximum local richness | Regional richness | Time of first colonisation (crown-stem ages) |
|---|---|---|---|---|
| Upper Middle America | 4 | 6 | 143 | 53.86–61.12 |
| Atlantic Forest Domain | 4 | 9 | 60 | 40.83–59.33 |
| Andes | 8 | 27 | 496 | 65.57–68.11 |
| West Indies | 7 | 20 | 206 | 48.53–61.31 |
| Amazonia-Guiana | 7 | 17 | 163 | 43.69–61.31 |
| Caribbean Coastal Forest | 8 | 8 | 23 | 30.98–39.15 |
| Lower Middle America | 12 | 22 | 92 | 28.80–37.56 |
| Chocó | 13 | 18 | 46 | 12.11–23.00 |

**Figure 2** Summary of local species richness of terraranan frogs in communities in Middle America and South America, showing that local species richness is higher in communities where species from both Middle and South American clades occur together (MASA, $n = 24$) relative to communities containing only species from Middle American clades (MA, $n = 9$) or only species from South American clades (SA, $n = 42$). Whiskers indicate ranges of values, and boxplots show quartile and median values. Results based on ln-transformed data: ANOVA: $r^2 = 0.181$, $F = 7.942$, $P = 0.001$; Tukey *post hoc* tests: MASA vs. MA: $P = 0.002$; MASA vs. SA: $P = 0.009$. Results for raw data: ANOVA: $r^2 = 0.169$, $F = 7.341$, $P = 0.001$; Tukey *post hoc* tests: MASA vs. MA: $P = 0.002$; MASA vs. SA: $P = 0.020$.

## RESULTS

Local richness was generally not related to climate. PCs 1–4 do not have significant relationship with richness among the 83 sites (PC1: $r^2 = 0.001$, $F = 0.096$, $P = 0.758$; PC2: $r^2 = 0.044$, $F = 3.734$, $P = 0.057$; PC3: $r^2 = 0.010$, $F = 0.791$, $P = 0.377$; PC4: $r^2 = 0.033$, $F = 2.793$, $P = 0.099$), nor does annual mean temperature (Bio1: $r^2 = 0.002$, $F = 0.127$, $P = 0.723$). However, there is a weak but significant relationship between annual precipitation and richness (Bio12: $r^2 = 0.071$, $F = 6.145$, $P = 0.015$).

Biogeographic reconstructions revealed that local richness in each region is generally unrelated to the estimated time terraranans have been present there (Table 1; Fig. S2) using stem and crown ages and mean and maximum local richness (stem, maximum: $r^2 = 0.001$, $F = 0.005$, $P = 0.944$; crown, mean: $r^2 = 0.343$, $F = 3.128$, $P = 0.127$; crown, maximum: $r^2 = 0.000$, $F = 0.003$, $P = 0.959$). However, we found a significant, negative relationship between stem age and mean local richness ($r^2 = 0.499$, $F = 5.978$, $P = 0.050$). Intriguingly, both Lower Middle America and Chocó have high mean local richness (Table 1), despite the relatively young clades in these regions, seemingly reflecting a dramatic increase in local richness in association with GABI. Indeed, the relationship becomes non-significant when these two regions are eliminated ($r^2 = 0.042$, $F = 0.174$, $P = 0.698$). Similarly, there is generally no significant relationship between local and regional richness, in part because of the high local richness in Lower Middle America and Chocó (Appendix S4). However, regional richness and time are significantly, positively related (Appendix S4).

Biogeographic reconstructions (Fig. S1) showed at least 10 invasions from South to Middle America, and four from Middle to South America (Table S14). Except for the early invasion of Middle America by Craugastoridae (53.9 Mya), most dispersal events involved single species (at least among those sampled here) and are associated with clades 31.3 to 6.7 million years old (but dispersal events may be younger than the age of these species).

Considering the 75 sites in the three categories relevant to the GABI [Middle American only (MA), South American only (SA), mixed (MASA)], local richness varied significantly among categories (ANOVA: $r^2 = 0.181$, $F = 7.942$, $P = 0.001$; Tukey *post hoc* tests: MASA vs. MA: $P = 0.002$; MASA vs. SA: $P = 0.009$). Thus, local richness was higher at sites where species from Middle and South American clades occurred together relative to sites where these clades occur alone (Fig. 2). These results reject the hypothesis of saturation.

These results are not explained by differences in sampling effort between regions (Appendix S5), nor by differences in richness of other frogs: ln-transformed local richness of non-terraranans is similar among categories (ANOVA: $r^2 = 0.008$, $F = 0.230$, $P = 0.795$, $n = 59$ sites). On average, terraranans make up 45% of anuran species across all sites (range = 2.5–100%; $n = 66$ sites) or 29% (range = 2.5–50%) excluding seven sites where only terraranans were found (test results are similar including or excluding these sites).

To test whether higher richness in MASA sites is explained by climate (given the weak but significant relationship

**Table 2** Testing for the possible impact of climate on analysis of community saturation, using ANCOVA to compare annual precipitation (Bio12) and community type (MA: containing species from Middle American clades only; SA: South American clades only; MASA: containing clades from both regions), and their interaction on local richness ($n = 75$ communities)

| Model parameters | d.f. | AIC | ΔAIC | Model probability | Cumulative probability |
|---|---|---|---|---|---|
| Community type alone | 4 | 172.306 | 0 | 0.615 | 0.615 |
| Community type + Bio12 | 5 | 173.622 | 1.316 | 0.319 | 0.934 |
| Community type + Bio12 + interaction | 7 | 176.992 | 4.686 | 0.059 | 0.993 |
| Bio12 alone | 3 | 181.202 | 8.896 | 0.007 | 1.000 |

Models are compared using the Akaike Information Criterion (AIC). The model containing only community type as an independent variable scored the best (lowest) AIC, whereas Bio12 alone had a model probability (Akaike weight) of only 0.007 and is therefore a poor predictor of species richness relative to community type.
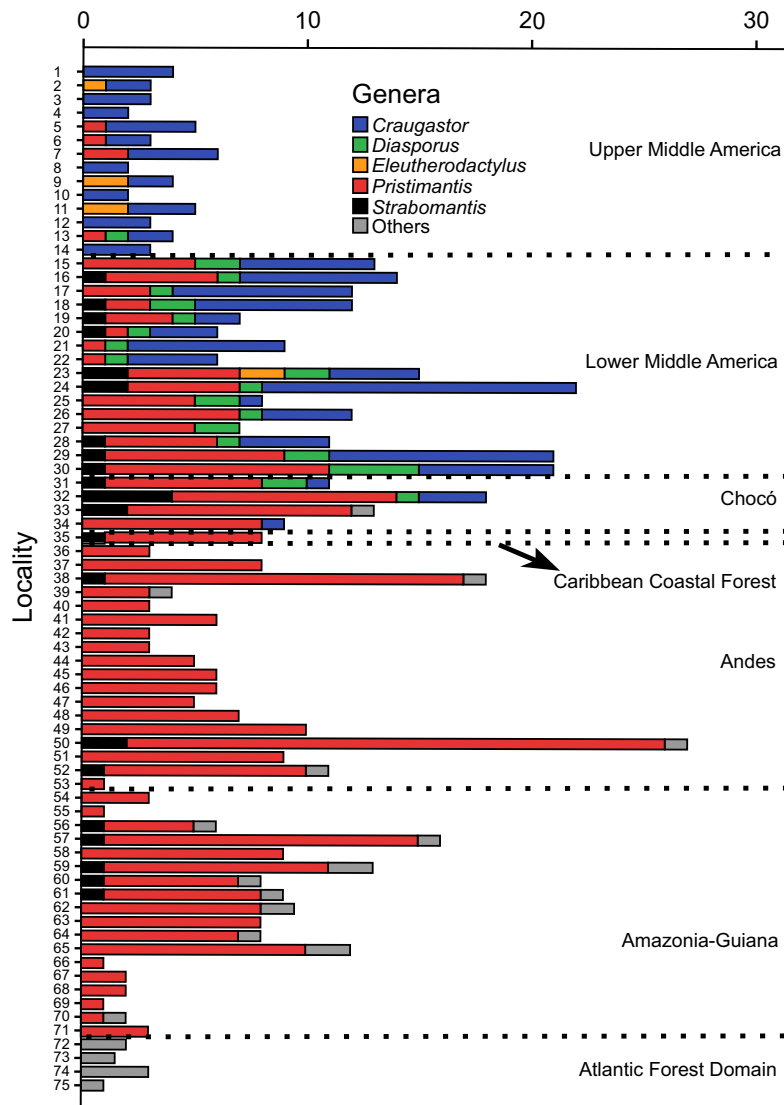
**Figure 3** Local species richness of terraranan frogs, shown by locality, region and genus, for 75 sites relevant to the GABI (i.e. excluding West Indian sites). Detailed locality information and species lists are provided in Tables S1 and S2. The category 'others' combines seven endemic South American genera with low species richness that did not participate directly in the GABI: *Adelophryne, Haddadus, Holoaden, Hypodactylus, Ischnocnema, Noblella* and *Oreobates*. We assumed that the genera *Craugastor, Diasporus* and *Eleutherodactylus* originated in Middle America, whereas *Pristimantis* and *Strabomantis* originated in South America (see Materials and Methods).

between annual precipitation and richness), we conducted an ANCOVA of the effects of annual precipitation, community type (MA, MASA, SA) and their interaction on local richness (Table 2). Richness was influenced most strongly by community type and least strongly by precipitation, and the model including both community type and precipitation is not supported over one based on community type alone ($\Delta$AIC = 1.3). Although the interaction of precipitation and community type has some impact on local richness (i.e. higher richness in wetter MASA communities), it is clear that the influence of precipitation is minor and does not explain differences in richness between community types.

There was no evidence that sympatry between clades from different regions reduced their local richness (Fig. 3). For each clade, we found similar richness for sites with only South American (SA) or Middle American (MA) clades relative to sites where clades from these two regions co-occur (MASA): *Craugastor* (ANOVA: $r^2 = 0.047$, $F = 1.493$, $P = 0.231$), *Pristimantis* (ANOVA: $r^2 = 0.045$, $F = 2.776$, $P = 0.101$), *Strabomantis* (ANOVA: $r^2 = 0.022$, $F = 0.434$, $P = 0.518$) and Eleutherodactylidae (ANOVA: $r^2 = 0.009$, $F = 0.199$, $P = 0.661$).

## DISCUSSION

In this study, we present a test for community saturation based on historical biogeography and local species richness. We apply this approach to a case study involving terraranan frogs and the GABI. Our results show no evidence of community saturation in this system. Instead, we find that richness is significantly higher in communities where clades

from Middle and South America occur together. Furthermore, we rule out other potential explanations for these patterns, such as climate, time and interactions with species in other frog clades. Regional-scale area does not explain the high local richness of mixed sites either, as Lower Middle America (Costa Rica, Panama) has a tiny area relative to Upper Middle America (Mexico to Nicaragua) and South America.

We emphasise that the basic approach used here could be applied to many other organisms and regions. Furthermore, even though our system included reciprocal interchange of lineages between regions, the test could be applied to simpler cases where one or more lineages invades a region where closely related and/or ecologically similar species are already present and have radiated. The hypothesis of saturation is rejected if higher local species richness is documented in invaded communities (relative to communities that lack these invading lineages), after controlling for potentially confounding factors such as climate. Thus, there should be literally thousands of natural systems to which this approach could be applied.

The approach used here is only one among many for testing community saturation (e.g. manipulative experiments, invasive species, local vs. regional richness comparisons, time vs. local richness, paleontological analyses of regional richness). We think that all of these approaches offer important insights that can help address this pivotal issue. The approach proposed here is advantageous in utilising natural systems and long time scales (millions of years). Furthermore, this approach may offer a more direct test of community saturation (i.e. can species be added?) than comparing regional vs. local richness or time vs. local richness among regions. Intriguingly, our results suggest that recent dispersal and lack of saturation might disrupt or even reverse the time vs. local richness relationship (see Results) and the local vs. regional richness relationship (Appendix S4), leading to high local richness in recently colonised regions with limited regional richness. Thus, the scenario described here might actually mislead these other tests of saturation.

Our approach should offer similar insights to those from the fossil record. However, we emphasise local richness, whereas paleontological studies have emphasised regional richness (e.g. Vermeij 1991; Patzkowski & Holland 2007; Tilman 2011). Furthermore, a potentially serious disadvantage of paleontological studies is that they rely on incomplete morphological data for estimates of phylogeny, biogeographic history and species richness. Estimates of species richness might be the most serious problem. Many species may be difficult to distinguish in the fossil record, and many paleontological studies therefore focus on family and genus-level richness and ignore species richness altogether (e.g. Marshall *et al.* 1982). Terraranans may offer an especially instructive example of the advantages of considering extant species: many terraranan species now placed in different families by molecular data were placed in the same genus by morphological data alone (Hedges *et al.* 2008). Thus, relying on the fossil record, invasions and subsequent co-occurrence of morphologically (and ecologically) similar species at the local scale might easily be overlooked, potentially confounding analyses of community saturation.

On the other hand, a potential disadvantage of our approach relative to paleontological analyses is that our approach does not directly address extinction. In contrast, a paleontological analysis can show that invasion of a region by one lineage caused the extinction of ecologically similar species in the incumbent lineage (e.g. Vermeij 1991; Patzkowski & Holland 2007). The balancing of species invasion with species extinction is an important component of community saturation. However, showing increased local species richness in present-day communities as a result of historical invasions rejects saturation, regardless of whether other species have gone extinct. It is possible that there were unsuccessful invasions due to competition and extinction, and that the successful colonisations caused extinctions in local communities. Nevertheless, it is clear that some colonisations were successful and significantly increased local richness. Furthermore, we find no significant decreases in the local richness of clades from one region when they co-occur with species from another region, a pattern which also suggests that successful colonisations occurred without incurring widespread local extinctions.

Another issue is that analyses of biogeographic colonisation and interchange might be biased against detecting saturated communities: if communities were truly saturated, then there might not be any successful colonisations at all. Thus, the presence of successful colonisations may be a priori evidence that recipient communities are unsaturated (e.g. Wiens 2011), regardless of local richness. However, it is possible that colonisations will be successful but will not increase local richness because of local extinctions of incumbent species (either due to competition or other factors). Nevertheless, given this concern, our approach may be more useful for revealing particular systems where saturation is absent, rather than as a completely unbiased test of saturation across all possible taxa and regions. In a similar vein, we emphasise that to rigorously support saturation, additional lines of evidence should be considered beyond the lack of increased local richness in invaded communities. We also note that we have not demonstrated strong interspecific interactions among terraranan species at the spatial scale considered here, but this is not inconsistent with the idea that these communities are unsaturated, and demonstrating such interactions should not be a requirement for testing saturation.

The major conclusion of our study is that Middle American communities are not saturated with terraranan species, and there are several potential explanations for this pattern. For example, there may have been shifts in microhabitats, body sizes, dietary resources or abundance in the incumbent species to compensate for invaders, or vice versa (note that the presence of such shifts would help explain how saturation was avoided, not provide support for saturation). However, ecological studies at sites where Middle and South American clades co-occur suggest that terraranan species are (overall) ecologically similar within and between these clades. For example, 14 terraranan species co-occur in primary forest leaf-litter at La Selva, Costa Rica (Lieberman 1986), including eight *Craugastor* (Middle American), five *Pristimantis* (South American) and one *Diasporus* (Middle American). These 14 species overlap broadly in microhabitat, and an analysis of stomach contents found only limited trophic niche partitioning among them (Lieberman 1986). A study of nocturnal

perch height in 12 species at this site showed that Middle and South American genera co-occur microsympatrically, with seven *Craugastor* and one *Strabomantis* species sharing lower perches, and three *Pristimantis* and one *Diasporus* species sharing higher perches (Miyamoto 1982). Overall, terraranan clades have radiated in both regions separately and seem to fill similar ecological roles in both (i.e. generally small bodied, terrestrial and arboreal, generalist insectivores with direct development; Duellman 1978; Lieberman 1986; Savage 2002; Hedges *et al.* 2008). There may be limits on how many species terraranan communities can contain, but species richness may not have accumulated long enough for saturation to be reached (e.g. Mouquet *et al.* 2003). Communities in Lower Middle America are among the most species-rich (Table 2). Interestingly, some Andean communities have slightly higher richness and are located where terraranans have occurred the longest. Another possible explanation is that the ecological, phylogenetic or spatial scale of our study is too coarse to detect saturation. A signature of saturation might be detected if we focused on a specific microhabitat and/or subset of terraranans, although this seems unlikely given the ecological results mentioned above. Furthermore, the scale of local communities used here is standard for studies of local richness in frogs (e.g. Wiens *et al.* 2011), and we note again that many species across the terraranan families are morphologically and ecologically very similar. The ecological similarity of many terraranan species might also provide an important explanation for the apparent lack of saturation: their ecological similarity may make it difficult for one species to competitively exclude another over finite time scales (see Leibold & McPeek (2006) for one example of this extensive literature). Some combination of these various explanations might also apply.

Finally, an important question that our study raises is whether community saturation is a reasonable expectation to begin with. Our results are consistent with those from other approaches in rejecting the idea that communities are generally saturated with species (reviews in Tilman 2011; Wiens 2011). Nevertheless, the macroevolutionary literature increasingly emphasises 'ecological limits' (e.g. Rabosky 2009; Rabosky *et al.* 2012), the idea that clades fail to grow over time due to competition for finite resources (but generally without data on local richness, even though competition must occur at the local scale). Given the mounting evidence to the contrary, we argue that community saturation (and related clade-level ideas) should not be simply assumed without relevant supporting data.

to be presented to the Department of Biological Sciences at the Universidad de los Andes.

## STATEMENT OF AUTHORSHIP

NRPS, AJC and JJW designed the study. NRPS, AJC and JJW performed analyses. NRPS, AJC and JJW wrote the paper.

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