

Process-Based Species Pools Reveal the Hidden Signature of Biotic Interactions Amid the Influence of Temperature Filtering

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ABSTRACT: A persistent challenge in ecology is to tease apart the influence of multiple processes acting simultaneously and interacting in complex ways to shape the structure of species assemblages. We implement a heuristic approach that relies on explicitly defining species pools and permits assessment of the relative influence of the main processes thought to shape assemblage structure: environmental filtering, dispersal limitations, and biotic interactions. We illustrate our approach using data on the assemblage composition and geographic distribution of hummingbirds, a comprehensive phylogeny and morphological traits. The implementation of several process-based species pool definitions in null models suggests that temperature—but not precipitation or dispersal limitation—acts as the main regional filter of assemblage structure. Incorporating this environmental filter directly into the definition of assemblage-specific species pools revealed an otherwise hidden pattern of phylogenetic evenness, indicating that biotic interactions might further influence hummingbird assemblage structure. Such hidden patterns of assemblage structure call for a reexamination of a multitude of phylogenetic- and trait-based studies that did not explicitly consider potentially important processes in their definition of the species pool. Our heuristic approach provides a transparent way to explore patterns and refine interpretations of the underlying causes of assemblage structure.

Keywords: regional species pool, scale, niche differentiation, dispersal limitation, community assembly.

Introduction

An enduring issue in ecology is that there are more potential processes that may account for the structure of species assemblages than there are resulting patterns (Mayfield and Levine 2010). As a result, processes shaping species assemblages remain elusive and often appear idiosyncratic (Lawton 1999; Vellend 2010). The species composition of an assemblage likely arises from a hierarchical set of stochastic and deterministic processes. Such processes include dispersal limitation and environmental filtering that influence which species can arrive at and tolerate conditions in a given location (Leibold et al. 2004; Vellend 2010) and biotic interactions that further refine assemblage composition (Weiher and Keddy 1999). One approach to assess the relative influence of these processes on assemblage composition is to compare empirical patterns of phylogenetic (Webb et al. 2002) and functional trait (Kraft et al. 2007) structure to patterns generated by a null model (Connor and Simberloff 1979; Gotelli and Graves 1996). This randomization approach typically derives the species pool from a list of species either recorded in a study or present within a predefined political or geographic unit. However, the former definition introduces biases against rare species that are unlikely to be sampled, and the latter conflates geographic proximity with environmental similarity (Warren et al. 2014). Here, we introduce an approach that remedies these issues and builds on recent analytical advances (Kembel 2009; Gotelli et al. 2010; Lessard et al. 2012*b*) by constructing species pools that are process based and probabilistic.

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Our heuristic null model approach defines an assemblage-specific species pool (fig. 1A)—the set of species that could potentially occur in a given location—on the basis of a process hypothesized to influence assemblage composition (Graves and Gotelli 1983, 1993; Gotelli et al. 2010). We generate the modeled expectation by refining the pool of all species that could potentially occur in a given assemblage to those more likely to occur under the process being considered (fig. 1B). As an example, previous studies have built environmentally filtered species pools to assess the influence of the environment on patterns of phylogenetic (Lessard et al. 2012b; Eiserhardt et al. 2013) and trait (Kraft et al. 2008; Algar et al. 2011) structure. By limiting the species pool to species that could tolerate the local environmental conditions, Kraft et al. (2008) found that patterns of trait filtering became weaker while patterns of trait evenness became stronger. This illustrates how the signature of environmental filtering might conceal that of competitive interactions. Later studies have extended this approach by comparing the outcome of several null models (Gotelli et al. 2010), each derived from a different process-based species pool (Lessard et al. 2012a, 2012b). Here, we use a similar comparative approach as a heuristic tool to explore the potential influence of multiple processes on patterns of phylogenetic and trait assemblage structure.

While process-based species pools are becoming more common in community ecology, there is no simple way to decide on the level of constraint used to define these pools. Broadly speaking, the level of constraint, usually chosen arbitrarily, defines the strength of the filtering imposed on the definition of the process-based species pool (Swenson et al. 2006; Algar et al. 2011; González-Caro et al. 2012; Belmaker and Jetz 2013) and can influence the resulting pattern of assemblage structure (Kraft et al. 2007). Given this issue, it is desirable to develop an approach enabling the examination of several species pool definitions over the entire range of possible levels of constraint (fig. 1C). For example, a pool could be defined on the basis of distance (a proxy for dispersal probability), where only species within a certain distance of a given assemblage are included. The constraint would then be the actual distance considered, which could range from very short (species in neighboring assemblages) to very long (all species of a given clade in the world). Moreover, an ideal approach would allow for comparing different types of filters (e.g., environmental and dispersal filters) on the same scale. Our proposed approach fulfills both of these criteria (fig. 1C).

Hummingbirds provide a useful test case for our approach because patterns of phylogenetic and trait structure appear to be influenced by the species pool definition (Parra et al. 2011; González-Caro et al. 2012). A single species pool based on species detected in a set of local communities yielded phylogenetic clustering at high elevations, which indicated that environmental filtering may have structured

these hummingbird assemblages (Graham et al. 2009). However, these patterns of phylogenetic structure were clade specific, with some clades—but not all—showing a pattern of evenness at high elevation (Parra et al. 2010). In addition, patterns of trait structure exhibit both clustering (body mass) and evenness (beak length) at high elevations (Graham et al. 2012). As a result, it remains unclear how filtering and biotic interactions combine to structure local assemblages, providing a test case for our approach (fig. 1).

We use our heuristic approach (fig. 1) together with phylogenetic- and trait-based inference to quantify the influence of the abiotic environment, dispersal limitations, and biotic interaction on the species composition of 248 Andean hummingbird assemblages. Specifically, our approach relies on assemblage-specific and process-based definition of species pools, derived from data on the global distribution of hummingbirds, to simulate assemblage structure. We compare deviations from the modeled expectation under various definitions of process-based species pools and levels of constraints (using a quantile approach). The process-based species pools are based on precipitation and temperature envelopes as well as on dispersal limitation (fig. 1C). We hypothesized that (1) when species pools are defined with a high level of constraint so that they include only species that can tolerate environmental conditions at that site, hummingbird communities would be phylogenetically evenly dispersed, which could be interpreted as the combined influence of temperature filtering and competitive interactions (Kraft et al. 2008). Specifically, since temperature explains much variation in hummingbird assemblage structure (Graham et al. 2009, 2012), we predicted that a temperature-based species pool would have the strongest influence on the perceived structure of hummingbird communities. Concomitantly, we hypothesized that (2) patterns of beak length dispersion, which might reflect competition for food resources (Stiles 2008; Graham et al. 2012; Maglianesi et al. 2014), would be evenly dispersed and that this pattern would become stronger after accounting for temperature filtering. Finally, we hypothesized that (3) patterns of body mass dispersion, a trait that is under strong selection pressure in cold Andean environments (Brown and Bowers 1985; Stiles 2008; Graham et al. 2012), would appear clustered when using a broadly defined species pool but random when using a species pool that accounts for temperature filtering.

Methods

Species Assemblage Data

We compiled a database of hummingbird assemblages (presence/absence data) across northwestern South America (fig. A1; figs. A1–A9 available online) using lists from published references in peer-reviewed journals, gray literature,

A. Assemblage-specific definition of species pools

DISPERSION FIELD



B. Probability-weighted resampling of process-based species pools

TEMPERATURE

PRECIPITATION

DISPERSAL

COST-DISTANCE



C. Constraint on the resampling of process-based species pools

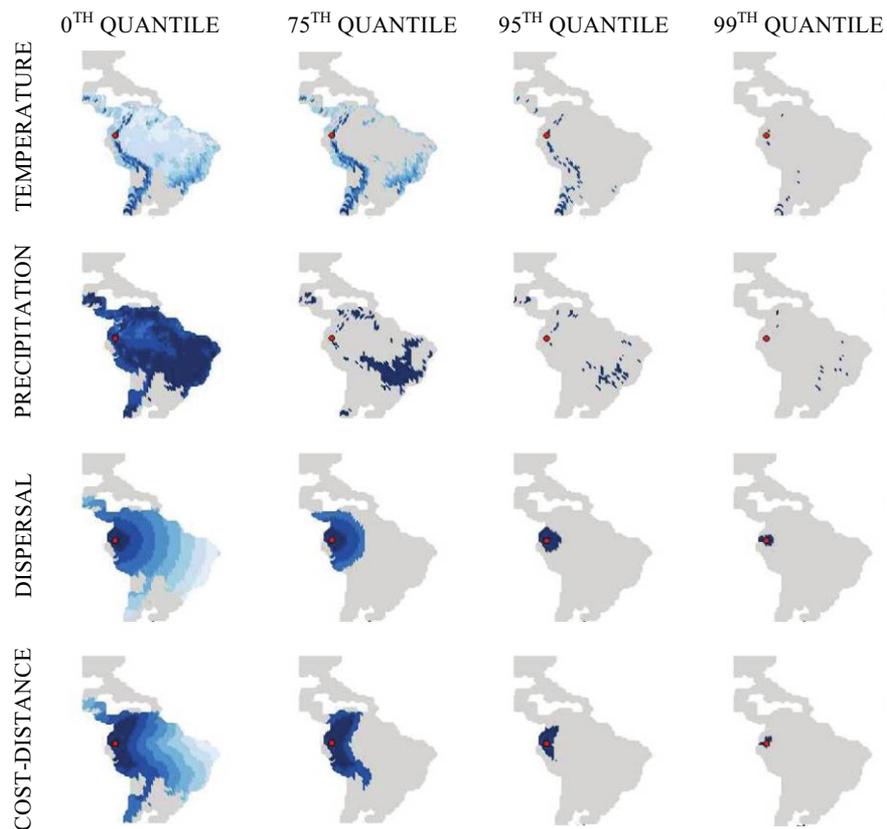


Figure 1: Construction of our process-based species pools is a three-step process. *A*, The largest possible species pool is defined separately for each focal assemblage (red circle) using the dispersion field approach. *B*, The probability of resampling a species in a different assemblage within the dispersion field is weighted by the distance between this assemblage and the focal assemblage, wherein distance is defined in environmental or geographical space. *C*, Constraints on the probability of resampling are imposed with varying strength.

and nonpublished reports to environmental organizations, including BirdLife International and Aves y Conservación. All georeferences of assemblages were checked using the elevation recorded and then confirmed using digital elevation data or gazeteers (for details, see Graham et al. 2009, 2012). We considered only mainland assemblages with more than three species and for which all members were represented in our phylogeny. In addition, to ensure that each assemblage had a consistently high level of species detection, we determined the average number of species in all assemblages within a given ecoregion to eliminate outlier assemblages. Specifically, we defined outliers as those outside 1.5 SD from the mean number of species per assemblage in a given ecoregion. Cases where outliers had a large number of species were at lodges where the use of feeders to attract birds result in species outside of their known ranges. Outliers with few species were assumed to be undersampled, because regional species richness of well-sampled communities tends to be similar under the same climatic conditions. This resulted in using 248 of the 290 localities across Ecuador and Colombia.

Phylogenetic Tree

We used a phylogenetic tree (fig. A2) constructed from DNA sequences covering six genetic loci (four nuclear and two mitochondrial) representing 284 hummingbird species and 15 outgroup species spanning four avian orders (McGuire et al. 2014). We then pruned the tree to include only the 281 species included in our global hummingbird distribution data. Sampling includes 101 of 105 currently recognized trochilid genera (lacking only three monotypic genera—*Anopetia*, *Hylonympha*, and *Sternoclyta*—and the bitypic genus *Augastes*, all with very restricted ranges). Time-calibrated Bayesian phylogenetic analyses were performed using BEAST v1.7.1 (Drummond and Rambaut 2007) with calibrations for divergence dating analyses using substitution rate priors (rather than fossil calibrations) based on rate estimates generated for Hawaiian honeycreepers (Lerner et al. 2011). The phylogenetic tree is deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.t897q> (Lessard et al. 2015).

Morphological Traits

We compiled measurements of body mass (in grams) and exposed culmen (in millimeters) from literature and unpublished data, including PhD dissertations and measurements taken in the field, for males and females of 334 species of hummingbirds. Here again, the data was pruned to match the phylogenetic and distributional data, such that 281 species were retained. For the species that were sampled in the

field, body mass was measured to the nearest 0.1 g with 10-g and 50-g Pesola spring balances, and exposed culmen was measured to the nearest 0.1 mm with dial calipers. Given that measurements do not encompass the entire geographic range for many species, intraspecific variation (V_w) was compared with interspecific variation (V_b) to assess whether intraspecific variation can affect our results. For the two traits, intraspecific variation corrected for small sample size (calculated as $[1 + 1/(4n)] \times V$, where n is the number of values and V the coefficient of variation; Sokal and Rohlf 1995) was lower than half of the interspecific variation (body mass: $V_w = 0.432$, $V_b = 0.104$; exposed culmen: $V_w = 0.392$, $V_b = 0.124$). On the basis of these results, we assume that intraspecific variation has a weak effect on our results. We excluded two species with extreme values for exposed culmen (*Ensifera ensifera*) and body mass (*Patagona gigas*) from the analysis, since their inclusion in an assemblage would inflate the average pairwise distance. Morphological trait data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.t897q> (Lessard et al. 2015).

Quantifying Patterns of Assemblage Structure

We estimated the phylogenetic and functional trait structure of each assemblage by calculating the net relatedness index (NRI). The NRI is a standardized index that measures the deviation between the observed mean pairwise distance (MPD) of species in an assemblage and a distribution of simulated MPD values. MPD is the mean distance between all possible pairs of species recorded in an assemblage, and it was calculated from matrices of pairwise phylogenetic distances or pairwise functional trait distances (Euclidean). A simulated MPD value is obtained by resampling a number of species equal to that in the observed assemblage from a given species pool. Resampling can be random or follow some constraints (see “Probability-Weighted Resampling of Process-Based Species Pools”). The NRI is calculated as the difference between the MPD of the observed assemblage and the mean of simulated MPD values, standardized (divided) by the standard deviation of simulated MPD values (Webb et al. 2002). Positive values of NRI (>1.96) indicate significant phylogenetic clustering, whereas negative values (<-1.96) indicate significant phylogenetic evenness.

A Process-Based Species Pool Framework

To assess the relative influence of the abiotic environment (temperature, precipitation) and dispersal limitation (distance, cost distance), we compare the phylogenetic structure of assemblages resulting from different species pool definitions (see fig. 1B). Then we progressively increase the level of constraint (filtering) imposed on each definition (see fig. 1C). We evaluate whether patterns of NRI

change for each species pool definition and with the level of constraint imposed. If NRI values change with the level of constraint for a given species pool definition, we hypothesize that the process being modeled influences assemblage composition. In other words, either communities exhibiting significant structure under an unconstrained pool become unstructured with increasing levels of constraint, or communities without structure under an unconstrained pool become structured with increasing levels of constraint. This is a heuristic approach wherein insight from the models comes from comparing them in combination rather than providing an absolute assessment of model performance.

Constructing Species Pools. There are three steps involved in the construction of our species pools for the purpose of process-based null model analyses (fig. 1). First, we define the universe of potential assemblage members, often referred to as the regional species pool (Cornell and Harrison 2014). For defining this broadest possible pool of species (fig. 1A), we use a macroecological approach that is assemblage specific (Gotelli and Graves 1996) and has an explicit and standardized definition (Graves and Rahbek 2005; Carstensen et al. 2013), which ensures that the method is tractable and reproducible. Second, we resample species from the broadly defined pool of species on the basis of a probability distribution (fig. 1B). This probability distribution is derived from site characteristics that represent a process of interest. Finally, we incrementally increase the level of constraint on the probability of resampling as a way to detect the influence of the process of interest on the observed pattern of assemblage structure (fig. 1C).

Macroecological Definition of Regional Species Pools. Most definitions of species pools for null model analyses of phylogenetic or trait dispersion use the list of species recorded in the study. However, this species pool definition often excludes species that occur in the study region but were not recorded in the sites surveyed in that particular study (also known as dark diversity; Pärtel et al. 2011). That is, a collection of local surveys rarely captures total regional diversity or species composition (Gotelli and Colwell 2001). As such, a number of species that could potentially colonize and persist in the focal assemblage are often excluded from the species pool definition (Carstensen et al. 2013). The inclusion of such species in the pool definition could affect perceived patterns of assemblage structure (e.g., NRI) if, for example, they represented a nonrandom subset of the phylogeny or trait space. To circumvent this issue, we use comprehensive data on the global distribution of hummingbirds rather than incidence data from local surveys to define species pools. Distributional data for species of hummingbirds were extracted from a comprehensive global

geographic range database for all land and nonpelagic species of birds (Rahbek et al. 2012). In this database, the geographic range of each species was mapped at a resolution of $1^\circ \times 1^\circ$ latitude-longitude grid cells following the approach outlined by Rahbek and Graves (2000), Brooks et al. (2001), Jetz and Rahbek (2002), and Fritz et al. (2012). Maps represent a conservative extent of occurrence of breeding ranges based on museum specimens, published sight records, and spatial distribution of habitats between documented records, which have subsequently been validated by ornithological experts. A complete list of references can be found in the supplementary material of Holt et al. (2013).

We delineated species pools explicitly using data on the global geographic distribution of hummingbird species (Rahbek et al. 2012). In particular, we used assemblage dispersion fields (Graves and Rahbek 2005) to determine which hummingbird species in the New World should be included in the species pool of a local assemblage. We generated a unique dispersion field for each local assemblage ($n = 248$) by overlaying the ranges of all species found in the grid cell containing that assemblage (fig. 1A). The dispersion field thus contains all hummingbird assemblages that share at least one species with the focal assemblage and provides a spatial representation of the region across which species in the focal assemblage have dispersed and been detected in recent history. The rationale behind using dispersion fields to define a broadscale species pool is that if two species could occupy a single focal assemblage, then either species could theoretically occupy all communities where one of the pair is present (Graves and Rahbek 2005; Borregaard and Rahbek 2010; Carstensen et al. 2013).

Variable Choice for Process-Based Species Pools. Multiple ecologically explicit definitions of the species pool can be used as a heuristic tool for evaluating the relative importance of multiple ecological processes. For example, one can weigh the probability of sampling a species from the pool on the basis of site-specific dispersal probability and/or the probability that a species could tolerate the local abiotic conditions (fig. 1B). We chose variables to generate our process-based species pools by considering hummingbird biology and biogeography of the region. Instead of testing the influence of a plethora of potentially important variables, we selected those variables that have been identified as most biologically meaningful for hummingbirds and appear to influence phylogenetic structure (Graham et al. 2009).

We used mean annual temperature (BIO1) and total annual precipitation (BIO12) from WorldClim (ver. 1.4; <http://www.worldclim.org/>; Hijmans et al. 2005) to model ecologically relevant species pools. Our study region is defined by large elevation (elevation is strongly correlated with temperature) and precipitation gradients. Hummingbirds have colonized all habitat types and elevations, but only a subset of

clades occurs at the highest and coldest elevations (Parra et al. 2010, 2011). In addition, temperature and precipitation have been shown to influence the composition and spatial turnover of hummingbird species in studies conducted in the same region (Graham et al. 2012; Weinstein et al. 2014). As such, the environmental parameters we chose should serve as meaningful environmental filters.

We chose geographic distance and cost distance as proxies for dispersal limitation. We included cost distance because the topographic complexity of the Andes influences genetic structure and species distributions within several groups (McRae et al. 2008; Wang et al. 2009), including hummingbirds (Chaves et al. 2011; Weinstein et al. 2014). We calculated least cost distances between each sampling locality and all grid cells on the dispersion field, using the R package *gdistance* (van Etten 2011). The algorithm calculates an ecologically meaningful measure of distance by assigning a cost to each grid cell on the basis of its environmental dissimilarity to the grid cell of origin. The algorithm then identifies the route that incurs the lowest cost between two points (McRae 2006). The route passes from grid cell to grid cell and corrects for elevational differences in the distance between grid cell centers. We defined the cost of passing through a grid cell as the difference in mean elevation between that grid cell and the grid cell of origin. Elevation is a strong proxy for environmental similarity, a predictor of taxonomic beta diversity, and possibly a strong dispersal boundary in hummingbirds (Parra et al. 2009, 2011; Chaves and Smith 2011). The probability weights were then calculated as the accumulated cost along the lowest cost path from that grid cell.

Probability-Weighted Resampling of Process-Based Species Pools. We compared the phylogenetic and trait structure of hummingbird assemblages using four different variables to refine species pools: (1) temperature, (2) precipitation, (3) distance, and (4) cost distance. We also considered several process-based species pools that integrated two different variables, in order to test for interaction between variables (i.e., temperature-dispersal pool, precipitation-dispersal pool, and temperature-precipitation pool). The weighting probabilities for these interaction models were obtained by multiplying probability weightings for each variable. However, we gained no new insights from these combined analyses, and we do not emphasize nor discuss these results.

The process-based algorithm used to refine species pools was implemented in a two-step process. First, the algorithm selected an assemblage within the dispersion field on the basis of a probability distribution. Second, the algorithm selected a species at random within that assemblage. Note that this two-step resampling procedure implicitly weighs the probability of sampling species by their observed incidence within the dispersion field. The probability of sam-

pling an assemblage from within the dispersion field was determined by a probability distribution reflecting the difference in temperature, precipitation, geographic distance, or elevation (cost distance) between that assemblage and the focal assemblage (fig. 1B). For example, using the distance-weighted species pool, assemblages located near the focal assemblage were more likely to be sampled than those located further away. For each of these variables, we created a pairwise similarity matrix comparing climatic conditions between each possible pair of hummingbird assemblages in the New World. For the distance-weighted species pool, we obtained the probability distribution by repeating the same procedure as for the climate-weighted species pool but using a pairwise matrix of Euclidean distances derived from the geographic coordinates of the centroid of all possible pairs of species assemblages (i.e., grid cells). Finally, we calculated the probability distribution for the cost distance-weighted species pool, using a procedure similar to that used for the distance-weighted species pool but with the additional implementation of cost penalization that accounts for dispersal barriers between species assemblages (described in previous section). We standardized probability distributions to lie between 0 and 1 by transforming each value, given a pairwise matrix of difference values χ_i , using the following equation:

$$\chi_i = \frac{\chi_{\max} - \chi_i}{\chi_{\max}}, \quad (1)$$

where χ_{\max} is the maximum value in matrix χ . All of the four matrices (one for each species pool definition) contained distance values for all possible pairs of grid cells (2,997 rows \times 2,997 columns). Note that for a given species pool definition (or environmental variable), χ_{\max} was always the same, no matter the location of the assemblage or the level of constraint on the resampling.

Constraints on the Resampling of Process-Based Species Pools. One challenge when defining probability weights for resampling the species pool is to decide on the level of constraints, since there is no clear way to identify a single appropriate constraint level for a given filter. Rather, different levels of constraint can be interpreted as the strength of filtering imposed on the definition of the species pool; thus, we evaluated the effects of species pool definition on NRI values across a range of constraint levels. For example, the effect of dispersal limitation might be detectable only if the preference toward resampling species in assemblages adjacent to the focal assemblage is very strong (i.e., if the probability of resampling an assemblage in the dispersion field decays faster than linearly with distance from the focal assemblage). It is possible to increase the effect of the sampling variable (i.e., filter) by limiting inclusion in the species pool to those assemblages with probability values above a

certain threshold (fig. 1C). We accomplished this by creating quantile probability distributions, wherein the X quantile of probability values was discarded and the remainder of probability values retained. If we use this approach with, for example, the distance-weighted species pool, a 0.95 quantile probability distribution would retain only the smallest 5% of Euclidean distances between the focal assemblage and all other assemblages in the dispersion field. In other words, the 5% of assemblages closest to the focal assemblage would be resampled. Here, we initially explored a broad spectrum of quantile values ($Q = 0, 0.25, 0.50, 0.75, 0.85, 0.90, 0.95, 0.99, 1$). However, changes in NRI were obvious only at higher level of constraints; therefore, we focused on a narrower spectrum of quantile values ($Q = 0, 0.75, 0.95, 0.99$). Note that probability distributions are not recalculated after applying the different quantile thresholds.

Sensitivity Analyses

Because patterns of phylogenetic and trait structure are known to be sensitive to various attributes of the species pool, we evaluated how such attributes varied among our species pool definitions and levels of constraint (Kraft et al. 2007). Specifically, we tested whether species pool size (total species richness), phylogenetic diversity, and mean range size of species in the pool were affected by our species pool definitions and level of constraint.

Testing for Niche Conservatism of Species Traits

A central assumption of community phylogenetics is that the phylogeny can predict community trait structure if the traits are phylogenetically conserved. While it is common to associate a high phylogenetic signal (e.g., Blomberg's K) with niche conservatism, there is little evidence supporting this relationship, given that different processes can produce similar values of phylogenetic signal (Revell et al. 2008). Here, instead of estimating the phylogenetic signal of each trait, we tested three models of trait evolution: Brownian motion (BM), which assumes that the correla-

tion structure among trait values is proportional to the extent of shared ancestry for pairs of species (Felsenstein 1973); Ornstein-Uhlenbeck (OU) model, which fits a random walk with a central tendency and with an attraction strength proportional to the parameter α (Hansen 1997; Butler and King 2004); and white noise, which assumes the data come from a single normal distribution with no covariance structure among species. We used the function `fitContinuous` from the R library `geiger` (Harmon et al. 2008). We compared the models using the second-order Akaike information criterion (AIC) and the Akaike weights as the weight of evidence for each model, given all the tested models (Burnham and Anderson 2002). Finding that a trait fits a model of white noise would not support niche conservatism, whereas an OU model, which is linked to stabilizing selection, would (Wiens et al. 2010). Support for a BM model does not permit rejecting the niche conservatism hypothesis if the rate parameter is low, since niche conservatism implies either strong stabilizing selection or a low rate of evolutionary change (Revell et al. 2008).

Results

We compared the outcomes of null model analyses of phylogenetic structure using different definitions of the species pool (fig. 1). Definitions of the species pool differed in two aspects: (1) the process of interest (i.e., temperature filtering, precipitation filtering, dispersal limitation, and the interactions thereof) and (2) the strength of the resampling constraint.

Our analyses of phylogenetic structure indicated that increasing the level of constraint on the temperature-weighted species pool had a stronger influence on the significance of NRI values than with any other species pool definitions (tables 1, A1; figs. 2, 3, A3; tables A1–A4 available online). In particular, the number of phylogenetically even communities ($NRI < -1.96$) increased from 13 under a loosely defined temperature pool ($Q = 0$; all assemblages resampled) to 59 under a highly constrained temperature pool ($Q = 0.99$; 1% of assemblages resampled).

Table 1: Impact of different species pool definitions and levels of constraint on the structure of hummingbird assemblages

Species pools	Phylogeny		Body mass		Beak length	
	Clustered	Even	Clustered	Even	Clustered	Even
Temperature	-1	46	11	8	-1	7
Precipitation	6	13	14	10	0	-2
Dispersal	7	8	9	2	2	-6
Cost distance	9	13	5	11	2	-7

Note: Shown are the differences in the number of local assemblages with significant net relatedness index (NRI) values (i.e., >1.96 for clustering and <-1.96 for evenness), calculated as the number of significant NRI values using a 0.99 quantile minus the number of significant values using a 0 quantile.

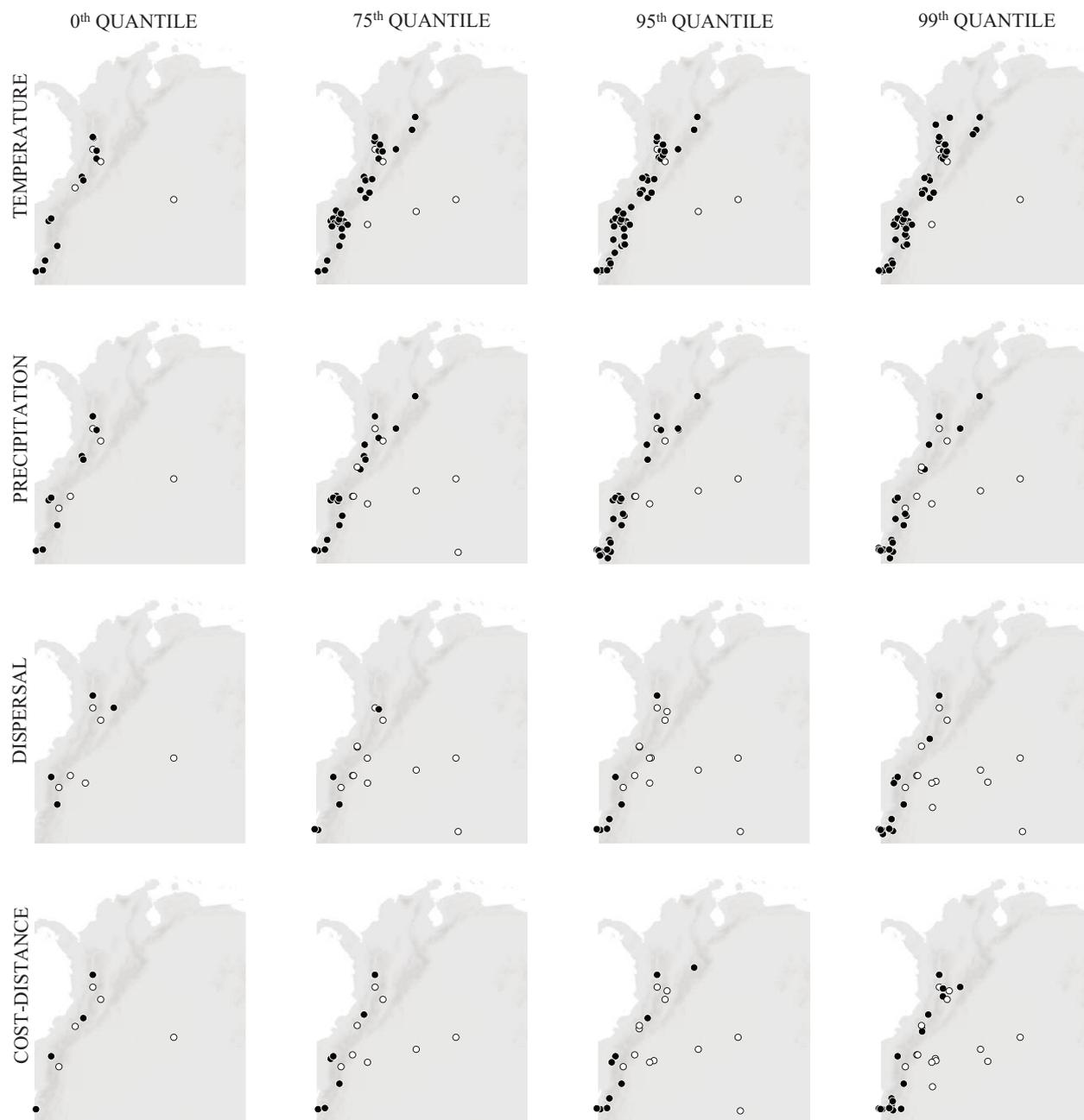


Figure 2: Phylogenetic structure of Andean hummingbird assemblages under four different species pool definitions. White circles indicate assemblages with significant phylogenetic clustering, and black circles indicate evenly dispersed assemblages. Assemblages with nonsignificant deviation from the simulated distribution of mean pairwise distance values are not shown (but see fig. 3). Results are shown for different species pool definitions and levels of constraint.

All of the 13 communities that were evenly dispersed at $Q = 0$ were even more evenly dispersed at $Q = 0.99$. The trend in NRI observed for temperature was stronger with a temperature-weighted species pool than with either a temperature dispersal pool or a temperature precipitation

pool (table A1; fig. A3). Under the temperature species pool, a phylogenetically even dispersion pattern was detected at many elevations along the gradient (fig. 3).

Our analyses of beak length dispersion showed that increasing the level of constraint had little influence on NRI

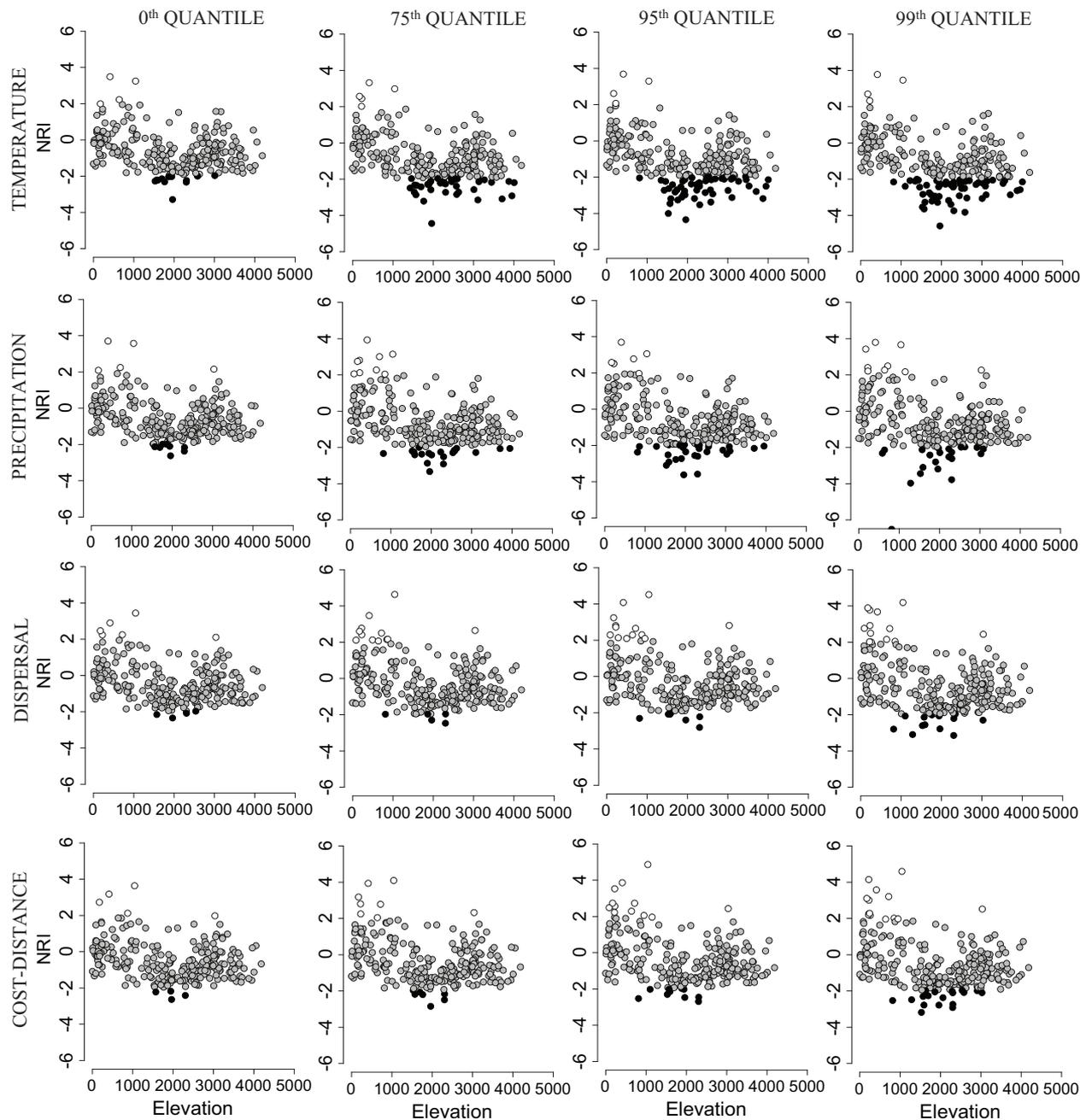


Figure 3: Phylogenetic structure of Andean hummingbird assemblages along an elevational gradient. White circles indicate assemblages with significant phylogenetic clustering, black circles indicate evenly dispersed assemblages, and gray circles indicate assemblages with nonsignificant deviation from the simulated distribution of mean pairwise distance values. Results are shown for different species pool definitions and levels of constraint. NRI, net relatedness index.

values, regardless of the species pool definition (tables 1, A1; figs. A3, A4, A6). Beak length was evenly dispersed in 3–28 communities, depending on species pool definition and constraint level, and showed clustered dispersion in almost none. Assemblages with an even pattern of beak length dispersion

were more common in the northern than the southern part of the Andes (fig. A4) and were detected all along the elevation gradient (fig. A6).

Our analyses of body mass dispersion showed that increasing the level of constraint had minimal influence on

NRI values, regardless of the species pool definition (tables 1, A1; figs. A3, A5, A7). Patterns of even versus clustered body mass dispersion were approximately equally frequent (table A1). Overall, assemblages that were clustered with respect to body mass tended to be more frequent at higher elevations (i.e., >2,000 m), whereas even dispersion was more widely detected at lower elevations (i.e., <3,000 m; fig. A7).

Kraft et al. (2007) showed that for the detection of significant NRI values, power increases with species pool size for communities created by filtering (i.e., clustering) but tends to decrease for communities created by niche differentiation (i.e., yielding a pattern of even dispersion). Here, we found that under the most constrained definition of the species pool, species pool size (total species richness) and phylogenetic diversity were slightly greater for the temperature pool than for other definitions (table A2). If anything, a more diverse species pool should decrease the probability of detecting even dispersion, which is the opposite of what we observed for phylogenetic structure and beak length dispersion but consistent with our result for body mass dispersion. Moreover, differences in NRI values between unconstrained ($Q = 0$) and highly constrained ($Q = 99$) species pool definitions were negatively related to changes in species richness and phylogenetic diversity of the species pool (fig. A8). Here, we would expect a positive relationship if changes in statistical power were the main driver of those patterns. The spatial extent of the species pool did not differ among species pool definitions (table A2).

A white noise model of trait evolution was discarded for all environmental and morphological traits included in our analyses (tables A3, A4), meaning we cannot reject the niche conservatism hypothesis for any of the traits. OU was the best-fitting model for average temperature, average precipitation, and range size (corrected AIC [AIC_c] weights = 0.99 in each case), which supports the niche conservatism hypothesis for these traits. However, BM was a better fit for body mass and length of beak length (AIC_c weights = 0.74 in each case) than OU (AIC_c weights = 0.26). An AIC_c weight of 0.26 for OU could suggest that stasis might have occurred in the evolution of these traits. However, the short phylogenetic half-life for these traits ($2.97E+15$ and $9.97E+15$) means that their evolution was likely governed by BM (table A4), and there is no tendency to move toward an optimum. Taken together, our results suggest that climate envelopes and range size are conserved, whereas morphological traits are not.

Discussion

We explored how various definitions of the species pool affected how we perceive the structure of hummingbird assemblages—and the processes shaping them—by creat-

ing species pools that accounted for environmental filtering, dispersal limitation, or both processes. Our results show that accounting for temperature filtering in the definition of the species pool has a stronger impact on assemblage structure than any other definitions. Specifically, we discovered an increasing number of assemblages with a phylogenetically even structure as we increased the level of constraint on the temperature filter but not when we repeated this procedure with other filters (i.e., precipitation or dispersal filters). We also found that a smaller number of communities exhibit either clustered patterns of body mass, as expected from environmental filtering, or evenly dispersed patterns of body mass and beak length, a pattern implicating niche differentiation. However, because patterns of phylogenetic and trait structure do not converge spatially, it remains challenging to infer the underlying coexistence mechanism. Nevertheless, our heuristic approach identified potential ecological processes acting simultaneously by revealing patterns of assemblage structure that would otherwise be hidden.

By explicitly accounting for the influence of environmental filtering in the definition of the species pool, we can strengthen the inference of local processes shaping community structure (Graves and Gotelli 1983; Zobel 1997). Further, by exploring a series of levels of constraint on that species pool definition, we can assess the magnitude by which environmental filtering influences the observed pattern. In our study, a large number of hummingbird assemblages in the Andes were phylogenetically even when the species pool was highly constrained to be environmentally filtered, but not otherwise. Specifically, we found that patterns of phylogenetic evenness were strongest when the species pool included only species that could tolerate the local abiotic conditions—and, in particular, annual mean temperatures—at the focal study site. This pattern was widespread at elevations above 1,000 m and suggests that regional environmental filtering and local niche differentiation jointly influence assemblage structure. This result builds on those of Graham et al. (2009), who studied the same hummingbird assemblages but restricted the species pool to the list of species recorded across study sites. Using their approach to define the species pool, they were able to detect only the influence of environmental filtering and not the possible role of competition in structuring high-elevation assemblages (for details, see fig. A9).

Although process-based definitions of the species pool enable us to refine our interpretation of phylogenetic structure, it remains challenging to infer the processes underpinning phylogenetic evenness. First, our interpretation of patterns of phylogenetic structure relies on the assumption that traits mediating environmental filtering (in the case of clustering) and coexistence (in the case of even dispersion) are phylogenetically conserved (Losos 2008; Mayfield and

Levine 2010). However, evidence for niche conservatism in body mass and beak length is weak, which prevents us from concluding that these traits underlie the generalized patterns of phylogenetic evenness. Results from niche conservatism analyses instead suggest that tolerance to abiotic conditions is evolutionarily conserved, whereas morphology is more labile. These results are consistent with the idea proposed by Silvertown et al. (2006), whereby traits that determine environmental associations (also known as β niche) evolve conservatively, whereas traits that enable coexistence (also known as α niche) are evolutionarily labile. Second, because the temperature niche is conserved in hummingbirds and we used temperature to define our species pools, it might be that particular clades are at least partly responsible for our observed patterns of evenness. Similar to our result, analyzing clades separately, Parra et al. (2011) found patterns of phylogenetic evenness at high elevation for one of the two clades that radiated in the Andes (i.e., for brilliants but not coquettes) and for emeralds, which are also somewhat common in midelevation assemblages. Taken together, these results suggest that conservatism in the evolution of climatic niches, environmental filtering, and competitive interactions might interact to shape hummingbird assemblages, at least at high- and midelevation sites dominated by particular clades.

Dispersal limitations could play a role in shaping the structure of hummingbird assemblages. Specifically, phylogenetic overdispersion could stem from allopatric speciation with insufficient time for dispersal to yield secondary sympatry. However, using two different approaches to model dispersal limitations in our species pools (dispersal pool and cost distance pool definitions), we found little support for the influence of this process on assemblage structure. Moreover, the low phylogenetic beta diversity observed among assemblages and across prominent geographic barriers suggests that dispersal limitation for Andean hummingbirds is unlikely (Weinstein et al. 2014).

We expected patterns of beak length (i.e., culmen length) dispersion to be increasingly even as we increased the level of constraint on the temperature species pool. In hummingbirds, the extreme metabolic demands of hovering flight lead to intense exploitative and interference competition for floral nectar resources (Feinsinger and Colwell 1978; Feinsinger et al. 1979; Altshuler and Dudley 2002). In addition, hummingbirds partition resources through differences in beak morphology (Feinsinger and Colwell 1978; Maglianesi et al. 2014; Vizentin-Bugoni et al. 2014). However, the signature of niche differentiation might emerge only after accounting for the influence of environmental filters. We indeed found that evenness in beak length was more prevalent than clustering, but this pattern was not necessarily stronger with a temperature-weighted species pool, and those assemblages exhibiting evenness in beak length were

usually not the same as those exhibiting phylogenetic evenness. Only 12% of phylogenetically even assemblages showed the same pattern for beak length (data not shown). This is perhaps not surprising, given the lack of strong phylogenetic conservatism observed in this trait. In addition, assemblages exhibiting evenness in beak length were found throughout all except the highest elevations, the opposite pattern of that found for phylogenetic evenness.

High-altitude hummingbirds are characterized by short and straight beaks (e.g., *Chalcostigma*, *Metallura*, *Ramphomicron*, *Eriocnemis*, *Oxygogon*, *Aglaeactis*), and the variation and range of beak lengths is narrower at high than at low elevations (data not shown), possibly reflecting an environmental filter related to coevolution with flowers (Wolf et al. 1976; Brown and Bowers 1985; Maglianesi et al. 2014). One possible explanation for the lack of consistency among patterns of phylogenetic and trait assemblage structure is that it may merely be a statistical artifact of having less power at high elevations to detect a significant deviation. Another is that species at high elevations are achieving co-occurrence through other means than character spacing, for example, by differentially using space or time (Wolf et al. 1976). Regardless, there are clear inconsistencies between patterns of trait dispersion and phylogenetic structure, which indicate a potential for further exploration and fine-tuning of this conceptual framework. It still remains unclear whether we should expect to find consistent patterns of trait and phylogenetic dispersion and for which (or all) traits.

We hypothesized that body mass, which relates to thermal tolerance in hummingbirds (Stiles 2008), would be clustered when the species pool was defined loosely but random after accounting for environmental filtering. Although we did find a certain number of clustered assemblages, clustering increased rather than decreased when the species pool included only those species that can tolerate the local abiotic conditions. In addition, we found that the number of assemblages exhibiting clustered body mass dispersion was approximately equal to the number of assemblages with even dispersion. Clustering of body mass tended to be more common at higher elevations (>2,000 m), consistent with the findings of Graham et al. (2012), whereas even dispersion was more common at lower elevations (<3,000 m). Hummingbirds tend to be large on average—and the range and variation in weights narrow—at high elevations. Thus, the pattern of clustering at high elevations, even with a constrained species pool, is surprising and might be taken as evidence of additional filters we have not yet considered. At lower elevations, even body mass dispersion in some lowland assemblages is consistent with the idea of niche partitioning—and there are clear examples of this segregation within clades (e.g., co-occurrence of large and small *Phaethornis*)—but does not match patterns of phylogenetic

dispersion in the lowlands. In sum, we found limited support for the hypothesis that body mass mediates the impact of environmental filtering on high-elevation assemblages of hummingbirds.

Our study reveals a clear discrepancy between results from phylogenetic and trait-based analyses, a result that is increasingly observed in studies of community phylogenetics (Kraft et al. 2008; Graham et al. 2012). This lack of consistency might suggest that traits other than the two we measured here enable niche differentiation among co-occurring species of hummingbirds. For example, it could be that niche differentiation involves character displacement in other traits such as beak curvature, wing shape, or tarsus length rather than the length of beaks, or a complex mix of morphological characters, as shown in other studies (Jönsson et al. 2012, 2015). Therefore, the combined use of phylogeny and traits, together with a process-based species pool definition, proved to be a powerful approach. Indeed, using this approach, 32% of our hummingbird assemblages were evenly dispersed on the basis of phylogeny or traits, and the strength of even dispersion increased when using a highly constrained temperature pool.

While there might not be one single acceptable species pool definition, there are a few considerations that will contribute to improving ecological inference derived from null model analyses (for an example, see fig. A9). First, it is important to evaluate the completeness of the species pool sampling or to obtain a complete regional species inventory (Gotelli and Graves 1996; Carstensen et al. 2013). It is commonplace to pool species lists from local surveys to define the species pool. However, a tally from local surveys most likely underestimates the richness and misrepresents the composition of the species pool (Pärtel et al. 2011), both of which can influence results from null models (Kraft et al. 2007; Kembel 2009). Second, the species pool should be defined explicitly rather than arbitrarily (Graves and Gotelli 1983; Zobel 1997; Graves and Rahbek 2005). To address these issues, we defined species pools using exhaustive information on the geographic distributions of species and resampled those pools on the basis of either environmental affinities or dispersal probabilities (Lessard et al. 2012a).

Our study illustrates the complexity of processes underlying patterns of assemblage structure and perhaps provides an explanation for much of the idiosyncrasy and contingency observed in ecology (Lawton 1999; Vellend 2010; Chase and Myers 2011; Lessard et al. 2012a). Our heuristic approach with multiple explicit species pool definitions is a first step in this direction. Our results, which suggest that both filtering and biotic interactions could influence the composition of hummingbird assemblages, are consistent with a more recent study that focused on patterns of morphological trait dispersion in the same hummingbird assem-

blages (Graham et al. 2012). The signature of biotic interactions might be visible only after accounting for environmental filtering, which potentially operates at a different spatial and temporal scale. Our results thus call for a re-examination of a multitude of null model-based studies published in recent years that have defined the species pool incompletely and/or arbitrarily.

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Left, *Calliphlox mitchelli*. Jardín, Antioquia, June 27, 2015. Center, *Lafresnaya lafresnayi*, the only nonhermit high-elevation species with a long and curved beak. Jardín, Antioquia, June 27, 2015. Right, *Discosura conversi*. This species represents a lineage from the coquette family that colonized the lowlands. La Unión, Carepa, Antioquia, Colombia, January 8, 2015. Photographs by Carlos Bran-Castrillón.