

# Contrasting patterns of phylogenetic assemblage structure along the elevational gradient for major hummingbird clades

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## ABSTRACT

**Aim** We evaluated the hypothesis that, given niche conservatism, relatedness of co-occurring hummingbird species of a given clade will increase at greater distances from the elevation where it originated. We also used prior knowledge of flight biomechanics and feeding specialization of hummingbird species (family Trochilidae) to evaluate which environmental variables were important predictors of changes in phylogenetic structure for each hummingbird clade.

#### Location Ecuador.

**Methods** We compiled species lists for 189 local hummingbird assemblages across major environmental gradients in Ecuador from a variety of published and non-published sources. For the entire family and each of the major hummingbird clades (hermits, emeralds, mangoes, coquettes and brilliants) we quantified the phylogenetic structure of each assemblage using the net relatedness index (NRI). This index calculates the standardized mean of all possible pairwise phylogenetic distances between co-occurring species. We related NRI for each clade to elevation, precipitation and vegetation-related variables using generalized additive models.

**Results** Our findings support the prediction of an increase in the co-occurrence of close relatives away from the elevation of origin at the family level and for assemblages of mangoes and brilliants. The opposite pattern was found for assemblages of coquettes and emeralds. For the hermits, variation in phylogenetic structure was not explained by elevation. Clades with high levels of feeding specialization (hermits and brilliants) always included a vegetation-related variable as an important predictor of change in phylogenetic structure.

**Main conclusions** We found no overall support for the conservatism and zone of origin hypotheses. Knowledge of each clade's natural history proved useful for predicting which environmental variables correlated with phylogenetic structure of local assemblages. Clades with the same elevation of origin appear to have radiated along the elevational gradient in association with different environmental factors.

## Keywords

Birds, community, Ecuador, environmental factors, hummingbirds, net relatedness index, niche conservatism, phylogenetic structure, Trochilidae, zone of origin.

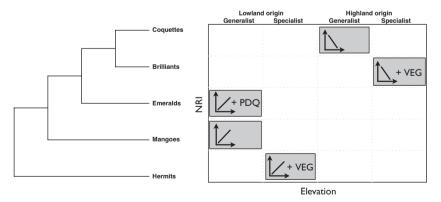
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# INTRODUCTION

Despite the large number of hypotheses that attempt to explain the contemporary distribution of species richness (Hawkins et al., 2003; Currie et al., 2004; Mittelbach et al., 2007; Field et al., 2009), few hypotheses address how phylogenetic relatedness of assemblages varies across space (Wiens & Donoghue, 2004; Ricklefs, 2006; Algar et al., 2009). The tropical niche conservatism hypothesis posits that phylogenetically conserved climatic tolerances combined with the tropical origin of many taxa explain the observed latitudinal diversity gradient in species richness (Ricklefs & Latham, 1992; Ackerly, 2003; Hawkins & Agrawal, 2005; Wiens et al., 2005; Hawkins et al., 2006). Algar et al. (2009) recently extended this hypothesis to evaluate how phylogenetic structure changes in relation to the zone of origin. They proposed that species richness should decrease and the co-occurrence of close relatives should increase in localities that are further removed from their zone of origin (also see Ricklefs, 2006). Here we evaluate changes in the phylogenetic composition of five hummingbird clades with zones of origin at distinct elevations. Additionally, phylogenetic structure might be responding to variation in other factors that vary along the elevational gradient. We use cladespecific ecomorphological traits to predict which axes of environmental variation should be relevant for changes in phylogenetic structure.

Graham *et al.* (2009) studied the phylogenetic structure of hummingbird assemblages across environmental gradients in Ecuador. They identified two environmental gradients where the phylogenetic structure of assemblages changed from even (i.e. distant relatives co-occur) to clustered (i.e. close relatives co-occur), one along the elevational gradient associated with the Andes and another along the western lowlands where precipitation and seasonality change dramatically (Sierra, 1999). At the family level, these patterns are consistent with the expectation from the niche conservatism hypothesis because hummingbirds most likely originated in the humid lowlands (Bleiweiss, 1998a; McGuire et al., 2007). Graham et al. (2009) interpreted these gradients in phylogenetic structure as consistent with environmental filtering at high elevations (i.e. aerodynamic challenges to flight at high elevations) or in the dry lowlands (i.e. water efficiency, seasonality of food availability), and competition in the wet lowlands. They suggested that these patterns of phylogenetic structure were a result of different clades responding in distinct ways to environmental gradients, but did not conduct clade-level analyses. We expect clade-level analyses to provide more insights into the ecological and evolutionary mechanisms that influence the observed variation in phylogenetic structure in hummingbird assemblages. Here, we evaluate five clades that have a relatively high number of co-occurring species in our study region: hermits (c. 17 species), mangoes (c. 12 species), emeralds (c. 27 species), brilliants (c. 30 species) and coquettes (c. 27 species; Fig. 1). Clade designation is based on plumage and trophic characteristics, as well as several anatomical and molecular studies (Zusi & Bentz, 1982; Bleiweiss et al., 1994, 1997; Stiles, 2008; McGuire et al., 2009).

Ancestral phylogenetic reconstructions indicate that these hummingbird clades most likely originated at different elevations (McGuire et al., 2007). The hermits, mangoes and emeralds probably had a lowland origin, while the coquettes and brilliants most likely originated within the Andes (McGuire et al., 2007). Assuming conservatism in hummingbird climatic tolerances and morphologies, an assumption generally supported by phylogenetic, biogeographical and morphological studies in this group (Bleiweiss, 1998a,b; Stiles, 2004, 2008), we expect that the co-occurrence of close relatives (i.e. phylogenetic clustering) should increase at greater distances from their elevation of origin (Fig. 1). Because of the energetic demands of flight in hummingbirds at high elevations and the conservatism in flight morphology (Altshuler et al., 2004; Altshuler & Dudley, 2006), we expect all clades to respond to this important axis of environmental variation. While the elevational gradient provides a challenge to hummingbirds, other factors might also be associated with variation



**Figure 1** Phylogenetic tree representing the backbone of relationships among the five major hummingbird clades used in the analyses: hermits, mangoes, emeralds, coquettes and brilliants. Boxes show the expectations of the relationship between net relatedness index (NRI) and elevation based on the most likely elevation of origin for each clade (McGuire *et al.*, 2007). Based on a broad categorization of clades into feeding generalists or specialists based on bill length, and results from previous studies (Graham *et al.*, 2009), we predict that in addition to elevation, other factors including precipitation of the driest quarter (PDQ) and vegetation-related variables (VEG) should be important in explaining variation in NRI for particular clades.

in patterns of relatedness (Graham *et al.*, 2009). For example, some species of emeralds are more common in dry regions than hummingbirds from other clades (Parra *et al.*, 2010). There is also evidence that hermits exhibit no morphological variation across elevation in wing-loading (body mass divided by wing area), a characteristic that presumably precludes them from occupying alpine environments (Stiles, 2004). Thus, other environmental factors, in addition to elevation, might influence variation in phylogenetic structure (Fig. 1).

Hummingbird clades exhibit ecomorphological traits that allow them to exploit different floral resources, which could also determine the types of environmental factors to which species respond (Rodríguez-Flores & Stiles, 2005; Stiles, 2008). Based on previous work on hummingbird morphology and behaviour (reviewed in Feinsinger & Colwell, 1978), we assume that species with long beaks (and therefore large body size and longer wings; see Stiles, 2008), whether curved or straight, are more specialized in terms of the flower resources used than short-billed hummingbirds. Hermits usually have long and curved bills (Schuchmann, 1999; Stiles, 2008; Temeles et al., 2010) and feed largely on understorey monocot herbs (Stiles, 1975; Rodríguez-Flores & Stiles, 2005). Thus, in addition to the elevational gradient, we expect hermits to respond to vegetation (Fig. 1). Mangoes occupy a broad range of habitats and their beak and wing morphologies are quite variable (Bleiweiss, 1998a; Stiles, 2008). Due to their generalist habits, we do not expect mango assemblages to respond strongly to changes in vegetation. Both the brilliants and coquettes have relatively straight bills, although brilliants are larger, have longer bills, smaller feet, and shorter tarsi than coquettes (Stiles, 2008). Because brilliants have longer beaks (Stiles, 2008), we assume they have more specialized feeding habits, and thus, like the hermits, should respond to gradients in vegetation or precipitation, in addition to elevation. Finally, the emeralds comprise a radiation of morphologically homogeneous birds mostly occupying the lowlands and often somewhat dry regions (Bleiweiss, 1998a). Emerald phylogenetic structure should not be driven by changes in vegetation because they have generalist feeding habits, but there is evidence that they might respond to changes in precipitation regimes (Parra et al., 2010).

We evaluate the prediction that there will be phylogenetic clustering away from the elevation of origin for assemblages of five hummingbird clades. We also assess whether additional factors are important in explaining the variation in phylogenetic structure for hummingbird clade assemblages in Ecuador (Fig. 1).

# MATERIALS AND METHODS

#### Study area

Our study encompasses Ecuador's entire mainland. Even though we recognize that this political boundary does not necessarily represent a bioregion, our main interest was to cover an area that included a variety of environmental conditions. Elevational gradients in the Andes span from sea level to more than 6000 m a.s.l. Further, the lowlands on either side of the Andes are distinct. The east is humid and aseasonal. In the west there is a gradient from humid in the north to dry in the south, a pattern caused by the interaction between the circulation of heat in the atmosphere and the Humboldt Current (Ruddiman, 2001). Variation in precipitation across elevational gradients can also influence patterns of diversity. For example, mountains that are dry at the base often have different diversity patterns than those that are wet at the base (McCain, 2005). Hence, the existence of multiple factors varying along the elevational gradient offers a unique opportunity to disentangle the relationship between diversity and environmental factors.

#### Assemblage composition

We compiled a database of hummingbird assemblages using lists (presence/absence data) from published references in peer-reviewed journals, grey literature, and non-published reports from environmental organizations, including BirdLife International and Aves & Conservación (see Appendix S1 in Supporting Information). All the lists were checked for their georeference, elevation and species composition. Both local experts and the authors ensured that each species in each assemblage was within its known elevation and geographical range. We also checked that the lists represented all or most of the species expected to be observed in those assemblages. The composition of hummingbird assemblages is known to vary across time and space (Feinsinger et al., 1988; Hilty, 1997). These lists generally include species that appear only during part of the year, but there is certainly variation in their extent of completion. Nevertheless, the measures of phylogenetic structure implemented here should be robust to this variation. The taxonomy was updated to reflect the current version of the South American Classification Committee (Remsen et al., 2008, version: 1 November 2008). The average area covered by sites was 4.2 km<sup>2</sup> (median: 1, range: 0.07–25) and the average recorded elevational range covered was c. 300 m (median: 200, range: 0-2500). Assemblage species richness was not related to either area or elevational range covered (Appendix S2). The area and elevational range reported in the studies where the lists come from does not always reflect the elevations or area actually sampled but were often intended to describe the general area where the inventory took place (e.g. Cotopaxi National Park, 3400-5897 m). In total, we accumulated data for 189 assemblages that included 126 species out of which 113 had phylogenetic information. Ecuador has 132 hummingbird species (Ridgely & Greenfield, 2001). Clades were well represented in these assemblages (hermits - 64 assemblages with more than one species represented, mangoes - 56, emeralds -87, coquettes -96, and brilliants -102).

## Phylogeny

We used a densely sampled phylogenetic estimate (166 of 324 species) for hummingbirds as the historical framework for comparative analyses. Phylogenetic relationships were inferred

using DNA sequences representing three nuclear genes [adenvlate kinase intron 5 (AK1), beta fibrinogen intron 7 (Bfib), and ornythin decarboxylase intron 6 (ODC)], and two mitochondrial genes [NADH dehydrogenase subunit 2 and 4 (ND2, ND4)], comprising 4906 aligned base pairs. All sequences have been stored in GenBank (accession numbers GU166869-GU167199). The phylogenetic estimate was based on partitioned Bayesian analysis (MRBAYES v. 3.1; Ronquist & Huelsenbeck, 2003) with separate partitions applied to each nuclear gene, as well as to each codon position within the mitochondrial genes and their flanking tRNAs (for a total of 12 partitions). Appropriate substitution models for each partition were determined using MODELTEST v. 3.06 (Posada & Crandall, 1998). The resulting tree is well resolved and supported with 141 of 164 nodes receiving posterior probabilities of 95% or greater. In terms of taxonomic coverage, taxa missing from our phylogeny are primarily from outside the region of interest for this study (e.g. Ecuador). Thus, our tree includes 113 of the 126 species for which we have assemblage occurrence data. Our data matrix includes complete data for each species except Phaethornis superciliosus (missing AK1), Chalcostigma stanleyi (missing AK1), Urosticte ruficrissa (missing ODC), Thalurania fannyi (missing AK1, ODC), and Hylocharis gravi (missing AK1, Bfib, ODC). To produce the ultrametric tree needed for assemblage structure analyses, we transformed the Bayesian consensus topology using nonparametric rate-smoothing (Sanderson, 1997) as implemented in TREEEDIT v. 1.0a10 (A. Rambaut, Edinburgh, UK).

## **Phylogenetic structure**

We calculated the net relatedness index (NRI) to estimate phylogenetic structure of local communities by clade. NRI compares the mean pairwise phylogenetic distance (MPD) among all pairwise combinations of species within an assemblage to the MPD of samples of the same size drawn at random from a source pool (in our case all species of a given clade within Ecuador) as follows (Webb *et al.*, 2002):

 $NRI_{sample} = -1 \times (MPD_{sample} - MPD_{rndsample}) / sd(MPD_{rndsample}),$ 

where 'rnd' refers to random and 'sd' refers to standard deviation. Null assemblages were generated under the independent swap algorithm (Gotelli & Entsminger, 2003). This algorithm is preferred to other methods because it maintains species' occurrence frequencies and assemblage richness (i.e. number of species per assemblage) while randomizing patterns of co-occurrence (Kembel & Hubbell, 2006; Kembel, 2009). We assessed the significance of each observed value of NRI by comparing it to the distribution of 999 NRIs calculated under the null model. Positive NRI values indicate phylogenetic clustering, and negative ones phylogenetic evenness. All analyses were carried out using PHYLOCOM v. 4.01.b (Webb *et al.*, 2008).

# **Contemporary environmental variables**

We chose a priori three factors that could influence patterns of phylogenetic structure: vegetation, which we expect is correlated with resource availability, and elevation and precipitation, which impose physiological limitations on hummingbird energetics. We used four variables representing these three factors: (1) elevation, (2) precipitation of the driest quarter, (3) complexity of forest structure, and (4) percentage tree cover. Elevation was taken from the digital elevation model supplied by the Shuttle Radar Topography Mission at 250-m resolution. The precipitation data used are interpolated from weather station records averaged between 1950 and 2000 (Hijmans et al., 2005). Even though these data have some deficiencies in topographically complex areas with low weather station coverage, the Andes are one of the most populated areas in Ecuador and many weather stations are placed along the elevational gradient. Therefore, we feel confident about using these data at the spatial resolution of our study. The latter two variables are derived from microwave data sent from QSCAT (Quick Scatterometer) and MODIS (Moderate Resolution Imaging Spectroradiometer), respectively, and are good predictors of bird composition in the Andes (Buermann et al., 2008). OSCAT is an active-sensor (radar) satellite originally developed for measuring wind speed and direction over the ocean, but is also useful for vegetation structure metrics (e.g. estimates of forest structure complexity, Buermann et al., 2008). We used the annual mean of radar backscatter with horizontal polarization based on average monthly QSCAT composites at 1-km resolution (Long et al., 2001). Percentage tree cover was generated from the 1-km resolution global 8-day MODIS and provides an indicator of tree cover and a surrogate for the spatial distribution of biomass density (Hansen et al., 2002).

#### Correlates of phylogenetic assemblage structure

To evaluate the relationship between environmental variables and NRI we ran generalized additive models (GAM) using the library мдсv v. 1.5-2 in R v. 2.8.0 (Wood, 2004, 2006, 2008). In order to estimate which variables had a significant contribution to the variation in NRI, we first started with the full model (i.e. including all four variables) and then dropped terms following three criteria specified by Wood (2006). First, we checked whether the estimated degrees of freedom from the smoothing function of any predictor variable were close to one, indicating that the relationship estimated between the predictor and NRI is linear. The degrees of freedom for each smoothing function are estimated using general cross validation. To prevent over-fitting, we also enforced simple relationships in the GAMs by minimizing the number of smoothing terms fitted. Second, we checked whether after fitting the GAM, zero was included in the confidence interval band throughout the range of the predictor variable. Third, we checked whether there was a drop in the general cross validation score after removing the term. If all three criteria applied, we dropped the term from the model. If the estimated degrees of freedom were close to one but the other two criteria did not apply, we replaced the smoothing parameter with a linear term following Wood (2006). As

composition of assemblages on opposite sides of the Andes is different, potentially for historical reasons (Kattan *et al.*, 2004; Thomassen *et al.*, 2010), we evaluated whether east/west slope was necessary as an explanatory variable. We ran all full GAMs with and without the inclusion of slope and used the change in AIC<sub>c</sub> (Akaike information criterion corrected for small sample size) as a criterion to evaluate the effect of this variable. Changes in AIC<sub>c</sub>  $\leq$  2 units were considered within the range of equally likely models. If the change in AIC<sub>c</sub> was above 2 units, we chose the model with lower AIC<sub>c</sub>.

Assemblages may not be independent. At relatively small spatial scales, individuals can disperse among assemblages, while at larger spatial scales the distribution of species is related to environmental variables. In order to evaluate the potential existence of spatial autocorrelation in the residuals of the fitted GAMs, we calculated both Moran's I and Geary's C among various distance classes (e.g. 0-5, 7.5-10 km), ensuring that at least five pairs of samples were used in each distance class. We used an inverse distance squared weight matrix and assumed a random distribution of points. Positive values of Moran's I indicate positive spatial autocorrelation and negative values indicate negative autocorrelation. Values between 0 and 1 of Geary's C indicate positive spatial autocorrelation, while values higher than 1 indicate negative spatial autocorrelation. We assessed the significance of Moran's I and Geary's C at each distance class and the significance of the correlogram after Bonferroni corrections using the program PASSAGE v. 1.1 (Rosenberg, 2001).

## RESULTS

The addition of slope as a covariate did not significantly improve the information content of the general additive models (changes in AIC<sub>c</sub> were all below two units, Table 1); therefore, we only ran GAMs for both slopes jointly. Spatial autocorrelation was significant for both Moran's I and Geary's C at the third distance class (10-20 km) for the residuals of the GAM for assemblages including only mangoes (I = -0.7, P = 0.02,C = 2.1, P = 0.03, Appendix S3). Both coefficients indicated a negative spatial autocorrelation, meaning that closer communities have very different residuals. There were two other instances in which only Moran's I was significant for a distance class. There was a positive autocorrelation for the residuals of the GAM for mango assemblages within the first distance class (0-5 km) (I = 1.5, P = 0.01) and a positive spatial autocorrelation for the residuals of the GAM for assemblages of hermits within the fourth distance class (30–40 km; I = 0.4, P = 0.04). No correlogram (i.e. including all distance classes) was significant after Bonferroni corrections and no other distance class showed significant autocorrelation in the residuals (Appendix S3). Given the inconsistent pattern of significance in Moran's I and Geary's C and the lack of significance after Bonferroni corrections, we concluded that our inferences are unlikely to be biased by spatial autocorrelation.

The relationship between NRI and elevation differed among clades and was not always as expected based on the zone of

**Table 1** Change in the Akaike information criterion corrected for small sample size (AIC<sub>c</sub>) when adding slope as a variable in the generalized additive models relating the net relatedness index to temperature, precipitation, elevation and vegetation variables in hummingbird assemblages in Ecuador. Changes below 2 units of AIC<sub>c</sub> were considered not significant. If the change was above 2 units, we picked the model with the lowest AIC<sub>c</sub>.

Clade	AIC <sub>c</sub>	AIC <sub>c</sub> (model + slope)	$\Delta AIC_c$		
All	557.4	557.4	0.06		
Coquettes	225.5	226.3	0.78		
Brilliants	225.8	227.5	1.67		
Hermits	111.2	112.8	1.63		
Mangoes	146.6	147.1	0.52		
Emeralds	176.0	174.8	1.23		

origin and conservatism hypothesis (Figs 2-7, Table 2). We show both the raw and predicted relationships between NRI and the environmental variables selected in the final GAM. Nevertheless, interpretations were based on the modelled relationship from the GAM (plots shown on the second column of Figs 2-7). When all hummingbirds were considered simultaneously, the expectation from the zone of origin hypothesis was supported; phylogenetic clustering occurred away from the elevational zone of origin (Fig. 2, also see Graham et al., 2009). When analyses were undertaken separately for each clade, this result was only seen in mangoes (Fig. 3, clustering at high elevations) and brilliants (Fig. 4, clustering at low elevations). Emeralds and coquettes exhibited the opposite pattern (clustering at elevation of origin; Figs 5 & 6, respectively). Elevation was not an important predictor of variation in NRI for hermits (Table 2).

In addition to elevation, precipitation and vegetation features were important predictors of the variation in NRI in specific clades (Table 2). Phylogenetic structure based on all hummingbird species (Fig. 2, Table 2) had a tendency to become more even at high values of forest structure (e.g. forest with high structural complexity), and high levels of precipitation of the driest quarter. Forest structure and precipitation of the driest quarter seemed to have very little effect on NRI at low values (Fig. 2). In the brilliants, NRI was positively related to precipitation of the driest quarter (Fig. 4). Variation in NRI showed a tendency for clustering at high levels of tree cover (Fig. 4) but not for low values of tree cover (Fig. 4). Emerald assemblages were phylogenetically even throughout the eastern side of the Andes (all values of NRI below zero) but relatively more even in humid lowlands (Fig. 5). On the western side of the Andes, emerald assemblages were phylogenetically clustered in areas with less precipitation of the driest quarter (e.g. sites with a very strong dry season, Fig. 5). Hermit assemblages tended to be phylogenetically even in areas with high levels of forest structural complexity, but relatively clustered in forests with high values of tree cover (Fig. 7, Table 2).

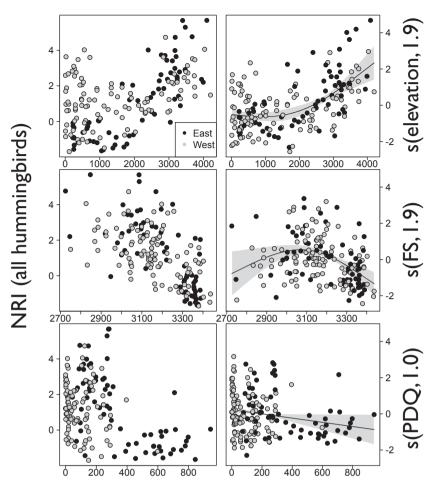


Figure 2 Observed relationship between net relatedness index (NRI) (considering all hummingbirds in assemblages in Ecuador) and values of environmental variables selected in the best generalized additive model (GAM) are plotted in the first column. Plots in the second column present the predicted relationship between NRI and the environmental variables from the best GAM (black line) showing 95% confidence interval of the prediction shaded in grey and partial residuals (points). Black and grey points represent assemblages (n = 174) on the east or west of the Andes, respectively. Partial residuals refer to the residuals obtained by dropping the term of interest while accounting for the other terms in the model. Labels on the y-axes in the second column of plots refer to the smoothed function (s) for the term of interest (elevation; FS, complexity of forest structure; PDQ, precipitation of the driest quarter) and the estimated degrees of freedom (following the term). The x-axes for each plot correspond to the term evaluated.

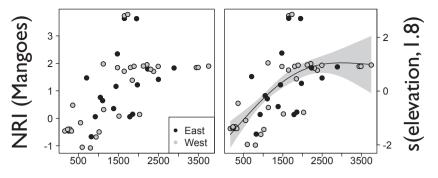
**Table 2** Results of the generalized additive models relating net relatedness index (NRI) based on all hummingbirds in local assemblages, and for each clade separately, to four environmental variables throughout Ecuador. Only results for variables that were not dropped from the model are shown. *F*-values (*F*) and their associated probabilities (*P*) are presented. Estimated degrees of freedom for each variable are presented in Figs 2–7. As measures of overall fit we present adjusted  $R^2$  and the deviance explained.

	All		Mangoes		Coquettes		Brilliants		Emeralds		Hermits	
	F	Р	F	Р	F	Р	F	Р	F	Р	F	Р
Elevation	19.61	0.00**	24.40	0.00**	34.55	0.00**	12.08	0.00**	15.01	0.00**		
PDQ	4.33	0.04*					10.06	0.00**	45.82	0.00**		
TC							4.17	0.02*			9.79	0.00**
FS	10.14	0.00**									4.34	0.02*
$R^2$	0.51		0.48		0.43		0.49		0.42		0.35	
Deviance (%)	52.50		49.70		44.10		51.80		43.50		40.10	
n	174		50		90		99		76		49	

PDQ, precipitation of the driest quarter; TC, tree cover; FS, forest structure; *n*, sample size (number of assemblages). \*P < 0.05; \*\*P < 0.01.

# DISCUSSION

The net relatedness index for each clade within the hummingbirds showed different responses to the same climatic and vegetation variables. First, we found no overall support for the conservatism and zone of origin hypotheses; species within assemblages from localities that are further removed from the ancestral elevation of origin were not consistently more closely related. In two clades (mangoes, brilliants) we found support for this prediction, while in two other clades (coquettes and emeralds) the patterns were opposite to those expected along the elevational gradient. In the hermits, NRI variation was not associated with elevation. Second, variation in NRI was predicted from variables potentially associated with the



**Figure 3** Observed relationship between net relatedness index (NRI) (considering only mangoes in assemblages in Ecuador) and values of environmental variables selected in the best generalized additive model (GAM) are plotted in the first column. Plots in the second column present the predicted relationship between NRI and the environmental variables from the best GAM (black line) showing 95% confidence interval of the prediction shaded in grey and partial residuals (points). Black and grey points represent assemblages (n = 50) on the east or west of the Andes, respectively. The label on the *y*-axis in the second column of plots refers to the smoothed function (s) for the term of interest (elevation) and the estimated degrees of freedom (following the term).

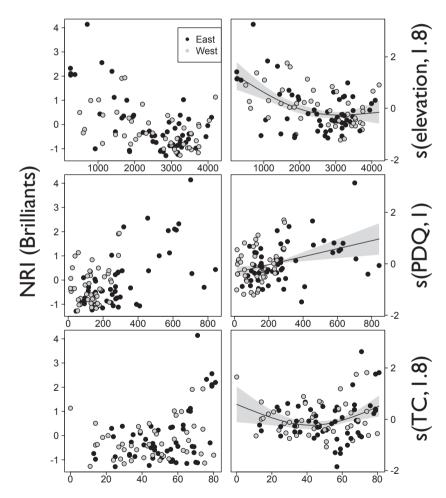
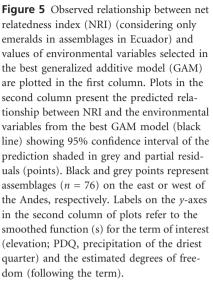
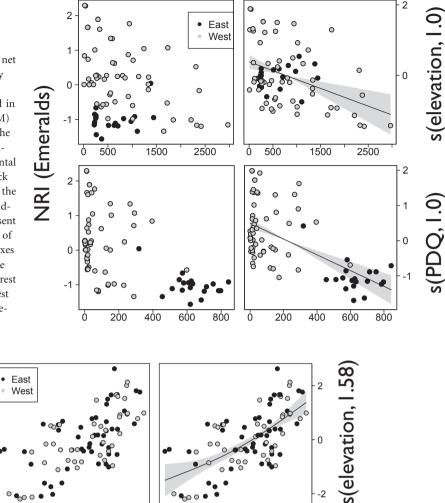


Figure 4 Observed relationship between net relatedness index (NRI) (considering only brilliants in assemblages in Ecuador) and values of environmental variables selected in the best generalized additive model (GAM) are plotted in the first column. Plots in the second column present the predicted relationship between NRI and the environmental variables from the best GAM (black line) showing 95% confidence interval of the prediction shaded in grey and partial residuals (points). Black and grey points represent assemblages (n = 99) on the east or west of the Andes, respectively. Labels on the y-axes in the second column of plots refer to the smoothed function (s) for the term of interest (elevation; PDQ, precipitation of the driest quarter; TC, percentage tree cover) and the estimated degrees of freedom (following the term).

distribution and availability of resources used by each hummingbird clade. Each clade has a generally distinct set of ecomorphological traits that partly defines how it uses resources (Stiles, 2008) in different habitat types. This information proved useful in predicting some of the factors associated with variation in phylogenetic structure. Information about the environment where a clade originated can be used to predict patterns of diversity away from this environment under the assumption of niche conservatism (Wiens & Graham, 2005; Mittelbach *et al.*, 2007; Wiens, 2007). This prediction has support in the literature (e.g. Hughes & Eastwood, 2006; Donoghue, 2008; Hawkins & DeVries, 2009;





**Figure 6** Observed relationship between net relatedness index (NRI) (considering only coquettes in assemblages in Ecuador) and values of environmental variables selected in the best generalized additive model (GAM) are plotted in the first column. Plots in the second column present the predicted relationship between NRI and the environmental variables from the best GAM (black line) showing 95% confidence interval of the prediction shaded in grey and partial residuals (points). Black and grey points represent assemblages (n = 90) on the east or west of the Andes, respectively. Labels on the *y*-axes in the second column of plots refer to the smoothed function (s) for the term of interest (elevation) and the estimated degrees of freedom (following the term).

4000

1000

20'00

3000

4000

3000

Pyron & Burbrink, 2009; Wiens *et al.*, 2009), although this support is inconclusive because similar patterns are predicted by other hypotheses (Mittelbach *et al.*, 2007). At the family level (i.e. using the entire phylogeny) assemblages became clustered the further they were from their most likely environmental zone of origin, the humid lowlands (Bleiweiss, 1998a; McGuire *et al.*, 2007), in support of the expectation from the niche conservatism and zone of origin hypotheses. In contrast, at the clade level, this expectation did not always hold. The only study, to our knowledge, that has examined how phylogenetic assemblage structure changes with environmental distance from the conditions where it originated found no support for the niche conservatism hypothesis (Algar *et al.*, 2009).

NRI (Coquettes

2

0

-1

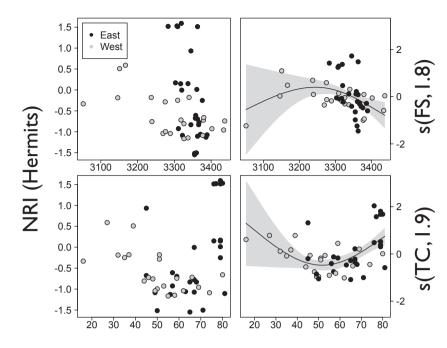
-2

1000

2000

Our findings could be interpreted in various ways. First, elevation is probably not the only factor determining the

phylogenetic structure of assemblages (Cavender-Bares et al., 2009). In fact, Graham et al. (2009) and Parra et al. (2010) provide evidence of other environmental factors to which hummingbirds might be responding, such as precipitation and seasonality. Second, changes in the diversification rates or the level of trait conservatism can modify the expected distribution of diversity across environmental gradients (Ricklefs, 2006; Wiens, 2007; Algar et al., 2009). For example, if the rate of trait evolution changes in some clades but not others, the general expectation of trait conservatism might not be warranted. Third, the interpretation of how the zone of origin influences phylogenetic assemblage structure and patterns of species cooccurrence might depend in part on the phylogenetic depth examined. For young clades, time may have been insufficient for a pattern to establish, and, for old clades, further diversification events away from the zone of origin might



erase the expected signal (see Buckley *et al.*, 2010). However, in our case, clades with the same age (brilliants and coquettes) showed contrasting results in terms of our expectations. Finally, we do not account for the uncertainty in the estimates of the elevations of origin (McGuire *et al.*, 2007).

Knowledge of the environmental zone of origin alone does not necessarily provide insight into which environmental factors are relevant for changes in phylogenetic structure. However, by incorporating information about the groups' ecomorphological traits (i.e. beak length as a predictor of feeding specialization), we were able to predict some of the environmental factors that were related to changes in phylogenetic structure. In the groups that we hypothesized as more specialized in terms of flower resources (i.e. the brilliants that have long straight bills and the hermits that have curved bills) variation in phylogenetic structure was explained by changes in vegetation-related features (i.e. forest structure and percentage tree cover). In contrast, in clades that were characterized by short-billed generalists (i.e. mangoes and coquettes), variation in phylogenetic structure was only related to elevational differences. In sum, our analyses indicate that variation in the phylogenetic relationships among co-occurring species is related to multiple environmental factors that often differ among clades within the hummingbirds depending on their patterns of resource use. This result is perhaps not surprising given that many groups have radiated to occupy new environments and variation in the degree of trait lability is apparent for different clades and species (Mueller et al., 2004; Evans et al., 2009; Ricklefs, 2010). However, few attempts have been made to relate the changes in the phylogenetic history of co-occurring species to environmental gradients in a given region. For instance, we observed that groups that shared a similar place of origin along the elevational gradient seemed to have radiated along this gradient in contrasting directions,

Figure 7 Observed relationship between net relatedness index (NRI) (considering only hermits in assemblages in Ecuador) and values of environmental variables selected in the best generalized additive model (GAM) are plotted in the first column. Plots in the second column present the predicted relationship between NRI and the environmental variables from the best GAM (black line) showing 95% confidence interval of the prediction shaded in grey and partial residuals (points). Black and grey points represent assemblages (n = 49) on the east or west of the Andes, respectively. Labels on the y-axes in the second column of plots refer to the smoothed function (s) for the term of interest (FS, complexity of forest structure; TC, percentage tree cover) and the estimated degrees of freedom (following the term).

following different environmental variables. By relating patterns of phylogenetic structure to environmental gradients for each clade we are able to evaluate how different clades diversified or moved into new environments, such as those present in mountains.

## ACKNOWLEDGEMENTS

The manuscript was improved by comments from the Graham lab. S. Baines, D. Strubbe, O. Broennimann and J. Knowlton provided advice in the methodology and editing. The National Science Foundation through DEB-563 0820490 provided funding for this work to C.H.G. and J.L.P., and DEB-0543556 and 0330750 to J.A.M. C.R. thanks the Danish National Research Foundation for supporting the Center of Macroecology, Evolution and Climate. We thank the organizers of the conference *Niche Evolution* held in Zurich on 3–4 July 2009.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** List of sources for hummingbird local inventories.

**Appendix S2** Relationships between assemblage species richness and area and elevation range covered.

**Appendix S3** Results of spatial autocorrelation in residuals of generalized additive models.

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# BIOSKETCH

**Juan L. Parra** is a post-doctoral researcher at the Department of Ecology and Evolution at Stony Brook University, New York. The focus of the research collaboration is to determine what factors influence composition of hummingbird communities. We combine geographical, environmental, phylogenetic and morphological data to evaluate a series of ecological (e.g. species interactions, environmental filtering) and evolutionary (e.g. trait evolution) hypotheses about species co-occurrence.

Author contributions: C.H.G. and J.L.P. conceived the ideas; C.H.G., C.R., J.A.M. and J.L.P. collected data and edited the manuscript; J.L.P. and J.A.M. analysed the data; J.L.P. and C.H.G. led the writing.

Editor: Peter Linder