

**Exploring the opportunities for divergence in
heterogeneous environments in the tropical Andes**

by

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DEDICATION

To both my families, the one I was luckily born in and the one I chose to form; especially to my parents and my wife, without whom I would not have been able to come this far.

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ABSTRACT

The high biological diversity associated with environmentally heterogeneous regions reflects the complex interactions between multiple evolutionary and ecological processes. Disentangling this interaction is crucial for understanding a wide variety of fundamental biological questions including the differential accumulation of species across the globe, the influence of geographic context on diversification processes, and the susceptibility of species to environmental change. In this dissertation, I integrate theoretical expectations with comprehensive analyses of species, genetic, and phenotypic variation across the hyper-diverse tropical Andes to help elucidate the role environmental heterogeneity plays in the generation and maintenance of diversity.

First, I explore the association between diversity and spatial and temporal heterogeneity. I show that environmental heterogeneity is a strong predictor of both species and phylogenetic diversity across the entire study region despite significant environmental differences among regions. My results indicate that this association is unlikely to be simply explained by passive accumulation of species or spatial autocorrelation. Instead, active differential diversification across zones may explain this pervasive association.

Second, I investigate if environmentally driven isolation and adaptive divergence drives population differentiation in this system using the soft-grass mouse as a model. Specifically, I test whether local adaptation is prevalent in this species. However, rather than providing strong support for local adaptation, my findings indicate environmental heterogeneity promotes neutral and phenotypic differentiation in this species through genetic drift facilitated by environmental isolation.

Third, I explore the extent of climatic niche differentiation in the soft-grass mouse to assess the role of environmental heterogeneity in promoting ecological specialization. Combining genetic and ecological analyses, I provide evidence of restricted differentiation of the climatic preferences of populations of this species. I show that in spite of marked genetic and geographic structure, this species maintains a common set of climatic tolerances. Initial exploration of plausible causes for this finding hints at selection for ecological broadness, reinforced by frequent range shifts.

Together, this dissertation offers a multi-faceted evaluation of the evolutionary consequences of inhabiting highly heterogeneous environments. Its findings demonstrate the significant role that geographic context plays in structuring diversity, bringing attention to the importance of system-specific characteristics in modulating these effects.

CHAPTER 1: Introduction

Biodiversity is unevenly distributed across the world with environmentally heterogeneous areas containing an overwhelming proportion of it. This pattern is pervasive across taxa, regions, and geographic scales (Simpson 1964, Distler et al. 2009, Dainese and Poldini 2012). Yet, while an increasing number of theoretical studies have examined plausible causes for this pattern (e.g., Levins 1964b, a, Brown and Pavlovic 1992, Debarre and Gandon 2011), definitive causal explanations have remained elusive. Despite significant advances, a thorough understanding of the role of environmental heterogeneity in the generation and maintenance of tropical mountain communities, known for their incredibly high species richness and high levels of endemism (Myers et al. 2000, Ruggiero and Hawkins 2008), is still far from being satisfactory. This limited understanding has complicated a comprehensive assessment of the evolutionary consequences of environmental change in these biologically important regions that accommodate major diversification foci and that represent a diversity source for adjacent regions (Kattan et al. 2004, Wiens 2007). Testing mechanistic hypotheses that link diversification processes to the amount of environmental heterogeneity in tropical mountains and surrounding areas is expected to provide valuable insights into species evolutionary dynamics and responses to environmental change in these systems.

One of the first authors to formally link diversity and environmental heterogeneity under an evolutionary context, Dobzhansky (1950) argued that coupled with stronger competition, increased habitat heterogeneity should favor species specialization. Dobzhansky (1950) also

linked temporal stability in geological time of a region to increased intensity of competition and opportunities for specialization, suggesting that more stable environments should offer greater opportunities for species to become locally adapted. Janzen (1967) also highlighted the importance of temporal stability and topographical environmental heterogeneity in facilitating species specialization; however, in contrast to Dobzhansky, Janzen focused on the ecological scale, and in particular the links between seasonality (or short-term temporal stability) and diversity. Since then, several researchers have continuously used these arguments to link the topographic and climatic complexity of tropical mountain systems with increased opportunities for allopatric and/or parapatric divergence (Endler 1977, Patton and Smith 1992, Schneider et al. 1999, Graham et al. 2004, Kozak and Wiens 2007, Cadena et al. 2012). For instance, in an extensive review of diversification studies in tropical systems across the globe, Moritz et al. (2000) suggested that recent climatic or geological instability coupled with high habitat heterogeneity results in high diversification rates in recently uplifted montane systems such as the tropical Andes. Despite the confirmation of these findings by later studies (e.g., Fjeldså and Rahbek 2006, Hughes and Eastwood 2006), the underlying processes and their mechanistic links with environmental heterogeneity remain understudied.

Further complicating advances in this area of research is the lack of consensus as to how exactly heterogeneity should be measured (Wiens 2000). Environmental heterogeneity in its simple form constitutes spatial and/or temporal variability in a defined area (Hopton 2006). However, the extent to which an environment is heterogeneous depends on the scale at which individual organisms perceive it (Hutchings et al. 2000, Begon et al. 2006). Furthermore, at different spatial and temporal scales, environmental heterogeneity may be linked to different

processes (Levins 1968, Hopton 2006). At broad geographic scales, environmental heterogeneity may reflect community assembly processes including environmental filtering and broad-scale competition (Webb et al. 2002, Graham and Fine 2008), whereas at small spatial scales it may primarily reflect small-scale biotic interactions (Ricklefs 1977, Snyder and Chesson 2004). At meso-scales, on the other hand, the effect of microevolutionary forces, including isolation, drift, and selection, may predominate (Brown and Pavlovic 1992, Fine et al. 2013). In addition, the study of the environmental heterogeneity-diversity association is further complicated by the fact that underlying mechanisms are most likely context-dependent (Lim 2010, Stevens 2011), varying for example with taxon-specific ecologies (e.g., dispersal abilities and habitat requirements), the geographic setting (e.g. geological age), and historical contingencies (e.g., biogeographic history).

Thus, given the complexity of the interactions involved and the extreme difficulty of experimental analyses on the evolutionary consequences of inhabiting heterogeneous environments, integrative approaches focused on testing specific *a priori* theoretical expectations are the most suitable alternative. In this dissertation, I follow this alternative to explore the relation between environmental heterogeneity and diversity in the tropical Andes and surrounding regions, using small terrestrial mammals as a model. I focus on this group of vertebrates because their limited vagilities and constrained geographic ranges over steep environmental gradients favor a close association between the diversity of these animals and local topographic and climatic heterogeneity. In the dissertation, I first analyze the impact of spatial and temporal heterogeneity on the community of small mammals inhabiting the Andes of Ecuador and surrounding regions (Chapter 2), and then I focus on the soft-grass mouse (*Akodon*

mollis) to explore in more detail the support for environmental heterogeneity in promoting local adaptation (Chapter 3) and facilitating ecological specialization (Chapter 4). By explicitly assessing how environmental heterogeneity impacts these animals, this dissertation provides the necessary background for further studies on the evolutionary consequences of past and predicted future environmental change. In this context, this dissertation offers valuable insights to better cope with the consequences of ongoing global warming and increases the knowledge of the highly threatened highland tropical Andean ecosystems and the challenges they may experience in the near future.

Are spatial and temporal environmental heterogeneity consistently associated with the species and phylogenetic diversity of small terrestrial mammals?

One particular approach to understanding the evolution of populations and species in heterogeneous regions is to disentangle the effect of heterogeneity on different components of diversity (Davies et al. 2007, Graham and Fine 2008). Because species diversity and phylogenetic diversity can arise independently under different processes (Davies and Buckley 2011, Fritz and Rahbek 2012), investigating their pattern of covariation with heterogeneity across multiple regions is expected to provide valuable insights into the processes involved. Because local phylogenetic diversity is expected to arise by the accumulation of independent evolutionary lineages caused by infrequent immigration or limited extinction (Davies and Buckley 2012, Fritz and Rahbek 2012), whereas local species diversity (i.e., richness) can originate by local speciation, lineage immigration, or limited extinction, areas of recent active diversification (i.e., diversity cradles; Stebbins 1974) should be characterized by high species diversity, but low phylogenetic diversity since average phylogenetic distance between species in

the community will be low. Areas with low extinction rates that have accumulated species that originated far back in time (i.e., diversity museums; Stebbins 1974) should, in contrast, be characterized by both high species and high phylogenetic diversity.

Taking advantage of these different expectations for species and phylogenetic diversity, in Chapter 2 I investigate three main questions: (1) is environmental heterogeneity a strong predictor of local species and phylogenetic diversity in the tropical Andes?; (2) does the strength, steepness, and slope of the association depend on the nature of environmental heterogeneity (i.e., spatial vs. temporal)?; and (3) does this association vary predictably between environmentally distinct regions? To address these questions, I use a combination of spatially-explicit regressions to investigate how the association varies over space. The results of these analyses indicate that species and phylogenetic diversity are decoupled. Greater species diversity is consistently associated with high spatial and low temporal environmental heterogeneity, with temporal heterogeneity having a steeper effect, whereas phylogenetic diversity is negatively associated with overall spatial heterogeneity, although it shows a positive association with spatial heterogeneity associated with local precipitation variability. These results were notably consistent (i.e., repeated) across regions. Taken together, these findings confirm that environmental heterogeneity is an important predictor of diversity and suggest that environmental filtering coupled with active in situ diversification might be the main drivers of diversification in this region.

Does environmental heterogeneity facilitate local adaptation in *A. mollis*?

Another suitable approach to investigate the mechanistic links between environmental heterogeneity and diversity is to directly evaluate the support for divergent selection vs. drift in species inhabiting heterogeneous environments. Since a prerequisite for adaptation and specialization is for species to respond to environmental heterogeneity (Levins 1964a), it is expected that the interaction between environmentally driven isolation, genetic drift, and adaptive selection drives population and species divergence (Slatkin 1985, Brown and Pavlovic 1992). Yet, the relative contributions of these micro-evolutionary forces depend on the specific geographic context of populations (Hanski et al. 2011, Blanquart et al. 2012, Messier et al. 2012). Specifically, as the grain of heterogeneity increases, the opportunity for local adaptation increases because the proximity of areas with contrasting selective regimes may facilitate adaptive peak shifts and the maintenance of effectively isolated populations (Hadany 2003, Hedrick 2006, Richter-Boix et al. 2013). However, as the grain of heterogeneity increases, patchiness of the environment also increases. This may result in reduced population sizes (Fahrig and Paloheimo 1988, Yackulic et al. 2011), and hence, local adaptation could be confounded by genetic drift (Kawecki and Ebert 2004, Uyeda et al. 2009). By assessing the evidence of local adaptation in wild populations inhabiting environmentally complex regions, this study aims to provide valuable insights into how environmental heterogeneity conditions the interactions between these forces.

In Chapter 3 I use this approach to test whether environmental heterogeneity promotes genetic and phenotypic divergence by (1) facilitating population isolation and (2) promoting adaptive divergence. In particular, I focus on contrasting patterns of neutral genetic and presumably adaptive skull and mandible morphological variation to explore how this variation is

structured along gradients of environmental resistance and environmental dissimilarity. In addition, I test for evidence of divergent selection by contrasting the degree of genetic and morphological differentiation between populations. The results of these analyses provide evidence for the importance of dispersal barriers in heterogeneous environments, but fail to recover evidence for adaptive divergence. Instead, my findings suggest that, as seen in other systems (Knowles and Richards 2005, Uyeda et al. 2009), genetic drift might be an important contributor to divergence in this species.

Does environmental heterogeneity influence the opportunities for ecological specialization in *A. mollis*?

The degree of specialization of ecological niches is conditioned by a diverse array of factors including the underlying genetic or developmental structure (Smith et al. 1985, Bradshaw 1991), species' population structure (Jakob et al. 2010, Eckhart et al. 2011), the relative strength of stabilizing selection vs. divergent selection (Futuyma and Moreno 1988, Ackerly 2003), ecological opportunity (Holt and Gaines 1992, Litsios et al. 2012), and the stability of environmental conditions (Hallsson and Björklund 2012, Grewe et al. 2013). Because the vast majority of these processes are in turn directly or indirectly influenced by the environmental context of species and populations, the opportunity for niche differentiation should be strongly linked to the degree of environmental heterogeneity individual species experience in their habitats (Kellermann et al. 2012, Grigg and Buckley 2013). From the perspective of climatic niche differentiation, in particular, the ability of species to become specialized to particular climates will depend on the breadth and variability of the climatic conditions experienced. If, for instance, populations face constant climates within their distributions, and if there is a trade-off

between tolerance broadness and overall performance (Levins 1968, Huey and Hertz 1984), intra-specific specialization should be prevalent in temporally stable and spatially variable climatic environments (Janzen 1967, Ghalambor et al. 2006). On the other hand, populations facing unstable or fine-grained climatic environments should preferentially evolve common, generalist climatic tolerances (Angilletta 2009). Thus, depending on the relative constitution of the environment, ecological adaptation and population differentiation may be more or less prevalent (Keller and Seehausen 2012).

In Chapter 4, I explore this association between climatic heterogeneity and the degree of climatic niche differentiation in *A. mollis* using a combination of genetic and ecological analyses to investigate current patterns of intra-specific climatic overlap between populations across the entire range of this species. These analyses indicate that limited intraspecific differentiation of the climatic niche of *A. mollis* and point towards an interaction between selection for wide tolerances and climatic instability as plausible responsible circumstances for this finding. Furthermore, these results imply that the suggested prevalence of ecological specialization in tropical mountains may need to be revised as different species likely show different responses to the environmental conditions of these systems.

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CHAPTER 2: The structuring of species and phylogenetic diversity in heterogeneous environments

ABSTRACT

Understanding the causes behind the pervasive association between diversity and environmental heterogeneity is critical to answering the long-standing question of why species diversity is unevenly distributed on Earth. However, the complex interactions involved and the infeasibility of empirical investigations call for correlative approaches focused on disentangling the confounding patterns that form part of this general association. Here we present such an approach that couples analyses on species and phylogenetic diversity with spatially-explicit association analyses across regions with different degrees of spatial and temporal environmental heterogeneity. Applying this approach to investigate the pattern of covariation between both components of diversity and meso-scale spatial and temporal heterogeneity, we demonstrate how it can be used to elucidate alternative plausible mechanisms, assess the particular contexts under which different mechanisms might be prevalent, and guide future research. Specifically, we explore the likelihood of different evolutionary hypotheses based on contrasting patterns of species and phylogenetic diversity of small terrestrial mammals in continental Ecuador. The results indicate that species and phylogenetic diversity are spatially uncoupled from each other and that although environmental heterogeneity is a strong predictor of both components of diversity, the corresponding associations differ. These findings point toward an important role of high spatial and low temporal heterogeneity in promoting disruptive selection, environmental specialization, and isolation in this mountain system. Future research will be needed on

evaluating the support for each factor. More generally, our results suggest that complementary processes acting at different levels of the environmental heterogeneity continuum can account for markedly different diversity patterns and community compositions across regions.

INTRODUCTION

Biodiversity is unevenly distributed across the world with great number of species concentrated in topographically complex areas. This pervasive pattern is thought to be driven by the greater environmental heterogeneity of mountains, which is expected to favor adaptation and speciation by increasing habitat diversity and isolation (Simpson 1964, Davies et al. 2007a, Badgley 2010). In support of this hypothesis, a well-documented association between environmental heterogeneity and species richness has been recovered for a wide variety of taxa and regions (Harner and Harper 1976, Qian and Ricklefs 2000, Kreft and Jetz 2007). Yet, most studies have exclusively focused on assessing the explanatory power of environmental heterogeneity on species richness patterns relative to other environmental factors such as available energy or area (e.g., Kerr and Packer 1997, Distler et al. 2009, Dainese and Poldini 2012), mostly over coarse geographic resolutions, which has made it difficult to uncover the generality and ecological and evolutionary mechanistic causes of this association. In addition, traditionally studies have exclusively focused on spatial heterogeneity even though topography clearly affects not only spatial, but also temporal components of heterogeneity.

To improve our understanding of the processes involved in structuring biodiversity in topographically complex regions, in this study we investigate the interaction between spatial and temporal environmental heterogeneity (EH, hereafter) and the diversity of small terrestrial

mammal in the tropical Andes – one of earth’s major biodiversity hotspots (Myers et al. 2000, Sarkar et al. 2009). Specifically, we analyze the correlation between two components of diversity, species diversity or richness (i.e., number of species in an area) and phylogenetic diversity (i.e., average phylogenetic distance between species in an area), and the amount of meso-scale spatial and temporal environmental heterogeneity of this region. In addition, taking advantage of the steep topographical gradient of the tropical Andes, we assess the consistency of these correlations across regions that are in close proximity, but that differ in their biogeographic histories and environmental configurations. In doing so, we evaluate the support for the previously proposed positive link between diversity and the habitat heterogeneity and seasonal stability of a region.

We investigate the association between diversity and environmental heterogeneity using a dataset of continental Ecuadorian small terrestrial mammals that include species richness and phylogenetic diversity data. We focus on the tropical Andes and surrounding lowlands in this area because previous global-scale studies on this association have consistently identified the high diversity of the tropical Andes as an outlier (Rahbek and Graves 2001, Fritz and Rahbek 2012), which suggests the importance of evolutionary processes acting at meso-scales, in particular those associated with its marked EH (Davies et al. 2007a). Specifically, we focus on tropical Andean small terrestrial mammals and their lowland counterparts because, given their low vagility and localized distributions, this species-rich group of animals is likely strongly affected by local evolutionary processes such as adaptation and population-level isolation (Ruggiero and Hawkins 2006). In addition, their occurrence thorough all habitats, with the only exception of permanently glaciated areas at high elevations, allows investigating the interaction

of spatial and temporal EH across environments with different magnitudes of these two components of EH. In particular, we compare the strength and steepness of the association between EH and diversity between the Andes and the western and eastern lowlands given the marked environmental differences between these regions. The Andes are characterized by high habitat patchiness, steep environmental gradients, and relative low seasonality (Graves and Rahbek 2005, Buytaert et al. 2006, Ruggiero and Hawkins 2006). The western lowlands, on the other hand, comprised two main zoogeographic regions, the notoriously humid Chocó region and the semiarid Tumbes region (Hershkovitz 1958), both of which are characterized by relative high habitat uniformity and high seasonality – the latter being more evident in the Tumbes region (Sierra 1999). Finally, the eastern lowlands are characterized by intermediate habitat heterogeneity, driven mostly by soil heterogeneity, and low seasonality (Tuomisto 2006, Fine et al. 2013).

Using this study system and a combination of modeling approaches that explicitly account for the spatial dependence of the data, we estimate the overall strength and steepness of the association between diversity and both components of EH (i.e., spatial and temporal), and determine if this association differs among the Andes and western and eastern lowlands. Specifically, we address whether meso-scale EH is strongly associated with local diversity in the tropical Andes, (2) if this association depends on the nature of EH (i.e., whether diversity is differentially associated with spatial and temporal EH), and (3) if the association varies in a predictable manner among regions in response to their different environmental configurations. The answers to these questions contribute to our understanding of the generation and maintenance of the high diversity of tropical mountains. More generally, this study provides valuable insights into long-standing questions about the accumulation of diversity in

topographically complex areas, and enhance our ability to forecast and cope with the impact of ongoing habitat disturbances that are modifying temporal and spatial patterns of EH (Manel et al. 2003, Thomassen et al. 2011).

METHODS

Data collection

Diversity estimates were derived from distribution data of species of Ecuadorian terrestrial small mammals, obtained from the “Mammals of the Western Hemisphere” database (Patterson et al. 2007). Specifically, we superimposed the distribution maps of all the species that inhabit Ecuador of 6 families of small terrestrial mammals (Caenolestidae, Cricetidae, Didelphidae, Echimyidae, Heteromyidae, and Soricidae) on a 1 km²-resolution digital map of continental Ecuador to determine the number and identity of species occurring in each 1 km² cell. The number of species (i.e., richness) was used directly as our estimate of species diversity. Phylogenetic diversity was estimated from the average pairwise patristic distance between each pair of species co-occurring in a cell (i.e., average sum of the lengths of the branches linking each pair of species in a phylogenetic tree; Fourment and Gibbs 2006). Patristic distances were calculated in R using the package *geiger* (Harmon et al. 2008) based on the mammalian super-tree of Fritz et al. (2009). Patristic distance, which is analogous to the average taxonomic distinctness index of Clarke and Warmick (1998), was chosen over the more commonly used Faith’s (1992) index because the later is dependent on richness (Schweiger et al. 2008) and hence could not be used as a separate response variable in our analyses.

Estimates of spatial and temporal EH for continental Ecuador were independently obtained. Spatial EH was estimated by combining the two commonly used metrics of EH (Davies et al. 2007b): climatic heterogeneity, measured as the amount of spatial variation in 19 climatic variables at 1km resolution taken from Worldclim database (Hijmans et al. 2005); and number of habitat types, measured from a grid summarizing vegetation types from the Global Land Cover Characterization Program (DeFries et al. 2000) (Table 2.S1). Local spatial EH for each climatic variable was calculated as the standard deviation of values within a moving-window zonal statistics calculation in ArcGIS v9.3 (ESRI 2009), whereas the number of unique vegetation categories within each window was used as the measure of spatial EH for the categorical vegetation layer. All zonal statistics calculations were based on a 10-km² neighborhood window; this value was chosen as a compromise between the spatial resolution of the data and a biological meaningful resolution for terrestrial small mammals – the ranges of small terrestrial mammals do not usually exceed 10 km² (Jones et al. 2009, Quirici et al. 2010, Sommaro et al. 2010) even though dispersal distances and home-range size vary widely across species. To reduce dimensionality of this dataset and avoid problems due to collinearity, we ran a principal component analysis (PCA) on the standardized results of the previous zonal calculations. Temporal environmental heterogeneity was measured as seasonal differences in temperature and precipitation, as summarized by 5 of the 19 climatic variables (Table 2.S1). Again, to reduce dimensionality and avoid collinearity issues, we computed a PCA on these 5 variables after standardizing them. From both PCAs we kept only the first three principal components (PCs), which accounted for approximately 91% and 99% of the spatial and temporal EH data, respectively (Table 2.S1).

Finally, we randomly sampled 100 map cells from the entire study area and extracted the diversity and EH estimations to these cells using ArcGIS v9.3 (ESRI 2009). To minimize any possible margin effect, the 100 selected cells did not include any border cells with less than 100% of their area covered. Using these 100 data points we assessed the degree of correlation between our two response variables (i.e., species and phylogenetic diversity: Pearson's $r = 0.00$, $p\text{-value} = 0.96$) as well as between all 6 predictor PCs. Since none of these variables were highly correlated with each other (the only exception being a moderately high correlation between PC3 of spatial EH and PC1 of temporal EH, Table 2.S2), we kept all variables.

Association Analyses

To investigate the association between diversity and EH, we ran 2 simultaneous autoregressive (SAR) regressions, one for species diversity and one for phylogenetic diversity. The set of EH PCs retained as predictors in each regression was determined based on the Akaike Information Criterion (AIC; Burnham and Anderson 2002). We chose this regression models that incorporate a spatial term, either in the model *per se* (SAR_{LAG}) or in the model residuals (SAR_{error}), to account for the lack of spatial independence in the data that compromises the estimation of model coefficients and p-values in non-spatial regressions (Lichstein et al. 2002, Werneck et al. 2012) (for details on these models see Diniz-Filho et al. 2003, Kissling and Carl 2008, Diniz-Filho et al. 2009). Because results were consistent regardless of the spatial error term used (i.e., SAR_{LAG} or SAR_{error}), here we report exclusively the results from SAR_{LAG} models. Finally, we verified the appropriateness of the models used running Lagrange multiplier tests for spatial autocorrelation (Anselin 1988, Anselin et al. 1996) for each regression.

In addition, to assess if the association between heterogeneity and diversity varies across regions given their different environmental configurations, we ran geographically weighted regressions (GWRs). GWRs fit locally weighted regressions at each observation location using subsets of neighbor observations, weighted according to their proximity to the focal location (Wheeler and Páez 2010). This procedure effectively allows parameters estimates to vary over space (Brunsdon et al. 1996, Fotheringham et al. 2002). Hence, by fitting GWRs for both components of diversity using the previously identified set of predictors, we were able to assess if model coefficients vary systematically by region. Both GWRs were based on a Gaussian-fixed kernel with bandwidth optimized by minimizing cross-validation (CV) scores (Fotheringham et al. 2002). Under this optimization algorithm the number of nearest neighbors is iteratively estimated following a residual-minimization criterion (i.e., CV) that basically provides a best fit model over the range of bandwidth values tested (Jacquez 2010). We then ran analyses of variance (ANOVAs) and Tukey's post hoc tests on the \log_e -transformed β coefficient estimates and R^2 values using region as the grouping factor. Regions were defined using a 1000m contour line and roughly corresponded to the three continental Ecuador regions (i.e., Andes, western lowlands, and eastern lowlands; Sierra 1999).

To evaluate the adequacy of all regressions we tested for violations of error assumptions (i.e., that errors are independent and identically distributed). To do this, we computed Shapiro-Wilk tests and created Q-Q and residuals vs. predicted plots to assess normality and homoscedasticity of errors (Faraway 2005). Also, we ran Moran's I correlograms (Lichstein et al. 2002) to test for spatial dependency of residuals and local Moran's I tests with a Benjamini and Hochberg's (1995) correction for multiple tests to identify possible regions of under or over-

prediction (Anselin 1995, Goovaerts and Jacquez 2004). All analyses were run in ArcGIS (ESRI 2009) and R (R Core Development Team 2012) using packages *spdep* and *spgwr* (Bivand 2013, Bivand and Yu 2013).

RESULTS

Species and phylogenetic diversity of terrestrial small mammals in continental Ecuador are found not to be spatially associated with each other, as neither are spatial and temporal EH. Species diversity is highest on the eastern lowlands, whereas phylogenetic diversity is highest in the western lowlands and eastern Andean foothills (Fig. 2.1a, b). The Andes region is identified as the most spatially heterogeneous region. This region has the highest scores on the spatial PC1, which is positively associated with all local variability estimates (Fig. 2.1c, Table 2.S1), and hence, could be interpreted as an overall spatial EH component. The western lowlands, on the other hand, have the highest temporal EH. This latter region possesses the highest scores on the temporal PC1, which mainly summarized precipitation seasonality (Fig. 2.1d, Table 2.S1). The rest of EH principal components have less defined spatial patterns, yet all of them differ among regions.

The spatially-explicit SAR and GWR regressions show consistent results. Under both models variation in species diversity is found to be explained by spatial and temporal EH ($R^2 = 0.55$), whereas variation in phylogenetic diversity is explained exclusively by spatial EH ($R^2 = 0.51$). Specifically, species diversity increases as precipitation seasonality (i.e., PC1 of temporal EH) decreases and precipitation spatial heterogeneity (i.e., PC2 of spatial EH) increases (Fig. 2.2a), whereas phylogenetic diversity increases as overall spatial heterogeneity (i.e., PC1 of

spatial EH) decreases and precipitation spatial heterogeneity (i.e., PC2 of spatial EH) increases (Fig. 2.2b). Under both regression types the β coefficients associated with all predictors were consistent, with the median of predictors' β coefficients in the GWR regressions closely resembling the estimates obtained under SAR models (Table 2.1) It is important to note that residuals of GWR models violated the assumption of spatial independence as the Moran's I analyses show that they are both globally and locally clustered (Table 2.1), which cautions against interpreting isolated local predictors' β coefficient values. In contrast, in none of the SAR regressions the assumption of independent and identically distributed residuals is violated (Table 2.1).

Variation in predictors' β coefficients is evident across the study region in both species and phylogenetic diversity GWR regressions (not shown). Variation in local R^2 across regions is also present, but less evident (Fig. 2.3). ANOVAs on these estimates show that the strength of the diversity-EH association significantly varies only for the western lowlands in the species diversity GWR (Fig. 2.4a), whereas differences in coefficients between regions are significant only in one instance (Fig. 2.4b, c), whereas.

DISCUSSION

Our analyses indicate that EH is a strong predictor of species and phylogenetic diversity across the entire study region, explaining over 50% of the variance in both components (Table 2.1). Specifically, our results indicate that species diversity increases as precipitation spatial EH increases and precipitation temporal EH decreases (Fig. 2.2a); supporting the expectation of opposite effects of spatial and temporal EH on diversity (see Introduction; Levins 1968, Hallsson

and Björklund 2012). Phylogenetic diversity also increases with precipitation spatial heterogeneity, but decreases with overall spatial heterogeneity (Fig. 2.2b). Being overall spatial EH and phylogenetic diversity significantly correlated with elevation (Pearson's correlation coefficients = 0.675 and -0.684, p-values = 0.02 and 0.01 for overall spatial EH and phylogenetic diversity, respectively), the negative association between these two components is likely the reflection of increased speciation in the Andes and associated foothills (see below for further discussion). The strong influence of precipitation heterogeneity on species and phylogenetic diversity, on the other hand, probably results from local differences in primary productivity and/or habitat structure among contiguous ecosystems (Pianka 1966, Hawkins et al. 2003). These meso-scale habitat differences are probably well captured by the notorious and highly irregular patterns of precipitation variability in these regions (Patterson et al. 1998, Buytaert et al. 2006, Fitzjarrald et al. 2008). Contrary to our expectation, these associations between EH and diversity are consistent across the 3 continental regions of Ecuador (i.e., Andes and western and eastern lowlands), as evidenced by the, for the most part, equally strong and steep association with diversity (Fig. 2.4). This spatial consistency in the EH-diversity association across the study area is especially noteworthy given the marked differences among these 3 regions in the amount of spatial and temporal EH across (Fig. 2.1), which are expected to influence the relative contributions of alternative underlying mechanisms behind local diversity, including those associated with EH, area, productivity, and ambient temperature (Kerr and Packer 1997, Hawkins et al. 2003). Taken together, these results support the overarching effect of EH processes determining the structure of diversity and indicate that variation in precipitation regimes and overall heterogeneity across habitats is of great importance in structuring diversity of small terrestrial mammals in both the tropical Andes and surrounding lowlands. Such a

pervasive effect of EH highlights the significant role that geographic context plays in the generation and maintenance of diversity (Losos 2010, Hanski et al. 2011), and suggests that as theoretically predicted EH is indeed a key component of adaptive diversification (Levins 1968, Kishi 2002).

EH and the structure of diversity

As a result of this consistent effect of EH across regions, the relative contribution of in-situ diversification and immigration in the Andes and surrounding lowlands likely differs, conditioning the diversity structure of these regions. As indicated by several theoretical studies, spatial and temporal EH should influence the likelihood of in-situ diversification. Spatial EH, in particular, is predicted to favor disruptive selection, ecological divergence, and local adaptation by presenting species with spatially variable selective environments (Vivian-Smith 1997, Moritz et al. 2000, Funk et al. 2006), and because spatial EH should also promote population isolation by limiting the establishment of mal-adapted migrants into locally-adapted demes (Cheviron and Brumfield 2009, Blanquart and Gandon 2011) and by increasing the difficulty of migration across environmentally different regions (Row et al. 2010; see also Chapter 3, Fig. 3.1). In addition, spatial EH should promote high evolvability (i.e., ability to adapt rapidly, which can lead to differentiation and speciation) if new habitats become available due to local extinctions or range expansions, opening the opportunity for adaptation races between colonizing individuals (Palmer and Feldman 2011, Campos and de Oliveira 2012). On the other hand, temporal heterogeneity has been predicted to deter in-situ diversification. This is because temporal EH should select for wider physiological tolerances and high dispersal, which in turn reduce the effectiveness of dispersal barriers and promotes high levels of gene flow among populations

(Janzen 1967, Ghalambor et al. 2006, Grewe et al. 2013) (see Fig. 3.1). In addition, temporal EH should effectively homogenize across-space environmental variability, reducing the effectiveness of disruptive selection and impeding the maintenance of locally adapted biotas (Levins 1964). In addition, temporal EH should create fluctuating selective environments that prevent species from specializing as the selective optimum becomes a movable target (Hallsson and Björklund 2012).

Tacking these theoretical predictions into consideration, the high overall and low precipitation EH of the Andes seems to be in part responsible for this region being an active center of in-situ diversification, as indicated by its low phylogenetic diversity relative to species diversity (Fig. 2.1a, b) (Davies and Buckley 2012). This is because, as predicted, high spatial EH should result in high in-situ speciation, which combined with environmental filtering caused by the physiological challenges of mountain environments (Mani 1968, Parra et al. 2011), should result in a high proportion of closely related species (i.e., with low phylogenetic distances between them). In line with this inference, previous studies have uncovered (1) genetic distances between sister taxa in the Andes being half as large as those between sister taxa in the lowlands (Moritz et al. 2000), (2) an accumulation of young lineages in these mountains (Fjeldså and Rahbek 2006, Sedano and Burns 2010), (3) impressively fast diversification rates for some of their inhabitants (Hughes and Eastwood 2006), and (4) evidence of environmental filtering limiting colonization of these mountains (Graham et al. 2009, Brehm et al. 2013). Moreover, our data shows that endemism in this region is almost 3 times greater than in the lowlands (approximately 28.6% of the species we recorded for the Andes are endemic to this region, whereas only 9.7% and 7.7% of the species in the eastern and western lowlands, respectively, are endemic to these regions), suggesting that the interpretation that the Andes are a diversification

center would be enhanced if only endemics were analyzed as endemics are not only numerically dominant in the Andes, but also of more recent origin (cf. Parada et al. 2013).

In contrast, the high phylogenetic diversity and low species richness of the western lowlands (Fig. 2.1a, b) suggest that, as predicted, the high precipitation seasonality limits *in-situ* diversification and that its diversity may be mostly driven by external immigration of phylogenetically distant species (Davies and Buckley 2012). This is likely associated with this region being composed of two distinct ecoregions, the xeric Tumbesian ecosystems to the south and the humid Chocó evergreen forests to the north. Immigrants into these two sets of ecosystems are likely conditioned by their distinct environments, as indicated by their different species compositions (Sierra 1999, Carrera et al. 2010). Because ecological requirements tend to be phylogenetically conserved (Losos 2008), immigrants into these two different environments should be phylogenetically distant. In turn, the eastern lowlands probably experience intermediate levels of diversification and high immigration in response to their more moderate levels of spatial and temporal EH, as suggested by its high species diversity and relatively high phylogenetic diversity (Fritz and Rahbek 2012, Fine et al. 2013).

The pervasiveness of the EH-diversity association

While our results point to a widespread role of EH in promoting or limiting diversity across regions regardless of environmental configuration, it remains to be seen whether EH has similar effects and explanatory power in other systems. On the one hand, the importance of spatial and temporal EH in promoting isolation and adaptation is likely to differ across taxa. In particular, more vagile taxa such as flying vertebrates are expected to show a weaker association

with local EH than small terrestrial mammals. On the other hand, in contrast to other mountain systems, the tropical Andes are relatively young, with an important portion of its uplift concentrated in the last 5-10 million years (Gregory-Wodzicki 2000, Garziona et al. 2008). Thus, the strong association recovered between EH and small terrestrial mammal diversity might be associated with the recent geological dynamic of the tropical Andes and its impact on surrounding areas (Hoorn 1993, Colinvaux et al. 1997, Gregory-Wodzicki 2000, Smith et al. 2005), which was probably enhanced during periods of climate change (Kreft and Jetz 2007, Alvarado-Serrano and Knowles In prep). The high degree of in-situ diversification recovered for the Andes, for example, may be caused by a combination of current patterns of EH in this region and the increased opportunities for differentiation in newly opened environments (Losos 2010) that arouse with the uplift of these mountains (Albert et al. 2006, Brumfield and Edwards 2007). The role of EH in promoting diversity in this system may also have been influenced by the impact of past climatic events, which disproportionally affected the Andes more than the adjacent lowlands (Iriando 1994, Colinvaux et al. 2001). It is likely, for instance, that climatic-induced distributional shifts may have significantly contributed to the genetic differentiation of populations as these populations migrate through a heterogeneous landscape (Knowles and Alvarado-Serrano 2010). These events in the tropical Andes that offered the opportunity for increased colonization and diversification could have been exploited by several of the mammal families included in the analysis, as several of the taxa included are relatively young and/or of relative recent appearance in South America (e.g., Cricetidae; Steppan et al. 2004, Parada et al. 2013). Therefore, it seems likely that the strong association we recovered between small terrestrial mammal diversity and EH most likely reflects ongoing processes as well as past environmental effects that are unique to this system and that may have potentially increased the

importance of EH on the prevalence of in-situ diversification in comparison to older areas where speciation may be limited by competition, limited ecological opportunities, or saturation (Burbrink and Pyron 2010, Yoder et al. 2010).

Testing this possibility would require new studies on the association of EH and species and phylogenetic diversity in other topographically complex and diverse systems. Although several studies have previously supported a strong association between EH and species diversity, measurements of EH and spatial-scale of analysis vary considerably across studies (e.g., Kreft and Jetz 2007, Londoño-Murcia et al. 2010, Rocchini et al. 2010). Besides, in contrast to this study, phylogenetic diversity has rarely been considered, making a straightforward comparison of our results with previous findings challenging. In this regard, this work offers a valuable baseline upon which the EH-diversity association could be investigated. Although the ability of testing theoretical predictions of the effects of spatial and temporal EH on diversification is unavoidably constrained by the nature of correlative analyses, the approach followed has the advantage not only being highly repeatable, objective, and biologically meaningful, but also of representing the true multitude of interacting processes that species encounter in nature given its focus on two different aspects of diversity and of incorporating information from multiple environmental layers to quantify spatial and temporal components of EH.

Conclusions

Although many challenges remain in mechanistically uncovering the role of EH in promoting or limiting diversity, the expansion of analyses to include phylogenetic information together with the dissection of heterogeneity into its spatial and temporal components provide

valuable insights into drivers of diversity. Evidence of a strong association between heterogeneity and diversity in all three regions of continental Ecuador highlights the consistent effect of EH on the prevalence of underlying processes along the EH continuum. In particular, the results of this study are in line with theoretical predictions that link high spatial and low temporal EH to opportunities for population and species divergence, although the strong EH-diversity recovered is likely not exclusively driven by this process. Our results suggest that meso-scale precipitation variability and associated ecogeographic variability have the potential to influence these processes by conditioning the opportunities for diversification in small terrestrial mammals. Yet, as previously recognized, the search for a unique mechanism for differences in biodiversity across disparate taxa and regions is meaningless; multiple interacting processes are certainly involved and their relative contribution likely depends on systems' idiosyncrasies (Hillebrand 2004). Thus, it is important to assess the generality of these findings, which could be done taking advantage of the baseline offered by this study. Of special interest would be the evaluation of the mechanisms, extent, and conditions under which EH may limit or favor species diversity. Future work including explicit modeling of the influence of EH on the opportunities for divergence will certainly be of value. Ultimately a better understanding of the EH-diversity association will improve our knowledge of diversification processes in highly diverse, topographically complex regions and the possible biological impacts of natural and human-induced changes in patterns of EH.

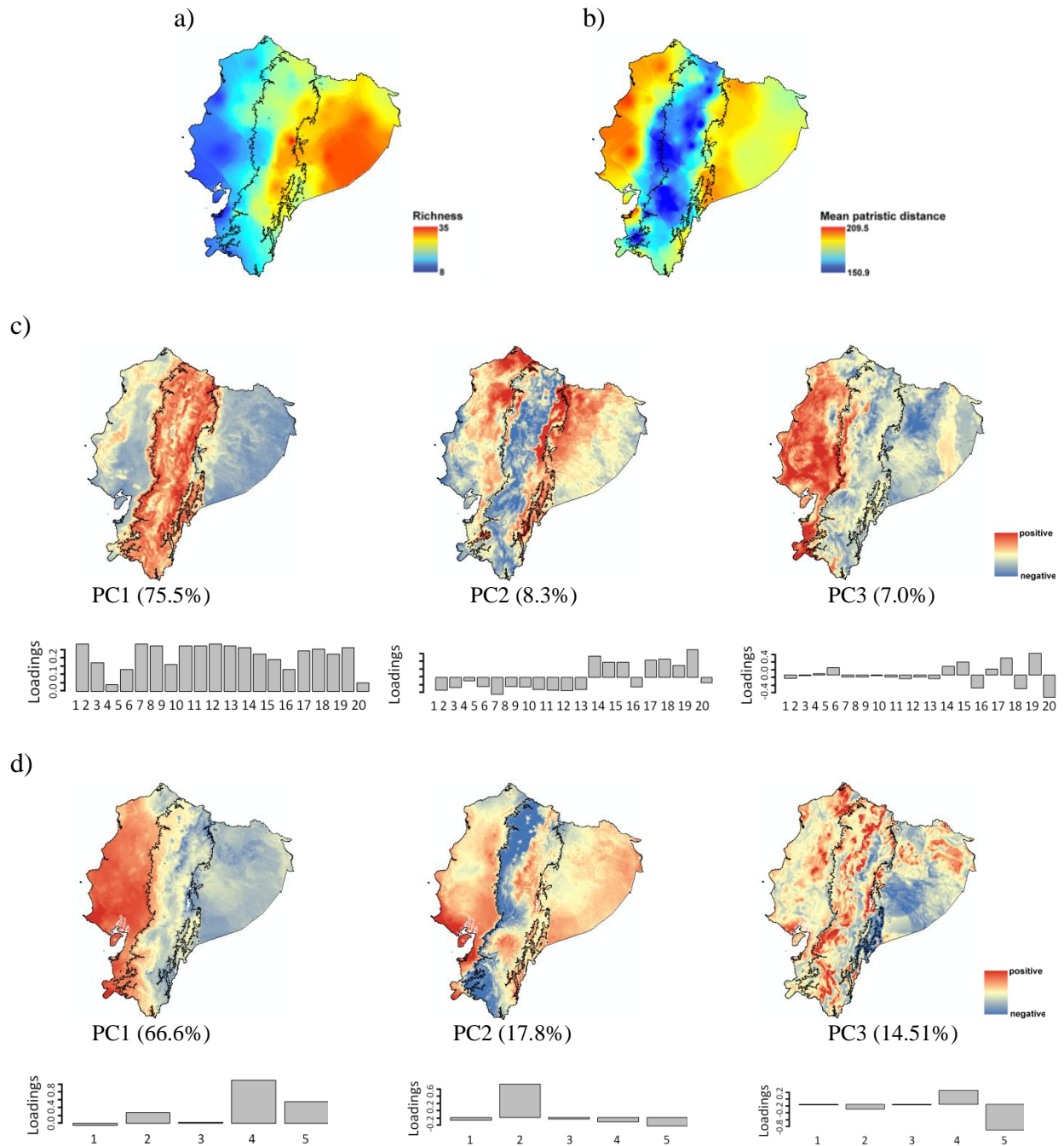
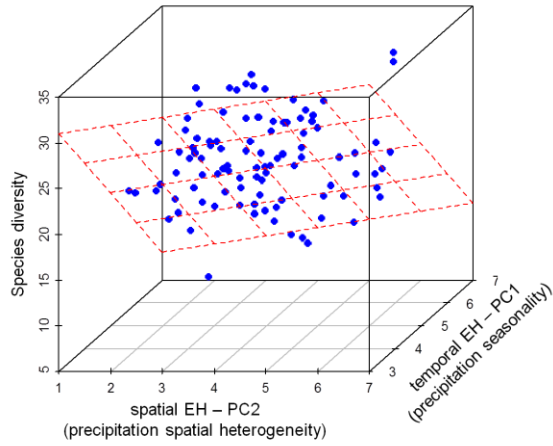


Figure 2.1. Geographic patterns of diversity and environmental heterogeneity. Interpolated species (a) and phylogenetic diversity (b) and grids of the estimated principal components of spatial (c) and temporal (d) environmental heterogeneity (see methods for details). Diversity was interpolated based on an Inverse Distance Weighting (IDW) model based on the 100 sampling points used in the analyses. Percentage of the variance explained and variable loadings associated with each EH principal component are presented under each PC map (for variables description and order see Table 2.S1). In all maps the 1000m contour line is shown to indicate the 3 regions of continental Ecuador: western lowlands (left), Andes (center), eastern lowlands (right).

a)



b)

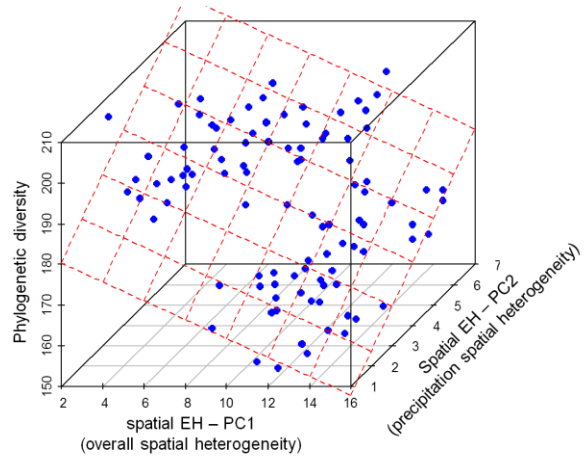
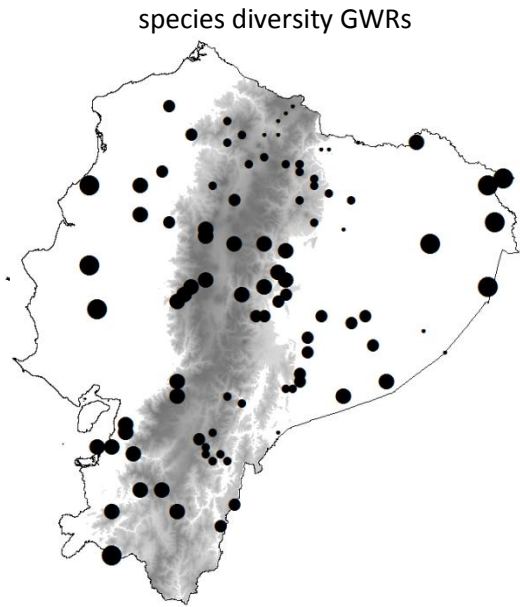


Figure 2.2. Results of SAR regressions.

Scheme of Spatial Autoregressive (SAR) models depicting the association between species (a) and phylogenetic diversity (b) and the components of environmental heterogeneity that are significant in these multivariate regressions (see Table 2.1). The slope of the regression plane (in red), depicts the steepness of the association (i.e., the predictor's β coefficient). The main environmental variation summarized by each component is given in parentheses (see Table 2.S1).

a)



b)

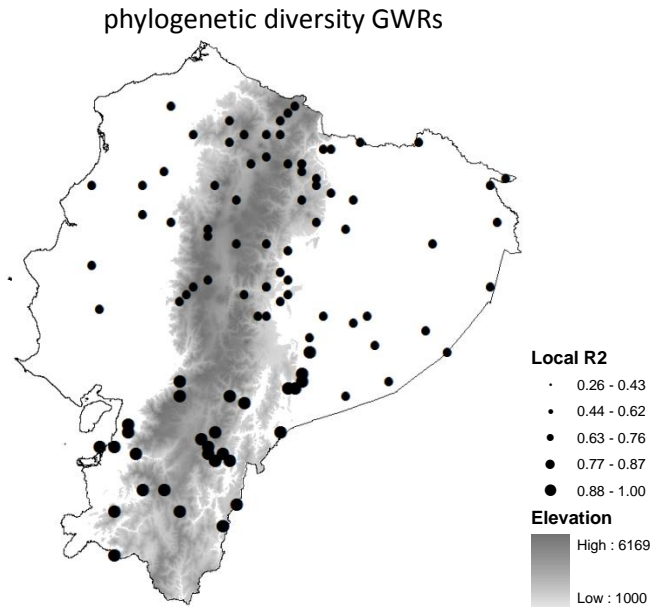
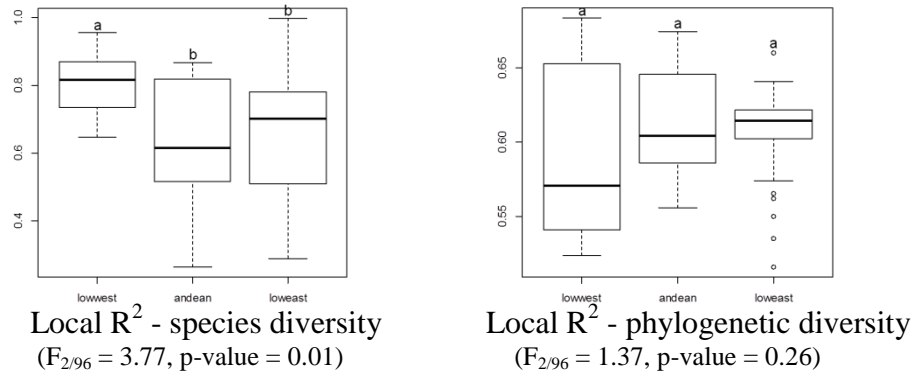
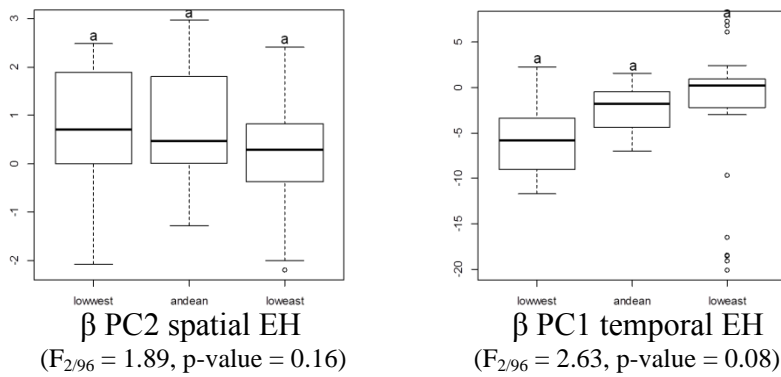


Figure 2.3. Spatial variation of explanatory power of local GWRs of diversity on EH. Variation of the local R^2 of geographically-weighted regressions (GWRs) of species diversity (a) and phylogenetic diversity (b) on spatial and temporal environmental heterogeneity (for exact model specification see Table 2.1). Note the roughly consistent predictive power across regions in both regressions.

a)



b)



c)

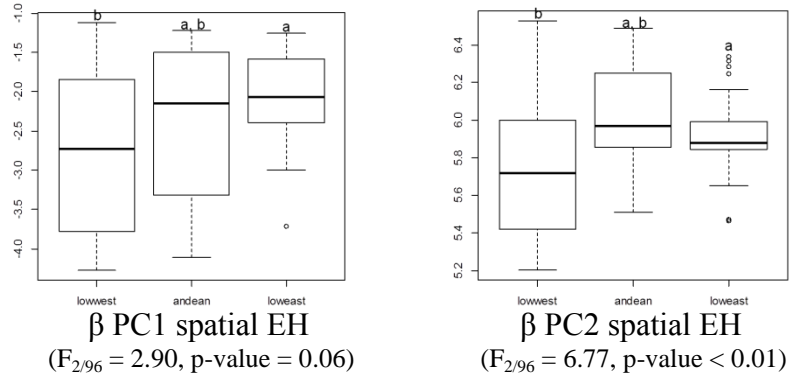


Figure 2.4. Across-regions comparison of the strength and steepness of the association between environmental heterogeneity and diversity.

Boxplots of local R^2 (a) and significant predictors' β coefficients estimated from geographically weighted regressions of species (b) and phylogenetic (c) diversity on EH. ANOVA results are given below each figure; letters are used to represent results of Tukey's post hoc tests. Variable loadings for all principal components (PCs) are provided in Table 2.S1.

Table 2.1. Summary of SAR and GWR models.

Spatial autoregressive (SAR) and geographically weighted regression (GWR) models are summarized by predictors' β coefficients (with standard error presented between square brackets) and results of analyses of residuals. For comparison, non-spatial ordinary least squares (OLS) regressions run on the same data are also shown for species (a) and phylogenetic diversity (b). Residuals normality was assessed using Shapiro-Wilk's tests, whereas their degree of spatial autocorrelation was assessed using Global Moran's I. One asterisk is used to indicate significant results with a p-value between 0.05 and 0.01, whereas two asterisks are used to indicate p-values smaller than 0.01. Variable loadings for all principal components (PCs) are provided in Table 2.S1. Abbreviations: SAR_(ERR): error spatial dependence model; SAR_(LAG): Lagrange spatial dependence model, sEH: spatial environmental heterogeneity; tEH: temporal environmental heterogeneity.

Model	R ²	Δ AIC ¹	Predictor coefficients						Residuals		
			sEH.PC1	sEH.PC2	sEH.PC3	tEH.PC1	tEH.PC2	tEH.PC3	Normality	Spatial autocorrelation	
(a) Response variable: species diversity											
OLS	0.55	-5.29		0.73* [0.33]			-5.97** [0.60]		-3.18** [0.83]	0.99	0.42**
SAR _(LAG)	0.75	-48.91		0.56* [0.25]			-2.62** [0.60]		-1.26 [0.66]	0.99	0.39
GWR	0.91	-156.53		0.49 (\pm 1.24) [0.83]			-1.67 (\pm 5.05) [2.24]		-1.51 (\pm 3.21) [2.18]	0.96*	0.03**
(b) Response variable: phylogenetic diversity											
OLS	0.51	-5.67	-2.26** [0.31]	5.76** [0.92]					-3.39 [2.31]	0.99	0.18**
SAR _(LAG)	0.57	-13.07	-1.73** [0.34]	4.53** [0.89]					-2.66 [2.16]	0.99	1.03
GWR	0.61	-195.72	0.00 (\pm 0.87) [0.39]	5.91 (\pm 0.28) [1.09]					-4.63 (\pm 1.15) [2.69]	0.99	0.13**

1. AIC difference with full OLS model including all 6 predictors.

Table 2.S1. Environmental variables used in the spatial and temporal heterogeneity PCAs.

The loadings of all variables on the first 3 principal components used in the analyses are provided. Abbreviations: l.s.d.: local standard deviation, max.: maximum, min.: minimum.

Spatial Heterogeneity PCA				Temporal heterogeneity PCA			
Variable	PC1	PC2	PC3	Variable	PC1	PC2	PC3
1. Annual mean temperature (l.s.d.)	0.28	-0.20	-0.06	1. Isothermality	-0.05	-0.08	-0.01
2. Mean diurnal range (l.s.d.)	0.17	-0.16	-0.01	2. Temperature seasonality	0.22	0.96	-0.15
3. Isothermality (l.s.d.)	0.04	-0.05	0.04	3. Temperature annual range	0.01	-0.04	-0.02
4. Temperature seasonality (l.s.d.)	0.13	-0.14	0.17	4. Precipitation seasonality	0.87	-0.12	0.48
5. Max. temperature warmest month (l.s.d.)	0.28	-0.26	-0.04	5. Precipitation Annual range	0.44	-0.23	-0.87
6. Min. temperature warmest month (l.s.d.)	0.27	-0.14	-0.04				
7. Temperature annual range (l.s.d.)	0.16	-0.15	-0.01				
8. Mean temperature wettest quarter (l.s.d.)	0.27	-0.19	-0.04				
9. Mean temperature driest quarter (l.s.d.)	0.27	-0.20	-0.08				
10. Mean temperature warmest quarter (l.s.d.)	0.28	-0.21	-0.04				
11. Mean temperature coldest quarter (l.s.d.)	0.27	-0.19	-0.07				
12. Annual precipitation (l.s.d.)	0.26	0.34	0.20				
13. Precipitation wettest month(l.s.d.)	0.22	0.24	0.30				
14. Precipitation driest month (l.s.d.)	0.19	0.24	-0.28				
15. Precipitation seasonality (l.s.d.)	0.13	-0.15	0.14				
16. Precipitation wettest quarter (l.s.d.)	0.24	0.27	0.39				
17. Precipitation driest quarter (l.s.d.)	0.25	0.29	-0.30				
18. Precipitation warmest quarter (l.s.d.)	0.22	0.19	0.49				
19. Precipitation coldest quarter (l.s.d.)	0.26	0.44	-0.49				
20. Vegetation types (local variety)	0.05	-0.08	0.01				
Variance explained	75.46	8.33	6.99		66.59	17.79	14.51

Table 2.S2. Pearson's correlation coefficients among predictors used in the regressions.

The only significant correlation is indicated by * (p-value < 0.001). Predictors kept in the minimum adequacy models for species and/or phylogenetic diversity are in bold.

	spatial EH PC1	spatial EH PC2	spatial EH PC3	temporal EH PC1	temporal EH PC1	temporal EH PC1
spatial EH PC1	-					
spatial EH PC2	0.00	-				
spatial EH PC3	0.07	0.07	-			
temporal EH PC1	0.03	-0.06	0.75*	-		
temporal EH PC2	-0.37	0.16	-0.24	-0.22	-	
temporal EH PC3	0.14	-0.12	-0.15	-0.09	0.06	-

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CHAPTER 3: Exploring the effect of environmental heterogeneity on isolation and local adaptation

ABSTRACT

An important goal in evolutionary biology is to understand the interplay between gene flow, genetic drift, and divergent selection because this interaction determines the likelihood of population and species divergence. Complex topographic regions offer a valuable opportunity to investigate this interaction, where environmental heterogeneity may affect the opportunity for local adaptation. Here we explore whether environmental heterogeneity promotes genetic and phenotypic differentiation, and whether evidence of local adaptation variation can be recovered in a tropical generalist mouse from the tropical Andes. We focus specifically in patterns of neutral genetic variation and skull and mandible morphological variation. The results show that despite abundant genetic and phenotypic variation in this species, adaptive divergence does not seem to be a major differentiation force in this species. Instead, genetic drift associated with environmentally driven isolation is recovered as a crucial process in this system. The implications of these findings, in particular, how this scenario fits within the general debate about the importance of climatic barriers in the diversification of montane tropical taxa are discussed.

INTRODUCTION

Understanding the role of environmental factors in promoting and maintaining population differentiation is crucial for improving our knowledge of the processes structuring genotypic,

phenotypic, and ultimately species diversity. Such understanding is fundamental not only for addressing how landscape configuration affects patterns of species diversity across the globe, but also how environmental changes may impact species evolutionary paths – currently an issue of pressing importance (Hansen et al. 2012). In particular, studying how the geographic/environmental contexts of populations influence the interaction between gene flow, genetic drift, and selection is of major concern in biology, because this interaction affects species' cohesiveness, population viability, propensity to local extirpation, and ultimately the likelihood of divergence or extinction (Kawecki and Ebert 2004, Gandon and Nuismer 2009, Uyeda et al. 2009, Yackulic et al. 2011). The relative contribution of these processes in natural systems remains under debate (Nei 2005, Bird et al. 2012).

The geographic/environmental contexts of species are expected to be a primary determinant of the balance between the forces promoting differentiation (i.e., drift, divergent selection) and those preventing it (i.e., gene flow, stabilizing selection) (Levins 1964, Slatkin 1985), and hence, the likelihood of divergence by local adaptation is expected to vary in a predictable manner in response to environmental differences. Homogeneous environments are expected to facilitate gene flow and stabilizing selection due to the absence of dispersal barriers and the presence of similar selective optima across space (Fig. 3.1). In contrast heterogeneous environments are expected to increase the likelihood of local adaptation and reduce the opportunity for gene flow (Fig. 3.1), especially if effective population sizes are large and the spatial scale of heterogeneity is coarse with respect to the dispersal ability of individuals (Levins 1968, Brown and Pavlovic 1992, Blanquart et al. 2012). This is because local adaptation would restrict the effectiveness of migration, and hence gene flow, because locally adapted migrants

would be at a selective disadvantage in new environments and, being specialized, would have more difficulty migrating through a heterogeneous landscape due to inhospitable habitats in intervening areas (Fig. 3.1) (Geffen et al. 2004, Blanquart et al. 2012). Hence, assessing the extent of adaptive divergence in wild populations inhabiting heterogeneous environments should improve our understanding of their sensitivity to ongoing environmental changes (Hansen et al. 2012) and provide valuable insights into why high species diversity is often concentrated in environmentally complex regions (Davies et al. 2007, Badgley 2010) because the likelihood of species divergence as well as the ability of species to cope with rapid environmental change differs significantly depending on the relative contribution of gene flow, selection, and genetic drift (Gavrilets 2003, Hendry et al. 2007).

Here we investigate the micro-evolutionary consequences of inhabiting an environmentally heterogeneous region in the soft grass mouse (*Akodon mollis*), a common inhabitant of the northern Andes. As a hotspot for speciation and endemism and a source of species for adjacent biomes (Hughes and Eastwood 2006, Santos et al. 2009), the northern Andes offer a great opportunity to study divergence processes because its complex topography and steep environmental gradients (Pearson and Ralph 1978, Patterson et al. 2006, Sarkinen et al. 2012) favor the existence of spatially structured species with populations inhabiting ecologically different environments (Chapter 2). In particular, the broad latitudinal and altitudinal range *A. mollis* within the Andes of Ecuador and northern Peru, limited vagility, high morphological variability, and spatially-structured genetic variation (Barnett 1999, Alvarado-Serrano 2005, Alvarado-Serrano et al. 2013) make this tropical mouse an ideal candidate for investigating the role of environmental heterogeneity in promoting population divergence. Here, we test 3 specific

predictions on the influence of environmental heterogeneity on opportunities for divergence using mitochondrial and genome-wide genetic variation to measure neutral genetic divergence (Avice et al. 1987, Morin et al. 2004, but see Galtier et al. 2009) and skull and mandible morphometric variation as a proxy for morphological adaptive divergence. Skull and mandible variation are likely targets of local adaptation given their fundamental role in food and sensory information acquirement and processing (Grieco and Rizk 2010, Santana et al. 2012), and their demonstrated correspondence with environmental variation (Cordero and Epps 2012, Marchán-Rivadeneira et al. 2012). Specifically, we test: (1) whether environmental landscape, specifically the friction it offers to individual movement (Slatkin 1987, McRae et al. 2005, Cushman et al. 2006), promotes both genetic (**prediction 1a**) and adaptive phenotypic (**prediction 1b**) differentiation; (2) whether environmental dissimilarity between habitats, by influencing the similarity of selective optima (Nosil et al. 2008, Räsänen and Hendry 2008, Hereford 2009), condition the amount of neutral genetic (**prediction 2a**) and adaptive phenotypic differentiation (**prediction 2b**); and (3) whether the degree of phenotypic differentiation exceeds that of neutral genetic differentiation (**prediction 3**), indicating divergent selection is prevalent in this system (Leinonen et al. 2013). In doing so, we provide a thorough assessment of the relative contribution of divergent selection and genetic drift to the patterns of genetic and morphological differentiation observed in this mouse, with the aim of improving our understanding of divergence processes in this threatened biodiversity hotspot (Londoño-Murcia et al. 2010).

METHODS

Data acquisition

Tissues, skulls, and mandibles were obtained from specimens sampled across the entire distribution of *A. mollis* following standard trapping methods for small terrestrial mammals (Wilson 1996) and observing American Society of Mammalogists' guidelines (Sikes et al. 2011) and University-approved procedures (UCUCA#10265-1). We supplemented our sampling with specimens from several natural history collections in Ecuador, Peru and the United States. The identification of all studied specimens was based on a thorough comparison with available morphological descriptions (Alvarado-Serrano 2005, Hershkovitz 1940, Myers et al. 1989) and photographs of the holotype. All specimens in our sample with locality information for which GPS coordinates were not available were georeferenced following a point-radius method that considers the uncertainty in the locality description (Wieczorek et al. 2004). Highly uncertain points were discarded. The vetted set of localities, which comprised 89 unique records, was used to estimate the environmental preferences of this species (see details below). For all other analyses localities within 5km from each other were considered as one population given the estimated mobility of *A. mollis* (Barnett 1999). Only populations with at least 6 individuals with genetic and morphometric data were analyzed, which resulted in a total of 15 single-locality populations (mean population size = 13 ± 3 individuals; Fig. 3.2).

Estimation of phenotypic differentiation

Morphological variability within *A. mollis* was assessed from 23 measurements of the skull and 4 of the mandible (Table 3.S1; for details see Fig. 1 in Alvarado-Serrano and D'Elía 2013) recorded to the nearest 0.01 mm using a digital caliper (UPM Model 111-513). All measurements were selected in accordance with the criteria proposed by Lestrel (2000) and were taken twice to reduce experimental error to acceptable limits (Gannon et al. 1992, Strauss and

Atanassov 2006). Only adult individuals, identified by the molar wear criteria proposed by Myers (1989), were included in the analyses to reduce experimental error due to non-linear allometric associations (Myers 1989, Myers et al. 1990). Individuals from different sexes were pooled together as no significant sexual dimorphism in the morphological measurements was recovered (Wilk's lambda $F_{27/199} = 0.71$, p-value = 0.85).

After \log_e -transforming all data to improve normality and homoscedasticity, we estimated missing data from partially damaged skulls/mandibles with less than 25% of measurements missing using an expectation-maximization algorithm (Strauss et al. 2003). Using the occipito-nasal length (ONL; Fig. 1 in Alvarado-Serrano and D'Elia 2013) as an indicator of overall skull size, we then size-standardize all 26 remaining measurements by individually regressing each measurement against ONL. This was done to account for growth-scaling variation that could obscure other morphological differences. A principal component analysis (PCA) was run based on the variance-covariance matrix of the 26 sets of residuals from these regressions. Only principal components (PCs) with eigenvalues above 1 were kept for further analyses (Kaiser's rule; Sokal and Rohlf 2003). A multivariate analysis of variance (MANOVAs) was performed to test for significant differences in PC scores among populations.

The degree of morphological differentiation between populations was estimated using P_{ST} , which is an analog to Q_{ST} estimated from phenotypic data that is commonly used in field studies in which calculating empirical heritabilities is not possible (Leinonen et al. 2013). For this calculation we used the formula: $P_{ST} = Vb / (Vb + 2h^2Vw)$; where Vb = phenotypic variance between populations, h = heritability, and Vw = phenotypic variance within populations.

Following Storz et al. 2002 (see also Antoniazza et al. 2010, Mobley et al. 2011), we estimated between and within population phenotypic variance using individual analyses of variance (ANOVAs) for each retained morphological principal component. Because no estimates of heritability exist for skull and mandible variation in *A. mollis*, we used a broad range of heritability values (from 0.1 to 1; see Arnqvist and Kolm 2010, Oneal and Knowles 2013), which span the range of empirical estimates for various morphological traits in other taxa (Leinonen et al. 2006, Manier et al. 2007), to assess the sensitivity of results to estimates of heritability (Pujol et al. 2008, Leinonen et al. 2013). Because results were not sensitive to the specific value of heritability used (see below) we only report results for $h^2 = 0.5$, a value that lies in the lower range of heritability estimates for phenotypic traits in several organisms (Falconer and Mackay 1989, Tamioso et al. 2012). Finally, once P_{ST} values were calculated for each principal component with an eigenvalue greater than 1, we estimated an overall P_{ST} value as a weighted average of the individual P_{ST} estimates for each component with weights determined by the proportion of the total morphological variance explained by each component. This provided a single estimate of inter-population morphological differentiation that accounted for over 71% of the total morphological variance in the data.

Estimation of genetic differentiation

Neutral genetic differentiation between populations was estimated using two different sets of molecular markers amplified from genomic DNA extracted from liver or muscle tissue using an extraction kit (QIAGEN). 800 bp of the cytochrome b gene were amplified in 121 individuals from 9 populations. Amplifications were performed in 25 μ L reactions following the protocol outlined by D'Elia (2003) with primers MVZ05 and MVZ16 (Smith and Patton 1993).

PCR products were purified with a purification kit (QIAGEN) and bidirectionally sequenced on an Applied Biosystem's 3730 XL DNA Sequencer at the University of Michigan DNA Core Sequencing Facility. Thirty four additional individuals' cytochrome b sequences from 4 populations were obtained from Alvarado-Serrano et al. (2013). Sequences were aligned using Sequencher v4.6 (Gene Codes 2006) and visually inspected in MacClade (Maddison and Maddison 2000). After verifying there were no departures from neutrality based on Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989) statistics (results not shown), pairwise F_{ST} were estimated using Arlequin v3.5 (Excoffier et al. 2005).

A subset of individuals, specifically 8 individuals of 12 populations, were used to construct a reduced representation library for next-generation sequencing following the protocol outlined by Parchman et al. (2012). After digestion with *EcoRI* and *MseI* restriction endonucleases, fragments were ligated to a short DNA sequence that include a 10bp barcode unique for each individual and an adaptor sequence for sequencing using Illumina technology. These ligated fragments were PCR-amplified and size-selected in a 2.5% agarose gel to retain only fragments between 300 and 400bp. The reduced library was sequenced in an Illumina Genome Analyzer IIx at the University of Michigan DNA Core Sequencing Facility. The sequenced reads were demultiplexed (i.e., classified based on barcodes) and filtered to assure a minimum average sequence Phred score of 32. The filtered data were then processed using the program STACKS v0.99.993 (Catchen et al. 2011), which is a program designed to identify and call single nucleotide polymorphisms (SNPs) – even in the absence of a reference genome – using a likelihood ratio test to distinguish true variants from sequencing errors (Emerson et al. 2010). Using this program we estimated pairwise F_{ST} values for all 12 populations based on a set

of 1216 genome-wide SNPs recovered (1 population, however, was later excluded because it lacked a minimum of 6 individuals with useful morphological data). Only SNPs present in at least 5 individuals of at least 8 of the 12 populations were included.

Association analyses

A series of Mantel and partial Mantel tests (Mantel 1967) and distance-based redundancy analyses (dbRDA; Anderson and Legendre 1999, McArdle and Anderson 2001) were used to test for a positive association between environmental friction and neutral genetic and phenotypic differentiation (i.e., **predictions 1a** and **1b**, respectively). Three separate grids of environmental resistance to dispersal were calculated for three different environmental variables, mean annual temperature and annual precipitation (from Worldclim, Hijmans et al. 2005) and vegetation type (from the BINU project, EcoCiencia 2004). We chose these three variables as proxy for overall environmental variation because of their known biological relevance in a wide variety of vertebrates (Graham et al. 2010, McCormack et al. 2010) and their tight correlation with other ecological variables affecting species relationship to their environment (Porter and Gates 1969). Resistance values for each cell in the 3 grids were calculated based on the assumption, commonly used in the realm of ecological niche models (Peterson et al. 2011), that the environmental suitability of a landscape for a species is reflected in the distribution of the species' sampling localities, and the assumption that dispersal difficulty is inversely associated with landscape suitability (see Knowles and Alvarado-Serrano 2010). Specifically, resistance values were determined based on histograms of the frequency with which different climatic conditions or vegetation types were represented among the 89 vetted individual localities. (i.e., areas where *A. mollis* is more frequently encountered, as judged by the number of unique

localities, received smaller resistant values than areas where this species is less represented; vegetation types and climatic conditions not inhabited by *A. mollis* received greater resistances). In the case of the two continuous variables, histograms' bin cut-offs followed standard-deviation increments from the sample distribution mean. It is important to note that alternative resistance parameterizations (e.g. based on distribution's natural breaks; Bolstad 2008) showed that results were not sensitive to the particular parameterization selected. Pairwise resistance values based on the 3 resistance grids were then calculated in Circuitscape v3.5.8 (McRae et al. 2005, McRae 2006) and tested for a correlation with pairwise F_{ST} and pairwise P_{ST} using the R package *vegan* (Oksanen et al. 2012). We run both Mantel and dbRDA analyses to verify consistency in the results because the validity of partial Mantel tests remains under discussion (Raufaste and Rousset 2001, Geffen et al. 2004). In all these analyses, we account for the effect of spatial autocorrelation in genetic, phenotypic, and environmental variation by incorporating as a covariate the Euclidian geographic distance separating populations.

The degree of environmental dissimilarity was characterized using data on 19 climatic variables (Worldclim, Hijmans et al. 2005) and vegetation type (BINU project, EcoCiencia 2004) for each locality. After standardizing these variables, we run a PCA based on the correlation matrix (appropriate for this type of data that include variables measured in very different units; Quinn and Keough 2002). We then calculated the pairwise environmental Euclidian distance between all population pairs based on the 4 PCs with eigenvalues above 1 (Kaiser's rule; Sokal and Rohlf 2003), which together accounted for over 96% of the inter-population environmental variance (Table 3.S2). The resulting environmental Euclidian distances (our measure of environmental dissimilarity) was tested for a correlation with pairwise

F_{ST} and pairwise P_{ST} using geographic distance as a covariate. In addition, we assessed the magnitude of the difference between P_{ST} and F_{ST} to test for the predicted species-wide signal of divergent selection (**prediction 3**).

RESULTS

Morphometric variation

The PCA on the 27 size-standardized variables summarizing morphometric variability in the skull and mandible of *A. mollis* results in 7 components with an eigenvalue greater than 1, which together explained 71.84% of the morphological variance (Table 3.S1). Individuals' scores on these components were normally distributed (Shapiro-Wilk tests result in p-values greater than 0.15 for all 7 components). A visual inspection of these scores indicates broad intra-population variability and substantial morphological overlap between populations. Nonetheless, significant morphological differentiation is recovered between populations (Wilk's lambda $F_{98/1052} = 6.38$, p-value < 0.01). Tukey's post-hoc tests on each of these principal components indicate that the significance is driven mainly by the differentiation between populations at opposite ends of the latitudinal gradient encompassed by our sampling (i.e., populations separated by long geographic distances) irrespective of environmental conditions (Fig. 3.3). P_{ST} estimates between populations are also relatively high (mean $P_{ST} = 0.23 \pm 0.12$), despite high variation within populations. Similar results were recovered across the range of heritability values tested (mean P_{ST} ranged from 0.28 ± 0.16 when $h^2 = 0.1$ to 0.17 ± 0.09 when $h^2 = 1$).

Genetic variation

Considerable genetic differentiation among populations was observed in cytochrome-b dataset (mean $F_{ST} = 0.74 \pm 0.23$) and the genomic data (mean $F_{ST} = 0.27 \pm 0.06$). The higher cytochrome-b F_{ST} values reflect more limited intra-population variation in the mitochondrial DNA compared to the genetic data. Despite these differences in the magnitude and variance of pairwise F_{ST} estimates obtained with both datasets, the genetic signal recovered is consistent, as demonstrated by the significant correlation between both matrices (Pearson's $r = 0.4$, p -value < 0.01 ; Fig. 3.S1). Accordingly, both mitochondrial and genetic datasets provided, for the most part, consistent results (see below).

Isolation and divergent-selection

Both, partial Mantel and dbRDA analyses identify a significant pattern of isolation by resistance (IBR; McRae 2006) for all 3 environmental variables tested (i.e., mean annual temperature, annual precipitation, and vegetation type), regardless of the molecular marker used (Table 3.1; Fig. 3.4a, b). An analogous pattern of differentiation by resistance is recovered in both Mantel and dbRDA analyses for the morphological data (Table 3.1; Fig. 3.4c). When spatial autocorrelation is considered (by incorporating geographic distance as covariate), however, the only associations that remain consistently significant across methods are those between genomic-based F_{ST} and the two environmental climate-based environmental resistances (i.e., those based on annual mean temperature and annual precipitation). The association between cytochrome data and the resistance based on the two climatic variables remains significant in the dbRDA for the cytochrome-b F_{ST} . In this regard, it is important to note that dbRDA is considered a more robust analysis than partial Mantel tests (Raufaste and Rousset 2001, Geffen et al. 2004).

In contrast, both Mantel and dbRDA analyses show that both genetic and phenotypic differentiation are not significantly correlated with environmental dissimilarity between populations, regardless of whether or not geographic distances is used as a covariate (Table 3.1, Fig. 3.5). Furthermore, *A. mollis* shows no structuring of morphological variation in response to environmental conditions (Fig. 3.3) and no evidence of divergent selection based on the P_{ST} - F_{ST} comparison. With only a few exceptions, pairwise P_{ST} estimates are significantly lower than pairwise F_{ST} estimates (Fig. 3.6a). A bootstrap analysis indicates that the species-wide median difference of P_{ST} and F_{ST} is either statistically indistinguishable from zero or P_{ST} is significantly smaller than F_{ST} for both cytochrome-b and genomic datasets, regardless of the heritability scalar used (Fig. 3.6b).

DISCUSSION

Our analyses of the association between environmental heterogeneity and genetic and morphological differentiation in *A. mollis* identify genetic isolation as the main consequence of inhabiting a heterogeneous environment in this species because our results support exclusively the theoretical prediction of genetic differentiation being promoted by the increased environmental resistance to dispersal of heterogeneous areas (**prediction 1a**; Fig. 3.4a, b). In contrast, no evidence of divergent selection or local adaptation is recovered, since no evidence of genetic isolation by adaptation (Nosil et al. 2008) (**prediction 2a**; Fig. 3.5a, b) or environmentally induced phenotypic differentiation (**predictions 1b and 2b**; Fig. 3.4c and 3.5c) is recovered, nor is there an excess of phenotypic differentiation in skull and mandible morphometric variation over neutral genetic differentiation (**prediction 3**; Fig. 3.6). These results suggest that genetic drift may be more important than local adaptation in this species; a

possibility that seems further supported by our findings of pronounced and broadly variable F_{ST} estimates across population pairs and geographic distances (Hutchison and Templeton 1999), and the lack of environmentally structured morphometric variation (Fig. 3.3) – expected under adaptively driven divergence (Hoekstra et al. 2005, Antoniazza et al. 2010).

The prevalence of genetic drift in this species, which is in contrast with the accumulating evidence in support of local adaptation in other tropical vertebrates facing similarly steep altitudinal gradients in the tropics (e.g., McKenzie et al. 2013, Wilson et al. 2013) highlights the significant role of genetic drift in genetic differentiation and phenotypic evolution (Lande 1976, Hershberg et al. 2008). In particular, our results suggest that skull and mandible morphological variation in this species may be less adaptively constrained than previously thought. Not only there is no effect of environmental differences in their degree of differentiation, but also there is limited evidence of specialization (Alvarado-Serrano et al. 2013). Yet, the finding that broad morphological variation characterizes individual populations (Fig. 3.3) suggests that genetic drift is not the sole responsible process for the morphological patterns observed because high levels of gene flow within individual populations would prevent within-population morphological differentiation. It is possible instead that reduced canalization and increased sensitivity to microenvironmental variation, not captured by the scale of our environmental data, (Hall et al. 2007) accompany genetic drift. Genetic differentiation, on the other hand, seems to be strictly determined by the connectivity between habitats (Table 3.1). This lack of an effect of environmental differences among populations, expected when local adaptation is prevalent (Nosil et al. 2008, Räsänen and Hendry 2008), further suggests that population differentiation in this species is strongly condition by the spatial configuration of its populations. Together, these

findings indicate that in species facing high levels of environmental heterogeneity, such as *A. mollis*, random differentiation processes may be an important counterpart to adaptive processes.

Implications of the prevalence environmental isolation

The primary role that climatic barriers seem to play in facilitating genetic and phenotypic differentiation in *A. mollis* highlights the importance of physiological or behavioral limits to migration in heterogeneous environments (e.g., Geffen et al. 2004, Row et al. 2010). The significant association between F_{ST} and temperature- and precipitation-based environmental resistance for both mitochondrial and genomic data is in agreement with a scenario in which the complex topography and low seasonality of tropical mountains (Mani 1968, Sarmiento 1986) provides multiple opportunities for restricted migration among small isolated population due to the inability of these isolated populations to evolve their climatic tolerances to overcome climatic dispersal barriers. Our finding of a likely dominant role of genetic drift expands this hypothesis by identifying a plausible mechanism that can account for the limited evolution of climatic preferences required by this hypothesis (Cooper et al. 2010). Yet, although the presence of long term climatic barriers, conditioned by conserved climatic preferences, has been found to be determinant in population differentiation in other environmentally heterogeneous systems (Knowles and Alvarado-Serrano 2010, He et al. 2013), such hypothesis has been rarely explored in the tropical Andes (but see Alvarado-Serrano and Knowles In prep).

To assess whether this scenario applies to *A. mollis* requires further analyses that go beyond the goal of this contribution. If proven valid, however, it would have important consequences for assessing the risk of ongoing climate change in this region. For example, the

prevalence of genetic drift recovered implies that heterogeneous environments may not only promote isolation, but also habitat patchiness since reduced population size should favor genetic drift over selection (Johansson et al. 2007, Leimu and Fischer 2008, Pickup et al. 2012). Thus, although the broad latitudinal and altitudinal range of *A. mollis* seems to suggest that this species faces reduced risk of extinction in light of future climate change, the results of this study cautions against such simple interpretation. The patchiness of its distribution, the strong isolation among its populations, and the prevalence of genetic drift, which may counteract the likelihood of adaptation to novel environments (see above), suggest that changes in the climatic landscape of *A. mollis* could potentially further decrease population connectivity while reducing populations' size and genetic variability (Gilpin and Soulé 1986, Templeton et al. 2001). Thus, despite being a generalist species with a wide distribution, this species might still become threatened by future climate change. Whether other tropical montane generalist species follow similar patterns of differentiation in response to the high heterogeneity, and hence, whether they might face similar conservation risks, remains to be seen.

Is adaptive divergence not prevalent in *A. mollis*?

While it is important to note that our results do not imply that local divergent selection is not acting at all on *A. mollis*, the limited evidence of adaptive differentiation in the skull and mandible of *A. mollis* is unexpected. Not only are skull and mandible tightly linked to environmental variation and the fitness of individuals (Monteiro et al. 2003, La Croix et al. 2011, Marchán-Rivadeneira et al. 2012), but there are also substantial differences in environmental pressures associated with the broad distribution range of *A. mollis*; including a rapid decrease in temperature, air pressure, and available oxygen with elevation (Alvarado-Serrano et al. 2013)

and significant changes in the biotic communities that *A. mollis* occupy across its range (Sarmiento 1986, Patterson et al. 1998).

The lack of support for divergent selection does not seem to be related to lack of distinct selective pressures given the high environmental heterogeneity characteristic of the northern Andes (Sarmiento 1986, Patterson et al. 1998) and the comparatively constrained distribution of *A. mollis*' populations (Barnett 1999). Nor does it seem to be related to high levels of gene flow swamping adaptive variations given the significant pattern of genetic isolation by resistance (Fig. 3.4a, b) and relative high F_{ST} estimates recovered for both sets of genetic markers. Lack of phenotypic variation is also an unlikely explanation given the broad morphometric variability observed within and between populations (Fig. 3.3) and the relatively high P_{ST} estimates recovered.

It is plausible that the lack of evidence for local adaptation is caused by selection for a generalist or plastic morphology (Alvarado-Serrano et al. 2013). In line with this possibility is the pattern of high intra-specific morphological variability recovered within individual populations (Fig. 3.3), as well as previous findings of plastic responses to elevation (Hammond et al. 2001), climate (Rezende et al. 2009), and diet (Myers 1996) in related mouse species. Under this hypothesis, plasticity may inhibit local adaptation by hiding genetic variants from the effect of selection (Baythavong and Stanton 2010, Pfennig et al. 2010). Testing this latter possibility, however, goes beyond the scope of this study as it requires common garden experiments, which are particularly challenging in this species. It is also possible that genetic drift might overcome divergent selection due to small effective population sizes caused by the

patchiness of species' ranges in heterogeneous environments (Johansson et al. 2007, Lamy et al. 2012). Support for this hypothesis remains equivocal (Hereford 2009), although evidence of populations and species divergence being driven by drift has been uncovered in several taxa (e.g., Ackermann and Cheverud 2004, Lehtonen et al. 2009). In line with this possibility, we recovered a species-wide median $P_{ST}-F_{ST}$ difference that is not significantly bigger than zero (Fig. 3.6; cf. Lehtonen et al. 2009, Lamy et al. 2012, Le Corre and Kremer 2012).

Conclusions

Our analyses of genetic and phenotypic differentiation in a heterogeneous environment using *A. mollis* as a model calls into question the general notion that local adaptation is prevalent in these systems. Our findings imply that environmental heterogeneity might exert contrasting forces on the effectiveness of divergent selection. On the one hand, our results suggest that environmental heterogeneity promotes isolation, potentially facilitating the effect of divergent selection by reducing the swamping effect of high levels of gene flow on adaptive variants. On the other hand, increased isolation also facilitates genetic drift, especially considering that the patchiness resultant from high levels of environmental heterogeneity may reduce population sizes (Pickup et al. 2012) and thus the effectiveness of selection (Dempster and Lerner 1954, Johansson et al. 2007). As a result of this interaction, population differentiation in this highly heterogeneous environment may be mostly driven, counter-intuitively, by drift. This finding, which is in line with the hypothesis that wild populations are often precluded from becoming locally adapted due to stochastic processes overcoming natural selection (Hereford 2009), has important consequences for the persistence of species and their response to environmental change. Under the circumstances suggested here for *A. mollis* (i.e., environmentally isolated

populations dominated by drift) climatic niche conservatism may be an important factor in facilitating species divergence in allopatry (Cadena et al. 2012).

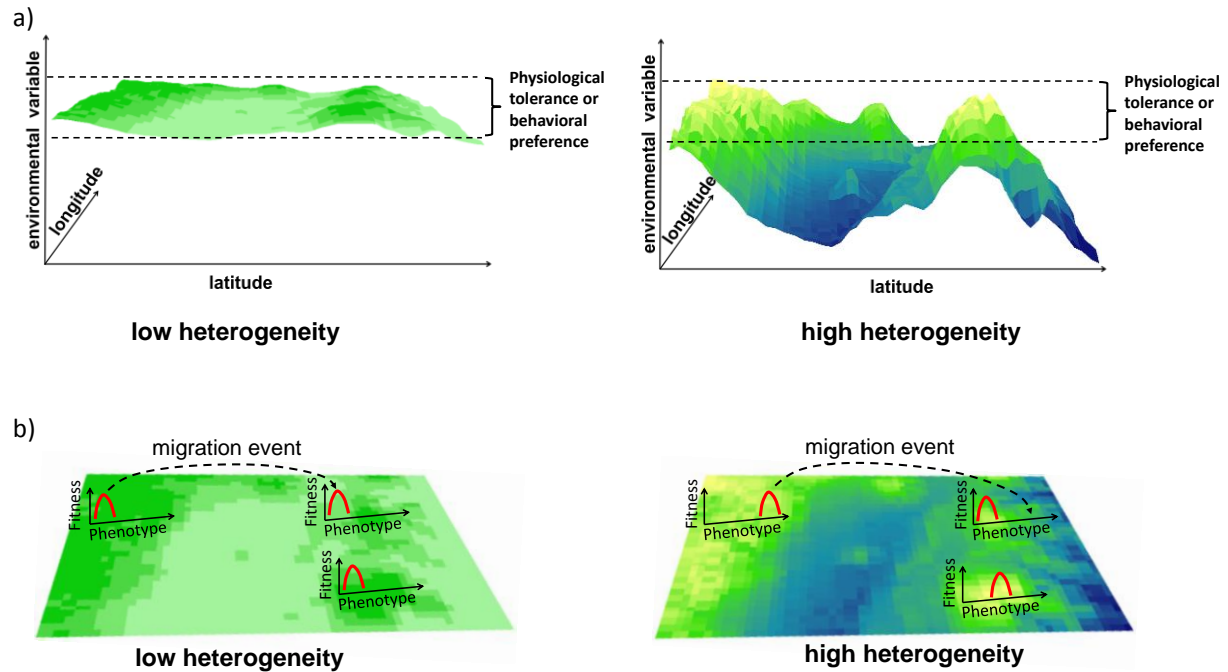


Figure 3.1. Theoretical role of environmental heterogeneity in promoting populations divergence.

The variability in the magnitude of environmental variables in heterogeneous regions makes more likely the existence of intervening areas with conditions outside the physiological tolerances or behavioral preferences of species (indicated by horizontal dashed lines) (a). These areas should limit gene flow between populations due to the increased difficulty of movement across the landscape. Similarly, the environmental variability among areas in heterogeneous regions increases the likelihood of different selective optima across space (indicated by red fitness curves) (b). These spatially variable selective optima should promote local adaptation and limit immigration of mal-adapted migrants from other locations.

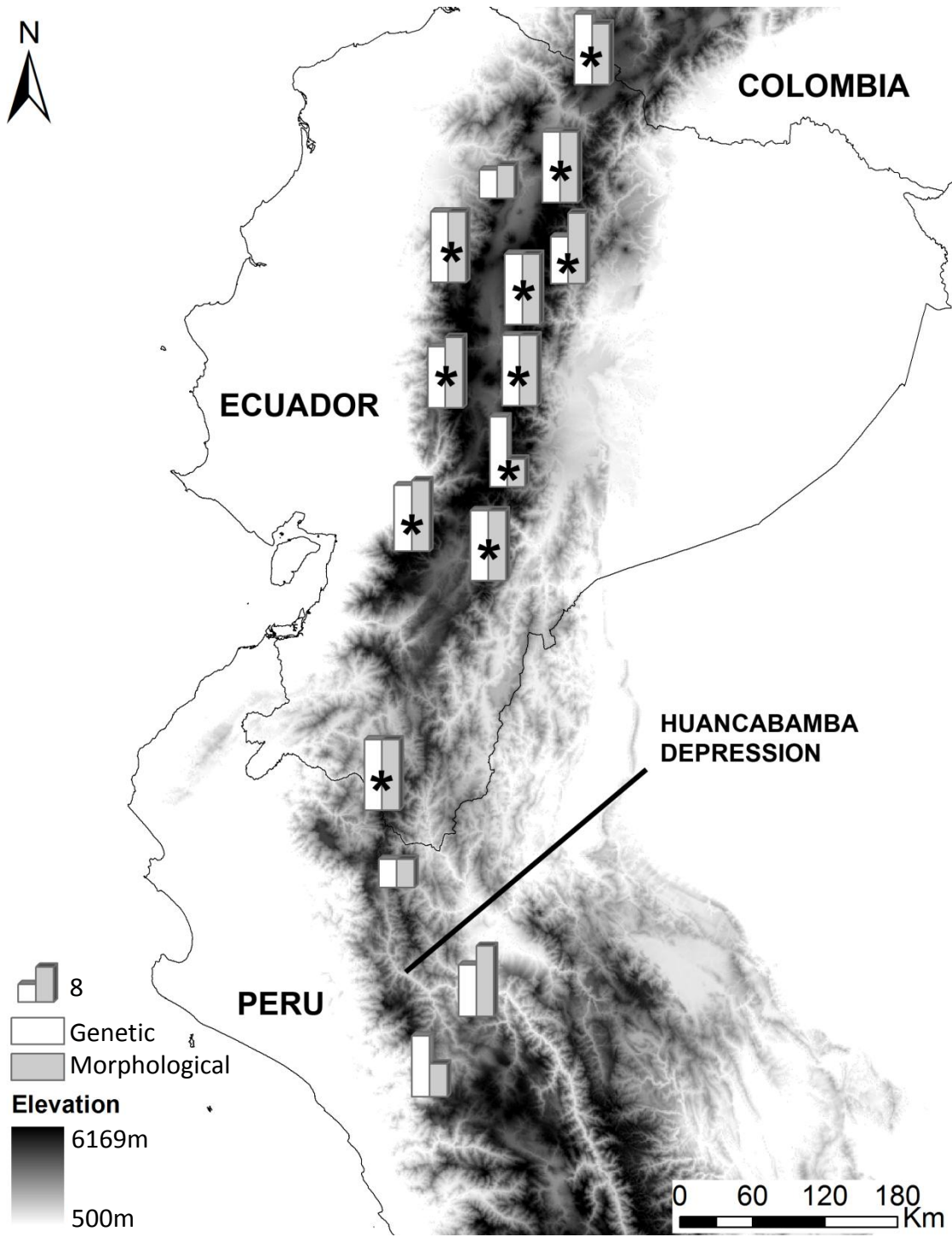


Figure 3.2. Distribution of sampling localities. Localities and sample sizes for genetic and morphological analyses are presented. The subsample populations used in the SNPs-based analyses are indicated with an asterisk. Note that the cytochrome-b dataset includes populations from around the Huancabamba depression, the lowest pass of the northern Andes (Weigend 2002).

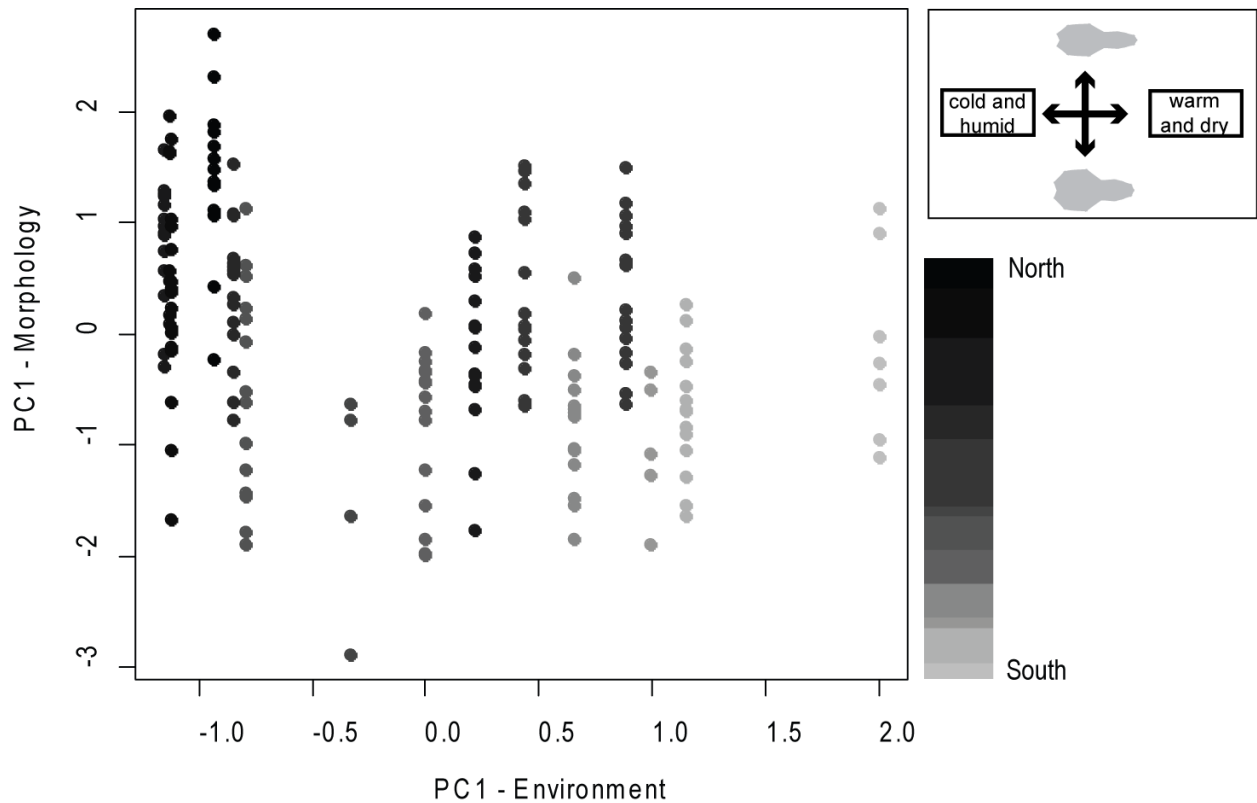


Figure 3.3. Pattern of morphological and environmental variation.

Comparison of morphological variation along the steep environmental gradient *Akodon mollis* inhabits, as summarized by the first principal components of morphological and environmental PCAs. The inset on the right presents a scheme of the variation summarized by these components based on variable loadings (see Tables S3.1, S3.2). Individuals are colored according to the latitude at which they were collected. Note that few evident differences between populations occur between populations from different latitudes, regardless of their environmental conditions.

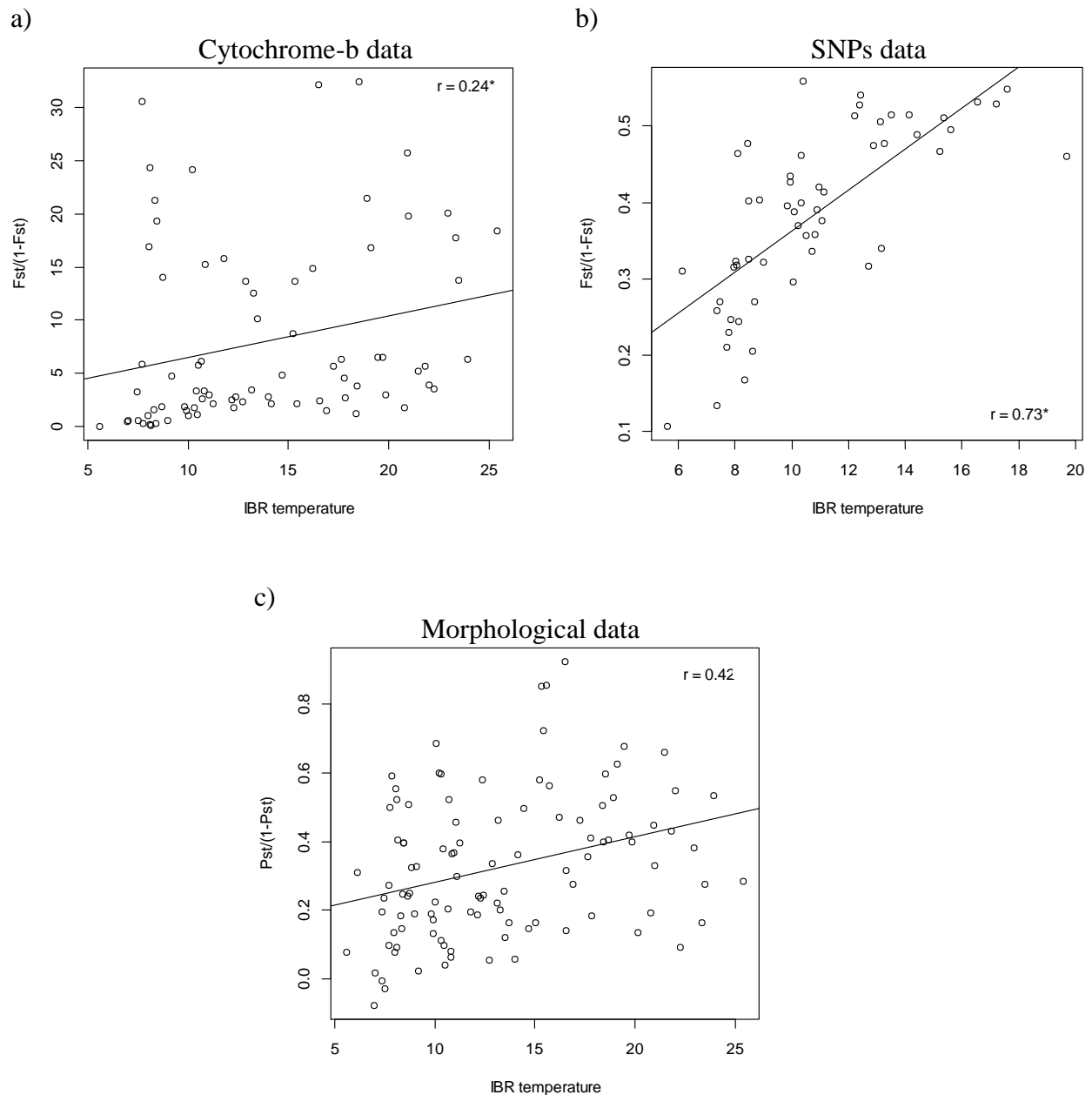


Figure 3.4. Association between environmental resistance and genetic and phenotypic differentiation.

Patterns of isolation by resistance for the genetic (a, b) and phenotypic (c) data. Only the isolation by resistance pattern based on mean annual temperature is shown because the results were similar when environmental resistance was calculated based on annual precipitation or vegetation type (see Table 3.1). Correlation coefficients are given for all analyses; an asterisk next to these coefficients indicates that the association remains significant after accounting for spatial autocorrelation. To facilitate interpretation linear regression lines are shown.

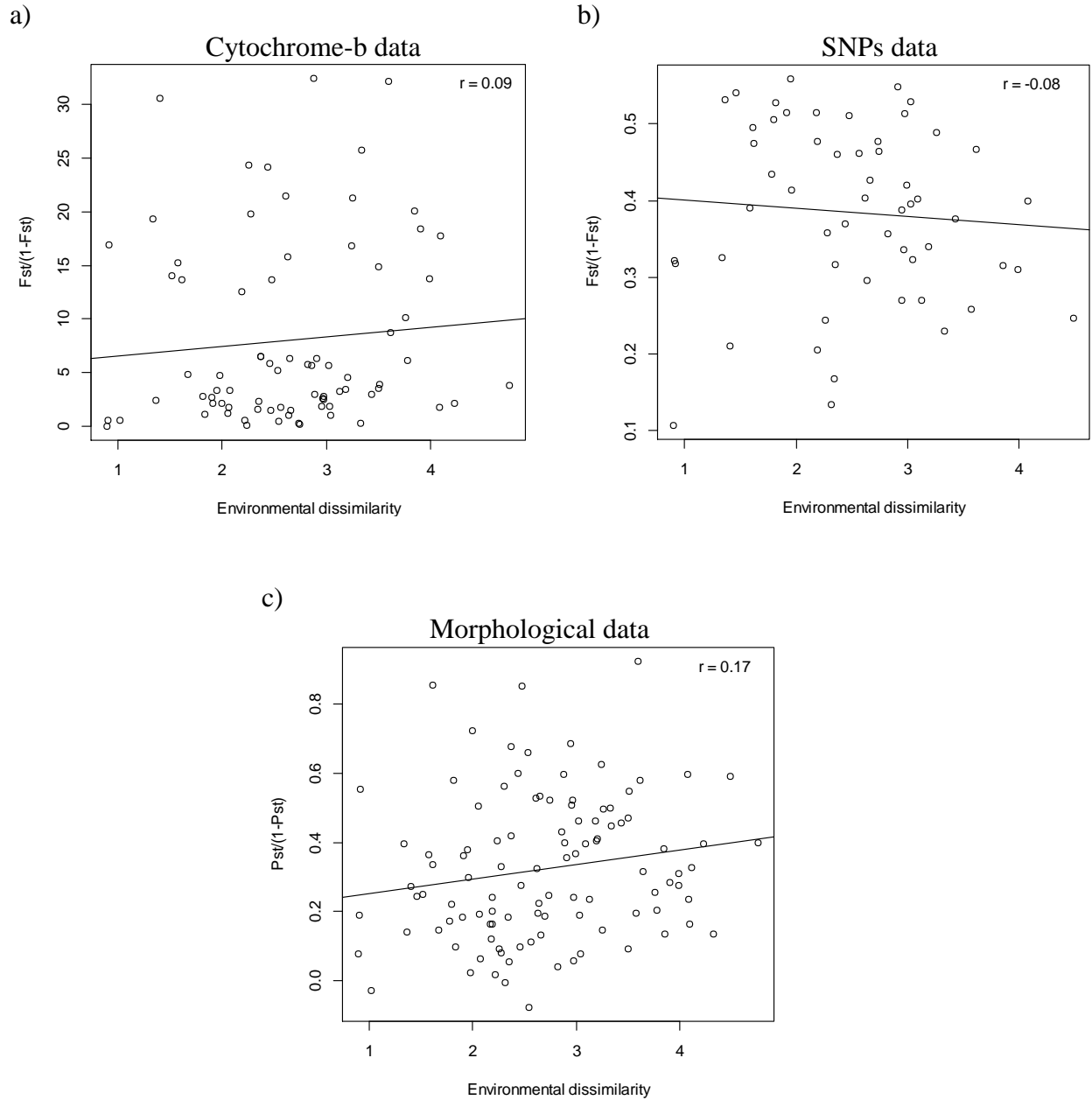


Figure 3.5. Association between environmental dissimilarity and genetic and phenotypic differentiation.

Patterns of genetic (a, b) and phenotypic (c) differentiation according to the Euclidean environmental distance separating populations (see methods for details). Correlation coefficients are given for all analyses; an asterisk next to these coefficients indicates that the association remains significant after accounting for spatial autocorrelation. To facilitate interpretation linear regression lines are shown.

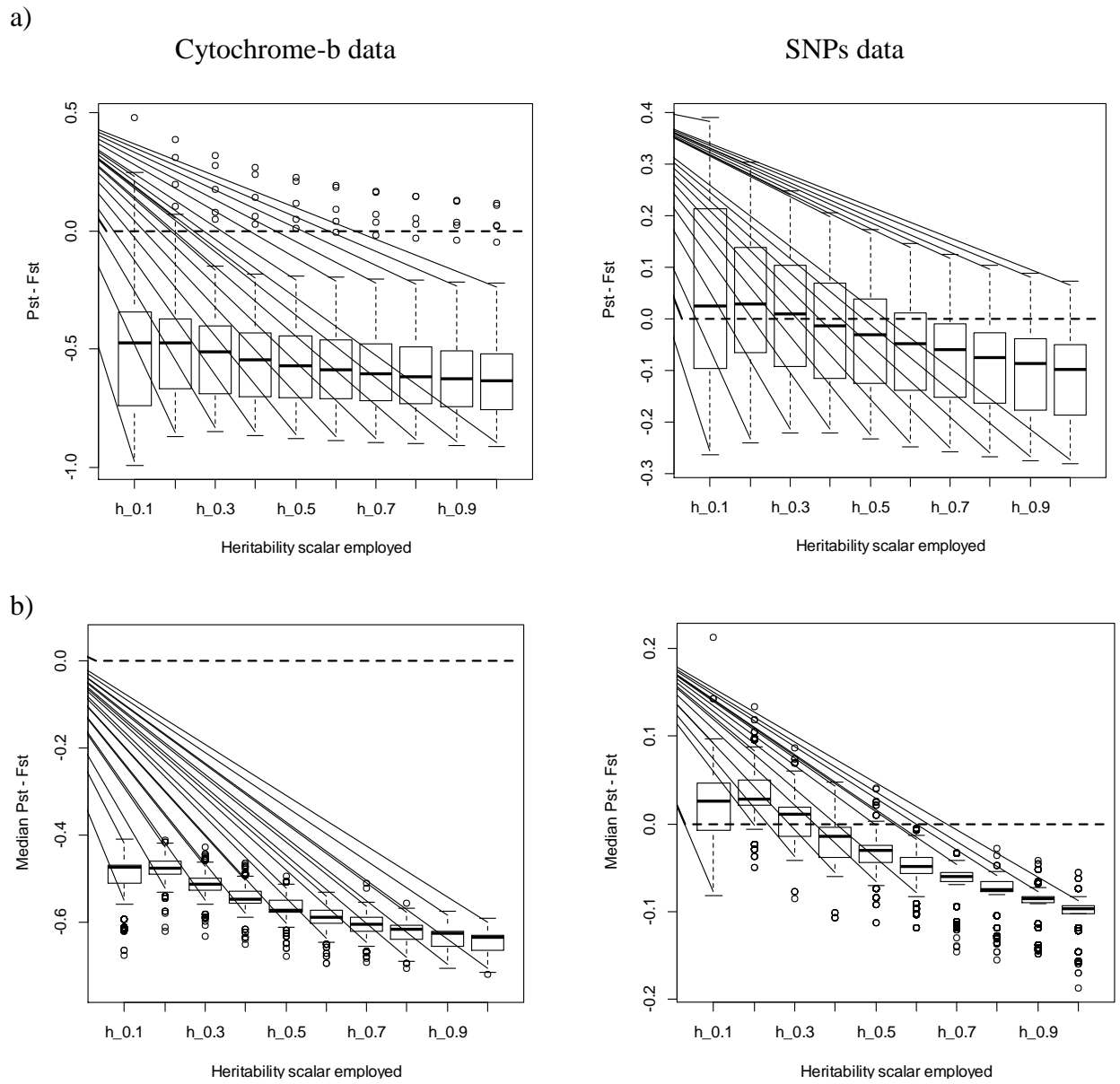


Figure 3.6. Difference between morphological and neutral genetic differentiation. The difference between P_{ST} minus F_{ST} is presented. Plots in the first row (a) show the estimated differences for all populations, whereas plots in the second row (b) show the results of a 1000-replicate bootstrap of the species-wide median difference. Note that irrespective of the heredity scalar used or molecular marker set, no significant positive species-wide difference is recovered.

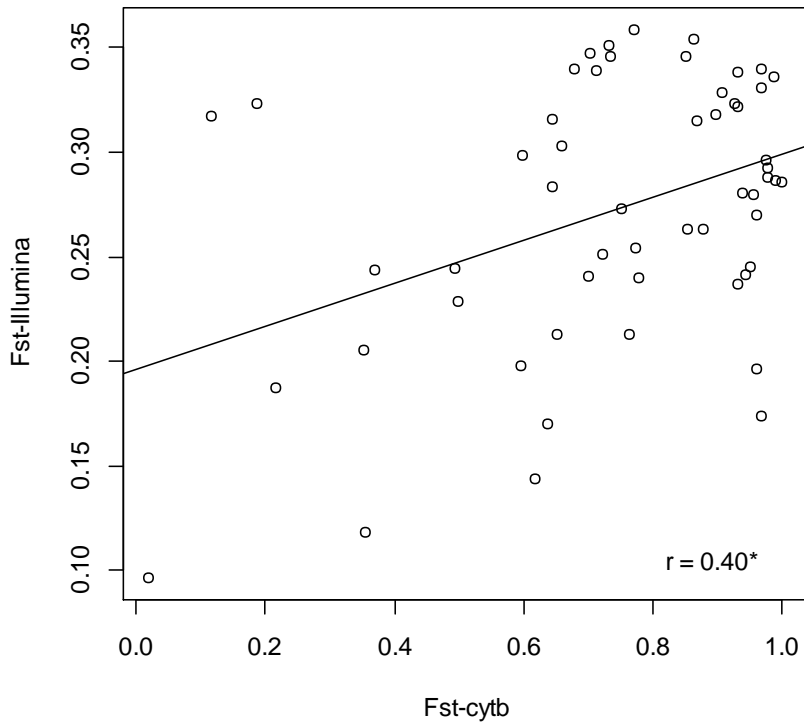


Figure S3.1. Comparison of genetic differentiation based on cytochrome-b and SNP data. F_{ST} values estimated either from cytochrome b sequences or from 1216 genome-wide SNPs are compared. This comparison involves 8 populations across the range of *A. mollis* for which we have both SNP and cytochrome b data.

Table 3.1. Assessment of predicted correlations.

Results of Mantel, partial Mantel, and dbRDA test. Results with and without geographic distance as a covariate (second line in parentheses) are presented. In analyses involving F_{ST} results are provided for both cytochrome-b and SNPs data (left and right of slash, respectively). Correlation coefficients that were identified as significant in mantel and dbRDA analyses are shown in bold. Abbreviations: Env.R.: environmental resistance; Env.D.: environmental dissimilarity distance.

Process	Test	Pearson's r	Mantel p-value	dbRDA p-value
Isolation effect				
Prediction 1a:				
Isolation by resistance (temperature friction)	$F_{ST} - \text{Env.R.}$	0.24 / 0.73 (-0.05 / 0.37)	0.03 / <0.01 (0.99 / 0.01)	0.03 / <0.01 (0.04 / 0.02)
Isolation by resistance (precipitation friction)	$F_{ST} - \text{Env.R.}$	0.26 / 0.73 (-0.04 / 0.35)	0.02 / <0.01 (0.97 / 0.02)	0.03 / <0.01 (0.05 / 0.02)
Isolation by resistance (vegetation friction)	$F_{ST} - \text{Env.R.}$	0.24 / 0.71 (-0.03 / 0.21)	0.04 / <0.01 (0.96 / 0.13)	0.04 / <0.01 (0.06 / 0.14)
Prediction 1b:				
Isolation by resistance (temperature friction)	$P_{ST} - \text{Env.R.}$	0.42 (-0.22)	<0.01 (0.94)	<0.01 (0.12)
Isolation by resistance (precipitation friction)	$P_{ST} - \text{Env.R.}$	0.43 (0.00)	<0.01 (0.50)	<0.01 (0.97)
Isolation by resistance (vegetation friction)	$P_{ST} - \text{Env.R.}$	0.40 (-0.19)	<0.01 (0.98)	<0.01 (0.09)
Divergent selection effect				
Prediction 2a:				
Isolation by adaptation	$F_{ST} - \text{Env.D.}$	0.09 / -0.08 (-0.02 / -0.05)	0.32 / 0.69 (0.54 / 0.625)	0.41 / 0.63 (0.81 / 0.70)
Prediction 2ba:				
Local adaptation	$P_{ST} - \text{Env.D.}$	0.17 (0.09)	0.07 (0.23)	0.07 (0.33)

Table 3.S1. Principal component loadings of size-standardized cranial and mandibular measurements.

The total variance explained by each principal component and the correlation of each of these components with latitude (Pearson's r correlation coefficient and p-value, in parentheses underneath) are provided. Abbreviations: ZB: zygomatic breadth; IB: interorbital breadth; LB: lambdoidal breadth; HBC: height of braincase; BZP: breadth of zygomatic plate; LD: length of diastema; LBP: length of bony palate; BBP: breadth of bony palate; PPL: postpalatal length; LIF: length of incisive foramina; BIF: breadth of incisive foramina; LM: length of maxillary toothrow; BM1: breadth of 1st upper molar; BPB: breadth of palatal bridge; LN: length of nasals; BN: breadth of nasals (BN); BB: breadth of braincase; HI: height of incisor; DI: depth of incisor; BOC: breadth of the occipital condyles; CIL: condylo-incisive length; ZIL: zygomatic internal length, ML: mandibular length; MH: mandibular height; IDL: inferior diastema length; IML: inferior molar toothrow length

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
ZB	.013	.001	.002	.002	-.002	.009	.002
IB	-.015	.022	.001	.010	-.001	.012	.002
LB	.023	.041	.006	.004	-.001	.030	-.011
HBC	-.005	.007	-.002	.003	-.002	.006	.002
BZP	.002	.003	-.005	-.002	-.004	.007	.003
LD	.083	-.005	-.033	.003	-.031	-.015	-.003
LBP	.024	-.014	.006	.002	.011	.000	.006
BBP	.022	.028	-.032	.004	.049	-.008	.007
PPL	.020	.003	.002	.011	-.002	.011	.004
LIF	.017	-.005	-.004	.002	.015	.000	.001
BIF	.002	-.005	.022	.000	-.014	-.006	.002
LM	.006	-.017	.032	.055	.007	-.013	-.005
BM1	-.002	.015	-.002	.004	-.005	.001	.006
BPB	-.002	.035	-.002	.022	-.011	-.008	.039
LN	.032	-.021	.013	.017	.009	.027	-.003
BN	-.003	.000	.005	-.010	.008	.001	-.004
BB	-.006	.006	.000	.004	-.007	.005	.004
HI	.033	.021	.051	-.030	.000	-.006	.014
DI	.013	.052	.019	.003	.004	-.025	-.027
BOC	.000	.002	.002	.004	.002	.006	.002
CIL	.012	-.013	.003	.004	.006	-.009	.001
ZIL	.021	-.006	-.001	.000	.004	-.010	-.001
ML	.013	.007	.002	-.003	.004	.011	.001
MH	.026	.011	.002	.001	-.006	.019	-.004
IDL	.031	-.027	.015	-.016	.019	.000	.015
IML	-.010	.013	-.005	.008	-.005	.003	.013
% Variance explained	21.18	14.54	10.49	8.05	7.03	5.86	4.69
Correlation with latitude	0.58 (<0.001)	-0.38 (<0.001)	-0.08 (0.286)	0.04 (0.605)	0.08 (0.303)	-0.01 (0.865)	0.17 (0.024)

Table 3.S2. Principal component loadings of the environmental PCA.
 Results for the 4 principal components with an eigenvalue greater than 1 in the analysis on 20 environmental variables.

Variable	PC1	PC2	PC3	PC4
Annual Mean Temperature	0.96	0.28	-0.04	-0.08
Mean Diurnal Temperature Range	0.93	-0.25	0.02	-0.03
Isothermality	0.05	-0.43	0.60	-0.62
Temperature Seasonality	0.36	0.27	-0.55	0.66
Maximum Temperature of Warmest Month	0.97	0.22	-0.06	-0.07
Minimum Temperature of Warmest Month	0.88	0.43	-0.04	-0.16
Temperature Annual Range	0.94	-0.19	-0.09	0.10
Mean Temperature of Wettest Quarter	0.97	0.23	-0.04	-0.04
Mean Temperature of Driest Quarter	0.93	0.33	-0.09	-0.13
Mean Temperature of Warmest Quarter	0.96	0.28	-0.06	-0.05
Mean Temperature of Coldest Quarter	0.96	0.27	-0.01	-0.12
Annual Precipitation	-0.44	0.86	0.24	-0.02
Precipitation of Wettest Month	0.34	0.68	0.58	0.18
Precipitation of Driest Month	-0.49	0.79	-0.23	-0.18
Precipitation Seasonality	0.73	-0.44	0.35	0.29
Precipitation of Wettest Quarter	0.05	0.74	0.64	0.05
Precipitation of Driest Quarter	-0.56	0.78	-0.20	-0.10
Precipitation of Warmest Quarter	-0.21	0.12	0.58	0.69
Precipitation of Coldest Quarter	-0.35	0.90	-0.18	-0.13
Vegetation type	-0.12	0.31	0.07	-0.01
% Variance explained	50.94	26.10	10.89	8.19

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CHAPTER 4: Investigating the degree of climatic niche differentiation in a tropical generalist inhabiting an environmentally heterogeneous region

ABSTRACT

Interest in the degree of specialization of populations' climatic tolerances has increased recently in light of the potential drastic effects of past climatic cycles and future climate change on species and ecosystems. Because the degree of intraspecific ecological niche differentiation has important consequences for how species respond to climate change, evaluating the extent of intraspecific climatic specialization is fundamental for assessing the likelihood of climatic-induced extinction or divergence. Yet, the extent of intraspecific differentiation in the climatic niche of the vast majority of species in the tropics, a region that holds most of Earth's diversity and that is considered to be highly vulnerable to climate change, has rarely been assessed; limiting our ability to evaluate how individual species' ecologies and geographic setting condition their response to climatic events. Here, we investigate the degree of intraspecific climatic differentiation in the soft-grass mouse (*Akodon mollis*) using a combination of complementary analyses that make use of genetic and ecological comparisons and ecological niche models. Our results identify limited intraspecific differentiation of the climatic niche of *A. mollis* in spite of the broad latitudinal and altitudinal range and limited vagility of this species, and the marked environmental differences among populations' habitats. This evidence of limited differentiation suggests that population divergence in this species may respond to processes other than ecological specialization across environmental gradients. With the aim of identifying venues

for future research that could directly test this possibility, the relative likelihood of four alternative explanations that could account for this pattern is discussed.

INTRODUCTION

The degree of intraspecific differentiation of physiological tolerances has important consequences for understanding several fundamental processes including the prevalence of alternative speciation processes (McCormack et al. 2010), the maintenance of species' geographic limits (Angert 2009), and the likelihood of species persistence (Jump et al. 2006). Stagnant climatic tolerances, for example, have been linked to allopatric divergence (Kozak and Wiens 2007), the inability of species to colonize new environments (Stephens and Wiens 2009), and higher extinction risk (Soto et al. 2010). Climatic specialization, on the other hand, has been suggested to be an important force facilitating divergence (Beukema et al. 2010). Hence, investigating the degree of differentiation of the climatic tolerances among populations of species experiencing disparate environments through their ranges is of great importance to elucidate the evolutionary consequences of climatic changes. In particular, in face of ongoing climate warming, improving our understanding of the extent of intraspecific climatic niche differentiation has become a fundamental concern (Moritz et al. 2000, Moritz et al. 2012).

Most analyses on the specialization of climatic tolerances to-date have been focused on temperate ectotherms (Ghalambor et al. 2006), limiting our ability to thoroughly evaluate the importance of climatic adaptation or lack thereof in promoting diversification in tropical ecosystems. For example, testing the hypothesis that stagnant climatic tolerances across species' ranges in conjunction with the more stable climate of the tropics increase the opportunity for

allopatric speciation in this region (Janzen 1967, Cadena et al. 2012) requires further understanding of the degree of intraspecific climatic specialization along altitudinal gradients in tropical mountains. Likewise, our ability to properly investigate the effect of past climatic fluctuations (Alvarado-Serrano and Knowles In prep) and to predict future species responses in these biodiversity hotspots is also currently hindered by the relative paucity of data.

Here, with the goal of contributing to the ongoing debate about the role of environmental specialization in promoting the accumulation of biodiversity in tropical regions, we take advantage of recent analytical developments to investigate patterns of climatic niche evolution in a tropical generalist species, the soft grass mouse (*Akodon mollis*). This species represents a great model system to study the likelihood of climatic niche differentiation in tropical species given its limited dispersal abilities and broad latitudinal and altitudinal distribution in the highly diverse tropical Andes (Barnett 1999, Alvarado-Serrano 2005). Thanks to the high spatial heterogeneity and low seasonality of this region, this mouse faces multiple potential dispersal barriers and a broad range of environmental conditions as a species, with individual populations facing disparate environmental conditions (Chapter 2). Hence, this mouse is a good candidate for experiencing increased opportunities for both allopatric differentiation in isolated populations, especially in association with climate-induced refugial distributions (Casner and Pycrz 2010, Alvarado-Serrano and Knowles In prep), and parapatric differentiation in response to steep environmental gradients (Endler 1982, Schneider et al. 1999), the likelihood of these two processes being conditioned by the strength of evolutionary constraints on its climatic preferences (Wiens and Graham 2005, Crisp and Cook 2012).

Specifically, we address the question of whether differentiation in the climatic tolerances of populations across the range of *A. mollis* is limited, as predicted under the hypothesis that phylogenetic constraints promotes diversification in the tropics due to allopatry around stable climatic barriers (Cadena et al. 2012), or whether it shows evidence of climatic specialization, as predicted under the hypothesis of diversification along ecological gradients (Endler 1977, Schneider et al. 1999). To address this question, we combined genetic and ecological analyses, and take advantage of the information ecological niche models (ENMs) can generate about species' abiotic preferences and tolerances (Peterson et al. 2011). It is important to note that because species climatic niches are complex and multidimensional (Emery et al. 2012), our characterization of environmental variation is only a proxy for the true climatic tolerances and preferences (Peterson et al. 2011). Nevertheless, our approach should capture, however imperfectly, the degree of intraspecific differentiation of the climatic niche. It is also important to note that we focus on intra-specific tolerances, as inter-specific analyses may confound differentiation that originated during speciation with differentiation accumulated afterwards (McCormack et al. 2010). By exploring the degree of intra-specific niche evolution in *A. mollis*, this study offers new insights into how the interaction between ecological opportunity and evolutionary constraints plays out in the likelihood of climatic niche differentiation in a tropical species.

METHODS

Sampling and data generation

To assess the extent of climatic niche differentiation in *Akodon mollis*, we compiled a sample of 260 individuals from 46 localities distributed along the entire geographic range of this

species (Appendix 4.1). The sample included individuals collected for this study as well as tissues that other researchers graciously shared with us and sequences obtained from Alvarado-Serrano et al. (2013). All collecting was done in accordance with procedures established by the American Society of Mammalogists (Sikes et al. 2011) and University of Michigan's Committee on Use and Care of Animals. We used this sample to quantify intra-specific genetic variability in this species and to characterize its fundamental realized climatic niche (*sensu* Peterson et al. 2011).

The quantification of intra-specific genetic variability relied on two complementary datasets derived from total genomic DNA that we extracted from liver or muscle tissues using a Qiagen's extraction kit under the manufacturer's protocol. The first, more extensive, dataset corresponded to mitochondrial DNA and was obtained by amplifying the first 800bp of the cytochrome b gene (*cyt-b* hereafter) with primers MVZ05 and MVZ16 (Smith and Patton 1993) using the protocol outlined by D'Elía (2003). The generated fragments were purified with a purification kit (QIAGEN) and sequenced at University of Michigan's Sequencing Core Facility. Resulting sequences were cleaned in Sequencher v4.6 (Gene Codes 2006) and aligned with Clustal W (Thompson et al. 1994) in MEGA v5.1 (Tamura et al. 2011) using the default values for all alignment parameters. The second dataset corresponded to a genome-wide panel of anonymous single nucleotide polymorphisms (SNPs) and was derived from a subsample of 96 individuals from 12 populations (*i.e.*, 8 individuals per population). The number of individuals per population and/or the quality of their DNA constrained our ability to include all populations in the Illumina library (details in Alvarado-Serrano and Knowles In prep). Briefly, a restriction-site-associated DNA (RAD) reduced representation library of pair-ended fragments was created

for each of the 96 individuals using the protocol outlined by Parchman et al. (2012). The pooled library, which included per-individual barcoded fragments, was then sequenced in an Illumina Genome Analyzer IIx at University of Michigan DNA Core Sequencing Facility. After demultiplexing (i.e., grouping sequence fragments according to the individual barcodes), we filtered out poor quality data using custom-made python scripts. The vetted sequences were then used to recover a panel of 1216 genome-wide SNPs for all 12 populations using the program STACKs v0.99993 (Catchen et al. 2011). This panel included only SNPs that were present in at least 5 of the 8 individuals per population and in at least 8 of the 12 populations sequenced. Finally, using Arlequin v3.5 (Excoffier et al. 2005) and STACKs v0.99993 (Catchen et al. 2011) we estimated pairwise F_{ST} based on the pairwise nucleotide differences per population for the mitochondrial and genomic datasets, respectively.

The characterization of the existing fundamental climatic niche (sensu Peterson et al. 2011) of *A. mollis* was done by associating the 46 sampling localities with their environmental conditions. To do this, we first established the precise geographic location of all localities by extracting collection coordinates when available from associated field catalogs or by georeferencing using a point-radius method (Wieczorek et al. 2004), with a maximum locality uncertainty radius tolerance of 4 km (probably a negligible distance given the resolution of our climatic data, 1 km², and their strong spatial autocorrelation; cf. Anderson and Raza 2010). We then associated each locality with its climatic conditions determined based on 19 bioclimatic variables (Hijmans et al. 2005) that have been shown to be biologically relevant in several previous studies (e.g., McCormack et al. 2010, Glor and Warren 2011). This was done using the

Extraction Tool in ArcGIS v9.3 (ESRI 2009). In addition, we identified the ecoregion in which each locality was located (ecoregions definition followed Olson et al. 2001).

Phylogeographic analyses

In contrast to the characterization of genetic differentiation (see below), the phylogeographic characterization relied exclusively on the cytochrome-b data, and hence, should be considered exploratory. After removing duplicate haplotypes, maximum likelihood (ML, Felsenstein 1981) and Bayesian inference (BI; Rannala and Yang 1996) analyses were carried out on the alignment matrix of cyt-b sequences, with missing characters (corresponding to less than 1% of the matrix) treated as unknowns. Sequences of the type species of the genus (*A. boliviensis*) and 3 additional species that belong to the same infra-generic phylogenetic clade as *A. mollis* (the aerosus group; Smith and Patton 2007), *A. aerosus*, *A. orophilus* and *A. torques*, were included as outgroups. The best fitting model of nucleotide substitution (GTR+I+G) for this matrix was selected with JModeltest (Posada 2008) based on the Akaike Information Criteria (AIC). The ML analysis was carried out in RAxML v7.2.8 (Stamatakis 2006) with the data partitioned by codon. First, 100 rapid bootstraps (Stamatakis et al. 2008) were run, followed by a final maximum likelihood search using 10 random bootstrap topologies as starting trees. The BI analysis was run in MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001) with site-specific rates for each of the 3 codon positions using two runs of four simultaneous Markov chains (1 cold, 3 heated, temperature = 0.20) each. This analysis was run for 50 million generations to assure convergence (i.e., average standard deviation of split frequencies ≤ 0.0001). The convergence of log-likelihood values was verified in Tracer v1.5 (Rambaut and Drummond 2009). All model parameters were left to be estimated by MrBayes using uniform interval priors for all of them

except base composition and GTR parameters, which followed a Dirichlet process prior. Trees were sampled every 1000 generations. Posterior probability estimates were based on the last 90% of the samples (the first 10% were discarded as burn-in). For both, ML and BI analyses, a 50% majority rule tree was computed. Topology support was estimated in the ML analysis by bootstrap resampling over 1000 replicates and by posterior nodal support in the BI analysis.

In addition, using the *cyt-b* data, we estimated the time to the most recent common ancestor (MRCA) across the phylogeny in BEAST v1.7.5 (Drummond and Rambaut 2007). We relied exclusively on the *cyt-b* data for this analysis because in contrast to SNPs, this mitochondrial marker has well-defined models of nucleotide substitutions, which are needed for this analysis. The estimation was performed using a log-normal relaxed molecular clock and a constant-population-size coalescent model. Because no fossil calibrations exist for our focus group (Pardiñas et al. 2002) – the oldest confirmed record of this or a presumably closely related species comes from the early Holocene (Fejfar et al. 1993) –, we let BEAST jointly estimate mutation rates and divergence times. For this estimation we used a normally distributed mutation rate prior (2.3 ± 0.5 substitutions per site per million years) based on a range of published estimates of MTDNA mutation rates for rodents and centered around a previous estimate of *cyt-b* mutation rate specific for Akodontini (Smith and Patton 1993). The substitution model used in this analysis was the same previously selected for this dataset (i.e., GTR+I+G). Three runs of 1×10^8 generations each were performed, with samples taken every 1×10^5 generations. Convergence to stable values (i.e., effective sample size > 200) was assessed in Tracer v1.5 (Rambaut and Drummond 2009). All three runs were then combined, discarding the first 10% of each run as burn-in, using LogCombiner v1.75 (Drummond et al. 2012). The combined file was

then used to generate a maximum clade credibility tree annotated with the median node ages using TreeAnnotator (Drummond et al. 2012).

Climatic niche differentiation analyses

First, we characterized the extent of overlap in the climatic preferences among populations and assessed if populations located in different ecoregions, which are a proxy for environmentally distinct areas (Olson et al. 2001), show significant climatic differences from each other. To do this, we performed a principal component analysis (PCA) on the correlation matrix of the standardized and \log_e -transformed climatic data of all 46 populations. We chose to \log_e -transform the data to improve normality. To control for spatial autocorrelation, we independently regressed the scores of all principal components with an eigenvalue greater than 1 (Kaiser's rule; Sokal and Rohlf 2003) onto the localities' latitudes and longitudes (McCormack et al. 2010) and used the regression residuals in the following analysis. We assessed differences among populations from different ecoregions by performing a multivariate analysis of variance (MANOVA) on the residuals from these regressions, with ecoregions as fixed factor. Significance in the ANOVAs was assessed by resampling using 10000 replicates. In addition, we assessed the correlation between populations' pairwise genetic differentiation, quantified by F_{ST} , and differentiation in their climatic environments, quantified by the pairwise Euclidean distance between all populations based on all PCs retained (i.e., those with an eigenvalue greater than 1). To do this, we ran partial Mantel tests (Mantel 1967) and distance-based redundancy analyses (dbRDA; Anderson and Legendre 1999, McArdle and Anderson 2001) on the original principal components (i.e., not the residuals) using both the mitochondrial and genomic F_{ST} estimates. Because of a significant pattern of isolation by distance for both datasets (p-value < 0.05 in

mitochondrial and genomic DNA analyses), we used the geographic distance separating populations as a covariate in these analyses.

We also analyzed the similarity between the climatic niches of populations from different ecoregions (Fig. 4.1) by contrasting ENMs independently generated for each group of samples. This later method has the advantage over the previous approach of considering the background environmental differences (resulting from differences in the location of populations) when comparing how different are the niches of two populations (McCormack et al. 2010). In these analyses, two ecoregions could not be included because of their small sample size, leaving 5 of the 7 ecoregions represented in the sample available for comparison. To minimize the effects of sampling bias (Hortal et al. 2008) we removed duplicate localities (i.e., those that fall within the same grid cell in our climatic data) using ENMTools (Warren et al. 2010). This reduced locality set was then partitioned by ecoregion and input together with the 19 bioclimatic variables into Maxent v3.3.3 (Phillips et al. 2006). Different extents were used for each ecoregion group as recommended by Anderson and Raza (2010). To select optimal running parameters we first ran sensitivity analyses for the number and type of features, the regularization parameter, and the set of climatic variables to retain. Based on the result of these sensitivity analyses (not shown) we chose to run our final models using linear and quadratic features, default regularization values, and to keep all 19 bioclimatic variables. Each final model was based on 10 bootstrap replicates, in each of which data were randomly divided into training and testing points. Model accuracy was evaluated on the test dataset for each replicate using two threshold-dependent, overall accuracy (Pearson 2010) and True Skill Statistic (TSS; Allouche et al. 2006), and two threshold-independent statistics, Area Under the Curve (AUC; Phillips and Dudik 2008) and partial

Receiver Operator Curve (pROC; Peterson et al. 2007). Using the ecoregion-specific ENMs, averaged out of the 10 replicates, we evaluated the extent of pairwise between-ecoregion group niche identity and overlap. This was done by calculating Schoener's D (Schoener 1968) and Warren's *I* and relative rank (RR) (Warren et al. 2008) statistics in ENMTools (Warren et al. 2010). In addition, to verify robustness of the results we used an alternative approach that quantifies niche divergence (McCormack et al. 2010) using a custom-created R script. Significance in all three tests was obtained over 1000 replicates.

RESULTS

Phylogeographic structure

Both the ML and Bayesian analyses on the cyt-b data consistently identify 5 well-supported, geographically structured clades (bootstrap support ≥ 98 , posterior probability ≥ 0.69) (Fig. 4.1). The only differences between ML and BI analyses lie in the degree of resolution of the terminal taxa and the relationships between clades. Specifically, a fairly well-supported nested pattern of relationships is observable among clades in the ML tree, whereas in the Bayesian tree the same clades form a basal polytomy. The maximum credibility tree in the divergence time analysis also showed the same 5 well-supported clades (Fig. 4.2). The estimated date of occurrence for the most recent common ancestor (MRCA) of *A. mollis* in this analysis was between 0.62 and 1.22 million years ago (95% highest posterior densities), an estimate that is in line with a recently proposed divergence time between *Akodon* and its closest relative *Deltamys* of approximately 2 to 3.2 million years ago (Parada et al. 2013).

Climatic niche differentiation

The three complementary approaches used to evaluate the extent of specialization of the climatic niche among *A. mollis* populations result in a similar pattern of lack of differentiation. First, after controlling for spatial autocorrelation, extensive overlap among populations is evident in scatterplots of all 4 PCs' with an eigenvalue greater than 1 (Fig. 4.3), which together accounted for almost 95% of the climatic niche variance within *A. mollis*. In agreement with this observation, the MANOVA identifies no significant differences in these components between ecoregion-based population groups (Wilks' $\lambda = 0.99$, $F_{4/40} = 0.11$, $p = 0.98$). Second, the correlation between pairwise Euclidean distances in these 4 residual PCs (see Methods) and pairwise genetic distances is no significant. This finding is robust to whether mitochondrial (correlation coefficient = 0.14, Mantel's p -value = 0.19, dbRDA's p -value = 0.10) or genomic data (correlation coefficient = 0.22, Mantel's p -value = 0.09, dbRDA's p -value = 0.13) are used to estimate F_{ST} (Fig. 4.4).

Similar results are found in the ENMs analyses. A comparison between the ENMs generated for each ecoregion-based population group shows limited niche divergence, irrespective of the statistic (D , I , or RR) or test (niche overlap or niche divergence) used (Table 4.1). There is evidence of significant niche divergence only in two comparisons and in the niche divergence analysis (McCormack et al. 2010), but not in the niche overlap analysis. It is important to note that although the accuracy of the ENMs constructed for each clade varied, all of them performed noticeable better than random, regardless of the statistic used to measure accuracy (not shown).

DISCUSSION

Analyses of climatic preferences based on current species' distributions have proven valuable for understanding the evolution of species' physiological tolerances (e.g., McCormack et al. 2010, Smith and Donoghue 2010). The majority of studies on this topic have focused in comparisons among species (Wiens et al. 2010); in contrast, our study integrates focuses on the degree of climatic specialization of populations along steep environmental gradients in a generalist with a broad latitudinal and altitudinal distribution in the tropical Andes. The suit of complementary analyses performed agrees on finding an overall lack of climatic niche differentiation in this species in spite of marked population structure in mitochondrial and genomic data (Fig. 4.4). Not only is there limited climatic structure across the entire distribution of *A. mollis*, once the effect of spatial autocorrelation is removed (Fig. 4.3), but the climatic niches of populations from different ecoregions predominantly show evidence of conservatism (i.e., populations from different ecoregions tend to inhabit regions climatically more similar to each other than their geographic backgrounds; Table 4.1). In agreement with this result, there is no significant association between genetic and climatic niche differentiation despite significant population structure.

Why climatic niche differentiation seems to be limited in this species, whereas in other mountain taxa (e.g., Evans et al. 2009, Schnitzler et al. 2012) including some distributed in the northern Andes (e.g., Graham et al. 2004), it remains to be explained. One alternative is that the resolution of our environmental variables fails to capture relevant aspects of the niche of *A. mollis*. Being a small terrestrial organism, microclimatic conditions may be more important for this species than the mesoscale conditions captured by our climatic variables (Peterson et al.

2011). Although it is important to note that previous studies on small terrestrial organisms (e.g., Kozak and Wiens 2010, Kalkvik et al. 2012, Wooten and Gibbs 2012) have found evidence of niche divergence using data at similar spatial resolutions, this possibility could not be ruled out. It is possible that divergence may become apparent at smaller spatial scales; yet, such finding seems unlikely given the broad environmental tolerance and lack of morphological specialization of this mouse (Alvarado-Serrano et al. 2013). Still, until studies at different scales become available, we limit our discussion to mesoclimatic niche differentiation in recognition that any conclusion about niche differentiation is scale-dependent (Losos 2008a).

Four alternative possible non-mutually exclusive explanations for the limited niche differentiation uncovered include: (1) ecological mismatch associated with insufficient time for specialization (Bennett 1997, Ackerly 2003), (2) ineffective contrasting directional selection among genetically cohesive populations (Futuyma 2010), (3) stabilizing selection on ancestral tolerances (Ackerly 2003), and (4) maintenance of wide tolerances in response to spatial and/or temporal environmental variability (Futuyma and Moreno 1988). Below we discuss each of these possibilities in light of our results and speculate on their likelihood in *A. mollis*. We then discuss the implications of our findings to the understanding of diversification in the northern Andes.

Alternative 1: Insufficient time for specialization

Under this alternative, the lack of intra-specific climatic specialization is caused by populations not being in equilibrium with their local environments. This scenario proposes that while ongoing climatic differentiation may indeed have started in response to differences in local environments, the time since populations live in their current environments has been insufficient

relative to rates of climatic niche evolution for differentiation to have become apparent (Crisp and Cook 2012). This explanation is based on the assumption that climatic preferences may change with difficulty due to severe physiological constraints (West et al. 2002), genostasis, complex genetic architecture, or developmental constraints on associated traits (Smith et al. 1985, Bradshaw 1991, Quesada et al. 2002, Futuyma 2010). Biotic limitations have also been proposed as probable evolutionary constraints on niches (Ricklefs 2010, Levy et al. 2012). In line with this hypothesis, there is accumulating evidence of species tracking habitats in response to climate change (Myers et al. 2005, Sommer and Zachos 2009). Yet, whether the time since the MRCA of *A. mollis* (estimated in our analysis to be between 0.62 and 1.22 Mya) has been too short for their climatic niche to have diverged is not known. Although evidence of rapid shifts of climatic preferences in similar or even shorter time spans exist (e.g., Dormann et al. 2010, Wooten and Gibbs 2012), with the most striking examples coming from species invasions (e.g., Broennimann et al. 2007), the likelihood of niche differentiation is likely species- and context-specific.

Alternatively, it could be that repeated cycles of range contraction and expansion have prevented the accumulation of differences in the climatic preferences of populations of *A. mollis*. Distributional shifts associated with Pleistocene glaciations in the tropical Andes, which resulted in snowline depressions of up to 1350m in some regions (Klein et al. 1999, Smith et al. 2005) and marked vegetation down-slope shifts, regardless of prevailing temperatures (Mora et al. 2002, Marchant et al. 2009), might have prevented populations from becoming locally adapted to their current environment (Bennett 1997). In this context, three consequences of climatic changes could have been important in limiting the effectiveness of selection for climatic differentiation:

(1) continuous fluctuations in the selective environment (Futuyma and Moreno 1988, Donaldson-Matasci et al. 2008), (2) effective population size fluctuations (Dempster and Lerner 1954), or (3) dilution of genetic variation if climatic-induced distributional shifts have facilitated population mixing in this montane species (Alvarado-Serrano and Knowles In prep). Testing these possibilities requires further analyses of the extent of past distributional shifts and their effect on populations before any solid conclusion can be attained. However, in light of present evidence, it seems that insufficient time might contribute to explain the lack of climatic differentiation in *A. mollis*.

Alternative 2: Ineffective contrasting directional selection

Local climatic specialization in species inhabiting heterogeneous environments could also be prevented by gene flow (Angilletta 2009). Under this scenario, spatially separate populations may be experiencing independent directional selection for different climatic optima; however, the effectiveness of selection is reduced by high migration between populations (Brown et al. 2001, Futuyma 2010). Evidence of such a constraining effect of gene flow on adaptive divergence in the absence of geographical barriers to dispersal is well documented (Räsänen and Hendry 2008 and references therein). Nonetheless, the high spatial heterogeneity and habitat patchiness of the northern Andes likely offers strong dispersal barriers for *A. mollis*, as evidenced by the strong pattern of environmentally driven isolation that characterizes this species (Chapter 3). Furthermore, the possibility of high levels of gene flow across the entire range of *A. mollis* is contradicted by the relatively high F_{ST} estimates recovered for both mitochondrial and genomic DNA (average $F_{ST} = 0.81$ and 0.28 for the *cyt-b* and SNP datasets,

respectively), making the hypothesis of ineffective directional selection due to gene flow unlikely.

Yet, given the finding that the major axis of genetic differentiation recovered corresponds to latitude, not altitude (Fig. 4.1), it is possible that gene flow across elevations might still prevent differentiation of the climatic niche in *A. mollis*. Because the major axis of environmental variation in the tropics corresponds to elevation (Mani 1968, Sarmiento 1986), populations latitudinally isolated from each other may experience similar selective regimes if they occur at similar elevations. In contrast, populations at similar latitudes but different elevations probably experience significantly different environments. Thus, limited isolation across elevations could result in ineffective selection. Nevertheless, average F_{ST} estimates among populations at similar latitudes but different elevations seem to contradict this possibility, although the limited sample prevents us from formally testing this possibility. Relatively high degree of inter-population genetic differentiation is present both across elevations and latitudes. Hence, ineffective contrasting selection seems an unlikely explanation for the lack of climatic niche differentiation across elevations and ecoregions (Fig. 4.3, Table 4.1), despite the marked climatic stratification of the northern Andes (Kozak and Wiens 2007, Cadena et al. 2012).

Alternative 3: Stabilizing selection

Stabilizing selection in response to biotic limits to habitat expansion has also been proposed as a force that may constrain climatic tolerances and other traits associated with the fit of organisms to their environment (Ackerly 2003, Pearman et al. 2008, Crisp and Cook 2012). Specifically, this hypothesis proposes that antagonistic biotic interactions, such as competition or

predation, not only prevent individuals from expanding to new environments, but also favor stabilizing selection on current tolerances. Under this scenario, which is an extension of the “jack of all trades master of none” argument (Levins 1968, MacArthur 1972), individuals that deviate from the selective optimum experience reduced fitness due to reduced competitive advantage (Cavender-Bares et al. 2009, Ricklefs 2010). In this context, it is the fitness cost of crossing between fitness peaks (Wright 1931) what prevents climatic niche differentiation. To date, however, there are few definite examples of differentiation constraints being likely caused by stabilizing selection (Leroi et al. 1994, Crisp and Cook 2012). Although this paucity of evidence may be partially associated with the difficulty of uncovering stabilizing selection among populations (Futuyma 1998, Ackerly 2003), it seems to suggest that the prevalence of this alternative is limited.

Whether biotic limitations causes stabilizing selection on current climatic preferences across the entire range of *A. mollis* is debatable. Although little is known about the natural history and ecological interactions of this species, the biotic communities with which *A. mollis* coexists most likely vary across its range, especially across elevations. Contrary to *A. mollis*, the majority of tropical species have a limited altitudinal range (Ruggiero et al. 1998). In particular, among small terrestrial mammals, which are the most likely competitors for *A. mollis*, there are few examples of species with broad elevation ranges (Pearson and Ralph 1978, Patterson et al. 2006). Additional indirect evidence against this possibility comes from the marked differences in the climatic conditions and presumably selective environments available for different populations of this species, which makes a single climatic fitness optimum upon which stabilizing selection can act unlikely – stabilizing selection is theoretically more efficient when

selecting for a single optimum (Travis 1989, Futuyma 1998). In addition, the high abundance of *A. mollis* and its numerical dominance among co-distributed terrestrial small mammals, and conspicuous habitat disturbance tolerance (Barnett 1999, Voss 2003, Alvarado-Serrano 2005), brings into question the hypothesis that biotic interactions alone may be the major force constraining its climatic niche. Nonetheless, definitively falsifying this hypothesis is not possible while the ecology of this species remains poorly known.

Alternative 4: Maintenance of wide tolerances

Another possible explanation for the lack of evidence of differentiation is that the climatic tolerances of individual populations are broad and overlap with that of the other populations. This alternative suggests that the maintenance of a spatially undifferentiated climatic niche is caused by selection for wide tolerances within individual populations in response to broad local spatial and/or temporal climatic variability. Under this scenario, populations facing constant climatic fluctuations or disparate climatic conditions within their dispersal capabilities would be at an evolutionary disadvantage if they became specialized as their overall fitness would be negatively impacted by local or temporal mismatches (Futuyma and Moreno 1988). Under such climatic circumstances organisms' response might involve sacrificing local and/or temporal fitness in favor of optimizing long-term fitness (i.e., selection for ecological generalists, Brown and Pavlovic 1992, Olofsson et al. 2009). Such a strategy would be particularly prone to evolve if there are not strong tradeoffs between being a climatic generalist and performance at local conditions (i.e., contrary to an “jack of all trades master of none” argument; Levins 1968; see above, MacArthur 1972); see above). Support for this later possibility has been compiled, at least, for thermal tolerances (Huey and Hertz 1984, Angilletta

2009 and references therein). A widely recognized example of such strategy is the proposed lack of climatic specialization in temperate species linked to year-around survival in these highly seasonal regions (Janzen 1967, Kozak and Wiens 2007). In a similar manner, the striking amplitude of daily thermal fluctuations in tropical mountains might represent an analogous strong selective force for broad thermal tolerances, especially considering that tropical montane individuals are less likely to mitigate daily fluctuations by migration or hibernation as their temperate counterparts do across seasons (Ghalambor et al. 2006). Alternatively, spatial climatic heterogeneity may in a similar manner promote the evolution of broad tolerances as organisms may maximize their fitness across environments by remaining tolerant to conditions in all environments they encounter on a regular basis (Brown and Pavlovic 1992, Baythavong 2011).

Considering the fluctuating, highly stratified, and heterogeneous climatic environment of the northern Andes (Sarmiento 1986, Sierra 1999), it seems plausible that selection for wide climatic tolerances might contribute to the lack of population differentiation in regards to their climatic tolerances. Yet, without detailed physiological analyses that directly explore the tolerance breadth of individuals from different populations as well as their ability to thrive in different climatic regimes, this hypothesis cannot be formally tested. Ideally, reciprocal transplant or common-garden experiments such as those performed on thistles (Becker et al. 2006) and lizards (Huey and Hertz 1984), respectively, would be needed to assess individuals' performance across the range of climatic conditions encompassed by the range of *A. mollis*. Alternatively, given the extreme difficulty of performing these type of experiments in this species, physiological models such as those implemented by Levy et al. (2012) could be used to explore the breadth of individuals' climatic tolerances (if this hypothesis were to be supported,

individuals from different populations should have comparable broad tolerances irrespective of their local environmental conditions). For now this possibility remains speculative.

Conclusions

In contrast to some previous studies that have found support for accelerated rates of niche divergence across species in tropical regions (Graham et al. 2004, Kozak and Wiens 2007), this study finds limited climatic niche differentiation among populations of a broadly distributed tropical species across environmentally dissimilar ecoregions, adding to the mounting evidence that indicates that climatic tolerances of tropical montane taxa are often conserved (Anciaes and Peterson 2009, Cadena et al. 2012). Whether these contrasting findings between studies, supporting or not supporting gradient specialization, reflect species-specific ecologies and geographic context (Cooper et al. 2011), as opposed to methodological differences among studies (Losos 2008b, Warren et al. 2008, Wiens 2008), remains an open question. Resolving this question requires an exploration of the possible mechanisms governing the degree of ecological differentiation in multiple species with contrasting ecologies. For now, our results on *A. mollis*, a relatively young species with an unusually broad latitudinal and altitudinal range compared to other tropical Andean species suggest limited ecological specialization across steep environmental gradients (see also Alvarado-Serrano et al. 2013). This finding further supports previous studies (Patton and Smith 1992, Casner and Pyrcz 2010) that challenge the hypothesis that diversifying selection along ecological gradients (Endler 1977, Schneider et al. 1999) is a common diversification force in the tropical Andes. It is important to recognize, however, that our results point to region- and species-specific characteristics being an important determinant of the likelihood of climatic niche differentiation. In particular in the case of *A. mollis*, it seems

plausible, although speculative at this point, that limited opportunity for differentiation caused by the continuous distributional shifts this species have faced since its origination (alternative 1 above) together with selection for a generalist strategy (alternative 4 above) may account for its spatially undifferentiated climatic niche. Further analyses, however, especially in tropical mountains, before general conclusions can be attained about the relative importance of the mechanisms involved, and the role that climatic divergence or lack thereof plays in early stages of speciation in this region.

Appendix 4.1 List of specimens used in the analyses.

The elevation and geographical coordinates (from georeferencing or collector's notes) of all populations used in the analyses are provided. Abbreviations: ACUNHC: Abilene Christian University, Natural History Collection; MUSM: Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Perú; QCAZ: Museo de Zoología, Sección Vertebrados, Pontificia Universidad Católica del Ecuador, Quito, Ecuador; TTU: Natural Science Research Laboratory, Texas Tech University, Lubbock, Texas. For specimens without a museum catalog number assigned, the collector number (in lowercase) is provided. Population index numbers are provided in square brackets, with those populations included in the Illumina library shown in bold (note that populations [14], [15], and [16] were pooled for purpose of this library given their geographic proximity).

ECUADOR:

AZUAY: **[1]** Parque Nacional El Cajas, Laguna La Toreadora: 3810m, -2.78, -79.22 (QCAZ 5793, 5963, 5965, 5969-5972, 5974, 5975, 5981, 5982, 5984-5986); **[2]** Patococha, antenas repetidoras de Pacifictel: 3300m, -3, -78.65 (QCAZ 4998-5000, 5003, 5005, 5006, 5008, 5010, 5011, 5015-5018); **[3]** Tinajillas: 2300m, -3.02, -78.58 (QCAZ 8341-8343, 8345, 8346, 8349);
BOLÍVAR: **[4]** Bosque Protector Cashca Totoras, 20 km SE de San Miguel: 3250m, -1.72, -78.97 ();
CARCHI: **[5]** Lagunas del Voladero, Reserva Ecológica El Ángel: 3900m, 0.69, -77.87 (QCAZ 4295, 4299, 4301, 4303, 4305, 4307, 4309, 4311, 4331, 4333, 4337, 4365, 4367); **[6]** Páramo del Artesón, Comuna La Esperanza: 3650m, 0.78, -77.91 (QCAZ 9798, 9800, 9802);
CHIMBORAZO: **[7]** Páramo vía Laguna Yahuarcocha: 3600m, -2.36, -78.83 (QCAZ 11251, 11252); **[8]** Parque Nacional Sangay: 3500m, -2.19, -78.49 (ACUNHC 1577-1587, 1591, 1595, 1596, 1602, 1603);
COTOPAXI: **[9]** Pucayacu: 1300m, -0.76, -79.06 (QCAZ 11583, 11584,

11593); **[10]** Sachapungo, Hacienda Sr. Cepeda, vía Chugchilán-Pucayacu: 3355m, -0.78, -78.94 (QCAZ 11250, 11253-11261, 11271-11275); **IMBABURA:** **[11]** Parque Recreacional Jerusalem: 2250m, 0, -78.36 (tel 2065); **[12]** Páramos de Angochagua, comunidad de Zuleta: 3600m, 0.27, -78.05 (QCAZ 11658-11660); **LOJA:** **[13]** A 2 km de Bellavista, en la vía Amaluza-Bellavista-Gonzanamá; Finca Sr. Tobías Conde: 1400m, -4.57, -79.45 (QCAZ 11303); **[14]** Galápagos, Cantón Quilanga: 1300m, -4.35, -79.43 (QCAZ 11852); **[15]** Guineo Grande, Cantón Celíca: 750m, -4.2, -80.04 (gri 1315, QCAZ 11853-11855); **[16]** La Extensa, Cantón Catamayo: 1300m, -4.04, -79.36 (QCAZ 12108); **[17]** Laguna Negra, Bosque Protector Colombo-Yacurí: 3250m, -4.71, -79.44 (QCAZ 11281-11291, 11297-11299, 11302); **MORONA SANTIAGO:** **[18]** Cerro Bosco, bosque aledaño a las antenas repetidoras de Pacifictel: 2400m, -3.01, -78.5 (QCAZ 4812, 4815, 4827, 4830, 4833, 4899); **[19]** Lagunas de Atillo: 3500m, -2.19, -78.52 (QCAZ 8407, 8408, 8412, 8415, 8417, 8418); **[20]** Maylas: 3200m, -2.97, -78.69 (QCAZ 8352,8357); **NAPO:** **[21]** Oyacachi, cantón el Chaco: 3700m, -0.19, -78.11 (QCAZ 5217, 5218, 5221, 5223, 5225, 5228, 5229, 5231, 5233, 5234, 5237, 5238, 5242, 5243, 5244); **[22]** Papallacta, bosque administrado por la fundación TERRA: 3400m, -0.33, -78.14 (QCAZ 4089, 4146, 4169, 4190, 8386, 8391, 8393, 8395, 8396, 8398); **PICHINCHA:** **[23]** Yanacocha, estribaciones del Guagua Pichincha, bosque administrado por la Fundación Jocotoco: 3600m, -0.12, -78.55 (QCAZ 5715, 5716); **TUNGURAHUA:** **[24]** 1 km S, 1.5 km E Baños, Runtún: 2300m, -1.41, -78.41 (TTU 85235, 85238, 85240-85242, 85244-85246, 85252); **[25]** 1.5 km S, 3 km W Baños, zona de lahares del Tungurahua: 2200m, -1.41, -78.41 (TTU 85250, 85253, 85257, 85259, 85261-85263, 85266-85268); **[26]** 4.75 km E Baños, Represa Agoyán: 2200m, -1.4, -78.38 (TTU 85476, 85481); **[27]** Parque Nacional Llanganates, Laguna de Pisayambo: 3600m, -1.1, -78.39 (QCAZ 5722-5732, 5734, 5749-5751).

PERU:

ANCASH: [28] Pallasca, Magistral, Laguna Llamacocha: 3530m, -8.25, -77.82 (vpt 2623, 2626); [29] Pallasca, Magistral, Quebrada Toldobamba: 3550m, -8.24, -77.83 (vpt 2644, 2648); [30] Pallasca, Quebrada Chaluacocha, Laguna Magullo Chico: 3816m, -8.26, -77.8 (vpt 2847, 2861); CAJAMARCA: [31] Chota, Querocoto, Campamento La Granja, T32: 1933m, -6.35, -79.12 (esp 433); [32] Contumaza, Bosque Cachil, entre Cascas y Contumaza: 2500m, -7.39, -78.78 (jaa 178, vpt 1675, 1680, 1690, 1696); [33] Cutervo, San Andrés de Cutervo, Cutervo National Park, 100 m over El Tragadero: 2969m, -6.25, -78.77 (llw 1086, 1088, 1100, 1102, 1119, 1122, 1125); [34] San Andrés de Cutervo, 4 km W San Andrés de Cutervo: 2350m, -6.26, -78.72 (jaa 136, vpt 1569, 1590, 1212); [35] San Ignacio, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary Buffer Zone), campamento 1: 2923m, -5.29, -79.34 (llw 1003, 1004, 1013); [36] San Ignacio, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary Buffer Zone), campamento 2: 1897m, -5.28, -79.32 (llw 1048, 1082); [37] San Ignacio, Tabaconas, Piedra Cueva in Cerro Coyona (Tabaconas-Namballe National Sanctuary), campamento 1: 3290m, -5.27, -79.27 (llw 926, 929, 930, 967, ucf 43); [38] San Ignacio, Tabaconas, Piedra Cueva in Cerro Coyona (Tabaconas-Namballe National Sanctuary), campamento 2: 3290m, -5.27, -79.27 (llw 976, 995); [39] Santa Cruz, 2 km E Montesecco: 2148m, -6.85, -79.09 (lhl 98, 106, llw 1238, 1241, 1249, 1273, 1274, 1279, rco 1035, vpt 1664); LA LIBERTAD: [40] Sánchez Carrión, Sanagorán: 2775m, -7.79, -78.14 (lhl 83, llw 1220, 1223, 1225, vpt 2263); PIURA: [41] Huancabamba, Canchaque: Agua Azul: 1345m, -5.36, -79.6 (ucf 220); [42] Huancabamba, Cuenca del Río Blanco, Campamento Nueva York: 3103m, -4.9, -79.37 (hq 523, 527, vpt 2545, 2546); [43] Huancabamba, Cuenca del Río Blanco, Campamento

Quebrada del Gallo: 2084m, -4.88, -79.34 (vpt 2580, 2583); [44] Huancabamba, Huaricancha:
1900m, -5.32, -79.41 (ucf 262); [45] Huancabamba, Minera Majaz, campamento Bomba
Quemada: 2464m, -4.89, -79.35 (vpt 3001, 3002); [46] Morropón, Portachuelo: 1940m, -5.03, -
79.91 (ucf 233).

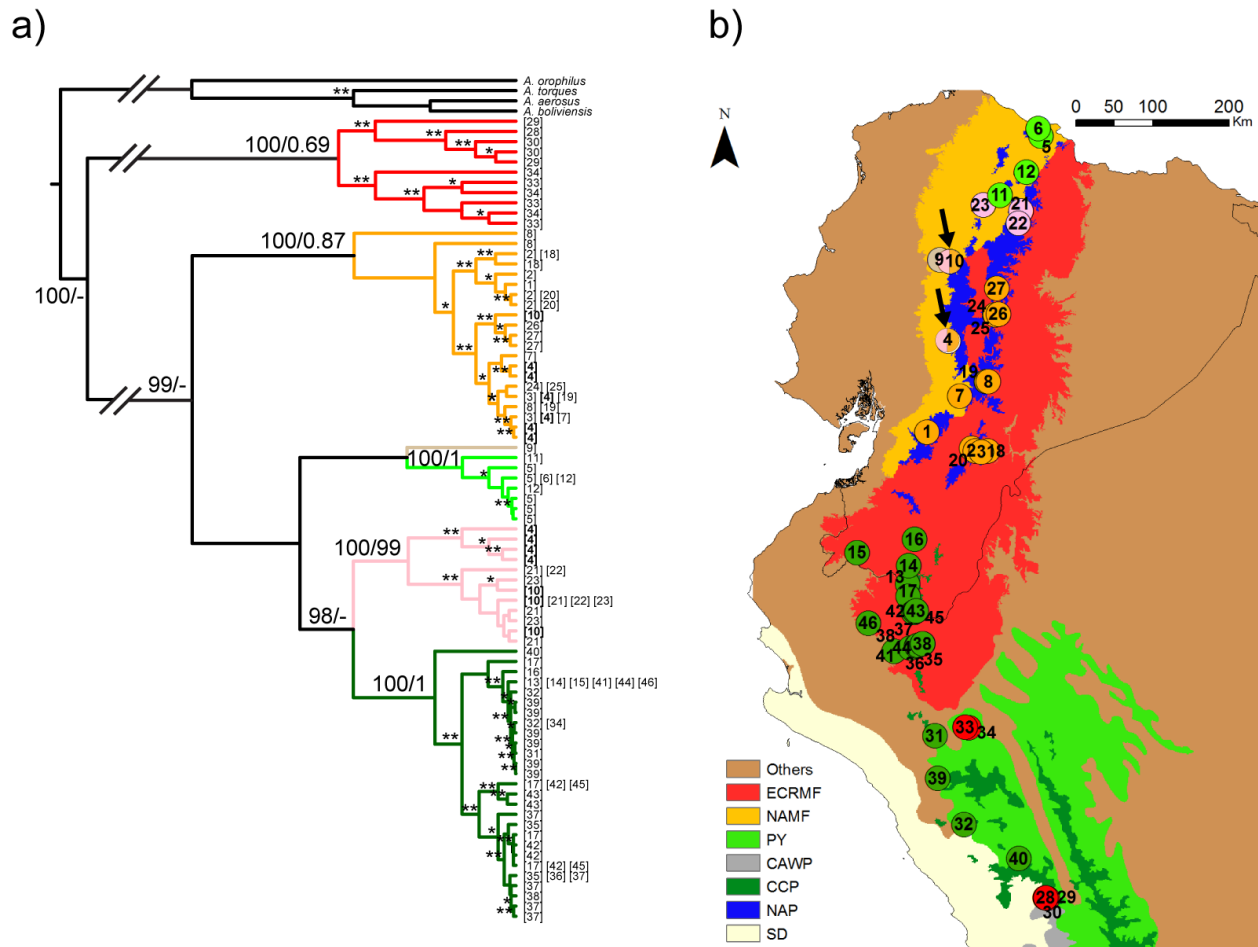


Figure 4.1. Phylogeographic structure of *Akodon mollis*.

The 50% majority rule maximum likelihood cytb tree is shown in (a), whereas the distribution of populations along the northern Andes is shown in (b). Phylogenetic support values are shown above branches, with ML bootstrap left to the slash and BI posterior probabilities right of it; only bootstrap values above 70 (*) and posterior probability values above 0.85 (**) are listed. The only 2 populations with individuals in more than one clade are highlighted in bold in (a) and marked by black arrows in (b). Ecoregions abbreviations: CCP: Cordillera Central Páramo; CAWP: Central Andes Wet Puna; ECRMF: Eastern Cordillera Real Montane Forest; NAMF: Northwestern Andean Forest; PY: Peruvian Yungas; NAP: North Andean Páramo; SD: Sechura Desert; Others: all other ecoregions that do not contain localities of *A. mollis*.

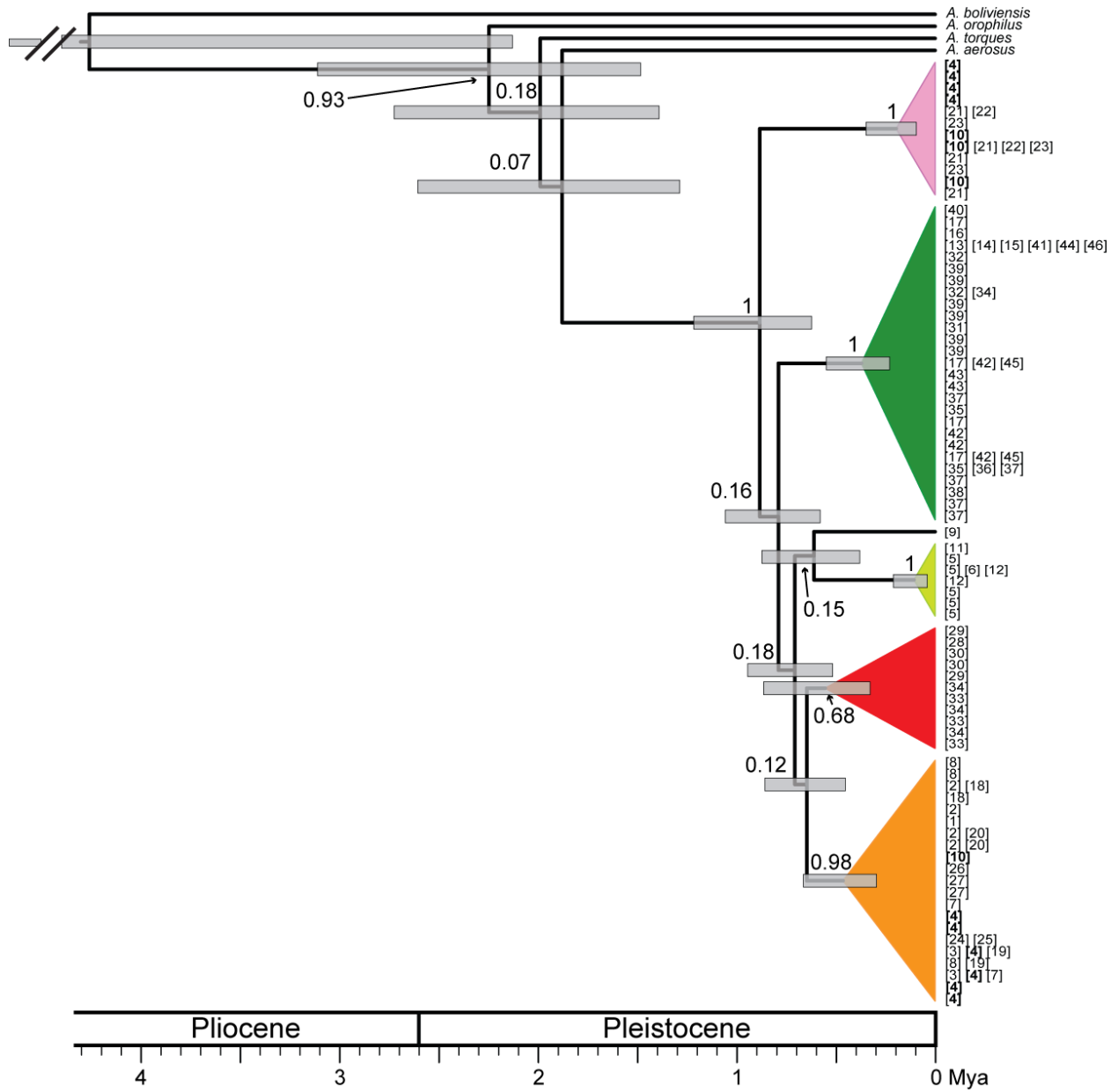


Figure 4.2. Timing of *Akodon mollis* diversification. Maximum credibility tree based on *cyt-b* data indicating estimated median node height and 95% highest posterior density credibility intervals (grey bars). Branch support is indicated as Bayesian posterior probability. Colors in the tree follow color branches in Fig. 4.1a.

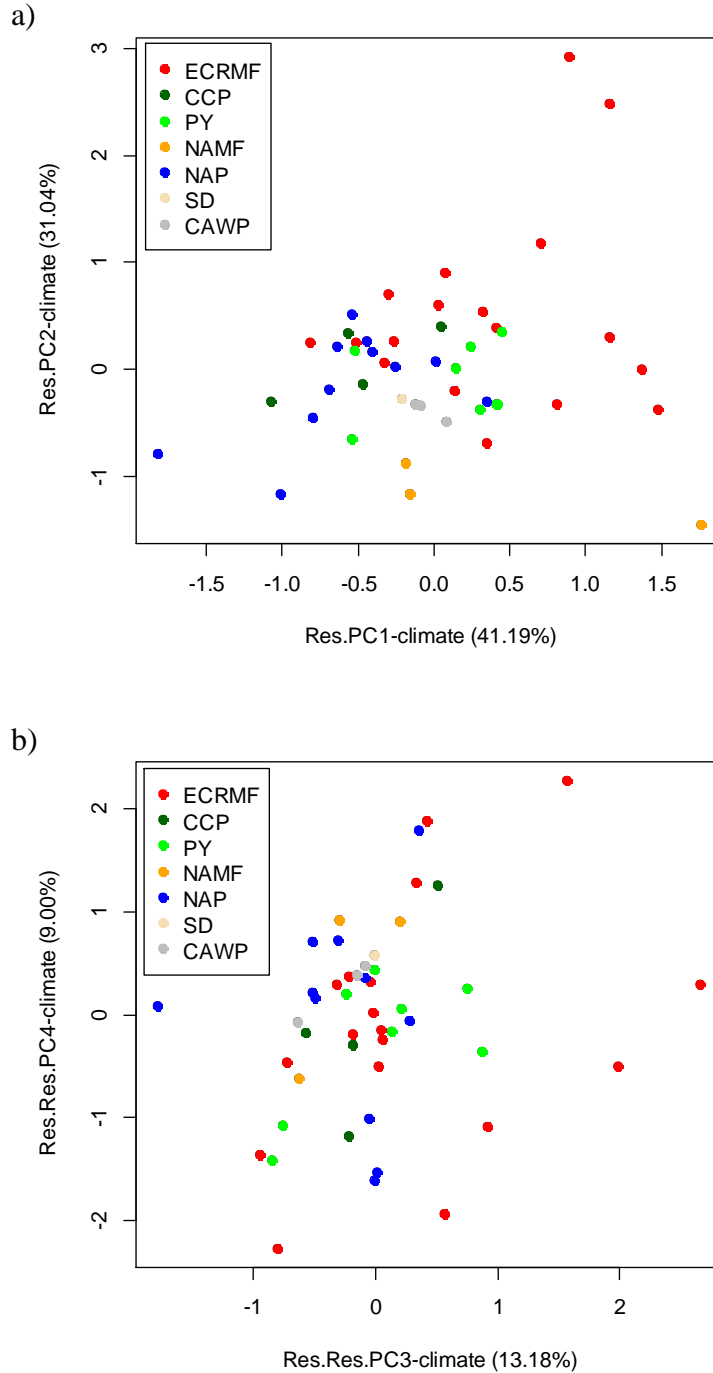


Figure 4.3. Variation in the climatic preferences of populations of *Akodon mollis*. Scatterplots of residual variation of the 1st and 2nd (a), and 3rd and 4th (b) PCs of a principal component analysis on the climatic environments of populations of *A. mollis*. Residuals were obtained from regressions of each PC against latitude and longitude to account for spatial autocorrelation. The variation explained by each component is given in parentheses. Populations are color-coded according to the ecoregion where they are located. Note the lack of differentiation among ecoregions (abbreviations follow Fig. 4.1).

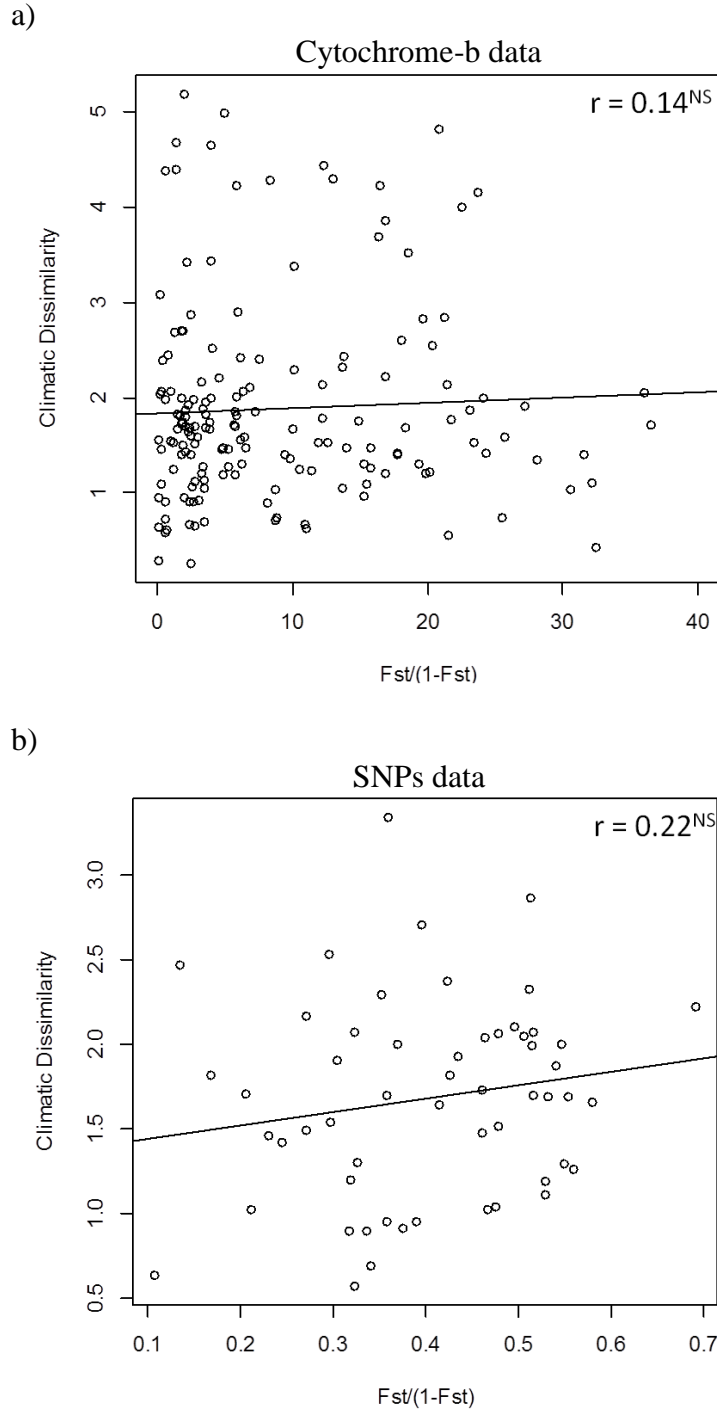


Figure 4.4. Association between genetic and climatic niche differentiation in *Akodon mollis*. Pattern of inter-population differentiation in the similarity of climatic niches according to their pairwise genetic distance. Results for mitochondrial (a) and genomic (b) datasets are shown (see methods for details). Correlation coefficients are given for all analyses; an asterisk next to these coefficients indicates that the association remains significant after accounting for spatial autocorrelation. To facilitate interpretation linear regression lines are shown.

Table 4.1. Similarity of ENMs of ecoregion-based population groups. Comparisons of the estimated ecological niche of populations grouped according to the ecoregion in which they are located. Niche similarity (Warren et al. 2008) results based on D statistic (results were similar to I and Relative Rank tests; not shown) are shown above the diagonal while niche divergence (McCormack et al. 2010) results are shown below the diagonal. Bold blue is used to indicate significant conservatism (i.e., comparisons in which niches from the two groups being compared are more similar to each other than their environmental backgrounds) while bold red is used to indicate significant niche differentiation. Ecoregions abbreviations follow Fig. 4.1.

Groups	CCP	ECRMF	NAMF	NAP	PY
CCP		0.42	0.14	0.48	0.59
ECRMF	3.11		0.22	0.17	0.56
NAMF	0.22	2.89		0.04	0.29
NAP	1.47	1.56	1.33		0.32
PY	0.89	2.22	0.67	0.67	

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CHAPTER 5: Conclusions

All together, this dissertation fills a knowledge gap on the concrete evolutionary consequences of inhabiting environmentally heterogeneous regions from the perspective of overall diversity (Chapter 2) as well as from the perspective of opportunity for differentiation of individual species (Chapters 3 and 4). In doing so, this work provides a testable link between populations and species divergence, offering new research venues to expand our understanding of the complex set of interactions structuring diversity in natural ecosystems. By highlighting likely evolutionary mechanisms at work, this dissertation provides new and valuable insights into the potential role of alternative diversification scenarios in heterogeneous environments that complement past theoretical work. The results of this dissertation shed light on the processes that generate biodiversity and that drive differences in species richness across the globe, and in particular, the reasons behind the incredible high diversity of the tropical Andean hotspot. Ultimately this knowledge represents an important tool for conservation by identifying fundamental factors required to maintain diverse communities.

Elucidating the role of alternative diversification scenarios acting on tropical ecosystems has been a long-standing goal in evolutionary biology (Moritz et al. 2000), dating back to the first naturalist expeditions during the seventeenth and eighteenth centuries (Mayr 1982). Since then a vast number of studies have focused on this question, resulting in a multiplicity of hypotheses (Willig et al. 2003), many of which revolve around the notion that environmental heterogeneity is a fundamental component (Hopton 2006). Yet, evidence for specific mechanistic

links has been elusive. Addressing this issue, the three empirical studies comprised by this dissertation explore how migration, differential adaptation to local habitats, selection, and genetic drift interact with environmental heterogeneity to promote or deter diversification. The first study, focused on assessing the association between spatial and temporal environmental heterogeneity and diversity in the tropical Andes, demonstrates that small terrestrial mammals' species and phylogenetic diversity in this region are strongly associated with environmental heterogeneity (with over 50% of the variance explained), and most likely linked to the opportunities for diversification offered by habitat heterogeneity. The second study, focused on evaluating the extent to which the heterogeneous landscape configuration of the tropical Andes deters migration and facilitates local adaptation, identifies environmentally driven isolation and genetic drift as most likely candidate drivers of population differentiation in the soft-grass mouse, *Akodon mollis*. Finally, the third study, which investigates the interaction between environmental heterogeneity of the tropical Andes and the degree of intra-specific differentiation of climatic niches, identifies evidence of a general, spatially unstructured the climatic niche in *A. mollis*. Taken together, these three studies suggest that an allopatric speciation mode (Patton and Smith 1992, Casner and Pyrcz 2010), as opposed to parapatric speciation along ecological gradients (Endler 1977, Schneider et al. 1999), is a more likely diversification scenario for the taxa studied.

In addition to uncovering potential diversification mechanisms and pointing specific research areas in need of more work, this dissertation suggests two main questions that require further investigation: (1) the specific role of species-specific ecologies in mediating the effects of

environmental heterogeneity, and (2) the impact that long-term climatic cycles have had in the differentiation of species across environmentally heterogeneous regions.

The importance of species-specific responses

Although this dissertation is focused on small terrestrial mammals, and in particular on one species of this group that has an unusually broad altitudinal and latitudinal range, its findings imply that species-specific natural history plays a preponderant role in modulating the effects of environmental heterogeneity given that the micro-evolutionary processes uncovered as important for this system depend directly on species' specific characteristics. For example, the likelihood of environmentally driven isolation is directly associated with the vagility of species, with more vagile species being less susceptible to be restricted by local environmental heterogeneity (Baythavong 2011). Similarly, the opportunity for ecological specialization depends on the population structure and biogeographic history of species (Futuyma and Moreno 1988, Williams et al. 2009). Yet, relatively few studies have explicitly addressed how individual species' ecologies interact with the geographic setting they occupy to generate and maintain diversity.

The methodological framework provided by this dissertation allows for explicit comparisons among species or groups of species with contrasting ecologies or that inhabit contrasting environments. In particular, two main comparisons seem the most fruitful in terms of advancing our general understanding of diversification processes. First, a direct comparison between temperate and tropical communities will allow testing the hypothesis that differences in the climatic regime between these regions is in part responsible for the differential accumulation of species between them (Kozak and Wiens 2007, Cadena et al. 2012). Although, the degree of

habitat heterogeneity may be comparable between temperate and tropical regions (Dobzhansky 1950, Rohde 1992), the impact of short-term climatic variability might be certainly different between these two regions (Janzen 1967, Ghalambor et al. 2006). Testing this prediction will require information on the correlation between spatial and temporal heterogeneity and diversity (Chapter 2) of ecologically equivalent species in both regions, together with an assessment of the degree of climatic niche differentiation (Chapter 4) in these species.

The second comparison of particular interest involves co-distributed species with distinct ecological affinities. In this context, comparing the evidence for local adaptation (Chapter 3) between species with similar habits but distinct environmental preferences (e.g., habitat-generalist vs. habitat-specialist), can inform previous hypotheses about the evolution of plasticity vs. local specialization in response to environmental heterogeneity (Donaldson-Matasci et al. 2008, Hollander 2008). A better understanding of these processes would allow for a more complete assessment of the ecological and evolutionary consequences of these alternatives (e.g., the maintenance of genotypic and phenotypic polymorphism; Van Valen 1965, Svanbäck and Schluter 2012). Hence, investigating the degree to which habitat fidelity evolves in response to environmental heterogeneity will offer valuable insights into the susceptibility of species to environmental change given that the ability to respond to climatic changes depends on the likelihood of species to evolve their tolerances.

The role of long-term temporal climatic variability

How important has been long-term climatic cycles in shaping the evolutionary trajectory of species across environmentally heterogeneous regions remains an opened question as most

studies to-date have been focused on high latitudes in the northern hemisphere (Lim 2010, Alvarado-Serrano and Knowles In prep-b). The findings of this dissertation suggest, nonetheless, that climatic-driven divergence is probably a major player in the tropical Andes. Of particular interest, is the effect of past climatic events in structuring diversity in this region as previous work has identified environmental heterogeneity as a primary influence on the genetic consequences of climate-induced distributional shifts (Knowles and Alvarado-Serrano 2010). Since the genetic consequences of climate change are expected to depend on the geographical configuration and topographic variation of regions (Hastenrath 1991, Scherrer and Korner 2011), elucidating the differences in how climatic cycles impacted species in the tropical Andes will provide further insights into how general are species responses to spatio-temporal environmental variability. A promising alternative to address this question is to couple explicit modeling of species responses to climate change with coalescent modeling under a statistical framework (Knowles and Alvarado-Serrano 2010, Alvarado-Serrano and Knowles In prep-a). In this regard, the evidence of limited climatic niche differentiation obtained in this dissertation (Chapter 4) is fundamental to validate the use of current distribution data to infer past distributional shifts.

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