Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains

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Speciation richness is greatest in the tropics, and much of this diversity is concentrated in mountains. Janzen proposed that reduced seasonal temperature variation selects for narrower thermal tolerances and limited dispersal along tropical elevation gradients [Janzen DH (1967) Am Nat 101:233–249]. These locally adapted traits should, in turn, promote reproductive isolation and higher speciation rates in tropical mountains compared with temperate ones. Here, we show that tropical and temperate montane stream invertebrates have diverged in thermal tolerance and dispersal capacity, two key traits that are drivers of isolation in montane populations. Tropical species in each of three insect clades have markedly narrower thermal tolerances and lower dispersal than temperate species, resulting in significantly greater population divergence, higher cryptic diversity, higher tropical speciation rates, and greater accumulation of species over time. Our study also indicates that tropical montane species, with narrower thermal tolerance and reduced dispersal ability, will be especially vulnerable to rapid climate change.

Significance

Over 50 years ago, Dan Janzen proposed an integrative framework relating latitudinal differences in climate variability to elevational trends in species diversity. We show that tropical species in three independent insect clades have (i) narrower thermal breadths, (ii) decreased dispersal and higher population structure, and (iii) higher cryptic diversity and speciation rates. This research tests all of the key predictions of Janzen’s hypothesis in related taxa. Our work advances the understanding of how climate variability shapes global diversity patterns, moving beyond simple correlations, to mechanistic links between climate, local adaptation, dispersal, and montane species richness.


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Data deposition: Restriction-site associated DNA sequences, SNP data, and detailed collection localities for all eight focal morphological taxonomic units are available in the Dryad Digital Repository, https://datadryad.org/ (doi:10.5061/dryad.7m8q). Specimens, locality, and sequence data have been accessioned in the Barcode of Life Database (DOI: dx.doi.org/10.5883/DS-ITADD) and in GenBank (MH838053–MH841890).

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measurements provides a unique test of the link between climate variability and trait-mediated diversification rates in tropical and temperate mountains (Dataset S1).

Results and Discussion
We found that stream temperature variation and the degree of overlap in temperature across elevation were greater in Colorado than in Ecuador, confirming the climatic context for regional differences in selection and diversification (13, 18) (Fig. 1). As predicted, tropical species, defined first by using morphology and then delimited by DNA barcoding (hereafter referred to as “barcoded species”), had narrower thermal breadths than their temperate counterparts (Fig. 2A). Specifically, when acclimated to a common temperature, temperate insects from a given elevation had on average a higher critical thermal maximum (CT\text{MAX}) and a lower critical thermal minimum (CT\text{MIN}) than phylogenetically paired tropical insects at the same elevation (Fig. 2A and SI Appendix, Table S1) (13). The broader thermal breadth (i.e., the difference between CT\text{MAX} and CT\text{MIN}) in temperate barcoded species is most pronounced at low elevations because temperate barcoded species exhibit significantly lower CT\text{MIN} values than tropical ones, despite being acclimated to the same temperature (Fig. 2A and SI Appendix, Table S1) (13).

We next characterized fine-scale gene flow along elevation gradients for four tropical and four temperate species complexes paired to represent all three insect clades. For gene flow analyses, we used morphological taxonomic units (MTUs)—i.e., species identified based on morphology alone—that often comprise species complexes. Both in Ecuador and in Colorado, the focal MTUs are known to harbor divergent lineages and/or undescribed diversity across their ranges. We chose MTUs for gene flow analyses because our goal was to quantify how much genetic differentiation and gene flow occur along the entire extent of the elevational gradient, and thus across boundaries of diversifying lineages and potential cryptic species in each region. We used double-digest restriction-site associated DNA sequencing (ddRADseq) (19) to genotype hundreds of individuals (316–990) at hundreds to thousands of SNP loci (419–4,544) (SI Appendix, Table S2). Clustering of genotypes into genetic demes revealed that genetic structure was lower in all MTUs from Colorado (mean = 2.75 demes, SD = 1.5) than in those from Ecuador (mean = 11.5 demes, SD = 3.9; SI Appendix, Fig. S1). Estimates of pairwise population genetic differentiation (F\text{ST}) and effective migration (N\text{m}) confirmed overall lower levels of dispersal and population connectivity along the tropical gradient. The ranges of F\text{ST} values between populations along the elevation gradient were lower in Colorado, indicating higher levels of interbreeding among temperate populations (Fig. 2B), and this finding was concordant with higher estimated migration rates among populations in Colorado than in Ecuador (P < 0.001) (Fig. 2C and SI Appendix, Fig. S2).

To be comparable, estimates of gene flow among populations in different regions must account for landscape features. We applied a linear mixed-effects model to quantify the effects of geographic distance and elevation on population genetic differentiation at each latitude. The best-fit model indicated significant interactions between latitude and geographic (Euclidean) distance (F\text{L,1114} = 1.86; P = 0.012) and latitude and elevation difference (F\text{L,1114} = 33.20; P < 0.001), confirming that elevation and geographic distance have greater effects on genetic differentiation among tropical stream insect populations compared with temperate ones (SI Appendix, Table S3). Combined, our population genomic results show extensive population structure and reduced dispersal rates in tropical taxa in each of the three clades, resulting from higher isolation-by-distance and isolation-by-elevation in tropical mountains.

To compare regional taxonomic richness, we surveyed all taxa in the three focal clades along our tropical and temperate elevation gradients and quantified the number of MTUs (11,433 specimens

Fig. 1. Variation in annual temperature overlap and stream temperature profiles for temperate and tropical streams. Annual maximum (squares) and minimum (circles) temperatures show that temperate streams (A) have greater annual temperature variation than tropical streams (B). Temperature profiles (daily average temperature over 1 y) at low, mid-, and high elevation streams show that temperate streams (C) show higher seasonal variance than tropical ones (D) at all elevations.
examined: Ecuador—4,511 and Colorado—6,922) and barcoded species (3,980 specimens barcoded: Ecuador—1,495; Colorado—2,485) (9) (Dataset S2). The difference between MTU and barcoded species at a survey site is a measure of cryptic diversity. Our surveys revealed higher MTU richness in Colorado than in Ecuador, but this pattern was reversed in two of the insect orders when taking cryptic diversity into account. Barcoded species richness was higher in Ecuador than in Colorado for Ephemeroptera (54 vs. 41 barcoded species) and Trichoptera (71 vs. 35 barcoded species), but not Plecoptera (14 vs. 36 barcoded species; Fig. 2F). This result underscores the importance of quantifying cryptic diversity in studies of richness, especially when comparing regions where species discovery and taxonomic knowledge are very different (20).

To test whether ancestral location (temperate or tropical) could explain among-clade differences in barcoded species diversity, we estimated Bayesian supertree phylogenies for the barcoded species sampled in our regional taxonomic surveys and inferred the location of the common ancestor for barcoded species in each clade (SI Appendix, Fig. S3). We found that the Trichoptera barcoded species in our study most likely had a tropical common ancestor, whereas the ancestors of Ephemeroptera and Plecoptera were temperate (SI Appendix, Fig. S3). Thus, the Trichoptera barcoded species that we sampled have had a longer history in the tropics, followed by the Ephemeroptera. In contrast, the Plecoptera barcoded species have had the shortest time to diversify in the tropics (21–24). Despite these differences in biogeographic history, we observed consistently larger increases in the number of barcoded species vs. MTUs in Ecuador compared with Colorado (Ephemeroptera: +350% vs. +40%; Plecoptera: +56% vs. +3%; Trichoptera: +137% vs. −3%; Fig. 2F), indicating higher levels of cryptic diversity in the tropics, irrespective of the time for tropical speciation in each clade.

Finally, to test for latitudinal differences in speciation rates, we used binary state speciation and extinction models (BiSSE).
(25). For the dataset including barcoded species for all three clades combined, the full BiSSE model with parameters estimated using Markov Chain Monte Carlo (MCMC) inferred a higher speciation rate for Ecuador compared with Colorado (Fig. 2D); the 95% credibility interval for the difference in speciation rates (Ecuador–Colorado) was positive and did not include zero (Fig. 2E and SI Appendix, Fig. S4). For barcoded species in each insect order, we also fit full BiSSE models to estimate parameter distributions using maximum likelihood and 20,000 post burn-in trees that were used to create the clade supertrees. We then compared rates across latitude by determining latitudinal differences in parameter estimates and examining the 95% credibility interval for these rate differences. For individual clades, we found consistent trends toward higher speciation rates in Ecuador compared with Colorado, with stronger effects for the Trichoptera and Plecoptera than the Ephemeroptera (SI Appendix, Fig. S5). Because these diversification analyses are based on a phylogeny of barcoded species, the estimates of speciation are not biased by regional differences in historical taxonomic effort and cryptic diversity; they show that the rate of origination of barcoded species is higher in the tropics independently of the number of barcoded species in each MTU in each region. Overall, our results demonstrate narrower thermal breadths, less gene flow, higher population divergences, higher cryptic diversity, and higher speciation rates in the tropics.

Empirical support for the CVH has been equivocal (2, 10), likely because most studies use range size as a proxy for dispersal capacity and latitude as a proxy for climatic variability, thereby disregarding important variance in organismal traits and local environmental fluctuations within latitudes (15, 26). Range sizes of organisms are a consequence of individual species’ responses to many environmental and biotic factors, as well as to historical contingencies (1, 27), and thus are imprecise proxies for selection on thermal tolerance and dispersal, the phenotypic traits that contribute directly to diversification (8). Our study shows that quantifying these key organismal traits provides a more mechanistic understanding of how populations diverge across environmental (selection) gradients (28, 29). We do not claim that other biotic factors, such as species competition and host-pathogen dynamics, are unimportant in determining the boundaries of species’ ranges; many studies clearly show that these factors are significant (30, 31). However, in the case of strong abiotic gradients, such as those found in mountains, the effects of climatic variability on local adaptation and dispersal are likely major drivers of diversification. Analyses of these organismal traits reveal commonalities and idiosyncrasies in rates of diversification among taxa, and they also have the potential to predict species’ responses to changing climate (32).

Our integrative approach provides the strongest evidence to date that reduced climate variability favors narrow thermal tolerance and reduced dispersal across elevational gradients, which in turn increase speciation rates. We were able to test the CVH by linking microevolutionary processes at the population genomic level to macroevolutionary patterns of species diversification, an approach that may be the key to testing the generality of the CVH in other taxonomic groups (2). Our study demonstrates the insights possible when integrating data on processes operating at different temporal scales—from local thermal adaptation, to ongoing gene flow among adjacent populations, to estimates of speciation that occurred in the distant past. For example, the magnitude of genetic divergence among populations within a species could be an indicator of the potential for speciation (2) because the likelihood of reproductive isolation increases with genetic distance (33). In fact, surveys in vertebrates show that the magnitude of population genetic differentiation within species parallels the species-richness gradients observed at a global scale (7, 34), a pattern expected if most incipient species reach species status. Our findings confirm that within-species processes of population divergence are indeed mirrored in among-species patterns of cryptic diversity and speciation rates at different latitudes. Furthermore, our results underscore how a focus on species richness alone can fail to reveal important aspects of global patterns in biodiversity. For example, we found Plecoptera species richness to be lower in the Andes compared with the Rockies; however, this recently arrived group is also speciating at a higher rate in the tropics, which may eventually produce greater species diversity.

Finally, our study has important implications for predicting the vulnerability of species to climate change. Global warming is projected to increase seasonal variability and alter daily maximum and minimum temperatures (35, 36), with potentially negative consequences for species’ persistence and future diversification (37). Tropical ectotherms persist close to the edge of their evolved thermal tolerances (38), and thus, paradoxically, the same traits that promote high speciation and diversity are the ones that render them especially vulnerable to rapid changes in thermal environments. Characterizing species in terms of traits that are directly linked to global warming (e.g., thermal tolerance) or that represent potential adaptive capacity to adjust (e.g., dispersal ability) provides a powerful framework for predicting evolutionary outcomes, species range shifts (39), and ecological changes (40) in natural communities across geographic gradients of projected climate change.

Methods

Study Area and Collection. To characterize temperature profiles at different elevations for streams in both regions, we measured stream temperature using Solinst temperature/level loggers in the Poudre River drainage in Colorado and HOBO level loggers in the Papallacta River drainage in Ecuador over a 12-mo period between 2013 and 2015. Loggers recorded temperature at 15-min intervals each day. We calculated daily average temperatures over the course of a year for each stream to characterize variation in stream temperature.

We collected aquatic insect samples from small (first–third order), minimally impacted montane streams at ~200-m elevation intervals from 1,556 to 3,478 meters above sea level (m asl) in Colorado (Cache la Poudre basin in the Front Range of the Rocky Mountains) (9) and 1,664–2,428 m asl in Ecuador (Napo basin in the Oriental Andean Cordillera) (41). To collect insects, we used D-frame nets with 500-μm mesh and searched under available substrates. From each sample, we separated all aquatic larvae of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). For estimates of regional diversity, we supplemented larval collections with adults collected from streamside vegetation. For measurements of gene flow, we subsampled phylogenetically paired species complexes with broad elevational distributions and sufficient sample sizes across sites (Dataset S1 and SI Appendix, Table S5). All specimens used for genetic analyses were field-preserved in 100% ethanol.

Measuring Latitudinal Differences in Thermal Tolerance. To test one of the primary predictions of the CVH, we measured the CT\text{MAX} and CT\text{MIN} temperatures of temperate and tropical barcoded species from various elevations (13). The CVH predicts that tropical species should have narrower thermal breadths, which was calculated as the difference between CT\text{MAX} and CT\text{MIN}. To account for the large seasonal differences at different latitudes, we conducted experiments in the Colorado Rockies between June and August, when stream temperatures at a given elevation were similar to those in the Ecuadorian Andes. We paired streams at the different latitudes by elevation and acclimated barcoded species from those streams for 48 h at the same temperature (SI Appendix, Table S1). For the experiments, we ramped temperature by 0.3 °C/min until we observed a loss of righting response, at which point individuals were placed in a recovery tank; only individuals that survived the treatment were included in the analysis (13). A critical aspect of this experimental design is that by acclimating barcoded species from similar elevations, but different latitudes, at the same acclimation temperature, we were able to control for environmental effects on measures of thermal breadth. Indeed, we could not acclimate species from all elevations to a single acclimation temperature because attempts to acclimate low elevation tropical species to cooler temperatures or high elevation tropical species to warmer temperatures resulted in obvious signs of thermal stress or mortality (13). To statistically control for evolutionary history while comparing thermal breadth values across latitude and thermal breadth across streams of different temperatures, we used...
phyletogenetic generalized least squares regression (42) fit with an Ornstein-Uhlenbeck model (43, 44) of trait evolution. We also calculated mean critical migration rates among populations for MTUs in each clade independently (47). We then calculated and examined the 95% credibility interval for latitudinal differences in species richness, we used the total number of barcoded species in Ecuador and Colorado, thus avoiding the underestimation of species richness in less-studied tropical faunas (9). Final identifications of all barcoded species are available in Dataset S2. Specimens, locality, and sequence data have been accessioned in the Barcode of Life Database (www.boldsystems.org/index.php/Public_SearchTerms?qquery=DS-TTADD) (60) and in GenBank (MH838053–MH841890).

**Historical Context for Latitudinal Differences in Species Richness.** We estimated a Bayesian phylogeny for the barcoded species included in our study. We estimated a tree constrained according to well-supported higher-level taxonomy groupings for the three clades (Dataset S3). In determining constraints, we applied a set of decision rules in cases of conflicting published phylogenetic relationships (9, 61). Each barcoded species is one tip in the phylogeny; thus we randomly chose among the longest available sequences for each barcoded species and aligned them in MAFFT v.7 (62) using strategy G-INS-i with offset value 0.1 and all other options set as default. Once aligned, we used JModelTest2 (63, 64) to select the best-fit nucleotide-substitution model based on the Akaike Information Criterion, which was GTR + Γ. We then conducted six runs each with 100,000,000 generations of Bayesian MCMC in BEAST v.2.3.2 (65). We modeled lineage-specific substitution rates using a relaxed clock with log-normally distributed rates (66–68) and diversification using a birth–death tree prior (69–71). For each run, we plotted the ln likelihood scores against generation time in Tracer v.1.6 (72) and examined the effective sample sizes for parameters to ensure that all six analyses reached stationarity. We combined the trees from each run into a consensus tree with the 50% majority rule and inferred species trees using the approximate likelihood-ratio test. For independent clad analysis, we used stochastic character mapping (73) to infer the most probable ancestral location and the relative historical presence of each clade in the tropics. We applied pattern-based stochastic character mapping (73) as opposed to other events-based methods because all of the taxa detected in our study are endemic to single continents (North or South America). We used maximum likelihood to fit equal-rates and all-rates-different transition matrices and compared them using a likelihood-ratio test. After determining the best-fit model, we used the R package phytools (74) to simulate 10,000 stochastic character maps for ancestral state coded as “tropical” or “temperate.” The results of stochastic mappings were summarized as pie charts indicating the posterior probability of the location of each ancestral node (SI Appendix, Fig. S3).

**Latitudinal Differences in Diversification.** For barcoded species from all three clades, we estimated and compared latitudinal diversification rates using BISEE (25) implemented in the R package diversieve (75). Although geographic state speciation and extinction models (GeoSSE) are suitable for modeling character-dependent diversification based on biogeography (76), we used BISEE rather than GeoSSE because the latter is not suitable for datasets containing species endemic to single continents (North or South America) (77, 78). For the analysis of all barcoded species together, we used a Bayesian MCMC version of BISEE designed to handle polytomies to fit the full model. We then calculated and examined the 95% credibility interval for latitudinal differences (Ecuador–Colorado) between parameter estimates. Lack of inclusion of zero in the 95% credibility interval indicated a significant difference in parameter estimates for Ecuador versus Colorado. For analyses of barcoded species in each clade, we determined the maximum-likelihood parameter estimates for the full BISEE model for each of the 20,000 post-burn-in trees summarized to create our superetrees and calculated the 95% credibility interval for latitudinal differences (Ecuador–Colorado) between parameter estimates.

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