

LETTER

Tropical forests are not flat: how mountains affect herbivore diversity

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Abstract

Ecologists debate whether tropical insect diversity is better explained by higher plant diversity or by host plant species specialization. However, plant–herbivore studies are primarily based in lowland rainforests (RF) thus excluding topographical effects on biodiversity. We examined turnover in *Eois* (Geometridae) communities across elevation by studying elevational transects in Costa Rica and Ecuador. We found four distinct *Eois* communities existing across the elevational gradients. Herbivore diversity was highest in montane forests (MF), whereas host plant diversity was highest in lowland RF. This was correlated with higher specialization and species richness of *Eois*/host plant species we found in MF. Based on these relationships, Neotropical *Eois* richness was estimated to range from 313 (only lowland RF considered) to 2034 (considering variation with elevation). We conclude that tropical herbivore diversity and diet breadth covary significantly with elevation and urge the inclusion of montane ecosystems in host specialization and arthropod diversity estimates.

Keywords

Biodiversity, diet breadth, *Eois*, herbivores, host plants, *Piper*, tropical elevation gradients.

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INTRODUCTION

How many species of insects exist? Erwin (1982) estimated global arthropod diversity to be *c.* 30 million species based on surveys of Coleoptera associated with the canopy of the tree *Luebea seemanii* (Malvaceae) in tropical dry forests of Panama. A key assumption of this estimate was that 14% of the species collected were monophagous on *L. seemanii*. This diversity estimate stimulated ample discussion about the global number of arthropod species (e.g., May 1990). One major criticism of Erwin's diversity estimate is the assumption of high levels of specialization, and this criticism has helped create a clear impetus for better measures of specialization and more empirical data that utilize these measures. Ødegaard (2000) introduced the effective

specialization model, in which host specificity is an inverse function of the number of host plants found per herbivore; utilizing this measure reduced the specialization estimate to 2–10%, resulting in a much smaller global estimate of insect diversity. Novotny *et al.* (2002) provided a thorough estimate of effective specialization by sampling plant–herbivore interactions and estimating the overlap across 45 host plant species in the lowland rainforest (RF) of Papua New Guinea. Following this estimate of diet breadth, Novotny *et al.* (2006) compared temperate and tropical plant–herbivore interactions and found no significant difference in host specialization of insect herbivores between Papua New Guinea and Eastern Europe. This lack of a latitudinal gradient in specialization supports the hypothesis that increased herbivore species richness is a

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direct result of higher host plant species richness (Novotny *et al.* 2006). An untested assumption of all specialization estimates is that monophagous herbivore richness per host plant species does not vary with elevation or latitude within an herbivore's host plant range, thus leaving uncertainty as to whether the plant–herbivore interactions included in these analyses only occur locally (Fox & Morrow 1981).

Two major components of global insect diversity estimates that can change with environmental variation are: (1) diet breadth and (2) herbivore diversity per plant species, or species packing (Lewinsohn *et al.* 2005). The most striking diversity pattern across environmental gradients is the strong increase in biodiversity with decreasing latitude, and one of the many predictions about this gradient is that there will be a positive correlation between diversity and habitat specialization (Hutchinson 1959; Dyer *et al.* 2007). Striking patterns can also be found across elevational gradients and associated temperature changes created by tropical mountains, which have produced juxtapositions of unique biotic communities, allowing for tests of associations between herbivore diversity, specialization and species packing (Janzen 1967). The differences in temperature that are characteristic of elevational gradients are known to influence ecological factors such as host plant quality, predation, parasitism and competition, which can restrict species distributions and host plant choice (Hodkinson 2005). Physiological constraints on metabolism may also reduce dispersal across thermal gradients, increasing genetic differentiation within altitudinal zones (Ghalambor *et al.* 2006) and creating substantial changes in plant–herbivore species composition. Both lowland and montane species have persisted in the Amazon basin under climatically stable conditions at least since the early Pleistocene (Colinvaux *et al.* 1996; Colinvaux & DeOliveira 2000), allowing time for highly specialized interactions among species to evolve.

In this study, we examine diversity patterns of an abundant moth genus, *Eois* Hübner (Geometridae), and its host plant genus, *Piper* L. (Piperaceae). First, we test the hypothesis that the number of species of *Eois* per host plant and the levels of *Eois* specialization differ between tropical lowland and highland forests at a local and regional scale. We then test whether *Piper* diversity is the best predictor of *Eois* diversity across elevational gradients (*sensu* Novotny *et al.* 2006; Lewinsohn & Roslin 2008), or if higher levels of host plant productivity and herbivore abundance are better predictors as proposed by Rosenzweig (1973) and Oksanen *et al.* (1981). Using the results from testing these hypotheses we then estimate overall Neotropical diversity of *Eois* species and then address whether estimates from lowland and montane tropical forests are adequate for global extrapolations of arthropod diversity (Erwin 1982; Ødegaard 2000; Novotny *et al.* 2002).

MATERIAL AND METHODS

Study system

We tested our hypotheses in multiple lowland and montane forests by examining interactions between the species rich herbivore genus, *Eois*, and its diverse and abundant host plant, *Piper*, along five independent elevational gradients: four in the Eastern Ecuadorean Andes and one on the Atlantic slopes of the Cordillera Central (Barva transect), Costa Rica. *Piper* (Piperaceae) has *c.* 2000 species throughout the tropics and is one of the most diverse lineages among basal Angiosperms (Jaramillo *et al.* 2008). *Piper* is predominantly an understory plant and is well represented in both lowland and montane Neotropical forests, where more than 700 species have been described (Jaramillo & Manos 2001). *Eois* (Lepidoptera, Geometridae, Larentiinae) comprises 257 valid described species worldwide, with 82% of them occurring in the Neotropics (Parsons *et al.* 1999). Neotropical lepidopteran larvae have narrow diet breadths (Dyer *et al.* 2007), and *Eois* is an exemplary genus with most species monophagous or oligophagous on a few species of *Piper* plants (Connahs *et al.* 2009). We decided to focus on the abundant and relatively well-studied *Piper–Eois* system across environmental gradients because it was possible to generate a large amount of accurate data for rigorous tests of our hypotheses. The system also allows for comparisons with diversity studies that have demonstrated high overlap in herbivore communities feeding on closely related host plant species (Novotny *et al.* 2008).

Study sites

Costa Rica

The data from this region were derived from caterpillar collections from several transects, including the following lowland sites (down to 100 m elevation) in Heredia Province, Costa Rica and multiple sites (up to 3280 m elevation) directly upslope from these: La Selva Biological Station, La Tirimbina Reserve, El Bejucó Field Station, Bijagual Ecological Reserve and Braulio Carrillo National Park. We also included data from adult *Eois* moth collections with light traps at the Barva transect (40–2730 m; Brehm *et al.* 2007; Fig. 1c; Table S1). The temperature lapse rate, e.g., along the Barva transect, is *c.* 5.5 °C per 1000 m (Brehm *et al.* 2007). For sampling sites location, see map on Figure S1a and Table S1.

Ecuador

We collected and reared caterpillars from 10 m diameter plots along three independent altitudinal gradients in the NE Andes ranging from 300 to 3200 m in elevation. Caterpillar collections were predominantly from three mountain ranges (Figure S1b; Table S1). To include independent elevational

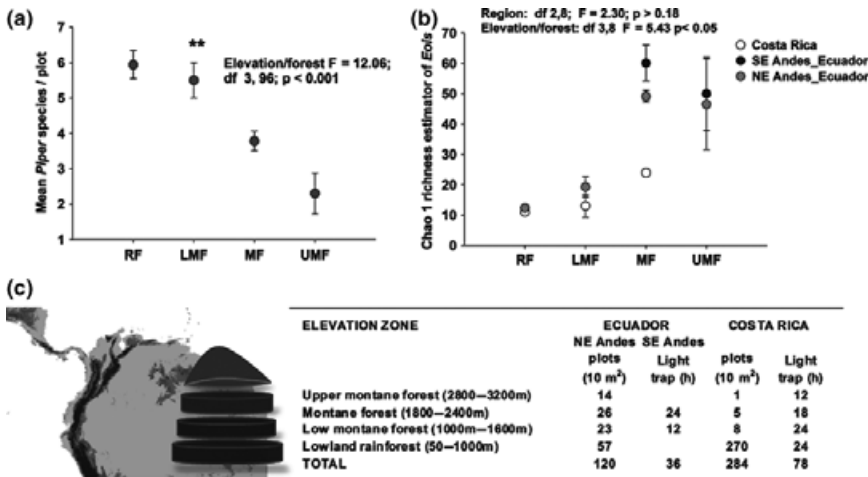


Figure 1 Patterns of species richness for *Piper* host plants vs. species richness of *Eois* among the four elevation zones. (a) *Piper* species; (b) *Eois* species richness is higher at montane and upper montane forest in Costa Rica and Ecuador (1800–3200 m; region $F = 2.30$, d.f. = 8, $P > 0.18$; elevation zone $F = 5.43$, d.f. = 8, $P < 0.05$). (c) Sample size within each elevation zone. Map shows location of mountains and sections across mountains that each elevation zone covers.

gradient studies and compare them with our rearing data, we added adult *Eois* collections along an altitudinal gradient in the SE Andes (Brehm *et al.* 2005; Table S1). The temperature in SE Ecuador decreases at a rate of *c.* 5.5 °C per 1000 m (Figure S2).

Methods

Standard 10 m diameter plots were established inside the forest at our sampling sites. Inside each plot, we tagged the base of all *Piper* plants present then harvested all of the leaves and stems of each individual plant, leaving the roots intact. We sampled an average of nine plots per 200 m interval along an elevational range of 50–3200 m. However, within the intervals of 800–1000 m and > 2400–2600 m we found only one location with accessible and suitable forest to sample; thus we exclude these elevations from our analyses. We kept each plant in a separate bag, identified by plot number and plant number, and transported samples to our laboratory and rearing centres at La Selva (50 m) in Costa Rica, Yanayacu biological station (2200 m), Oyacachi (3200 m) and Jatun Sacha (400 m) in Ecuador. Each plant was weighed and identified to morphospecies. We then counted all leaves, measured the leaf area of three leaves per plant, estimated per cent herbivory per leaf and carefully surveyed for caterpillars or other insects. At each rearing facility, caterpillars were reared following previously published protocols (Gentry & Dyer 2002; see Table S1 for collection site details). *Piper* diversity and leaf area data were obtained from these plots along an altitudinal gradient in the NE Andes of Ecuador.

We used data from 13 807 *Piper*–*Eois* feeding relationships, which we reared to adults, feeding on the same host plant on which they were collected on. Initial identifications of *Eois* and *Piper* were made by parataxonomists and

then confirmed by taxonomic specialists, J. Miller (Smithsonian) and G. Brehm (Universität Jena) for *Eois* and E. Tepe (University of Utah) for *Piper*. Voucher specimens of moths and plants were deposited at the Smithsonian Institute (Washington, DC, USA), the Missouri Botanical Garden (St. Louis, MO, USA), InBio (Costa Rica), the Museo Nacional de Ciencias Naturales (Ecuador) and other collaborating institutions (see <http://caterpillars.unr.edu/> for a list). Our database provides the most complete record of this specialized interaction, including different geographical sites and up to 12 years of systematic or haphazard collection of *Eois* caterpillars on *Piper* (Fig. 1c; Table S1).

To compare elevational patterns of *Eois* diversity in the SE Andes of Ecuador and the Barva transect, Costa Rica, we include data from Brehm *et al.* (2005, 2007). In these studies, *Eois* adults were manually collected from 18:30 to 21:30 with two 15 W blacklight tubes. *Eois* adults were recorded predominantly in the forest understory where their host plants grow (Brehm 2007). Caterpillar sampling could miss certain *Eois* species not specialized on *Piper*, and this is counterbalanced by adult sampling that could miss certain *Eois* species specialized on rare and local *Piper* species. We therefore assume that sampling of larvae and adults per site will provide qualitatively similar patterns of estimated species richness.

Analyses

We used light trapped adult moths and plot based collection of *Eois* caterpillars to study patterns of diversity across elevation gradients. For the herbivore–host plant analyses, we used reared *Eois* collected from plots (details on sampling effort for adults and caterpillars are displayed in Fig. 1c). To determine whether natural breaks in the *Eois* communities exist along elevational gradients, we constructed a matrix of all *Eois* species found in each plot across

the complete elevational gradient in the NE Andes. We used multiple stage community comparisons with the Morisita index. This two stage probabilistic approach allows for a simultaneous comparison among communities sharing more than two species. To date, this is the most robust method for comparing overlap between various samples simultaneously (Chao *et al.* 2008). We compared similarity among plots across all elevations (q value = 56) and it provided low resolution (0.1). Similarity values among individual plots are displayed on Table S2a. We then made comparisons between contiguous *Eois* communities at every 200 m (q value = 11), and separated the *Eois* communities based on whether contiguous 200 m communities had more than 80% turnover (i.e., similarity values ≤ 0.19 ; Table S2b). We used the program SPADE for all the species turnover analyses, setting the number of bootstraps to 200. Bootstrapping was used in this program to simulate the asymptote of variance for the species sampled per site (Chao & Shen 2010). There were four elevation zones determined by this analysis (see results) that correspond to the following types of forest: (1) RF (0–1000 m), (2) lower montane forest (LMF) (1000–1600 m), (3) MF (1800–2400 m) and upper montane forest (UMF) (2600–3200 m). These forests and associated elevations were the basis of separation for all the subsequent analyses of *Piper* and *Eois* diversity across elevation. Changes of *Piper* diversity across elevation categories were compared utilizing analysis of variance (ANOVA), with *Piper* richness as a dependent variable. We also computed a linear regression of *Piper* and *Eois* species richness per plot and elevation.

To determine if *Piper* and *Eois* diversity differed among the elevation zones, we conducted two separate analysis of covariance (ANCOVA); these analyses used different subsets of data, one with *Piper* species richness as a response variable, elevation as a predictor variable and *Piper* abundance as a covariate. In the second ANCOVA, we used *Eois* species richness per plot as a response variable, elevation zone as an independent variable and *Eois* abundance as a covariate. To test if these differences occurred at a regional level we performed a two way ANOVA using the Chao 1 estimator of species richness as a response variable, elevation zone (with four levels: RF, LMF, MF and UMF) and region (with three levels: the Barva transect, Costa Rica, the SE and the NE Andes, Ecuador as independent variables) as predictor variables. Chao 1 estimate of species richness for each region and elevation zone was calculated using SPADE (Chao & Shen 2010).

To calculate the degree of specialization, we compared the number of *Eois* species from *Piper* species for which we had sampled at least 700 m² of leaf area from the forests and excluded data with ≤ 2 observations (see Table S6 and S7 for details on sampled leaf area/*Piper* and *Eois*/*Piper* records). From these data, we counted the number of

monophagous *Eois* per *Piper* species and the total number of host plant species per elevation zone. Because specialization is subject to change with low sample size of herbivores per host plant (Novotny *et al.* 2002), we performed a sensitivity analysis to study how vulnerable our estimate of *Eois* diet breadth was to differences in sample size (i.e., changes in *Eois* abundance per species). We first performed several ANOVAs using elevation zone as an independent variable and monophagous *Eois* species as the response variable; five versions of this response variable were created by computing number of monophagous *Eois* species when excluding from the data any *Eois* species with fewer than 3, 5, 10, 15 and 20 successful rearings for each elevation zone. Additionally, we conducted an ANCOVA with monophagous species of *Eois* as a response variable, elevation zones as a predictor variable and each series of *Eois* monophagous species data as a covariate.

As specialization across geographic gradients has been defined and measured in different ways (Novotny *et al.* 2006; Dyer *et al.* 2007), we included a second measure of host specialization. We calculated a two stage probabilistic Morisita index of *Eois* beta-diversity among host plant species present within each elevation zone as a measurement of the overlap of *Eois* species between *Piper* species (Chao *et al.* 2008). To quantify the variation in *Eois* species richness for each host plant species (i.e., species packing), we calculated the total number of *Eois* species found on each *Piper* species and the nonparametric Chao 1 estimator or *Eois* diversity for each *Piper*. Species packing and *Eois* overlap between *Piper* species were calculated using SPADE (Chao & Shen 2010).

To examine biogeographical differences in specialization, we tested the hypothesis that the lowlands of two different regions (Costa Rica and NE Ecuador) are characterized by differences in the average number of monophagous *Eois* species and *Eois* species richness per host plant species (i.e., species packing). We utilized multivariate analysis of variance (MANOVA) for this test, with region as the independent variable and the measures of *Eois* species richness as response variables.

We also utilized MANOVA to compare number of monophagous *Eois* species and species packing (response variables) in RF, LMF, MF and UMF. To analyse overlap of *Eois* communities among *Piper* species between lowland RF, we used *t*-test; to study *Eois* overlap among *Piper* species within elevation zones we utilized one way analysis of variance (ANOVA).

We used path analysis (Shipley 2000) to test two models of hypothesized causal relationships among *Eois* species richness, *Eois* abundance, *Piper* diversity and biomass per plot across elevation (300–3200 m). For these models, we used caterpillars sampled in 10 m plots across a continuous gradient of elevation in Ecuador. The first model tested the hypothesis

that *Piper* diversity decreases with increased elevation (Marquis 2004), with consequent decreases in *Eois* diversity (Lewinsohn & Roslin 2008). The second model tested the hypothesis that high host plant biomass causes higher *Eois* abundance and associated species richness, i.e., the ecosystem exploitation hypothesis which predicts that resource availability determines herbivore and predator diversity (Rosenzweig 1973; Oksanen *et al.* 1981). We used SAS 9.1 (SAS 2003) for two way ANOVA, ANCOVA, MANOVA and path analyses.

We used methods outlined by Ødegaard (2000) and Novotny *et al.* (2002) to calculate *Eois* species richness in the Neotropics and how sensitive this estimate is to geographical variation in species packing and specialization. We first used the Chao 1 estimator of *Eois* species richness for each host plant species within each elevation zone (A). For the measurement of effective specialization, we used two approaches: (1) dissimilarity among *Eois* communities between *Piper* species (B_1) and (2) the mean number of monophagous *Eois* found within each elevation zone (B_2). We used Jaramillo & Manos' (2001) estimate of Neotropical *Piper* species richness (C). To account for the differences in area represented by each Neotropical elevation zone, we used mountain area determined by the UNEP map (Edwards *et al.* 2010) and estimated the proportion of area covered by each elevation zone (D). We then estimated *Eois* richness using each elevation zone as the single location from which estimates are extrapolated by multiplying the number of herbivores per host plant (A), the specialization estimates (B_1 or B_2), the number of host plants recorded for the Neotropics (C) and the proportion of land area each elevation zone covers in the Neotropics (D). We contrasted species packing and specialization estimates from a single elevation zone and the mean value across elevation for these estimates.

RESULTS

Biogeographical regions and effects of elevation

For all our transects, we found four distinct elevation bands corresponding to distinct forest habitats: RF, LMF, MF and UMF. The highest *Eois* species turnover was between LMF and MF (mean Morisita similarity value $c.$ 0.016), followed by the turnover between MF and UMF (mean Morisita similarity value $c.$ 0.02), and the turnover between RF and LMF (mean Morisita similarity value $c.$ 0.11). Moreover, turnover of *Eois* species within elevation zones was higher among UMF and lower among lowland RF (Figure S3). All similarity values are displayed in Figure S3 and Table S2a,b. When we compared alpha diversity among the four elevation zones, *Piper* diversity peaked in lowland RF (i.e., at low elevations of 0–1000 m, Fig. 1a; $F = 12.06$, d.f. = 96, $P < 0.001$) and declined linearly with elevation

(*Piper* richness = $8.14 - 0.0021 \times \text{elevation}$; $r^2 = 0.46$, $P < 0.001$, Figure S4a). ANCOVA results for *Piper* species richness across elevation zones revealed no significant interaction between *Piper* abundance and elevation (Table - S3a). The slope of *Eois* diversity regressed against elevation was positive but not significant ($r^2 = 0.15$, $P > 0.3$). However, *Eois* diversity was higher in MF and UMF in Costa Rica, and the NE, SE Andes of Ecuador (Fig. 1b; ANOVA for region $F = 2.50$, d.f. = 8, $P > 0.18$; ANOVA for elevation $F = 5.43$, d.f. = 8, $P < 0.05$). Furthermore, *Eois* diversity peaked in MF for various diversity measures in the NE Andes of Ecuador (Figure S4b). ANCOVA results for *Eois* species richness across elevation zones showed no significant interaction between *Eois* abundance and elevation (Fig. 1b; Table S3a).

We found that lowland tropical RF (0–1000 m in Fig. 2a) had low herbivore diversity values, with no significant differences (Wilk's lambda = 0.88, $F = 3.56$, d.f. = 25, $P < 0.07$) among biogeographical regions for *Eois* species richness in each host plant species (i.e., species packing) and host effective specialization (Fig. 2a; Table S3b). *Eois* species richness, species packing per host plant species, and proportion of monophagous species was higher in LMF, MF and UMF (Wilk's lambda = 0.67, $F = 7.16$, d.f. = 25, $P < 0.0001$). We found diversity of monophagous *Eois* was dependent on sample size (Fig. 3). However, the differences among elevation zones remained significant even when the minimum number of records for each *Eois* species was set to 15 and the rest of the data were discarded (Fig. 3; Table S4). We also compared the overlap of *Eois* communities among *Piper* species in lowland RF of Ecuador and Costa Rica with the overlap of *Eois* communities among *Piper* species in LMF, MF and UMF. We found no significant difference between the lowland RF of Ecuador and Costa Rica (T -test = 1.64, $P > 0.12$, Fig. 2c). However, *Eois* species overlap between *Piper* species decreased with elevation ($F = 28.75$, d.f. = 38, $P < 0.0001$; Fig. 2d, dissimilarity matrices in Table S5).

Correlates of herbivore diversity across elevation

Piper species richness alone did not explain variance in *Eois* diversity across elevation. This is demonstrated by the poor fit of the path analysis model with increases in *Piper* richness causing increases in *Eois* species richness (Fig. 4a, $\chi^2 = 6.21$, d.f. = 2, $P < 0.04$). We found a better fit for the model that supports the resource availability hypothesis (Fig. 4b; $\chi^2 = 1.07$, d.f. = 2, $P > 0.59$; r^2 for *Piper* richness = 0.38 and r^2 for *Eois* richness = 0.72). Elevation had a positive effect on *Eois* richness (0.28 in Fig. 4b); then *Piper* biomass had a positive effect on *Eois* species richness (0.23 in Fig. 4b). Resource availability hypothesis is also supported by the strong path coefficient between *Eois* species richness and *Eois* abundance found for both models (0.61 in Fig. 4b). Further-

Figure 2 Specialization of *Eois* species on *Piper* host plants. (a) Mean numbers (± 1 SEM) of monophagous *Eois* species and Chao 1 estimate of *Eois* richness per *Piper* species in the rainforests of two different biogeographical regions: Costa Rica and Ecuador. (b) The same specialization parameters for rainforest, lower montane forest, montane forest and upper montane forest of the NE Andes of Ecuador. (c) *Eois* species overlap between *Piper* species for the rainforest of two different biogeographical regions: Costa Rica and Ecuador. (d) *Eois* overlap between *Piper* species for rainforest, lower montane forest, montane forest and upper montane forest of the NE Andes of Ecuador.

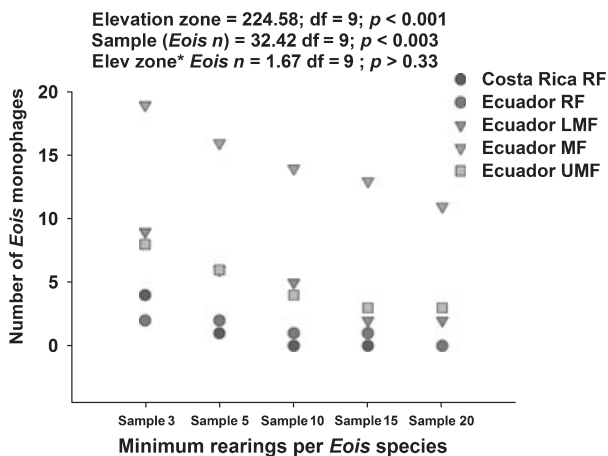
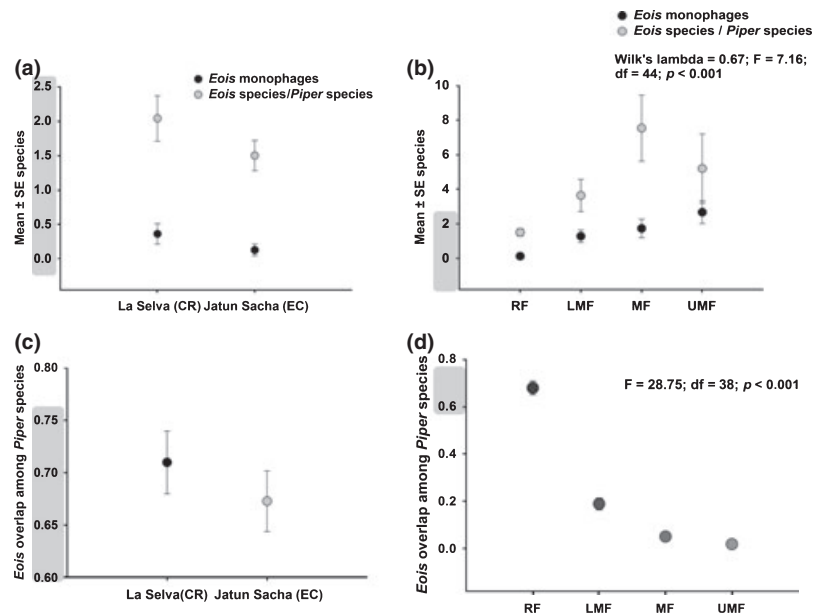


Figure 3 Sensitivity analysis for the measurement of number of monophagous *Eois* species. Samples 3–20 include all the *Eois* species with a minimum of 3, 5, 10, 15 and 20 successful rearings respectively.

more, the path analysis supports the hypothesis that elevation has a strong negative effect on *Piper* species richness (-0.62 in Fig. 4b); however, *Piper* species richness did not have a strong effect on *Eois* species richness (-0.08 in Fig. 4b).

Estimating *Eois* Neotropical diversity

The key variables that are traditionally used to extrapolate insect diversity from a focal study are: (1) the number of herbivore species per host plant (or species packing per host plant), (2) specialization (or a measurement of dissimilarity of herbivore communities between plant species) and (3) host plant species richness. Our results show elevational

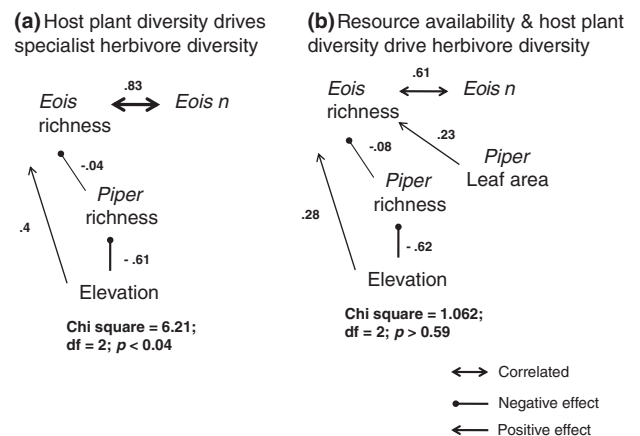


Figure 4 Path analysis models examining two alternative hypotheses behind gradients in herbivore diversity. (a) Host plant diversity drives diversity of specialist herbivores, (b) resource availability and host plant diversity drive herbivore diversity. Standardized path coefficients are adjacent to each line; bullet-headed lines indicate a negative effect, while arrows indicate a positive effect.

variation in all three variables. Diversity estimates change depending on whether plant–herbivore interactions are studied in lowland RF (0–1000 m), LMF (1000–1600 m), MF (1800–2400 m) or UMF (2600–3200 m; Table 1). For example, estimations based solely on data from lowland RF, yield an *Eois* turnover rate among *Piper* species of 0.26 ± 0.13 , while the overall turnover rate among *Piper* species across the complete elevational gradient was 0.83 ± 0.001 . Thus, the probability of finding the same species of *Eois* among several *Piper* species is 0.74; this probability decreases to 0.17 when (Fig. 2d) *Piper* species across elevational gradients are considered. Similar differ-

ences were obtained comparing monophagous *Eois* in RF (0.22 ± 0.08) and the mean number of monophagous *Eois* across the complete elevation gradient (1.56 ± 0.22). Furthermore, we found significant differences between RF *Eois* species richness per *Piper* species (1.72 ± 0.19 at elevations from 50 to 1000 m vs. 3.5 ± 0.59 when including elevations from 50 to 3200 m). Considering that there are 700 described species of *Piper* in the Neotropics, our calculations including lowland RF, LMF, MF and UMF yielded an estimate of 2034 Neotropical species of *Eois*. If these estimates were drawn exclusively from lowland RF records, we would have estimated Neotropical diversity of *Eois* to be a total of 313 species (Table 1).

DISCUSSION

Our results and analyses yield novel conclusions for understanding patterns of tropical herbivore diversity. First, we found a peak of herbivore diversity in MF, despite the fact that the diversity of their host plants peaked in the lowlands and decreased with elevation. These differences between plant and herbivore diversity were in part due to the higher number of monophagous herbivores and higher alpha diversity of *Eois* per host plant species in MF.

Furthermore, the turnover of *Eois* species between host plants was lower in montane ecosystems and higher in lowland RF from Costa Rica and Ecuador. We found differences between *Eois* communities associated with lowland RF, LMF, MF and UMF, which is consistent with the elevation zones found in a comprehensive review of community turnover in tropical MF (Bruijnzeel *et al.* 2010). Interestingly, differences in plant–herbivore interactions changed over short distances (*c.* 50 km) from 400 to over 2000 m in elevation (Fig. 2b; Table S3b), but there were no differences in herbivore species richness or specialization between lowlands (0–400 m) of two biogeographically distinct sites separated by *c.* 1500 km (Fig. 2a; Table S3b). This suggests that environmental conditions, such as temperature, which remain largely constant in lowland tropical forests but change at a fast rate with elevation (Figure S2) are important for the generation or maintenance of diversity. Moreover, *Eois* diversity was higher in MF than in RF in Ecuador and Costa Rica, and this was correlated with changes in both diet breadth and number of *Eois* species interacting with *Piper*. *Eois* were also more abundant in MF than in RF, thus montane *Eois* could be better sampled than RF *Eois*. However, even 12 years of sampling and rearing of 1512 *Eois* specimens in La Selva, Costa Rica,

Table 1 Estimates of the number of Neotropical *Eois* species using number of *Eois* species/*Piper* species and the *Eois* turnover rate between *Piper* species

Estimates from:	Lowland Neotropics (0–1000 m)	Lower montane Neotropics (1000–1600 m)	Montane Neotropics (1800–2400 m)	Upper montane Neotropics (2600–3200 m)	Neotropical estimates using only rainforest mean values, including elevations from 50 to 3200 m
A. Number of <i>Eois</i> species/ <i>Piper</i> species	1.72 ± 0.19	3.64 ± 0.93	7.54 ± 1.92	5.20 ± 1.5	3.5 ± 0.59
B ₁ . <i>Eois</i> turnover rate between <i>Piper</i> species	0.26 ± 0.13	0.81 ± 0.025	0.95 ± 0.02	0.98 ± 0.004	0.83 ± 0.01
B ₂ . Mean monophagous <i>Eois</i>	0.22 ± 0.08	1.28 ± 0.36	1.73 ± 0.54	2.6 ± 2	1.56 ± 0.22
C. <i>Piper</i> species reported for the Neotropics	700	700	700	700	700
D. Proportion of area (km ²) each elevation zone covers in the Neotropics	0.86	0.1029	0.1148	0.06	
E. Estimated number of Neotropical <i>Eois</i> species with the estimated decrease in <i>Piper</i> richness with elevation ($A \times B_1 \times C \times D$)	~269	~212	~578	~214	313 2034
F. Estimated number of Neotropical <i>Eois</i> species with the estimated decrease in <i>Piper</i> richness with elevation ($A \times B_2 \times C \times D$)	~228	~335	~1048	~569	265 3822

First columns demonstrate geographical variation and its influence on the estimates and the last column represents the mean *Eois* estimate of species richness and the overall turnover rate of *Eois* between *Piper* species. Neotropical *Piper* estimates come from Jaramillo & Manos (2001). Bold numbers indicate final calculations with different specialization measurements.

Dyer & Gentry (2010), still failed to match the number of species found in the MF of the Barva transect or the eastern Andes of Ecuador (Fig. 1b, Brehm *et al.* 2007).

In this study, we found that high abundance of *Eois*, high resource availability in the form of *Piper* leaf area, and a narrow diet breadth of *Eois* are correlated with the high *Eois* species richness at MF (see effects of *Piper* biomass and *Eois* abundance on *Eois* richness, Fig. 4b). Large population sizes of *Eois* species and lower predation at cooler montane and UMF (Rodríguez-Castañeda 2009) suggest that differences in diversity may reflect lower extinction rates of *Eois* in montane vs. lowland RF. There is evidence that tropical montane species have persisted throughout South and Central America since the start of the Pleistocene (MacVean & Schuster 1981; Colinvaux *et al.* 1996; Colinvaux & De Oliveira 2000). *Eois* diversification in the SE Andes of Ecuador is thought to date back to the Oligocene or early Miocene with highest rates of diversification within the Miocene (Strutzenberger *et al.* 2010). In tropical species affected by post-glacial warming, upslope shifts in their ranges are more likely to occur than latitudinal shifts (Colwell *et al.* 2008). Thus, tropical mountains may have acted as a refuge in which highly specialized species with low dispersal abilities thrived during Pleistocene glacial oscillations (Jansson 2003).

Eois diversity is highest from 1800 to 2000 m; this pattern has been observed for other genera of Neotropical Geometridae, one of the most diverse lineages of the Lepidoptera (Brehm 2010), and other organisms, such as small mammals (McCain 2005) and epiphytes (Cardelús *et al.* 2006). Surprisingly, tropical studies of plant–herbivore interactions rarely include mountains. A literature review demonstrated only 15% of plant–herbivore–predator studies ($n = 200$) included tropical sites above 750 m (Rodríguez-Castañeda 2009). Although it is thought that there is a mid-domain trend in species distributions across altitudinal gradients (reviewed by Rahbek 2005), empirical studies have not yet unravelled the complex ecological mechanisms generating such patterns. For the variation in *Eois* diversity, we have examined three mechanisms driving herbivore diversity proposed by Lewinsohn & Roslin (2008). We found that while herbivore diet specialization and species packing did not change across biogeographical regions, these parameters changed substantially with elevation. Combining these results with measures of lepidopteran diet breadth for temperate vs. tropical geometrids (Dyer *et al.* 2007) provides support for the hypothesis that lepidopteran herbivores across the Americas exhibit narrowest diet breadth in the tropical mountains.

Our rearing data allowed for statistical tests of the following two hypotheses: (1) increased plant diversity causes increases in herbivore diversity (Novotny *et al.* 2006; Lewinsohn & Roslin 2008), and (2) diet breadth declines

with elevation resulting in increasing *Eois* species richness (Dyer *et al.* 2007; Lewinsohn & Roslin 2008). Path analyses supported the hypothesis that factors in addition to increased host plant species richness are responsible for increases in herbivore species richness. The path model that best fits the data included effects of host plant biomass and herbivore abundance on species richness (Fig. 3b), corroborating other studies that have demonstrated that geographical variation is a key determinant of herbivore diversity (Condon *et al.* 2008). Our model also provides evidence for the hypothesis that narrow diet breadth in habitats with larger host plant biomass enhances co-occurrence of abundant species in tropical MF.

We extrapolated our results to overall Neotropical *Eois* species richness to examine the implications of the revised assumptions for previously published arthropod diversity estimates (Erwin 1982; Odegaard *et al.* 2000; Novotny *et al.* 2002). Including specialization and number of herbivore species per host plant from LMF, MF and UMF significantly increased a specific diversity estimate compared to estimates using only lowland RF data (Table 1). The differences in estimated diversity suggest geographical variation in the tropics, especially across elevation, should be considered in future estimates of arthropod diversity. There are 257 valid described *Eois* species (Parsons *et al.* 1999), thus our estimates indicate only *c.* 13% of the species have been described. Not a surprising result since on a single study area in the SE Andes of Ecuador 130 *Eois* species have been found to coexist along one altitudinal gradient – which is half of all described species for this genus. Of these, 30 cryptic species were uncovered (Strutzenberger 2009). Therefore, estimates such as 2034 for the number of *Eois* species are feasible.

Tropical elevation gradients present an excellent array of abiotic conditions and associated species ranges, similar to those seen across latitudinal gradients. We found four distinct elevation zones with respect to diversity of plant–herbivore interactions, including a strong gradient of herbivore specialization on host plants. Our results demonstrate that, studies of biodiversity will clearly benefit from a greater focus on beta diversity, host specificity and herbivore species packing across different spatial and topographic scales. Moreover, inclusion of topographical variation in biodiversity estimates will more effectively guide conservation policy and management.

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SUPPORTING INFORMATION

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

Figure S1 Map with all *Piper–Eois* plots.

Figure S2 Changes in temperature as elevation increases (SE Ecuador).

Figure S3 Similarity values of *Eois* communities within each elevation zone: rain forest (0–1000 m), low montane forest (1000–1600 m), montane forest (1800–2400 m) and upper montane forest (2600–3200 m).

Figure S4 (a) Linear regression of *Piper* diversity across elevation gradient. (b) Exponential Shannon and inverse Simpson index for *Eois* diversity/plot at each elevation zone. RF: rain forest (50–1000 m), LMF: lower montane forest (1000–1600 m), MF: montane forest (1800–2400 m) and UMF: upper montane forest (2800–3200 m). Subsequent columns add the number of plots or hours sampled at each elevation zones.

Table S1 Location, elevation and description of sampling transects.

Table S2 (a) Matrix with similarity values for *Eois* communities among plots ($q = 56$); mean similarity index 0.01 ± 0.04 . (b) Matrix with similarity values for *Eois* communities among 200 m intervals across elevation ($q = 11$). *Eois* communities, similarity values between elevation zones: rain forest (50–1000 m), lower montane forest (1000–1600 m), montane forest (1800–2400 m) and upper montane forest (2800–3200 m). Subsequent columns add the number of plots or hours sampled at each elevation zones.

Table S3 (a) Analysis of covariance (ANCOVA) for *Piper* and *Eois* species richness per plot between elevation zones: rain forest (50–1000 m), lower montane forest (1000–1600 m), montane forest (1800–2400 m) and upper montane forest (2800–3200 m). Using *Piper* and *Eois* abundance as covariates. (b) *Eois* diversity across biogeographical regions and across elevation. Multivariate analysis of variance (MANOVA) of overall effect of treatment on monophagous *Eois* and species packing (measured as *Eois* species richness per host plant species).

Table S4 Count of *Eois* monophages. Results from five ANOVA with elevation zone as a predicting variable and number of monophagous *Eois* as a response variable. The different ANOVAs include different rules for data inclusion. With the most inclusive being *Eois* species that had more than three successful rearings and the less inclusive *Eois* species that had more than 20 successful rearings at the four elevation zones.

Table S5 Similarity matrices of *Eois* species between *Piper* species in rainforest, lower montane forest, montane forest and upper montane forests.

Table S6 Records of Ecuador and Costa Rica reared to adults.

Table S7 *Piper* species names and the leaf area surveyed for each species.

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