THE INFLUENCE OF SPATIOTEMPORAL CLIMATE VARIATION ON INSECT SPECIES RICHNESS AND TRAITS

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Abstract

Anthropogenic climate change is having major impacts on global biodiversity, with effects to habitats, traits, and communities as a whole. Studying how species have changed, and will change, in response to changing climates is a critical area of research. In this thesis, I aim to better understand the response of our largest group of animals, insects, to changes in climate through time and space. I investigate two areas of possible species' responses: species richness (Chapter 1 & 2) and trait changes (Chapter 3). In chapter 1, I used a meta-analysis to determine whether there is a global pattern of insect species richness change with elevation, and what climatic factor(s) are associated with it. I found that insect richness plateaus at low elevations and then as elevation increases richness declines, associated with mean annual temperature and temperature seasonality. In chapter 2, I used collections of one of the largest groups of insects, weevils, to determine how species richness changes with elevation in an area at high risk of climatic change: tropical montane environments. I found that in this region, spring temperature, spring humidity, and surface area are associated with an increase in species richness across elevation. In chapter 3, I used museum specimens to look at how Canadian butterfly morphology is responding to climate change. I found that, since the 1950s, butterfly species have gotten paler and larger, but not in response to warming temperature, suggesting the importance of other factors like nutrition. Together, these findings help us to understand how insects are responding to changes in climate. Future research should focus on quantitative and experimental approaches to test these patterns and hopefully help to make better policy and conservation decisions in the future.

Résumé

La biodiversité est en train de changer, avec des effets prévisibles sur les habitats, les traits de vie, et les communautés dans leur ensemble. L'étude de la manière dont les espèces ont changé et changeront, en réponse aux changements climatiques est un domaine de recherche essentiel. Dans cette thèse, je cherche à mieux comprendre la réaction des insectes, notre plus grand groupe d'animaux, aux changements climatiques, dans le temps et dans l'espace. J'étudie les réponses possibles des espèces sous deux formes: la richesse des espèces (chapitres 1 et 2) et les changements de traits (chapitre 3). Dans le chapitre 1, j'ai utilisé une approche méta-analytique pour déterminer comment les modèles mondiaux de richesse en espèces d'insectes changent avec l'altitude. J'ai constaté que la richesse atteint un plateau à faible altitude et décline ensuite, sous l'effet de la température annuelle moyenne et de la saisonnalité de la température. Dans le chapitre 2, je me suis concentré sur un système plus local afin de déterminer comment la richesse en espèces évolue avec l'altitude dans une zone à haut risque de changement climatique (environnements montagnards néotropicaux). J'ai découvert que dans les régions néotropicales, la température et l'humidité printanières, ainsi que l'aire de surface, entraînent une augmentation de la richesse en espèces en fonction de l'altitude parmis un groupe de coléoptères, les charançons. Dans le chapitre 3, j'ai utilisé une approche basée sur les traits pour étudier la façon dont les espèces de papillons canadiens réagissent au changement climatique. J'ai constaté que, depuis les années 1950, les espèces de papillons réagissent temporellement par une diminution de la mélanisation des ailes, ce qui indique qu'en réponse aux conditions climatiques, les traits biologiques changent également. Ces trois chapitres nous aident à comprendre comment les insectes réagissent aux changements climatiques. Les recherches futures devraient se concentrer sur des approches quantitatives et expérimentales pour tester ces modèles et, espérons-le, aider à prendre de meilleures décisions en matière de politique et de conservation à l'avenir

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General Introduction

Insect biodiversity and climate change

Anthropogenic climate change is having major impacts on global biodiversity. Global mean temperatures have increased 1.5°C since industrialization began, with additional increases to the frequency of extreme climatic events like droughts, floods, and fires (Wagner et al. 2021, Pörtner et al. 2022). Recent research has sounded alarm bells about how our largest group of animals, insects, are responding to the changing climates (Scudder 2017, Lister and Garcia 2018, Sánchez-Bayo and Wyckhuys 2019, Harvey et al. 2023). The impacts of climate change on insect biodiversity is widespread and complex, involving many different ecological and evolutionary responses (Rodrigues and Beldade 2020, Yang et al. 2021).

A review of historical insect population reports by Sánchez-Bayo & Wyckhuys (2019) found that 40% of the world's insect species could be at risk of extinction. Declines have been observed in numerous taxa where species losses are occurring on a far faster timescale than speciation can occur (Barnosky et al. 2011, Warren et al. 2021). Insect declines could have far reaching implications for ecological communities and ecosystems. For example, fewer pollinators can result in the reduction of critical pollination services for many plants (Mathiasson and Rehan 2020), and fewer insects have led to declines in upper trophic levels who rely on insects as a valuable food source (e.g., birds; Tallamy and Gregory Shriver 2021).

Insect declines are being driven by many factors including climate change (Lister and Garcia 2018, Halsch et al. 2021, Raven and Wagner 2021). Gradual long-term climate change and extreme events are driving insect declines through creating new unsuitable climatic conditions (Wagner et al. 2021, Wudu et al. 2023). In addition to changing climates, insects are declining from agricultural intensification (Raven and Wagner 2021, Outhwaite et al. 2022). Chemical fertilizers and pesticides create lethal effects on insects, and agricultural intensification destroys natural habitat through development, displacing insects and their resources (Sánchez-Bayo and Wyckhuys 2019, Serrão et al. 2022). The two main drivers of insect declines, climate change and agricultural intensification, are intrinsically linked. Climate change has increased agricultural pests and climatic extremes are rough on crops, thus creating increased need for fertilizers, pesticides, and more habitat destruction for increasing farmland (Raven and Wagner 2021). It is important to note that while insect declines are commonly reported, not all species are declining (Crossley et al. 2020), such as some pest species which show population increases (Wilson and Fox 2021).

Many insect species have been shifting their ranges to track climate shifts (Pereira et al. 2010). In general, species distributions are shifting upwards to the cooler climates found at higher elevations and poleward to latitudes where they find refuge in response to warming temperatures (McCain and Garfinkel 2021, Ramalho et al. 2023). These range shifts have impacted species' interactions and community structure to a significant degree (Kharouba et al. 2018, Wang et al. 2023). While many species have shifted their ranges to track climate shifts, there is variation in the type and degree of response, like range extensions versus range contractions (McCain and Garfinkel 2021). There is also evidence that some groups are not moving at all, like butterflies in Europe (McCain and Garfinkel 2021).

Other insect species' responses to climate change include morphological changes to tolerate new conditions caused by climate change (Kellermann and van Heerwaarden

2019). For example, longer growing seasons have led to larger body sizes in a variety of insect taxa (Siepielski et al. 2019), and hotter summers are linked to paler insects which are able to reflect more heat (Clusella Trullas and Nielsen 2020). These changes have occurred due to a combination of phenotypic plasticity and adaptation (Bonamour et al. 2019). Changes to morphological traits have resulted in species existing in new climatic niche spaces. For example, Wenda et al. (2021) found that size differences between various butterfly species facilitated their persistence in cooler, higher elevations in response to climate change in tropical China.

Amongst the generalizations about how climate change will impact insect biodiversity there is much uncertainty. I know most about the impacts of climate change on insect species that are important medically (e.g. mosquitos), economically (e.g. mountain pine beetle), or are charismatic (e.g. monarchs), but even for these groups there are large gaps in the research such as from geographic biases (Miličić et al. 2021, Harvey et al. 2023). For many, if not most insect species there is very little information on how and why they will respond, and have responded, to changing climates (Donaldson et al. 2016).

Elevation gradients

Mountains are important areas for biodiversity research because of the interaction between elevation and climate. As elevation increases, temperature decreases, precipitation increases, and seasonality increases (McCain and Grytness 2010, Perrigo et al. 2019). This rapid change in abiotic factors often creates high turnover of fauna across a small geographic space, allowing for analysis of how animals are dispersed over space and how these patterns relate to climate (García-Robledo et al. 2016, Perrigo et al. 2019, Mamantov et al. 2021). Species across mountains can be highly specialized to these narrow climatic bands, and therefore are at risk from even small degrees of climate change (McCain and Garfinkel 2021, Ramalho et al. 2023). Due to the changes in environmental and climate factors across elevation, biodiversity generally decreases as elevation increases, though not always linearly, and this prediction varies greatly across taxa and biomes (Sanders and Rahbek 2012, Kinlock et al. 2018, Saupe et al. 2019).

When historical data (i.e., <1980) is missing, elevation gradients can be used as space-for-time-substitutions to better understand climate change impacts on biodiversity. This approach is based on the hypothesis that biotic-climate relationships observed over space are causal and therefore, the relationship will be the same over time (Wogan and Wang 2018). For example, spatial gradients like latitude or elevation display changes in temperature from cool (high latitude or elevation) to hot (lower latitude or elevation), similar to what is expected through time with climate change in some regions (cooler to warmer) (Lovell et al. 2023). In these instances, space can be used as a substitute for time to examine how species are predicted to respond to future climate change (Wogan & Wang 2017; Kharouba & Williams 2024). In some cases space-for-time substitutions have been shown to be relatively similar to temporal investigations (Blois et al. 2013, Wogan and Wang 2018, Lovell et al. 2023).

Documentation and conservation of understudied taxa

Many places on earth do not yet have comprehensive estimates of biodiversity. There are approximately 1.5 million living species formally described, with an estimated 7 million more species on earth that are not yet named or described (Liu et al. 2022). One of the reasons for this is because invertebrates are historically understudied (Donaldson et al. 2016, Stahl et al. 2020). This is due to various factors, including societal preferences (Troudet et al. 2017). Understudied taxonomic groups, like most insects, are at risk of a silent extinction, where species disappear without knowing they were at risk, or even what they were (Ellison et al. 2021).

The impacts of declines in these understudied taxa are not well understood. Despite this limited understanding, literature reviews suggest that the number of biodiversity estimate studies are declining (Craven et al. 2019), with theoretical research outweighing these basic empirical studies (McCain and Garfinkel 2021). For areas of the world that are well sampled and well understood, the biodiversity estimates that we have may not be useful to the questions being asked (e.g. how do we assess the functioning of an ecosystem if we only have an abundance estimate?) (Rapacciuolo et al. 2019, McCain and Garfinkel 2021). Additionally, the biodiversity estimate that we have may not be comparable to other locations (e.g. how can we compare a species rich area to an area of high abundance?) (Rapacciuolo et al. 2019, McCain and Garfinkel 2021). These difficult questions and comparisons of biodiversity estimates are critical to understanding the importance of different ecosystems and making general conservation decisions across regions and taxa.

A baseline understanding of biodiversity is still needed in many parts of the world to understand how and where organisms exist, and how that may change in the future (Butchart et al. 2010, Asaad et al. 2017). Conservation measures are useless if we do not first create a baseline from which to assess progress (Lawton et al. (1998); Rapacciuolo et al. 2019, Kharouba et al. 2019, McCain and Garfinkel 2021). Furthermore, simple estimates of species richness can therefore allow for further tests to be done in the future.

Thesis objectives

My PhD research is motivated by the desire to better understand insect ecology with the hope of being able mitigate threats to insects from climate change. To do so, I investigate how multiple insect groups respond to climate through space and time at various scales. I investigate two types of ecological responses: species richness and morphological traits (colour paleness and body size).

In Chapter 1, I conduct a meta-analysis to determine if there is a global pattern in insect species richness change with elevation. In Chapter 2, I use data collections from the Leaf Litter Arthropods of MesoAmerica (LLAMA project) to focus on a region at high risk of climatic change (neotropical montane environments). In this chapter I determine how weevil species richness changes with elevation, and what factors are associated with the pattern. In chapter 3, I image museum specimens to quantify how colour paleness and body size of Canadian butterfly species are responding to climate change.

Chapter 1

30 years of terrestrial insect richness patterns across elevation: What have we learned? A global meta-analysis

The following thesis chapter is a modified version of a manuscript that is accepted at the *Journal of Animal Ecology*. The article was modified to meet thesis formatting requirements. My contributions to this thesis chapter are the conceptions of ideas, design of methodologies, data collection, data analysis, writing, and editing.

Article Citation: S. J. Dolson & H. M. Kharouba. 30 years of terrestrial insect richness patterns across elevation: What have we learned? A global meta-analysis. In press at *Journal of Animal Ecology* (*JAE-2023-00693*).

1.1 Abstract

Understanding elevation variation in biodiversity is a classic question in ecology and has implications for understanding climate change impacts on mountain ecosystems. While insects are the largest group of animals, the global trend in insect species richness with elevation is unknown. To date, single studies and taxa-specific syntheses have provided no single picture, finding variable patterns of insect richness with elevation. A global synthesis across systems would provide a better understanding of how insect species richness changes with elevation, and the possible environmental correlates of those patterns. I used published studies of terrestrial insect elevation gradients from 1990-2020 to ask: How does insect species richness change with elevation, and which environmental factors best explain this relationship statistically? With 1487 sites spanning 151 species richness-elevation gradients from 80 studies from 4 diverse insect taxonomic groups and 5 biomes, I found that overall proportional richness reached a low elevation plateau and then decreased. I also show that mean annual temperature and seasonality best explained this trend. Amongst these conclusions, I suggest best practices and areas of interest for the future of insect richness elevation studies, including underrepresented groups, geographic areas, and more standardized methods.

1.2 Contributions to the Advancement of Knowledge

Ecological patterns across elevation have been long-studied in ecology. To date, most of these studies have focused on terrestrial plants and well-studied animals. Previous syntheses for these groups show clear taxa-specific patterns (McCain & Grytnes, 2010). Despite being the largest group of animals, the global trend in insect species richness with elevation is unknown. To date, single studies and taxa-specific syntheses have provided no consensus. For example, a synthesis of one group of moths found species richness peaked at mid-elevations (Beck et al. 2017), but in other groups, like ants, richness is found to decline with elevation (Smith 2015). My study was conducted across a broad spatial scale with a large dataset for many taxonomic groups which allowed us to rigorously evaluate the species richness-elevation pattern, a fundamental question in ecology. Despite this long history of research, richness-elevation studies across regions and taxa continue to be done today, but without a synthesis of this knowledge questions remain. My goal was therefore to ask: has a consistent pattern been found across insects? I found that overall proportional richness reached a low elevation plateau and then decreased. I also show that mean annual temperature and seasonality are associated with this trend.

1.3 Introduction

Understanding spatial variation in biodiversity is a fundamental question in ecology and has implications for understanding climate change impacts on ecosystems (e.g. Huston 1979). Species are particularly vulnerable to extirpation from climate change in ecosystems across elevation gradients (Nogués-Bravo et al. 2007, Elsen and Tingley 2015, Perrigo et al. 2019, Mamantov et al. 2021). Mountain systems may be warming faster than other areas (Nogués-Bravo et al. 2007; Shah et al. 2020; Pepin et al. 2022), and furthermore, geographic barriers at the tops and bottoms of mountains (e.g., water bodies and increased fragmentation of habitats) mean that organisms cannot migrate higher or lower to track shifts in abiotic conditions (Colwell et al. 2008, Antonelli et al. 2018, McCain and Garfinkel 2021). Understanding how and why species richness is related to elevation can help conservation managers prioritize areas that are most likely to be vulnerable to climate change impacts (Perrigo et al. 2019).

Elevation patterns in species richness have been studied extensively in many taxonomic groups over recent decades (Sanders and Rahbek 2012, Rahbek et al. 2019). For some groups, global patterns of species richness have been observed, and can be classified into four general patterns: decreasing richness, low elevation plateau with decreasing richness, low elevation plateau with a mid-elevation peak in richness, and mid-elevation peak in richness (Figure 1.1; Colwell et al. 2004, McCain & Grytnes 2010). Studies show that the species richness of small mammals peaks at mid-elevations, whereas reptile richness typically decreases with elevation (McCain & Grytnes 2010). Comparatively, there is variability in the richness~elevation relationship for other groups. For example, in bats and birds, patterns of both decreasing richness and mid-elevation peaks in richness have been found (McCain & Grytnes 2010).

The evidence for a single elevation pattern of species richness for the largest group of animals, the insects, is ambiguous. The two most recent syntheses for this group were done by McCoy (1990) and Werenkraut & Ruggiero (2011). Using 20 studies, McCoy (1990) found weak, taxa-specific patterns, but no overall trend. Werenkraut & Ruggiero (2011) assessed all arthropods, found a predominance of hump-shaped patterns through visual assessment, and encouraged more robust tests of these patterns. More recently, Beck et al. (2017) in a meta-analysis on geometrid moths, found that the majority of studies found a mid-elevation peak in moth species richness. Similarly, Longino & Branstetter (2019) found a low elevation peak in species richness across Central American ants. However, there is also evidence for decreases in insect species richness with elevation from individual studies (e.g., Szewcyk & McCain 2016) and a global meta-analysis on ants

(Smith 2015). Considered together, the evidence to date does not provide a clear picture of how insect species richness varies across elevation gradients.

Here I synthesize 30 years of published insect richness and elevation studies to determine whether there is a global trend in the insect species richness-elevation relationship. I then ask whether any resulting pattern is robust to differences across studies in geography (e.g., biome or latitude), taxa, methodology (e.g., highest elevation sampled), and which environmental factors are correlated with the richness-elevation relationship (Table 1.1). For example, habitat complexity typically decreases across large mountains in temperate biomes but increases across smaller mountains in tropical biomes (McCain & Grytnes, 2010; Smith 2018). This mechanism may lead to negative richness-elevation relationships in temperate biomes but positive relationships in tropical biomes. Patterns may also differ for insect taxonomic groups based on variation in physiological and ecological traits (for example, both Lepidoptera and beetles are speciose and widespread, but Lepidoptera are more specialized in their ecology than beetles; Peters et al. 2016). Methodological factors can impact how many, and where, species are found (McCain & Grytnes 2010). Finally, I test some of the environmental factors most frequently associated with richness to find what factors are related to the pattern. For example, I considered climatic factors like temperature, precipitation, and seasonality, which are known to be important in triggering life events or for general homeostasis for insects (Fick & Hijmans 2017), as well as a vegetation index, which quantifies greenness and is thus important for insect habitat quality (Sweet et al. 2015).

1.4 Materials and Methods

1.4.1 Literature search

To determine whether there is a global trend in the insect species richness-elevation relationship, I completed a systematic literature review in June 2019 using the Web of Science database with the following search terms: "TS = (insect AND richness AND elevation)". Follow-up searches were conducted using the following keywords: “arthropod”, “diversity”, and “altitude”. The final search was conducted in April 2020. I reviewed articles published from 1990-2023. This timeframe was chosen because it captures new studies since the McCoy (1990) meta-analysis evaluating insect richness-elevation gradients, and because there are relatively few studies done before 1990. This search generated approximately 1000 studies. Reviews on a previous version of this manuscript suggested an additional round of searching for manuscripts, so out of due diligence I performed another search to check for additional papers in February 2023. Furthermore, I went through the papers cited by Beck et al. (2017), and Werenkraut & Ruggiero (2011).

I then only included studies that (1) surveyed insects, (2) published or shared terrestrial insect species richness data across elevation, and (3) used elevation as an independent factor affecting richness (e.g., an elevation transect was used). These three criteria led to the exclusion of studies using pooled elevation and richness data that was evidently collected across several mountain ranges or regions without being separated into different gradients. In addition, I required that studies (4) surveyed at least five elevation sites, (5) had no obvious sign of elevation bias in sampling intensity or methods, and (6) used some measure of taxonomic or morphological species richness (taxonomic species names, morphospecies, or barcode index numbers (BINs)). Based on these criteria, I found 80 suitable papers (Figure 1.2, Appendix 1.1).

1.4.2 Data structure

To maximize sample size, I took steps to differentiate studies to the smallest possible unit: a single gradient (elevation transect) per insect order. First, sampling collections that occurred across different elevation gradients within the same study (30% of all studies) were divided into separate gradients for analysis. On average, each study comprised two gradients, with a mean of seven sites per gradient. Second, studies that included multiple taxa (5% of all studies), were divided into separate gradients according to insect order.

1.4.3 Data collection and covariates

I extracted richness and elevation data from each site directly from the papers or contacted the authors to request the data. For each gradient, I extracted or calculated three types of covariates (Table 1.1): (1) taxonomic: order-level taxonomic identification; (2) location: biome and latitude (latitude and biome were taken from the paper or estimated based on location information provided); and (3) methodological: highest elevation sampled.

1.4.4 Environmental dataset

To test some of the most frequently hypothesized drivers of richness to find what variables are associated with the pattern, I extracted site-level abiotic and biotic data for all gradients with geographic coordinates for all sites. For 33 gradients from 20 studies, I was able to extract latitude and longitude and environmental data.

I chose variables that are widely considered to influence species richness, vary across elevation, and for which data were available for all gradients. Broad scale climate variables such as mean annual temperature, total annual precipitation, and temperature seasonality are all important variables for general homeostasis for insects (Andrew & Hughes 2005, Beck et al. 2017). I extracted these at the 30 second (1 km^2) resolution from WorldClim2 (Fick and Hijmans 2017) in R version 4.0.3 (R Core Team 2013) using the "Raster" package (Hijmans and Etten 2020). WorldClim computes temperature seasonality as the standard deviation of the mean annual temperature estimate (standard deviation x 100).

Normalized difference vegetation index (NDVI) quantifies vegetation greenness and is used to assess plant density (Table 1.1). NDVI is associated with species richness because it tends to be correlated with biotic production and plant diversity (Fernandez-Tizon et al. 2020). These factors represent resources for insects (Fernandez-Tizon et al. 2020) and therefore positively impact insect biomass (Sweet et al. 2015). I extracted NDVI through ArcGIS 10.8.1 using a Landsat thematic mapper dataset LC8NDVI (*U.S. Geological Survey*). To estimate NDVI that mostly closely matches the seasonal time of insect collection, I extracted a single NDVI value from the month and year that was nearest to the sampling of the final collections (*U.S. Geological Survey*).

1.4.5 Statistical analysis

The analysis was divided into two sections: (1) assessing the overall richness-elevation relationship through statistical analysis and visual classification to determine the overall proportional change in richness across elevation; (2) evaluating the influence of methodological and environmental factors on this pattern to determine what environmental variables are associated with the richness-elevation relationship. I used linear mixed-effects models throughout the analysis. Mixed effects models were performed in R (R Core Team 2013) using the “lme4” package (Bates et al. 2015). I used Maximum Likelihood estimation when comparing models with the same random effects and optimized the model using “optim” from “lme4” (Bates et al. 2015). In final models, I used restricted maximum likelihood (REML) for parameter estimation. Model selection was based on Akaike’s Information Criterion (e.g., dAIC > 2) with the final models being those that were the most parsimonious (e.g., lowest AIC) (Burnham and Anderson 2002), and p-values from analysis of variance tests (ANOVA). To minimize scale differences between gradients, species richness was z-transformed within each gradient ((x_i – mean)/standard deviation). The choice to z-standardize the variables was to ensure that we eliminate the bias associated with different taxa and/or studies having hugely different richness variables that would skew the overall pattern (e.g., investigating a Colopteran assemblage will likely show much higher richness variables than a Lepidopteran assemblage). Therefore, species richness patterns we present represent proportional richness changes rather than absolute values. I report both the conditional R^2 ($R^2_{(C)}$) (it accounts for variance explained by both the fixed and random effects), and the marginal R^2 ($R^2_{(M)}$) (it accounts for variance explained by the fixed effects) using the R package “MuMIn” (Nakagawa and Schielzeth 2013, Barton 2022). All statistical analysis were done in R version 4.0.3 (R Core Team 2013).

i) Richness-elevation relationship

I analyzed the relationship between richness and elevation in two steps. First, I determined the overall relationship between species richness and elevation. I tested for the presence of a peak in the richness-elevation relationship by evaluating whether the fit of a quadratic model was better than a linear model based on dAIC. To account for the non-independence of data points along each elevation gradients and among multiple gradients in the same study (30% of studies), I included study and gradient as random effects, with gradient nested within study. Slopes were also allowed to vary among studies and gradients.

Second, I investigated whether the overall richness-elevation relationship was correlated with geographical, ecological, and methodological factors. I considered: elevation, biome, absolute latitude, taxa, and highest elevation site sampled. To test for multicollinearity, I used variance inflation factor (VIF) and used the cut-off value of VIF < 5. No variables were eliminated based on multicollinearity. The random effect structure was the same as described above.

For model selection, I used the "dredge" function of the R package "MuMIn". Dredge uses combinations of fixed effect and static random effect structure to provide the best fit of the model, as assessed by AIC, while maintaining the ability to include the largest number of predictors that may be important (Barton 2022). From dredge, I included the combination of predictors (elevation, highest elevation sampled, and latitude) that remained within dAIC < 2 of the intercept-only model (Appendix 1.2; Barton 2022). I determined the significance of each covariate in the final model using an ANOVA.

ii) Richness-environment associations

To evaluate richness-environment associations, I considered two models: 1) richness as a function of the environment; and 2) environment as a function of elevation. A separate model was fit for each environmental variable ($n = 4$) to avoid overfitting the model. All environmental variables were Z-transformed to allow direct comparisons of effect size (i.e., slope) across models. For all models, I tested for the presence of a quadratic relationship. I included gradient ID nested within sample ID as a random effect. Not every elevation site had environmental data associated with it (likely due to proximity to water), and so models were slightly reduced (temperature: $n = 301$, precipitation: $n = 293$, seasonality: $n = 293$, NDVI: $n = 316$)

I also fit richness as a function of elevation in this smaller subset using the same methods described in the previous section.

1.5 Results

1.5.1 Database description

Our search resulted in 1487 sampling sites representing 151 species richness-elevation gradients from 80 studies. The most common biome in the database was tropical (49%, 74/151 gradients; Figure 1.2, Appendix 1.3), and the most common taxonomic group was Lepidoptera (31%, 47/151 gradients; Appendix 1.3).

1.5.2 Richness-elevation relationship

The overall proportional richness-elevation relationship was best explained by a polynomial order 2 function (quadratic) (slope = $1.1\ E^{-5}$ ($1.2\ E^{-4}$ SE), quadratic coefficient = $-2.0\ E^{-7}$ ($4.0\ E^{-8}$ SE), $t_{1,1335} = -5.3$, $p < 0.01$, $R^2_{(C)} = 0.41$, $R^2_{(M)} = 0.16$; Table 1.1; Figure 1.3). The quadratic model was a better fit than a linear model (dAIC = -16.8). This quadratic pattern is consistent with a low-elevation plateau with decreasing proportional species richness (LP; Figure 1.1C; Figure 1.3).

Total gradient richness was greater on gradients with higher elevations sampled (slope = 3.8 E^{-4} (4.7 E^{-8} SE), $t_{1,139} = 8.2$, $p < 0.01$; Table 1.2). None of the other covariates (biome, absolute latitude, or taxa) explained variation in richness after accounting for elevation (Table 1.2).

1.5.3 Richness-environment relationship

Not all environmental predictors varied with elevation. Temperature decreased with elevation (slope = -9.2 E^{-4} (1.0 E^{-4} SE), $t_{1,266} = -9.0$, $p < 0.01$, $R^2_{(C)} = 0.77$, $R^2_{(M)} = 0.47$; Figure 1.4A). There was no relationship between elevation and temperature seasonality (slope = -3.4 E^{-4} (1.8 E^{-4} SE); $t_{1,258} = -1.9$, $p = 0.06$, $R^2_{(C)} = 0.55$, $R^2_{(M)} = 0.08$; Figure 1.4C), precipitation (slope = 6.9 E^{-5} (2.3 E^{-4} SE); $t_{1,258} = 0.30$, $p = 0.76$, $R^2_{(C)} = 0.73$, $R^2_{(M)} < 0.01$), or NDVI (slope = -1.3 E^{-4} (1.6 E^{-4} SE), $t_{1,281} = -0.81$, $p = 0.42$, $R^2_{(C)} = 0.48$, $R^2_{(M)} = 0.01$; Appendix 1.4).

The environmental variables that best predicted proportional species richness were mean annual temperature and temperature seasonality. Richness increased with mean annual temperature (slope = 0.21 (0.06 SE); $t_{1,266} = 3.4$, $p < 0.01$, $R^2_{(C)} = 0.59$, $R^2_{(M)} = 0.03$; Figure 1.4B), and seasonality (slope = 0.18 (0.05 SE); $t_{1,258} = 3.3$, $p < 0.01$, $R^2_{(C)} = 0.55$, $R^2_{(M)} = 0.02$; Figure 1.4D) but was not predicted by NDVI (slope = -0.09 (0.10 SE), $t_{281} = -0.89$, $p = 0.37$, $R^2_{(C)} = 0.16$, $R^2_{(M)} = 0.01$), or total annual precipitation (slope = 0.06 (0.06 SE), $t_{258} = 0.93$, $p = 0.35$, $R^2_{(C)} = 0.59$, $R^2_{(M)} = 2.1$ E^{-3}).

In this smaller dataset of gradients with environmental data, there was no relationship between elevation and proportional richness (slope = 3.9 E^{-6} (2.0 E^{-4} SE), $t_{1,281}$ = 0.02, p = 0.98; $R^2_{(C)}$ = 0.63, $R^2_{(M)}$ = 9.9 E^{-6}; Appendix 1.5).

1.6 Discussion

Despite decades of research on animals along elevation gradients, there has not been a global synthesis of patterns of insect species richness with elevation. Here I sought to determine whether there is a global trend in the insect species richness-elevation relationship. I found that overall proportional insect richness followed a low elevation plateau, where richness continued steadily around one value, and then decreased (i.e., LP), represented by a portion of a quadratic relationship. I tested some of the most frequently hypothesized predictors of richness to find what variables are associated with the pattern. I found that both mean annual temperature and temperature seasonality were associated with proportional species richness across elevation.

I found that overall proportional insect species richness followed a low elevation plateau with a decrease in species richness. This quadratic-type pattern is consistent with many richness-elevation studies that found mid-elevation peaks in richness, including geometrid moths (Beck et al. 2017), a literature review on insects (McCoy 1990), and other taxa including plants and small mammals (McCain & Grytnes 2010). Low elevation plateau with decreasing richness is one of the less common patterns in elevation richness found, with only a few documented cases in birds, reptiles, plants, and arthropods (McCain & Grytnes 2010; Werenkraut & Ruggiero 2011). This pattern could be less common because of general challenges in quantifying the exact shape of the pattern.

The hypothesis that there is a peaked relationship (Figure 1.1BCD) in insect richness across elevation is difficult to reject with confidence because the shape of the relationship depends on the percent or portion of the gradient sampled (Rahbek 2005, Werenkraut & Ruggiero 2011). For example, a mid-elevation peak in richness may look like a linear decrease in richness if only the top half of a gradient was sampled (Nogués-Bravo et al. 2008). Indeed, I found that gradients with higher sampled elevations had higher overall proportional species richness. This is likely due to studies with higher sampled elevations having surveyed more of the gradient and therefore greater variation and total richness.

Further, estimates of diversity will almost always increase with spatial scale (Chase et al. 2018). For example, a larger or higher mountain has more space, and therefore just by chance will likely have more species (more immigration, more niches, less disturbance, etc.; MacArthur & Wilson 1967). If a higher elevation was sampled, it likely represented a larger mountain and therefore more species. It is also challenging to define the base of a mountain, especially when they are part of a larger mountain range, or not a clearly defined conical mountain. Many of the gradients in this study likely do not have clear mountain bases, or areas that plateau and can be deemed the base, so it is also likely that portions of the lowest elevation sites were not sampled at all. Without better sampling at lower elevations and consistent methodology among studies, it is difficult to know why I found a low elevation plateau pattern. These artefacts of elevation studies can be hard to avoid and I encourage future researchers to keep this in mind when planning surveys.

I found that elevation variation in proportional species richness was most correlated with temperature and seasonality. There were higher proportional species at warmer elevations and elevations with greater seasonal variation in temperature. Warmer temperatures may result in higher species richness through higher metabolic rates and therefore faster ecological and evolutionary processes (e.g., carrying capacity, speciation rates; Brown et al. 2004). While seasonality was related to richness, seasonality was not found to vary across elevation in this global analysis likely due to large variation in seasonality across regions. For example, at low elevations, seasonality is high in temperate regions (50°C annual temperature range) but low in tropical environments (20°C annual temperature range) (Sheldon et al. 2018). Increased seasonality could be associated with higher species richness because of the greater range of potential optimal conditions available with predictable seasonal oscillations in temperature, resulting in a greater number of adaptive strategies and thus more species (Tonkin et al. 2017). Additionally, temperature and seasonality likely both contributed indirectly by limiting the physiology, or ranges of individuals (Fernandez-Tizon et al. 2020).

Despite temperature being most favorable at low elevations (i.e., warmer), I found a low-elevation plateau in richness rather than richness being highest at the lowest elevations. There are a few potential explanations. First, it could be that other factors constrain richness at lower elevations. Though temperature and seasonality were the strongest correlates of proportional richness, they were a poor fit (Figure 1.4). There was a large range in seasonality at low elevations, reducing its predictability of richness at lower elevations. It could also be that mid-elevations have the largest number of overlapping species' distributions just by chance due to boundaries at bottoms and tops of elevation gradients (such as water, disturbance, or no further land; i.e., the mid-domain effect (MDE); Colwell & Lees 2000, Colwell et al. 2004, Letten et al. 2013). Empirical support for the hypothesis has shown MDE as a likely contributor to mid-elevation peaks in richness across taxa (Colwell et al. 2004, Dunn et al. 2007, Colwell et al. 2016, Macek et al. 2021). The MDE is difficult to test in a large scale study such as ours because a strong test of the hypothesis requires distribution data of each species or their landscape—data that were not available in these community-level studies (Colwell et al. 2004, Dunn et al. 2007).

Second, evolutionary and historical effects could play a key role in predicting where species are and the environmental factors they experience. There is evidence supporting this hypothesis across mountain systems where species towards the richness peaks in elevation are more phylogenetically closely related than expected by chance (Machac et al. 2011, Smith et al. 2014, Dolson et al. 2021). It remains difficult to determine how this translates to richness-elevation patterns (McCain & Grytnes 2010). Nonetheless, it is likely that in addition to current abiotic conditions, historic niche constraints could be structuring these communities (e.g., niche conservatism; Dolson et al. 2021).

Alternatively, it could be that I have underestimated richness from low to mid-elevations and that the 'true' pattern is a linear decrease with elevation (Figure 1.1), which would be more consistent with the richness-environment associations I found. Low elevation sites are often under-sampled due to the human disturbance of many lowland sites (Nogués-Bravo et al. 2008), leading to an underestimation of richness at lower elevations.

I found no statistical evidence that either total annual precipitation or NDVI influenced proportional species richness. It could be that both variables are acting on a more regional or local scale, and thus not an important environmental association at a coarser resolution and larger spatial extent such as ours. Both variables are known to have differing effects on insect communities depending on more regional/local factors like proximity to oceans, insularity, latitude, on coasts, or with disturbance (for NDVI) (Körner 2007). For example, islands typically receive higher rainfall than mainland populations based on the surrounding oceans. Given this global analysis with coarser data, I was less likely to detect potentially important local-scale variation. Following recent work (Beck et al. 2017; Bärtschi et al. 2019; Dolson et al. 2021), I encourage future researchers to investigate whether there are any broad-scale patterns in the way these local-scale factors influence insect species richness.

While I found an overall trend in how insect species richness changes across elevation, I found three gaps in this knowledge: some insect taxonomic groups (e.g., Hemiptera or Orthoptera) and some particular regions (e.g., North America, Africa) were underrepresented, and a lack of standardization in methodology across studies was evident. With gaps in the knowledge of species diversity, the ability to conserve or even understand diversity will be limited. Therefore, I propose that future researchers should focus on three research areas and/or approaches. First, researchers should use collection methods that are broad and encourage by-catch, like leaf-litter sifting, pitfalls, or malaise traps (Smith et al. 2014, Spears and Ramirez 2015). Though specialized methods are beneficial for detecting rare species, broad methods will help to target a wide array of taxa including ones that were underrepresented here. Alternatively, researchers could focus on underrepresented groups and use more specialized methods (e.g., sweep netting or branch beating [Moir et al. 2005]). Methods like DNA barcoding and Barcode Index Numbers could help to get a coarse estimate of species richness, given the taxonomic impediments associated with underrepresented and undersampled taxonomic groups (Dolson et al. 2021).

Next, to facilitate future comparisons across gradients, when possible, researchers should survey the same gradient for different taxa or standardize sampling regimes across multiple gradients when surveying the same taxa. Examples of replicative studies and programs already in place include Lifeplan (www2.helsinki.fi/en/projects/lifeplan), BOLD (www.boldsystems.org/), and GBIF (www.gbif.org/). More standardized methods across regions and gradients would enable more direct comparisons of how certain regions or taxa relate to environmental variation. Finally, more studies are needed in North America, Oceania, and Africa, and in alpine and desert biomes, which were the least sampled regions and biomes. With species in high elevation habitats at high risk of being extirpated due to climate change throughout the world (Nogués-Bravo et al. 2007), documenting patterns of richness across elevation in these often drier regions will be needed for robust predictions of climate change impacts on these communities. I hope that a better understanding of montane insect communities will help to conserve these environments in the context of climate change.

1.7 Tables

Table 1.1. The rationale and type of data for all covariates that were extracted for each gradient, other than elevation and richness, and the environmental variables used in my environmental dataset.

	Covariate	**Variable description**	**Rationale**	**Reference**
Taxonomic	Taxa	Order-level taxonomic identification, four categories: Hymenoptera, Lepidoptera, Diptera, Coleoptera	Taxa will respond to abiotic factors differently based on differences in physiological and ecological traits.	Staunton *et al.* 2011, Peters *et al.* 2016
Location	Latitude	Absolute latitude of the elevation gradient	Abiotic and biotic factors vary with latitude (e.g., hotter in low latitudes, colder in high latitudes). Consequently, there are global patterns of species richness with latitude.	Gaston 2007
	Biome	Five general biome categories: Tropical, Temperate, Mediterranean, Desert, and Alpine	Biomes are large scale zones of biotic and abiotic conditions, and thus are defined by the flora and fauna that exist within them. Therefore, species in different biomes may respond to elevation differently.	Stein *et al.* 2014
Methodological	Highest elevation sampled	Highest site that was sampled (masl)	A measure of how large the elevation gradient was, and possibly how much abiotic change occurs. For example, smaller mountains typically have less variation in abiotic factors.	Lomolino 2001
Environmental	Normalized difference vegetation index (NDVI)	Measure of greenness through surface reflectance satellite images	NDVI quantifies vegetation greenness and is therefore a good proxy for biotic production and plant density in areas.	U.S. Geological Survey
	Mean annual temperature	Mean temperature at each site (°C)	Given its importance in maintaining homeostasis, temperature is a key mechanism influencing species richness, especially for ectotherms like insects.	Fick & Hijmans 2017; González-Tokman *et al.* 2020
	Seasonality	Standard deviation of mean temperature x 100	Seasonality has been shown to be related to species richness through habitat heterogeneity and specializations to individual seasons within and across systems	González-Tokman *et al.* 2020
	Total annual precipitation	Total precipitation at each site (mm)	Like temperature, precipitation is important for maintaining homeostasis	Fick & Hijmans 2017

Table 1.2. The relative importance of different methdological and location factors correlated with insect species richness along elevation gradients in the final model. Shown are the results for the full dataset (n = 151 gradients) and the temperature dataset (n = 33 gradients). Significant covariates are bolded ($p < 0.05$). The response variable in all models was species richness. In the full dataset, the linear model has no covariates, the quadratic model had highest elevation sampled and latitude as covariates. In the environmental dataset, the linear and quadratic model had no covariates. The linear models with environmental variables were run as separate models to avoid overfitting.

Dataset	Model Type	Predictor	Coefficient (Standard Error)	t-Value	Denominator DF*	P-Value	R^2 (Conditional, Marginal)
Full	Linear	**Elevation**	**-5.0 E^{-4} (8.6 E^{-5})**	**-5.8**	**1337**	**<0.01**	**0.48, 0.15**
	Quadratic	Elevation	1.1 E^{-5} (1.2 E^{-4})	0.09	1335	0.93	**0.41, 0.16**
		Elevation^2	**-2.0 E^{-7} (4.0 E^{-8})**	**-5.3**	**1335**	**<0.01**	
		Highest	**3.8 E^{-4} (4.7 E^{-8})**	**8.2**	**139**	**<0.01**	
		Latitude	2.2 E^{-3} (2.3 E^{-3})	0.98	1335	0.33	
Environmental	Linear**	Elevation	3.9 E^{-5} (2.0 E^{-6})	0.02	281	0.98	0.63, 9.9 E^{-6}
		Temperature	**0.21 (0.06)**	**3.4**	**266**	**<0.01**	**0.59, 0.03**
		Precipitation	0.06 (0.06)	0.93	258	0.35	0.59, 0.01
		Temperature seasonality	**0.18 (0.05)**	**3.3**	**258**	**<0.01**	**0.55, 0.02**
		NDVI	-0.09 (0.10)	-0.89	281	0.37	0.16, 8.7 E^{-3}
	Quadratic	Elevation	3.7 E^{-4} (3.0 E^{-4})	1.2	280	0.23	0.62, 0.01
		Elevation^2	-1.1 E^{-7} (1.0 E^{-7})	-1.6	280	0.11	

*Numerator was 1 in all cases
**Environmental dataset model with environmental predictors run individually to avoid overfitting.

1.8 Figures

Figure 1.1. Global patterns of species richness with elevation can be classified into four general patterns: (A) decreasing richness (LD), (B) low elevation plateau, with low-elevation peak in richness (LPMP), (C) low elevation plateau with decreasing richness (LP), and (D) mid-elevation peak in richness (MP) (McCain & Grytnes, 2010).

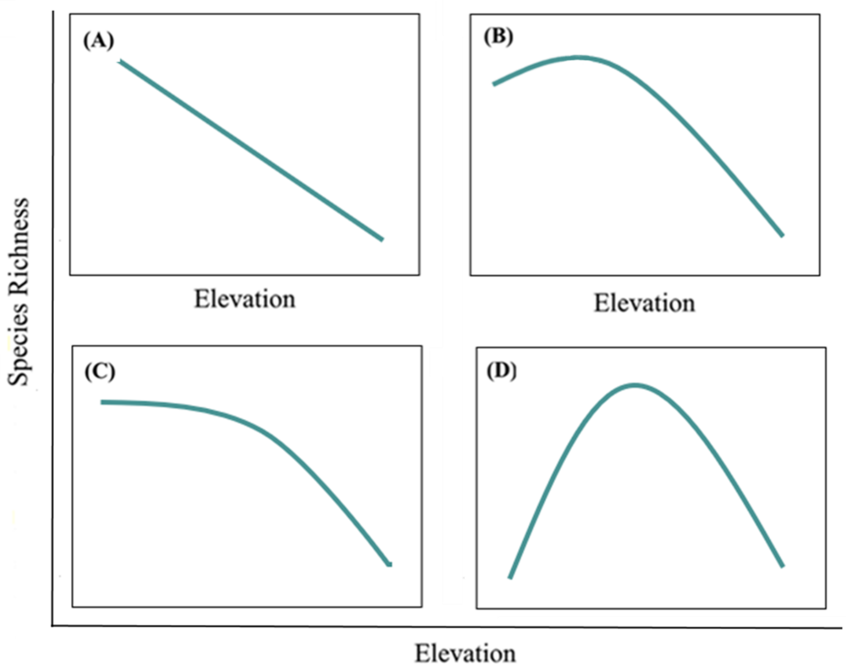


Figure 1.2. Locations of the 151 gradients from 80 elevation studies of insect species richness. The 33 elevation gradients (20 studies) from which I extracted environmental data are represented by triangle symbols. The map shows which biome each site represents. Latitude and longitude were estimated for each study based on sampling localities. Gradients that reached very high elevations or were located within large mountain ranges or at high latitudes were designated Alpine.

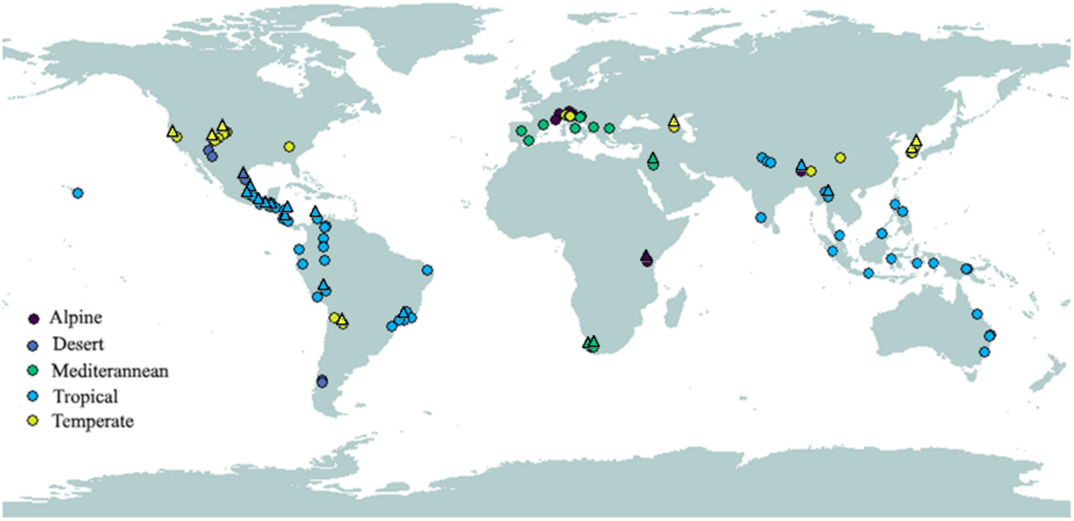


Figure 1.3. The relationship between z-standardized insect species richness and elevation (meters above sea level – masl) across all 151 elevation gradients. Coloured points and thin lines represent individual gradients. Individual fit lines were produced by predicting the fits of the random effects based on the overall model. The overall line of best fit across all gradients is shown in black, with the grey shading indicating the 95% prediction interval (slope = 1.1 E^{-5} (1.2 E^{-4} SE), quadratic coefficient = -2.0 E^{-7} (4.0 E^{-8} SE), $t_{1,1335}$ = -5.3, $p < 0.01$, $R^2_{(C)} = 0.41$, $R^2_{(M)} = 0.16$).

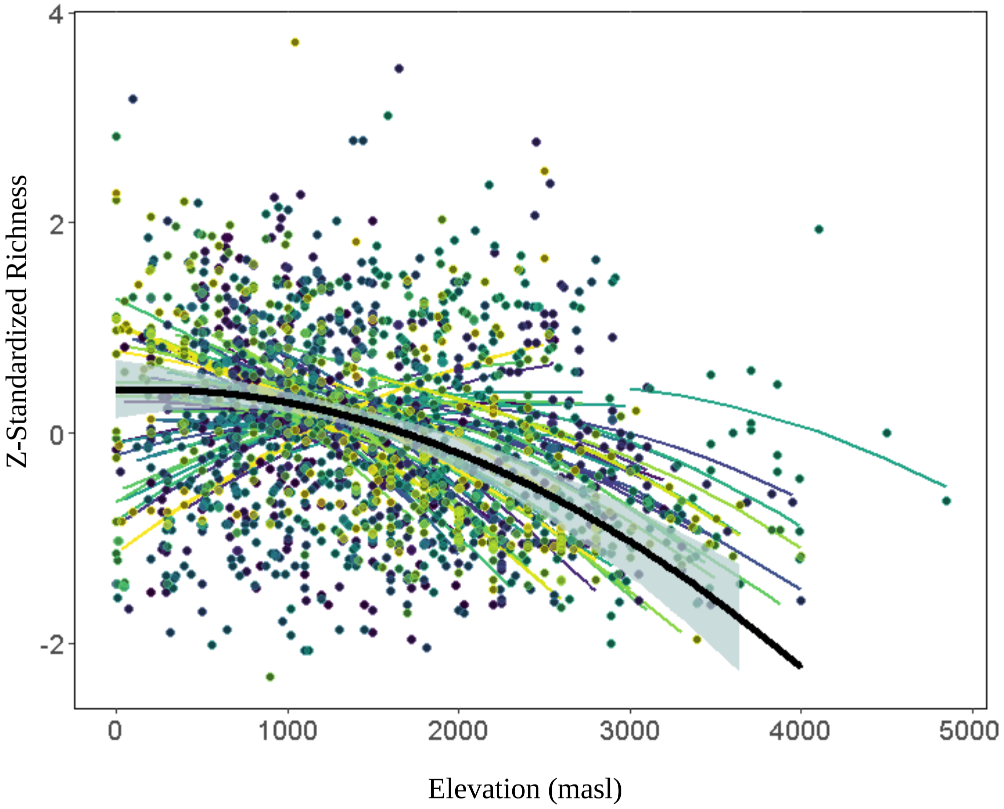
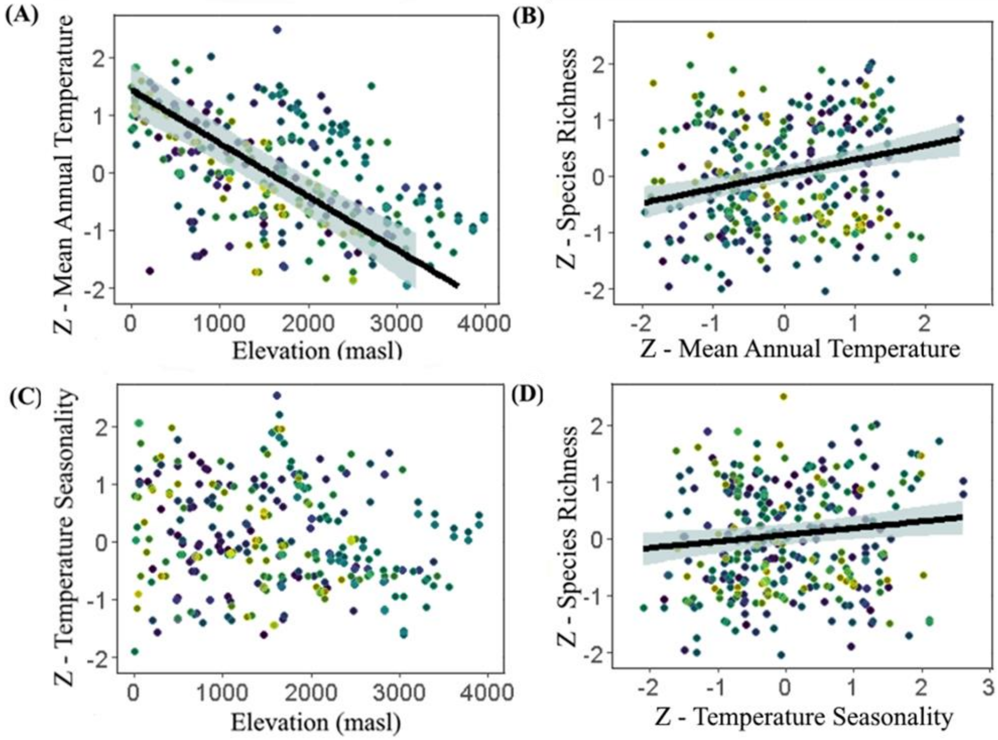


Figure 1.4. The richness-environment relationships (n = 33 gradients). First column represents the environment-elevation relationship and the second column represents the richness-environment relationship with mean annual temperature shown in the top row and temperature seasonality shown in the bottom row. (A) Z-standardized temperature decreased with elevation (slope = -9.2 E^{-4} (1.0 E^{-4} SE), $t_{1,266}$ = -9.0, $p < 0.01$, $R^2_{(C)}$ = 0.77, $R^2_{(M)}$= 0.47). (B) Z-standardized species richness increased with z-standardized mean annual temperature (slope = 0.21 (0.06 SE); $t_{1,266}$ = 3.4, $p < 0.01$, $R^2_{(C)}$= 0.59, $R^2_{(M)}$ = 0.03). (C) Z-standardized temperature seasonality was not related to elevation (slope = -3.4 E^{-4} (1.8 E^{-4} SE); $t_{1,258}$ = -1.9, p = 0.06, $R^2_{(C)}$ = 0.55, $R^2_{(M)}$ = 0.08). (D) Z-standardized species richness increases with z-standardized temperature seasonality (slope = 0.18 (0.05 SE), $t_{1,258}$ = 3.3, $p < 0.01$, $R^2_{(C)}$ = 0.55, $R^2_{(M)}$ = 0.02). In all panels, coloured points indicate different gradients, best fit lines are shown in black, and the grey shading indicates the 95% prediction interval. There is no relationship in between seasonality and elevation (C) so no best fit line is shown.



Chapter 2

Higher weevil (Coleoptera: Curculionidae) species diversity in cooler, wetter elevations in Central America

The following thesis chapter includes a portion of data and analysis which contributed to a chapter for the book “Insect Decline and Conservation in the Neotropics” edited by J. L. León-Cortés and A. Córdoba-Aguilar. This chapter was completed in collaboration with Dr. Robert Anderson at the Museum of Nature, Canada. The following thesis chapter includes additional data from other regions and more analysis. My contributions to this thesis chapter are the conceptions of ideas, design of methodologies, data collection, data analysis, writing, and editing.

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2.1 Abstract

Studies on elevation gradients in tropical montane ecosystems of central America have shown a variety of patterns in insect diversity. It remains unclear whether this variation is related to ecological strategy or diversity of the group as hyperdiverse groups remain poorly studied. Here I use a large dataset of over 32,000 specimens across 25 sites in the tropical mountain forests of Central America to investigate elevational patterns in richness and abundance and the environmental variables associated with them (such as temperature, humidity, seasonality, surface area, or primary production) in one of the largest groups of insects: the weevils (Coleoptera: Curculionidae). From sea level to 2500 m, I found that both morphospecies richness and abundance was highest in high elevation forests. This study represents one of the few instances of a group of animals which increases in species richness with elevation. This finding suggests that general diversity patterns along elevational gradients may not be as applicable for hyperdiverse groups. Elevations with cooler spring temperature, smaller surface area, and higher relative humidity were associated with higher richness. Climate change in this region is predicted to lead to warmer and drier forests, threatening the high diversity of those high elevation forests.

2.2 Contributions to the Advancement of Knowledge

A variety of richness-elevation patterns have been found across tropical montane ecosystems. It is unclear if this variation is due to differences in taxa, ecology, or diversity because hyperdiverse groups are poorly studied here. Therefore, I evaluated richness patterns in weevils, a hyperdiverse group of insects, across elevation in Central

America. I found that weevil richness increased linearly with elevation. This study represents one of the few instances of a group of animals which increases in species richness with elevation. These findings contribute evidence to the presence of unique patterns across elevation, indicating that diversity patterns along elevational gradients may not be as applicable for hyperdiverse groups. I also found that elevations with cooler spring temperature, smaller surface area, and higher relative humidity were associated with higher richness. This helps to understand more clearly these unique, cool and moist high elevation environments which are often thought to be less diverse.

2.3 Introduction

Most of the world's biodiversity is in the tropics, yet we have a limited understanding of the ecology, biology, and distribution of most tropical species (Barlow et al. 2018, Janzen and Hallwachs 2019). Tropical organisms are predicted to be more at risk from climate change than temperate species (García-Robledo et al. 2016, Jørgensen et al. 2022) due to environmental (e.g., naturally lower climatic variability in the tropics) and biological (e.g., narrower thermal tolerances, closer proximity to their thermal optima) factors. Smaller climatic changes have had, and will have, disproportionately larger effect on tropical species compared to temperate species, pushing them to, or beyond, the limits of their range or thermal capacities (García-Robledo et al. 2016, Sheldon et al. 2018, Perrigo et al. 2019, Mamantov et al. 2021, McCain and Garfinkel 2021). The limited understanding of tropical species means that the implications of these impacts for these communities and ecosystems are unclear (Mora et al. 2013, Barlow et al. 2018, Smith et al. 2023).

In addition to climate change, there are strong climate gradients across space, particularly across elevation, which leads to variation in the insect communities found across them. A recent global meta-analysis demonstrated that insect richness is on average highest at low elevations and then after a plateau begins to decrease with elevation (Chapter 1). However, there was unexplained variability in this pattern across gradients and studies (Chapter 1). In the tropics especially, studies have found a variety of insect richness responses to elevation. For example, there has been a lot of variation across taxonomic groups in the tropics: mid-elevation richness peaks have been found in ants and moths (Smith et al. 2014, Bärtschi et al. 2019, Longino and Branstetter 2019), decreases in richness in moths and scarab beetles (Rodríguez-Castañeda et al. 2010, Noriega and Realpe 2018), but richness increases in two hyperdiverse taxa: rove beetles and wasps (Mumladze et al. 2017; Dolson et al. 2021), or no relationship such as in spiders (Dolson et al. 2020). This variation makes it difficult to understand how insects in this region will respond to changing climates. For example, groups with high richness at the cool and wet peaks of these mountains (like the wasps - Mumladze et al. (2017)) are at risk because climate change will bring warmer and drier climates upwards, shrinking and eliminating habitats at the peaks. Groups with highest richness at the warm and dry base of these mountains (like moths - Noriega and Realpe (2018)) may thrive with climatic change and/or have the potential to track shifting climates up the mountains (García-Robledo et al. 2016, Jørgensen et al. 2022).

Variation in the insect richness patterns across gradients at the global scale (Chapter 1) could also be related to system- or scale differences in the key environmental factors associated with this pattern. At a broad scale, climatic factors like temperature and seasonality are often found to be the key factors that influence elevational variation in species richness and abundance. Factors like precipitation or humidity may be more important in the tropics than in other biomes because in general the total amount of precipitation and humidity is much greater (Delhey 2019). For leaf-litter inhabiting insects like weevils, vegetation biomass may determine their local distribution due to resource and habitat availability (Fernández-Tizón et al. 2020, Liao et al. 2023). At global scales it can also be difficult to test some hypotheses due to data availability. For example, the amount of surface area available can be a key factor influencing species richness (species-area hypothesis) due to the increase in niches, resources, and colonization in larger areas (MacArthur and Wilson 1967, Smith et al. 2023). However, quantifying surface area at broad scales is difficult because it requires spatial data on surrounding habitats and dispersal distances of taxa.

Here I investigate elevational patterns in richness and abundance, and the factors underlying these patterns, in one of the largest groups of insects—the weevils (Coleoptera: Curculionidae). I focus on tropical Central America which is an area at high risk of the effects of climate change (Mora et al. 2013, Shah et al. 2020). Weevils are one of the largest families of beetles; yet for most species within this group, we do not know the general patterns of their distribution and diversity (Marshall 2006, Cardoso et al. 2011). Weevils play a critical role in many ecosystems (e.g., as herbivores, prey, or pollinators) and are economically relevant as pests and/or pest management species (Anderson and Ashe 2000, Marshall 2006). Understanding weevil diversity patterns across tropical elevation gradients is needed before weevils are lost due to environmental change (Faith 1992, Anderson and Ashe 2000).

I use weevil specimens systematically collected through the Leaf Litter Arthropods of Mesoamerica project (LLAMA) across a tropical Central American elevation gradient to ask: How does weevil species richness and abundance change across this gradient? What environmental factors are related to these patterns? With limited knowledge about weevils in this area and diversity patterns found in this region, it is difficult to predict what patterns they may follow or what factors are related to this pattern. For the most related study on a similar hyperdiverse group of beetles, staphylinid beetles, researchers showed an uncommon pattern of richness increasing with elevation, related to temperature and precipitation (Dolson et al. 2021).

2.4 Methods

2.4.1 Data collection and processing

To determine how weevil species richness and abundance change across this gradient, I used data from the Leaf Litter Arthropods of Mesoamerica (LLAMA) project (</sites.google.com/site/longinollama>; (Longino et al. 2014, Longino and Branstetter 2019)). Leaf-litter weevils were collected by LLAMA in the springs (April-June) of 2008-2011 across an elevation gradient spanning Guatemala (9 sites), Mexico (9 sites), and Nicaragua (7 sites) (n = 25 sites; Figure 2.1).

The aim of LLAMA was to evaluate elevational changes in leaf litter communities in undisturbed forests. However, it was challenging to find a continuous transect of undisturbed forest across elevation because disturbance varies with elevation in this region. Sampling sites were thus chosen with varying elevations in each country instead of a single continuous linear gradient. Sites were remote patches of forest and ranged from close to sea level to 2500 masl. There were nine low- (<1000 masl), nine mid- (1100 – 1600 masl), and seven high-elevation (1900 – 2500 masl) sites (n= 25).

The spatial delineation of each “site” is based on the sampling regime. Sampling at each site involved two transects of 50 miniwinkler samples (each representing 1 m^2 of wet forest floor litter) taken at 5 m intervals for a total of 100 samples at each site across 500 m. Miniwinkler sifters are commonly used for insects and work by separating moving leaf-litter inhabitants from decaying leaf-litter through variously sized mesh screens. Therefore, “site” refers to one elevation measurement that contains pooled samples from two 500 m transects of 100-point measurements. Samples were retained in whirlpac bags in ethanol and subsequently sorted down to weevils. More details of collection and processing methods can be found in Longino et al. (2014).

2.4.2 Weevils

Weevils were mounted and sorted to morphospecies (morphological based delineation of species). Morphospecies were classified based on differences in external morphology and in some cases based on differences in the structure of male genitalia. Morphospecies were generally able to be assigned to genus and given a unique identifier but in some cases, no generic placement was possible so both a genus and morphospecies number was assigned. Abundance was measured as the total number of individuals per site. All specimens are vouchered in the invertebrate collection of the Canadian Museum of Nature, Ottawa, Ontario, Canada.

2.4.3 Environmental variables

To determine what environmental variables are related to these richness patterns, I extracted site-level environmental data from multiple sources (Appendix 2.1). To avoid model overfitting, I focused on the variables most likely to have an impact on richness based on their known association with weevils, or other insects, and based on Central American forests.

Richness is known to be associated with temperature in a variety of ways (e.g., every species has its own thermal response curve) (Andrew and Hughes 2005, McCain and Grytness 2010, González-Tokman et al. 2020). I selected spring temperature because it reflects the conditions when these beetles are active and were collected (Longino et al. 2014). In this region, spring coincides with the start of the rainy season, which has cooler weather, more frequent rainfall, and generally more plant biomass than the dry season (Janzen 2000, Rodríguez-Castañeda et al. 2010). I extracted and calculated average ground level temperature across April, May, and June at point locations (10 m^2) from each elevation site coordinates using the package NicheMapR in R version 4.0.3 (R Core Team 2013, Kearney et al. 2021).

Additionally, I considered how temperature changes over the year (i.e., seasonality). Seasonality has been shown to increase species richness through habitat heterogeneity and ecological specialization to individual seasons within and across systems (Janzen 1967, Andrew and Hughes 2004). I extracted seasonality at the 30 second (1 km^2) resolution from Worldclim (Fick and Hijmans 2017) in R version 4.0.3 (R Core Team 2013) using the “Raster” package (Hijmans and Etten 2020). Temperature seasonality was calculated using the standard deviation of the mean annual temperature estimate (standard deviation x 100).

Relative humidity was selected because it changes rapidly with elevation in Central America and could be a limitation to insect distributions (Marshall 2006, Chen et al. 2019). I predicted that richness would increase with humidity based on previous studies of tropical beetles which show that high moisture is related to richness (Dolson et al. 2021, Smith et al. 2023). I extracted and calculated average ground level humidity across April, May, and June at point locations from each elevation site coordinates using the package NicheMapR in R version 4.0.3 (R Core Team 2013, Kearney et al. 2021).

Gross primary production (GPP) has been shown to be associated with increased animal species richness because it is a measure of plant biomass and diversity (Cusens et al. 2012, Rafique et al. 2016, Liao et al. 2023). I extracted GPP through ArcGIS 10.8.1 using a Landsat thematic mapper dataset MOD17A2H v006 (*U.S. Geological Survey*). I extracted the data available from 2009 (mean year of weevil collection) at 500 m^2 resolution (*U.S. Geological Survey*).

Finally, I tested the species-area hypothesis (MacArthur and Wilson 1967, Fattorini 2014). To determine the surface area available to organisms in each elevation band, I used a digital elevation model (DEM) created in 2000 (*U.S. Geological Survey*). I created 100 m elevation bands around each elevation collection site, such as 250 – 350 m for a site at 200 masl and extracted the surface area in these bands within a 100 km radius of the collection site (Beck et al. 2017, Dolson et al. 2020). The 100 km radius was selected based on 1) previous literature (Beck et al. 2017, Dolson et al. 2020), and 2) an assumption that, while we do not know the dispersal distances of these weevils, estimates of wind dispersed insects dispersal can be in the 100s of kms (Gandiaga & James 2023).

2.4.4 Statistical analysis

To determine how weevil species richness and abundance change across this gradient, I used a generalized linear model with a negative binomial error distribution to account for the right-skewed distribution of richness and abundance. I first tested for a peaked relationship, a common elevational pattern in animal species richness (McCain & Grytness, 2010; Chapter 1). To do this, I compared the fit of a linear model to a quadratic model based on Akaike's Information Criterion (AICc). The best model was the one that was the most parsimonious (e.g., model with the lowest AIC) and/or was more than 2 AIC units lower than the next best model (Appendix 2.2).

To test how various environmental factors influenced elevational variation in morphospecies richness and abundance, avoid collinearity, and to maximize my power, I considered the influence of environmental variables individually in separate models (n = 5) (Appendix 2.3). I found that morphospecies richness and abundance were strongly correlated using a general linear model using the log of abundance (due to the skewed abundance values - see results). Therefore, I only assessed the effect of environmental variables on richness. For each environmental variable, I fit two types of models: 1) environmental variable as a function of elevation; and 2) richness as a function of environmental variable. For all models, I tested for linearity, normality, and homoscedasticity. All models were best fit with a linear function (Appendix 2.2). For model 1, I used a normal distribution, and for model 2 I used a negative binomial error distribution based on the error distribution of richness. For the richness-environment models (i.e., model 2), I applied a Bonferroni correction to account for the testing of multiple environmental variables.

Because of the relatively broad geographic distribution of the sites (Southern Mexico to Nicaragua; Figure 2.1), I included latitude as a covariate in all models. I tested for collinearity amongst latitude and covariates in the models using variance inflation factor tests (VIF) in R (R Core Team 2013) using the "car" package (Fox and Weisberg 2019). Latitude was significantly correlated with seasonality and humidity (VIF > 5), so site was not included as a covariate in these models (Table 2.1).

All environmental variables were Z-transformed ((x_i – mean)/standard deviation) to allow direct comparisons of effect size. Surface area was log transformed before it was Z-transformed to meet the assumptions of the GLM.

All statistical analysis were done in R version 4.0.3 (R Core Team 2013).

2.5 Results

2.5.1 Data summary

A total of 32,023 weevil specimens were collected from 13 genera representing 761 individual morphospecies from Mexico, Guatemala, and Nicaragua. The most abundant and species-rich genus was *Eurhoptus* (9200 specimens (29%), 121 species (16%)). There were 213 species (28%) that were identified as singletons (i.e., present only once in my dataset; Figure 2.2; Appendix 2.5).

2.5.2 Richness and abundance patterns

Morphospecies richness and abundance were positively linearly related (slope = 0.47 (0.06 SE), $t_{1,24} = 7.8$, $p < 0.001$; Appendix 2.4). Morphospecies richness increased linearly with elevation (slope = 8.3 E^{-4} (8.7 E^{-5} SE), $t_{1,23} = 9.5$, $p < 0.001$; Figure 2.3A) and with latitude (slope = 0.23 (0.05 SE), $t_{1,23} = 4.6$, $p < 0.001$). Abundance increased linearly with elevation (slope = 1.6 E^{-3} (1.2 E^{-4} SE), $t_{1,23} = 13.1$, $p < 0.001$; Figure 2.3B), but not with latitude (slope = 0.11 (0.07 SE), $t_{1,23} = 1.6$, $p = 0.11$).

2.5.3 Environment-elevation relationships

There was elevational variation in most of the environmental variables. Spring temperature (slope = -1.1 E^{-3} (9.2 E^{-4} SE), $t_{1,23} = -11$, $p < 0.001$; Figure 2.4A), surface area (slope = -1.1 E^{-3} (1.3 E^{-4} SE), $t_{1,23} = -8.9$, $p < 0.001$; Figure 2.4C), and seasonality (slope = -6.5 E^{-4} (s2.3 E^{-4} SE), $t_{1,24} = -2.3$, $p = 0.01$) decreased with elevation, whereas humidity increased with elevation (slope = 7.2 E^{-4} (1.7 E^{-4} SE), $t_{1,24} = 4.2$, $p <0.001$; Figure 2.4E). There was no elevational variation in GPP (slope = -2.3 E^{-4} (2.7 E^{-4} SE), $t_{1,23} = -0.86$, $p = 0.40$). The elevations surveyed in the LLAMA project increased with latitude (Table 2.1).

2.5.4 Richness-environment relationships

Variation in morphospecies richness was best predicted by decreasing spring temperature (slope = -0.72 (0.08 SE), $t_{1,23} = -9.3$, $p < 0.001$; Figure 2.4B). It was also predicted by decreasing surface area (slope = -0.58 (0.10 SE), $t_{1,23} = -5.8$, $p < 0.001$; Figure 2.4D), and increasing humidity (slope = 0.41 (0.15 SE), $t_{1,24} = 2.7$, $p < 0.001$; Figure 2.4F). Morphospecies richness was not determined by seasonality (slope = -0.30 (0.14 SE), $t_{1,24} = -2.2$, $p = 0.30$), or GPP (slope = -0.09 (0.13 SE), $t_{1,23} = -0.69$, $p = 1$). Latitude was a significant covariate in almost all models (Table 2.1).

2.6 Discussion

I used over 32,000 weevil specimens across Central America to investigate how weevil species richness and abundance change across an elevational gradient. I predicted weevils would increase in richness with elevation like other hyperdiverse taxa in these regions like the rove beetles and wasps (Mumladze et al. 2017; Dolson et al. 2021). In accordance with these predictions, high elevation forests have the highest number of individuals and morphospecies of weevils. I found that low spring temperature and high relative humidity were associated with the richness pattern, factors that are currently shifting due to climate change (Shah et al. 2020).

The positive linear richness-elevation pattern I detected is in contrast to assumptions and previous evidence for some groups that tropical high-elevation forests are lower in animal species richness than surrounding areas (Anderson and Ashe 2000, Janzen and Hallwachs 2019). For example, studies based on the same samples of the LLAMA project as this study show that ant richness and abundance decline with elevation (Longino et al. 2014, Longino and Branstetter 2019). However, recently there has been more support for the positive linear pattern in other taxa in tropical regions: rove beetles (Dolson et al. 2021), wasps (Peck et al. 2008, Mumladze et al. 2017), and dance flies (Chatelain et al. 2018). It could be that historically, insects in high elevation forests were simply not documented enough to fully understand how diverse these areas are. Alternatively, it is possible that in other areas a linear increase in richness may only represent a portion of the "true" richness-elevation pattern. For example, if the mountains extended higher, and/or into drier regions, richness may begin to decrease and thus the "true" relationship may be non-linear (Chapter 1). However, in the LLAMA project, sampling was completed in environments that are representative of the highest peaks in the region, so this is unlikely to be the case here.

In addition to high richness and abundance at high elevations, I found a high proportion of singletons (species present only once) across all samples – 28% (Figure 2.2) suggesting rarity or endism. This is not unexpected, as tropical areas have long been known to contain disproportionately high amounts of rare species compared to temperate environments (Brown 2014, (Zizka et al. 2018; Antonelli 2022). The environmental heterogeneity and general fragmentation from other environments that occurs across tropical mountains creates extreme niche segregation, high beta diversity, and thus specialization to these environments (Soininen et al. 2018, Smith et al. 2023).

My rarefaction curve (Appendix 2.5) did not show an asymptote, indicating that with each new sample I found new morphospecies. This means that although I had over 32,000 specimens, these areas were still not sufficiently sampled to confidently state that I found all weevil species. This could mean that at any, or all, of the sampling locations the richness values could be much higher. However, high singleton frequency and rarefaction curves with no asymptote are typical of hyperdiverse areas like the tropics (Coddington et al. 2009, Zizka et al. 2018). To determine with certainty that these morphospecies are rare (few individuals) versus endemic (restricted to a small geographic area), future research in these regions should focus on: (1) sampling areas that are geographically closer together to enable measurements of β-diversity; 2) improving estimates of average dispersal capacity amongst weevils.

2.6.1 Environmental associates of weevil richness-elevation patterns

Spring temperature was the most important predictor of morphospecies richness, accounting for 81% of variation in richness. Temperature is often associated with richness across elevational gradients (Beck et al. 2017, González-Tokman et al. 2020). However, I found higher richness in areas with cooler springs, opposite to what most studies find (Smith 2015, Beck et al. 2017, Chapter 1). It could be that spring in low elevation areas is too hot, or just not optimal, for many weevil species. On average, spring temperature in lower elevation sites was 10°C hotter than the upper elevation sites. Studies have found that the critical thermal maxima of insect species (i.e., above or below which motor function control is lost) is one of the main determinants of their elevational distributions in the tropics (Polato et al. 2018, Amundrud and Srivastava 2020, Bota-Sierra et al. 2022).

I also found that higher relative humidity was associated with higher morphospecies richness. Humidity is known to be important for insects for maintaining homeostasis (Marshall 2006, Chen et al. 2019), though it is not commonly found to be related to species richness across elevation (Longino and Colwell 2011, Beck et al. 2017). The other studies may not have found a strong influence of humidity on richness if 1) the richness-elevation relationship was non-linear but humidity-elevation was linear, 2) richness is more closely related to actual evapotranspiration and not a proxy for it (though this is uncommonly tested across elevation and so this prediction is unclear (McCain & Grytness, 2010, Beck et al. 2017)), and/or 3) humidity is collinear with other environmental factors and thus teasing out its exact effect was difficult.

Morphospecies richness was correlated with surface area and latitude. Richness was negatively related to surface area, which is opposite to what is predicted by the species-area hypothesis (MacArthur and Wilson 1967). A similar result was found in this region by Dolson et al. (2021) with rove beetle diversity. The most likely reason for my finding is that richness is more strongly associated with environmental factors that vary across elevation rather than the habitat space itself. For example, I found that species richness was more strongly related to temperature than area (Table 2.1), that temperature decreases with elevation (Table 2.1), and that there was collinearity between temperature and surface area (Appendix 2.3). Another possible explanation is that during the Pleistocene, these high elevation cool and wet cloud forests extended across the full elevation ranges of these mountains but are now vertically isolated to mountain peaks due to climatic warming over recent Millenia (Ramirez-Barahona and Eguiarte 2013). Therefore, high richness may be relegated to small areas at high elevations as a legacy effect of the species-area hypothesis. However, there is not a lot of evidence supporting this hypothesis (Ramirez-Barahona and Eguiarte 2014). Finally, latitude was positively related to richness and elevation in most models (Table 2.1). This result is also not unexpected, as the sites spanned a latitudinal range of 4.8° (Figure 2.1). This was a limitation of my study since I cannot entirely separate the effects of the environmental factors on richness from latitude.

One factor I could not test due to a lack of data is human disturbance/land use changes. Lowland sites are almost always more disturbed and developed compared to higher elevation areas because of where humans have settled and the ease of visiting and developing in these geographic areas (Lomolino 2001). This can affect the number of species that would be present in these regions in various ways. For example, if low elevations have high disturbance and thus fewer species, the “true” pattern may have been higher richness at lower elevations, more so resembling a low elevation plateau, or mid-elevation trough in species richness (Murphy and Remanuk 2014). Additionally, low elevation sites can sometimes be under-sampled because continuous areas of undisturbed forest are hard to find for ecological sampling, thus leading to an underestimation of richness at lower elevations (Nogués-Bravo et al. 2008). Therefore, it is possible that one of the reasons I found low richness across lowland sites was due to disturbance or land use changes. However, I do not anticipate this to have altered my results substantially since sites were specifically selected to be undisturbed and undeveloped.

2.6.2 Conclusions

In this study, I predicted that weevils would increase in richness with elevation and will be associated with local factors like temperature and humidity. I found that tropical high elevation forests are diverse with weevils. Morphospecies in these forests thrive in cooler springs with high humidity. The availability of these cool and wet forests are predicted to decrease with climate change (Halsch et al. 2021, Antonelli 2022). Surface temperatures in the tropics are predicted to increase in the future (Zeh et al. 2012). Humidity is predicted to decrease through the upslope movement of the clouds (Laurance et al. 2011). As these high elevation species likely exist nowhere else, the loss of these environments could lead to a dramatic decline in the unique weevil diversity that exists there. Future investigations ought to measure the causal relationships between environmental factors and richness, and further document and move towards protecting this diversity while it still exists.

2.7 Tables

Table 2.1. The relative importance of various environmental factors on weevil morphospecies richness and how they vary across elevation. Each linear model is seperated by horizontal lines, and some include latitude as a covariate. Richness is the response variable in the first set of models, and elevation is the independent variable in the second set. P-values for the richness models are the values after a Bonferroni correction. Latitude is included as a covariate in all models except with humidity and seasonality due to collinearity. Significant covariates are bolded ($p < 0.05$). All environmental variables were Z-transformed ((x_i – mean)/standard deviation).

	Variables	**Slope (Standard Error)**	**t - Score**	**DF**	**P - Value**
Richness	**Temperature**	**-0.72 (0.08)**	**-9.3**	**23**	**<0.001**
	Latitude	**0.31 (0.05)**	**6.0**		**<0.001**
	Log(Area)	**-0.58 (0.10)**	**-5.8**	**23**	**<0.001**
	Latitude	**0.22 (0.07)**	**3.2**		**0.001**
	Humidity	**0.41 (0.15)**	**2.7**	**24**	**<0.001**
	Seasonality	-0.30 (0.14)	-2.3	24	0.3
	GPP	-0.09 (0.13)	-0.69	23	0.99
	Latitude	**0.22 (0.09)**	**2.2**		**0.04**
Elevation	**Humidity**	**7.3 E^{-4} (1.7 E^{-4})**	**4.2**	**24**	**<0.001**
	Log(Area)	**-1.1 E^{-3} (1.3 E^{-4})**	**-8.9**	**23**	**<0.001**
	Latitude	0.04 (0.07)	0.52		0.6
	Temperature	**-1.1 E^{-3} (9.2 E^{-4})**	**-11**	**23**	**<0.001**
	Latitude	**0.14 (0.05)**	**2.9**		**0.009**
	Seasonality	**-6.5 E^{-4} (2.3 E^{-4})**	**-2.8**	**24**	**0.01**
	GPP	-2.3 E^{-4} (2.7 E^{-4})	-0.86	23	0.40
	Latitude	0.08 (0.15)	0.59		0.56

2.8 Figures

Figure 2.1. The 25 study sites (orange points) used in this study collected through the LLAMA project across Central America in Mexico (n = 9), Guatemala (n = 9), and Nicaragua (n = 7). This topographic map shows higher elevations with darker green colouring. Map created with R package “ggmap” (Kahle and Wickham 2013).

Figure 2.2. The frequency distribution of number of specimens per morphospecies.

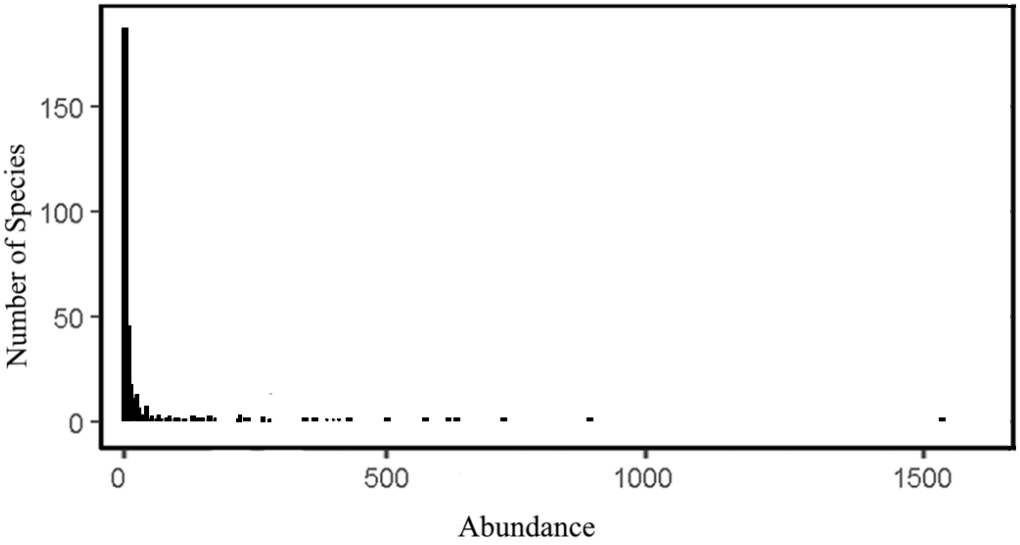


Figure 2.3 (A) Morphospecies richness linearly increased with elevation (slope = 8.3 E^{-4} (8.7 E^{-5} SE), $t_{1,23}$ = 9.5, p < 0.001), (B) Abundance linearly increased with elevation (slope = 1.6 E^{-3} (1.2 E^{-4} SE), $t_{1,23}$ = 13.1, p < 0.001). Predicted best fit from simple linear model is shown in black for all panels, with the grey shading indicating the 95% prediction interval (n = 25 sites).

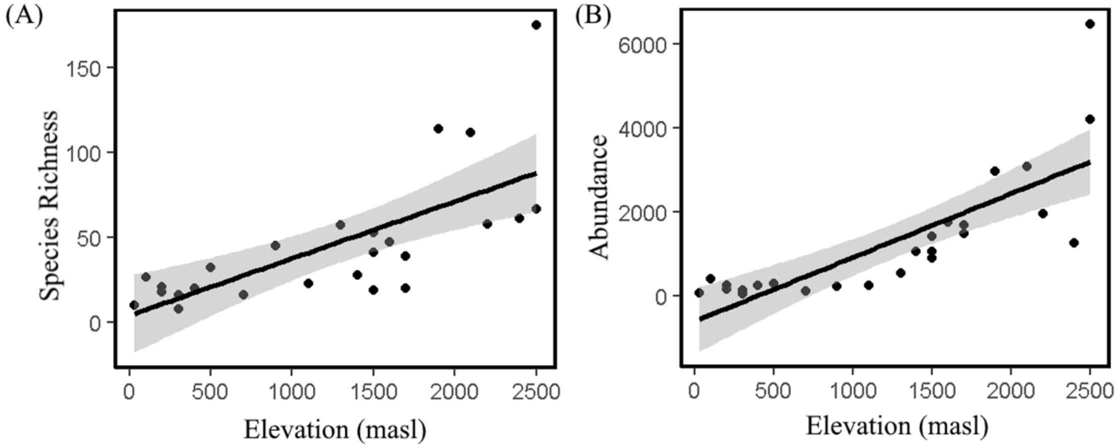
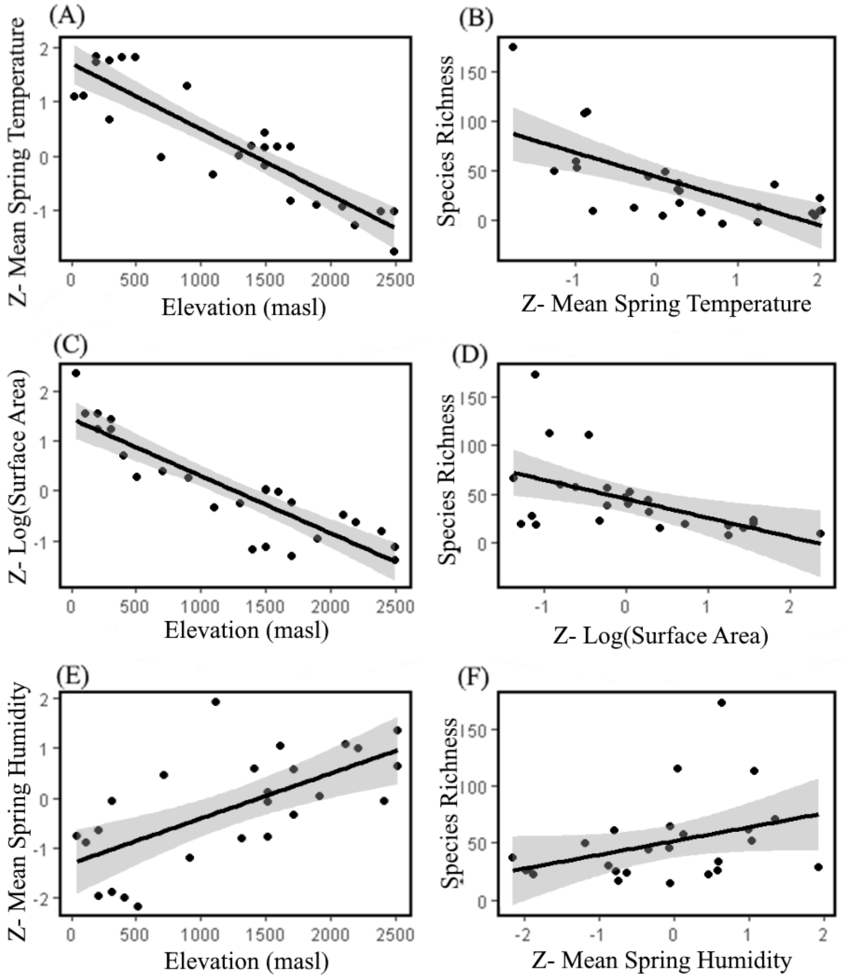


Figure 2.4. The relationship between environmental factors and elevation (first column), and morphospecies richness (second column). Predicted best fit from simple linear model is shown in black for all panels, with the grey shading indicating the 95% prediction interval (n = 25 sites). The relationship between elevation and (A) spring temperature (slope = -1.1 E^{-3} (9.2 E^{-4} SE), $t_{1,23}$ = -11, $p < 0.001$), (C) Log(surface area) (slope = -1.1 E^{-3} (1.3 E^{-4} SE), $t_{1,23}$ = -8.9, $p < 0.001$), and (E) humidity (slope = 7.2 E^{-4} (1.7 E^{-4} SE), $t_{1,24}$ = 4.2, $p < 0.001$; Figure 2.4E). The relationship between elevational variation in morphospecies and (B) spring temperature (slope = -0.72 (0.08 SE), $t_{1,23}$ = -9.3, $p < 0.001$), (D) surface area (slope = -0.58 (0.10 SE), $t_{1,23}$ = -5.8, $p < 0.001$), and (F) humidity (slope = 0.41 (0.15 SE), $t_{1,24}$ = 2.7, $p < 0.001$).



Chapter 3

Butterflies became paler and bigger over recent decades in Canada

My contributions to this thesis chapter are the conceptions of ideas, design of methodologies, data collection, data analysis, writing, and editing.

3.1 Abstract

Climate change has had widespread impacts on species' morphologies thus far. Colour is an important morphological trait for ectotherms due to its role in protection from predation and maintenance of homeostasis. The thermal melanism hypothesis predicts that paler individuals have higher fitness in warmer environments because the colour paleness of an individual determines its solar absorption. Body size is also linked to thermoregulation, since larger bodies gain and lose heat more slowly. Consequently, ectotherms should be changing in paleness and size over recent decades in response to climatic warming, yet few studies have tested this. Here, I use a spatially and temporally extensive data set from a natural history collection to examine how wing colour paleness and body size has changed in 10 widespread butterfly species (Lepidoptera) across Canada. Across 991 specimens and 54 years, I did not find support for the thermal melanism hypothesis using spatial-temporal variation in temperature. Despite the lack of a direct relationship with temperature, wing colour became paler over time. Similarly, body size (measured as forewing length) became larger over time but did not vary directly with temperature. Therefore, the morphology of some butterflies across Canada has changed over the past 50 years, but it remains unclear what is influencing the changes. Identifying these underlying factors will be critical for prioritizing conservation actions.

3.2 Contributions to the Advancement of Knowledge

This is one of the first studies to test the thermal melanism hypothesis in insects over time. In the past, studies have mainly focused on spatial patterns with temperature as a proxy for temporal patterns. Here, I used a dataset of 10 species of butterflies across 54 years to examine how wing colour and body size has changed in Canada. I did not find support for the thermal melanism hypothesis using spatial-temporal variation in temperature. However, both wing colour and body size changed over time. These findings contribute evidence to how insects are responding to climate change and that other factors, instead of commonly used metrics of temperature, could be driving these changes.

3.3 Introduction

Colour paleness or darkness of animal body parts is thought to be a key trait in the response of species to climate change due to its role in thermoregulation. The thermal melanism hypothesis postulates that the colour paleness of an individual determines its solar absorption and therefore influences its fitness (Watt 1968, Clusella Trullas et al. 2007). More specifically, that darker colour increases solar absorption of an individual, leading to faster heating, and shorter time to reach optimal temperature. Optimal temperature plays a direct role in fitness for ectotherms like butterflies, since general thermoregulation is necessary for flight – a critical part of food searching, finding mates, camouflage, and egg laying (Dufour et al. 2018).

One commonly tested prediction of the hypothesis is that there should be more darker individuals or species than paler individuals/species in cooler areas, and vice versa in warmer areas (Clusella Trullas et al. 2007). Support for this prediction has been found for ectotherms across geographic temperature gradients within and across species. Darker individuals have been linked to cooler and higher elevations relative to paler individuals in several species (e.g. snake *Naja nivea* (Barends & Scholtz 2024), lizard *Sceloporus grammicus* (Gonzalez-Morales et al. 2024), grasshopper *Phaulacridium vittatum* (Harris et al. 2013), wasp *Agelaia pallipes* (de Souza et al. 2020), and butterfly *Pieris canidia* (Gautam and Kunte 2020)). Interspecific examples have found that European butterfly and dragonfly assemblages (Zeuss et al. 2014) and North American butterfly assemblages (Stelbrink et al. 2019) have darker wing and body colours in cooler areas. Similarly, Bishop et al. (2016) and Xing et al. (2018) found that ant and moth species' assemblages (respectively) are darker at cooler/higher elevations than lower elevations. Clusella Trullas et al. (2008) found that lizards were paler in areas with more solar radiation and higher temperatures. Evidence of darker pigmentation have been found in colder more northern latitudes in snakes (Martinez-Freiria et al. 2020). These spatial studies demonstrate potential support for a causal relationship between colour paleness and temperature, however it remains unclear if this pattern has led to climate change-driven changes in colour paleness.

Another prediction of the thermal melanism hypothesis is that climatic warming will lead to increasing paleness in ectotherms over time (Delhey et al. 2020); yet few studies have tested this. Consistent with the hypothesis, Brakefield and De Jong (2011) and Zvereva et al. (2019) found decreases in the darker morphs of two beetle species over two decades in Europe. In contrast, MacLean et al. (2019) found inconsistent support for this hypothesis: there were both region-dependent increases and decreases in wing melanism over 60 years in the butterfly *Colias meadii* in the American Rocky Mountains. Without additional studies, it is uncertain if there are any general patterns in how climate change is influencing ectotherm colour paleness (Clusella Trullas and Nielsen 2020).

Here, I sought to test the thermal melanism hypothesis in a taxon known for its colour – the butterflies. I use museum specimens from across Canada over a 54-year period to test the prediction that butterfly wing colour has become paler in response to climate warming. Museum records have been successfully used to detect variation in colour paleness of butterflies (MacLean et al. 2019). I tested the hypothesis using: (i) spatial variation in temperature; ii) spatio-temporal variation in temperature; and iii) trends in paleness over time.

I additionally examined the role of body size because body size is also linked to thermoregulation. An implicit assumption of the thermal melanism hypothesis is that there is a relationship between colour paleness and body size (Clusella Trullas et al. 2007): larger ectotherm bodies gain and lose heat more slowly than smaller bodies (Gardner et al. 2011). Higher melanism (i.e., darker colour) provides an advantage in larger ectotherms because it takes longer for larger bodied organisms to reach their ideal temperatures, and darker individuals gain heat more quickly (Clusella Trullas et al. 2007). Despite this implied relationship between colour paleness and body size, support for this relationship remains unclear (Goldenberg et al. 2022). Colour has been found to be darker with larger body size in assemblages of beetles (Schweiger and Beierkuhnlein 2016), ants (Bishop et al. 2016), and noctuid moths (Heidrich et al. 2021). However, the opposite pattern (i.e., larger bodies and paler colour) has also been found in butterfly species such as *Pararge aegeria* (Taylor-Cox et al. 2020) and *Colias meadii* (MacLean et al. 2019). This is thought to arise because larger ectotherms may need greater reflectance (paler bodies) to avoid overheating (Clusella Trullas et al. 2007).

In response to climate warming, the direction and magnitude of change in body size for ectotherms has been variable over the past few decades (Sheridan and Bickford 2011, Tseng et al. 2018, Wonglersak et al. 2021, Ryding et al. 2021). However, across butterfly species there have been consistent increases in body size with warming (Fenberg et al. 2016, Davies 2019, MacLean et al. 2019), likely due to extended growing and foraging opportunities (Blanckenhorn and Demont 2006). Here I evaluate how body size related to temperature and colour paleness by (i) using size as a covariate in the colour paleness-temperature relationship; ii) body size as a function of spatial variation in temperature;; and iii) body size as a function of spatial-temporal variation in temperature; and iv) change in body size over recent decades.

I will test the thermal melanism hypothesis by evaluating both butterfly colour paleness and butterfly body size with time and temperature. I predict that butterfly colour paleness will increase over time and with temperature. I additionally predict that body size will increase over time and with temperature.

3.4 Methods

3.4.1 Species and collections

I used butterfly specimens from 1950-2004 housed at the Canadian National Collection (CNC) in Ottawa, Canada. I chose species that are: i) native to Canada; ii) abundant in the collection since 1950 (i.e., more than 30 records); and iii) larger than 20 mm mean wingspan to exclude microleps - a group of Lepidoptera that are small with cryptic taxonomy. Microleps are known to have a low capacity for heat retention through basking, and therefore are not predicted to have responsiveness in wing melanisation (De Keyser et al. 2015). I focused on years after 1950 to avoid mid-century changes in collection patterns (Kharouba et al. 2014), but still capture variation in colour paleness before the early 1980s when it is generally accepted that climate began to change more strongly (Pörtner et al. 2022). No species were eliminated based on lack of information on the specimen (i.e., date or locality information).

In cases where the latitude and longitude of the specimen were not known (~5% of specimens), I geolocated them based on other locality information in the collection notes (e.g., Cape Breton Highlands National Park, North Mountain, 2km W of Radio Tower). I matched the collection location to map coordinates through Google Earth or ArcGIS 10.8.1. I estimate that the maximum location uncertainty for the geolocated coordinates is ~5 km since locality information from collection notes was quite detailed. There were no duplications from the same location and same date.

Preliminary analysis showed sex-based differences in colour paleness and size for most species. To limit the influence of sexual dimorphism, I restricted my analysis to males which were better sampled in the collection.

In total, there were 991 specimens from 1950-2004 (n = 54 years) for 10 species (Table 3.1; Figure 3.1). There were specimens from the provinces of British Columbia, Yukon, Alberta, Ontario, and Quebec. I had specimens from 4 families and 7 genera: Pieridae (*Pieris* and *Colias*), Papilionidae (*Parnassius* and *Papilio*), Lycaenidae (*Cupido* and *Lyceana*), and Hesperiidae (*Hesperia*). The mean number of specimens per species was 100 (41 SD), the mean number of sites per species was 34 (14 SD), and the mean number of years per species was 42 (8 SD).

3.4.2 Colour paleness measurements

To photograph each specimen, I used a mounted DSLR camera with standardized lighting, an all white background, and a scale bar. Each specimen was photographed dorsally and laterally to measure wing colour paleness on the side that the species basks on (Table 3.1). Butterfly colour is structural and therefore does not break down over time (Prum et al. 2006), so there is minimal risk of colour changes post-collection in my specimens.

To measure wing colour paleness, I calculated a grey level from black (0 units of colour) to white (255 units of colour). Using ImageJ, I calculated a grey level value for the full forewing (Figure 3.2). Using a grey level to calculate paleness has been used before and is known to reflect the proportion of melanic scales on the wing (Zeuss et al. 2014). I used the forewing based on previous research, and based on how the butterflies are preserved it is the only wing that is fully visible (MacLean et al. 2019).

3.4.3 Temperature

For a temperature estimate, I used mean summer temperature (June-August inclusively). I examined the influence of maximum summer temperature, mean spring temperature, and mean winter temperature on wing colour paleness, and found no differences between the temperature metric used (Appendix 3.1). Therefore, I used mean summer temperature to match the season the butterflies are active as adults and based on what was used in previous research (Stelbrink et al. 2019). I extracted mean summer temperature of the location-year for each specimen from ClimateNA which provides historical and current weather station data gridded to 500 m^2 across North America (Harris et al. 2020).

3.4.4 Covariates

Colour is predicted to be darker at cooler, higher elevations compared to warmer, low elevations (Bishop et al. 2016, Dufour et al. 2018), so I extracted elevation data of the location for each specimen through the “elevatr” package in R at a resolution of 5 m^2 (Hollister et al. 2023).

To test for the influence of body size on the paleness~temperature relationship, I measured forewing length on each specimen. Forewing length is widely used and known to be a proxy for body size in butterflies (Figure 3.2) (Brehm et al. 2019; Verma & Arya 2023).

To account for possible seasonal changes in paleness over the growing season, such as from seasonal patterns in temperature change or different generations of butterflies (Davies 2019, Novella-Fernandez et al. 2023), I tested for the importance of the day of year the specimen was collected in the model. The number of generations per summer per species ranged from 1-5 generations.

Finally, I examined the importance of latitude. Latitude is known to influence butterfly size (Gardner et al. 2011, Zeuss et al. 2014), and colour paleness (Stelbrink et al. 2019).

3.4.5 Statistical Analysis

Overview

In total, I fit eight models (Appendix 3.2). I first evaluated how temperature has changed since 1950 (model 1). To test the thermal melanism hypothesis, I considered three models: i) colour paleness as a function of temperature (model 2), ii) colour paleness as a function solely of spatial variation in temperature (model 3), and iii) colour paleness as a function of time (model 4).

To evaluate the influence of body size on the colour-temperature relationship, I considered two models: i) forewing length as a function of temperature (model 5), and ii) forewing length as a function of time (model 6). To test for a potential trade-off between the two traits, I evaluated the correlation between colour paleness and forewing length (model 7). Finally, I wanted to see if darker species respond to temperature more strongly than paler species, so I fit the sensitivity of paleness to temperature as a function of the mean colour paleness of each species (model 8).

I used mixed-effects modelling ("lme4" package (Bates et al. 2015)) for all models except model 8. I used a restricted maximum likelihood (REML) for parameter estimation. Slope and intercept were allowed to vary within the random effect structure. For the mixed models, I report both the conditional R^2 ($R^2_{(C)}$) (it accounts for variance explained by both the fixed and random effects), and the marginal R^2 (it accounts for variance explained by the fixed effects) using the R package "MuMIn" (Nakagawa and Schielzeth 2013, Barton 2022).

For five of the trait models (models 2-6) I tested for collinearity amongst covariates (various amounts from 3 - 4 – Appendix 3.2) through variance inflation factor tests (VIF) using the "car" package (Fox and Weisberg 2019). I selected a VIF cutoff of 5 to balance the complexity of the model and collinearity of the variables. I did not remove any variables due to collinearity (Appendix 3.3).

All statistical analysis were done in R version 4.0.3 (R Core Team 2013).

Model 1 – Temperature change over time

To evaluate how temperature has changed over the years (i.e., temperature as a function of year), I randomly selected 25 sites across species and extracted mean summer temperature for every year from 1950-2004 (n = 1285). First, I evaluated if the relationship between temperature and time was non-linear by comparing a linear and non-linear model with a hinge point at 1980 when temperature is known to have increased (Pörtner et al. 2022). To do so, I used the R package "segmented" (Muggeo 2008). Given no difference between models, I went forward with the linear model (Appendix 3.4). I included location as a random effect to account for multiple years of data per site. I tested for and accounted for temporal autocorrelation by specifying the correlated within-group error structure with the lme function using an autoregressive order of one. Both latitude and longitude were included as covariates because of the broad spatial extent of the location occurrences (Figure 3.1).

Model 2 – Colour-temperature relationship

To test if colour paleness changes with mean summer temperature, I included latitude, forewing length, day of year, and elevation as covariates (n = 991 specimens).

I accounted for temporal autocorrelation by 1) using year as a random effect in the final model, and 2) specifying the correlation error structure with the lme function using an autoregressive order of one. I used species as a random effect in the model to account for multiple observations per species.

Model 3 – Colour-temperature relationship across space

I evaluated colour paleness as a function of spatial variation in mean summer temperature by averaging colour paleness and temperature across years per species per location (n = 306 unique species-location values). I used the same model structure as listed above for model 2, but without 1) year as a random effect, and 2) any correlation structure to maintain power.

Model 4 – Colour-time relationship

To determine if colour paleness changed over the past 54 years, I included latitude, forewing length, day of year, and elevation as covariates (n = 991 specimens). In the full model, I tested for the presence of spatial autocorrelation in the relationship between paleness and time using Morans I value and found that residuals of colour paleness values were spatially autocorrelated (Morans I p-value < 0.01). To account for this, I 1) used location ID as a random effect in the final model, and 2) specified the correlated error structure using a gaussian spatial correlation structure. Location ID was derived from a concatenation of latitude and longitude of each specimen rounded to the second decimal place (equivalent to rounding to 1 km). Across all species there were 243 unique locations. I used species as a random effect in the model to account for multiple observations per species.

Model 5 & 6 – Body size change with temperature and over time

To determine how body size (measured through forewing length) was related to mean summer temperature and year, I maintained the same correlation and random structure as listed above (models 3 & 4), with changes to the covariates included (n = 991 specimens in both models). As covariates, I included latitude, day of year, elevation.

Model 7 – Colour-body size relationship

To evaluate how colour paleness was related to forewing length, I fit colour paleness as a function of forewing length and included species as a random effect to account for interspecific species differences (n = 991).

Model 8 – Sensitivity of paleness to temperature

To determine if the mean colour paleness of each species influenced the strength of the relationship between paleness and temperature, I fit a simple linear model with mean colour paleness of each species as a function of slope from a simple linear model of colour-temperature fit for each species (n = 10).

3.5 Results

On average, mean summer temperature increased over the 54-year period by 0.01 °C/year (0.002 SE), $t_{1,1260}$ = 8.1, p < 0.001; Figure 3.3), and with longitude from west to east (slope = 0.10°C/°longitude (0.04 SE), $t_{1,20}$= 2.8, p =0.01).

Wing colour paleness was not related to mean summer temperature (slope = -0.11 units of colour/ °C (0.27 SE), $t_{1,791}$ = -0.41, p = 0.68; Table 3.2; Figure 3.4) or any of the covariates (Table 3.2). Wing colour paleness was not related to mean summer temperature across space (slope = -1.1 units of colour/ °C (0.51 SE), $t_{1,291}$ = -0.20, p = 0.84). Interestingly, wing colour paleness increased over time (slope = 0.18 units of colour/ year (0.04 SE), $t_{1,654}$ = 4.3, $p < 0.01$; Table 3.2; Figure 3.4) and decreased over the growing season (slope = -0.05 units of colour/day (0.02 SE), $t_{1,654}$ = -2.5, p = 0.01; Table 3.2).

Butterfly forewing length was not related to mean summer temperature (slope = 0.01 mm/ °C (0.03 SE), $t_{1,792}$ = 0.47, p = 0.64; Table 3.2; Figure 3.4) but increased over the time period (slope = 0.02 mm/ year (8.2 E^{-3} SE), $t_{1,655}$ = 2.9, $p < 0.01$; Table 3.2; Figure 3.4). In the year model, length decreased with latitude (slope = -0.14 mm/° latitude (0.03 SE), $t_{1,655}$ = -4.1, $p < 0.01$; Table 3.2) and elevation (slope = -6.5 E^{-4} mm/m (3.1 E^{-4} SE), $t_{1,655}$ = -2.1, p = 0.04; Table 3.2).

Butterfly wing colour paleness was not related to forewing length (slope = 2.6 E^{-3} units of colour/mm (0.01 SE), $t_{1,980}$ = 0.18, p = 0.85; Appendix 3.5).

There was a weak relationship between species' paleness and the sensitivity of paleness to temperature: the paleness of paler species was less sensitive to temperature than darker species (slope = -15 (7.2 SE), $t_{1,8}$ = -2.1, p = 0.06; Figure 3.6).

3.6 Discussion

Using museum specimens from across Canada, I tested the thermal melanisms hypothesis by evaluating the prediction that variation in colour across time and space are functions of changes in temperature. I did not find consistent support for the hypothesis: wing colour paleness was not related to temperature. However, I did find that butterfly wing colour became paler over the past 54 years. Similarly, I did not find a relationship between wing length and temperature but found that butterflies have gotten bigger over the past 54 years. These results suggest that wing colour paleness and wing length are responding to multiple factors, and that there is a need to examine the influence of other factors in more detail.

3.6.1 Butterfly wings became paler over recent decades

The wings of butterfly species in this study became paler over recent decades. This result is consistent with evidence from other studies that have found decreases in the darker morphs of two beetle species over time in Europe (Brakefield and De Jong 2011; Zvereva et al. 2019), but not with MacLean et al. (2019) which found region-dependent changes in the butterfly *Colias meadii* over time in the American Rocky Mountains. Unlike these studies, the colour paleness change I found was not consistent with spatio-temporal variation in temperature. It could be that 1) colour paleness is causally related to variation in temperature in these species but in this dataset I did not detect such a relationship, 2) the thermal melanism hypothesis is not supported at this scale, or 3) colour paleness is not causally related to temperature.

In the first scenario, it is possible that it was difficult for me to detect an overall relationship between paleness and mean summer temperature because of the presence of interannual variation in temperature. There is evidence that interannual variation in temperature can constrain the degree of change in wing colour paleness in response to mean temperature trends (Kingsolver and Buckley 2015). This is because the strength or direction of selection from temperature changes may be too weak year-to-year to generate detectable change, but over a long enough time period this weak but consistent selection can generate a detectable trend (Clusella Trullas et al. 2007, Siepielski et al. 2019).

As digitization has become more prominent over recent decades, museum collections have steadily declined past the 1980's in favor of photographs (especially for butterflies) and eventually citizen science (Fischer et al. 2021, Davis et al. 2023). This change affected my total number of specimens and species. The main drawback of this limitation is that it is difficult to assess the generality of the pattern I found. In particular, it would have been beneficial to have more dark species to detect changes in these darker species or assess more robustly if the pattern changes depending on average paleness. However, with more species spread across more families, it could have been beneficial to use a phylogenetic correction to determine if the patterns I found are due to how related the species are. Further, the lack of specimens effected the timeframe that I was able to evaluate. Indeed, I had fewer records after the 1980s, and almost none after the 2000s (Figure 3.4). Temperature changes have substantially increased since the early 1980s (Pörtner et al. 2022), so if temperature is a mechanism in this relationship, temperature changes before the 1980s might not have been a big enough selective pressure on paleness. I join previous calls that increased investments in natural history collections are needed (Balke et al. 2013; Kharouba et al. 2019; Mason & De Bello, 2013).

In the second scenario, it could be that thermoregulation is not the main selective pressure affecting butterfly wing colour paleness at this scale. There are many other hypothesized determinants of colour paleness, such as precipitation/moisture (darker cuticle improves desiccation resistance and increases disease resistance in wet areas), UV radiation (melanin pigments can protect against higher UV exposure), and fire frequency (increased fires can create directional selection for camouflaged darker individuals) (summarized by Clusella Trullas and Nielsen (2020)). However, most of these hypotheses would predict darker rather than paler individuals (as I have observed here) with climatic change. For example, more extreme heat events from climate change are increasing desiccation risks for many organisms, precipitation is increasing across Canada, and the frequency of wildfires in Canada has increased with climate change (Coogan et al. 2019, Zhang et al. 2019, Pörtner et al. 2022). Finally, UV and/or solar radiation has not changed substantially in Canada over recent decades, so solar radiation is unlikely to be associated with the paleness changes I observe here (Bernhard et al. 2020).

I propose two alternative reasons for butterfly wings becoming paler over recent decades. First, with the conversion of natural areas to farmland, and widespread deforestation since the 1950s in Canada, a lot of forested habitats have been converted to open, exposed, habitats (CFS 2016). This has benefited butterfly species in Canada that prefer open habitats (Clark et al. 2007, Tzortzakaki et al. 2019, Lewthwaite and Mooers 2022). Ectotherms using these open areas are exposed to more sun and heat fluctuations compared to covered areas (Li et al. 2015, Barnes et al. 2023). So if temperature and/or solar exposure are a mechanism here, it could be that paler colouration (and higher reflectance) provides an advantage in open, more exposed areas (Kleckova and Klecka 2016). Future research should evaluate: 1) if overall butterfly colour paleness differs between open and covered habitats, and 2) if land use changes over time have led to morphological changes (like colour paleness) in butterfly assemblages due to sun exposure, because this could have broad implications for how animals are being affected by anthropogenic changes.

Second, butterfly wing colour could have gotten paler because of decreased nutrient levels in plants. Lower nutrient levels in food, like low protein, can limit the development of melanin pigments in butterflies (i.e., decrease darkness) (Lee et al. 2008). This has been shown to have occurred in a few Lepidopteran species (summarized by Lee et al. (2008)). The magnitude and direction of impacts of climate change on plant nutrient levels is unclear, but plant nutrient availability has been shown to be changing due to drought, extreme temperatures, and increased carbon dioxide (Heckathorn et al. 2020). Since all the species I studied are mainly herbivorous in the larval stage (Layberry et al. 1998), a decline in plant nutrient levels over recent decades could have decreased melanin pigments. Future research should explore the relationship between habitat type and plant nutrient levels, melanism, and climate change.

3.6.2 Butterflies became larger over recent decades

I found that butterfly body size, measured as forewing length, became bigger over time. My finding is consistent with other studies of butterfly species (Fenberg et al. 2016, Davies 2019, MacLean et al. 2019). While I did not find that temperature was linked to this change, this finding is not entirely unexpected as longer seasons from climatic change can provide extended growing and foraging opportunities (Hill et al. 2021) and several environmental factors can influence body size (e.g., latitude) (Chown and Jaco Klok 2003, Horne et al. 2015).

3.6.3 The relationship between colour paleness and body size

I did not find a relationship between wing length and wing colour paleness across species (Appendix 3.5). This is in contrast to some studies that have found that darker individuals are larger (Bishop et al. 2016, Schweiger and Beierkuhnlein 2016), but many thermal melanism hypothesis studies do not report the relationship between size and paleness (Brakefield and De Jong 2011, MacLean et al. 2019, Zvereva et al. 2019). Therefore, it is difficult to know if there is a known widespread relationship between colour paleness and body size. Again, no relationship between body size and wing colour paleness is not entirely unexpected because the relationship between body size and colour paleness can be confounded by many intra- and inter-specific selection patterns of predation, mating success, and reproductive success (Clusella Trullas et al. 2007, Siepielski et al. 2019). More work is needed to disentangle the complexities of the relationship between body size, colour paleness, and climate change.

3.6.4 Sensitivity of paleness to temperature

I found that the wing colour paleness of paler-winged butterfly species was weakly less sensitive to temperature than darker-winged species (Figure 3.6). This makes sense, as it is known that darker species experience more rapid changes in temperature, but paler butterflies can reflect light/heat more efficiently (Ashe-Jepson et al. 2023). Therefore, the thermal melanism hypothesis may be acting more strongly (or only) on darker species. Furthermore, paler species have less initial pigmentation to respond to environmental changes, and so it is possible that this minimal degree of pigmentation is selected for. Some level of melanism is important as it protects against cell damage from UV radiation, provides camouflage, and pathogen resistance (Roulin 2014). Therefore, I suggest that for paler species, the benefit of retaining some melanism outweighs the cost of possible thermoregulatory disadvantage associated with that melanism. Future research should tease out the mechanisms behind this pattern and the fitness consequences of organisms becoming paler.

3.6.5 Conclusions

Butterfly wing colour has gotten paler and wings have gotten larger since 1954. Regardless of the underlying mechanism(s), butterflies are experiencing morphological change which could impact how they interact with their environment. Changes to colour and body size more generally could result in differences to mate attraction or predator avoidance if the colour changes have affected their ability to be detected by other individuals (Dufour et al. 2018). Regardless of mechanism, my results suggest that butterflies are undergoing large scale morphological changes.

3.7 Tables

Table 3.1. The 10 Canadian butterfly species considered in this study, ordered by decreasing wing colour paleness (i.e., pale to dark).

Species	Family	Photograph Angle	Mean Length (SD) (mm)	Mean Colour (SD)	Sample Size
Pieris oleracea 	Pieridae	Ventral	23 (1.6)	180 (0.2)	102
Pontia occidentalis 	Pieridae	Dorsal	23 (1.7)	171 (0.2)	73
Parnassius smintheus 	Papilionidae	Dorsal	31 (2.4)	161 (0.2)	34
Colias nastes 	Pieridae	Ventral	20 (1.7)	137 (0.2)	120
Colias eurytheme 	Pieridae	Ventral	26 (2.1)	135 (0.2)	95
Cupido amyntula 	Lycaenidae	Dorsal	13 (1.2)	132 (0.1)	176
Colias philodice 	Pieridae	Ventral	24 (2.6)	127 (0.3)	44
Lyceana helloides 	Lycaenidae	Dorsal	15 (0.9)	101 (0.1)	120
Hesperia comma 	Hesperiidae	Dorsal	14 (0.8)	101 (0.1)	123
Papilio canadensis 	Papilionidae	Dorsal	47 (3.0)	83 (0.3)	114

Table 3.2. Relationship between butterfly wing colour paleness, forewing length, and environmental factors. Model numbers refer to model numbers in text, each one is a mixed effect model with covariates. DOY stands for day of year. Bolded terms represent a significant term in the model. Degrees of freedom (DF) is shown for the main predictor in each model – the first variable in the list. Marginal and conditional R^2 are shown. Marginal R^2 accounts for variance in the fixed effects and conditional R^2 accounts for variance explained by both the fixed and random effects.

Model	Predictor	Coefficient (Standard Error)	t-value	DF	P-value	Marginal R^2	Conditional R^2
Model 2: Colour paleness ~ Temperature	Temperature	-0.11 (0.27)	-0.41	791	0.68	6.0 E^{-3}	0.9
	Latitude	0.31 (0.19)	1.6		0.11		
	Length	0.19 (0.22)	0.88		0.38		
	DOY	-0.02 (0.02)	-0.84		0.40		
	Elevation	1.8 E^{-3} (1.4 E^{-3})	1.3		0.21		
Model 3: Colour paleness ~ spatial temperature	Temperature	-0.1 (0.51)	-0.2	291	0.84	0.01	0.87
	Latitude	0.29 (0.26)	1.1		0.26		
	Length	-0.07 (0.35)	-0.21		0.84		
	DOY	**-0.06 (0.03)**	**-2.2**		**0.03**		
	Elevation	6.4 E^{-4} (2.2 E^{-3})	0.29		0.77		
Model 4: Colour paleness ~ Year	**Year**	**0.18 (0.04)**	**4.3**	654	**<0.01**	8.6 E^{-3}	0.92
	Latitude	0.15 (0.16)	0.93		0.35		
	Length	0.04 (0.21)	0.18		0.86		
	DOY	**-0.05 (0.02)**	**-2.5**		**0.01**		
	Elevation	1.1 E^{-3} (1.6 E^{-3})	0.66		0.51		
Model 5: Length ~ Temperature	Temperature	0.01 (0.03)	0.47	792	0.64	1.0 E^{-3}	0.96
	Latitude	-0.03 (0.03)	-1.6		0.11		
	DOY	1.6 E^{-3} (2.1 E^{-3})	0.75		0.46		
	Elevation	-2.3 E^{-4} (1.3 E^{-4})	-1.7		0.08		
Model 6: Length ~ Year	**Year**	**0.02 (8.2 E^{-3})**	2.9	655	**<0.01**	0.01	0.99
	Latitude	**-0.14 (0.03)**	-4.1		**<0.01**		
	DOY	6.9 E^{-4} (2.7 E^{-3})	0.25		0.80		
	Elevation	**-6.5 E^{-4} (3.1 E^{-4})**	-2.1		**0.04**		

3.8 Figures

Figure 3.1. Distribution of specimens (n = 991) from the 10 butterfly species I analyzed across Canada. The legend shows the species.

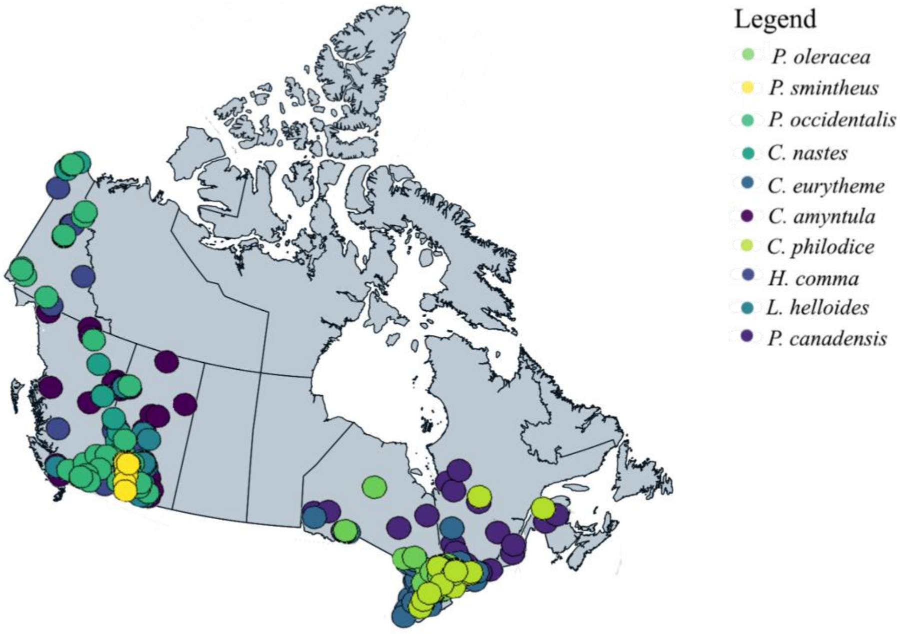


Figure 3.2. Image of *Papilio canadensis* measurements. The red highlighting indicates one of the butterfly's forewings, which was the area used for wing colour paleness measurement. Forewing length was measured from the base of the forewing to the tip, indicated by the red line.

Figure 3.3. Changes in mean summer temperature across my study period of 1950-2004 (n = 25 randomly selected sites). The overall line of best fit across sites is shown in black, with the grey shading indicating the 95% prediction interval. Grey lines represent inter-annual site-level variation in temperature (random effects). Over 54 years, mean summer temperature increased by 0.5 °C (0.01 °C/year (0.002 SE), $t_{1,1260} = 8.1$, $p < 0.01$)

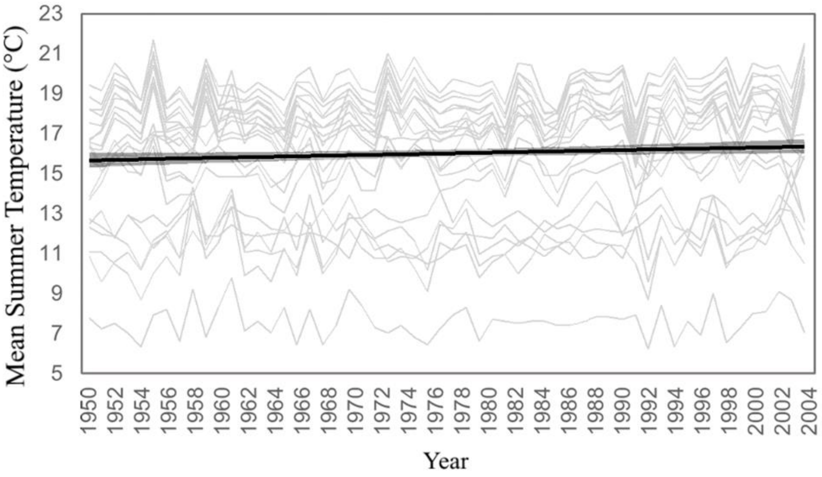


Figure 3.4. The relationship between butterfly wing colour paleness, forewing length, and mean summer temperature and year. The first column (panels A, C) shows the effects of mean summer temperature on both traits and the second column (panels B, D) shows the effects of year on both traits. The relationship between butterfly wing colour paleness and (A) mean summer temperature (slope = -0.11 units of colour/ °C (0.27 SE), $t_{1,791} = -0.41$, $p = 0.68$), and (B) year (slope = 0.18 units of colour/ year (0.04 SE), $t_{1,654} = 4.3$, $p < 0.01$). The relationship between forewing length and (C) mean summer temperature (slope = 0.01 mm/ °C (0.03 SE), $t_{1,792} = 0.47$, $p = 0.64$), and (D) year (slope = 0.02 mm/ year ($8.2\ E^{-3}$ SE), $t_{1,655} = 2.9$, $p < 0.01$). Larger units of colour represent paler wing colour. The overall line of best fit is shown in black for models with a significant trend, with the grey shading indicating the 95% prediction interval. Coloured lines and points represent the estimated relationship for each individual species based on random effects (n = 10 species).

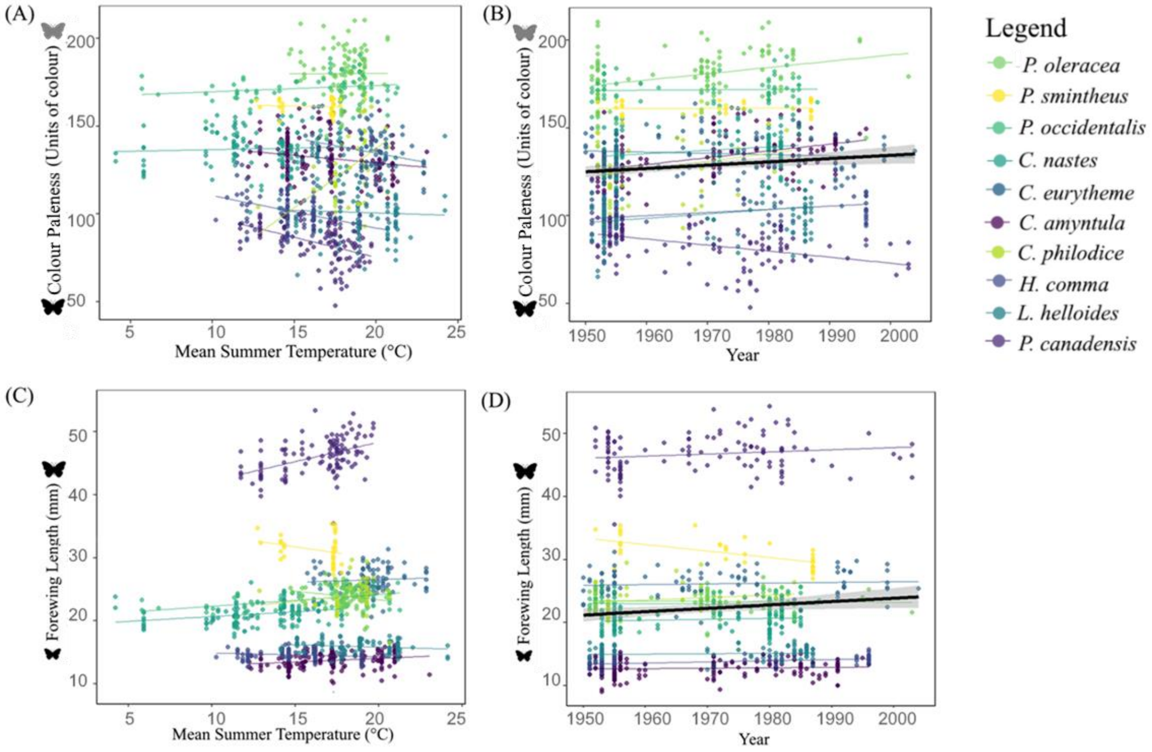


Figure 3.5. Colour paleness as a function of spatial variation in mean summer temperature calculated from a linear model (n = 306) (slope = -1.1 units of colour/ °C (0.51 SE), $t_{1,291}$ = -0.20, p = 0.84).

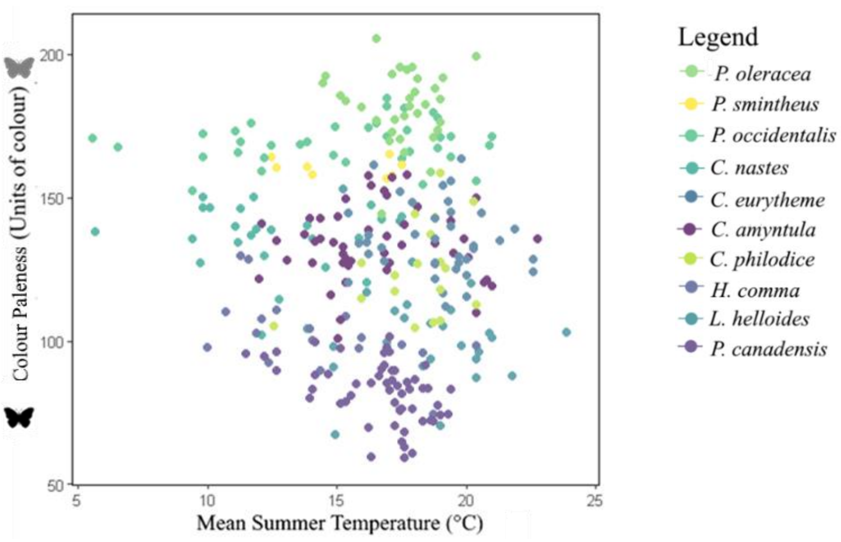
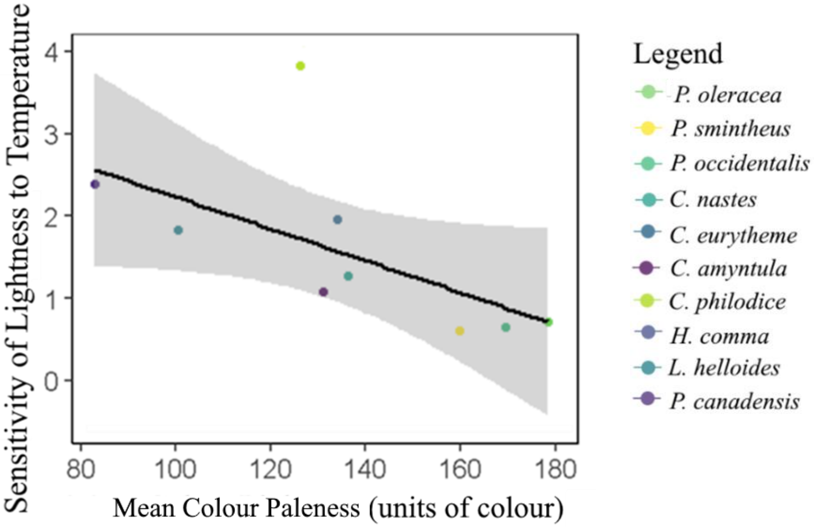


Figure 3.6. The relationship between the sensitivity of paleness to temperature and mean species' colour paleness (n = 10 species) (slope = -15 (7.2 SE), $t_{1,8}$ = -2.1, p = 0.06). A simple linear model with slope from a simple linear paleness-temperature model for each species and mean colour paleness of each species. The overall line of best fit is shown in black, with the grey shading indicating the 95% prediction interval.



General Conclusion

The impacts of climate change on insect biodiversity are widespread and complex, involving many different ecological and evolutionary responses (Halsch et al. 2021, Pörtner et al. 2022). Studying how species have changed, and will change, in response to changing climates is a critical area of research. In this thesis, I contributed several key findings to this area. In chapter 1, I found a global elevation pattern in insect species richness: richness plateaued at low elevations and then declined with elevation. This was related primarily to mean annual temperature and temperature seasonality. In chapter 2, I found that in the mountainous tropics of Central America, spring temperature, spring humidity, and surface area drove an increase in weevil species richness across elevation. These first two chapters help us to better understand the main environmental associates of species richness across mountainous landscapes. In Chapter 3, I found that butterfly species in Canada are becoming paler and larger over time, but the climatic mechanisms behind these patterns are still unclear. These three chapters help us to understand how insects are responding to climatic changes and represent findings that can hopefully improve future policy and conservation decisions.

Summary of Chapter 1

I used 80 studies with 1487 sites from 1990-2020 to determine how insect richness changes across elevation globally. I found that overall insect richness reached a low elevation plateau and then decreased. I also showed that mean annual temperature and seasonality best explained this trend. This contributes to research about insect communities and the factors associated with them by summarizing findings over 30 years and identifying the next steps in this field.

With the addition of my meta-analysis, we now know the elevational-richness patterns in reptiles, birds, plants, small mammals, bats, and terrestrial insects (McCain & Grytness, 2010). However, this does not nearly represent a comprehensive list of life on earth. Similar richness-elevation studies in understudied taxa, and meta-analyses in well studied groups, are needed to understand these patterns and the environmental factors associated with them. Therefore, I encourage future research to focus on other speciose taxa for which we do not know their elevation patterns.

Communities that occur across elevation gradients are at high risk of climatic changes altering their environments (Pepin et al. 2022). As such, there are two areas of future research that I propose. The first being: 1) How are specific shifting along elevation gradients in response to climatic change? Richness-elevation studies can be used to track if elevational range shifts are occurring by sampling species elevational distributions through time. A recent meta-analysis by McCain & Garfinkel (2021) of elevation range shifts in insects found that, in general, insect ranges are shifting to higher elevations. However, they concluded that there was too little data to make further conclusions about how other insect or non-insect assemblages are responding to climate change. Richness-elevation studies like the ones I synthesized provide only a point estimate of species distributions in time. However, I urge researchers in this field to consider two of the following: First, in well-studied regions (like North America or Europe), digitize any historical records of insect distributions to add to the data on insect range shifts over time. Some of the studies I used in my analysis are in areas that are common for ecological research and thus it is possible that historical data on insect distributions are available in these regions but are not yet digitized or available. Second, I encourage researchers to resample these gradients in future years. With future sampling of these regions, these studies in my analysis at least can provide a baseline of where insect species existed at that time to compare to.

Further, I encourage future research to investigate: 2) How well are insect communities across elevation gradients protected from future climatic changes or anthropogenic disturbances/development? Despite mountains being incredibly important in the generation and maintenance of biodiversity (Perrigo et al. 2019), only 19% of mountainous areas globally are considered protected (Jacobs et al. 2023). With this information, one could make conclusions about what percentage of insect communities are being protected, what portions of the richness-elevation pattern are protected, and what new geographic areas should be considered for protection.

Summary of Chapter 2

I digitized and identified 32,000 weevil specimens collected across 25 elevation sites in Central America to determine how weevil richness and abundance changes with elevation, and what factors are related to this pattern. From sea level to 2500 m, I found that richness linearly increases, indicating that neotropical high elevation environments are more diverse with weevils than lower elevations. Spring temperature, humidity, and habitat surface area were the main environmental associates of this pattern. This chapter contributes to understanding how insect diversity changes across elevation gradients and provides insight into how climate change will impact these areas.

From a conservation perspective I found novel and concerning results. Weevils were most rich and abundant towards the cool and moist peaks of these tropical mountains. Climate change is predicted to shift warmer and drier climates upwards, potentially eliminating the areas in which weevils are thriving (Anderson and Ashe 2000, Ghalambor et al. 2006, McCain and Garfinkel 2021). While I found high richness of weevils in highly elevated, cool and moist environments across elevation, I cannot say for certain that these environmental factors are driving the high richness seen here. This is important to understand why there is such high diversity in these regions, and what that may mean for the future. To directly link factors like temperature to changes in weevil communities, experimental manipulations are required. For example, one could evaluate thermal thresholds for neotropical weevils across elevation using critical thermal maxima (For example see: Amundrud & Srivastava, 2020). This thermal threshold would allow us to determine if weevils are already at the edge of their climatic range.

Considering these conclusions and the fact that my data only extends to 2011, it is important to determine if these weevil species have remained the same, changed through range shifts, and/or been eliminated from climatic changes. Past research in similar areas have shown that insect communities have shifted about 100 m upward since the 1980's, such as in Ecuadorian ground beetles (Moret et al. 2016). I am not aware of any historical reports from these areas that sampled weevils, but hopefully future research will resample these same areas using the same methods present in my study to see if there have been any notable shifts in these weevil communities.

High richness present in high elevation cloud forests may be critical to the conservation of insects in general. High species richness in these high elevation tropical communities contains high phylogenetic and trait diversity (Dolson et al. 2021). These metrics of diversity are important in maintaining the resilience of a community to climate change as they allow a mechanism for future adaptation (Cadotte et al. 2015). Since phylogenetic and trait diversity are important metrics in conservation terms, one could ask: how much phylogenetic or trait diversity is contained within the high elevation weevil communities in Central America? This question could be answered through trait-based approaches or by DNA sampling these weevils and creating a phylogeny to determine phylogenetic diversity or even evolutionary history (See: Hulshof et al. 2024; J. Li et al. 2021).

Summary of Chapter 3

I used butterfly species collected over 50 years across Canada to determine if butterfly colour paleness and body size has changed with climatic warming. I found that butterflies have gotten paler and larger over recent decades, but increasing mean summer temperature does not explain this temporal trait pattern.

This study used natural history collections and as such, I was limited by the number of specimens and species that met my criteria for analysis. Thus, it is unclear whether the patterns I found here are applicable to other habitats, regions, and different species. In my search for specimens, I contacted many museum collections and curators (e.g. Smithsonian Museum of Natural History, Strickland Entomological Museum, Royal Saskatchewan Museum, Lyman Entomological Museum) and did not find any other species and/or specimens that fit my selection criteria. However, this does not mean that these do not exist. Many, if not most, entomology collections are not digitised and therefore, the data and specimens that exist within them are not yet available for broader access (Mason and De Bello 2013, Balke et al. 2013, Kharouba et al. 2019). Thus, it is likely that there are more species that fit my criteria across collections in Canada, or elsewhere, and I hope that they are located through further research or digitization.

One of the reasons I was limited by the number of specimens is because I used a novel approach to test the thermal melanism hypothesis by evaluating intra-specific changes in colour paleness over time. Most of the previous work on the thermal melanism hypothesis has used inter-specific trends to evaluate the prediction that darker individuals should inhabit cooler areas. While this tests the prediction that darker individuals should inhabit cooler areas, it ignores variation within species which is a stronger test of the thermal melanism hypothesis. However, amongst these challenges, one could use interspecific methods to test the hypothesis in another way and ask: Have butterfly communities become paler over time based on distribution changes? Using the same methods outlined by studies like Stelbrink et al. (2019) and Zeuss et al. (2014), one could assess the colour paleness of butterfly species in a region today (such as across Europe by Stelbrink et al. (2019)), and compare it to the same region decades previous, using known range shifts of butterfly species in Europe (Warren et al. 2021, Ulrich et al. 2023, Sunde et al. 2023). Here one could assess not only if colour paleness of species in certain regions is changing, but also if there are differences in how paler or darker species are moving in response to climatic shifts.

I hope that future research focuses on directly testing the drivers of colour paleness over time. I did not find a relationship between colour paleness and the proposed mechanism of colour paleness – temporal changes in temperature. To directly determine the driver of colour paleness over time, rearing experiments should be conducted on these same Canadian butterflies under different conditions. For example, to determine if nutrition changes are determining colour paleness, one could rear butterflies at various nutrition levels and measure paleness of adults (See: Jaumann & Snell-Rood, 2019).

Overall Conclusion

My work has shown that insects are responding to climatic factors over space and time in a variety of ways. We see that some insects across elevation respond to climatic factors like temperature (in different ways), and that butterflies in Canada are becoming paler over time. My elevation research (Chapter 1 and 2) has provided insight into where insect species are currently located, and what climatic factors they are responding to. My butterfly morphological trait research (Chapter 3) shows us that colour is changing through time, but the drivers of this change have yet to be determined.

With global insect populations declining (Wagner et al. 2021), understanding of spatiotemporal climate variation and how it impacts species richness, abundance, and traits is imperative to determining the exact drivers of these declines. This thesis helps us to understand these patterns and how insects will respond to climate change. This work has also demonstrated that while insects are responding to changing climate, the patterns of responses are complex, and there is much more that needs to be done to protect vulnerable ecosystems. Losses of insect assemblages will have profound consequences on surrounding ecosystems and taxa that rely on them and the window of action amongst these rapid changes is small. I hope that in knowing these patterns, future research will utilize this information to contribute directly to policy change and environmental conservation.

Supplementary Materials Chapter 1

Appendix 1.1. Study code, corresponding manuscript citation, and DOI of all papers included in my meta-analysis.

Study ID	DOI	Citation
MS001	0.1111/j.1365-2699.1997.00137.x	Anderson, A (1997) " Functional groups and patterns of organization in North American ant communities: a comparison with Australia" Journal of Biogeography 24:433-460
MS002	10.1111/jbi.12616	Ashton, L A (2016) " Vertical stratification of moths across elevation and latitude " Journal of Biogeography 49:59-69
MS003	10.1111/aec.12309	Ashton, L A, Odell E H, Burwell C J, Maunsell S C, Nakamura A, McDonald W J F, Kitching R L (2016) " Altitudinal patterns of moth diversity in tropical and subtropical Australian rainforests " Austral Ecology 41:197-208
MS004	10.1111/aec.12309	Ashton, L A, S Maunsell, D Bito, D Putland (2011) " Macrolepidopteran assemblages along an altitudinal gradient in subtropical rainforest - exploring indicators of climate change " Memoirs of Queensland Museum 55:375-389
MS005	10.1111/j.1366-9516.2004.00101.x	Axmacher, J C, G Holtmann, L Scheurmann, G Brehm, K Muller-Hohenstein, K Fielder (2004) " Diversity of geometrid moths (Lepidoptera: Geometridae) along an Afrotropical elevational rainforest transect " Diversity and Distributions 10:293-302
MS006	10.1111/icad.12395	Baudino F, Werenkraut V, Ruggiero A (2019) " Early recovery of beetle richness-elevation relationship and their environmental correlates after a major volcanic event in northwestern Patagonia, Argentina " Insect Conservation and DiversityNA:NA-NA

MS007	10.1016/j.baae.2010.08.009	Beck, J, Altermtt F, Hagmann R, Lang S (2010) " Seasonality in the altitude-diversity pattern of alpine moths " Basic and Applied Ecology11:714-722
MS008	10.1111/j.1466-8238.2009.00447.x	Beck, J, Kitching, I J (2009) “Drivers of moth species richness on tropical altitudinal gradients: a cross-regional comparison” Global Ecology and Biogeography 18: 361-371
MS009	10.1016/j.aspen.2011.12.003	Bhardwaj, M, Uniyal V P, Sanyal A K, Singh A P (2012) " Butterfly communities along an elevational gradient in the Tons valley, Western Himalayas: Implications of rapid assessment for insect conservation " Journal of Asia-Pacific Entomology 15:207-217
MS010		Bharti, H, Y P Sharma, M Bharti, M Pfeiffer (2013) " Ant species richness, endemicity and functional groups, along an elevational gradient in the Himalayas " Asian Myrmecology5:79-101
MS011	10.1111/j.1365-2699.2005.01336.x	Botes, A, M A McGeoch, H G Robertson, A Niekerk, H P Davids, S L Chown (2006) " Ants, altitude and change in the northern Cape Floristic Region " Journal of Biogeography33:71-90
MS012	10.1111/j.1442-9993.2007.01681.x	Botes, A, M A McGeoch, S L Chown (2007) " Ground-dwelling beetle assemblages in the northern Cape Floristic Region: patterns, correlates, and implications " Austral Ecology 32:210-224
MS013	10.3897/zookeys.547.9723	Bouzan, A M, V Flinte, M V Macedo, R F Monteiro (2015) " Elevation and temporal distributions of Chrysomelidae in southeast Brazil with emphasis on the Galerucinae " ZooKeys547:103-117
MS014	10.1371/journal.pone.0150327	Brehm, G, Hebert P D N, Colwell R K, Adams M, Bodner F, Friedemann K, Mockel L, Fielder

		K (2016) " Turning Up the Heat on a Hotspot: DNA Barcodes Reveal 80% More Species of Geometrid Moths along an Andean Elevational Gradient " Plos One11:e0150327-
MS015	10.1111/j.1466-8238.2006.00281.x	Brehm, G, R K Colwell, J Kluge (2007) " The role of environment and mid-domain effect on moth species richness along a tropical elevational gradient " Global Ecology and Biogeography 16:205-219
MS016	10.1034/j.1600-0587.2003.03498.x	Brehm, G, Sussenbach D, Fielder K (2003) " Unique Elevational Diversity Patterns of Geometrid Moths in an Andean Montane " Ecography26:456-466
MS017	10.1017/S0266467499000802	Bruhl, C A, M Mohamed, K E Linsenmair (1999) " Altitudinal distribution of leaf litter ants along a transect in primary forests on Mount Kinabalu, Sabah, Malaysia " Journal of tropical Ecology 15:265-277
MS018	10.1371/journal.pone.0108207	Carneiro, E, O H H Mielke, M M Casagrande, K Field (2014) " Community Structure of Skipper Butterflies (Lepidoptera, Hesperiidae) along Elevational Gradients in Brazilian Atlantic Forest Reflects Vegetation Type Rather than Altitude " Plos One9:e108207-
MS019	10.1111/btp.12548	Chatelain P, Plant A, Soulier-Perkins A, Daugeron C (2018) " Diversity increases with elevation: empidine dance flies (Diptera, Empididae) challenge a predominant pattern " Biotropica 50:633-640
MS020	10.1016/j.aspen.2013.06.002	Choi, S, An J (2013) " What we know and do not know about moth diversity from seven-year-monitoring in Mt. Jirisan National Park, South Korea " Journal of Asia-Pacific Entomology16:401-409
MS021	10.1007/s11284-017-1555-z	Choi, S, P P Thein (2018) " Distribution breadth and species turnover of night-flying

		beetles and moths on different mainland and island mountains " Ecological Research33:237-247
MS022	10.1007/s00442-018-4169-4	Corcos, D, P Cerretti, M Mei, A V Taglianti, D Paniccia, G Santoiemma, A De Biase, L Marini (2018) " Predator and parasitoid insects along elevational gradients: role of temperature and habitat diversity " Oecologia188:193-202
MS023	10.1111/ddi.12763	da Silva P G, J M Lobo, M C Hensen, F Z Viz-de-Mello, M I M Hernandez (2018) " Turnover and nestedness in subtropical dung beetle assemblages along an elevational gradient " Diversity and Distributions 24:1277-1290
MS024	10.1007/s10531-019-01745-4	Dale E J, Kitching R L, Thebaud C, Maunsell S C, Ashton L A (2019) " Moths in the Pyrénées: spatio-temporal patterns and indicators of elevational assemblages " Biodiversity and Conservation 28:1593-1610
MS025	10.1657/1938-4246-44.4.423	Despland, E, Humire R, San Martin S, (2012) " Species Richness and Phenology of Butterflies Along an Altitude Gradient in the Desert of Northern " Arctic, Antarctic, and Alpine Research44:423-431
MS026	10.1002/ecs2.3984	Dewan, S, N J Sanders, K Archarya (2022) " Turnover in butterfly communities and traits along an elevational gradient in the eastern himalaya, India " Ecosphere 13:e3984
MS027	10.1111/ecog.05427	Dolson, S J, Loewen E, Jones K, Jacobs S R, Solis A, Hallwachs W, Brunke A J, Janzen D H, Smith M A (2020) " Diversity and phylogenetic community structure across elevation during climate change in a family of hyperdiverse neotropical beetles (Staphylinidae) " Ecography 44:740-752
MS028		Dolson, S J, R A Anderson, H M Kharouba - Unpublished

MS029	10.1093/jmedent/45.4.800	Eisen, L, Bolling B G, Blair C D, Beaty B J, Moore C G (2008) " Mosquito species richness, composition, and abundance along habitat-climate-elevation gradients in the Northern Colorado Front Range " Journal of Medical Entomology 45:800-811
MS030	10.1111/j.2007.0906-7590.04818.x	Escobar F, Halffter G, Arellano L (2007) " From forest to pasture: an evalution of the influence of environment and biogeography on the structure of dung beetle (Scarabaeinae) assemblages along three altitudinal gradints in the Neotropical region " Ecography 30:193-208
MS031	10.1111/j.1466-822x.2005.00161.x	Escobar, F, J M Lobo, G. Halffter (2005) " Altitudinal variation of dung beetle (Scarabaeidae: Scarabaeinae) assemblages in the Colombian Andes " Global Ecology and Biogeography 14:327-337
MS032	0.1007/s10531-014-0738-y	Fattorini, S (2014) " Disentangling the effects of available area, mid-domain constraints, and species environmental tolerance on the altitudinal distribution of tenebrionid beetles in a Mediterranean area " Biodiversity and Conservation 23:2545-2560
MS033	10.1007/978-3-540-73526-7	Fiedler, K, G Brehm, N Hilt, D Sussenbach, C L Hauser (2008) " Variation of diversity patterns across moth familias along a tropical alititudinal gradient " Book Chapter in: Gradients in a Tropical Mountain Ecosystem of Ecuador
MS034	10.1111/icad.12456	Finnem S, K Sam, M, Leponce, Y Basset, D Drew, M K Schutze, et al. (2021) " Assemblages of fruit flies (Diptera: Tephritidae) along an elevational gradient in the rainforests of Papua New Guinea " Insect conservation and diversity 14:348-355

MS035	10.1371/journal.pone.0204787	Flores, O, J Seoane, V Hevia, F M Azcarate (2018) " Spatial patterns of species richness and nestedness in ant assemblages along an elevational gradient in a Mediterranean mountain range " Plos One13:e0204787-
MS036	10.1073/pnas.0909686107	Forister, M L, A C McCall, N J Sanders, A M Shapiro (2009) " Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity " PNAS 107:2088-2092
MS037	10.1111/jbi.13710	Gebert F, Steffan-Dewenter I, Moretto P, Peters M K (2020) " Climate rather than dung resources predict dung beetle abundance and diversity along elevational and land use gradients on Mt. Kilimanjaro " Journal of Biogeography 47:371-381
MS038	10.1111/btp.12263	Gillette, P N, K K Ennis, G Dominguez Martinez, S M Philpott (2015) " Changes in Species Richness, Abundance, and Composition of Arboreal Twig-nesting Ants Along an Elevational Gradient in Coffee Landscapes " Biotropica47:712-722
MS039	10.7717/peerj.4117	Gonzalez-Reyes, A X, Corronca J A, Rodriguez-Artigas S M (2017) " Changes of arthropod diversity across an altitudinal ecoregional zonation in Northwestern Argentina " PeerJ 5:e4117-
MS040	10.1603/022.038.0611	Hackenberger, B K, D Jaric, S Krcmar (2009) " Distribution of Tabanids (Diptera: Tabanidae) Along a Two-Sided Altitudinal Transect " Environmental Entomology38:1600-1607
MS041	10.1111/aen.12114	Hall, C R, C J Burwell, A Nakamura, R L Kitching (2015) " Altitudinal variation of parasitic Hymenoptera assemblges in Australian subtropical rainforest " Austral Ecology54:246-258

MS042	10.1017/S0266467405003056	Hilt, N, Brehm G, Fiedler K (2006) " Diversity and ensembly composition o geometrid moths along a successional gradient in the Ecuadorian Andes " Journal of Tropical Ecology22:155-166
MS043	10.1098/rspb.2012.1581	Hoiss B, Kraussss J, Potts S G, Roberts S, Steffan-Dewenter I (2012) " Altitude acts as an environmental filter on phylogenetic composition, traits, and diversity in bee communities " Proceedings of the Royal Society B 279:4447-4456
MS044		Karaman, M (2011) " Zoogeography, diversity and altitudinal distribution of ants (Hymenoptera: Formicidae) in the mediterranean and the oro-Mediterranean parts of Montenegro " North Western Journal of Zoology7:26-34
MS045	10.1111/j.1466-8238.2007.00319.x	Kubota, U, R D Loyola, A M Almeida, D A Carvalho, T M Lewinsohn (2007) " Body size and host range co-determine the altitudinal distribution of Neotropical tephritid flies " Global Ecology and Biogeography 16:632-639
MS046	10.1038/s41598-018-23210-y 1	Lefebvre, V, C Villemant, C Fontaine, C Daugeron (2018) " Altitudinal, temporal and trophic partitioning of flower-visitors in Alpine communities " Scientific Reports8:4706-
MS047	10.1656/1528-	Lessard, J, R R Dunn, C R Parker, N J Sanders (2007) " Rarity and diversity in forest ant assemblages of Great Smoky Mountains National Park " BioOne 6:215-228
MS048	10.14411/eje.2007.069	Lobo, J M, E Chehlarov, B Guerguiev (2007) " Variation in dung beetle (Coleoptera: Scaraboidea) assemblages with altitude in the Bulgarian Rhodopes Mountains: A comparison " European Journal of Entomology 104:489-495

MS049	10.1603/0013-8746(2000)093[0115:BAEFAT]2.0.CO;2	Lobo, J M, G Halffter (2000) " Biogeographical and Ecological Factors affecting the altitudinal variation of Mountainous communities of copeophagous beetles (Coleoptera: Scarabaeoidea): a comparitive study " Annals of the Entomological Society of America 93:115-126
MS050	10.1002/ecs2.2798	Longino, J T, M G Brantstetter, P S Ward (2019) " Ant diversity patterns across tropical elevation gradients: effects of sampling method and subcommunty " Ecosphere 10:e02798
MS051	10.1890/ES10-00200.1	Longino, J T, R K Colwell (2011) " Density compensation, species composition, and richness of ants on a neotropical elevational gradient " Ecosphere 2:1-20
MS052	10.1016/j.aspen.2021.08.009	Marathe, A, K Shanker, J Krishnaswamy, D R Priyadarsanan (2021) " Species and functional group composition of ant communities across an elevational gradient in the Eastern Himalaya " 24:1244-1250
MS053	10.1016/j.baae.2012.09.003	Marini, L, M Quaranta, P Fontana, J C Biesmeijer, R Bommarco (2012) " Landscape context and elevation affect pollinator communities in intensive apple orchards " Basic and Applied Ecology12:681-689
MS054	10.3897/zookeys.147.2047	Maveety, S A, R A Browne, T L Erwin (2011) " Carabidae diversity along an altitudinal gradient in a Peruvian clod forest (Coleoptera) " ZooKeys 147:651-666
MS055	10.1080/01650521.2013.873266	Maveety, S A, R A Browne, T L Erwin (2013) " Carabid beetle diversity and community composition as related to altitude and seasonality in Andean forests " Studies on Neotropical Fauna and Environment 48:165-174

MS056	10.21425/F5FBG47013	McCain, C (2020) " Another rejection of the more-individuals-hypothesis: Carrion beetles (Silphidae, Coleoptera) in the Southern Rocky Mountains " Frontiers of Biogeography 13:e47013
MS057	10.1371/journal.pone.0135210	Meyer III, W M, J A Eble, K Franklin, R B McManus, S L Brantley, J Henkel, P E Marek, W E Hall, C A Olson, R McInroy, E M B Loaiza, R C Brusca, W Moore (2015) " Ground-Dwelling Arthropod Communities of a Sky Island Mountain Range in Southeastern Arizona, USA: Obtaining a Baseline for Assessing the Effects of Climate Change " Plos One10:e0135210-
MS058	10.1080/11956860.2017.1324717	Mumladze, L, W Ulrichc, Z Asanidze, and G Japoshvili (2017) " An inverse elevational species richness gradient of Caucasian vascular plants and Encyrtidae (Hymenoptera, Chalcidoidea) " Ecoscience 24:75-79
MS059	10.1093/ee/nvy133	Noreiga, J A, E Realpe (2018) " Altitudinal turnover of species in a Neotropical peripheral mountain system: A case study with dung beetles (Coleoptera|: Aphodiinae and Scarabaeinae) " Environmental Entomology 47:1376-1387
MS060	10.1093/ee/nvy133	Noriega, J A, Realpe E (2018) " Altitudinal turnover of species in a Neotropical peripheral mountain system: A case study with dung beetles (Coleoptera: Aphodiinae and Scarabaeinae) " Environmental Entomology 47:1376-1387
MS061	10.1111/btp.12539	Orivel, J, P Klimes, V Novotny, and M Leponce (2018) " Resource use and food preferences in understory ant communities along a complete elevational gradient in Papua New Guinea " Biotropica 50:641-648

MS062	10.1007/s10530-008-9218-1	Peck, P W, P C Banko, M Schwarzfeld, M Euaparadorn, K W Brinck (2008) " Alien dominance of the parasitoid wasp community along an elevation gradient on Hawaii island " Biological Invasions10:1441-1455
MS063	10.1111/jbi.14217	Perez-Toledo, G, J E Valenzuela-Gonzalez, C E Moreno, F Villalobos, R R Silva (2021) " Patterns and drivers of leaf-litter ant diversity along a tropical elevational gradient in Mexico " Journal of Biogeography 48:2512-2523
MS064	10.1371/journal.pone.0182054	Perillo, L N, F S Neves, Y Antonini, R P Martins (2017) " Compositional changes in bee and wasp communities along Neotropical mountain altitudinal gradient " Plos One 12:e0182054
MS065	10.1111/jbi.12384	Peters, M K, A Mayr, J Roder, N J Sanders, I Steffan-Dewenter (2014) " Variation in nutrient use in ant assemblages along an extensive elevational gradient on Mt Kilimanjaro " Journal of Biogeography 41:2245-2255
MS066	10.1007/s13744-012-0031-2	Pyrcz, T W, R Garlacz (2012) " The Presence–Absence Situation and Its Impact on the Assemblage Structure and Interspecific Relations of Pronophilina Butterflies in the Venezuelan Andes (Lepidoptera: Nymphalidae) " Neotropical Entomology 41:186-195
MS067	10.1046/j.1466-822X.2002.00285.x	Pyrcz, T W, Wojtusiak J (2002) " The Vertical Distribution of Pronophiline Butterflies (Nymphalidae, Satyrinae) along anElevational Transect in Monte Zerpa (Cordillera de Mérida, Venezuela) with Remarks onTheir Diversity and Parapatric Distribution " Global Ecology and Biogeography 11:211-221
MS068	10.1111/j.1466-822x.2006.00281.x	Pyrcz, T W, Wojtusiak J (2007) " The role of environment and mid-domain effect on moth

		species richness along a tropical elevational gradient " Global Ecology and Biogeography 16:205-219
MS069	10.1016/j.rbe.2018.12.005	Ribeiro, D G, R Silvestre, B R Garcete-Barrett (2019) " Diversity of wasps (Hymenoptera: Aculeata: Vespidae) along an altitudinal gradient of Atlantic Forest in Itatiaia National Park, Brazil " Revista Brasileira de Entomologia 63:22-29
MS070	10.1111/j.1461-0248.2010.01525.x	Rodriguez-Castaneda G, Dyer L A, Brehm G, Connahs H, Forkner R E, Walla T R (2010) " Tropical forests are not flat: How mountains affect herbivore diversity " Ecology Letters 13:1348-1357
MS071		Romero-Alcaraz, E, J M Avila (2000) " Effect of elevation and type of habitat on the abundance and diversity of Scarabaeoid dung beetle assemblages in a Mediterranean area from Southern Iberian Peninsula " Zoological Studies39:351-359
MS072		Samson, D A, E A Rickart, P C Gonzales (1997) " Ant diversity and abunance along an elevational gradient in the Phillipines " Biotropica 29:349-363
MS073	10.3897/zookeys.611.9608	Sánchez-Reyes, U J, S Niño-Maldonado, L Barrientos-Lozano, S W Clark, and R W Jones (2016) " Faunistic patterns of leaf beetles (Coleoptera, Chrysomelidae) within elevational and temporal gradients in Sierra de San Carlos, Mexico " ZooKeys 611:11-56
MS074	10.1673/031.008.6901	Sau, T K, P J Vineesh, K V Vinod (2008) " Diversity of forest litter-inhabiting ants along elevations in the Wayanad region of the Western Ghats " Journal of Insect Science 8:NA-NA

MS075	10.14411/eje.2019.034	Senyuz Y, Lobo J M, Dinder K (2019) " Altitudinal gradient in species richness and composition of dung beetles (Coleoptera: Scarabaeidae) in an eastern Euro-Mediterranean locality: Functional, seasonal and habitat infl uences " European Journal of Entomology116:309-319
MS076	10.3897/zoologia.36.e32938	Sublett, C A, Cook J L, Janovec J P (2019) " Species richness and community composition of sphingid moths (Lepidoptera: Sphingidae) along an elevational gradient in southeast Peru " Zoologia 36:e32938-
MS077	10.1111/ecog.04115	Szewcyzk, T M, McCain C M (2018) " Disentangling elevational richness: a multi-scale hierarchical Bayesian occupancy model of Colorado ant communities " Ecography 42:977-988
MS078	10.1007/s11676-016-0212-7	Thein P P, Choi S (2016) " Forest insect assemblages attracted to light trap on two high mountains (Mt. Jirisan and Mt. Hallasan) in South Korea " Journal of Forestry Research 27:1203-1210
MS079	10.1080/15230430.2018.1475951	Winkler, M, P Illmer, P Querner, B M Fischer, K Hofmann, A Lamprecht, N Praeg, J Schied, K Steinbauer, H Pauli (2018) " Side by side? Vascular plant, invertebrate, and microorganism distribution patterns along an alpine to nival elevation gradient " Arctic, Antarctic, and Alpine Research50:e1475951-
MS080	10.1371/journal.pone.0069177	Yu, X, T Luo, H Zhou (2013) " Elevational Gradient in Species Richness Pattern of Epigaeic Beetles and Underlying Mechanisms at East Slope of Balang Mountain in Southwestern China " Plos One 8:e69177

Appendix 1.2. Measures of all possible predictors and predictor combinations to determine the best mixed effects model. Completed through the “dredge” function in the package “MuMln”. The final model (bolded below) contained covariates of highest elevation sampled and latitude.

Intercept	**Biome**	**Elevation**	**Elevation^2**	**Highest**	**Latitude**	**Taxa**	**DF**	**logLik**	**AICc**	**Delta**	**Weight**
-0.3			0.0	0.0			10.0	-2029.3	4078.7	0.0	0.4
-0.4			**0.0**	**0.0**	**0.0**		**11.0**	**-2029.0**	**4080.1**	**1.4**	**0.2**
-0.3		0.0	0.0	0.0			11.0	-2029.3	4080.7	2.0	0.1
-0.1	+		0.0	0.0			14.0	-2026.9	4082.1	3.4	0.1
-0.4		0.0	0.0	0.0	0.0		12.0	-2029.0	4082.1	3.5	0.1
-0.3			0.0	0.0		+	13.0	-2028.1	4082.4	3.7	0.1
-0.2	+		0.0	0.0	0.0		15.0	-2026.2	4082.8	4.1	0.0
-0.3			0.0	0.0	0.0	+	14.0	-2027.9	4084.0	5.3	0.0
-0.1	+	0.0	0.0	0.0			15.0	-2026.9	4084.2	5.5	0.0
-0.2		0.0	0.0	0.0		+	14.0	-2028.1	4084.4	5.7	0.0
-0.2	+	0.0	0.0	0.0	0.0		16.0	-2026.3	4084.9	6.2	0.0
-0.3		0.0	0.0	0.0	0.0	+	15.0	-2027.8	4086.0	7.3	0.0
0.0	+		0.0	0.0		+	17.0	-2025.9	4086.2	7.5	0.0
-0.1	+		0.0	0.0	0.0	+	18.0	-2025.2	4086.8	8.1	0.0
0.0	+	0.0	0.0	0.0		+	18.0	-2025.9	4088.3	9.6	0.0
-0.1	+	0.0	0.0	0.0	0.0	+	19.0	-2025.2	4088.9	10.2	0.0
0.0		0.0		0.0			10.0	-2040.1	4100.4	21.7	0.0
-0.1		0.0		0.0	0.0		11.0	-2039.7	4101.6	22.9	0.0
0.1		0.0		0.0		+	13.0	-2038.8	4103.9	25.2	0.0
0.2	+	0.0		0.0			14.0	-2038.0	4104.2	25.6	0.0
0.0		0.0		0.0	0.0	+	14.0	-2038.5	4105.2	26.5	0.0
0.2	+	0.0		0.0	0.0		15.0	-2037.8	4105.8	27.1	0.0
0.3	+	0.0		0.0		+	17.0	-2036.8	4107.9	29.2	0.0
0.2	+	0.0		0.0	0.0	+	18.0	-2036.5	4109.4	30.7	0.0
0.8	+		0.0				13.0	-2048.5	4123.3	44.6	0.0
0.7	+	0.0	0.0				14.0	-2048.3	4124.8	46.1	0.0
0.8	+		0.0		0.0		14.0	-2048.5	4125.3	46.6	0.0
0.8	+	0.0	0.0		0.0		15.0	-2048.3	4126.8	48.1	0.0

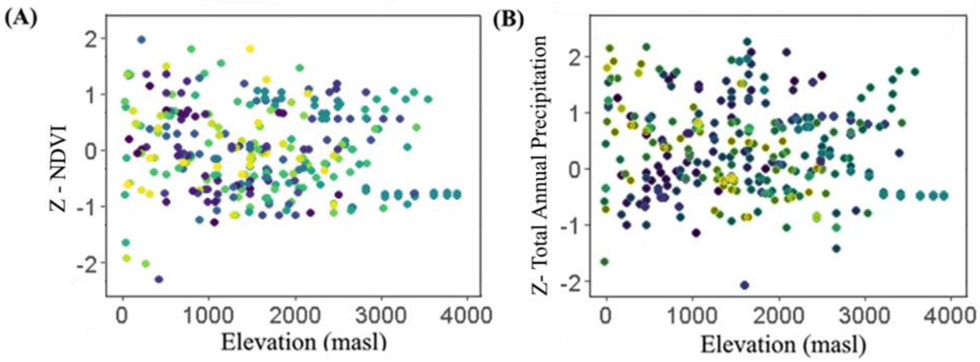
0.9	+		0.0			+	16.0	-2047.3	4126.9	48.2	0.0
0.5			0.0				9.0	-2054.6	4127.4	48.7	0.0
0.6			0.0		0.0		10.0	-2053.8	4127.7	49.1	0.0
0.8	+	0.0	0.0			+	17.0	-2047.1	4128.5	49.8	0.0
0.9	+		0.0		0.0	+	17.0	-2047.3	4129.0	50.3	0.0
0.4		0.0	0.0				10.0	-2054.5	4129.2	50.5	0.0
0.5		0.0	0.0		0.0		11.0	-2053.7	4129.5	50.8	0.0
0.8	+	0.0	0.0		0.0	+	18.0	-2047.0	4130.5	51.8	0.0
0.5			0.0			+	12.0	-2053.7	4131.5	52.9	0.0
0.6			0.0		0.0	+	13.0	-2052.9	4132.0	53.3	0.0
0.5		0.0	0.0			+	13.0	-2053.6	4133.4	54.7	0.0
0.6		0.0	0.0		0.0	+	14.0	-2052.8	4133.8	55.1	0.0
1.2	+	0.0					13.0	-2059.0	4144.3	65.6	0.0
-0.6				0.0			9.0	-2063.4	4144.9	66.2	0.0
0.8		0.0					9.0	-2063.9	4146.0	67.3	0.0
1.2	+	0.0			0.0		14.0	-2058.9	4146.0	67.3	0.0
-0.7				0.0	0.0		10.0	-2063.2	4146.5	67.8	0.0
0.9		0.0			0.0		10.0	-2063.3	4146.8	68.1	0.0
1.2	+	0.0				+	16.0	-2057.7	4147.8	69.1	0.0
-0.4	+			0.0			13.0	-2061.2	4148.6	69.9	0.0
-0.6				0.0		+	12.0	-2062.5	4149.2	70.5	0.0
1.3	+	0.0			0.0	+	17.0	-2057.6	4149.7	71.0	0.0
-0.4	+			0.0	0.0		14.0	-2061.0	4150.2	71.5	0.0
0.9		0.0				+	12.0	-2063.0	4150.2	71.5	0.0
-0.6				0.0	0.0	+	13.0	-2062.3	4150.9	72.2	0.0
1.0		0.0			0.0	+	13.0	-2062.4	4151.1	72.4	0.0
-0.3	+			0.0		+	16.0	-2060.3	4152.9	74.2	0.0
-0.4	+			0.0	0.0	+	17.0	-2060.0	4154.4	75.7	0.0
0.4	+						12.0	-2074.6	4173.3	94.6	0.0
0.1							8.0	-2079.3	4174.7	96.0	0.0
0.5	+				0.0		13.0	-2074.5	4175.3	96.6	0.0
0.2					0.0		9.0	-2078.9	4175.8	97.1	0.0
0.5	+					+	15.0	-2073.4	4177.2	98.5	0.0
0.5	+				0.0	+	16.0	-2073.4	4179.2	100.5	0.0

0.2		+	11.0	-2078.5	4179.2	100.6	0.0
0.2	0.0	+	12.0	-2078.1	4180.4	101.8	0.0

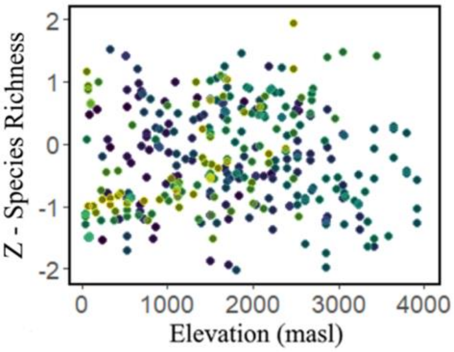
Appendix 1.3. A breakdown of all 151 elevation gradients included in my meta-analysis by biome and taxa.

		Full Dataset		Environmental Dataset	
Category		Number of Gradients	Percent	Number of Gradients	Percent
Biome	Tropical	74	49	17	52
	Temperate	48	32	11	33
	Mediterranean	13	9	3	9
	Alpine	10	7	1	3
	Desert	6	4	1	3
Taxa	Lepidoptera	47	31	6	18
	Hymenoptera	38	25	16	48
	Coleoptera	42	28	9	27
	Diptera	24	16	2	6

Appendix 1.4. Environmental factors with elevation, including (a) NDVI (slope = -1.3 E^{-4} (1.6 E^{-4} SE), $t_{1,281}$ = -0.81, p = 0.42, $R^2_{(C)}$ = 0.48, $R^2_{(M)}$ = 0.01), (b) precipitation (slope = 6.9 E^{-5} (2.3 E^{-4} SE); $t_{1,258}$ = 0.30, p = 0.76, $R^2_{(C)}$ = 0.73, $R^2_{(M)}$ < 0.01).



Appendix 1.5. Species richness in the smaller environmental dataset was not related to elevation (slope = 3.9 E^{-6} (2.0 E^{-4} SE), $t_{1,281}$ = 0.02, p = 0.98; $R^2_{(C)}$ = 0.63, $R^2_{(M)}$ = 9.9 E^{-6}).



Supplementary Materials: Chapter 2

Appendix 2.1. Data sources for all covariates used in analysis.

Variable	Year of Data Availability	Source of data	Size	Link
Gross Primary Production	2009	MODIS Global - Earth Explorer	500 m^2	https://catalog.data.gov
Mean Spring Humidity	2009 Spring	NicheMapR	Point measurement	doi/10.1111/2041-210X.13330
Mean Spring Temp	2009 Spring	NicheMapR	Point measurement	doi/10.1111/2041-210X.13330
Area	2000	GPTOPO30 - Earth Explorer	1 km^2	https://www.usgs.gov/centers/eros
Temperature Seasonality	2000	Worldclim	1 km^2	https://www.worldclim.org/

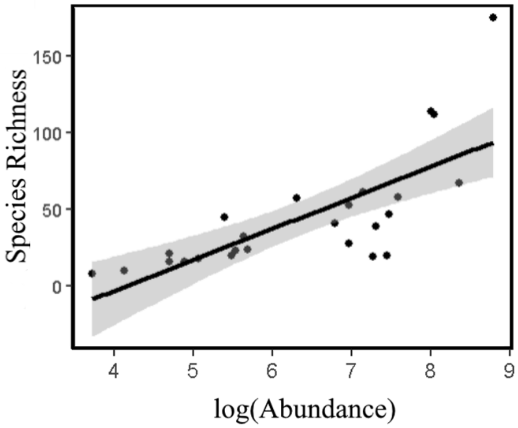
Appendix 2.2. The comparison between linear and quadratic models for the richness-elevation relationship. We determined the best fit based on Akaike’s Information Criterion (AICc). The final model was the one that was the most parsimonious (e.g., model with the lowest AIC – linear model).

Model	**Variables**	**Slope (Standard Error)**	**t - Score**	**DF**	**P - Value**	**AIC**
Quadratic	**Richness**	**7.2 E^{-4} (3.4 E^{-4})**	**2.1**	**23**	**0.03**	201
	Richness^2	4.1 E^{-8} (1.3 E^{-7})	0.3		0.74	
Linear	**Richness**	**8.3 E^{-4} (8.7 E^{-5})**	**9.5**	**24**	**<0.001**	199

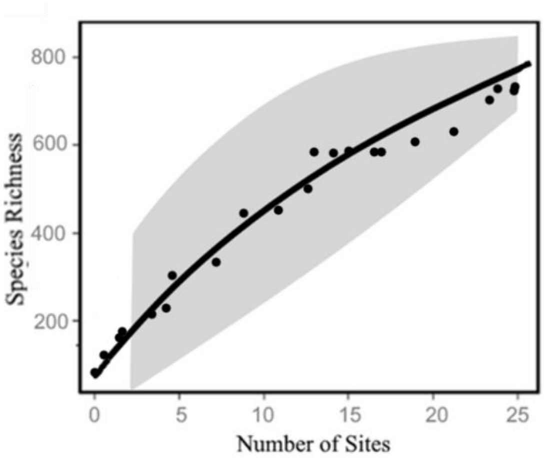
Appendix 2.3. Collinearity amongst environmental variables assessed through variance inflation factor (VIF). VIF test was done from a linear model with all the below variables and species richness. Typically, a VIF value over 4 or 5 is considered collinear (Zuur et al. 2009).

Variable	VIF
Elevation	11.9
Temperature	9.91
Area	6.14
Seasonality	2.65
Humidity	4.64
GPP	1.18

Appendix 2.4. The relationship between morphospecies richness and abundance (slope = 0.47 (0.06 SE), $t_{1,24}$ = 7.8, $p < 0.001$). Abundance was log transformed due to the data being right-skewed. The overall line of best fit is shown in black with the grey shading indicating the 95% prediction interval (n = 25 sites).



Appendix 2.5. Rarefaction curve of the number of morphospecies accumulated per site sampled, across 25 sites. The overall rarefaction curve is shown in black with the grey shading indicating the 95% prediction interval.



Supplementary Materials: Chapter 3

Appendix 3.1. To demonstrate that there is no difference between the temperature metrics being used, I show a simple linear regression between mean colour paleness of butterflies and different temperature metrics.

Temperature Metric	Coefficient (Standard Error)	F-value	DF	p-value
Average Spring Temperature	-0.96 (0.18)	-5.3	990	<0.001
Average Summer Temperature	-1.6 (0.31)	-5.3	990	<0.001
Average Winter Temperature	-0.83 (0.13)	-6.2	990	<0.001
Maximum Summer Temperature	0.92 (0.24)	-3.8	990	<0.001

Appendix 3.2. In total, I fit 8 models to determine how butterfly wing colour paleness and body size change in response to climate change.

Model	Predictor	Random effect	Number of observations
Model 1: Temperature	Year Latitude Longitude	Site	1285
Model 2: Colour paleness	Temperature Latitude Length DOY Elevation	Species Year	991
Model 3: Colour paleness	Temperature Latitude Length DOY Elevation	Species Location	306
Model 4: Colour paleness	Year Latitude Length DOY Elevation	Species Location	991
Model 5: Length	Temperature Latitude DOY Elevation	Species Year	991
Model 6: Length	Year Latitude DOY Elevation	Species Location	991
Model 7: Colour paleness	Length	Species	991
Model 8: Colour paleness	Slope	NA	10

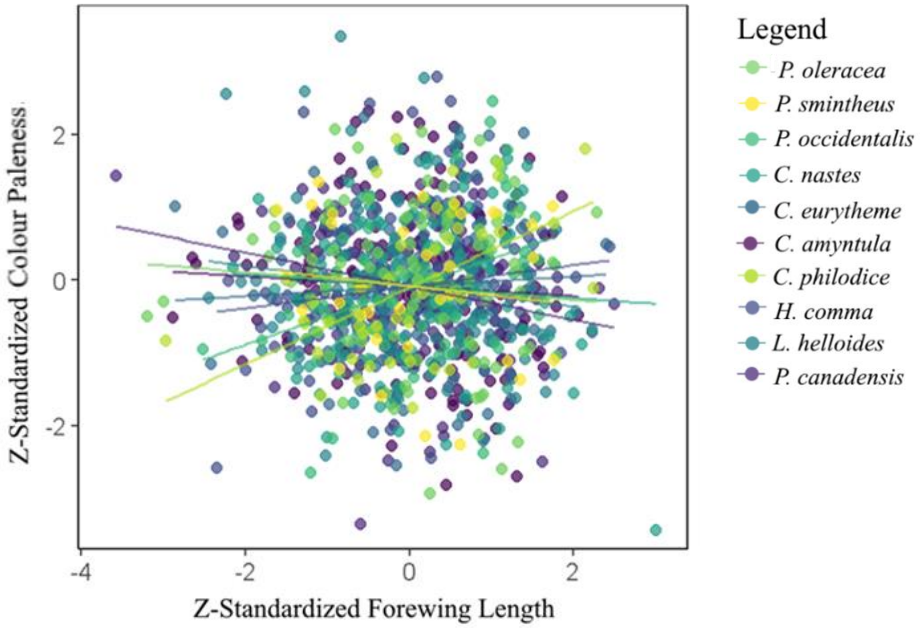
Appendix 3.3. Collinearity amongst covariates in trait models tested through variance inflation factor tests (VIF). I selected a VIF cutoff of 5 to balance the complexity of the model and collinearity of the variables. I did not remove any variables due to collinearity.

Model	Predictor	VIF
Model 2: Colour paleness ~ Temperature	Temperature	1.6
	Latitude	1.9
	Length	1.0
	DOY	1.0
	Elevation	1.4
Model 3: Colour paleness ~ Temperature	Temperature	1.8
	Latitude	1.9
	Length	1.0
	DOY	1.0
	Elevation	1.3
Model 4: Colour paleness ~ Year	Year	1.1
	Latitude	1.3
	Length	1.0
	DOY	1.1
	Elevation	1.2
Model 5: Length ~ Temperature	Temperature	1.5
	Latitude	1.7
	DOY	1.0
	Elevation	1.4
Model 6: Length ~ Year	Year	1.0
	Latitude	1.2
	DOY	1.0
	Elevation	1.1

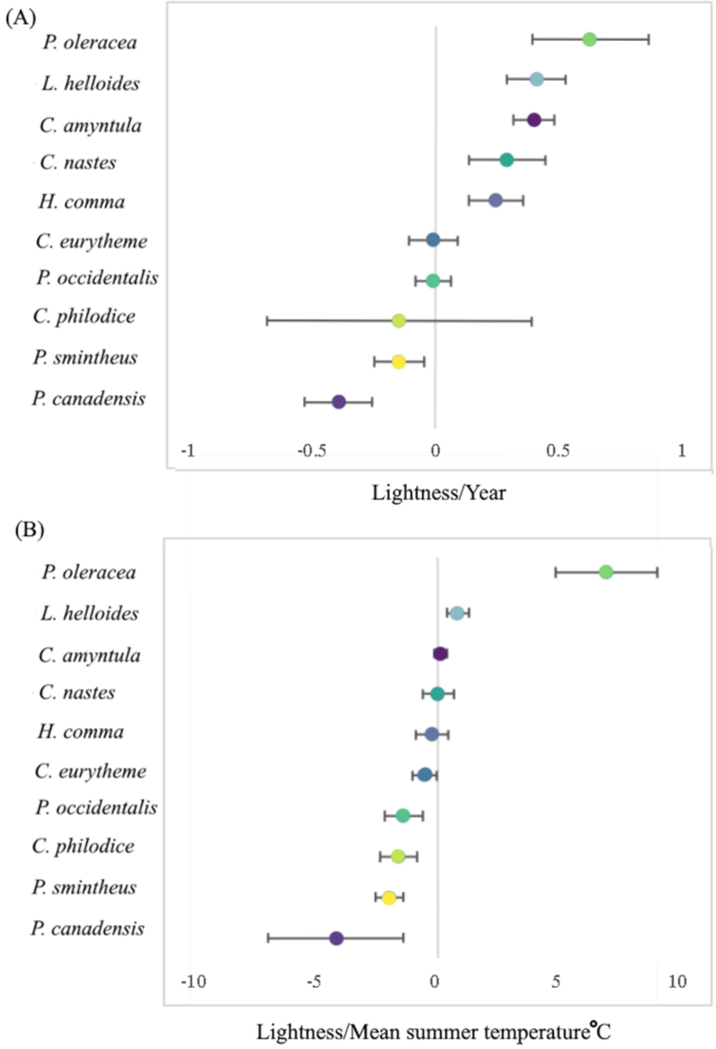
Appendix 3.4. The relationship between temperature and time in a linear, and non-linear model with a hinge point at 1980 when temperature is known to have increased.

Model	Predictor	Coefficient (Standard Error)	F-value	DF	p-value	AIC
Temperature ~ Year	Year	0.01 (0.01)	2.2	1282	0.03	6766
Temperature ~ Year Hinge at 1980	Year pre 1980	0.003 (0.01)	0.27	1280	0.79	6769
	Year post 1980	0.02 (0.03)	0.97			

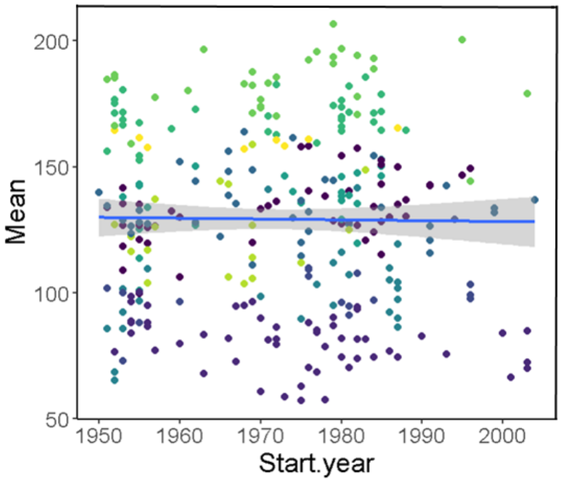
Appendix 3.5. To determine the relationship between colour paleness and forewing length, I Z-transformed ((x_i – mean)/standard deviation) both variables to evaluate the pattern regardless of overall size and colour paleness differences between species. Standardized colour paleness was not related to standardized forewing length (slope = 2.6 E^{-3} units of colour/mm (0.01 SE), $t_{1,980}$ = 0.18, p = 0.85). Coloured lines represent models from each individual species based on random effects.



Appendix 3.6. Slope of colour paleness over (A) year and (B) mean summer temperature for all 10 species. Error bars represent standard error.



Appendix 3.7. I used a reduced model to examine if the difference in climate over time has impacted colour paleness by evaluating the mean colour paleness per year, per species, with year. I used the same model structure as listed above for model 4, but without 1) location as a random effect, and 2) any correlation structure to maintain power. I additionally added a covariate of average temperature from my latest year of collection (2004), subtracted by the temperature of the year of collection per species per location. Colour paleness changed over time in this reduced dataset (0.23 (0.06 SE), $t_{1,290} = 4.0$, $p < 0.001$), and day of year was the only significant covariate (-0.09 (0.02 SE), $t_{1,290} = -3.4$, $p < 0.001$).



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