ECOGRAPHY

Research

Noctuid and geometrid moth assemblages show divergent elevational gradients in body size and color lightness

Lea Heidrich, Stefan Pinkert, Roland Brandl, Claus Bässler, Hermann Hacker, Nicolas Roth, Annika Busse, Jörg Müller and Nicolas Friess

L. Heidrich (https://orcid.org/0000-0002-3229-4758) ✉ (lea.heidrich@gmx.de), S. Pinkert (https://orcid.org/0000-0002-8348-2337) and R. Brandl, Faculty of Biology, Dept of Ecology – Animal Ecology, Philipps-Universität Marburg, Marburg, Germany. SP also at: Yale Univ., Ecology and Evolutionary Biology, New Haven, CT, USA. – LH, H. Hacker and J. Müller (https://orcid.org/0000-0002-1409-1586), Ökologische Station Fabrikschleichach, Dept of Animal Ecology and Tropical Biology, Univ. of Würzburg, Germany. – C. Bässler (https://orcid.org/0000-0001-8177-8997), Goethe Univ. Frankfurt, Faculty of Biological Sciences, Inst. for Ecology, Evolution and Diversity, Dept of Conservation Biology, Frankfurt am Main, Germany. – CB, JM and A. Busse, Bavarian Forest National Park, Grafenau, Germany. – N. Roth, Bern Univ. of Applied Sciences School of Agricultural Forest and Food Sciences, Zollikofen, Switzerland; and, Swiss Federal Research Inst. WSL, Birmensdorf, Switzerland. – N. Friess, Faculty of Geography, Environmental Informatics, Marburg.

Previous macroecological studies have suggested that larger and darker insects are favored in cold environments and that the importance of body size and color for the absorption of solar radiation is not limited to diurnal insects. However, whether these effects hold true for local communities and are consistent across taxonomic groups and sampling years remains unexplored. This study examined the variations in body size and color lightness of the two major families of nocturnal moths, Geometridae and Noctuidae, along an elevational gradient of 700 m in Southern Germany. An assemblage-based analysis was performed using community-weighted means and a fourth-corner analysis to test for variations in color and body size among communities as a function of elevation. This was followed by a species-level analysis to test whether species occurrence and abundance along an elevation gradient were related to these traits, after controlling for host plant availability. In both 2007 and 2016, noctuid moth assemblages became larger and darker with increasing elevation, whereas geometrids showed an opposite trend in terms of color lightness and no clear trend in body size. In single species models, the abundance of geometrids, but not of noctuids, was driven by habitat availability. In turn, the abundance of dark-colored noctuids, but not geometrids increased with elevation. While body size and color lightness affect insect physiology and the ability to cope with harsh conditions, divergent trait–environment relationships between both families underline that findings of coarse-scale studies are not necessarily transferable to finer scales. Local abundance and occurrence of noctuids are shaped by morphological traits, whereas that of geometrids are rather shaped by local habitat availability, which can modify their trait–environment-relationship. We discuss potential explanations such as taxon-specific flight characteristics and the effect of microclimatic conditions.

Keywords: insects, color lightness, body size, elevation, habitat availability, flight characteristics

Introduction

Macrophysiological approaches aim to understand the importance of trait variation for the distribution and abundance of species along environmental gradients (Chown and Gaston 2016). The key assumption of this conceptional framework is that trait–environment interactions scale from individual advantages in terms of growth, fecundity or survival, over local patterns in abundance and distribution, to larger taxonomic and spatial scales (Gaston et al. 2009). Hence large-scale patterns in average traits of species assemblages should be indicative of a higher physiological performance of individuals with certain traits in a given environment (Gaston et al. 2009). Such inferences provide a powerful starting point for improving mechanistic predictions of species responses to climate change (Violle et al. 2014, Urban et al. 2016). However, for the sake of identifying generalities, macroecological approaches necessarily reduce biological detail, that is they integrate over local factors that might limit the realized niche (McGill 2019), such as habitat availability (Platts et al. 2019), species interactions (Wiens 2011) or microclimatic variation (Hof et al. 2011). Thus, the question is whether the trait–environment correlations observed at a macroscale can indeed contribute to predictions of the responses of organisms to environmental change at smaller spatial and temporal scales (Law et al. 2020).

For highly diverse groups such as insects, trait-based generalizations offer a promising tool to overcome the taxonomic impediments in studying organismal responses to environmental changes (Wong et al. 2019). Most insects are ectothermes and thus rely on external heat sources such as solar radiation to reach their thermal optimum. Therefore, functional traits that influence an insect's heat budget, particularly body size and color lightness, are often the focus of trait-based approaches (Chown et al. 2004). Large bodies are assumed to be advantageous in colder climates because the body surface-to-volume ratio decreases with size. This means that larger species can raise their body temperature above the ambient temperature because of the slower loss of heat gained by solar radiation through the body surface ('heat conservation hypothesis;' Stone 1993, Zamora-Camacho et al. 2014). Furthermore, ectotherms develop faster in warm regions but tend to reach a smaller adult body size than in colder regions ('temperature size rule', Atkinson and Sibly 1997). In addition to body size, the color lightness of a body surface influences the absorption of solar radiation. Dark organisms heat up faster than their light-colored counterparts at a given level of solar irradiance (Watt 1968), ultimately leading to a fitness advantage in colder environments ('thermal melanism hypothesis', Clusella Trullas et al. 2007). Moreover, correlations between temperature and insect color lightness may be the product of pleiotropic linkages of color lightness to life history, which depends on temperature itself (Umbers et al. 2013, Clusella-Trullas and Nielsen 2020).

In diurnal insects, traits related to the uptake and storage of external heat provide a direct mechanistic link to the environmental temperature. Although not directly exposed to solar radiation during their periods of activity, their nocturnal counterparts nonetheless exhibit distinct spatial patterns in both size and color. For instance, the mean color lightness of assemblages of geometrid moths was shown to decrease with increasing latitude across Europe (Heidrich et al. 2018) and along elevational gradients (local assessments; Xing et al. 2018). Increases in body size along elevational gradients have been found in assemblages of tropical geometrid and arctiid moths (Brehm et al. 2019) and in assemblages of temperate moths in the Alps (Beck et al. 2016). These repeated observations linking color lightness and body size to thermal gradients in nocturnal insects suggest that these relationships are of a more fundamental and general nature than merely serving to regulate the uptake and retention of heat gained by solar radiation (Heidrich et al. 2018, Brehm et al. 2019, but see the discussion in Beck et al. 2016).

Recent studies also show that the sensitivity of a species towards changes in thermal conditions varies among families (Bladon et al. 2020). The different families within Lepidoptera display a variety of ecological adaptations and differ in their thermal strategies (Heinrich 2013), which could subsequently affect the relationship between functional traits and the environment. For example, some diurnal butterflies heat up on warm surfaces through body contact (Heinrich 2013). Such additional thermoregulatory mechanisms, which do not rely on color, might explain why not all butterfly families have uniform color lightness–temperature relationships (Stelbrink et al. 2019). Just like their diurnal counterparts, moths differ in their flight-performance and sensitivity to temperature (Heinrich 2013). Some moths such as Sphingidae and Noctuidae shiver to raise their body temperatures above the ambient temperature to take off ('hot moths'), while other families rely on low wing load ('cool moths'; Heinrich 2013). Hence, the question arises whether the linkage between color lightness and body size to thermal gradients found in macroecological studies are general enough to be also observed at smaller scales, where biotic factors become more important, but also whether patterns found in one family can be transferred to another.

In this study, we used standardized monitoring data for the years 2007 and 2016 to investigate the variation of body size and color lightness along a local elevational gradient in two abundant moth families with distinct thermal strategies: geometrids and noctuids. Our analysis tested for consistency across families and sampling years. Furthermore, we assessed whether the traits affect local abundance and the habitat occupancy of species of these families after accounting for host plant availability as a biotic driver of abundance and occupancy (Friess et al. 2017, Pinkert et al. 2020). If a larger body and darker color are generally beneficial in colder climates, then body size should increase and color lightness decrease with increasing elevation.

Methods

Study area

The study was conducted at the northern and southern parts of the Bavarian Forest National Park, in the German part of the Bohemian Forest, in southeastern Germany, described in detail in Bässler et al. (2015). In 2007 and 2016, 27 sites, arranged along four transects following an elevational gradient, were sampled. The elevational gradient ranges from 660 m to 1368 m a.s.l. Annual temperature ranged from 4.1°C at higher elevations to 7.8°C at lower elevations, with 2016 on average being 0.2°C colder than 2007 (Supporting information). Precipitation ranged from 1119 mm at lower elevations to 2212 mm at higher elevations, with 2016 being generally drier than 2007 (Supporting information). The National Park is dominated by mixed mountain forests of spruce *Picea abies*, European beech *Fagus sylvatica* and fir *Abies alba*. At lower elevations, *F. sylvatica* dominates. At elevations > 1150 m a.s.l., spruce dominates but low proportions of mountain ash *Sorbus aucuparia* and European beech *F. sylvatica* are present as well. At this elevation, canopy cover of some of the plots has been substantially decreased by windthrow events (Hilmers et al. 2018).

Sampling

On each plot, light traps were set up one night per month from June to August in 2007 and 2016, respectively, during nights with optimal weather conditions (excluding nights with full moon or rain). The light traps consisted of 12 V, 15 W superarctinic UV-lights controlled by a twilight-sensor and operated by a 12 V, 15 Ah battery. The traps were emptied the next day and their contents were frozen until individuals belonging to *Noctuidae*, *Geometridae*, *Arctiidae*, *Lymantriidae*, *Erebidae*, *Lasiocampidae*, *Sphingidae*, *Drepanidae*, *Notodontidae*, *Nolidae*, *Hepialidae* and *Cossidae* were determined to species level. The overall catch consisted of 20 668 individuals of 342 species. The two families used in our study comprised 38% and 36% of the species and 51% and 26% of the individuals (Geometridae and Noctuidae, respectively).

Morphological traits

Recently published information on wing spans (for simplicity hereafter called 'body size') was used as a proxy for species body size and extracted from Potocký et al. (2018). The color lightness of the considered species was estimated by scanning color plates in 'The Macrolepidoptera of Germany' (Segerer et al. 2011). The color plates consist of photographs from museum specimens, which were taken by G. Ronkay and G.M. László, who also led the technical realization of the book (Acknowledgements in 'The Macrolepidoptera of Germany'). G.R., G.M.L. and the publishers are renown lepidopterologists, so that we presume that the images represent the variation of natural coloration of the specimens. The plates were scanned at 300 dpi with RGB color space with an scanner. Subsequently, the images of the specimens were separated and their backgrounds removed. The mean gray values of all pixels was calculated for each image. If more than one image was available per species, their mean gray values (ranging from 0 to 1; absolute black to pure white) were averaged. Only images of dorsal viewpoint were selected.

Host plant availability

The effect of host plant availability on the abundance of moth species was taken into account by including information on the occurrence of larval host plants into the analysis. Host plants listed in Pearse and Altermatt (2013) were complemented by those given in Ebert (1994, 1997, 2001, 2003), Koch and Heinicke (1996), Ebert and Steiner (1997, 1998), Hacker and Müller (2006), and Steiner et al. (2014). If only the genus or family of potential host plants was given, all host species belonging to this genus and family were considered. If one source did not list the species–plant interactions on the level of genus or family, but only categorized the species as 'polyphagous', this source was excluded as long the other ones listed the interactions at a finer taxonomic resolution. Otherwise, the whole species was excluded from the analysis on the species level. It total, this led to the exclusion of ten species in the species-level analysis.

The sites' suitability for a certain species is not solely based on the occurrence of larval host plants. Other factors might include the sites openness, the occurrence of tree sap or, in case of nectar-feeding moths, the occurrence of night-flowering plants. However, adult and larval food plants overlap to some extent (Altermatt and Pearse 2011) and preferred habitat openness is often associated with the species' host plant preferences. Naturally, open habitats harbor more herb-and grass-feeding moths than closed habitats, in which primarily tree and shrub feeding moths thrive (Supporting information). We are thus confident that the occurrence of host plants covers the most important aspect of habitat availability.

At all study sites, the scale suggested by Londo (1976) was used to estimate the coverage of each plant species within a radius of 8 m around the center of the site (~200 m²) at four vertical layers (> 15 m; 5–15 m; 1–5 m and < 1 m), once in 2006 (used for the moths sampled in 2007) and once in 2016. The percentages of all layers were averaged per plant species, site and year, followed by a logit transformation to achieve normality. For each moth species and year, the coverage of all potential host plants was summed per site to obtain an estimate of host plant availability.

Statistical analysis

To investigate whether body size and color lightness influence the community composition and abundance of species along an elevational gradient, we performed three types of analyses. First, to enable comparisons between our results and those of previous large-scale studies, patterns in body size and color lightness of assemblages were investigated. For

geometrid and for noctuid species, community-weighted means (cwm) of body size and color lightness were calculated based on the species abundances at each site in 2007 and in 2016. The relationship between community-weighted means and elevation was assessed using linear models. We wanted to test whether the community weighted means of each family and year combination showed significant clines along elevation. We thus estimated group-specific slopes of the unique combinations by including elevation in interaction with year and family, but not as main effect. Assuming, that the community weighted means could generally differ between the years and families, respectively, both factors were included as main effects to allow for different intercepts between the factor levels. Prior to analysis, elevation was centered to estimate the average values for the grouping factors:

Model 1: cwm ~ family + year + family : year : elevation

In a second step, we evaluated whether the family-specific slopes change significantly between sampling years. Thus, we also included the interaction-term of family and elevation which now estimates the slope observed in 2007 for geometrids and noctuids, respectively, and then tests for differences to 2016 for each family:

Model 2 : cwm ~ family + year + family : elevation

+ year : family : elevation

Finally, we evaluated whether the slopes differ between both families across both years. Thus, we included the interaction between elevation and the factor family together with the respective main effects. Year was included as a fixed effect to account for general differences between the year, but not in the interaction term:

Model 3 : cwm ~ family + year + elevation + family : elevation

See the Supporting information for the model with all interactions and main effects.

One of the shortcomings of assemblage approaches is that only trait averages and not trait variations among co-occurring species are taken into account (Peres-Neto et al. 2017). This underrates the total variance and can lead to an overestimation of the correlation, in studies using community-weighted means (Peres-Neto et al. 2017). Furthermore, community-weighted means do not provide information whether species distributions along an environmental gradient are causally linked to species traits (Peres-Neto et al. 2017). Thus, we used also a fourth-corner analysis, as implemented in the *ade4* package (Dray and Dufour 2007). The fourth-corner approach allows direct testing of the interactions between the distribution of environmental variables p along the sites n and the traits q of the species m by connecting them via the species per site matrix, $n \times m$ (Peres-Neto et al. 2017, ter Braak 2017). In this study, the analysis was conducted using

the species–site ($n \times m$) matrices for both moth families in both years, a table of the elevation per site ($n \times p$) and a table of the trait value per species ($m \times q$). The fourth-corner statistic measures the link between these matrices and thus allows inferences about an unknown 'fourth corner,' which is the trait–environment interaction matrix $p \times q$ (ter Braak 2017). This link, $p \times q$, is determined as a Pearson correlation coefficient, with its significance assessed by bootstrapping. Here, both site and species values were permuted (default setting of the 'fourthcorner' function) 999 times and the two outputs were combined, as other procedures are prone to inflated type I statistical errors (ter Braak 2017). We performed the fourth corner analysis for each family and year combination.

As a third approach, the occurrence and abundance of species were modeled using generalized linear mixed models as implemented in the *glmmTMB* package (Brooks et al. 2017). We included only species occurring in more than three plots per year and for which information about their preferred host plant is available, resulting in 60 species which had adequate occurrences. The interaction of elevation with each of the two traits as predictor variables was used to assess whether body size and color lightness influence the abundance of species along an elevational gradient. As variations in abundance along elevational gradients can be hump- or U-shaped (Choi and An 2013), the independent effect of elevation on species abundance was modeled using a general additive model, as implemented in the *mgcv*-package ver. 1.8 (Wood 2017, Supporting information). This allowed a visual determination of non-linear patterns. Since no clear hump- or U-shaped pattern was evident (Supporting information), only the linear term of elevation was included. Family and year were added both as fixed and as interaction terms to obtain group-wise estimates, and host plant availability was included as a fixed factor. The models also contained a random factor of the species identity. Prior to the analysis, all independent variables were centered. A negative-binomial error distribution (quadratic parametrization) with truncated zero inflation was used to model both the likelihood of occurrence and the effect on abundance.

Because we wanted to test whether patterns are consistent between sampling events, this analysis was initially conducted for the sites which were equally sampled three times in both years ('balanced data set'). However, an additional 15 sites located between the National Park and the Danube River were also sampled in 2016 to elongate the gradient to 297 m a.s.l. (Fig. 1). These expanded the range of temperature and precipitation covered to 10.36°C and 908 mm, respectively and added 27 new geometrid moth species and 24 noctuid moth species to the data. Subsequently, we made use of these additional data to compare the effect of analysing short versus long environmental gradients ('full data set'). We repeated the community weighted mean analysis, the fourth corner analysis and the species-level analysis for the long elevational gradient sampled in 2016 only, thus excluding year as factor. All species sampled in 2016 were included in the community weighted mean analysis and the fourth corner analysis. In the species-level model, however, we included only those species
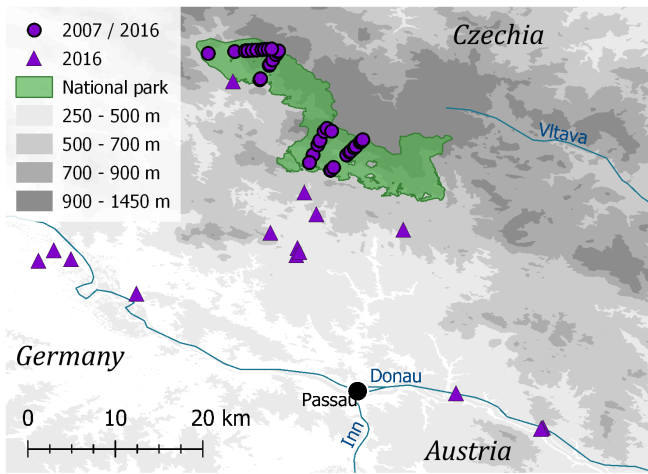
Figure 1. Map of the sampling locations. The borders of the National park are shown in green. Gray shading revers to the elevation, with light-shaded areas indicating low elevations, dark-shaded areas indicating high elevations. Triangular markings show the sites sampled in 2016 only, point markings represent the sites sampled in both 2016 and 2007.

already used in the analysis of the balanced data to increase comparability between both data sets.

The statistical analyses can be reproduced via the R-Script at <https://github.com/Friessn/Ecography_Moths_Elevation>.

Results

In 2007, Geometridae were represented by 4718 individuals of 87 species and Noctuidae by 2424 individuals of 77 species. In 2016, Geometridae were represented by 5724 individuals of 116 species and Noctuidae by 3006 individuals of 112 species, respectively (full gradient). From those, 4083 individuals 83 species of Geometridae and 2168 individuals of 73 species of Noctuidae were included in the community weighted mean and fourth corner analysis of balanced data.

The wing spans of the considered species ranged from 16 mm (*Eupithecia tenuiata*, Geometridae) to 55 mm (*Eurois occulta*, Noctuidae) with a mean ± standard deviation of 31.76 ± 8.17 mm. The wing spans of geometrid moth species were on average 8.5 mm smaller than those of noctuid moth species (Welch two sample t-test: t = −9.60, df = 248.47, p < 0.001; Fig. 2A). Color lightness values ranged from 0.34 (*Noctua janthina*, Noctuidae) to 0.82 (*Scopula immutata*, Geometridae), with a mean of 0.54 ± 0.10. Geometrid moth species were on average 0.14 gray values lighter in color than noctuid species (t = 14.55, df = 247.51, p < 0.001, Fig. 2B). Geometrid moth assemblages in 2007 and 2016 did not differ with respect to body size (t = 1.33, p = 0.188) or color lightness (t = −0.570, p = 0.571). The mean body size of noctuid assemblages did not differ between the two years (t = 0.73, p = 0.464), but these moths were on average lighter in 2016 than in 2007 (t-test, t = −2.567, p = 0.013, Fig. 3).

With increasing elevation, the mean body size of geometrid assemblages did not change clearly, but the moths became lighter colored, with a significantly less steep slope in 2016 (Table 1). By contrast, noctuid assemblages at higher elevations were on average larger and darker. These trends were consistent across the two sampling years (Table 1, Fig. 3). The results of the fourth-corner analysis were similar to those of the assemblage approach in the case of noctuid moths, as both showed an increase in body size (r = 0.36 in 2007, r = 0.35 in 2016) and a decrease in color lightness with elevation (r = −0.36 in 2007, r = −0.29 in 2016, Table 2). In geometrid moths, neither the relationship between body size and elevation nor that between color lightness and elevation was significant (Table 2).

Geometrid moths were more abundant (2007 only) and more likely to occur at sites with high host plant availability in both 2007 and 2016. Host plant availability had no significant effect on occurrence nor abundance of noctuid moths. In contrast, the distribution of noctuid moths along elevation was affected by color lightness in both years. Specifically, light-colored noctuid moths had a steeper decline in abundance with increasing elevation, whereas the distribution
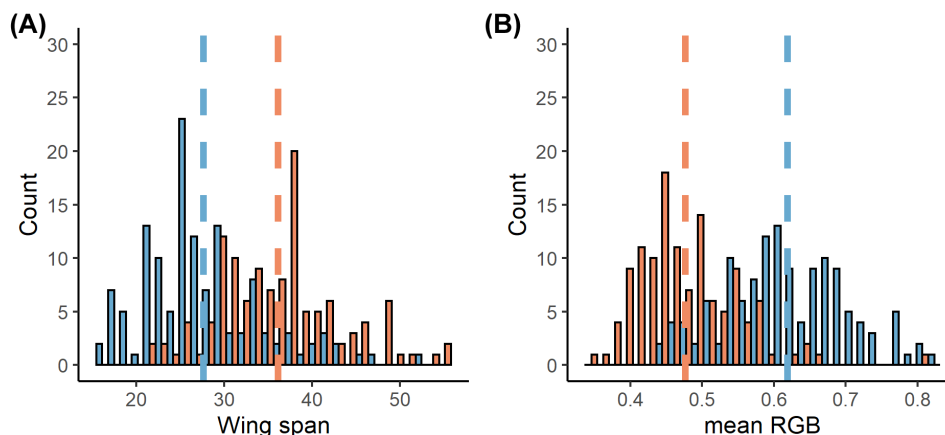

Figure 2. Histograms of (A) wing span and (B) color lightness of moth species of the two families investigated in this study: Geometridae (blue) and Noctuidae (orange). Color lightness (mean RGB) ranges from 0 (black) to 1 (white). Dashed lines represent the mean values.
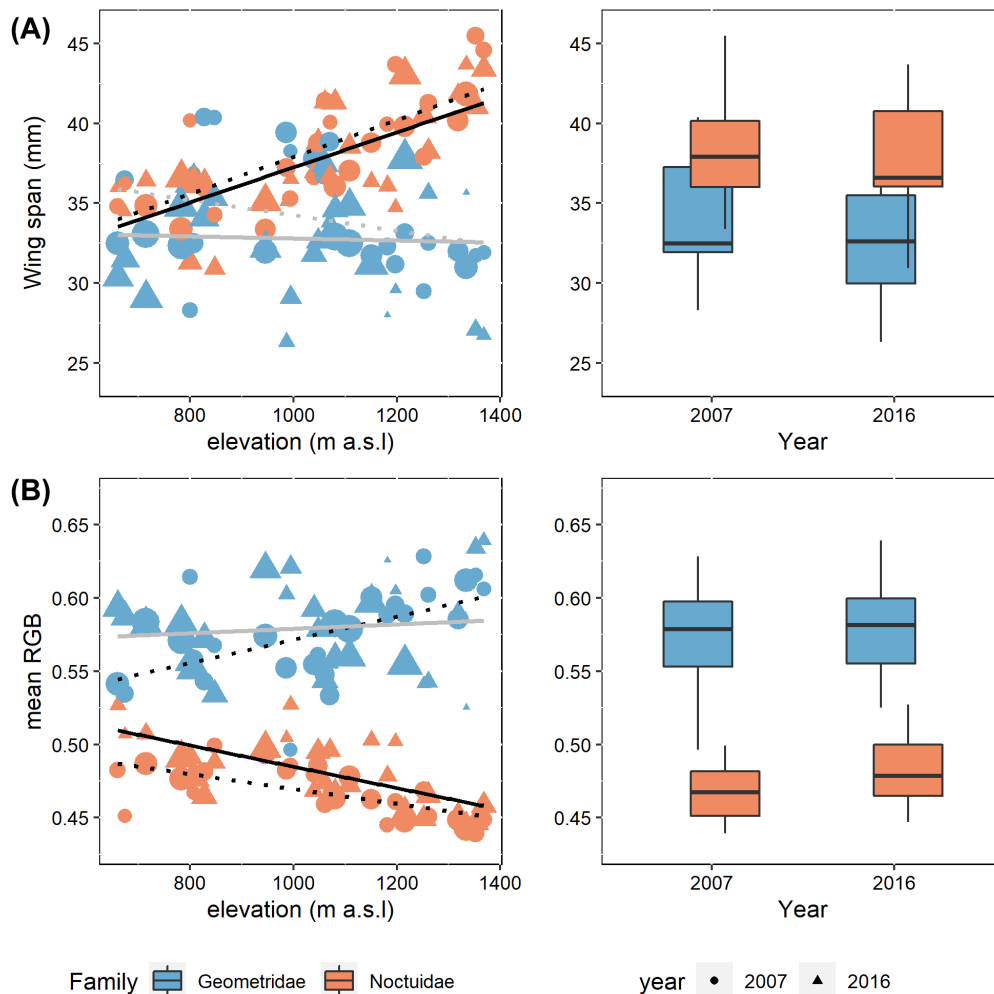
Figure 3. Scatterplots of the relationships of abundance-weighted community trait means of the (A) body size and (B) color lightness of assemblages of geometrid (blue) and noctuid (orange) species with increasing elevation above sea level. The mean values are shown in the plots on the right. Dotted regression lines and circles indicate the results for 2007, and solid regression lines and triangles the results for 2016. Species trait values in the assemblage were weighted according to species abundances. Note that for species of the family Geometridae the relationship between mean body size and elevation was non-significant in both years (gray lines).

of geometrid moths was not affected. Large bodied noctuid moths were more likely to occur at high elevation in 2016 (Table 3).

The inclusion of additional study sites in lower elevation that were only sampled in 2016 reversed the relationship between community weighted means of geometrid moths and elevation in that year, showing now a significant decline in average color lightness and an increase in body size. For noctuid moths, signs did not change but the effect of elevation on average color lightness became non-significant (Supporting information). These trends were also apparent in the fourth-corner analysis. Again, signs of the relationships between traits and elevation changed in geometrid moths, though the effect was not significant, whereas in noctuid moths, trends became non-significant (Supporting information). For the 60 species used in the species-level analysis, expanding the covered gradient only led to slight changes; the effect of color lightness on the abundance along elevation

became non-significant for noctuid moths (Supporting information).

Discussion

Strength and direction of the clines in body size and color lightness along elevation were inconsistent among moth families. The predicted pattern, darker coloration and larger body size in colder environments, which is often observed in macroecological studies of several diurnal ectothermic insect taxa (body size: Shelomi and Zeuss 2017, Zeuss et al. 2017; color lightness: Zeuss et al. 2014, Bishop et al. 2016, Pinkert et al. 2017, Stelbrink et al. 2019), occurred only in noctuid moths. In geometrid moths these trait–environment relationships were either non-significant (body size) or the opposite of what has been documented by previous large-scale studies on this family (Heidrich et al. 2018, Xing et al. 2018, Brehm et al. 2019).

Table 1. Results of linear regression modeling of community-weighted means of body size and color lightness against elevation (centered) for two moth families, Geometridae and Noctuidae, and the sampling years 2007 and 2016. Full estimates and statistical summary are given for model 1, which estimated slopes for each family × year combination, after including year and family as fixed factors. Note, that the p-values of the slope estimates thus indicate whether the slope itself is significantly different from zero. Whether the slope differs significantly between the sampling years (family-specific, Δyear, model 2) and between the families (Δfamily, model 3) is indicated by t- and p-values given at the right-hand-side of the table. Estimates (Est) and the corresponding standard errors (SE) indicate a response to changes per 100 m. Significant effects are shown in bold.

		Model 1				Model 2	Model 3
		Est	SE	t-value	Pr(>|t|)	ΔYear	ΔFamily
Wing span							
Balanced data	Intercept	**33.9**	**0.50**	**67.9**	**< 0.001**		
	FamilyNoc	**4.61**	**0.58**	**8.00**	**< 0.001**		
	Year2016	−0.98	0.58	−1.70	0.092		
	Geo:2007:elevation	−0.48	0.27	−1.76	0.079	t=1.08, p=0.28	t=5.16, p < 0.001
	Geo:2016:elevation	−0.06	0.27	−0.24	0.813		
	Noc:2007:elevation	**1.15**	**0.27**	**4.25**	**< 0.001**	t=−0.15, p=0.88	
	Noc:2016:elevation	**1.09**	**0.27**	**4.03**	**< 0.001**		
meanRGB							
Balanced data	Intercept	**0.572**	**0.004**	**145.5**	**< 0.001**		
	FamilyNoc	**−0.102**	**0.005**	**−22.50**	**< 0.001**		
	Year2016	**0.009**	**0.005**	**2.114**	**0.036**		
	Geo:2007:elevation	**0.008**	**0.002**	**3.707**	**< 0.001**	t=−2.12, p=0.03	t=−5.02, p < 0.001
	Geo:2016:elevation	0.002	0.002	0.703	0.483		
	Noc:2007:elevation	**−0.005**	**0.002**	**−2.372**	**0.020**	t=−0.73, p=0.47	
	Noc:2016:elevation	**−0.007**	**0.002**	**−3.403**	**< 0.001**		

The results of the species-level model indicated no relationships between body size or color lightness and the distribution of geometrid moths. Instead, occurrence and abundance of geometrid moths are positively associated with host plant availability. Previous studies have shown that host plant availability has strong effects on the distribution and abundance of herbivorous insects (Friess et al. 2017), but our results indicate that the strength of this effect differs between the two considered families. Like other biotic factors, the relative importance of host plant availability tends to increase with decreasing scale (Schweiger and Beierkuhnlein 2016a). For example, across an elongated thermal gradient (Supporting information) – and thus an increased probability that the ends of the climatic niche of geometrid moths will be covered – patterns in average body size and color lightness follow the expectations. However, it is unclear why, on the local scale, host plant availability more strongly impacts geometrid than noctuid moths.

Potential effects of differing flight energetics

The relative importance of host plant availability can be modified by an organism's mobility, as a low mobility is associated with a strong relationship between the host plant and organismal abundance (Curtis et al. 2015). Geometrid moths have a low wing load, which reduces their energetic costs for flying (Utrio 1995). However, this energetically cheap flight comes at the cost of a poor flight performance, so that the occurrence and abundance of geometrid moths might depend on resource availability. Noctuid moths, however, have a significantly higher wing load and wing beat frequency (Casey and Joos 1983) and may thus be less dependent on local host plant availability. Nonetheless, they need to shiver to raise their body temperatures above the ambient temperature (Heinrich 2013) to take off ('hot moths', Utrio 1995). This pre-flight warm up together with the high wing load is energetically costly, especially if ambient temperatures are low (Casey and Joos 1983). Larger body sizes reduce the loss of body temperature and may therefore benefit noctuid moths.

In addition, pleiotropic linkages between body size and color lightness (Schweiger and Beierkuhnlein 2016b, San-Jose and Roulin 2018) could also lead to darker noctuid moth assemblages at high elevations. Although temperature had the highest independent effect on community weighted means from all environmental variables covarying with elevation (Supporting information), other factors than

Table 2. Results of the fourth-corner analysis of Geometridae and Noctuidae for both 2007 and 2016. R is the Pearson correlation between single traits and elevation. The significance of the fourth-corner statistic was determined via permutations of site and species values based on 9999 permutations. Significant effects are shown in bold.

	Geometridae				Noctuidae			
	2007		2016		2007		2016	
	R	p-value	R	p-value	R	p-value	R	p-value
Elevation × Body size	−0.065	0.671	0.056	0.561	**0.363**	**0.023**	**0.347**	**0.01**3
Elevation × Color lightness	0.120	0.455	−0.004	0.957	**−0.360**	**0.017**	**−0.287**	**0.044**

Table 3. Species-level analysis to test the effect of host plant availability on species abundances and whether species distributions along an elevation gradient are mediated by traits. The results are reported as the t-and z-values. The model had a truncated negative-binomial error distribution. The conditional part represents the effect on abundance, and the zero-inflated part the probability of the absence of a species. The interactions of the sampling year (2007, 2016) and family (Geo = Geometridae, Noc = Noctuidae) were included to test whether the trends were consistent across taxonomic groups and repeatable between two sampling events. Significant effects are shown in bold.

| | | | | Geometridae | | | | Noctuidae | | | |
| | | | | 2007 | | 2016 | | 2007 | | 2016 | |
		z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
Conditional model	Family	−1.45	0.148								
	Year	1.60	0.109								
	Host plant			**3.60**	**< 0.001**	0.58	0.565	1.22	0.264	−0.82	0.415
	Elevation			0.73	0.467	−1.85	0.065	−0.95	0.342	**−2.78**	**0.005**
	Elevation:Wingspan			0.21	0.835	−0.86	0.392	1.88	0.061	0.59	0.554
	Elevation:MeanRGB			0.71	0.480	−0.12	0.908	**−2.16**	**0.031**	**−2.07**	**0.039**
Zero-inflated model	Family	−0.61	0.545								
	Year	4.69	<0.001								
	Host plant			**−2.42**	**0.016**	**−4.01**	**<0.001**	−1.61	0.107	−1.31	0.192
	Elevation			1.81	0.071	**5.79**	**<0.001**	1.31	0.192	**2.79**	**0.005**
	Elevation:Wingspan			−1.08	0.278	**−2.36**	**0.018**	−1.66	0.097	**−2.51**	**0.012**
	Elevation:MeanRGB			−0.75	0.453	−0.95	0.342	1.82	0.068	1.82	0.069

temperature may instead lead to the observed clines along an elevation. Elevation could filter towards more robust and long-living species, usually associated with larger body size (Beck et al. 2016), which could also affect color lightness, given that melanin production is linked to several life history traits (Clusella-Trullas and Nielsen 2020). The differences in the two families with respect to their life histories, with most noctuid moths overwintering in the larval stage, whereas geometrid moths overwinter as pupae (Supporting information), could thus add to the difference in the observed trait patterns. In addition, differences in respect to ecology could play a role; first, noctuid moths have a higher share of species preferring open habitats, while in geometrid moths only a fraction can be found outside the woodland (Supporting information). Though woodland and open habitat species do not show significant differences in color lightness and body size within the families, the investment in traits related to robustness might have enabled noctuid moths to forage in the open, unsheltered habitat found at higher elevations. Adult noctuid moths forage on a higher number of nectar plants than geometrid moths (Supporting information), which could decrease the relative importance of larval host plants as a biotic factor for occurrence and abundance. However more rigorous conclusions of the mechanisms underlying body size and color lightness variation in insects and the effect of those variations on species distributions, are hampered by a lack of life-history and ecological data as well as abundance data over longer times and higher temporal resolution (Clusella-Trullas and Nielsen 2020).

Limitations and potential extensions of the study

While we had a lower spatial grain in comparison to macroecological studies, the taxonomic unit mostly remained unchanged, such that intraspecific variations in color lightness and body size could not be considered in our analysis. Nevertheless, it is a premise of trait-based ecological studies that trait–environment relationships can be scaled up from the individual to the ecosystem (Enquist et al. 2015). Consequently, the use of mean values from the literature may lower the power of predictions, especially if turnover between species is low (Yang et al. 2018). For instance, Xing et al. (2016) found support for the thermal melanism hypothesis at the individual level but not at the species level. Hence, future studies on smaller scales should, whenever possible, include intraspecific variations to improve the power of their predictions. Advancements in the field of digital image processing via deep learning algorithms may facilitate the generation of data from local collections (Wu et al. 2019).

The use of elevation as a proxy for a thermal gradient has its shortcomings, as not only do other environmental factors covary, but the decrease in temperature along the elevation gradient is also not necessarily uniform. Sinks of cold air occurring within the mixed montane zone (800–900 m a.s.l.) can strongly favor the occurrence of cold-adapted species (Bässler et al. 2010) and in the case of geometrid moths may be responsible for the low average community-weighted

means of color lightness within the respective elevational range. If cold-air sinks are at the lower border of the elevational gradient and the elevational gradient is not long enough to compensate for their presence, the result may ultimately be a change in the sign of the slope of trait–elevation clines. This again points to the importance of microclimate in assessing trait–environment relationships (Hof et al. 2011). Hence, experimental studies that additionally control for microclimate would help gaining insights into the effect of color lightness and body size on the performance of species.

Conclusion

Our study showed that color lightness and body size affect the physiology and shape the occurrence and abundance of noctuid moths along elevation. However, the lack of such patterns in geometrid moths shows that such trait–environment relationships are inconsistent and underline that coarse-scale patterns are not necessarily transferable to smaller scales, where local environmental factors such as the host plant availability can modify occurrence and abundance patterns. The strength of this modification is likely to be based on taxon-specific flight capacities, though also other factors, such as thermoregulatory propensities and life history, might play a role. Thus, one should be careful to not overestimate the relative importance of single functional traits on the performance of species on small regional scales. Rather, our study shows the importance of validating macroecological patterns on local environmental gradients, incorporating abundance data into the analysis whenever possible. A good database on which gradients and for which taxonomic groups macroecological patterns are valid will significantly deepen our understanding of macrophysiological processes in the future. Such an understanding will allow us to make reliable predictions about how target species will respond to environmental change.

Authors contributions

Lea Heidrich: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Investigation (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (equal). **Stefan Pinkert**: Conceptualization (equal); Formal analysis (equal); Writing – original draft (lead); Writing – review and editing (equal). **Roland Brandl**: Conceptualization (supporting); Formal analysis (supporting); Funding acquisition (equal); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (equal). **Claus Bässler**: Funding acquisition (equal); Project administration (equal); Writing – original draft (supporting); Writing – review and editing (equal). **Hermann Hacker**: Data curation (equal); Investigation (equal); Writing – original draft (supporting). **Nicolas Roth**: Data curation (supporting); Writing – original draft (supporting); Writing – review and editing (equal). **Annika Busse**: Data curation (supporting); Resources (supporting); Writing – review and editing (equal). **Jörg Muller**: Conceptualization (equal); Data curation (equal); Funding acquisition (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (equal). **Nicolas Friess**: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Investigation (equal); Supervision (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (equal).

Transparent Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/ecog.05558>.

Data availability statement

The data supporting this study are available from Github together with the accompanying R code at <https://github.com/Friessn/Ecography_Moths_Elevation>.

References

Altermatt, F. and Pearse, I. S. 2011. Similarity and specialization of the larval versus adult diet of European butterflies and moths. – Am. Nat. 178: 372–382.

Atkinson, D. and Sibly, R. M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. – Trends Ecol. Evol. 12: 235–239.

Bässler, C. et al. 2010. Detection of climate-sensitive zones and identification of climate change indicators: a case study from the Bavarian Forest National Park. – Folia Geobot. 45: 163–182.

Bässler, C. et al. 2015. The BIOKLIM Project in the National Park Bavarian Forest: lessons from a biodiversity survey. – Silva Gabreta 21: 81–93.

Beck, J. et al. 2016. Patterns or mechanisms? Bergmann's and Rapoport's rule in moths along an elevational gradient. – Community Ecol. 17: 137–148.

Bishop, T. R. et al. 2016. Ant assemblages have darker and larger members in cold environments. – Global Ecol. Biogeogr. 25: 1489–1499.

Bladon, A. J. et al. 2020. How butterflies keep their cool: physical and ecological traits influence thermoregulatory ability and population trends. – J. Anim. Ecol. 89: 2440–2450.

Brehm, G. et al. 2019. Moth body size increases with elevation along a complete tropical elevational gradient for two hyperdiverse clades. – Ecography 42: 632–642.

Brooks, M. E. et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. – R J. 9: 378–400.

Casey, T. M. and Joos, B. A. 1983. Morphometrics, conductance, thoracic temperature and flight energetics of noctuid and geometrid Moths. – Physiol. Zool. 56: 160–173.

Choi, S.-W. and An, J.-S. 2013. Altitudinal distribution of moths (Lepidoptera) in Mt Jirisan National Park, South Korea. – Eur. J. Entomol. 107: 229–245.

Chown, S. L. and Gaston, K. J. 2016. Macrophysiology – progress and prospects. – Funct. Ecol. 30: 330–344.

Chown, S. L. et al. 2004. Macrophysiology: large-scale patterns in physiological traits and their ecological implications. – Funct. Ecol. 18: 159–167.

Clusella-Trullas, S. et al. 2007. Thermal melanism in ectotherms. – J. Therm. Biol. 32: 235–245.

Clusella-Trullas, S. and Nielsen, M. 2020. The evolution of insect body coloration under changing climates. – Curr. Opin. Insect Sci. 41: 25–32.

Curtis, R. J. et al. 2015. Butterfly abundance is determined by food availability and is mediated by species traits. – J. Appl. Ecol. 52: 1676–1684.

Dray, S. and Dufour, A.-B. 2007. The ade4 package: implementing the duality diagram for ecologists. – J. Stat. Softw. 22: 1–20.

Ebert, G. 1994. Die Schmetterlinge Baden-Württembergs, Bd.4, Nachtfalter II. – Verlag Eugen Ulmer.

Ebert, G. 1997. Die Schmetterlinge Baden-Württembergs, Bd.5, Nachtfalter: Biotop- und Artenschutz, Bestandssituation, Rote Liste. Glasflügler (Sesiidae), Bärenspinner (Arctiidae), Eulen (Noctuidae), 1. Teil. – Verlag Eugen Ulmer.

Ebert, G. 2001. Die Schmetterlinge Baden-Württembergs, Bd 8, Nachtfalter. – Verlag Eugen Ulmer.

Ebert, G. 2003. Die Schmetterlinge Baden-Württembergs Bd 9 – Nachtfalter VII. – Verlag Eugen Ulmer.

Ebert, G. and Steiner, A. 1997. Die Schmetterlinge Baden-Württembergs, Bd.6, Nachtfalter: Eulen (Noctuidae). 2. Teil. – Verlag Eugen Ulmer.

Ebert, G. and Steiner, A. 1998. Die Schmetterlinge Baden-Württembergs, Bd.7, Nachtfalter. – Verlag Eugen Ulmer.

Enquist, B. J. et al. 2015. Scaling from traits to ecosystems: developing a general trait driver theory via integrating trait-based and metabolic scaling theories. – Adv. Ecol. Res. 52: 249–318.

Friess, N. et al. 2017. Habitat availability drives the distribution–abundance relationship in phytophagous true bugs in managed grasslands. – Ecology 98: 2561–2573.

Gaston, K. J. et al. 2009. Macrophysiology: a conceptual reunification. – Am. Nat. 174: 595–612.

Hacker, H. and Müller, J. 2006. Die Schmetterlinge der bayerischen Naturwaldreservate: eine Charakterisierung der süddeutschen Waldlebensraumtypen anhand der Lepidoptera (Insecta). – Arbeitsgemeinschaft Bayerischer Entomologen.

Heidrich, L. et al. 2018. The dark side of Lepidoptera: colour lightness of geometrid moths decreases with increasing latitude. – Global Ecol. Biogeogr. 27: 407–416.

Heinrich, B. 2013. The hot-blooded insects: strategies and mechanisms of thermoregulation. – Springer Science & Business Media.

Hilmers, T. et al. 2018. Biodiversity along temperate forest succession. – J. Appl. Ecol. 55: 2756–2766.

Hof, C. et al. 2011. Rethinking species' ability to cope with rapid climate change. – Global Change Biol. 17: 2987–2990.

Koch, M. and Heinicke, W. 1996. Wir bestimmen. Schmetterlinge. Tagfalter, Eulen, Schwärmer, Spinner, Spanner. – Neumann Verlag Radebeul.

Law, S. J. et al. 2020. Darker ants dominate the canopy: testing macroecological hypotheses for patterns in colour along a microclimatic gradient. – J. Anim. Ecol. 89: 347–359.

Londo, G. 1976. The decimal scale for releves of permanent quadrats. – Vegetatio 33: 61–64.

McGill, B. J. 2019. The what, how and why of doing macroecology. – Global Ecol. Biogeogr. 28: 6–17.

Pearse, I. S. and Altermatt, F. 2013. Extinction cascades partially estimate herbivore losses in a complete Lepidoptera–plant food web. – Ecology 94: 1785–1794.

Peres-Neto, P. R. et al. 2017. Linking trait variation to the environment: critical issues with community-weighted mean correlation resolved by the fourth-corner approach. – Ecography 40: 806–816.

Pinkert, S. et al. 2017. Colour lightness of dragonfly assemblages across North America and Europe. – Ecography 40: 1110–1117.

Pinkert, S. et al. 2020. Mobility costs and energy uptake mediate the effects of morphological traits on species' distribution and abundance. – Ecology 101: e03121.

Platts, P. J. et al. 2019. Habitat availability explains variation in climate-driven range shifts across multiple taxonomic groups. – Sci. Rep. 9: 1–10.

Potocký, P. et al. 2018. Life-history traits of central European moths: gradients of variation and their association with rarity and threats. – Insect Conserv. Divers. 11: 493–505.

San-Jose, L. M. and Roulin, A. 2018. Toward understanding the repeated occurrence of associations between melanin-based coloration and multiple phenotypes. – Am. Nat. 192: 111–130.

Schweiger, A. H. and Beierkuhnlein, C. 2016a. Scale dependence of temperature as an abiotic driver of species' distributions. – Global Ecol. Biogeogr. 25: 1013–1021.

Schweiger, A. H. and Beierkuhnlein, C. 2016b. Size dependency in colour patterns of Western Palearctic carabids. – Ecography 39: 846–857.

Segerer, A. et al. 2011. Die Großschmetterlinge Deutschlands. The Macrolepidoptera of Germany. – Goecke & Evers.

Shelomi, M. and Zeuss, D. 2017. Bergmann's and Allen's rules in Native European and Mediterranean Phasmatodea. – Front. Ecol. Evol. 5: 25.

Steiner, A. et al. 2014. Die Nachtfalter Deutschlands – Ein Feldführer. – BugBook Publishing.

Stelbrink, P. et al. 2019. Colour lightness of butterfly assemblages across North America and Europe. – Sci. Rep. 9: 1–10.

Stone, G. N. 1993. Endothermy in the solitary bee *Anthophora plumipes*: independent measures of thermoregulatory ability, costs of warm-up and the role of body size. – J. Exp. Biol. 174: 299–320

ter Braak, C. J. F. 2017. Fourth-corner correlation is a score test statistic in a log-linear trait–environment model that is useful in permutation testing. – Environ. Ecol. Stat. 24: 219–242.

Umbers, K. D. L. et al. 2013. Colour in insect thermoregulation: empirical and theoretical tests in the colour-changing grasshopper, *Kosciuscola tristis*. – J. Insect Physiol. 59: 81–90.

Urban, M. C. et al. 2016. Improving the forecast for biodiversity under climate change. – Science 353: 1113–1124.

Utrio, P. 1995. On flight temperatures and foraging strategies of nocturnal moths. – Baptria 20: 113–122.

Violle, C. et al. 2014. The emergence and promise of functional biogeography. – Proc. Natl Acad. Sci. USA 111: 13690–13696.

Watt, W. B. 1968. Adaptive significance of pigment polymorphisms in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. – Evolution 22: 437–458.

Wiens, J. J. 2011. The niche, biogeography and species interactions. – Phil. Trans. R. Soc. B 366: 2336–2350.

Wong, M. K. L. et al. 2019. Trait-based ecology of terrestrial arthropods. – Biol. Rev. 94: 999–1022.

Wood, S. N. 2017. Generalized additive models: an introduction with R, 2nd edn. – Chapman and Hall/CRC.

Wu, S. et al. 2019. Artificial intelligence reveals environmental constraints on colour diversity in insects. – Nat. Commun. 10: 4554.

Xing, S. et al. 2016. Cool habitats support darker and bigger butterflies in Australian tropical forests. – Ecol. Evol. 6: 8062–8074.

Xing, S. et al. 2018. Colors of night: climate–morphology relationships of geometrid moths along spatial gradients in southwestern China. – Oecologia 188: 537–546.

Yang, J. et al. 2018. Why functional traits do not predict tree demographic rates. – Trends Ecol. Evol. 33: 326–336.

Zamora-Camacho, F. J. et al. 2014. Bergmann's rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-m elevational gradient. – J. Evol. Biol. 27: 2820–2828.

Zeuss, D. et al. 2014. Global warming favours light-coloured insects in Europe. – Nat. Commun. 5: 3874.

Zeuss, D. et al. 2017. Environmental drivers of voltinism and body size in insect assemblages across Europe. – Global Ecol. Biogeogr. 26: 154–165.