



# Colors of night: climate–morphology relationships of geometrid moths along spatial gradients in southwestern China

Shuang Xing<sup>1,2</sup> · Timothy C. Bonebrake<sup>1</sup> · Louise A. Ashton<sup>1,3,4</sup> · Roger L. Kitching<sup>2,4</sup> · Min Cao<sup>2</sup> · Zhenhua Sun<sup>2</sup> · Jennifer Chee Ho<sup>5</sup> · Akihiro Nakamura<sup>2,4</sup> 

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## Abstract

Color lightness of insects is an important ecological trait affecting their performance through multiple functions such as thermoregulation, UV protection and disease resistance. The geographical pattern of color lightness in diurnal insects are relatively well understood and largely driven by thermal melanism through the enhancement of insect activity. In nocturnal insects, however, the ecological function of color lightness in response to climatic factors is poorly understood, particularly at small spatial scales. In this study, we investigated color lightness of nocturnal moth assemblages along environmental gradients. Using geometrid moths collected with comparable methodologies (light trapping), we examined assemblage-level changes in color lightness across elevational gradients and vertical strata (canopy vs understory) across three climatically different locations in Yunnan, China. The results showed that moths are darker in color at higher elevations. Such patterns are most apparent in canopy assemblages. In addition, the strength of the elevational pattern on color lightness varied across location, being most pronounced in the canopy of the subalpine site. These patterns are likely driven by UV protection and/or thermoregulation. Our study highlights the importance of abiotic factors such as temperature and solar radiation in structuring morphological patterns of nocturnal ectothermic assemblages along elevational gradients of climatically harsh environments.

**Keywords** Morphology · Solar radiation · Temperature · Nocturnal · Insect

## Introduction

Body color is an important ecological trait of insects, which plays an essential role in their survival and reproduction (Kingsolver 1995; Ellers and Boggs 2004; Wittkopp and Beldade 2009). In particular, color lightness has been recognized as a key biophysical property for insects and ectotherms in general, with three dominant hypotheses commonly proposed for explaining patterns of color lightness (Wilson et al. 2001; True 2003; Clusella-Trullas et al. 2007; Bastide et al. 2014; Heidrich et al. 2018). First, the thermal melanism hypothesis (TMH) states that organisms can benefit from dark coloration in cold environments by absorbing heat more efficiently (Porter and Gates 1969; Clusella-Trullas et al. 2007). One assumption particularly applicable to diurnal organisms is that darker organisms, relative to their more lightly colored counterparts, should be able to inhabit cooler environments and extend activity time in thermally limited environments (Watt 1968; Ellers and Boggs 2004; Clusella-Trullas et al. 2007, 2008; Zeuss et al. 2014; Bishop et al. 2016). Second, the UV-protection hypothesis (UVH)

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✉ Akihiro Nakamura  
a.nakamura@xtbg.ac.cn

- <sup>1</sup> School of Biological Sciences, The University of Hong Kong, Hong Kong, China
- <sup>2</sup> CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan, China
- <sup>3</sup> Life Sciences Department, Natural History Museum, South Kensington, London, UK
- <sup>4</sup> Environmental Futures Research Institute and Griffith School of Environment and Science, Griffith University, Nathan, QLD, Australia
- <sup>5</sup> Department of Physical and Environmental Sciences, University of Toronto, Toronto, Canada

proposes that increase in melanin pigment can effectively reduce cell damage from UV-B radiation and, therefore, may increase the survival rate of organisms exposed to strong solar radiation (Gunn 1998; Bastide et al. 2014). For this reason, darker organisms may survive better at high elevations or low latitudes exposed to strong insolation with high levels of UV-radiation (Dalrymple et al. 2017). Third, the disease resistance hypothesis (DRH) suggests that an increase in melanin may enhance disease resistance of insects by strengthening the cuticle against incursions by parasites or pathogens, by being directly toxic to microorganisms or by being associated with an increase in phenoloxidase activity (Wilson et al. 2001; True 2003; Krams et al. 2016). Organisms should, therefore, benefit from darker coloration within environments that are most vulnerable to pathogen infection, such as warm and humid conditions that are ideal for pathogen growth and reproduction (Walstad et al. 1970; Harvell et al. 2002; Burt and Ichida 2004; Heidrich et al. 2018).

Given their sensitivity to ambient temperature and solar radiation along with their relatively well known taxonomy and biology, butterflies have been one of the most commonly used insect groups when investigating environmental clines in color lightness (Kingsolver 1983, 1985; Ellers and Boggs 2004; Dalrymple et al. 2017). Accumulating evidence suggests that climatic patterns of butterfly color lightness mostly support the TMH (Kingsolver 1983; Ellers and Boggs 2002; Zeuss et al. 2014; Xing et al. 2016, 2018). Butterflies, however, make up only a small proportion of the order Lepidoptera (Scoble 1992; Regier et al. 2013). Unlike mostly diurnal butterflies, a minority of moth species is active during daytime hours (either facultatively or obligately) and most moth species are night-fliers (Scoble 1992; Kawahara et al. 2018). Whether color lightness of nocturnal species of Lepidoptera responds consistently to climatic factors remains unclear.

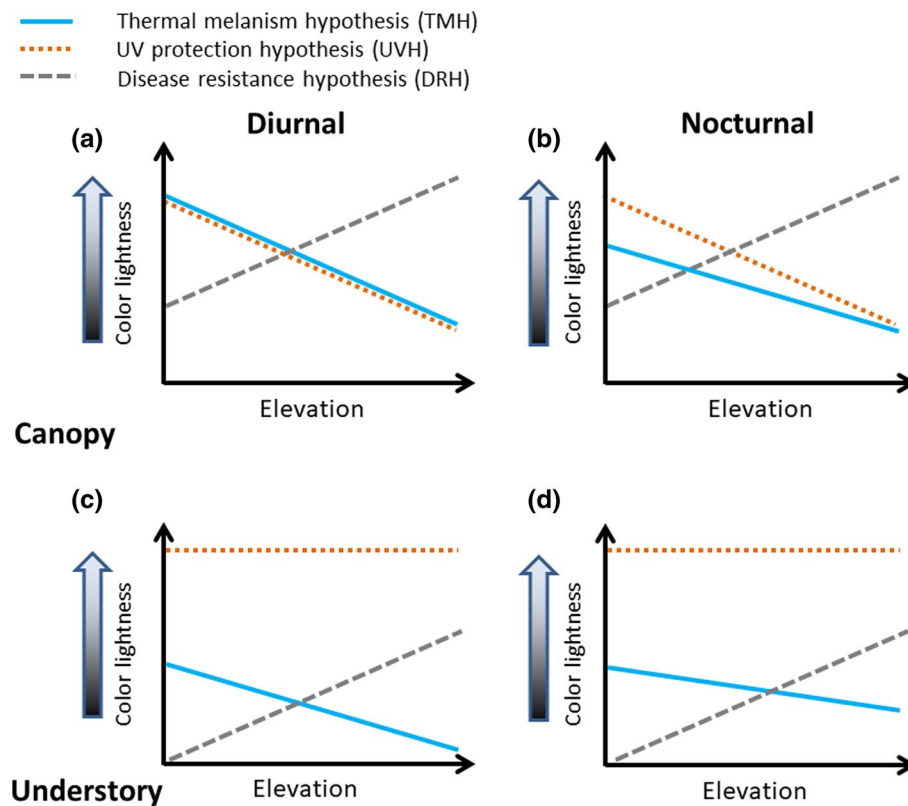
Dark coloration may facilitate the absorption of solar radiation into body heat, which increases overall activity potential for diurnal individuals (Clusella-Trullas et al. 2007). Such benefit, however, may be less important for nocturnal moths, which are inactive during the day. On the other hand, efficiency in transferring solar energy into body heat gain may shorten the pre-reproduction period of adult stages (e.g., oogenesis and egg maturation in females), enabling earlier initiation of breeding activity, and ultimately benefit their reproductive performance (Kitching 1977; Ellers and Boggs 2004; Stuart-Fox et al. 2017). In such cases, selection may favor traits that enhance efficiency in heat gain, especially in thermally limited environments. The TMH, however, should be less important for nocturnal moths than diurnal butterflies as their efficiency for solar absorption is not directly linked with their activity (Heidrich et al. 2018). In addition, nocturnal moths resting in less shady habitats with rich sunlight will be vulnerable to strong UV-radiation. Melanism, therefore, may be favored by selection for its

protective function against harmful UV-radiation. Finally, as disease resistance should be independent of activity time, this function of melanism should also apply to nocturnal moths, and with higher relative importance than their diurnal counterparts (Heidrich et al. 2018).

The variations in climate presented along elevational gradients provide a good opportunity to test these hypotheses. Although temperature consistently decreases with increasing elevation, solar radiation is expected to increase with elevation (Blumthaler et al. 1997; Hodkinson 2005). Accordingly, both the TMH and UVH would predict decreases in body color lightness towards higher elevations (Fig. 1). In contrast, the DRH predicts that body color lightness increases with elevation, as disease resistance becomes less important at higher elevations (Fig. 1) where parasitism and pathogen infections may decline (Hodkinson 2005; Heidrich et al. 2018). Nevertheless, this prediction comes with high uncertainty due to the difficulties in predicting general humidity patterns across elevations (Hodkinson 2005).

As well as being sensitive to elevational gradients, vertical stratification of moth assemblages has been addressed across the globe, indicating the importance of considering the vertical dimension in addition to other spatial patterns (Ashton et al. 2016a). The difference in microclimates of canopies and understories will provide additional insights into the understanding of environment–morphology relationships (Bohlman et al. 1995; Scheffers et al. 2013; Scheffers and Williams 2018). In general, the solar radiation of forest understories is largely buffered by dense, higher forest layers (Endler 1993; Rich et al. 1993; Jankowski et al. 2013), generating cooler and wetter microclimates in the forest understory compared with the canopy (Bohlman et al. 1995; Scheffers et al. 2013). Hence, if color lightness patterns are mostly explained by the TMH or DRH, understory moths should be darker, living under relatively cool and humid conditions: in contrast, if selection is largely explained by the UVH, canopy moths should be darker as a result of being exposed to stronger solar radiation (Fig. 1). Moreover, we expect different elevational patterns in color lightness between canopy and understory assemblages. As solar radiation is relatively weak in the forest understory, the role of thermal melanism and UV-protection should be less important for understory moths. We predict, therefore, that canopy moths will exhibit steeper declines in color lightness with increasing elevation driven by the TMH or UVH (Fig. 1).

The predicted patterns are also expected to vary between diurnal and nocturnal insects, with smaller elevational effects in nocturnal assemblages driven by the TMH (Fig. 1). In contrast, effects of both the UVH and DRH may not vary between nocturnal and diurnal insects as these two hypotheses operate independent of activity times. Such hypothetical differences (and similarities) in predicted patterns and underlying selection pressures between diurnal and



**Fig. 1** Hypothetical illustrations of color lightness patterns across elevational gradients according to the three hypotheses (the TMH, UVH and DRH, see text). Comparisons are made between diurnal (a, c) and nocturnal (b, d) insects within the forest canopy (a, b) and understory (c, d) strata. The TMH (blue solid lines) predicts that the insects are darker in the understory where temperature is expected to be cooler than the canopy, and elevational patterns of color lightness will be weaker in the understory. Moreover, elevational clines in

color lightness should be less apparent for nocturnal compared with diurnal insects. The UVH (orange dotted lines) predicts darker insects in the canopy and at higher elevations, with a negligible elevational effect on understory insects as most solar radiation is buffered. The DRH (grey dashed lines) predicts that understory insects are darker in more humid understory, and low, warmer elevations where the risk of infection by parasites or pathogens may be higher (color figure online)

nocturnal groups highlight the importance of investigating color lightness of nocturnal in addition to diurnal insects.

In this study, we focused on the family Geometridae, which exhibits high diversity in terms of species and morphology (Scoble 1992; Abraham et al. 2001; Zou et al. 2016) and is sensitive to environmental gradients (Brehm et al. 2003; Axmacher et al. 2004; Chen et al. 2009; Zou et al. 2016). Moth assemblages have rarely been sampled systematically in tropical Asia, especially with samples from both canopy and understory strata. Here, we used moth specimens collected by Ashton et al. (2016b), who reported the patterns of assemblage composition of moths from both canopy and understory in forests along three elevational gradients established within tropical, subtropical and subalpine climatic regions of southwestern China, to examine vertical and elevational patterns of nocturnal moth color lightness. We investigated which of the three hypotheses best explain mean color lightness of nocturnal moth assemblages along elevational and vertical gradients across different climatic

regions. We hypothesized that: (1) decrease in mean color lightness of moth assemblages with increasing elevation would follow the TMH and UVH, whereas the opposite pattern would be expected by the DRH; (2) lighter color for canopy than understory moth assemblages would follow the TMH and DRH, whereas the opposite would be expected by the UVH; and (3) stronger elevational effect (i.e., steeper slopes) on color lightness of canopy moths compared with understory moths would follow the TMH and UVH, whereas no difference in elevational effect would be expected following the DRH (Fig. 1).

## Materials and methods

### Field sampling

Moth assemblages were collected by LAA, RLK, MC, ZS and AN (Ashton et al. 2016b) between 05 July 2012 and 24

July 2012 in Mengla (21.4°N, 101°3'E: tropical), 01 July 2011 and 20 July 2011 in Ailaoshan (24.2°N, 101°2'E: subtropical) and 09 August 2012 and 22 August 2012 in Lijiang (27.1°N, 100°1'E: subalpine) all in Yunnan province, southwestern China. The inter-site distances between neighboring locations from South to North is about 300 km in each case (see Fig. 1 of Ashton et al. 2016b). Within each of the three locations, elevational transects were established across a 600 m elevational span. Within each transect, four elevational bands were defined, separated by about 200 m in vertical distance (800, 1000, 1200, 1400 m in Mengla; 2000, 2200, 2400, 2600 m in Ailaoshan; and, 3200, 3400, 3600, 3800 m in Lijiang). Within each elevational band, five 20 × 20 m sampling plots were carefully chosen to avoid visible earlier disturbance and to keep aspect and slope consistent across plots. The relevant survey permits were obtained from the administration bureau of the local nature reserves.

Pennsylvania-style light traps (Southwood and Henderson 2000), modified for ground and canopy sampling in, often wet, forests were used to sample canopy and understory moth assemblages at each plot following the same protocols as other moth studies at different locations globally (Ashton et al. 2016a, b). The traps were run at Mengla and Ailaoshan for three nights and at Lijiang for two nights or until at least 100 individuals were encountered in each canopy and understory sample (Ashton et al. 2016b). The samples collected on different nights were pooled together as representative of the moth assemblages for each stratum (canopy or understory) at a plot. In total, 4 (elevational bands) × 2 (canopy and understory) × 5 (plots) = 40 samples were obtained from each of the three elevational transects, making a total of 120 replicate samples. Specimens of geometrid moths collected during sampling were mounted and identified to morphospecies and a representative collection was made for all morphospecies encountered. Air temperature data were measured in both canopy and understory strata at each plot using thermologgers (DS1923 Hygrochron iButton, Maxim, CA, USA) for a year after sampling. Temperature data for 34 samples (ten from canopy and 24 from understory) were obtained successfully in this fashion (Table S1). The temperature data covered most of the elevational and vertical strata except in Mengla where data loggers from all the canopy and the 1200 m locations (both canopy and understory) could not be retrieved. We used temperature data from between July and August, which represents the wet summer period (when moths were sampled). Since mean, maximum and minimum hourly air temperature in daytime (7:00–20:00) and nighttime (21:00–6:00) were highly correlated ( $R^2 > 0.98$ ), we used mean hourly temperature to represent the air temperature of each sampled stratum and elevation.

## Moth morphology analysis

Because collections were sorted to morphospecies in Ashton et al. (2016b), the variation in intraspecific morphological traits was assumed to be subtle relative to interspecific morphological variability. We focused, therefore, on color lightness of moths at the interspecific level (i.e., among morphospecies) by choosing one of the best preserved moth specimens of each morphospecies series in the Ashton et al. (2016b) collection to represent the color lightness of that taxon. We took photos of the specimens using a Nikon D3300 camera with a flash under standardized settings (exposure time: 1/125 s, ISO speed: 100, aperture: F/14, vertical distance from specimen: 45 cm). We then used Adobe Photoshop CC 2014 software to determine the color lightness of each morphospecies, following a similar protocol for color lightness analysis in Zeuss et al. (2014) and Xing et al. (2016, 2018). Since the region of geometrid moths exposed to solar radiation will usually be the forewing plus the dorsal side of body (as forewing and thorax are always exposed given the characteristic 'triangular' resting pose of the vast majority of Geometridae), we analyzed color lightness of the forewing and thorax together as representative of the color lightness of the area exposed. We selected the relevant region and analyzed the color lightness using the average option under the blur function listed in the Filter menu. The mean values of red, green and blue channels of the selected region were recorded as color lightness values, ranging between 0 (darkest) and 255 (lightest) (Fig. S1). The mean color lightness of the entire geometrid assemblages was calculated by weighting the relative abundance of morphospecies in each sample, so color lightness of more abundant morphospecies had higher weight. In this way, we considered the changes in relative abundance of morphospecies in contributing to mean traits at the level of the entire assemblage for each sample (Bishop et al. 2016; Xing et al. 2018). For comparison, we also calculated mean color lightness without this weighting (Table S1). We used only mean (rather than median) color lightness of moth assemblages, as they were highly correlated (correlation coefficient 0.92,  $P < 0.001$ ).

## Statistical analysis

To test how temperature varied across elevation and vertical stratum (canopy vs understory), we used a linear mixed effects model (LMM) with restricted maximum likelihood (REML). We treated mean hourly air temperature as the response variable (excluding all data from Mengla where incomplete temperature data were retrieved: overall,  $n = 29$ ) with location as a random intercept, and incorporated elevation, vertical stratum (canopy/understory) as well as the interactive effects of elevation and vertical stratum as fixed

factors using the lme function in the R package nlme (R v.3.1-122, Pinheiro et al. 2015). Elevation was treated as a continuous predictor variable, and vertical stratum as a categorical predictor variable within the model. We calculated marginal  $R^2$  and conditional  $R^2$  for the linear mixed effects model using the r.squaredGLMM function in the R package MuMIn (R v.1.15.6, Barton 2016).

We then tested whether or not the elevational and vertical signals in color lightness were significant when the effect of location was controlled for. To do this, we conducted a similar LMM analysis which treated mean color lightness of moth assemblages ( $n = 120$  samples) as the response variable, location as a random intercept, and elevation and vertical stratum (canopy/understory) as well as their interactive effects as fixed factors. We conducted this LMM separately for color lightness using both abundance-weighted and abundance-unweighted data. Furthermore, we examined the elevational effect on canopy and understory strata separately. To this end, we split our database into canopy and understory assemblages and applied LMM with location as random intercept and elevation as the fixed factor for each vertical stratum. We then conducted post hoc simple linear regressions to investigate the effect of elevation for each vertical stratum in each location with Bonferroni correction for multiple tests. To examine further the temperature effect on mean color lightness of moth assemblages, we conducted ordinary linear regressions to test the relationships between moth color lightness and mean hourly air temperature. We only included samples where temperature data were available ( $n = 34$ ). All analyses were conducted in R v.3.2.3 (R Core Team 2015).

## Results

Air temperature in Ailaoshan and Lijiang significantly decreased with increasing elevation and exhibited slightly stronger elevational patterns in the canopy than the understory (Table 1; Fig. S2). We extracted color lightness from 1164 morphospecies, representing a total of 19,846 moth individuals, across the three locations. Results of the linear mixed effects model showed that elevation significantly negatively affected moth color lightness, indicating moth assemblages were darker at higher elevations. The effect of elevation, however, was not significant when mean color lightness of moth assemblages was unweighted by abundance. No significant effects of vertical stratum or the interactions between elevation and vertical stratum were found on moth color lightness either when weighted or unweighted by abundance (Table 2). When we investigated color lightness of canopy and understory moths separately, the effect of elevation was significant for canopy moth assemblages when weighted or unweighted by abundance, but the effect was not significant (and the trend subtle) for understory assemblages (Table 3).

The post hoc linear regression analysis showed that elevational patterns of moth color lightness varied across different locations with the elevational effect only significant for the canopy moth assemblages in Lijiang (Fig. 2; Table 4). In addition, our ordinary linear regression indicated that mean color lightness of moth assemblages was significantly positively correlated with mean hourly air

**Table 1** Results of the analysis using the linear mixed effect model with location as a random effect, showing the estimated coefficients of elevation and vertical stratification as well as their interactions on mean hourly temperature ( $n = 29$ , excluding incomplete Mengla data)

Response	Model factor	Estimate	Std. error	t value	Pr (>  t )
Temperature ( $R^2$ marginal=0.87, $R^2$ conditional=0.97)	Elevation	<b>-5.81E-3</b>	6.21E-4	-9.36	<0.001
	Canopy/understory	2.37	1.35	1.76	0.09
	Elevation × canopy/understory	<b>-1.17E-3</b>	4.98E-4	-2.34	0.03

Coefficients with significant  $P$  values are shown in bold

**Table 2** Results of the analysis using linear mixed effect models with location as a random effect, showing the estimated coefficients of elevation, vertical stratification and their interactions on weighted (by abundance of moth morphospecies) and unweighted color lightness of moth assemblages ( $n = 120$ )

Response	Model factor	Estimate	Std. Error	t value	Pr(> t )
Color lightness (weighted) ( $R^2$ marginal=0.50, $R^2$ conditional=0.78)	Elevation	<b>-8.00E-3</b>	2.04E-3	-3.93	<0.001
	Canopy/understory	3.75	2.57	1.46	0.15
	Elevation × canopy/understory	-8.20E-4	1.02E-3	-0.80	0.42
Color lightness (unweighted) ( $R^2$ marginal=0.09, $R^2$ conditional=0.80)	Elevation	-2.07E-3	1.53E-3	-1.36	0.18
	Canopy/understory	1.27	1.77	0.72	0.47
	Elevation × canopy/understory	-7.70E-4	7.04E-4	-1.09	0.28

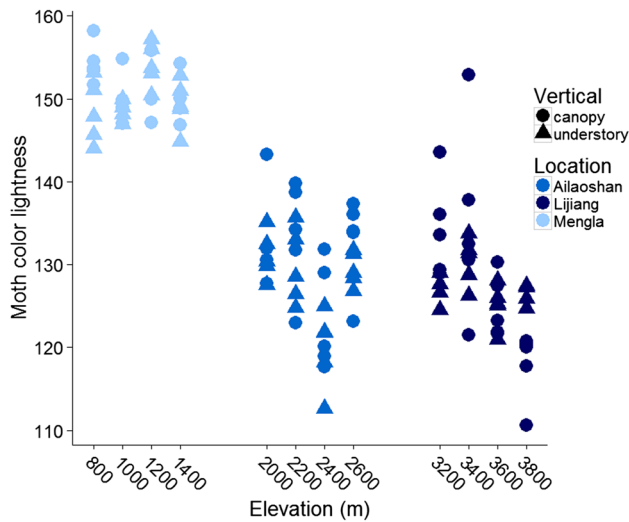
Coefficients with significant  $P$  values are shown in bold

**Table 3** Results of the post hoc analysis using linear mixed effect model with location as a random effect, showing the estimated coefficients of elevation on weighted (by abundance of moth morphospe-

cies) and unweighted color lightness of canopy ( $n=60$ ) and understory moth assemblages ( $n=60$ )

Vertical stratum	Response (Color lightness)	Estimate	Std. Error	t value	Pr(> t )	R <sup>2</sup> marginal	R <sup>2</sup> conditional
Canopy	Weighted	<b>-0.01</b>	2.71E-3	-4.26	<0.001	0.62	0.80
	Unweighted	<b>-6.03E-3</b>	1.96E-3	-3.08	3.00E-3	0.45	0.77
Understory	Weighted	-3.73E-3	2.25E-3	-1.66	0.10	0.11	0.86
	Unweighted	1.02E-3	1.78E-3	0.57	0.57	8.77E-3	0.91

Coefficients with significant  $P$  values are shown in bold



**Fig. 2** Mean color lightness of moth assemblages (weighted by abundances of morphospecies) in the canopy (circle) and understory (triangle) strata, along the elevational gradients of the three locations ( $n=120$ ). Colors of points indicate the location of moth assemblages: light blue for tropical Mengla; medium blue for subtropical Ailaoshan; and dark blue for subalpine Lijiang (color figure online)

temperature, with lighter assemblages associated with warmer air temperatures (Fig. 3;  $R^2 = 0.59$ ,  $F_{1,32} = 47.72$ ,  $P < 0.001$ ).

### Discussion

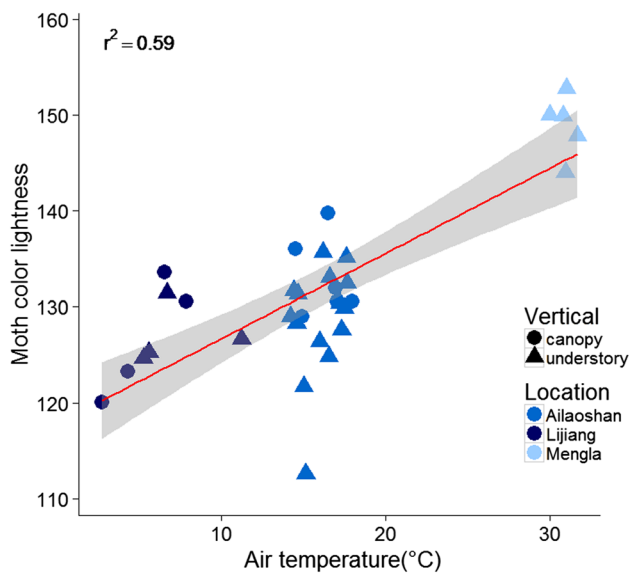
We found that mean color lightness of geometrid moths decreases with increasing elevation across the three locations at the level of the multi-species assemblage. These results support the predictions of the TMH and the UVH, but not the DRH. This finding is similar to that identified for diurnal insects (Zeuss et al. 2014; Bishop et al. 2016; Pinkert et al. 2017; Xing et al. 2018) and a recent large-scale analysis of European geometrid moths (Heidrich et al. 2018). We found that the elevational patterns were most apparent among canopy assemblages, which are more exposed to intense solar radiation than understory moths, and in the subalpine location where moths are likely to experience stronger solar radiation and colder temperatures than other locations.

Vertical stratification in species composition of moth assemblages has been found to be almost universal across latitude and elevation (Ashton et al. 2016a). Unlike species composition, we found no significant differences in color lightness between canopy and understory assemblages, when elevational effects were controlled for, indicating a lack of strong environmental selection for color lightness at the vertical scale. We initially expected the ambient temperature to be lower in the understory than the canopy due to the lack of solar radiation. Temperatures, however, were not significantly different between the canopy and ground (Table 1), suggesting an overall thermal uniformity

**Table 4** Results of the post hoc linear regression analyses, showing estimated coefficients of elevation on color lightness of moth assemblages in the canopy and understory levels within each of the three study locations

Location	Vertical stratum	Estimate	Std. error	t value	Pr(> t )	R <sup>2</sup>
Mengla	Canopy	-5.02E-3	3.18E-3	-1.58	0.13	0.07
	Understory	4.03E-3	3.47E-3	1.16	0.26	0.02
Ailaoshan	Canopy	-4.91E-3	7.22E-3	-0.68	0.51	-0.03
	Understory	-7.40E-3	5.60E-3	-1.32	0.20	0.04
Lijiang	Canopy	<b>-0.03</b>	7.19E-3	-4.12	<0.001	0.46
	Understory	-4.77E-3	2.80E-3	-1.70	0.11	0.09

Coefficients with significant  $P$  values are shown in bold (threshold of significance after Bonferroni correction:  $0.05/6$  tests = 0.008)



**Fig. 3** Correlation of mean color lightness of moth assemblages (weighted by abundances of species) and hourly average air temperature ( $n=34$ ) in the canopy (circle) and understory (triangle) strata. Colors of points indicate the location of moth assemblages: light blue for tropical Mengla; medium blue for subtropical Ailaoshan; and dark blue for subalpine Lijiang. Modeled trend line (red line) ( $P < 0.001$ ,  $R^2 = 0.59$ ) with standard errors (grey area) is also shown (color figure online)

across strata in our study sites. The absence of contrasting temperature regimes at the vertical scales in our sites limited our ability to differentiate the effects of TMH and UVH on moth color lightness. In this respect, the paucity of temperature data from our fully tropical sites likely reduced the power of our analysis.

Though we found no significant interactive effects of elevation and vertical stratum on moth color lightness, our separate analyses for each stratum demonstrated a significant elevational signal for canopy but not for understory assemblages, indicating a differential vertical response of moth color lightness across elevations. Such discrepancies between canopy and understory might be explained by the stronger elevational trend of temperatures in the canopies than in the understories (Table 1), which may result in higher selective pressure from temperature on color lightness, supporting the TMH. In addition, the different elevational responses in moth color lightness across vertical dimensions are also in line with the prediction of UVH. Moths inhabiting the treetops are likely to be more exposed to direct or diffuse sunlight, and, therefore, may encounter higher risk of UV-damage at higher elevations. As stronger solar radiation may also strengthen thermal melanism, the TMH is still a plausible explanation. In contrast, buffered solar radiation by multiple higher forest layers in the understory may largely reduce the risk of UV harm and thermal effect of color lightness for understory

moths (Rich et al. 1993), resulting in the ambiguous elevational cline in their color lightness.

The strengths of the relationships between color lightness and elevation varied across locations, with the greatest influence of elevation in the canopy of subalpine Lijiang. The particular sensitivity of the color lightness in this location may be explained conjointly by limitations in thermal resources and the strength of solar radiation. First, the temperature at the highest elevation (3800 m) dipped below 0 °C, even in summer (Fig. S2). This may represent a fatal temperature for many moth species (Heath and Adams 1967; Heinrich 1987). Moreover, convective heat loss tends to be greater at higher elevations, even after factoring in the empirical decrease in reduced air density (Dillon et al. 2006). In such conditions, survival of geometrid moths, as small-sized ectotherms, is likely to be challenging considering their physiological and behavioral limitations for thermoregulation (Beck et al. 2017). More effective absorption of solar energy from darker coloration has the potential to enhance survival and reproductive performance at higher elevations (Ellers and Boggs 2004). At the highest location, Lijiang moths are most exposed to harmful UV, especially in the canopy. Accordingly, the dark coloration for UV-protection should increase the diurnal survival rate of the insects, especially when avoidance by position adjustment while resting is not feasible without attracting additional risks (e.g., predation, desiccation) (True 2003; Bastide et al. 2014). As such, dark coloration may well improve the fitness of high-elevation moths through both thermal adaptation and UV-protection. Distinguishing between effects of UV-protection and heat absorption and quantifying their relative importance on moth color lightness, however, remain a challenge—additional studies exploring canopy and understory strata with distinct differences in temperatures (where understories are significantly cooler than canopies) may provide valuable insights (e.g., Fig. 1).

Unlike subalpine Lijiang where temperature and solar radiation are suggested as the main selective pressures on color lightness, the climate of subtropical Ailaoshan may overall be more suitable for moths. In consequence, elevation may not have filtered moth species based on their sensitivity to abiotic factors to the same degree. This may explain the lack of a clear elevational signal for Ailaoshan (Table 4). In addition, the deviation of moth color lightness from the regression line (Fig. 3) and the variance of color lightness among morphospecies (Fig. S3) were both greater in Ailaoshan than the other two locations, which may indicate relaxed or absent selection pressure on moth color lightness. The ambient understory temperature in Mengla did not vary across elevations within the location (Fig. S2, but the results need to be interpreted with caution, as some temperature data were missing), which makes it difficult to interpret how moth color lightness may be influenced by

climatic factors in this fully tropical, lowland locality. The relatively uniform high color lightness observed in Mengla moths is clearly contradictory to the predictions of the DRH (Fig. 1), which predicts darker assemblages in warm and humid tropical lowland locations. Such a pattern might be explained by the TMH, which predicts lighter coloration in hot climates to avoid overheating, but further experimental and field studies are needed to test this prediction and to compare whether selective pressure is higher due to cold or hot limitation.

Here, we have focused on a single morphological trait evident in adult moths. Other morphological traits such as body size/mass and thickness of setae may also be important in explaining how the environment shapes trait distributions within communities (Church 1960; Heath and Adams 1967; Bartholomew and Casey 1978; Zeuss et al. 2017). In addition, selection pressures acting on moth color lightness such as predation were not considered in this study, but potentially, shed light on distribution patterns of color lightness (Sargent 1966; Boardman et al. 1974; Majerus 1998). Dark coloration, for example, may be better for camouflage against predators in the dark forest understory (Lev-Yadun et al. 2004; Cheng et al. 2018). Such camouflage function might be particularly important in environments with high-predation risk, such as in tropical rainforest (Roslin et al. 2017). Experiments examining multiple morphological characteristics at different life stages and considering diel variation are needed to clarify the relative importance of morphology in determining survival and reproduction of insects (Goulson 1994; Lindstedt et al. 2009; Battisti et al. 2015). Furthermore, as we used morphospecies in our study, we cannot infer the evolutionary history of moth color lightness for these sites. Future work including phylogenetic frameworks in trait analysis may provide important evolutionary perspectives on current distribution patterns of morphological traits for nocturnal insects (Zou et al. 2016). The existence of increasingly robust phylogenies for the Geometridae (Sihvonen et al. 2011; Ounap et al. 2016) makes this an interesting prospect for future work on the existing collections.

In this study, we have tested different hypotheses that may explain color lightness of geometrid moths based on assemblage-level distribution patterns along elevational and vertical dimensions. Our study provides some of the first evidence supporting the thermal melanism and the UV-protection hypotheses, but not the disease resistance hypothesis for nocturnal insect assemblages in the tropics. Although we did not detect clear vertical stratification of moth color lightness in this study, we did observe differential responses in color lightness to elevation of canopy and ground moth assemblages, with a statistically supported decrease in mean color lightness only in canopy assemblages. This pattern is similar to that observed in diurnal insect groups. These

findings highlight the role of temperature and solar radiation in affecting the degree of color lightness of nocturnal insect assemblages, especially in climatically harsh environments where organisms are under strong selective pressures from abiotic factors. Our study further emphasizes the importance of considering verticality and diel activity when investigating morphology–climate relationships for future studies.

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