ORIGINAL ARTICLE

Adult thermoregulatory behaviour does not provide, by itself, an adaptive explanation for the reflectance–climate relationship (Bogert's pattern) in Iberian butterflies

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Abstract

1. The association of darker, less reflective insect wings with cooler environments (Bogert's rule) is thought to be related to adult thermoregulation, but the adaptive explanation and the implications for sensitivity to climate warming are yet to be tested. We re-evaluate the pattern for butterflies using finer resolution data than in previous approaches, both geographically and morphologically, and test its correlation with recent evidence of impacts of warming on butterflies.

2. We compared reflectance–climate relationships at different grid sizes, selected the best subset of reflectance measurements and tested the contribution of the species basking mode, the phylogenetic structure of the data and the correlation between reflectance and published abundance or altitudinal shifts. We used standardised RGB (Red, Green, Blue) values from 222 species from the Iberian Peninsula, and regional mean temperature and precipitation data from the study area (10 and 50-km resolutions) and Europe (50-km resolution).

3. Correlations between reflectance and temperature increased at finer geographical and morphological resolutions. However, the butterfly basking mode did not improve the statistical explanation of the pattern. Reflectance shows a strong phylogenetic structure, while variance partitioning indicated a poor pure contribution of the climate variables in the reflectance–climate correlation.

4. Overall, mean temperature and precipitation were only modest predictors of butterfly reflectance. No correlation between reflectance and recent abundance or altitudinal shifts was found using the hypothesised best estimates of reflectance. Although significant correlations between butterfly shading and altitudinal shifts were found for two of the reflectance measurements, this is interpreted as weak, probably artifactual evidence on the predictive power of this relationship.

5. The strong phylogenetic pattern of the reflectance and the low fraction of the reflectance measures analysed suggest that tests for alternative explanations are still needed to shed light on the meaning of the colour–environment relationships in butterflies, which probably are of a complex nature. From an adaptive point of view, unravelling the basis of Bogert's pattern in butterflies requires a closer,

habitat-level approach and alternative variables to adult thermoregulatory behaviour to be tested.

INTRODUCTION

In the current context of global change and the ecological effects of warming (Lovejoy & Hannah, 2019), understanding the precise nature of the link between the biological functions of organisms and climate should assist in the interpretation and even the prediction of organisms' responses to ongoing environmental change (Buckley et al., 2010). Negative climate impacts should be expected for small ectotherm insects such as the butterflies (Lepidoptera, Papilionoidea) (Hill et al., 2021; Kingsolver et al., 2013) as already documented from the European area (Settele et al., 2008; Warren et al., 2021). These should be even more pronounced in temperate areas (Kingsolver et al., 2013) such as the Southwest Mediterranean, where severe shifts in temperature and precipitation conditions are expected (Giorgi & Lionello, 2008; Hertig & Jacobeit, 2008). However, the nature of the negative impacts of warming may be difficult to identify. Temperature shifts might have varied indirect effects on insect populations, but also direct ones related to their small body size (which imposes low thermal inertia: Huey et al., 2019), for example, through growth rates (Roitberg & Mangel, 2016) or due to the costs of behavioural thermoregulation (Kerr et al., 2015).

Recent comparative research on butterflies shows evidence of a link between the wing and body reflectance and climate, either at a macroecological scale (Kang et al., 2021a; Stelbrink et al., 2019; Zeuss et al., 2014), at the habitat one (Xing et al., 2016) or close to this (on a 5-km resolution: Munro et al., 2019). This is supported both from measurements done on printed colour plates (Stelbrink et al., 2019; Zeuss et al., 2014) or taken from real, collection specimens (Kang et al., 2021a, 2021b; Munro et al., 2019; Xing et al., 2016). This is interesting as it suggests that climate variables are good correlates of the optimal butterfly colouration (Stelbrink et al., 2019; Zeuss et al., 2014), hence reflectance could be a predictor of potential use in, for example, modelling and forecasting species responses to increasing temperatures (Millien et al., 2006). However, the causes of the relationship between reflectance and environmental temperature remain speculative. The results of the phylogenetically driven analysis of the European butterflies by Kang et al. (2021a) support the interspecific tendency of ectotherms to have darker bodies in comparatively colder areas, known as Bogert's rule (Bogert, 1949; L. Hill & Taylor, 1933; Lomolino et al., 2016, Trullas et al., 2007). This eco-geographic pattern is often implicitly interpreted as advantageous for thermoregulation with further examples from insects other than butterflies such as springtails (Collembola) (Rapoport, 1969) and dragonflies (Odonata) (Pinkert et al., 2017; Zeuss et al., 2014). After Kang et al. (2021a) an alternative, less supported explanation, for the European butterflies is Gloger's pattern. This 'rule' (brighter colour in warm and moist environments) was originally proposed for endotherms based on a higher deposition rate of the melanin-derived pigments in tropical habitats as the proximate explanation (Rensch, 1938; see discussion by Delhey, 2019).

An evolutionary explanation at the macroecological scale does not necessarily reflect present interactions at the habitat or microhabitat level, thus determining the relevance of reflectance as an ecological trait in butterflies that involves a twofold problem: first, evaluating the predictive ability (in terms of forecasting capacity) of climate with that trait as the response. Testing the current geographic variation of butterfly colouration in terms of the mean species climatic values (as in the references quoted above) is feasible, although under the assumption that present spatial variation is not exactly equivalent to temporal variation (Kerr et al., 2011). The second part of the problem involves clarifying the nature of the reflectance–climate relationship. We hypothesise that a 'finer dissection' of the issue is possible by increasing the accuracy and resolution of the data used, from two points of view: the accuracy of geographic location and morphology. From the point of view of geographic accuracy, based on Xing et al. (2016), we would expect that the statistical association between reflectance and climate increases at higher spatial (or other, see below) resolution. We test this by re-assessing the relation between climate and reflectance: (1) working on a regional scale (the Iberian Peninsula instead of Europe), (2) increasing the geographic accuracy of the climate estimates (on a 10-km grid basis instead of 50 km as used by Kang et al., 2021a) and (3) adopting more accurate morphological data to specifically test the role of adult thermoregulatory behaviour.

From the morphological point of view, former authors have wisely used the butterfly reflectances of the body and the wing bases since, due to the low thermal conductivity of the wings, these areas are the most relevant for heating (Kammer & Bracchi, 1973). However, the adult butterflies expose themselves to the sun in three main basking postures: lateral, dorsal and reflection (Clench, 1966; Kingsolver, 1987, 1988). In lateral basking the ventral wing surfaces are exposed to the light, while in dorsal and reflection basking the dorsum of the insect receives the incident radiation. Moreover, not all parts of each wing are exposed to the incident light on the living butterfly, because the two wings often overlap to a large extent (Figure 1a,b). Therefore, measurements taken in a non-selective way from a pinned specimen may be misleading. If the relationship between reflectance and climate is linked to adult thermoregulation, we would expect a specific response pattern with a stronger correlation between temperature and shading on the appropriate parts of the wings: those exposed to the light according to the species basking posture. Thus, measuring reflectance measurements in those parts of the wing and body is necessary: we hypothesise that if reflectance is estimated in the specific parts exposed by each butterfly species depending of its specific basking behaviour, the correlations with the climate variables should increase.
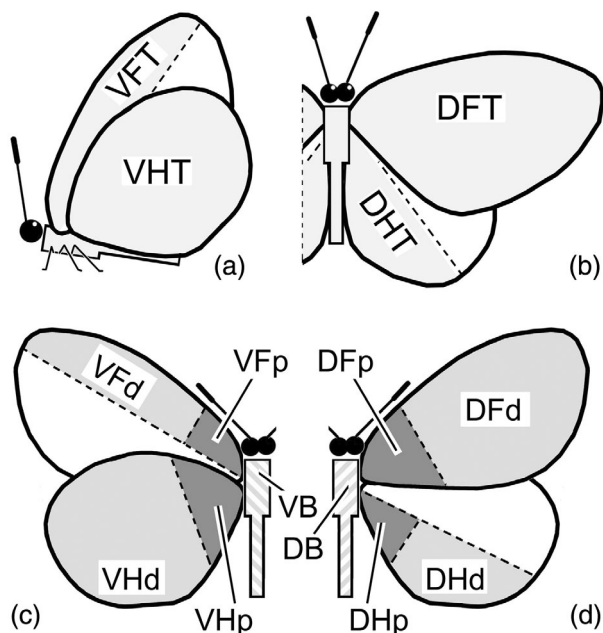
BUTTERFLY REFLECTANCE, BASKING BEHAVIOUR AND CLIMATE

Ecological
Entomology

Royal
Entomological
Society

3

FIGURE 1 Idealised representations of the posture of a butterfly during lateral (a) and dorsal basking behaviour (b), and the paths selected for measurement on the ventral (c) and dorsal (d) aspects of the pinned butterflies. The list of measurements and abbreviations is detailed in the text. B/F/H, body, forewing, hindwing; D/V, dorsal, ventral; p/d/T, proximal, distal, total.

The kind of comparisons we propose require basic statistical techniques such as correlation and regression. Control for phylogenetic autocorrelation in studies involving cross-species comparisons is required when evolutionary explanations are the objective (Freckleton et al., 2002; Harvey & Pagel, 1991), but shared ancestry should not be a matter of concern when the purpose is the prediction from variables with no or very low phylogenetic structure (Desdevises et al., 2003; Legendre & Legendre, 2012; Uyeda et al., 2018). Hence, the amount of phylogenetic structuring of the data must be determined as a first step in any case. Besides measuring this (as described below) we performed part of our analyses both with, and without regard to phylogenetic relations, as well as estimated the contribution of phylogeny itself to the relationships of relevance for this study.

So, in summary, besides (1) providing a table of standardised reflectance measurements of the parts effectively exposed during basking for the Iberian butterflies (visible range of the light spectrum), our objectives are: (2) comparing the strength of the relationship between climate (temperature and precipitation) and reflectance at two different geographical resolutions, (3) testing the role of adult basking modes on the reflectance–temperature relationship as potential support for a behaviourally mediated explanation, (4) measuring the 'non-phylogenetic' fraction of the relationships between climate and butterfly shading and finally (5) testing the correlation between butterfly reflectance and published, recent demographic or altitudinal trends in Iberian or European butterflies.

Under the assumption that the link between colour and climate of butterflies has a functional basis at the habitat scale and that this

derives from adult thermoregulation, we expect correlations to increase when estimated at the regional context and at the highest resolution, with specific combinations of the reflectance measures yielding the best results and a relevant fraction of non-phylogenetic load on the temperature–reflectance relationship, as well as a correlation between reflectance and species trends.

METHODS

Butterfly specimens

The measurements were done on pinned individuals of 222 butterfly species (families Papilionidae, Hesperiidae, Pieridae, Riodinidae, Lycaenidae and Nymphalidae) from the Iberian Peninsula (the continental territories of Spain and Portugal). This sample represents ca. 95% of the species in that area (235 species after García-Barros et al., 2013 updated after Bolotov et al., 2021; Hinojosa et al., 2022; Lukhtanov & Pazhenkova, 2021; Vila et al., 2018; Wiemers et al., 2018; Zhang et al., 2020). The species list includes five pairs of cryptic or closely related species whose detailed geographic distribution requires a thorough reassessment; we merged these species pairs into single 'morphospecies', at the cost of a loss of resolution in the analyses. Unfortunately, such cases include widespread species in the genera *Polyommatus*, *Leptidea*, *Melitaea* and *Spialia* (in addition to the sources quoted above, see: Dincă et al., 2015; Hernández-Roldán et al., 2016; Muñoz Sariot & Sánchez Mesa, 2019, Platania et al., 2020; Sañudo-Restrepo et al., 2013). The complete species list and additional comments are available in Table S2. The specimens were selected from the collection at the authors' department, complemented by a small number (<10) of additional samples from a private collection (J. L. Hernández-Roldán). One member of each sex (eventually two) were sampled and their average values for each trait were adopted. For three species only one male was available; we kept their data to represent the species' values.

Reflectance measurements

The set specimens were photographed in a dim room with an Olympus E-500 camera (focal distance = 50.5 cm, ISO (sensitivity, International Organization for Standardization) = 125, speed = 1/5″, F = 16) with all options set to manual, grey balance adjusted to a DGC-100 neutral grey card and two daylight bulbs (Osram Dulux, 36 W, 3000 K) fixed at 30 cm and 45° from the target, with a neutral grey background (8 cm below the target). Each image included a metric scale and a standard colour chart with seven paths of known average RGB values ranging from 243.67 (white) through five intermediate grey shades (RGB = 200, 160, 121, 67, 85) to black (RGB = 52) (AIC (Akaike Information Criterion) PhD Target, Robin Myers Imaging: www.rmimaging.com) (see sample image in Figure S1).

We measured forewing length and 26 shade measurements (average RGB on the visible spectrum) on each specimen (Figure 1), in

TABLE 1 Abbreviations and definitions of the variables.

Variable	Aspect	Part	Reflectance measurements (except for FWL)
FWL	–	Forewing	Wing length (base to apex, mm)
DFT	D	Forewing	Whole wing
DFp	D	Forewing	Proximal area
DFd	D	Forewing	Distal area
DHT	D	Hindwing	Whole posterior (anal) half
DHp	D	Hindwing	Proximal part of the posterior half
DHd	D	Hindwing	Distal part of the posterior half
DB	D	Body	Body, head and eyes excluded.
D(Tp + B)	D	Combined	Proximal wing areas and body
DT	D	Combined	Dorsal, whole insect (DFT, DHT and DB together)
DTp	D	Both wings	Joint proximal section of the two wings (DFp, DHp)
DTd	D	Both wings	Joint distal section of the two wings (DFd, DHd)
VFT	V	Forewing	Whole anterior (costal)
VFp	V	Forewing	Proximal (basal) and anterior part
VFd	V	Forewing	Distal, anterior part
VHT	V	Hindwing	Whole hindwing
VHp	V	Hindwing	Proximal area
VHd	V	Hindwing	Distal area
VB	V	Body	Ventral area, pin and eyes excluded
V(Tp + B)	D + V	Combined	Ventral, proximal wing areas and body
VT	V	Combined	Ventral reflectance of the whole insect (VFT, VHT and VB measured together)
VTp	V	Both wings	Proximal forewing and hindwing areas (VFp, VHp)
VTd	V	Both wings	Distal forewing and hindwing areas (VFd, VHd)
Mean	D + V	Combined	Overall mean (average of DT and VT)
p_Mean	D + V	Both wings	Average of all the proximal wing areas
p_Optimum	D + V	Combined	Weighted average of the proximal wing areas and body, with the dorsal values for dorsal basking species and the ventral ones for lateral basking species

Note: Except for FWL (wing length) the data represent total reflectance (RGB, visible light spectrum) of the parts involved taken dorsally or ventrally from the wings (forewing, hindwing or both wings), the body or the wings and the body (combined). Any combinations of single measurements represent the weighted average of them, based on their known areas.

Abbreviations: B/F/H, body, forewing, hindwing; D/V, dorsal, ventral; p/d/T, proximal, distal, total.

addition to seven measurements done on the reference paths of the colour card. Ten measurements represented the 'primary' zones (i.e., those exposed to the sun during basking by a living insect, such as the proximal [basal] or distal surfaces of each wing [Figure 1c,d, see details and abbreviations in next paragraph], while the remaining ones represent combinations of them [e.g., the total fore or hind wing reflectance]). Such combinations are weighted averages based on the relative surfaces of the parts involved. The delimitation of the primary areas implies a degree of oversimplification because the exact posture may vary across species as well as in the same individual according to varied circumstances. The basal part of the wing was set to the 25% portion of the maximum wing length, and the sections exposed/ hidden during basking were estimated as 50% of the wing width by reference to the line perpendicular to the maximum wing length.

We used a combination of letters to designate the wing and body areas measured, where: D/V = dorsal/ventral; B/F/H = body, forewing, hindwing; and p/d/T = proximal, distal, total (e.g., DFT is the total reflectance of the exposed path of the forewing, equivalent to DFp plus DFd). See Figure 1 for reference. The list of measurements and abbreviations is presented in Table 1.

The reflectance in the visible light spectrum was measured on the photographed specimens with *ImageJ* (Schneider et al., 2012). The reflectance values were then corrected by regression of the seven observed RGB values from the reference colour card on the expected ones. Among several curvilinear models tested (using *CurveExpert Basic*: Hyams, 2009), a third-degree polynomial rendered the best results ($r > 0.998$ in all instances). Linear re-scaling was then applied, ensuring that the observed grey tones were virtually identical to the

BUTTERFLY REFLECTANCE, BASKING BEHAVIOUR AND CLIMATE

Ecological
Entomology

Royal
Entomological
Society

5

reference ones. The same correction was then applied to the wing and body values in each digital image.

Climate and basking behaviour

The mean temperature and precipitation values of the species were based on the species geographic distributions in two geographic contexts (Iberia, Europe) at two geographic scales: the 50×50 km MGRS grid (Europe and the Iberian Peninsula) and the 10×10 km MGRS grid (for the Iberian data). The European species mean temperature and mean precipitation data were gathered from Schweiger et al. (2014). Although comparable data from Platania et al. (2020) may be more precise for the members of some pairs of closely related species isolated by taxonomists in recent years, we retained the information in Schweiger et al. (2014) to facilitate comparison with other studies having used the same source of European climate data (such as Kang et al., 2021a, 2021b). The Iberian climate data came from WorldClim version 2.1 (Fick & Hijmans, 2017, resolution ca. 1 km^2, 1970–2000). The WorldClim values were averaged for the 50×50 km and the 10×10 km Iberian cells. On this basis, the species mean temperature and precipitation were calculated as the average value from the subset of cells occupied by each species. We used the pooled presence data for the period 1900–2020 (updated from García-Barros et al., 2004: www.geobrink.uclm.es), even when this period is longer than that covered by WorldClim data. We believe that this approach is reasonable as far as the detailed distribution data on the Iberian butterflies still requires improvement (García-Barros et al., 2022) and most of the information dates after 1970 (the mean date of the records is 1989). Our estimates largely coincide with those recently used by Mingarro et al. (2021) to estimate the butterfly community temperature and precipitation indexes for the same area (the correlation between both sources is $r = 0.973$ for temperature and $r = 0.892$ for precipitation, $p < 0.001$ in both instances).

The typical basking postures of the species involved were obtained from Middleton-Welling et al. (2020): dorsal, reflection and lateral basking, with the first two recoded as dorsal (since in both cases the light falls on the dorsum).

Butterfly shade and demographic or altitudinal change

We tested the bivariate correlations between each of the reflectance variables (as well as the basking mode and adult size, represented by forewing length) with known published altitudinal or demographic trends. Forward stepwise regression was applied when more than one significant effect was detected. Additionally, we built a composite reflectance index representing the best statistical explanation of temperature in terms of the reflectance variables (based on the 10-km resolution Iberian data since, as detailed below, this grid size resulted in the highest correlations). To do this we estimated a model with temperature as the dependent variable and the reflectance values as predictors, fit by multiple regression (GLM approach, normal link

function, forward stepwise). The predicted values from this model (subsequently termed 'BestPredictor'; see Results) represent a linear, weighted combination of the reflectance variables with the strongest possible linear correlation with temperature. The trend data were gathered from four sources in the literature and include only those butterfly species from which a statistically significant trend has been reported and are represented in our data set. These sources were: (1) Twenty-three species with significant altitudinal shifts in the Sierra de Guadarrama (north of Madrid, Spain), from Wilson et al. (2005). These authors fit an altitudinal model to each species in two periods of time (decade of 1970 and 2000) to identify the temporal shifts in altitude. We quantified the shifts as the differences between each species altitude along that period, separately for the minimum and maximum elevations. (2) Sixty species with significant population trends (i.e., increasing or decreasing adult abundance) in Catalonia (NE Spain) over the last two decades, from the *Catalonian Butterfly Monitoring Scheme* (Stefanescu, 2021). These data were reported as a weighted density of adult numbers standardised for the number of counts and stations, the rBMS index (Schmucki et al., 2022). (3) The long-term demographic trends from 30 butterflies in the United Kingdom, from the comprehensive data in the UK Butterfly Monitoring Scheme as reported by Brereton et al. (2020); these data represent temporal trends of adult densities expressed as an index, which we used after squared-root transformation. Finally, (4) the significant trends of adult abundance from 12 species as measured by the *European Butterfly Indicator for Grassland Species* (van Swaay et al., 2019 and methodological details therein). These are comparable to those of the two previous sources (2, 3 above) although they focus specifically on grassland species and are presented as qualitative descriptors (stable, moderate decline, strong decline).

Statistical procedures

The data were log$_{10}$-transformed in the first step to approach normality. Standard (least squares, OLS) regression, analysis of the variance (ANOVA) and analysis of the covariance (ANCOVA) without controlling for phylogenetic relatedness were carried out and cross-checked with SPSS IBM Statistics (IBM, 2019) and Statistica (StatSoft, 2004). ANCOVA was specifically applied to test whether our hypothesised optimum combination of exposed areas had the strongest relation with temperature depending on the species basking behaviour, that is, DB + DTp for dorsal baskers and VB + VTp for lateral baskers.

In contrast to analyses with a merely predictive purpose, evolutionary approaches (namely, to determine the ultimate nature of the climate–reflectance relationship) require a phylogenetic framework (Freckleton et al., 2002; Harvey & Pagel, 1991; Revell, 2010). We calculated the pertinent regression and correlation analyses under Phylogenetic Generalized Least Squares models (PGLS) with *caper* (Orme et al., 2022) under the maximum likelihood estimate for lambda, and ANCOVA with *multcomp* (Hothorn et al., 2020) in the R environment (R Core Team, 2020). The tree topology and ultrametric branch lengths were taken from the European-level butterfly phylogeny by Wiemers et al. (2020). The

phylogenetic signal in the data was measured as Pagel's lambda (Pagel, 1999) as recommended by several authors (Freckleton et al., 2002; Molina-Venegas & Rodríguez, 2017; Münkemüller et al., 2012), with the package *phylosignal* (Keck et al., 2016).

Moreover, decoupling the effect of the 'pure' ecological, 'pure' phylogenetic and shared (ecological + phylogenetic) components of the reflectance–climate relationships and their shared effect (the last probably representing phylogenetic conservatism: Desdevises et al., 2003; Diniz-Filho et al., 2012) we applied the method of performed variance partitioning (Legendre & Legendre, 2012) to four representative reflectance variables (DHp, VHp, BestPredictor and Mean) and the Iberian temperature and precipitation data at 10×10 km resolution. We followed the procedure described by Desdevises et al. (2003) and Legendre and Legendre (2012) using the R packages *wcmdscale* and *varpart* (in *vegan*: Oksanen et al., 2022). We submitted the phylogenetic distance matrix to a principal coordinate analysis (PCoA). The generated eigenvectors were then selected by forward stepwise regression with each of the analysed reflectance variables as the problem variable. The set of eigenvectors selected was used to represent phylogeny in the variance partitioning analysis.

The relationships between butterfly trends from the literature and temperature were tested using Pearson's correlation except for the Grassland Species Indicator species where a rank correlation (Kendall's Tau) was applied. For the reasons given above, no correction for phylogenetic effects was applied in this section. We evaluated the bivariate relationship between temperature (Iberian data, 10-km resolution) and our 'BestPredictor' described in the former section, but also tested the remaining reflectance variables. When this was justified, multiple regression was attempted.

RESULTS

There was a clear co-variation of reflectance values within the ventral and dorsal subsets, much more marked for the ventral shades (VFp VFd, VHp, VHd, VB) than for the dorsal ones (Figure 2; Table S3). This pattern was exacerbated in the PGLS results, where the similarity among the ventral values was comparatively tighter while the

opposite was observed in the dorsal (D) values. Within each subset, the body values (DB, VB) remained the most independent.

For the 10 primary reflectance variables, the ventral aspect was brighter than the dorsal one (i.e., DFp, DFd, DHp, DHd and DB vs. VFp, VFd, VHp, VHd and Vb; after pairwise tests = 16.0 > T > 4.9, $p < 0.001$). The proximal wing sections were darker than the distal ones, although significantly so only for the hindwing (DHp < DHd, $T = 2.60$, $p < 0.01$; VHp < VHd, $T = 5.17$, $p < 0.001$). This was
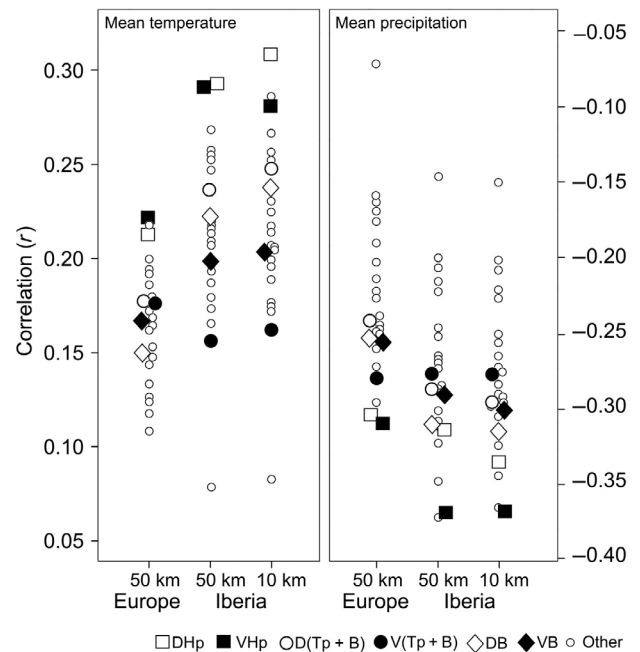
FIGURE 3 Graphical summary of the correlations between the shade measurements (primary variables and weighted averages described in the text) and the mean temperature and precipitation from the same subset of species and climatic data from Europe (50×50 km), and Iberia (50×50 km and 10×10 km resolutions) to show the general increase of correlations that accompanies the increase in geographic resolution. Six variables of special interest for the discussion have been highlighted with specific symbols (full details in Table S5).
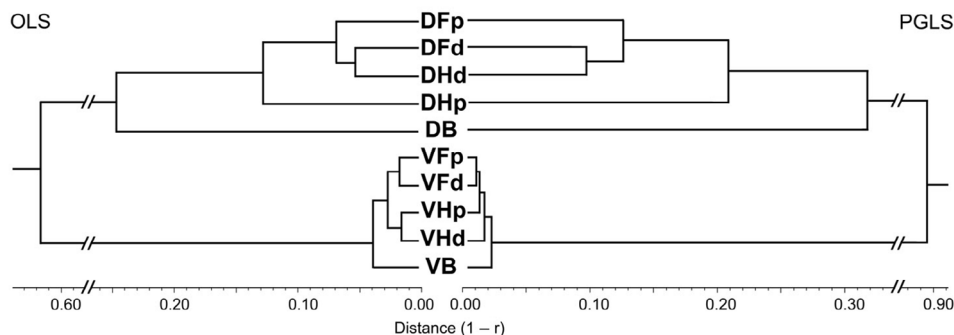
FIGURE 2 Distance among the basic measurements of reflectance in the wings and body, based on the correlation matrix (1 − Pearson *r*) and UPGM joining algorithm. Abbreviations as in Figure 1, see text. OLS, Ordinary Least Squares regression; PGLS, Phylogenetic Generalized Least Squares models.

BUTTERFLY REFLECTANCE, BASKING BEHAVIOUR AND CLIMATE

Ecological
Entomology

Royal
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Society

7

confirmed by multiple ANOVA and applied within the dorsal and lateral basking species (Table S4).

Under Ordinary Least Squares (OLS) regression statistics all the reflectance values (Figures 3 and 4, full details in Table S5) were positively correlated with temperature and negatively with precipitation, most of the time significantly (142 out of 150 scores) although with low r values (absolute values ranked between 0.11 and 0.37 except for the non-significant cases). As shown in Figure 3 the correlations of reflectance with the climatic variables increased moving to finer resolutions: Europe 50 km–Iberia 50 km–Iberia 10 km, with precipitation showing stronger effects than temperature. The best bivariate correlates of temperature were VHp (the ventral, proximal area of the hindwing) and DHp (the dorsal, proximal area of the hindwing) (Figure 4a,b). From that point of view none of the combined estimates of reflectance outperformed the primary measures mentioned above, either from the wings (DTp, DTd, HTp, HTd, p_Mean, p_Optimum) or from the wings and body (DT, VD, Mean); the same was true for our presumed best estimates: D(Tp + B) and V(Tp + B) (Figure 4c,d).

PGLS correlations largely followed those stated above, although with comparatively lower values overall (on average, 73% of the OLS r values) except for the ventral proximal wing reflectances (VFp, VFd,

TABLE 2 Summary of the final models fitted by multiple regression to select the best subset of reflectance variables as predictors for the mean Temperature and mean Precipitation (Iberian data, 10 × 10 km resolution), with phylogenetic effects accounted for Phylogenetic Generalized Least Squares (PGLS) or not Ordinary Least Squares (OLS).

Parameter	Temperature, OLS	Temperature, PGLS	Precipitation, OLS	Precipitation, PGLS
Intercept	−0.406***	0.608**	1.310***	3.596***
DHp	0.274***	0.254**	−1.112***	−0.325***
VHp	1.056***	0.990**	−0.141***	−0.416***
VHd	−1.089***	−1.005***	–	–
VFp	–	–	−0.141***	0.359**
Model statistics				
AIC	−312.513	−299.246	−427.867	−268.944
R^2_{adj}	0.204***	0.151***	0.202***	0.194***
$F_{3,218}$	19.875***	14.120***	16.696***	11.68***

Note: The upper part of the table shows the coefficients for the intercept and the variables selected; the lower three lines summarise the model fit statistics. –, variable not selected for the model.
Abbreviation: AIC, Akaike Information Criterion.
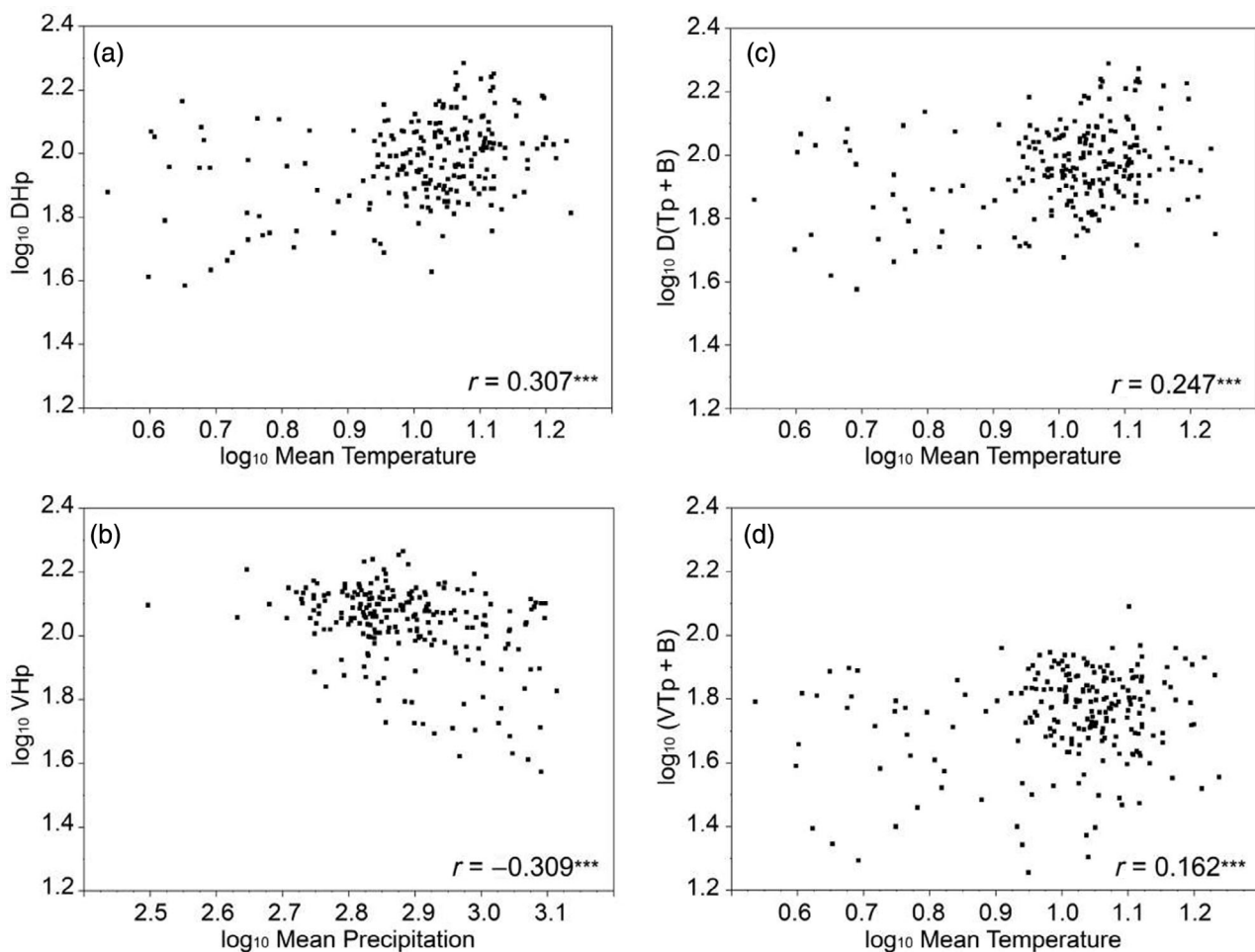***$p < 0.001$; **$p < 0.01$.

FIGURE 4 Scatter plots showing the relationships between the mean temperature and precipitation and single measures of primary reflectance, DHp and VHp (a, b) and with two combined estimates expected to show a good correlation with temperature based on adult basking behaviour (c, d). The correlation values (r) and significance are shown. $P<0.001$.

TABLE 3 Summary of the results of four individual analyses of the covariance (columns) to identify the effects of temperature, basking mode and their interaction, on four measurements of reflectance in the proximal areas of the wing (dorsal and ventral, forewing and hindwing).

Wing	Forewing	Hindwing	Forewing	Hindwing
OLS				
Wing side	Dorsal	Dorsal	Ventral	Ventral
Dependent variable	DFp	DHp	VFp	VHp
Intercept	1.748***	1.819***	1.890***	1.763***
Temperature (Iberia, 10 km)	0.236***	0.316***	0.182**	0.272***
Basking mode	0.016^{ns}	0.143^{a,}**	0.008^{ns}	0.014^{ns}
Basking mode × Temperature	0.084^{ns}	0.004^{ns}	0.079^{ns}	0.022^{ns}
Model $F_{3,218}$	5.340**	12.946***	3.534*	8.231***
PGLS				
Wing side	Dorsal	Dorsal	Ventral	Ventral
Dependent variable	DFp	DHp	VFp	VHp
Intercept	1.818***	1.883***	1.992***	1.848***
Temperature (Iberia, 10 km)	0.090*	0.153***	0.087^{ns}	0.119^{ns}
Basking mode	0.057^{ns}	0.076^{ns}	0.010^{ns}	0.005^{ns}
Basking mode × Temperature	0.121^{ns}	0.048^{ns}	0.014^{ns}	0.087^{ns}
Model $F_{3,218}$	4.055*	6.171***	0.279^{ns}	1.237^{ns}

Note: The analyses were done on the raw, log-transformed data (Ordinary Least Squares [OLS], above; further details in Table S4) and controlling for phylogenetic effects (Phylogenetic Generalized Least Squares [PGLS], below). No significant interactions between temperature and basking behaviour were identified.

[a]Dorsal and reflection baskers have significantly darker proximal, dorsal wing surfaces.

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; $^{ns}p > 0.05$.

VHp, VHd), which were higher than the least squares ones (between 1% and 3%) (Table S6).

The best OLS explanatory model for temperature (Iberian data, 10 × 10 km resolution) from stepwise multiple regression included the variables DHp, VHp (with positive coefficients) and VHd (with negative sign) (Table 2). Although the negative value of VHd suggested a complementary effect of VHp and VHd (e.g., the contrast between the two parts of the hindwing underside), this was not confirmed by testing the ratio VHd/VHp (as $\log_{10}$VHd − $\log_{10}$-VHp) in the regression, which rendered a slightly lower fit ($R^2_{adj} = 0.208$, $p < 0.001$, AIC = 313.539, cf. Table 2). The predicted values from the model selected were retained and used (as 'BestPredictor') to test the relationships between butterfly reflectance and the demographic or altitudinal trends reported.

Even though we found that the proximal wing areas included the best correlates of temperature, the ANCOVAs on these variables (Table 3) did not identify any significant interactions between the basking mode and temperature. Moreover, the basking mode had no

significant effects except for DHp (where the significance shifted to marginal under the PGLS approach). Incidentally, the basking posture was related to the Iberian temperature data (50 and 10-km resolution, only after OLS results) suggesting higher proportions of dorsal basking species in the warmer squares (see Tables S5 and S6).
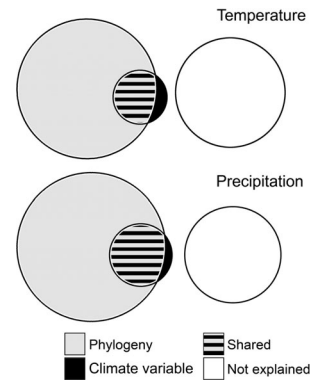

FIGURE 5 Unique ('pure') and shared contributions of phylogeny and the climate variables (temperature, above; precipitation, below) to explain the variation of VHp (the reflectance of the proximal, ventral portion of the hindwing) in terms of r^2 from partitioning of the variation (variance partitioning) analysis. The black area in each graph represents the 'pure' contribution of the climate variable. See Table S8 for detailed statistics.

TABLE 4 Comparison of the explanations for temperature and precipitation (Pearson *r*, Iberian data 10 × 10 km resolution) provided by our data (a) and the measurements (UV + VIS) by Kang et al. (2021b) (b) for six variables and 199 species comparable in both data sets.

	Temperature		Precipitation		
	(a) This study	(b) Kang et al.	(a) This study	(b) Kang et al.	r (a − b)
DB (R)	**0.334*****	0.255***	**−0.401*****	−0.360***	0.649***
DT (R)	**0.196****	0.174*	−0.249***	**−0.285*****	0.792***
DTp (R)	**0.290*****	0.223**	**−0.358*****	−0.286***	0.728***
VB (R)	0.232**	**0.303*****	−0.305***	**−0.340*****	0.750***
VT (R)	**0.226****	0.181*	**−0.315*****	−0.293***	0.793***
VTp (R)	**0.298*****	0.284***	**−0.364*****	−0.360***	0.761***
Best model (R^2)	**0.107*****	0.087***	**0.156*****	0.145***	–
Best model (vars.)	~(+DB)	~(+VB)	~(−DB)	~(−DB + VTp)	–

Note: The two lower rows summarise the best explanation of the climate variable (temperature or precipitation) in terms of the reflectance variables (from multivariate forward stepwise selection regression): adjusted R^2 and variables selected. The highest correlation in each pair compared (a − b) is marked in bold. The correlations between the reflectance estimates from the variables selected in the two studies are shown in the right column. Significance follows the notation.

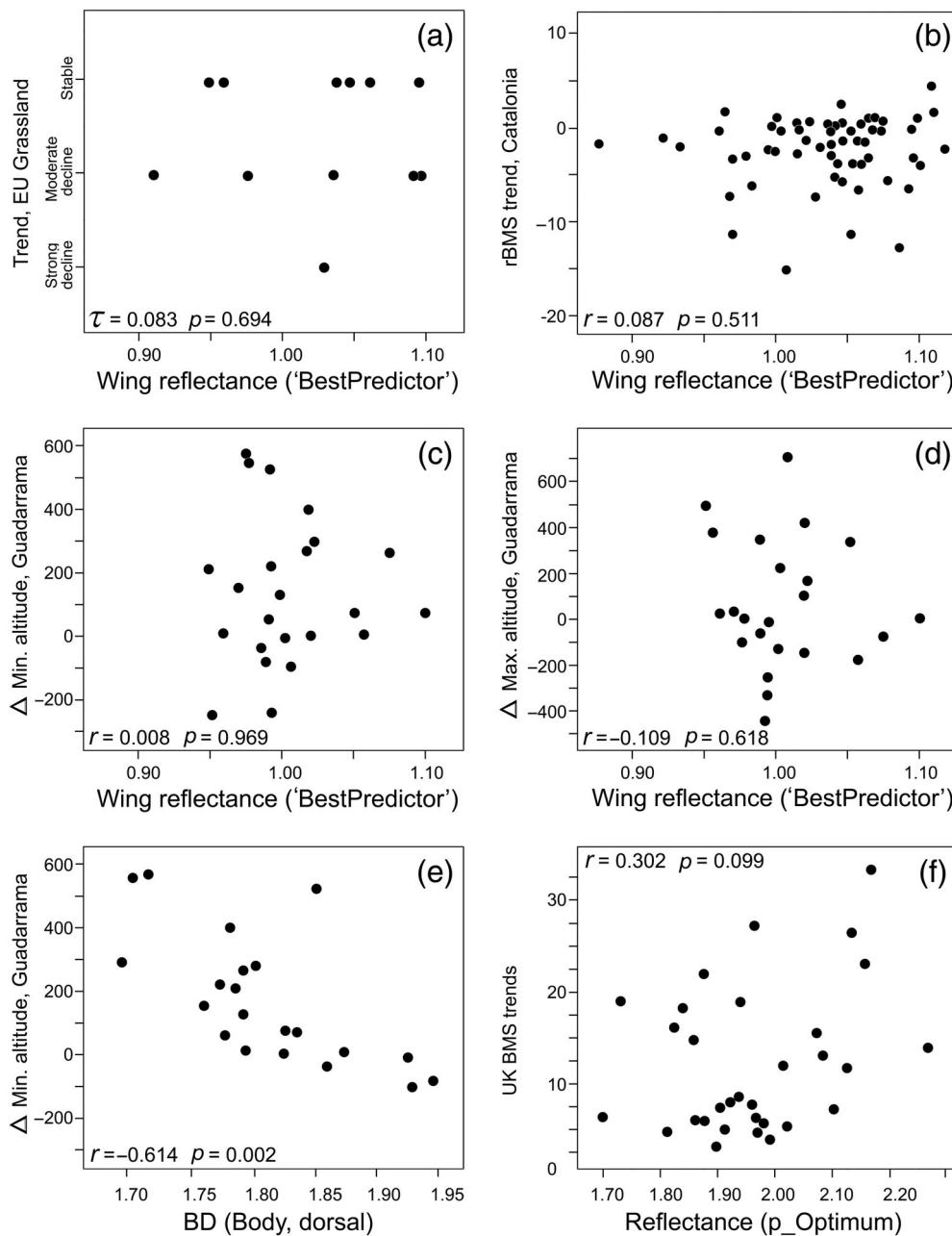*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

FIGURE 6 Dispersion graphs showing the relationship between butterfly reflectance and species significant changes in altitude or abundance from selected sources: (a) species in the Grassland Indicator Index (Europe); (b) significant population trends in Catalonia; shifts in minimum (c, e) and maximum altitude (d) in the Sierra de Guadarrama and trends detected from the UK Butterfly Monitoring Scheme (f). (a)–(d) are based in the 'BestPredictor' index of reflectance (see text) while (e) and (f) show examples of a significant and a nearly significant relationship based in other reflectance measures. r = Pearson's correlation coefficient, τ = Kendall's Tau for rank correlation. Data sources specified in the text, and full correlation matrix in Table S9.

The reflectance variables showed significant lambda values (0.67–0.9, all $p < 0.001$; Table S7), indicating a meaningful phylogenetic structuring. In contrast, the phylogenetic signal was non-significant or low (0.10–0.17) for the climatic variables. The results of variance partitioning for VHp (a comparatively good correlate of temperature) in terms of climate and phylogeny are illustrated in Figure 5 (further variables are analysed in the same way in Table S8). In short, climatic variables per se had very small contributions to the

explanation of reflectance (0.3%–3% of the variance). The largest part of the variation was explained by phylogeny alone (50%–77%), followed by the shared effects of phylogeny and climate (4%–18%).

To better assess the relative performance of our measurements compared to those previously published, we used the data provided by Kang et al. (2021b). This was possible for 199 species and for six measurements that were approximately equivalent in both data sets: DB and VB from the body; DT, DTp, VT and VTp from the body and

wings combined (Figure 1). We also estimated the correlations between these measurements and the mean temperature and precipitation (10 × 10 km Iberian data), the best model from forward stepwise selection for these two climate variables and the paired correlations between the two subsets of data.

As detailed in Table 4, the reflectance values of the two sources were correlated, our values often providing the best correlation with the climate variable (except for VB—temperature and DT/VB—precipitation).

We calculated the correlations between reflectance and the published abundance or altitudinal species trends using the 'BestPredictor' scores (the predicted values from the best fit model described above). These values represent the best possible linear combination of the reflectance values as a single correlate of temperature. The correlations were low, in all instances non-significant ($r < 0.11$, $p > 0.05$, see Figure 6 and Table S9). One strong outlier was omitted from the Catalonian data, the *Araschnia levana* (L., 1758) (Lepidoptera: Nymphalidae). Including this species had no relevant effect ($r = 0.15$, $p = 0.268$). The result for the British butterfly trends was $r = 0.075$ ($p = 0.693$), again with no significant differences when one outlying species was included ($r = 0.283$, $p = 0.123$); this was the large blue, *Phengaris arion* (L., 1758) (Lepidoptera: Lycaenidae), a butterfly subject to specific management after its extinction and subsequent reintroduction decades ago (Thomas et al., 2009). Testing the remaining reflectance variables (primary or composed; full details in Table S9) rendered similar, non-significant results with one exception: the shifts in the minimum altitude of the Sierra de Guadarrama butterflies (1970s–2000, from Wilson et al., 2005; Figure 6e) were negatively correlated with DHp ($r = -0.42$, $p < 0.05$) and BD ($r = -0.61$, $p < 0.01$), while for these data the basking type had a significant effect ($F = 5.60$, $p < 0.05$; Table S9). Given the inter-correlation between these variables, a multivariate stepwise selection approach (details not presented) retained only BD. This implies that changes in the minimum elevational range were consistent with the expectations, that is, more pronounced upward shifts in butterflies with darker dorsal body scale cover (BD) and darker dorsal, hindwing base (DHp) as well as with dorsal basking posture. A marginally significant correlation between the composite p_Optimum and the UK BMS trends was also detected (Figure 6f).

DISCUSSION

Regardless of the approach used, our results demonstrate clear patterns of co-variation involving the dorsal and ventral subsets of reflectance measurements, with higher consistency from the ventral group. Body reflectance values remained relatively independent in each of the two groups. We suspect that, although reflectance is generally well preserved in collection specimens (Munro et al., 2019), a loss of body cover scales is to be expected from the process of manipulation and mounting, thus increasing the exposure of the dark pigmented cuticle. Also, the ventral aspect of the body (as recorded in the photographs) does not exactly correspond to the lateral (pleural) area which the butterfly exposes to the sun. We accept that our sample sizes are short, and that a reliable reflectance index should provide better coverage of the colour

variation present in every butterfly species. However, the number of individuals per species used here compares favourably with those used in recent similar studies (Kang et al., 2021a; Stelbrink et al., 2019; Zeuss et al., 2014). We acknowledge the primarily prospective nature of our study, which should help to identify the best reference body parts to be used in subsequent research on this subject.

Both after OLS or PGLS regression, our results confirmed those reported by Stelbrink et al. (2019), Kang et al. (2021a) and Zeuss et al. (2014), with reflectance being positively correlated with temperature and negatively with precipitation (i.e., lighter butterflies in warm, dry habitats and dark ones in cool, moist ones). Irrespective of the method, the hind wing surface provides the best correlates of temperature. Moreover, the magnitude of the correlations between shading and climate varied in the way expected with higher scores in the most local geographic context (Iberia vs. Europe), at the highest geographic resolution (10 vs. 50-km grid) and by reference to the specific parts of the wings exposed to the sun by the adults (instead of, e.g., the whole basal area of the wing). This strongly supports a causal link between butterfly colour and the species climatic niche at the mid-to-low scale, where temperature or precipitation plays a role. In short, our results are consistent with Bogert's pattern as stated by previous authors; Gloger's pattern (brighter pigmentation in warm and moist habitats), partly supported by Kang et al. (2021a), is difficult to test in the south European context where, as a rule, temperature and precipitation are negatively correlated.

As a first conclusion, our interpretation is in favour of Bogert's pattern, but: is adult heating behaviour the adaptive explanation? Apparently not, judging from the RGB values of the visible spectrum. Our hypothetical 'best' combinations of measurements for the dorsal (dorsal and reflection) and lateral basking species rendered suboptimal statistical explanations of the climate variables. No significant interactions between brightness and the adult basking mode were detected by ANCOVAs with temperature as the problem variable (and reflection and basking mode as the independent ones). This is consistent with the fact that interspecific analyses on moths (Heidrich et al., 2018; Xing et al., 2018), where sun basking is not the rule, also showed the trend between the dark-light colour gradient and the cool-warm environment. What matters here is the strong phylogenetic pattern of the reflectance measurements (as documented by the lambda values) combined with variance partitioning results that show an almost residual 'pure' explanation by temperature or precipitation (adaptation in a strict sense), implying a moderate combined explanation of phylogeny and climate (phylogenetic inertia, e.g., Freckleton et al., 2002), and a strictly dominating phylogenetic component which might represent niche conservatism (Blomberg et al., 2003; but cf. Münkemüller et al., 2015 and Revell et al., 2008). This allows for the possibility that any alternative set of phylogenetically structured life history traits may provide alternative explanations for butterfly brightness patterns, including the preference for habitat features such as, for example, forest or open habitat types (Endler, 1993; Xing et al., 2016; see also Roulin, 2014). As stated by Munro et al. (2019) varied selective forces may have modelled colour in a day-living, flying insect (see review by Cuthill et al., 2017). Even if the observed patterns fit the expectations for warming behaviour (Ellers & Boggs, 2004; Kammer & Bracchi, 1973;

BUTTERFLY REFLECTANCE, BASKING BEHAVIOUR AND CLIMATE

Ecological
Entomology

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Entomological
Society

11

Wasserthal, 1983), we may even speculate that such co-variation is compatible with a non-adaptive (mechanistic) explanation. For instance, the ventral surfaces of butterflies were on average more reflective than the corresponding dorsal ones while, in each wing, the proximal areas were comparatively darker implying a generalised proximal-distal gradient of pigment deposition (of the kind of the 'global determinant patterns': Nijhout, 1990) along the basal-central-marginal wing areas (Nijhout, 1991 and references therein).

Finally, within the limits imposed by the limited quantitative evidence on butterfly demographic trends and altitudinal shifts tested here (Brereton et al., 2020; Stefanescu, 2021; van Swaay et al., 2019; Wilson et al., 2005), there was no correlation between the selected documented changes and the best correlate of temperature from combined reflectance measurements, although two of the reflectance measurements varied consistently with the shifts in the minimum altitude in the Sierra de Guadarrama (Wilson et al., 2005). In consequence, although range contractions related to the species climatic niches have been documented from the European butterflies and other insect taxa in the area (Bladon et al., 2020; Calosi et al., 2008; Engelhardt et al., 2022; G. M. Hill et al., 2021), there is only marginal support for a direct impact of warming on butterfly population changes via their wing and body reflectance (unlike for European dragonflies: Zeuss et al., 2014). Recent population changes for butterflies in Catalonia (NE in our study area) were best explained by the species habitat preferences (namely, for woodland or open habitats: Stefanescu et al., 2011) and phenological specialisation (Colom et al., 2022). Although such effects might be masked by other variables or may be buffered by microhabitat selection by the butterflies (Bladon et al., 2020), the low (although significant) correlations between climate variables and reflectance, together with the remaining results discussed above, tend not to support butterfly reflectance by itself as a relevant ecological predictor in these insects.

Thus, we may conclude that (1) without report to phylogeny, the relationship between reflectance (in the visible light spectrum) and climate (positive correlation with temperature, negative with precipitation, compatible with Bogert's pattern) is supported, and that (2) this relationship is strengthened at closer geographic distances and when focused on the 'target' parts of the insect's body (as expected from thermoregulatory behaviour), suggesting a habitat-level interaction. This remains to be tested for the whole of European butterflies, where distribution data at a finer scale than 10×10 km are likely to be available. After all, a 100-km^2 area may cover varied temperature and humidity conditions with ample chances for the butterflies, as flying insects, to select for their suitable microhabitat conditions. (3) In contrast, we found no evidence that this relationship is mediated by the adult basking posture. We are aware that recent studies demonstrate that reflectance in the near-infrared spectrum (NIR) provides a better explanation for the climate–reflectance relationship (Kang et al., 2021a; Munro et al., 2019). However, besides the general evidence that the body and basal wing areas are more relevant to warming, a test of NIR values of the specific areas exposed by each type of basking mode remains to be done. (4) From an evolutionary point of view, the PGLS results confirm the former statements, with weaker

statistical support. The strong phylogenetic pattern of the reflectance and the low fraction of the reflectance measures analysed suggest that tests for alternative explanations are still needed to shed light on the meaning of the colour–environment relationships in butterflies, which probably are of a complex nature. And (5) perhaps in consistence with the moderate (although significant) correlations between reflectance and temperature, there is only marginal evidence for a relationship between reflectance and recent demographic or altitudinal changes documented in the recent literature. Although some positive evidence arose from the elevation shifts in the Sierra de Guadarrama, the fact that this applied only to two of the several reflectance variables (and not to those representing the best combinations as a response to temperature) suggest that these results, though interesting, might be artifactual and require confirmation. From this and the former statements, butterfly reflectance does not by itself represent a strong predictor for changes in population numbers, attributable to warming. It is likely that this can be re-assessed in the short term, for example, with more local climate data and long-term temporal series of individual counts such as those from monitoring schemes (e.g., the European Butterfly Monitoring Scheme: www.butterfly-monitoring.net).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in DRYAD at https://doi.org/10.5061/dryad.1g1jwsv0q.

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REFERENCES

Bladon, A., Lewis, M., Bladon, E., Buckton, S., Corbett, S., Ewing, S. et al. (2020) How butterflies keep their cool: physical and ecological traits influence thermoregulatory ability and population trends. *Journal of Animal Ecology*, 89(11), 2440–2450. Available from: https://doi.org/10.1111/1365-2656.13319

Blomberg, S.P., Garland, T.J. & Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57, 717–745. Available from: https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Bogert, C. (1949) Thermoregulation in reptiles, a factor in evolution. *Evolution*, 3(3), 195–211. Available from: https://doi.org/10.2307/2405558

Bolotov, I., Gofarov, M., Gorbach, V.V., Kolosova, Y.S., Zheludkova, A.A., Kondakov, A.V. et al. (2021) *Parnassius nebrodensis*: a threatened but neglected Apollo butterfly species from Southern Europe (Lepidoptera: Papilionidae). *Ecologica Montenegrina*, 40, 140–163. Available from: https://doi.org/10.37828/em.2021.40.13

Brereton, T.M., Botham, M.S., Middlebrook, I., Randle, Z., Noble, D., Harris, S. et al. (2020) *United Kingdom Butterfly Monitoring Scheme report for 2019*. UK Centre for Ecology & Hydrology, Butterfly Conservation, British Trust for Ornithology and Joint Nature Conservation Committee. Wareham, Dorset, United Kingdom. Available at: www.ukbms.org/reportsandpublications [Accessed 11th June 2022].

Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J. & Sears, M.W. (2010) Can mechanism inform species' distribution models? *Ecology Letters*, 13, 1041–1054. Available from: https://doi.org/10.1111/j.1461-0248.2010.01479.x

Calosi, P., Bilton, D.T. & Spicer, J.I. (2008) Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters*, 4(1), 99–102. Available from: https://doi.org/10.1098/rsbl.2007.0408

Clench, H.K. (1966) Behavioral thermoregulation in butterflies. *Ecology*, 47(6), 1021–1034. Available from: https://doi.org/10.2307/1935649

Colom, P., Ninyerola, M., Pons, X., Traveset, A. & Stefanescu, C. (2022) Phenological sensitivity and seasonal variability explain climate-driven trends in Mediterranean butterflies. *Proceedings of the Royal Society B: Biological Sciences*, 289(1973), 20220251. Available from: https://doi.org/10.1098/rspb.2022.0251

Cuthill, I.C., Allen, W.L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M.E. et al. (2017) The biology of color. *Science*, 357(6350), eaan0221.

Delhey, K. (2019) A review of Gloger's rule, an ecogeographical rule of colour: definitions, interpretations and evidence. *Biological Reviews*, 94(4), 1294–1316. Available from: https://doi.org/10.1111/brv.12503

Desdevises, Y., Legendre, P., Azouzi, L. & Morand, S. (2003) Quantifying phylogenetically structured environmental variation. *Evolution*, 57(11), 2647–2652. Available from: https://doi.org/10.1111/j.0014-3820.2003.tb01508.x

Dincǎ, V., Montagud, S., Talavera, G., Hernández-Roldán, J., Munguira, M.L., García-Barros, E. et al. (2015) DNA barcode reference library for Iberian butterflies enables a continental-scale preview of potential cryptic diversity. *Scientific Reports*, 5, 12395. Available from: https://doi.org/10.1038/srep12395

Diniz-Filho, J.A.F., Bini, L.M., Rangel, T.F., Morales-Castilla, I., Olalla-Tárraga, M.A., Rodríguez, M.A. et al. (2012) On the selection of phylogenetic eigenvectors for ecological analyses. *Ecography*, 35, 239–249. Available from: https://doi.org/10.1111/j.1600-0587.2011.06949.x

Ellers, J. & Boggs, C.L. (2004) Functional ecological implications of intra-specific differences in wing melanization in *Colias* butterflies. *Biological Journal of the Linnean Society*, 82(1), 79–87. Available from: https://doi.org/10.1111/j.1095-8312.2004.00319.x

Endler, J. (1993) The color of light in forests and its implications. *Ecological Monographs*, 63(1), 1–27. Available from: https://doi.org/10.2307/2937121

Engelhardt, E.K., Biber, M.F., Dolek, M., Fartmann, T., Hochkirch, A., Leidinger, J. et al. (2022) Consistent signals of a warming climate in occupancy changes of three insect taxa over 40 years in central Europe. *Global Change Biology*, 28, 3998–4012. Available from: https://doi.org/10.1111/gcb.16200

Fick, S.E. & Hijmans, R.J. (2017) WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302–4315. Available from: https://doi.org/10.1002/joc.5086

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. *The American Naturalist*, 160(6), 712–726. Available from: https://doi.org/10.1086/343873

García-Barros, E., Cancela, J.P., Lobo, J.M., Munguira, M.L. & Romo, H. (2022) Forecasts of butterfly future richness change in the southwest Mediterranean. The role of sampling effort and non-climatic variables. *Journal of Insect Conservation*, 26(3), 639–650. Available from: https://doi.org/10.1007/s10841-022-00406-2

García-Barros, E., Munguira, M., Stefanescu, C. & Vives, A. (2013) Lepidoptera: Papilionoidea. In: Ramos, M.A., Alba, J., Bellés, X., Gosálbez, J., Guerra, A., Macpherson, E. et al. (Eds.) *Fauna Iberica*, Vol. 37. Madrid: Museo Nacional de Ciencias Naturales-CSIC.

García-Barros, E., Munguira, M.L., Martín, J., Romo, H., Garcia-Pereira, P. & Maravalhas, E.S. (2004) Atlas de las mariposas diurnas de la Península Ibérica e islas Baleares (Lepidoptera: Papilionoidea & Hesperioidea). *Monografías SEA*, 11, 1–228.

Giorgi, F. & Lionello, P. (2008) Climate change projections for the Mediterranean region. *Global and Planetary Change*, 63, 90–104. Available from: https://doi.org/10.1016/j.gloplacha.2007.09.005

Harvey, P.H. & Pagel, M.D. (1991) *The comparative method in evolutionary biology*. Oxford: Oxford University Press.

Heidrich, L., Friess, N., Fiedler, K., Brändle, M., Hausmann, A., Brandl, R. et al. (2018) The dark side of Lepidoptera: colour lightness of geometrid moths decreases with increasing latitude. *Global Ecology and Biogeography*, 27(4), 407–416. Available from: https://doi.org/10.1111/geb.12703

Hernández-Roldán, J.L., Dapporto, L., Dincǎ, V., Vicente, J.C., Hornett, E.A., Šíchová, J. et al. (2016) Integrative analyses unveil speciation linked to host plant shift in *Spialia* butterflies. *Molecular Ecology*, 25, 4267–4284. Available from: https://doi.org/10.1111/mec.13756

Hertig, E. & Jacobeit, J. (2008) Downscaling future climate change: temperature scenarios for the Mediterranean area. *Global and Planetary Change*, 63, 127–131. Available from: https://doi.org/10.1016/j.gloplacha.2007.09.003

Hill, G.M., Kawahara, A.Y., Daniels, J.C., Bateman, C.C. & Scheffers, B.R. (2021) Climate change effects on animal ecology: butterflies and moths as a case study. *Biological Reviews*, 96(5), 2113–2126. Available from: https://doi.org/10.1111/brv.12746

Hill, L. & Taylor, H. (1933) Locusts in sunlight. *Nature*, 132(3329), 276. Available from: https://doi.org/10.1038/132276a0

Hinojosa, J., János, T., Monasterio, Y., Mesa, L., Sariot, M., Jiménez, R. et al. (2022) Integrative taxonomy reveals a new *Melitaea* (Lepidoptera: Nymphalidae) species widely distributed in the Iberian Peninsula. *Insect Systematics and Diversity*, 6, 1–9. Available from: https://doi.org/10.1093/isd/ixac004

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A. & Scheibe, S. (2020) *Package 'multcomp' (Simultaneous Inference in General Parametric Models)*. Available at: www.multcomp.R-forge.R-project.org

Huey, R.B. & Kingsolver, J.G. (2019) Climate warming, resource availability, and the metabolic meltdown of ectotherms. *The American Naturalist*, 194(6), E140–E150. Available from: https://doi.org/10.1086/705679

BUTTERFLY REFLECTANCE, BASKING BEHAVIOUR AND CLIMATE

Ecological
Entomology

Royal
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Society

13

Hyams, C. (2009) *CurveExpert Version 1.40. A curve fitting system for Windows*. Available at: www.curveexpert.net

IBM. (2019) *IBM SPSS statistics version 26.0. For windows*. Armonk, NY: IBM Corporation. Available at: www.ibm.com

Kammer, A.E. & Bracchi, J. (1973) Role of the wings in the absorption of radiant energy by a butterfly. *Comparative Biochemistry and Physiology Part A*, 45, 1057–1063. Available from: https://doi.org/10.1016/0300-9629(73)90342-3

Kang, C., Lim, S., Won Young, L., Choi, Y., Stuart-Fox, D. & Huertas, B. (2021a) Climate predicts both visible and near-infrared reflectance in butterflies. *Ecology Letters*, 24(9), 1869–1879. Available from: https://doi.org/10.1111/ele.13821

Kang, C., Lim, S., Won Young, L., Choi, Y., Stuart-Fox, D. & Huertas, B. (2021b) Data for butterfly near-infrared adaptation research. *Dryad*, Dataset. Available from: https://doi.org/10.5061/dryad.cjsxksn5j

Keck, F., Rimet, F., Bouchez, A. & Franc, A. (2016) phylosignal: an R package to measure, test, and explore the phylogenetic signal. *Ecology and Evolution*, 6(9), 2774–2780. Available from: https://doi.org/10.1002/ece3.2051

Kerr, J.T., Kulkarni, M. & Algar, A. (2011) Integrating theory and predictive modeling for conservation research. In: Drew, C.A., Wiersma, Y.F. & Huettmann, F. (Eds.) *Predictive species and habitat modeling in landscape ecology, 9, concepts and applications*. New York, NY: Springer, pp. 9–29. Available from: https://doi.org/10.1007/978-1-4419-7390-0_2

Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M. et al. (2015) Climate change on bumblebees converge across continents. *Science*, 349, 177–180. Available from: https://doi.org/10.1126/science.aaa7031

Kingsolver, J.G. (1987) Evolution and coadaptation of thermoregulatory behavior and wing pigmentation pattern in pierid butterflies. *Evolution*, 41(3), 472–490. Available from: https://doi.org/10.2307/2409250

Kingsolver, J.G. (1988) Thermoregulation, flight, and the evolution of wing pattern in pierid butterflies: the topography of adaptive landscapes. *American Zoologist*, 28, 899–912. Available from: https://doi.org/10.1093/icb/28.3.899

Kingsolver, J.G., Diamond, S.E. & Buckley, L.B. (2013) Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, 27(6), 1415–1423. Available from: https://doi.org/10.1111/1365-2435.12145

Legendre, P. & Legendre, L. (2012) *Numerical ecology*, 3rd edition, Amsterdam: Elsevier.

Lomolino, M.V., Riddle, B.R. & Whittaker, R.J. (2016) *Biogeography: biological diversity through space and time*, 5th edition, Sunderland, MA: Sinauer Associates.

Lovejoy, T.E. & Hannah, L. (Eds.). (2019) *Biodiversity and climate change: transforming the biosphere*. New Haven and London: Yale University Press.

Lukhtanov, V. & Pazhenkova, E. (2021) The Taxa of the *Hyponephele lycaon – H. lupina* species complex (Lepidoptera, Nymphalidae, Satyrinae): deep DNA barcode divergence despite morphological similarity. *Folia Biologica*, 69(1), 11–21. Available from: https://doi.org/10.3409/fb_69-1.02

Middleton-Welling, J., Dapporto, L., García-Barros, E., Wiemers, M., Nowicki, P., Plazio, E. et al. (2020) A new comprehensive trait database of European and Maghreb butterflies, Papilionoidea. *Scientific Data*, 7, 351. Available from: https://doi.org/10.1038/s41597-020-00697-7

Millien, V., Lyons, S.K., Oolson, L., Smith, F., Wilson, A. & Yom-Tov, Y. (2006) Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters*, 9(7), 853–869. Available from: https://doi.org/10.1111/j.1461-0248.2006.00928.x

Mingarro, M., Cancela, J.P., Burón-Ugarte, A., García-Barros, E., Munguira, M.L., Romo, H. et al. (2021) Butterfly communities track climatic variation over space but not time in the Iberian Peninsula. *Insect Conservation and Diversity*, 14(5), 647–660. Available from: https://doi.org/10.1111/icad.12498

Molina-Venegas, R. & Rodríguez, M.A. (2017) Revisiting phylogenetic signal; strong or negligible impacts of polytomies and branch length information? *BMC Evolutionary Biology*, 17, 53. Available from: https://doi.org/10.1186/s12862-017-0898-y

Münkemüller, T., Boucher, F.C., Thuiller, W. & Lavergne, S. (2015) Phylogenetic niche conservatism – common pitfalls and ways forward. *Functional Ecology*, 29(5), 627–639. Available from: https://doi.org/10.1111/1365-2435.12388

Münkemüller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffers, K. et al. (2012) How to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, 3(4), 743–756. Available from: https://doi.org/10.1111/j.2041-210X.2012.00196.x

Muñoz Sariot, M.G. & Sánchez Mesa, L. (2019) *Melitaea ornata pseudornata* nuevo nombre de reemplazo para *Melitaea ornata baetica* Muñoz Sariot & Sánchez Mesa, 2019 (Lepidoptera: Nymphalidae). *Arquivos Entomolóxicos*, 21, 83–84.

Munro, J., Medina, I., Walker, K., Moussali, A., Kearney, M., Dyer, A. et al. (2019) Climate is a strong predictor of near-infrared reflectance but a poor predictor of colour in butterflies. *Proceedings of the Royal Society B*, 286(1898), 20190234. Available from: https://doi.org/10.1098/rspb.2019.0234

Nijhout, H.F. (1990) A comprehensive model for colour pattern formation in butterflies. *Proceedings of the Royal Society of London B: Biological Sciences*, 239, 81–113. Available from: https://doi.org/10.1098/rspb.1990.000S9

Nijhout, H.F. (1991) *The development and evolution of butterfly wing patterns*. Washington, DC: Smithsonian Institution Press.

Oksanen, J., Simpson, G.L., Guillaume Blanchet, F., Kindt, R., Legendre, P., Minchin, P.R. et al. (2022) *vegan: Community ecology package*. Available at: www.CRAN.R-project.org/package=vegan

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. et al. (2022) *caper: comparative analyses of phylogenetics and evolution in R*. Available at: www.CRAN.R-project.org/package=caper

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884. Available from: https://doi.org/10.1038/44766

Pinkert, S., Brandl, R. & Zeuss, D. (2017) Colour lightness of dragonfly assemblages across North America and Europe. *Ecography*, 40(9), 1110–1117. Available from: https://doi.org/10.1111/ecog.02578

Platania, L., Menchetti, M., Dincă, V., Corbella, C., Kay-Lavelle, I., Vila, R. et al. (2020) Assigning occurrence data to cryptic taxa improves climatic niche assessments: biodecrypt, a new tool tested on European butterflies. *Global Ecology and Biogeography*, 29, 1852–1865. Available from: https://doi.org/10.1111/geb.13154

R Core Team. (2020) *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: www.r-project.org/index.html

Rapoport, E. (1969) Gloger's rule and pigmentation of Collembola. *Evolution*, 23, 622–626. Available from: https://doi.org/10.2307/2406857

Rensch, B. (1938) Some problems of geographical variation and species-formation. *Proceedings of the Linnean Society of London*, 150, 275–285. Available from: https://doi.org/10.1111/j.1095-8312.1938.tb00182k.x

Revell, L.J. (2010) Phylogenetic signal and linear regression on species data. *Methods in Ecology and Evolution*, 1, 319–329. Available from: https://doi.org/10.1111/j.2041-210X.2010.00044.x

Revell, L.J., Harmon, L.J. & Collar, D.C. (2008) Phylogenetic signal, evolutionary process, and rate. *Systematic Biology*, 57(4), 591–601. Available from: https://doi.org/10.1080/10635150802302427

Roitberg, B.D. & Mangel, M. (2016) Cold snaps, heatwaves, and arthropod growth. *Ecological Entomology*, 41(6), 653–659. Available from: https://doi.org/10.1111/een.12324

Roulin, A. (2014) Melanin-based colour polymorphism responding to climate change. *Global Change Biology*, 20(11), 3344–3350. Available from: https://doi.org/10.1111/gcb.12594

Sañudo-Restrepo, C.P., Dincă, V., Talavera, G. & Vila, R. (2013) Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). *Molecular Phylogenetics and Evolution*, 66(1), 369–379. Available from: https://doi.org/10.1016/j.ympev.2012.10.010

Schmucki, R., Harrower, C.A. & Dennis, E.B. (2022) *rbms: computing generalised abundance indices for butterfly monitoring count data*. R package version 1.1.3. Available at: https://github.com/RetoSchmucki/rbms

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675. Available from: https://doi.org/10.1038/nmeth.2089

Schweiger, O., Harpke, A., Wiemers, M. & Settele, J. (2014) CLIMBER: climatic niche characteristics of the butterflies in Europe. *Zookeys*, 367, 65–84. Available from: https://doi.org/10.3897/zookeys.367.6185

Settele, J., Kudrna, O., Harpke, A., Kühn, I., van Swaay, C., Verovnik, R. et al. (2008) Climate risk atlas of European butterflies. *BioRisk*, 1 (Special Issue), 1–710. Available from: https://doi.org/10.3897/biorisk.1

StatSoft, Inc. (2004) *STATISTICA, data analysis software system, version 7*. Tulsa, OK: StatSoft Inc.

Stefanescu, C. (2021) Resum de les temporades 2019 i 2020. *Cynthia*, 16, 10–13.

Stefanescu, C., Torre, I., Jubany, J. & Páramo, F. (2011) Recent trends in butterfly populations from north-east Spain and Andorra in the light of habitat and climate change. *Journal of Insect Conservation*, 15, 83–93. Available from: https://doi.org/10.1007/s10841-010-9325-z

Stelbrink, P., Pinkert, S., Brunzel, S., Kerr, J., Wheat, C.W., Brandl, R. et al. (2019) Colour lightness of butterfly assemblages across North America and Europe. *Scientific Reports*, 9(1), 1–10. Available from: https://doi.org/10.1038/s41598-018-36761-x

Thomas, J.A., Simcox, D.J. & Clarke, R.T. (2009) Successful conservation of a threatened *Maculinea* butterfly. *Science*, 325, 80–83. Available from: https://doi.org/10.1126/science.1175726

Trullas, S., Van Wyk, J. & Spotila, J. (2007) Thermal melanism in ectotherms. *Journal of Thermal Biology*, 32(5), 235–245. Available from: https://doi.org/10.1016/j.jtherbio.2007.01.013

Uyeda, J.C., Zenil-Ferguson, R. & Pennell, M.W. (2018) Rethinking phylogenetic comparative methods. *Systematic Biology*, 67(6), 1091–1109. Available from: https://doi.org/10.1093/sysbio/syy031

Van Swaay, C.A.M., Dennis, E.B., Schmucki, R., Sevilleja, C.G., Balalaikins, M., Botham, M. et al. (2019) *The EU butterfly indicator for Grassland species: 1990–2017: technical report*. Butterfly ConservationEurope & ABLE/eBMS, Wageningen (Netherlands) and Bangor, Gwynedd (United Kindom) p. 23. Available at: www.butterfly-monitoring.net

Vila, R., Stefanescu, C. & Sesma, J.M. (2018) *Guia de les papallones diürnes de Catalunya*. Bellaterra: Lynx edicions.

Warren, M.S., Maes, D., van Swaay, C.A.M., Goffart, P., Van Dyck, H., Bourn, N.A.D. et al. (2021) The decline of butterflies in Europe: problems, significance, and possible solutions. *PNAS*, 118(2), e2002551117. Available from: https://doi.org/10.1073/pnas.2002551117

Wasserthal, L.T. (1983) Haemolymph flows in the wings of pierid butterflies visualized by vital staining (Insecta, Lepidoptera). *Zoomorphology*, 103(3), 177–192. Available from: https://doi.org/10.1007/BF00310476

Wiemers, M., Balletto, E., Dincă, V., Fric, Z.F., Lamas, G., Lukhtanov, V. et al. (2018) An updated checklist of the 838 European Butterflies (Lepidoptera, Papilionoidea). *ZooKeys*, 811, 9–45.

Wiemers, M., Chazot, N., Wheat, C.W., Schweiger, O. & Wahlberg, N. (2020) A complete time-calibrated multi-gene phylogeny of the European butterflies. *ZooKeys*, 938, 97–124. Available from: https://doi.org/10.3897/zookeys.938.50878

Wilson, R.J., Gutiérrez, D., Gutiérrez, J., Martínez, D., Agudo, R. & Monserrat, V.J. (2005) Changes to the elevational limits and extent of species ranges associated with climate change. *Ecology Letters*, 8(11), 1138–1146. Available from: https://doi.org/10.1111/j.1461-0248.2005.00824.x

Xing, S., Bonebrake, T., Ashton, L.A., Kitching, R.L., Cao, M., Chee Ho, J. et al. (2018) Colors of night: climate-morphology relationships of geometrid moths along spatial gradients in southwestern China. *Oecologia*, 188, 537–546. Available from: https://doi.org/10.1007/s00442-018-4219-y

Xing, S., Bonebrake, T., Tang, C., Pickett, E., Cheng, W., Greenspan, S. et al. (2016) Cool habitats support darker and bigger butterflies in Australian tropical forests. *Ecology and Evolution*, 6, 8062–8074. Available from: https://doi.org/10.1002/ece3.2464

Zeuss, D., Brandl, R., Brändle, M., Rahbeck, C. & Brunzel, S. (2014) Global warming favours light-coloured insects in Europe. *Nature Communications*, 5, 3874. Available from: https://doi.org/10.1038/ncomms4874

Zhang, J., Brockmann, E., Cong, Q., Shen, J. & Grishin, N.V. (2020) A genomic perspective on the taxonomy of the subtribe Carcharodina (Lepidoptera: Hesperiidae: Carcharodini). *Zootaxa*, 4748(1):182–194. Available from: https://doi.org/10.11646/zootaxa.4748.1.10

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Sample photograph showing one specimen set as detailed in the text (female of *Argynnis pandora*).

Table S2. List of species: species binomial, sample sizes and notes on nomenclature.

Table S3. Matrix of correlations between reflectance measurements, Ordinary Least Squares and Phylogenetic Generalized Least Squares values.

Table S4. Summary of the main differences between reflectance values (log transformed) according to the anatomical position and adult basking type, ANOVA results.

Table S5. Bivariate correlations between reflectance and climate, without correction for phylogenetic relations (Ordinary Least Squares).

Table S6. Bivariate correlations between reflectance and climate, from Phylogenetic Generalized Least Squares analyses.

Table S7. Lambda values (phylogenetic signal) for the reflectance and the climate variables.

Table S8. Results of variance partitioning for the explanatory power of the four selected measures of reflectance (DHp, VHp, BestPredictor and Mean) on Temperature and Precipitation.

Table S9. Relationships between recent altitudinal or demographic trends from four published sources and the reflectance variables plus forewing length and adult basking mode.