Ecological Entomology

Ecological Entomology (2019), **44**, 389–396 DOI: 10.1111/een.12714

Takeoff temperatures in *Melitaea cinxia* butterflies from latitudinal and elevational range limits: a potential adaptation to solar irradiance

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Abstract. 1. This study provides evidence that a heliophilic butterfly, the Glanville fritillary (*Melitaea cinxia*) has adapted differently to environmental variation across latitudes and elevations.

2. In cool air, basking *M. cinxia* orient themselves perpendicular to the sun's rays to gain heat and take off. During flight, solar heating is reduced because orientation perpendicular to the sun is no longer possible and convective cooling occurs. Consequently, *M. cinxia* have been shown to suffer net heat loss in flight, even in full sunshine. When flight duration is restricted in this way, the takeoff temperature becomes an important thermal adaptation.

3. Using a thermal imaging camera, takeoff temperatures were measured in experimental butterflies. Butterflies from the northern range limit in Finland took flight at slightly hotter temperatures than butterflies from the southern limit in Spain, and much hotter than butterflies from the elevational limit (1900–2300 m) in the French Alps. Butterflies from low-elevation populations in southern France also took off much hotter than did the nearby Alpine population.

4. These results suggest that the influence of elevation is different from that of latitude in more respects than ambient temperature. Values of solar irradiance in the butterflies' flight season in each region show that insects from the coolest habitats, Finland and the Alps, experienced similar solar irradiance during basking, but that Finns experienced much lower irradiance in flight. This difference may have favored Finnish butterflies evolving higher takeoff temperatures than Alpine butterflies that also flew in cool air but benefited from more intense radiant energy after takeoff.

Key words. Butterfly, climate change adaptation, range limits, solar irradiance, takeoff temperature, thermal imaging, thoracic temperature.

Introduction

As projections of species' responses to climate change acquire greater urgency (Urban, 2015), they are also acquiring greater complexity. Nadeau *et al.* (2017) considered how spatial and temporal variability of the climate, both now and in the past, might influence both dispersal and thermal tolerance. Badik *et al.* (2015) examined how within-year variation in timing and intensity of precipitation predicted changes in species richness across an elevational transect. Other models derive predictions by combining experimental measurements of physiological responses with climate envelopes of current distributions (Kearney & Porter, 2009; Araujo *et al.*, 2013; Sunday *et al.*, 2014). Despite these increasingly sophisticated approaches to climate data, species distribution models (SDMs) still calculate each species' climate space from bioclimatic variables and use this information to predict latitudinal and elevational range shifts (e.g. Jueterbock *et al.*, 2016).

In some cases, regional temperature changes suffice to account quantitatively for observed range shifts (Parmesan, 1996; Crozier, 2004). In other examples, changes in precipitation are

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more important than changes in temperature; for example, they account for range shifts of North American trees, explaining general trends for westward shifts of angiosperms (Fei *et al.*, 2017). Where temperature is the most important factor, projections of shifts in range, abundance, or demography generally carry the implicit assumption that responses to changes of ambient temperature will be similar regardless of whether those changes are measured along latitudinal or elevational gradients (Parmesan, 1996; Devictor *et al.*, 2012). However, operating body temperatures of heliophilic poikilotherms are strongly affected by solar irradiance, which varies differently with elevation and latitude, as we shall illustrate. We might therefore expect that temperature data alone will fail to explain how thermal adaptations of these species vary across species' ranges, and that influences of elevation and latitude might differ.

Here, we begin to address this question by seeking local adaptation of an easily measured thermal adaptation, body temperature at spontaneous takeoff, in a heliophilic insect, the Glanville fritillary butterfly (*Melitaea cinxia*) sampled from its elevational and latitudinal range extremes.

The ability of heliophilic insects to regulate body temperature allows them to be active in places and at times from which they would otherwise be excluded (Heinrich, 1995), with the result that they can be found at higher altitudes and latitudes than might be expected of any thermophilic poikilotherm. In Sunday *et al.*'s (2014) meta-analysis of cold tolerance across all poikilotherms, studies of insects extended to higher latitudes (> 60°) than those of reptiles or amphibians. The Dingy (or improbable) fritillary (*Boloria improba*), for example, has its equatorial range limit in northern Finland and does not occur at low elevation where the Fennoscandian mainland meets the Arctic Ocean (Lafranchis, 2004). Even more improbably, five butterfly species in three different families were recorded as residents at Lake Hazen in Nunavut, northern Canada, at latitude 81.5°N (Kevan, 1972).

Thoracic temperatures of arctic-alpine and temperate zone butterflies must exceed a lower threshold for performance of controlled flight and are crucial for dispersal ability, predator avoidance, foraging, mate finding, fecundity and oviposition (Watt, 1968; Kingsolver & Watt, 1983; Kemp & Krockenberger, 2002; Berwaerts *et al.*, 2008; Velde *et al.*, 2011).When air temperatures are cool, both temperate zone and arctic-alpine butterflies cycle through periods of activity and inactivity: in sunshine, they bask, thermoregulate, and fly; when a cloud passes across the sun they alight and quickly become dormant. During flight in cool weather, small butterflies, including our study insects, lose heat even in full sunshine and must alight frequently to bask and rewarm (Mattila, 2015). In these conditions, takeoff temperature, the thoracic temperature at which individuals take flight in the absence of specific flight-inducing stimuli, such as a predator or competitor, must strongly affect the overall proportion of time spent flying. Hence, takeoff temperature is a likely target of natural selection associated with climatic variation.

Interspecific variation in takeoff temperature can be extreme. For example, Neve and Hall (2016) reported that the thoracic temperatures of Australian butterflies at spontaneous takeoff ranged from 13.4 to 46.3 °C. The variation observed among congeners is also substantial: *Colias* in Colorado flew at higher body temperatures than those in Alaska, whereas within Colorado, a low-elevation species flew at higher temperatures than its high-elevation congener (Kingsolver, 1983; Heinrich, 1993; MacLean *et al.*, 2016).

Melitaea cinxia is well known ecologically, behaviourally and genetically (Hanski, 2011). Previous studies of intraspecific variation in takeoff temperature of this species have examined plastic responses of Finnish insects to the environment and within-population differences among genotypes and between sexes (Saastamoinen & Hanski, 2008; Mattila, 2015). Here, we complement these studies by reporting takeoff temperatures at the elevational and latitudinal extremes of the species' range, with Finland included as the northern range limit.

Melitaea cinxia is non-migratory, with levels of gene flow and genetic variation that permit adaptation to local climatic conditions. Even within the relatively small area (*c*. 50 × 40 km) of the intensively studied Finnish metapopulation of *M. cinxia*, habitat patches varied in heat-shock protein and in phosphoglucose isomerase (Pgi) genotype. Hsp70 genotype was variable and associated with takeoff temperature, while Pgi variation interacted with temperature to affect flight metabolic rate, body temperature in flight, and dispersal likelihood (Niitepold *et al.*, 2009; Niitepold, 2010; Mattila, 2015).

On a larger scale, the most recent common ancestor of populations at the species' latitudinal range limits existed at least 500 000 generations ago (Wahlberg & Saccheri, 2007). Given that local adaptation to climate can apparently occur within the Åland Islands, we had a strong expectation that these range-limit populations will differ in traits that adapt them to local climate. This expectation was fulfilled: traits relevant to climate adaptation, such as the constitutive level of heat-shock protein Hsp21.4 (Advani *et al.*, 2016) and frequency of alleles affecting tracheal development and oxygen delivery (Marden *et al.*, 2013), do differ between populations at the species' latitudinal range limits.

Materials and methods

At low elevations in Europe, *M. cinxia* is distributed between approximately 41.8°N in Catalunya in northern Spain and 60.2°N in the Åland Islands in southern Finland (Lafranchis, 2004); it is also found at high elevations further south, as far south as the Atlas Mountains in Morocco. The elevational range of the butterfly at mid-latitude is from sea level to 2350 m in the Alps (Lafranchis, 2004), with occasional individuals at higher elevations.

Wild-caught female *M. cinxia* provided eggs, and additional egg clutches were found in the field. We considered each egg clutch to be an independent sample from its population, and analysis assumes this independence. Although we could not control for maternal effects, all individuals tested underwent development in near-identical conditions at the University of Texas at Austin, albeit at different times, given the length of this study. This included feeding larvae on a combination of *Plantago lanceolata*, *Plantago alpina* and *Veronica spicata*, in Petri dishes, at room temperature (22 °C), under growth lights. Winter diapause lasted 3–4 months in a climate-controlled cold room (4 °C). Once the adult butterflies eclosed, they were kept in separate cages and were fed daily with a honey and water solution.

Table 1. Environmental variables for the sites used in the study: geographical locality information, elevation, peak flight date, average daytime temperature at peak flight (°C), and, where calculated, noon clear-sky irradiance (W m^{-2}) at peak flight for flying and basking insects.

Region	Population	Latitude (°)	Longitude (°)	Elevation (m)	Peak flight date	Average daytime temperature (°C)	Flight irradiance (W m^{-2})	Basking irradiance (W m^{-2})
Spain	Sils	41.800	2.730	73	15 May	18.5	1090	1182
	Seva	41.836	2.288	685	20 May	17.2	1101	1185
	Can Terrer	41.890	2.707	145	15 May	18.2		
	Sales de Llierca	42.238	2.657	27	1 May†	17.6	1058	1186
Southern France	Montpellier	43.580	3.947	3	10 July‡	25.7		
	Prades	43.725	3.869	77	10 July‡	25.4	1092	1170
	Cazevielle	43.770	3.825	294	15 July‡	24.3	1088	1171
	La Pourcaresse	43.771	3.749	278	15 July‡	24.6		
Alps	Laus de Cervieres	44.856	6.730	1850	20 June	14.8	1102	1183
	Col de Granon	44.963	6.599	2300	30 June	15.3	1101	1184
Isle of Wight	Compton Chine	50.663	−1.478	5	15 June	16.1	1039	1164
Finland	Åland	60.173	19.781	15	25 June	13.9	927	1144

†First generation of two.

‡Second generation of two. Other populations are almost completely univoltine, with a single generation per year.

We tested individuals from five geographically separate regions representing the latitudinal and elevational extremes of the species' range.

To make an elevational comparison, we sampled two regions: (i) the elevational limit at 1900–2350 m in the French Alps (two populations, 12 families, 45 individuals); and (ii) low-elevation southern French sites at 50–250 m elevation, around 180 km from the Alpine sites (four populations, nine families, 13 individuals).

To compare insects from different latitudes and similar (low) elevation we sampled three regions: (i) the low-elevation southern range limit in Catalunya (Spain) (four populations, 19 families, 38 individuals); (ii) the northern range limit in the Åland Islands (Finland) (four populations, eight families, 24 individuals – but populations were lumped; population identity was not retained with each family); and (iii) the northern range limit in the Isle of Wight (U.K.) (one population, 11 families, 15 individuals).

These regions encompass the climate extremes experienced by *M. cinxia*. Table 1 shows geographical positions of the study populations within the regions, their elevations and relevant data on flight dates and climatic variables.

Alpine *M. cinxia* are univoltine (one generation year^{-1}), flying in June and early July, while the low-elevation southern French butterflies are bivoltine, usually flying in April–May and again in July (Table 1). The elevational comparison undertaken here was between Alpine and second-generation French butterflies that normally fly at approximately the same time of year. The latitudinal comparison was between insects that would normally fly in April/May in Spain and June in Finland (Table 1), so only by raising them in the laboratory were we able to test them side by side.

Thermal images

To capture thermal images, we used a camera (MikroScan 7515 Thermal Imager, Santa Clara, California) that visualises infrared (IR) energy emitted by an object as a colour thermal image. The camera also incorporates a background compensation feature, to remove errors caused by IR radiation from background objects. One manually sets the camera according to the emissivity of the subject, which is the extent to which it reflects, absorbs and transmits IR energy. As part of the calibration, the camera allows the operator to establish the emissivity of a single point within the field of view. The emissivity of the butterfly thorax was determined as 0.95, consistent with the value found by Palmer *et al.* (2004), and the exact value used for *M. cinxia* by Mattila (2015). We set the camera to 0.95 emissivity for the entire set of experiments. We also kept the ambient compensation settings of the camera at a constant temperature of 20 °C and an object distance of 35 cm.

We might expect that heating of the flight muscles during basking would be delayed relative to external heating recorded by the thermal camera. To assess this possibility, we used a small sample of individuals ($n = 4$) to investigate the relationship between the thermal images of the thorax and the internal temperature of the butterfly by inserting a temperature probe (MT-29/1B Insect Probe, Type T, Copper-Constantan Thermocouple, Clifton, New Jersey) into the side of the thorax. The probe provided continuous measurements of the internal thoracic temperature of the butterfly as it warmed up. We measured internal thoracic temperatures at the same time that each thermal image was taken.

Testing of takeoff temperature

Trials were conducted in a climate-controlled greenhouse in full sun, with ambient temperature close to 20 °C. Only one butterfly at a time was tested. It was observed anecdotally that recent feeding reduced the tendency to fly, hence we refrained from testing within 3 h after feeding. Prior to each trial, the test butterfly was cooled in the shade next to an air conditioning vent. It was then taken out of its cage and allowed to bask in full sunlight on a white card. The white card was chosen to minimise absorption of heat by the surface, and the card was also cooled

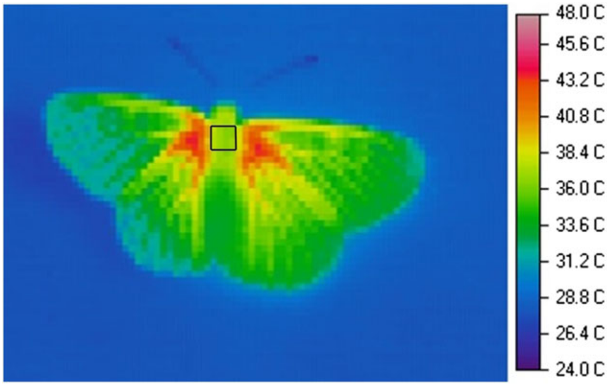

Fig. 1. A sample thermal image captured by the camera. The black square indicates the area used to calculate the average thoracic temperature. [Colour figure can be viewed at wileyonlinelibrary.com].

so as to not contribute to insect warming. Typically, a butterfly would start the test with wings closed, and then, after a few seconds in the sun, spread its wings into a dorsal basking posture, orient itself such that the plane of solar radiation was perpendicular to its body, gradually warm up, and then either take spontaneous flight or close its wings, preventing further heating. Thermal images were taken manually every few seconds.

Data were included if they met the following criteria during trials (if criteria were not met, the data were excluded): (i) the butterfly began by basking in constant direct sunlight and continued to do so until just before takeoff; (ii) the butterfly stayed on the same spot on the card, from the time it was placed there until the time it took off (behaviours violating this requirement included flapping of the wings while warming up, walking around the card while warming up, or closing the wings above the body after heating up instead of taking off); (iii) a usable thermal image was captured no more than 5 s before the butterfly took off (this was the image used for final analysis of the takeoff temperature).

Thermal image analysis

Thermal image analysis was conducted using MIKROSPEC 4.0 software (Santa Clara, California). This program divides the thermal image into a series of pixels, with each pixel assigned a temperature (Fig. 1). We obtained two measures of thoracic temperature at takeoff. First, our ‘multipixel’ value was an average temperature of as many pixels as possible covering the thorax. This value was calculated from a square grid of pixels such as that shown in Fig. 1 (black square), typically varying from nine (3 × 3 pixels) to 16 (4 × 4 pixels). Our second measure was the temperature of the hottest pixel in the image taken before takeoff.

Environmental variables

The average daytime temperature during the season(s) when adult butterflies were flying was calculated for each region by taking averages of these data from all the collecting sites/populations within the region. The data used for this calculation were gathered from the European Commission Joint Research Centre (2012). Solar irradiance was calculated for us by John Frederick, using his own algorithm (Frederick & Lubin, 1988; Frederick & Liao, 2005). For each study site, he provided estimates of irradiance received at noon by insects either flying (oriented horizontally) or basking (oriented perpendicular to the sun’s rays) (Table 1).

Statistical analyses

Statistical analyses were conducted using SPSS (v. 20). Average takeoff temperature per individual was calculated using the data gathered from repeat trials (number of trials varied between 1 to 6 per individual). A nested ANOVA (with individual nested within family, nested within region) was then used to estimate differences among regions. No significant differences were found among populations within a region. Therefore, where regions contained more than one population, different populations within that region were pooled. For pairwise comparisons between regions, the least significant difference (LSD) method was used. A linear regression analysis was conducted for the subset of individuals that had been weighed, plotting individual mass against average takeoff temperature. An ANOVA was used for within-region comparison of the sexes with regard to their thoracic temperatures at takeoff.

Results

Relationship between thermal camera data and internal thoracic temperature

As basking began, the internal thoracic temperature measured by the thermocouple was cooler than the external thoracic temperature measured by the camera. As the butterfly warmed, the gap between these temperatures became smaller, and eventually the external temperature recorded by the camera was identical or very close to that recorded by the temperature probe (Table S1).

Differences among regions in thoracic takeoff temperature

Overall analysis using the ‘multipixel’ measure showed significant heterogeneity of thoracic temperatures at takeoff among the five geographic regions (nested ANOVA: $F = 2.921$, d.f. = 4, $P = 0.026$). Pairwise comparisons among the different regions found three significant differences (Fig. 2). Finnish butterflies took off at hotter temperatures than those from the Alps (LSD: SE = 0.453, $P = 0.016$). Southern French insects had hotter takeoff temperatures than those from nearby Alpine populations (LSD: SE = 0.534, $P = 0.004$), and also hotter than insects from the Isle of Wight (LSD: SE = 0.624, $P = 0.032$).

Overall analysis using the ‘hottest pixel’ measure (Fig. 3) also found significant heterogeneity among regions (ANOVA: $F = 2.616$, d.f. = 4, $P = 0.042$). The three significant differences found in the ‘multipixel’ measure were again

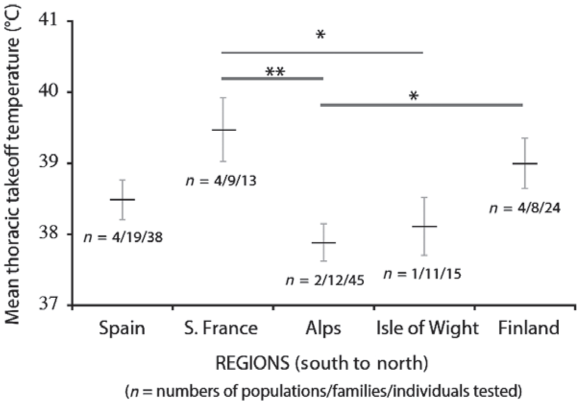

Fig. 2. Differences among regions in mean thoracic temperature at takeoff. For each region the numbers in the body of the figure show the number of populations/families/individuals tested. $*P < 0.05$, $**P < 0.01$ (see Table S2 for exact levels of significance).

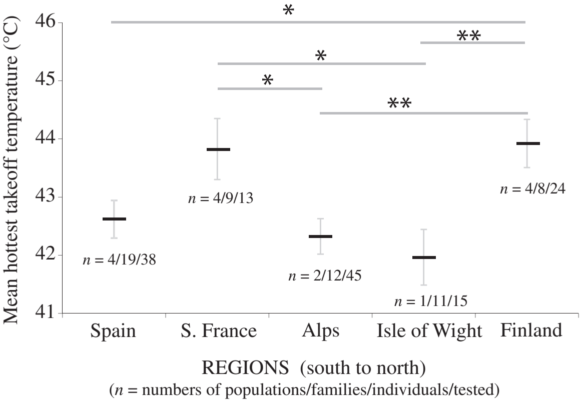

Fig. 3. Differences among regions in temperature of hottest pixel at takeoff. For each region the numbers in the body of the figure show the number of populations/families/individuals tested. $*P < 0.05$, $**P < 0.01$ (see Table S2 for exact levels of significance).

found among 'hottest pixels', with the difference between Finland and Alps acquiring greater significance. In addition, two more comparisons were significant with the hottest pixel measure: Finnish butterflies took off with hotter 'hottest pixels' than those from both Isle of Wight and Spain (Fig. 3).

Unsurprisingly, there is no disagreement between the 'hottest pixel' and 'multipixel' measures in the direction of interpopulation differences; the difference is in the number of those differences that achieve statistical significance. We know of no biological reason to expect a higher number of interpopulation comparisons to be significant using the 'hottest pixel' measure, so this difference may be accidental. In the absence of further knowledge, we place greatest trust in the comparisons that were significant by both measures.

Effects of sex and body mass

For individuals for which we had takeoff temperature data as well as mass data, a regression of multipixel takeoff temperature against body mass lacked significance both when the sexes were pooled ($R^2 = 0.055$, $N = 26$ individuals, $F = 1.385$, $P = 0.251$), and when they were analysed separately (females: $R^2 = 0.04$, $N = 11$ individuals, $F = 0.377$, $P = 0.554$; males: $R^2 = 0.005$, $N = 15$ individuals, $F = 0.070$, $P = 0.796$).

Males and females did not differ significantly in multipixel takeoff temperature, except in the Finnish (Åland Islands) population (ANOVA: $N = 24$ individuals, 13 females and 11 males; $F = 5.271$, d.f. = 1, $P = 0.032$), where the females took off at cooler temperatures than males.

Discussion

Differences in takeoff temperature by latitude and elevation

Non-migratory butterfly species such as *M. cinxia* can have ranges encompassing very different climates. Such species might be expected to adapt genetically to their local climates, adaptation that can be illuminated by reciprocal transplants (Van Dyck & Holveck, 2016) or by comparing individuals raised under the same conditions but sourced from regions in different parts of the species' range. The present study found evidence of local adaptations in a simple but important trait, body temperature at spontaneous takeoff.

The range of variation in takeoff temperature was not great, which is unsurprising in view of the evolutionary conservatism of thermal traits in general (Buckley & Kingsolver, 2012; Kellerman *et al.*, 2012; Araujo *et al.*, 2013). However, takeoff temperature did vary significantly among regions. We found significant regional differences between individuals from southern French sites at the elevational extremes, with cooler takeoff temperatures for the insects from high elevations. However, despite climatic differences between the latitudinal extremes during the seasons when the butterflies fly (Table 1), and despite known latitudinal trends in insect thermal tolerances (Lancaster, 2016), we found no consistent effect of latitude. Although U.K. (Isle of Wight) insects did take flight at cooler thoracic temperatures than southern French butterflies, there was no general trend for insects from northern regions to take off at cooler body temperatures than those from southern regions. In particular, Finnish insects took off at slightly higher temperatures than those from the southern range limit in Spain, a difference that achieved significance when we used the 'hottest pixel' measure.

Potential explanation for high takeoff temperatures in cool climates

If butterflies were unable to evolve efficient flight at low body temperatures, those in cooler climates could be under stronger selection to increase their flight durations by taking off at very high temperatures. Heinrich (1986) observed that *Coenonympha inornata* lost 10 °C during each flight, and they extended their flight durations by taking off at much higher temperatures than the minimum needed for active flight. Net heat loss in flight has also been observed in Finnish *M. cinxia*, which cooled at mean rates of 0.2–0.4 °C s^{-1} when flying in their natural environment (Mattila, 2015).

The hypothesis that takeoff temperatures should be high in cool climates may account for the hot takeoffs of Finnish insects, but not for the cool takeoffs of Alpine butterflies that operate in air averaging only 1–1.5 °C warmer than in Finland (Table 1). However, although solar heating is hardly different between the Alps and Finland for basking butterflies, the Alpine butterflies receive considerably higher solar irradiance when flying in sunshine (1102 W m^{-2}, vs. 927 W m^{-2} in Finland). This high input of radiant heat to flying insects should allow the Alpine butterflies to take off at relatively cool thoracic temperatures. Hence, we begin to suspect that differences in radiant energy may be as important to these insects as differences in ambient air temperature. However, response to solar irradiation will not explain all our results; in particular, the high takeoff temperatures of low-elevation butterflies in southern France are unlikely to be needed to extend flight duration.

The observation of high takeoff temperatures of Finnish butterflies in the current study is not the only result to show apparently paradoxical inter-site variation of thermal adaptation in butterflies. Vrba *et al.* (2012) found that overwintering larvae of *Erebia* butterflies from higher elevations were less cold-tolerant than those from lower elevations, the opposite of the simple expectation that denizens of colder climates should be more cold-tolerant. These authors suggested that low-elevation larvae may have experienced the most extreme low temperatures if the high mountain insects had been protected by insulating snow. Another interesting avenue of research would be to compare flight behaviour in habitats with similar solar irradiance when the sun is shining, but with significant differences in other weather variables (clouds, wind, etc.). Clearly, even though butterflies are relatively well-known poikilotherms, we do not yet have enough information to understand their adaptations to local climate.

Effects of sex and mass

We found no effect of sex on takeoff temperature, except in insects from Finland: Finnish males took off at a mean thoracic temperature of 39.8 °C, significantly hotter than females at 38.1 °C. Mattila (2015), using a protocol that differed from ours in several respects, found a nonsignificant difference in the opposite direction, and much lower mean takeoff temperatures in both sexes (31 °C in males vs. 31.9 °C in females).

Saastamoinen and Hanski (2008), also working with Finnish populations of *M. cinxia*, found a significant difference between the sexes in body surface temperature of butterflies captured during flight, with an average thoracic temperature in males of 28.4 °C, and 30.1 °C in females. This measure reflects temperatures at takeoff, rates of cooling in flight and flight durations. It is not explained by sex-specific rates of cooling in flight, as females cooled faster than males (Mattila, 2015). We suggest that it would be informative to measure body temperatures immediately after voluntary alighting, i.e. the temperatures at which insects flying in cool air decide to alight and bask, or are forced to do so.

One might expect larger butterflies to require higher thoracic temperatures at takeoff. This effect was found in a comparison among species (Neve & Hall, 2016). Berwaerts and Van Dyck (2004), working with *Pararge aegeria*, found that lighter males with high relative thoracic mass had higher performance than males with low relative thoracic mass. However, we found no effect of mass on takeoff temperature. Mattila (2015), working with Finnish populations of *M. cinxia*, found that large males took off at significantly lower thoracic temperature than small males, but there were no differences among females of different mass.

Relevance to climate warming

Acclimation, adaptation, dispersal and behavioural modification all play a part in responses to global warming (Deutsch *et al.*, 2008), as species shift their ranges in latitude and elevation (Parmesan, 2006; Singer, 2017; Socolar *et al.*, 2017). Detailed studies of physiological mechanisms will improve our projections of climate change impacts (Pörtner & Farrell, 2008). However, where it is legal, the most direct evidence to assess coming range shifts can be gleaned from translocating organisms outside their current ranges (Crozier, 2004; Pelini *et al.*, 2009). We hope that further studies of *M. cinxia* will be useful in illuminating differences between the thermal effects of elevation and latitude that might apply to small poikilotherms in general, and hence contribute to improving our ability to predict the effects of climate change.

By its very nature, takeoff temperature must be classed as a thermal adaptation. Within the set of populations in our current study, environmental differences associated with elevation seem to have been more important in shaping this adaptation than those associated with latitude. The suggestion from these results is that, in addition to climate, solar irradiance may be influential. Differences among habitats in features other than ambient temperature may prove to be significant drivers of thermal adaptations in poikilotherms.

Acknowledgements

Saskya van Nouhuys, Jeremy Thomas and Constanti Stefanescu helped in collecting samples, and John Frederick made the calculations of solar irradiance. The University of Texas Division of Statistics and Scientific Computation helped with statistical analyses. Nils Ryrholm and Larry Gilbert shared insights into the importance of radiant energy in shaping butterfly responses to climate change. The research was supported by funding from the University of Texas at Austin, Section of Integrative Biology, to NKA and CP. We thank the anonymous reviewers for their constructive inputs which significantly improved our manuscript. The authors declare that there were no conflicts of interest in conducting the research.

Author contributions

All authors participated in collecting and rearing insects, and in writing the manuscript. NKA took the thermal images, performed the analyses and wrote the first draft of the manuscript as part of his PhD dissertation at the University of Texas at Austin.

Supporting Information

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

Table S1. Comparisons between the internal temperature of the butterfly (recorded by a temperature probe), and the external temperature (recorded by a thermal camera), as the butterfly warms up.

Table S2. Exact levels of significance of inter-region comparisons of takeoff temperatures measured as mean thoracic temperature/hottest pixel temperature

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Accepted 22 November 2018

Associate Editor: Robert Wilson