# Thermoregulation of adult alpine butterflies: microhabitat choice is more important than physical differences

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**Abstract**

Efficient thermoregulation is crucial for animals living under changing climatic and weather conditions. We used a thermal camera to measure thermoregulation of six co-occurring species of *Erebia* (Lepidoptera: Nymphalidae) mountain butterflies in a laboratory experiment with artificial light and heating source. We studied wild individuals to test whether physical characteristics (body size, wing loading) are responsible for the interspecific differences in thermoregulation detected previously under natural conditions in European Alps. We revealed that physical characteristics had a small effect on explaining interspecific differences compared to distinct thermoregulatory differences recorded in the field. Our results show that larger butterflies, with higher weight and wing loading, heated up more slowly but reached the same asymptotic body temperature as smaller butterflies. Altogether, our results suggest that differences in body temperatures among *Erebia* species observed in the field are likely caused mainly by species-specific differences in microhabitat use and confirm the role of active thermoregulation in adult butterflies.

**Keywords**

alpine species, ecophysiology, habitat use, *Lepidoptera*, thermal camera, thermoregulation

## 1. Introduction

Thermal ecophysiology of butterflies is driven by interactions between the environment and species-specific physical (morphology, physiology) and behavioural characteristics. Important morphological traits for butterfly thermoregulation include body size (Heinrich, 1986; Kleckova et al., 2014), wing loading (i.e., the ratio of body weight over wing area) and colouration (Kingsolver, 1983). Behaviourally, insects also thermoregulate by specific body postures (Tsai et al., 2020) or by microhabitat choice (Kleckova & Klecka, 2016). Adult butterflies typically increase their body temperature over the ambient air temperature by basking postures (Heinrich, 1995; Kingsolver, 1985a) and by conduction from warm stones or soil (Kemp & Krockenberger, 2002; Kingsolver, 1985b; Kleckova et al., 2014). Conversely, body temperature can be decreased by a shift to a cooler microhabitat (Dreisig, 1995; Shreeve, 1984; Wickman, 1988). Hence, thermoregulation in butterflies is likely driven by a combination of behaviour, morphological and physiological traits.

Our knowledge about the interactions between behavioural and physiological responses to climate change is generally scarce in insects (Abram et al., 2017) because behavioural and physiological responses are difficult to disentangle by field observations (e.g. Lawson,et al., 2014a). However, it has been established that adult butterflies respond to short-term environmental changes by active thermoregulation based on their species-specific physiological optima (Bladon et al., 2020; Kleckova et al., 2014; Kleckova & Klecka, 2016). Such physiological optima are, in turn, also affected by morphological traits, which affect the choice of ambient temperature and daily activity patterns of different species of butterflies (Kingsolver, 1983) and other insects, such as beetles (Gallego et al., 2018). Similar variation has been observed also at the inter-population level in butterflies (Nève & Després, 2020) and intra-population level in grasshoppers (Forsman, 2000). Moreover, it has been shown that thermoregulatory behaviour of butterflies prevents overheating of living cells distributed in the wings veins (Tsai et al., 2020). Such studies of interactions between behavioural responses, morphological and physiological traits have provided novel insights into the importance of insect thermoregulatory strategies. However, we still know little about the main traits involved in thermoregulation of butterflies occurring in extreme environments such as alpine regions.

To respond to local climatic conditions, butterfly species may evolve different physiological optima (MacLean et al., 2016), disperse to optimal climates (Konvicka et al., 2003) or change their thermoregulatory behaviour, e.g., by shifts of microhabitat use (Kirkpatrick & Sheldon, 2022; Wilson et al., 2015). Species-specific physiological optima evolved under the selection pressures exerted by the environment during the whole life cycle (Radchuk et al., 2013; Willmer, 1982), forming the species' climatic niches (Klečková et al., 2022; Pitteloud et al., 2017). Ultimately, strong selection pressures of local conditions may result in local adaptation of adult thermoregulatory traits across climatic gradients, as has been reported in *Melitaea cinxia* butterflies (Advani et al., 2019; Mattila, 2015). Still, the importance of active behaviour, physical constraints and possible adaptations to local conditions are not fully resolved.

To disentangle the roles of behavioural responses and physical differences in thermoregulation, we studied adult *Erebia* (Nymphalidae, Satyrinae) butterflies occurring in sympatry in alpine environments. In the mountains, microclimatic conditions experienced by butterflies are changing rapidly (Stuhldreher & Fartmann, 2018) and alpine species including *Erebia* are endangered by climate warming (Konvicka et al., 2021; Sistri et al., 2022). *Erebia* butterflies have low mobility (Polic et al., 2014; Slamova et al., 2013), which probably leads to slower uphill shifts in response to climate warming compared to mobile generalist species (Rödder et al., 2021). On the other hand, at least some populations of high-altitude *Erebia* specialists are able to use suitable habitats at altitudes below their climatic optimum (Cizek et al., 2003). Our previous work suggests that inter-specific differences in thermoregulation of co-occurring closely related *Erebia* species might have been driven mainly by microhabitat use and body size (Kleckova et al., 2014), but it is unclear what the main thermoregulation strategy is. To test the relative effects of species identity (a proxy for possible physiological differences) and morphology (individual weight and wing-loading), we carried out laboratory measurements of body temperature heating rates under an artificial source of radiation. This allows a better comparison of the importance of morphological traits and microhabitat choice. Our hypothesis is that different thermal conditions along the elevational gradient and different microhabitats inhabited by individual species have driven divergent physiological constraints. We expected that larger individuals and individuals with higher wing loading would heat up more slowly than smaller individuals, as detected in *Thymelicus lineola* (Lepidoptera: Hesperiidae) (Pivnick & McNeil, 1986). Finally, we asked whether microhabitat choice is the primary thermoregulatory mechanism by comparing data from the laboratory heating experiments to field measurements of body temperatures (Kleckova et al., 2014).

## 2. Material and methods

### 2.1. Study site and species

We collected adult butterflies of six species of *Erebia* in the Austrian Alps, close to the town of Sölden, Tirol (1900-2020 m a.s.l., 46°57'37"N 11°01'49"E) and in Hochgurgl (2150 m a.s.l., 46°54'6"N, 11°03'14"E, 7 km away). The butterflies have brown wings with orange spots. Species differ in their habitat preferences, altitude of occurrence, body size, and phenology. Rocky habitats above the treeline host *E. pandrose* (Borkhausen, 1788) and *E. cassioides* (Reiner & Hochenwarth, 1792). Sub-alpine and alpine grassy meadows above the treeline are inhabited by *E. alberganus* (de Prunner, 1798) and *E. melampus* (Fuessly, 1775). Subalpine meadows around the treeline and woodland clearings below the treeline are inhabited by *E. euryale* (Esper, 1805) and *E. ligea* (Linnaeus, 1758). Regarding size differences, *E. cassioides* and *E. melampus* are small (forewing length less than 20 mm), whereas the other four species are large (forewing length above 20 mm). Three early flying species, *E. alberganus*, *E. melampus* and *E. pandrose*, were caught between 27$^{th}$ and 29$^{th}$ June 2011. Three late flying species, *E. ligea*, *E. cassioides* and *E. euryale*, were caught between 2$^{nd}$ and 4$^{th}$ August 2011. The butterflies were collected by hand netting and stored alive in envelopes inside a cooling box with suitable humidity provided by wet tissues. We transported the specimens to the

facilities of the Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic, for laboratory experiments.

## 2.2. Laboratory measurements of body heating

Before the experiments, the butterflies were acclimatised overnight, for at least 15 hours, in a net cage in a climate-controlled room (20°C) with 16 h day and 8 h night photoperiod. Butterflies were fed ad libitum by 20% sugar solution applied on the net. We measured the heating rate using a thermal camera (FLIR P660, Flir Systems) in a climate-controlled room. The thermal camera was mounted on a tripod 0.60 m above a flat wooden arena where the butterfly was placed. We used wood as material for the arena because it kept a relatively stable surface temperature during the experiments. On the camera, we set-up the emissivity to 0.85, humidity to 66 %, and object distance to 0.6 m for all individual specimens. The emissivity value compensates for the reflection, transmission, and absorption of IR energy and corresponds to a conservative estimate for insect cuticle, e.g. (Verdu et al., 2012). The humidity value corresponds to the average air humidity in the experimental room (range 56-82%).

The acclimatised butterflies were first cooled at 5 °C for 10 minutes. Then, using cold forceps, they were placed in the middle of the experimental arena under the two halogen light bulbs (18 W and 160 lm) situated 25 cm above the arena. The butterfly surface body temperature was recorded every 20 seconds for 10 minutes (Figure 1). All individuals were sitting during the experiment. Each individual was measured only once. The temperature in the middle of the mesothorax was recorded using FLIR QuickReport©1.2 software. The air-conditioning system in the experimental room created a strong draft, so we kept it turned off for the duration of the measurements. Consequently, the temperature in the experimental room fluctuated from 17.5 to 21.1 °C. To account for these fluctuations in the statistical analysis, room temperature ($T_{room}$) and *humidity* was measured just before each trial.

After the thermal camera measurements, the individual butterflies were sacrificed using ethyl-acetate. We obtained the weight (mg) of fresh individuals using a laboratory balance (precision 0.0001 g). We also photographed the left side of wings of every individual and calculated the wing area in the software ImageJ v.1.46 (Rasband, 1997) and multiplied by two to obtain an estimate of the total wing area. We calculated wing loading (mg/cm$^2$) of every individual as the ratio of body weight (mg) over wing area (cm$^2$). In total, we measured the heating rate in 78 individuals (Table 1).

## 2.3 Statistical analyses

*Heating curves and species effects*

All analyses and data visualisation were done in R version 4.2.0 (R Core Team, 2022). First, we analysed heating curves, describing the increase of body temperature $T_b$ in time during basking. The curves were analysed using nonlinear mixed effects models (NLME) with

Gaussian error distribution in the nlme package for R (Pinheiro et al., 2022). The heating curve was estimated by two alternative models:

1. following the modified asymptotic exponential equation $T_b = T_s\,(1–e^{-b\,(time-c)})$, where $T_s$ is the estimated asymptotic temperature reached by an individual. The slope of the asymptotic exponential curve ($b$) describes the heating rate of the body temperature increase in time until it reaches asymptotic body temperature $T_s$.

2. following the modified Michaelis-Menten equation $T_b = T_s((time-c)/(time+K-c))$, where the parameter $T_s$ is the same as above and $K$ is analogous to the Michaelis constant in classic Michaelis-Menten equation, but here $K+c$ is the time when $T_b$ reaches 50 % of the final $T_s$.

The parameter $c$ is included in both models to account for the fact that the initial $T_b$ at $time$=0 was >0 °C (Figure 2). Specifically, $c$ is the estimate of how long it would take for the butterfly to increase its body temperature from $T_b$=0 °C to the real initial $T_b$ (which varied among individuals) under the experimental conditions.

We compared the fit of the two models without any random effects and with two alternative random effect structures: the random effect of species and the random effect of individual nested within species. The individual identity was used to account for possible differences of the heating rate ($b$) and asymptotic body temperature ($T_s$) in different individuals. The model fits were compared by the Akaike information criterion (AIC).

*Body weight and wing loading effects*

We tested how the shape of the heating curves depends on individual body mass and wing loading. We used generalised linear mixed effect models (GLMM) in the lme4 (Bates et al., 2015) and lmerTest libraries for R (Kuznetsova et al., 2017). For every individual, we extracted the estimates of parameters of asymptotic temperature $T_s$ and heating rate $b$ from the best fitting NLME model (i.e., modified asymptotic exponential, see Table 2). Then, we correlated the dependence of individual-level $T_s$ or $b$ on individual body weight and wing-loading. As additional predictors in the model, we included the $T_{room}$ temperature and *humidity* in the experimental room recorded during individual measurements. We also compared mean body weight and wing-loading among species using generalised linear models (GLM) with Gamma distribution and log link function.

*Thermoregulation in the laboratory vs. in the field*

We intended to compare species-specific values of the asymptotic temperature ($T_s$) reached in laboratory conditions with body temperatures ($T_{b_field}$) reached in the field (Kleckova et al., 2014). Because the range of ambient temperatures experienced in the laboratory differed from the range in the field, we estimated the standardised asymptotic body temperature $T_s$ and the standardised body temperature in the field $T_{b_field}$. The standardised $T_s$ and $T_{b_field}$ temperatures

were estimated for 20 °C of ambient laboratory ($T_{room}$) and for 20 °C of the two field temperatures, $T_{air}$ (air temperature at breast height) and $T_{microhabitat}$ (microhabitat temperature) experienced by individual butterflies in the spot where an individual was sitting (see Kleckova et al., 2014). For the laboratory measurements, the estimate of $T_s$ at ambient temperature 20°C was calculated from a generalised linear model $T_s \sim T_{room} + species$ fitted to $T_s$ estimates for all individuals (see above). We used the *predict()* function in R to obtain the estimate of $T_s$ at ambient temperature 20°C for each species. For the field observations, we again used the *predict()* function to infer $T_{b_field}$ at ambient temperature 20 °C for each species. The dependence of body temperature $T_{b_field}$ on both the ambient air ($T_{a_field}$) and the microhabitat ($T_{m_field}$) temperature was obtained from a previous study (Kleckova et al., 2014). Finally, we tested the relationship between the standardised $T_s$ in the laboratory and the standardised $T_{b_field}$ reached in the field for individual species by fitting a generalised linear model with Gaussian error distribution.

## 3. Results

### 3.1. Heating curves and species effect

The increase of body temperature $T_b$ in the studied *Erebia* butterflies was better described by the modified asymptotic exponential model (AIC = 583.8), which includes the heating rate parameter *b*, compared to the modified Michaelis-Menten model (AIC = 1051.3) (Table 2). The modified asymptotic exponential model predicted that the asymptotic temperature $T_s$ is slightly more rapidly reached compared to the modified Michaelis-Menten model (Figure 2 and Table 2). The variability of the individual heating curves was best explained by a model including the random effect of individual identity nested within species (Table 2). However, differences in the shape of the heating curves among species were modest (Figure 3).

### 3.2. Body weight and wing loading effects

The six species differed in their body weight and wing loading (Table 1, GLM, $P < 10^{-8}$ in both cases), although there was considerable intraspecific variation. As expected, wing loading and body weight were strongly positively correlated (Pearson's correlation coefficient = 0.84, $t_{46}$ = 10.373, $P < 10^{-12}$). Thermoregulation at the individual level was affected by both measures of body size. The heating rate *b* decreased with increasing body mass (GLMM, F = 7.17, P = 0.0105) and wing loading (GLMM, F = 4.40, P = 0.0419) (Figure 4). On the other hand, the asymptotic temperature $T_s$ was independent of body mass (GLMM, F = 0.05, P = 0.8209) and wing loading (GLMM, F = 0.16, P = 0.6877). The results, thus, demonstrated that larger butterflies heated up more slowly but reached the same asymptotic temperature as smaller butterflies. The GLMMs also included the effect of air temperature and humidity measured at the time of each observation. While $T_s$ increased with the increasing room temperature (F = 46.53, P= <0.0001) and humidity (F = 5.12, P = 0.0294), the heating rate increased with the increasing room temperature (F = 13.82, P = 0.0011) and was not significantly affected by air humidity (F = 3.68, P = 0.0612).

3.3. Thermoregulation in the laboratory vs. in the field

The standardised asymptotic temperatures $T_s$ measured in the laboratory (Figure 5) were not correlated with the standardised body temperature reached in field ($T_{b_field}$). There was no statistically significant relationship regardless of whether $T_{b_field}$ was standardised for air temperature $T_{a_field}$ (GLM, F = 0.41, P = 0.5580) or for microhabitat temperature $T_{m_field}$ (F = 0.84, P = 0.4107). It is noteworthy that the two species preferring rocky habitats, *E. cassioides* and *E. pandrose*, kept the highest body temperatures in the field despite their intermediate asymptotic temperatures measured in the laboratory (Figure 5). Also, the subalpine species *E. alberganus* and *E. melampus* kept higher body temperatures than the woodland species *E. euryale* and *E. ligea* measured both in the field and in the laboratory.

## 4. Discussion

4.1. Species and individual effects on thermoregulation under laboratory conditions

The species-specific thermoregulation differences in laboratory conditions were modest (Figure 3), but still detectable (Table 2). The better fit of the individual heating curves by models including the random effect of individual nested in species (Table 2) indicates that there is a noticeable effect of individual-level traits (e.g., species-specific physiological responses) in thermoregulation. However, the observed higher heating rate *b* in individuals with lower body weight and wing-loading regardless of species identity (Figure 4) points toward a major role of morphological traits. In other words, larger butterflies having higher wing loading heated up more slowly than small species. Our results are in line with (Pivnick & McNeil, 1986), who observed that males of *Thymelicus lineola* (Lepidoptera: Hesperiidae) heated up faster than females, the latter having a higher wing-loading.

In the field, we previously detected a pattern with smaller species reaching higher body temperatures at low ambient temperatures compared to larger species (Kleckova et al., 2014). Thus, our original expectation was that larger species and individuals would accumulate higher body temperatures in the laboratory, as it was also recorded in the butterfly species *Polyommatus icarus* (Lepidoptera: Lycaenidae) (Keyser et al., 2015). Our results, nonetheless, did not support such an expectation in *Erebia*, as we found no relationship between asymptotic temperatures $T_s$ and body size under laboratory conditions. That larger *Erebia* species did not reach higher body temperatures under laboratory conditions might be partly explained by the low light intensity used in our experimental setup (45 W/m$^2$, but solar flux is around 1000 W/m$^2$ (Sterhov & Loshkarev, 2019)). Future experiments should consider the importance of sufficient light intensity because larger butterflies might be able to use their wings as “solar collectors” in natural conditions, but this was not possible to test in our experiment.

Other traits not included in our study, such as fur thickness on the body, may affect heating rates and body temperatures of butterflies. In the laboratory conditions, a small-bodied species, *E. melampus*, warmed up faster than the remaining species (Fig. 3), unlike the other small species, *Erebia cassiodes.* This may be caused by the fact that *E. cassioides* has more “furry”

(modified scales) wings than *E. melampus*. We argue that the fur may provide better insulation in cold conditions (Kingsolver, 1988), but it may also slow down heating. Low heating effectiveness can be caused also by different wing emissivity, reflectance and absorbance (Lou et al., 2021; Schmitz, 1994).

4.2. Habitat use

A previous study on thermoregulatory strategies in the field demonstrated that woodland *Erebia* species kept lower body temperatures compared to species of open and rocky habitats (Kleckova et al., 2014). In congruence, the smallest difference in asymptotic temperature *Ts* reached in the laboratory and $T_{b_field}$ under field conditions was found for the woodland species *E. ligea*. This indicates low buffering ability of this woodland species. Similarly, small differences in body temperatures measured in the field and in the laboratory were detected in another woodland butterfly *Hamaeris lucina* (Riodinidae) (Bladon et al., 2020; Hayes et al., 2019). In contrast, the greatest difference between $T_s$ and $T_{b_field}$ was observed for species inhabiting rocky habitats, *E. cassioides* and *E. pandrose*, indicating their strong ability to thermoregulate by microclimate choice.

4.3. Heating curves were better described by the modified asymptotic exponential model

There is no consensus in the literature about the most appropriate model for fitting the heating curves describing the increase of body temperature $T_b$ in time in insects. An exponential model was used to describe ectotherm thermoregulation, for example, to compare thermoregulation of colour morphs in the chameleon grasshopper (Umbers et al., 2013) and to infer the increase of internal body temperature in dung-beetles (Amore et al., 2017). Another model used previously is the Michaelis-Menten equation, used e.g. to describe heating curves of a sea snail by Muñoz et al., 2005. Complex models based on detailed physiological measurements of heat transfer within animal bodies and between them and their environment have been developed (Dreisig, 1984; Dzialowski & O'Connor, 2001), but they are impractical for use in ecological studies where detailed physiological parameters are difficult to obtain. In *Erebia* butterflies, the modified asymptotic exponential model explained the heating of body temperature better than the modified Michaelis-Menten model (Table 2).

## 5. Conclusions

Studying the interactions of insect physical traits and their behavioural responses to environmental factors has the potential to contribute to understanding and predicting the impacts of climate change on biodiversity. When behavioural thermoregulation was excluded under laboratory conditions, six closely related *Erebia* species differed minimally in thermoregulation, as measured by heating rate curves (Figure 3). We only detected a negative effect of individual body size on the rate of heating, but no effect on the asymptotic body temperate. Thus, the thermal conditions along the elevational gradient and different habitats have not resulted in divergent physiological constraints among species as we hypothesised, at least at the adult life stage. However, in the field, when adult butterflies have the opportunity

to actively seek out suitable microclimates, the species differed significantly in thermoregulation (Kleckova et al., 2014). This points toward a more prominent role of behavioural thermoregulation in these closely related butterflies. The key role of behaviour in thermoregulation has been shown also in a territorial butterfly (Rutowski et al., 1994) and in butterflies exposed to extreme temperatures (Tsai et al., 2020). The importance of behavioural thermoregulation found in this study suggests that the adult life stage is not under direct thermal selection induced by ongoing climate change, in agreement with (Buckley et al., 2015). Thus, under increased anthropogenic pressure on *Erebia* populations, it is necessary to safeguard a variety of microhabitats which can facilitate efficient behavioural thermoregulation of adults and increased survival of less mobile developmental stages (Abarca et al., 2019). This is in line with previous calls for management of mountain habitats supporting environmental heterogeneity (Habel et al., 2022) to increase species survival under ongoing climatic and other human-induced environmental changes (Lawson et al. 2014b).

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## Tables

**Table 1. The list of study species of the butterfly genus *Erebia***. For each species, we provide information about its typical habitat, the number of individuals used for the lab measurements (N), their mean body weight, total wing area, and wing loading (body weight/wing area). Species are sorted according to their body weight from lightest to heaviest.

| Species | Habitat | N | Body weight (mg) | Wing area (cm2) | Wing loading (mg/cm2) |
|---|---|---|---|---|---|
| | | | mean (SD) | mean (SD) | mean (SD) |
| *E. melampus* | Grassland | 11 | 24.4 (4.17) | 7.82 (0.544) | 3.12 (0.402) |
| *E. cassioides* | Rocks | 8 | 34.4 (7.01) | 10.95 (1.019) | 3.15 (0.682) |
| *E. euryale* | Woodland | 10 | 38.5 (5.02) | 15.45 (1.349) | 2.50 (0.359) |
| *E. ligea* | Woodland | 9 | 50.1 (21.76) | 17.94 (1.222) | 2.74 (0.949) |
| *E. alberganus* | Grassland | 9 | 54.0 (11.55) | 12.90 (1.398) | 4.16 (0.636) |
| *E. pandrose* | Rocks | 11 | 68 (14.51) | 13.58 (1.385) | 5.08 (1.286) |

**Table 2. The comparison of the fit of the modified asymptotic exponential and Michaelis-Menten models describing butterfly heating under laboratory conditions.** The models describe the increase of body temperature ($T_b$) in *time* in 78 individuals of *Erebia* butterflies, which heated up under an artificial source of heating and radiation. Their $T_b$ was measured by thermal camera. The non-linear mixed effect models included the random effects of individual nested in species and the random effect of species. The best model is highlighted **in bold**. Parameters describe maximum body temperature reached during heating (asymptotic temperature $T_s$), heating rate (*b)*, Michaelis-Menten constant (*K*), and the estimated time it would take for the butterfly to increase its body temperature from $T_b$=0 °C to the actual initial $T_b$ (c).

| | | Parameter estimates (fixed effects) | | | | | |
|---|---|---|---|---|---|---|---|
| **Model** | **Random effects** | **$T_s$ (SE)** | ***b* (SE)** | ***K* (SE)** | ***c* (SE)** | **AIC** | **ΔAIC** |
| **modified asymptotic exponential** | **Individual nested in species** | **21.00 (0.271)** | **0.687 (0.056)** | | **-1.188 (0.152)** | **583.8** | **0** |
| | Species | 20.93 (0.291) | 0.695 (0.061) | | -1.138 (0.141) | 2375.5 | 1791.7 |
| | None | 20.94 (0.037) | 0.660 (0.013) | | -1.140 (0.033) | 3410 | 2826.2 |
| modified Michaelis-Menten | Individual nested in species | 22.73 (0.334) | | 0.637 (0.073) | -0.559 (0.082) | 1051.3 | 467.5 |
| | Species | 22.46 (0.377) | | 0.587 (0.077) | -0.514 (0.078) | 2485.1 | 1901.3 |
| | None | 22.48 (0.068) | | 0.579 (0.018) | -0.507 (0.020) | 3422.2 | 2838.4 |

**Figures**

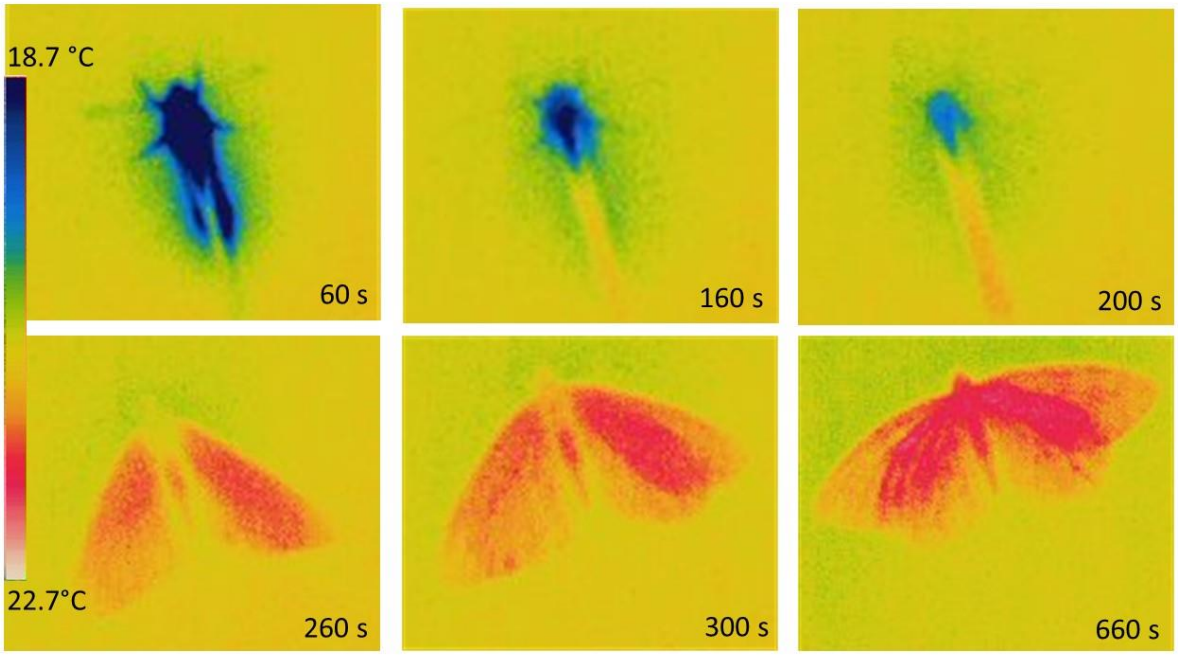


**Figure 1.** An example image from the thermal camera displaying the heating of an individual *Erebia* butterfly in *time* (s), blue indicates colder, red warmer temperatures.

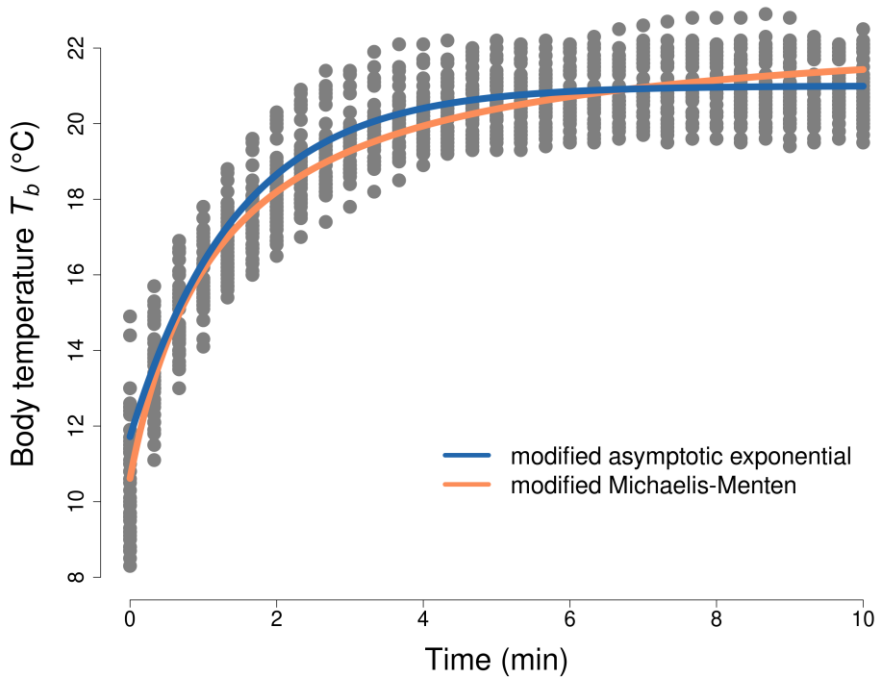


**Figure 2.** The comparison of the fit of the modified asymptotic exponential and Michaelis-Menten models describing the heating of individuals of *Erebia* butterflies under laboratory conditions. Data from all individuals of all species are pooled in the plot (grey dots represent measurements of individual body temperature $T_b$ in *time*). The modified asymptotic exponential model fits the temporal changes of body temperature during heating of the butterflies better than the modified Michaelis-Menten model (ΔAIC = 467.5, see Table 2 for details).

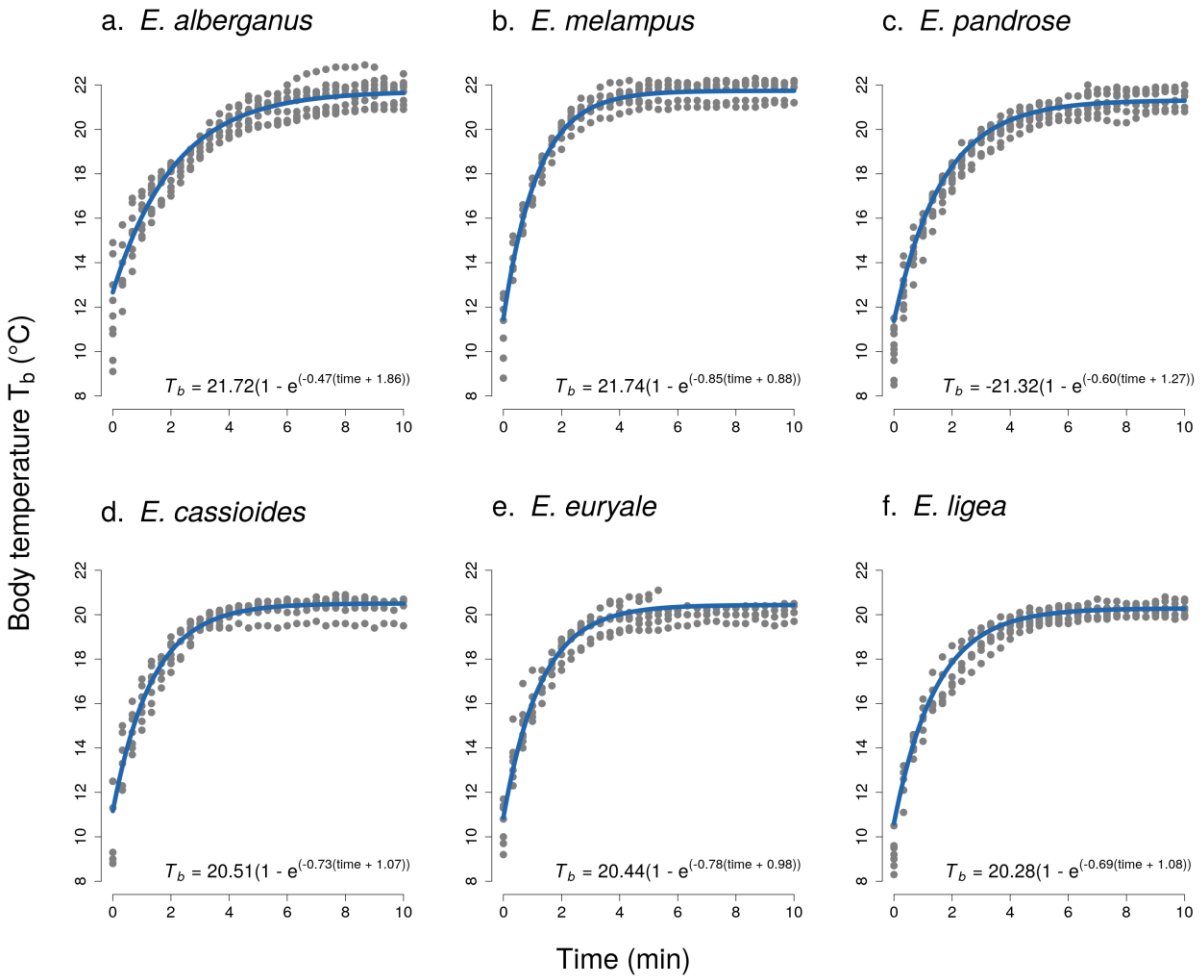


**Figure 3.** Heating of six *Erebia* butterfly species under artificial source of heat and radiation under laboratory conditions. The lines display the fit of the modified asymptotic exponential model. The species-specific parameters of the model were estimated by a nonlinear mixed effects model with individual and species identity included as nested random effects. The parameter *b* describes the heating rate, i.e. the increase of the body temperature ($T_b$) in *time* until it reaches the asymptotic body temperature ($T_s$). The parameter *c* is the estimate of how long it would take to increase the body temperature from $T_b$=0 °C to the real initial $T_b$. Equations describing the fitted curves with species-specific parameter estimates are added.

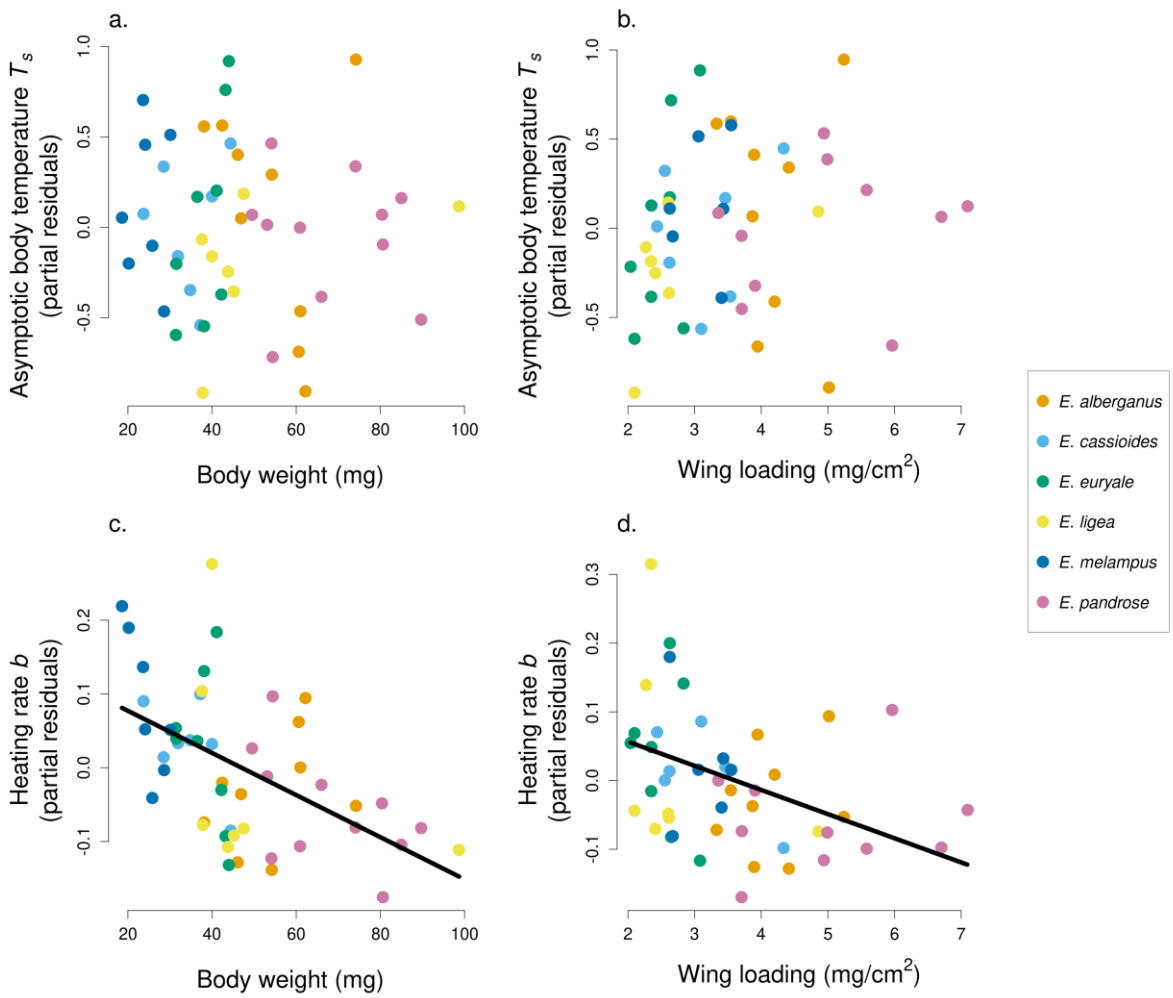


**Figure 4.** The effect of body weight and wing loading on the asymptotic body temperature ($T_s$) and on the heating rate ($b$) of individuals of six *Erebia* species. The $T_s$ and $b$ parameters describing heating of individuals under laboratory conditions were inferred from the modified asymptotic exponential models (see Figure 3). The y-axis shows partial residuals from a model which also included the room temperature and humidity at the time of each measurement. Each dot represents one individual, grouped into species according to colour.

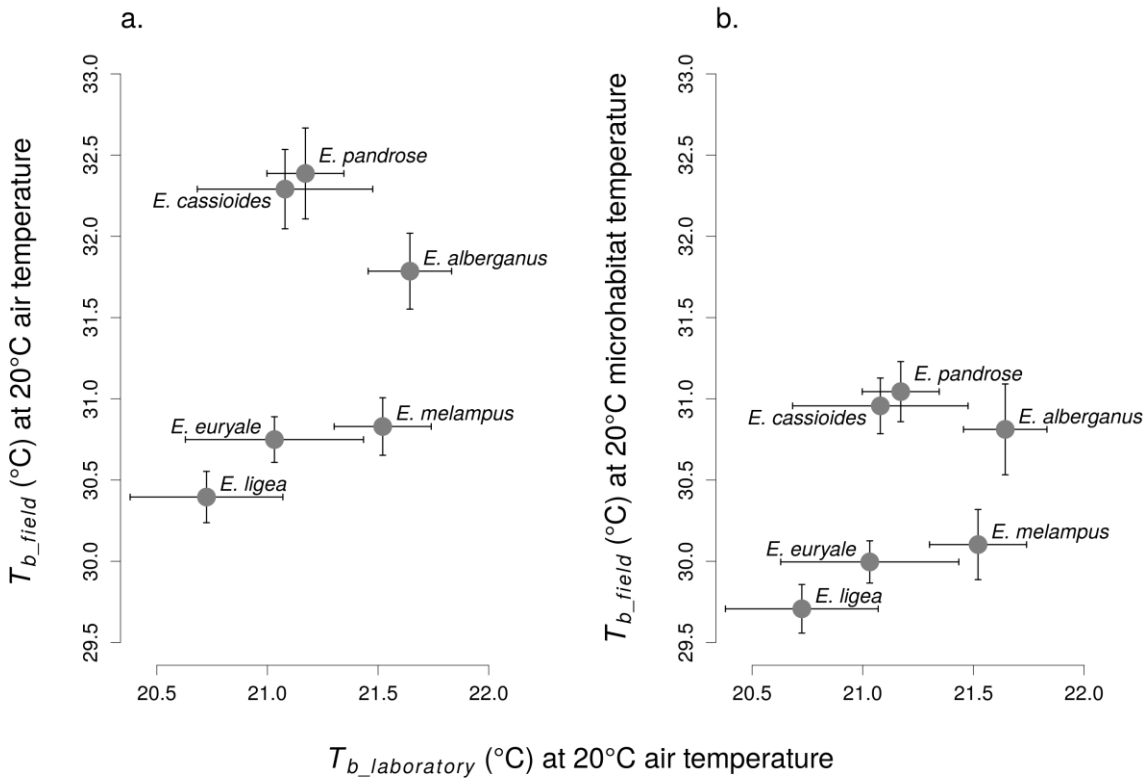


**Figure 5.** The correlation between *Erebia* butterfly species body temperatures reached under laboratory conditions ($T_{b_laboratory}$) and mean body temperatures $T_{b_field}$ measured in the field at 20°C of ambient air (panel a.) and microhabitat (panel b.) temperature. See the Methods section for more details. Estimates of $T_b$ values are accompanied by standard errors.