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Facing the heat: thermoregulation and behaviour of lowland species of a cold-dwelling butterfly genus *Erebia*

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Abstract

Understanding the potential of animals to quickly respond to changing temperatures is imperative for predicting the effects of climate change on biodiversity. Ectotherms, such as insects, use behavioural thermoregulation to keep their body temperature within suitable limits. Behavioural thermoregulation may be particularly important at warm margins of species occurrence where populations are sensitive to increasing air temperatures. In the field, we studied thermal requirements and behavioural thermoregulation by microhabitat choice and by switching among daily activities in low-altitude populations of the Satyrinae butterflies *Erebia aethiops*, *E. euryale* and *E. medusa*. We compared the relationship of individual body temperature with air and microhabitat temperatures for the low-altitude *Erebia* species to our data on seven mountain species, including a high-altitude population of *E. euryale*, studied in the Alps. *E. aethiops* and the low-altitude population of *E. euryale* kept lower body temperatures than the other species and showed signs of overheating. Adults of a lowland species *E. medusa* seemed well adapted to warm climate of a subxerotherm locality. Temperature-dependence of different daily activities also differed between the three lowland populations and the mountain species. Overall, our results suggest that lowland species and populations of *Erebia* butterflies are likely more severely threatened by ongoing climate changes than mountain species. Because of the ability of butterflies to actively search for appropriate microclimate and different requirements of individual species, we highlight the importance of sustaining habitat heterogeneity to protect individual species and entire assemblages.

Introduction

Ongoing climate changes induce range shifts of many animals and plants [1, 2], however species responses to climate change are individualistic. Animals diversely adjust their behaviour [3, 4], phenology [5], acclimate [6] or adapt by rapid evolutionary changes [3, 7, 8]. These adaptive responses to environmental changes together contribute to the demarcation of current species distribution ranges and also induce diversification. Selective pressure of large environmental changes leads to shifts of allele frequencies of genes responsible for physiological functions [7] and creates a phylogenetic signal in genus-level phylogenies [9, 10].

At short time scales, behavioural plasticity of ectotherms enables them to keep their body temperature at the optimal level and thus to optimize functioning of their

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physiological processes [4]. Behavioural thermoregulation is mainly represented by changes in timing of daily activities or exploration of various (micro)habitats [11–13]. Especially, the ability to use locally suitable microhabitats facilitates species survival under ongoing climate change [4]. Ectotherms search for warmer microclimate at the cold margins [12, 14] and decrease activity or search for shade at the warm margins of occurrence [15–18]. The comparison of species responses at contrasting climatic range margins, such as low- vs. high-altitude margins, may empower effective targeting of conservation activities especially in declining species with limited potential for range shift [2, 19, 20].

Despite the great behavioural plasticity of many species, physiological constraints can block behavioural compensation of climate change. For example, tropical lizards of forest interior suffer from higher risk of overheating than their open-habitat congeners [21]. Under climate warming scenario, open-habitat species have a potential to explore novel habitat types in the forest interior by adjusting their microhabitat use while forest-dwellers have a little chance of doing so because they already occupy the coolest available microhabitats [21]. Despite many obvious advantages of behavioural plasticity as a fast way to respond to environmental changes [4], behavioural thermoregulation could also limit physiological adaptation, which is necessary for species' long-term survival [6, 22, 23]. This so called Bogert effect has not yet been conclusively proven, but represents an important possibility with implications for predicting climate change effects.

Organisms living in high altitudes were among the first to attract considerable attention to the issues of species survival under climate change because of apparent range shifts along the altitudinal gradient [1, 24, 25]. However, recent studies report that lowland fauna seems to be even more sensitive to ongoing climate change then mountain one. Detailed studies of internal traits such as immune system [26], thermoregulation [21] or thermal limits [27] surprisingly report higher sensitivity of lowland species to increasing temperatures. Moreover, also extrinsic factors such as existence of extremely diverse (micro)habitats and topographies [2, 14, 28] or feasibility of uphill shifts along the altitudinal gradient [2], support long-term survival of species in mountains. On the contrary, lowlands provide more homogeneous environment, in addition, they are fully exposed to human activities such as agriculture, forestry or urbanization. In concordance, large comparative study by [2] showed that mountain assemblages are more stable, i.e. less vulnerable, than lowland ones in relation to climate change.

Here, we focus on thermal ecology and microclimate utilisation at high- and low-altitude margins of occurrence in adult butterflies of the genus *Erebia*. Alpine *Erebia* species are well adapted to extreme high-altitude environment, where they are able to maximize activity during short periods of favourable weather and they do not seem to be at risk of overheating in a warming environment [14]. However, the situation in aberrant lowland species of this mostly mountain genus is not known. Thus, we examined thermoregulatory strategies in adults of two lowland species at upper thermal margins of their occurrence and of a low-altitude mountain population of a montane-belt species. We compared our findings with data on seven alpine species reported in [14]. We report considerable differences in body temperatures, microhabitat utilisation, and contrasting temperature-dependence of different activities between low- and high-altitude butterflies. We discuss the role of behavioural thermoregulation for coping with ongoing climate change and conclude by a prediction that lowland species of *Erebia* butterflies will face more serious threat than their alpine relatives.

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Materials and Methods

Study group and study sites

The genus *Erebia* Dalman, 1816 is a popular group in studies of eco-physiological adaptations [14, 29] and biogeography [30–32] as well a subject of increasing interest from the conservation perspective [14, 18, 33–36]. In our study, we compared thermoregulation of seven alpine species occurring at high altitudes in European Alps (see [14] for more details) with thermoregulation of a low-altitude population of a mountain species *E. euryale* (Esper, 1805) and two aberrant lowland species, *E. aethiops* (Esper, 1777) and *E. medusa* (Fabricius, 1787).

Mountain species The seven alpine species *Erebia alberganus* (Prunner, 1798), *E. euryale* (Esper, 1805), *E. ligea* (Linnaeus, 1758), *E. melampus* (Fuessly, 1775), *E. montana* (de Prunner, 1798), *E. pandrose* (Borkhausen, 1788) and *E. cassioides* (Reiner and Hohenwarth, 1792) were studied in Austrian Alps, Tirol close to the town of Sölden in an altitude 1500-1800 m a.s.l. (see [14] for more details). All these species occur in montane and alpine vegetation belts [37]. Another, low-altitude population of *E. euryale* was studied in Šumava Mts., Czech Republic, close to Borová Lada village, in the area of a former village Zahrádky (13.68032°E,48.97362°N, altitude 930 m a.s.l.). *E. euryale* inhabits clearings and road margins within spruce forest in the area and was frequently observed nectaring on nearby pastures. The species *E. euryale* was thus represented by two populations; by a high-altitude population from Austrian Alps (*E. euryale*-Alps) and by a low-altitude population from Šumava Mts., Czech Republic (*E. euryale*-CZ).

Lowland species *E. aethiops* and *E. medusa* inhabit a rather wide range of altitudes and occur also in mountains and sub-alpine zone [37]. Both lowland species originated during Miocene diversification of a European clade of the genus *Erebia* and they are members of a phylogenetically distinct species group [32]. Their conservation status is Least Concern for Europe [38], but both species experienced local declines throughout their ranges [39, 40] attributed to habitat alteration [18, 36]. Moreover, both species are hypothesized to be negatively affected by ongoing climate change [17, 41]. We studied their lowland populations in an ex-military area Vyšný in the vicinity of a town Český Krumlov, SW Czech Republic (48°49'N, 14°1'E, altitude 550 m). The area is formed by a mosaic of dry grasslands, shrubs and woodlands. Both species co-occur in this subxerotherm area, probably on their upper thermal limits. *E. aethiops* (flight period: mid-June to August) is a woodland species, which inhabits forest edges, small grassland patches and sparse woodlands within the area [17, 18], while *E. medusa* (flight period: May to mid-July) is found in more open habitats - xeric grasslands with scattered shrubs.

Measurements of body, microhabitat and air temperatures

Butterfly body temperature T_b was measured by a hypodermic micro-needle probe (0.3 mm in diameter) within 5 seconds after capture and values were recorded on Physitemp thermometer (model BAT-12) during August 2010 (*E. aethiops*), May 2012 (*E. medusa*), July 2012 (*E. aethiops*, *E. euryale*-CZ) and August 2012 (*E. euryale*-CZ). The alpine species were measured during their flight periods in 2010 and 2011 (see [14]). Butterflies were collected by a net during random walks within the study area during the entire daytime activity period (between 9 a.m. and 6 p.m.). During a measurement, a butterfly was shaded and the microprobe was inserted into its thorax. The same thermometer was used to measure microclimate T_m and air T_a temperatures. Microclimate temperature T_m was measured immediately after T_b measurement at the

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place where the butterfly was located before the capture (approximately 3 mm above substrate in the case of sitting or nectaring butterflies). Air temperature T_a was measured at 1.5 m above ground by a shaded thermometer. Further, we classified individual behaviour prior to capture into three categories: sitting, nectaring and flight. All data are on Czech butterflies are available in Supplementary File S1, data from Austria are available in [14] (Appendix A).

Data analyses

The aim of our analyses was to compare thermoregulation of related butterflies occurring in mountains and in lowlands. All analyses were conducted in R 3.0.2 [42]. First, we tested the dependence of body temperature T_b on microhabitat T_m and air T_a temperatures using Generalized Additive Models (GAMs) with cubic splines and maximal complexity of the fitted relationship set to $k = 5$ d.f. in mgcv 1.8-4 package for R [43]. The two relationships ($T_b \sim T_a$ and $T_b \sim T_m$) were fitted for individual species (lowland and alpine populations of *E. euryale* were analysed separately). Next, to obtain standardized measures of species thermoregulatory abilities, we defined two related variables: body-to-air temperature excess $T_b - T_a$ and body-to-microhabitat temperature excess $T_b - T_m$. Then, we modelled the dependence of $T_b - T_a$ and $T_m - T_a$ on centered values of T_a and T_m, respectively, by GAMs, again separately for the two *E. euryale* populations and the two lowland species. The values of T_a and T_m were centered by subtracting the mean values of T_a and T_m, respectively, to ensure biologically meaningful intercepts in the analyses (without centering, the intercepts would show the air or microhabitat temperature excess when T_a or T_m, respectively, is zero) and to remove possible slope–intercept correlation (see [14] for more details). Data on alpine species reported by [14] were re-analysed the same way to facilitate comparison with the lowland species (original conclusions of [14] were not affected).

We also searched for differences in active behavioural thermoregulation between lowland and mountain species. We estimated the difference between microhabitat and air temperatures $T_m - T_a$ for settling and nectaring individuals (i.e. potentially actively thermoregulating) of each low-altitude and mountain species (separately for the two populations of *E. euryale*). Then, we used t-tests to test whether lowland species and *E. euryale* population also search for similarly warm microclimates as the alpine species [14].

Last, we tested how the proportion of settling, nectaring and flying individuals for individual species depends on air temperatures T_a using GAMs with quasibinomial distribution. This analysis was conducted for the three types of behaviour and all lowland and mountain species/populations separately.

Results

The two lowland species, *E. medusa* and *E. aethiops*, experienced higher maximal and higher minimal air temperatures T_a than the seven mountain species studied (Fig. 1, Fig. 2A, Table 1). Low-altitude population of *E. euryale*-CZ experienced similar maximal air temperatures T_a but ca 5°C higher minimal air temperatures T_a compared to the alpine population of *E. euryale* (Fig. 2A). In spite of these differences in experienced air temperatures T_a, experienced maximal microhabitat temperatures T_m did not differ in species across different altitudes (Fig. 2B). Thus, alpine species were able to detect warm microclimate to compensate for the experienced low air temperatures. Minimal microhabitat temperatures T_m were higher in the lowland species corresponding to generally warmer lowland environment. Body temperatures T_b increased approximately linearly with increasing air temperatures T_a as well as with

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increasing microhabitat temperatures T_m in all species (Fig. 1, Fig. 2A, 2B, Table 2). However, low altitude population of *E. euryale*-CZ and *E. aethiops* kept lower body temperatures T_b at similar air temperatures T_a than all other species, i.e. species from the Alps and also lowland *E. medusa* (Fig. 2A, 2B). Especially *E. aethiops* kept its body temperature only several °C above the air temperature T_a and had T_b almost equal to T_a at high T_a values (Fig. 1).

Table 1. Overview of temperature data. Summary of measured values for the two lowland species *Erebia aethiops* and *E. medusa* and for low- and high-altitude populations of *E. euryale* are shown; data for the remaining alpine congeners are available in [14]. $N(F, M)$ = the number of indiviudals measured (females, males); T_a = air temperature; T_m = microhabitat temperature; T_b = body temperature.

Species	**Location**	**Flight period**	$N(F, M)$		T_a	T_m	T_b
E. aethiops	Czech Republic; lowland	July - mid August	161 (33, 128)	mean	23.44	23.60	29.42
				median	23.10	23.80	29.80
				range	16.15-31.15	15.6-32.00	19.30-37.20
E. euryale-CZ	Czech Republic; lowland	July - mid August	104 (33, 71)	mean	22.80	23.09	29.36
				median	23.05	23.30	29.05
				range	18.80-26.35	18.30-29.50	22.20-36.00
E. euryale-Alps	Austria; timberline, the Alps	July - mid August	254 (79, 175)	mean	19.67	21.02	30.57
				median	20.00	21.00	30.95
				range	12.00-25.00	11.20-31.00	22.00-38.30
E. medusa	Czech Republic; lowland	May - June	154 (19, 135)	mean	24.70	25.80	34.14
				median	25.11	25.40	33.90
				range	19.28-29.82	18.10-32.60	28.10-39.20

The inter-species thermoregulatory differences become more obvious if we compare the dependence of body-to-air temperature excess $T_b - T_a$ and body-to-microhabitat temperature excess $T_b - T_m$ on air T_a and microhabitat T_m temperatures, respectively. The body-to-air temperature excess $T_b - T_a$ at similar air temperatures T_a was lower in lowland *E. aethiops* and *E. euryale*-CZ compared to species from the Alps and lowland *E. medusa* (Fig. 2C). This suggests that the lowland population of *E. euryale*-CZ and *E. aethiops* had lower optima of body temperature and heated up less effectively, i.e. that they had lower tendency to heat up at given air temperatures T_a than the other species. Contrary, lowland *E. medusa* had thermoregulatory characteristics similar to the alpine species. Dependence of the body-to-air temperature excess $T_b - T_a$ on air temperature T_a was nearly linear in species from the Alps and in *E. medusa* (Fig. 2). These species increased their body temperatures more at low than at high air temperatures, which suggest active thermoregulation by microclimate choice or body posture adjustment under low air temperatures. In *E. euryale*-CZ, the body-to-air temperature excess decreased with growing temperature linearly, but at high air temperatures, we observed abrupt enhancement of the body-to-air temperature excess; this might be a sign of overheating but the number of observations at high temperatures was rather low. In *E. aethiops*, we observed a hump-shaped dependence of the body-to-air temperature excess on air temperature. It slowly increased its body-to-air temperature excess $T_b - T_a$ in a range of air temperatures 15-20°C and, after reaching the optimum around $T_a = 21$°C, its body-to-air temperature excess $T_b - T_a$ decreased again (Fig. 2C). It seems, that *E. aethiops* thus has a narrow temperature range for activity compared to the other species.

Similarly as we observed for the dependence of the air temperature excess $T_b - T_a$ on air temperatures T_a, *E. aethiops* showed a hump-shaped dependence of its microhabitat temperature excess $T_b - T_m$ on microhabitat temperatures T_m it experienced. The relationship between $T_b - T_m$ and T_m in the remaining species also paralleled our

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findings about the dependence of $T_b - T_a$ on T_a (Fig. 1, Fig. 2, Table 2), which shows that the relationships reported did not arise from temperature-dependent microhabitat choice.

Regarding the thermoregulatory behaviour during sitting or nectaring, *E. aethiops* and the low-altitude population of *E. euryale*-CZ did not search for warm microclimates as much as the other species did (Fig. 3, Table 3). In *E. medusa*, the difference between microhabitat and air temperatures $T_m - T_a$ during sitting and nectaring was more similar to the alpine species. Moreover, *E. medusa* explored microhabitat temperatures up to 32°C (Fig. 3C) and thus, it has the highest thermal tolerance to high temperatures from all species studied. Except of *E. aethiops*, *Erebia* species searched for warmer microclimates then ambient air temperatures, i.e. $T_m > T_a$ (Fig. 3). *E. aethiops* explored microhabitats which were similarly warm as the air (Fig. 3B), suggesting heat-avoidance behaviour. High-altitude population of *E. euryale* searched for microhabitats on average 2.00 °C warmer than the air, while its low-altitude population was found in microhabitats on average only 0.64 °C warmer then the air.

Proportion of settling, nectaring or flying individuals was dependent on air temperature T_a at least for one of these behavioural categories in *E. aethiops*, *E. euryale*-Alps, *E. ligea*, *E. montana* and *E. medusa* (Table 3, Fig. 4). Settling (i.e. various forms of basking behavior and resting) was the most frequent behaviour under low air temperatures (Fig. 4). Frequency of nectaring increased with increasing air temperatures in species from the Alps, but displayed hump shape in *E. aethiops* and *E. medusa*. Frequency of flying was nearly constant in all species, with the exception of *E. aethiops* which displayed increase of the proportion of flying individuals with increasing air temperature.

Discussion

Thermoregulatory strategies at thermal margins

Behaviour has a potential to modify physiological responses to evolutionary pressures such as ongoing changes of climate [6, 44, 45]. Our results demonstrate that *E. aethiops* and *E. medusa*, two aberrant butterfly species of mostly cold-dwelling butterfly genus, diversified in their thermoregulatory strategies and also in their tolerance to high-temperatures, in *E. medusa*, or the lack of it, in *E. aethiops* (Fig. 2). We hypothesize that this differentiation of thermoregulatory strategies was driven by contrasting habitat preferences of the two lowland *Erebia* species, similarly as has been observed for Mediterranean cicadas [46, 47], tropical lizards [21] or mountain species of the genus *Erebia* [14]. *E. aethiops*, which inhabits heterogeneous forest-steppe environment [18], did not actively enhance its body temperature and suffered by overheating (see also [17]). Contrary, *E. medusa*, a grassland species, flourished in warm climate of the subxerotherm locality. It effectively heated up under low air temperatures and linearly enhanced its body temperature up to 39°C; i.e., higher than body temperatures of all its congeners we studied (see [14]). These differences illustrate that species with ancestral cold-climate preference [32] have the potential to adjust to environmental conditions in warm lowlands and that behavioural traits, such as habitat use, determine species thermal limits and affect their future perspectives under predicted climate warming.

Small ectotherms have to rely on behaviour such as shade seeking or decrease of activity to avoid overheating at their thermal margins [4]. *E. aethiops*, the species suffering by overheating, was frequently observed flying in the shadow of trees during the warmest part of the day [17]. In congruence, it was the only one of all *Erebia* species studied which showed a lack of selectivity for microhabitats warmer than air

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Table 2. Body temperature depends on air and microhabitat temperatures. Results of generalized additive models (GAM) testing the dependence of body-to-air temperature excess $T_b - T_a$ on air temperature T_a and body-to-microhabitat temperature excess $T_b - T_m$ on microhabitat temperature T_m for individual *Erebia* species. The fitted relationships are visualized in Fig. 1 and Fig. 2. Estimated d.f. describes the complexity of the fitted relationship; e.d.f. = 1 for a linear relationship, e.d.f. >1 for a non-linear relationship.

Dependence type and species	Location	Deviance explained (%)	Estimated d.f.	F	p-value
$\boldsymbol{T_b \sim T_a}$					
E. aethiops	lowland	72.1%	3.508	105.2	$< 10^{-6}$
E. medusa	lowland	66.80%	1	305.7	$< 10^{-6}$
E. euryale-CZ	lowland	29.8%	3.376	10.21	$< 10^{-6}$
E. euryale-Alps	alpine	38.60%	3.122	42.9	$< 10^{-6}$
E. alberganus	alpine	33.80%	1.632	28.28	$< 10^{-6}$
E. ligea	alpine	41.00%	3.38	34	$< 10^{-6}$
E. melampus	alpine	27.5%	1.773	25.96	$< 10^{-6}$
E. montana	alpine	35.70%	2.905	23.33	$< 10^{-6}$
E. pandrose	alpine	49.20%	3.381	25.49	$< 10^{-6}$
E. cassioides	alpine	32.4%	3.066	16.69	$< 10^{-6}$
$\boldsymbol{T_b \sim T_m}$					
E. aethiops	lowland	76.80%	3.523	133.5	$< 10^{-6}$
E. medusa	lowland	66.6%	3.05	83.98	$< 10^{-6}$
E. euryale-CZ	lowland	36.4%	1.784	25.64	$< 10^{-6}$
E. euryale-Alps	alpine	49.2%	1	244.4	$< 10^{-6}$
E. alberganus	alpine	37.9%	1	69.01	$< 10^{-6}$
E. ligea	alpine	37.3%	1	115	$< 10^{-6}$
E. melampus	alpine	37.4%	3.472	23.45	$< 10^{-6}$
E. montana	alpine	34.9%	2.156	27.79	$< 10^{-6}$
E. pandrose	alpine	47.10%	2.01	35.76	$< 10^{-6}$
E. cassioides	alpine	37.60%	3.451	20.17	$< 10^{-6}$
$\boldsymbol{(T_b - T_a) \sim T_a}$					
E. aethiops	lowland	34.60%	3.508	21.55	$< 10^{-6}$
E. medusa	lowland	27.70%	1	58.17	$< 10^{-6}$
E. euryale-CZ	lowland	19.8%	3.376	5.568	0.0006
E. euryale-Alps	alpine	31.10%	3.122	30.69	$< 10^{-6}$
E. alberganus	alpine	23.1%	1.632	16.5	$< 10^{-6}$
E. ligea	alpine	49.30%	3.38	47.99	$< 10^{-6}$
E. melampus	alpine	13.90%	1.773	10.73	2.7*10-5
E. montana	alpine	17.2%	2.905	8.604	1.4*10-5
E. pandrose	alpine	17.30%	3.381	5.527	0.0007
E. cassioides	alpine	39.1%	3.066	22.7	$< 10^{-6}$
$\boldsymbol{(T_b - T_m) \sim T_m}$					
E. aethiops	lowland	27.20%	3.523	14.39	$< 10^{-6}$
E. medusa	lowland	45.7%	3.05	35.16	$< 10^{-6}$
E. euryale-CZ	lowland	6.37%	1.784	2.605	0.0728
E. euryale-Alps	alpine	37.2%	1	149.2	$< 10^{-6}$
E. alberganus	alpine	29.70%	1	47.79	$< 10^{-6}$
E. ligea	alpine	39.8%	1	127.5	$< 10^{-6}$
E. melampus	alpine	31.80%	3.472	18.3	$< 10^{-6}$
E. montana	alpine	44.2%	2.156	41.42	$< 10^{-6}$
E. pandrose	alpine	51.30%	2.01	42.45	$< 10^{-6}$
E. cassioides	alpine	69.2%	3.451	75.58	$< 10^{-6}$

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temperature (Fig. 3). This suggests heat-avoidance behaviour in *E. aethiops*. Furthermore, *E. aethiops* was the only *Erebia* species in which the proportion of flying individuals increased at the highest air temperatures (Fig. 4). We argue that this behaviour represents a tendency to actively decrease its body temperature by convective cooling in the shadow, which follows e.g. nectaring on warm sun-exposed flowers. In other words, its individuals probably had to fly more frequently between warm (to search for nectar sources or mates) and cold microhabitats (to decrease their body temperatures). During the lowest air temperatures experienced (15-20°C), *E. aethiops* mainly settled on vegetation. These air temperature are suitable for activity of the other Erebia species; alpine relatives were found in flight at temperatures as low as 12°C [14]. We argue, that settling during temperatures potentially suitable for activity was conditioned by resting in a colder microclimate under shrubs and trees [17] and by the absence of behavioural enhancement of body temperature in this species. *E. aethiops* started to nectar at mid-range of experienced temperatures (20-25°C) in congruence to the temperatures in which other *Erebia* species maximized their nectaring activities. However, both lowland species experienced much higher maximal temperatures (around 30°C) than their mountain congeners (Fig. 4). The extreme temperatures obviously limited nectaring of both lowland species. *E. aethiops* reached the peak of its nectaring activity around 24°C and *E. medusa* around 26°C; again, this difference between the lowland species should reflect their different thermal tolerances. The decrease of nectaring activities of the lowland species during the warmest periods of the day could be caused by desiccation of nectar or by the need of the butterflies to prevent overheating. Thus, the timing of diurnal activities contributes to the *Erebia* thermoregulation together with microhabitat choice [17, 48] and facilitates species survival on their range margins.

Behavioural thermoregulation and adaptation to climate changes

Long-term persistence in changing climate requires adaptation; i.e. adjustments of physiological mechanisms responsible for thermal tolerance of species. It is possible that behavioural thermoregulation, which is a potent short-term solution for dealing with a changing climate, constrains the adaptive potential of such plastic species [6, 22, 23]. Buckley et al. [6] provided evidence of this, so called Bogert effect, in lizards where they showed that populations from different parts of the lizard's distribution have conserved preferred body temperature and thermal minima and maxima. This suggests that the lizards have limited potential to physiologically adapt to climate changes because their efficient behavioural thermoregulation buffers the selective pressure [6]. *Erebia* butterflies also thermoregulate behaviourally but their thermoregulatory strategies and preferred body temperatures are not conserved according to our data; i.e. individual species have different thermoregulatory requirements and we found no support for the Bogert effect in *Erebia*. This is indicative of high potential for evolutionary responses to climate change within the genus. In their detailed study of Anolis lizards, Munoz et al. [44] found that cold tolerance evolves faster than heat tolerance because selection on upper thermal limits is weakened by active thermoregulation more than selection on lower thermal limits. A possible explanation for the lack of conservatism of thermal ecology of *Erebia* species is that they need to feed on nectar and flowering plants are typically abundant in open sunny habitats which offer limited possibility for behavioural thermoregulation. This should create a selective pressure on the evolution of tolerance to higher temperatures in a warming climate. Moreover, butterflies of the genus *Erebia* conserved their extraordinary diversity in unstable temperate areas during extreme climatic fluctuations of Pleistocene [32] and thus, evolutionary plasticity of their

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Table 3. Proportion of settling, nectaring, and flying *Erebia* butterflies depends on air temperature. Results of generalized additive models (GAM) testing the dependence of the proportion of settling, nectaring and flying individuals of butterflies of the genus *Erebia* on the air temperature Ta. The fitted relationships are visualized in Fig. 4. Estimated d.f. describes the complexity of the fitted relationship; e.d.f. = 1 for a linear relationship, e.d.f. >1 for a non-linear relationship.

Species	Location	Deviance explained (%)	Estimated d.f.	F	p
Settling					
E. aethiops	lowland	5.50%	1.799	4.723	0.0108
E. medusa	lowland	1.54%	1.541	1.208	0.2890
E. euryale-CZ	lowland	0.14%	1.004	0.140	0.7110
E. euryale-Alps	alpine	4.86%	1.798	7.224	0.0010
E. alberganus	alpine	0.05%	1	0.056	0.8130
E. ligea	alpine	17.60%	1.771	16.07	$< 10^{-5}$
E. melampus	alpine	0.73%	1	1.117	0.2920
E. montana	alpine	7.58%	1.329	7.584	0.0024
E. pandrose	alpine	3.57%	1	3.463	0.0656
E. cassioides	alpine	1.21%	1	1.599	0.2080
Nectaring					
E. aethiops	lowland	8.84%	1.912	4.46	0.0132
E. medusa	lowland	23.70%	1.906	4.933	0.0085
E. euryale-CZ	lowland	0.08%	1	0.081	0.7770
E. euryale-Alps	alpine	5.31%	1	12.52	0.0005
E. alberganus	alpine	3.63%	1.744	1.316	0.2690
E. ligea	alpine	8.10%	1.299	10.64	0.0003
E. melampus	alpine	2.62%	1.72	1.168	0.3080
E. montana	alpine	9.22%	1.038	10.79	0.0011
E. pandrose	alpine	0.00%	1	0	1.0000
E. cassioides	alpine	0.45%	1.181	0.279	0.6640
Flight					
E. aethiops	lowland	4.22%	1	6.593	0.0111
E. medusa	lowland	0.40%	1	0.604	0.4380
E. euryale-CZ	lowland	0.22%	1	0.221	0.6400
E. euryale-Alps	alpine	2.39%	1.756	2.174	0.1170
E. alberganus	alpine	0.02%	1	0.027	0.8700
E. ligea	alpine	0.46%	1.253	0.153	0.7860
E. melampus	alpine	1.26%	1	1.922	0.1680
E. montana	alpine	0.10%	1	0.138	0.7110
E. pandrose	alpine	3.57%	1	3.463	0.0656
E. cassioides	alpine	1.38%	1	1.809	0.1810

thermal niches is a plausible mechanism facilitating their long-term survival.

Changing climatic conditions affect phenotypes of local populations [49] or higher taxonomical units [10, 21]. In *Erebia* butterflies, thermoregulation was affected also by local adjustments of geographically segregated populations. We noticed differences in thermal niches among the two populations of *E. euryale*. The low-altitude population *E. euryale*-CZ (900m a.s.l.) kept lower body temperatures T_b than the high-altitude population *E. euryale*-Alps (1800m a.s.l.) (Fig. 2). This suggest, that the lowland population is more "lazy" in its effort to heat up and waits with its activities for periods of the day with optimal air temperature. In a similar case in grasshoppers, Samietz et al. [50] found that individuals increased their body temperatures via mobility and

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basking more at higher altitudes and demonstrated that this was due to local adaptation. The observed difference between *E. euryale* populations could be caused by local adaptation or by phenotypic plasticity. Transplant or laboratory experiments are necessary to resolve the relative contributions of these interacting factors [50, 51]. Phenotypic plasticity of thermoregulaory mechanisms should facilitate species survival in unstable conditions, such as in temperate mountains during climatic oscillations, but on the other hand, it could block speciation and evolution of the thermal niche [6]. Local adaptation contributed to the evolution of thermoregulatory specialization in ectotherms such as lizards [21, 44, 52], grasshoppers [50] or butterflies [53, 54]. It is likely that this mechanism operates also in *Erebia*. It is important to note that main selective agents driving the evolution of thermoregulatory strategies are temperature extremes, rather than long-term means. In their review, [55] concluded that maximal temperatures seem to be more limiting than minimal temperatures in terrestrial ectotherms. In congruence, we noticed limitation by high ambient temperatures in *E. aethiops* and *E. euryale* adults. However, the effect of high temperatures may differ across developmental stages. The overwintering stages, larvae, are more likely to be affected by other factors associated with climate change such as changes of snow cover [29, 35]. The role of local adaptation and plasticity across all developmental stages [56] will be an important focus of future studies.

Habitat heterogeneity and species survival in a changing climate

On the example of *Erebia* butterflies we demonstrated the potential of behaviour for dealing with climatic changes. *Erebia* butterflies kept their thermal optima mainly by shifts in microhabitat exploration, but also by shifts in timing of daily activities. However, their survival under changing climate is primarily determined by availability of various microhabitats providing variety of microclimates. In lowland butterflies, habitat heterogeneity provided colder microclimates, whereas mountain species explored warmer microhabitats to enhance their body temperature [14]. Woodland resident *E. aethiops* [18], is more endangered by a combination of landscape homogenization and climate change in comparison to alpine species or *E. medusa*. It seems plausible, that woodland species are generally more sensitive to high temperatures because of their lower thermal optima [14] and lack of opportunities for adjusting to warming of their environment behaviourally by microhabitat shifts, e.g. by more intensive shade seeking [21]. It could be expected that under climate warming, *E. medusa* would retract to woodland edges and to woodland patches which provide shade. Indeed, the species inhabits these habitats in some parts of its range [39]. It remains to be tested whether local habitat preferences correlate with local climate.

Because of its heat tolerance and potential for habitat shifts, *E. medusa* will likely be less endangered by overheating during ongoing climatic change than *E. aethiops*. Nevertheless, our study focused on thermal strategies of adult stages, but the knowledge of dormant stages (in butterflies represented by larvae, pupae or eggs), which can not behaviourally thermoregulate, is necessary to reveal main selective pressures [45, 57, 58]. Still, the behaviour of active stages could predetermine environmental conditions experienced by dormant stages. For example, in a climate-sensitive butterfly *Euphydryas editha*, different populations experienced diverse thermal environments because of differences in adult phenology and egg placement by ovipositioning females [13]. Similarly, in *Erebia* butterflies the location of oviposition sites corresponding to female habitat choice can predetermine the conditions experienced by overwintering larvae. To target conservation activities for particular species, detailed knowledge of requirements of all developmental stages is necessary [56].

Habitat heterogeneity is a key to successful conservation of individual species and entire communities, because it maximises the chance of ecthoterms to find suitable

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microclimate and also to obtain resources needed for their activities. Lowland species are at risk from high temperatures, but they could shift into more shaded places in forests. However, the structure of forests in intensively used European landscapes often does not provide the required resources because the forests are too closed [18]. Because of species-specific responses to changes of environmental conditions [1, 19], habitat heterogeneity should mitigate the effect of climate change at the level of whole communities. Thus, active management supporting habitat heterogeneity could facilitate species survival under climate change [59]. Whereas mountain landscape provides heterogeneity by its rugged terrain, especially in their subalpine and alpine zones, lowlands are more prone to landscape homogenization [60], both because of more flat terrain and higher human pressure. Increased conservation management efforts should thus focus on low-altitude margins of species occurrence in montane zones and lowland areas.

Supporting Information

S1 Text

Raw data. The file contains measurements of body temperature, air temperature, microhabitat temperature, and behaviour prior to capture of *Erebia aethiops*, *E. euryale*, and *E. medusa* studied in the Czech Republic.

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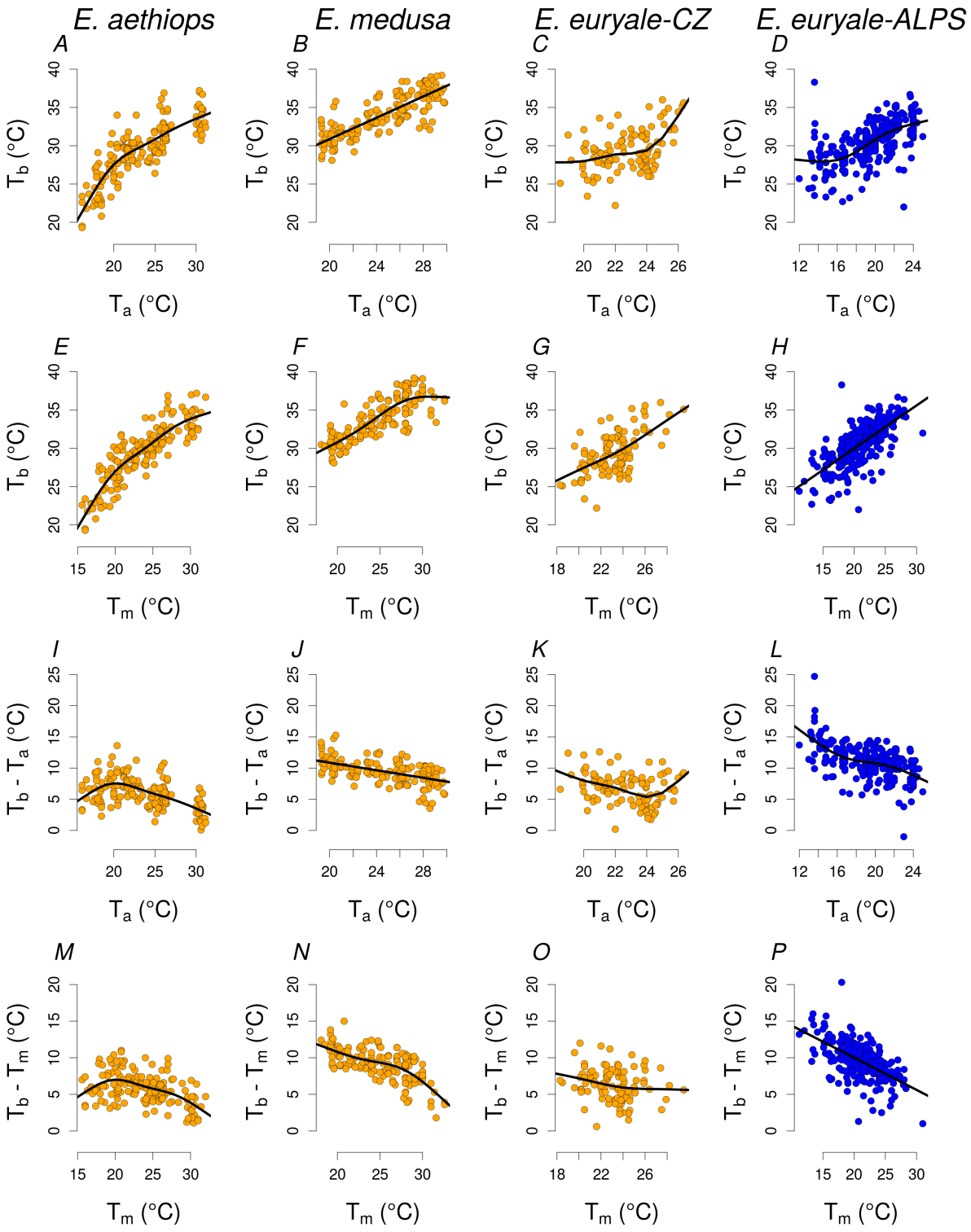

Figure 1. Body temperature depends on air and microhabitat temperatures. The relationship between body temperature T_b and air temperature T_a (A-D), body temperature and microhabitat temperature T_m (E-H), body-to-air temperature excess $T_b - T_a$ and T_a (I-L), and body-to-microhabitat temperature excess $T_b - T_m$ and T_m (M-P) in two lowland species of *Erebia* butterflies and in low- (*E. euryale*-CZ) and high-altitude (*E. euryale*-Alps) populations of a mountain species *E. euryale*. See Table 2 for detailed results of statistical tests.

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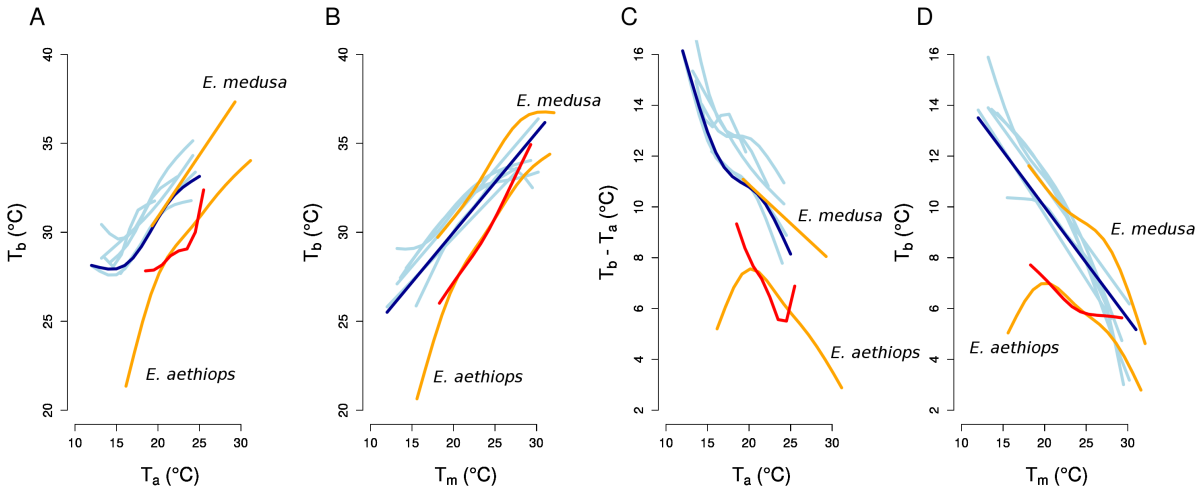

Figure 2. Comparison of body temperatures of lowland and alpine species and populations. The dependence of body-to-air temperature excess $T_b - T_a$ and body-to-microhabitat temperature excess $T_m - T_a$ on T_a and T_m , respectively, of low-altitude (orange lines) and high-altitude (blue lines) species of *Erebia* butterflies fitted by generalized additive models. Two populations of *E. euryale* are shown in red (low-altitude) and dark blue (high-altitude). Only the fitted lines are shown to facilitate comparison. T_a = air temperature, T_m = microhabitat temperature. See Table 2 for detailed results of statistical tests.

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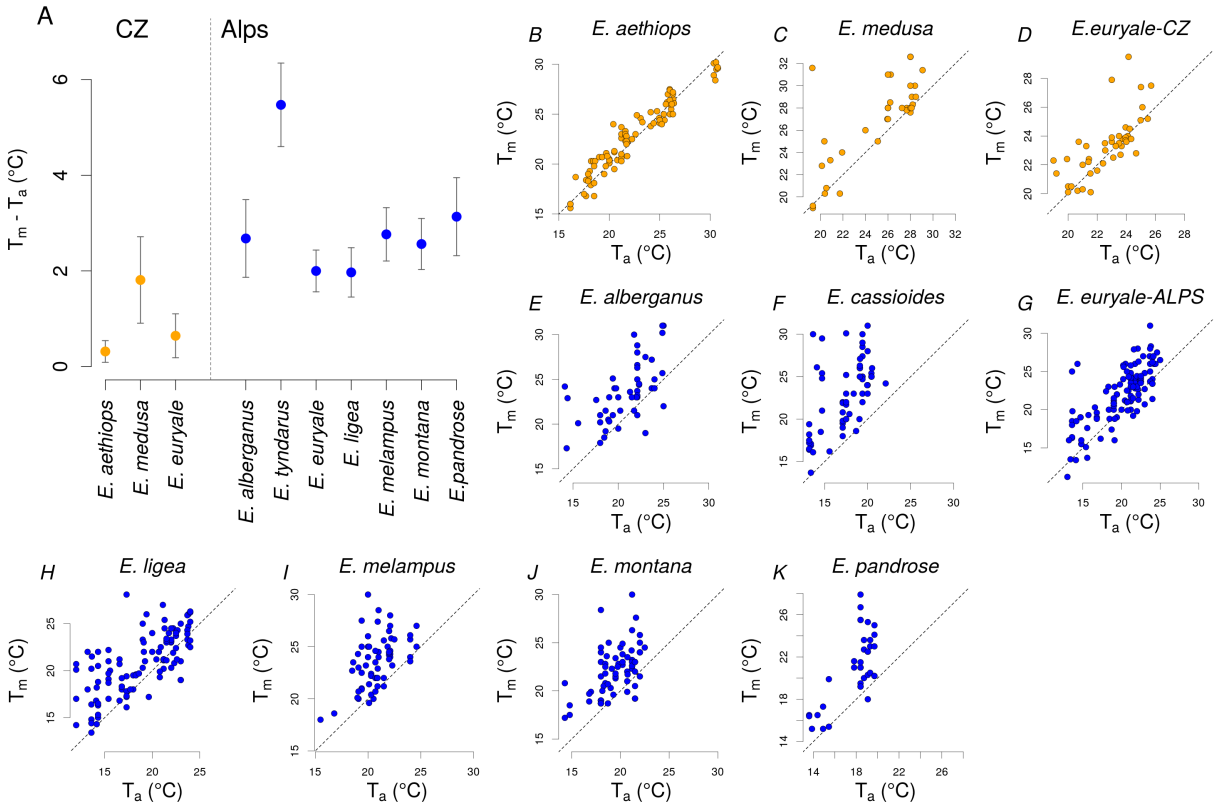

Figure 3. Behavioural thermoregulation is manifested by selectivity for microhabitats with temperature differing from the air temperature. Mean difference between microhabitat and air temperature $T_m - T_a$ for individual species; vertical bars denote 95% confidence intervals (A). Data for settling and nectaring individuals of lowland (orange circles) and mountain (blue circles) *Erebia* butterflies are shown. The dependence of microhabitat temperature T_m on air temperature T_a for individual species and populations of lowland (B-D) and alpine (E-K) butterflies. Dotted lines show $T_m = T_a$.

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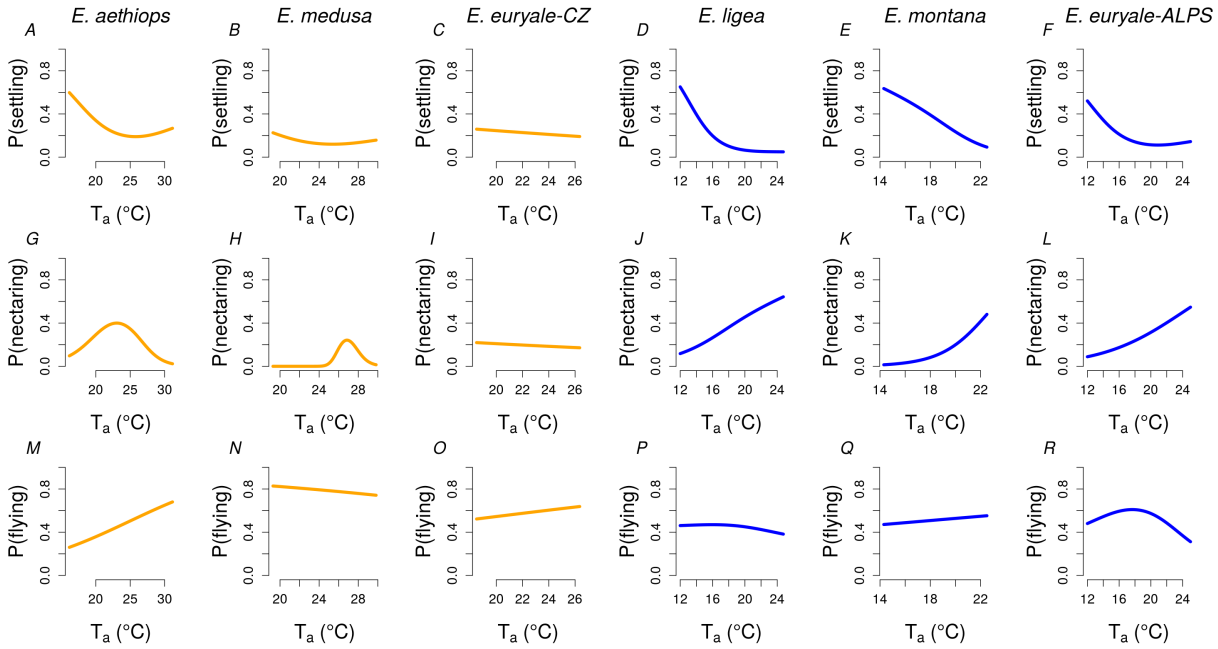

Figure 4. Main types of behaviour are affected by the air temperature. The dependence of the proportion of settling, nectaring and flying individuals on air temperature T_a in individual species of *Erebia* butterflies as estimated by generalized additive models (GAM). Only species with at least some significant trends are displayed; full results of the GAMs are shown in Table 3.