EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **113**: 571–578, 2016
doi: 10.14411/eje.2016.077
ORIGINAL ARTICLE

Variation of thorax flight temperature among twenty Australian butterflies (Lepidoptera: Papilionidae, Nymphalidae, Pieridae, Hesperiidae, Lycaenidae)

Gabriel NÈVE[1,2] **and Casey HALL**[3]

[1] Aix-Marseille Univ., Avignon Univ., IMBE, CNRS, IRD, 3 place Victor Hugo, 13331 Marseille Cedex 3, France;
e-mail: gabriel.neve@imbe.fr

[2] School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

[3] School of Biological Sciences, University of Adelaide, South Australia 5005, Australia; e-mail: casey.hall@adelaide.edu.au

Key words. Lepidoptera, Papilionidae, Nymphalidae, Pieridae, Hesperiidae, Lycaenidae, thorax flight temperature, wing loading, infra red thermometer, warming-up rate, physical constraint, allometry

Abstract. Thermal requirements for flight in butterflies is determined by a combination of external factors, behaviour and physical constraints. Thorax temperature of 152 butterflies was monitored with an infra-red thermometer in controlled laboratory conditions. The temperature at take-off varied from 13.4°C, for a female *Heteronympha merope* to 46.3°C, for a female *Junonia villida*. *Heteronympha merope,* an understorey species, had the lowest recorded take-off temperatures, with females flying at a much lower thorax temperatures than males. Among the tested butterfly species, warming-up rate was positively correlated with take-off temperature and negatively with body mass. Wing loading is a major variable in determining the thorax flight temperature. Butterflies with the highest wing-loadings experienced the highest thorax temperatures at take-off. A notable exception to this rule is *Trapezites symmomus*, the only Hesperiidae of our data set, which had thorax flight temperatures of 31.5°C and 34.5°C, well within the range of the observed butterflies, despite a wing load ca. five times higher. The high thorax temperature recorded in *J. villida* is probably linked to its high flight speed. The results highlight the importance of physical constraints such as body size on the thermal requirements for flight across a range of butterfly species.

INTRODUCTION

Butterflies, being facultative endotherms (Bartholomew, 1981), rely on both external factors and internally produced heat for the maintenance of their body temperature. The thorax temperature depends on ambient temperature, irradiant heat gained by basking behaviour and heat generated by muscle movements, while heat loss occurs through body irradiance and air convection around the body (Wickman, 2009). Thermoregulation in insects is therefore dependant ultimately on behaviour, either directly through shivering or basking, or indirectly by moving to or away from sunlit patches (Kingsolver, 1985a). Butterflies of the genera *Papilio*, *Colias* and *Pieris*, need a thorax temperature between 28 and 42°C for flight, while rigorous flight is restricted to thorax temperatures between 33 and 38°C (Kingsolver, 1985b; Srygley & Chai, 1990). The lowest recorded flight thorax temperatures are found in large species, such as *Parnassius phoebus*, which may fly with a thorax temperature of 17 to 20°C (Guppy, 1986).

The wing loading, defined as $p_w = m/S$, where m is the total individual mass and S the total wing surface is a major variable determining the energy required for insect flight (Heinrich, 1986). Compared with other insects, butterflies have much lower wing loading values, suggesting that wing size and shape in butterflies evolved not only for flight functions, but also for other functions such as thermoregulation, sexual behaviour, mimicry and camouflage (Dudley, 1991). Wing loading generally increases with body mass in butterflies (Heinrich, 1986; Dudley, 1990), but skipper butterflies (Hesperiidae) are heavier than other butterflies of the same wing surface (see below). Species with a high wing loading require faster wing beats, and hence a higher thorax flight temperature (Bartholomew & Casey, 1978). Thorax flight temperature in moths (Noctuidae and Geometridae) has been reported as correlated with wing loading (Bartholomew & Heinrich, 1973) or with mass (Casey & Joos, 1983). In butterflies, the data are sparse. As wing loading and mass are usually correlated, it is difficult to disentangle these two effects (Dudley, 1990; Heinrich, 1986).

The aims of this study were to document variation of thorax temperature among the studied species, and to test the effect of wing loading and mass on thorax flight temperature. Interspecific variation in thorax temperature needed for flight can have a significant impact on species fitness. For example, the ability to fly at lower temperatures may lead to more time available for dispersal and searching for mates and food (Kingsolver, 1983). Conversely, the more thorax temperature increases above ambient air temperature, the greater the risk of overheating, potentially leading to reduced survival and fitness. For example, in *Colias* butterflies, a thorax temperature of above 40°C causes individuals to cease flying to avoid overheating (Kingsolver & Watt, 1983). With the predicted increase in global temperatures and frequency of extreme temperatures, it is increasingly important to understand physical constraints on thermal tolerances which ultimately impact species fitness and dispersal ability.

Within a locality, the temperatures experienced by individual butterflies will largely depend on both their habitat preferences and timing of flight activity. Generally, species inhabiting forests will experience more stable conditions than species found in open habitats. A previous study, carried out in Mediterranean France (Nève, 2010), suggested that forest habitat species show lower thorax flight temperatures compared to open habitat species because of the lower temperature of the forest floor relative to open habitats. A secondary aim of the present study was to test this hypothesis with the available Australian species.

MATERIAL AND METHODS

A total of 152 butterflies were captured with hand nets (Upton & Mantle, 2010) in the field, 85 near Adelaide (mainly Bedford Park 35°01´S, 138°34´E and Wotton Scrub, 34°59´S, 138°47´E, South Australia) and 67 at Cedar Creek, 27°49´S, 153°11´E (Queensland) (Table 1); only *Pieris rapae* was collected at both locations. The tested specimens belonged to twenty species, mostly from open habitat, or linked with the canopy (e.g. *Graphium sarpedon*). Only four species were specialist understorey species (pers. observ., Braby, 2000): *Trapezites symmomus*, *Melanitis leda*, *Heteronympha merope* and *Nacaduba kurava*.

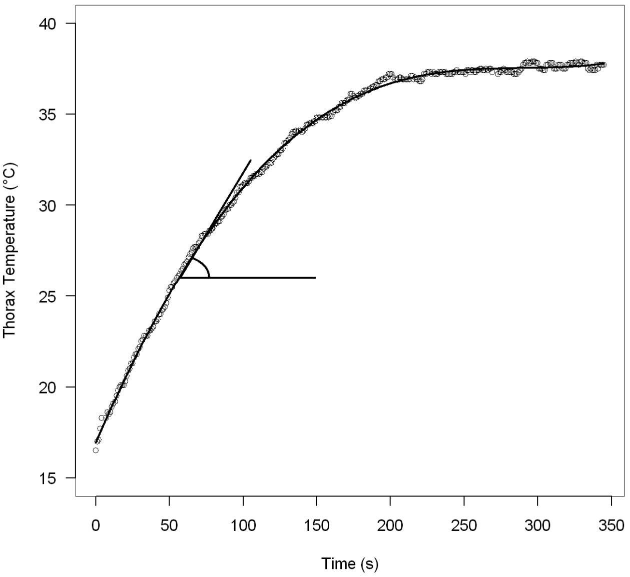

Fig. 1. Example of individual warming up process, here a male *Pieris rapae*. The solid line indicates the 4th degree function fitted to the raw data. Its derivative at y = 26°C gives the warming up rate at this temperature, here 0.13°C/s, indicated by the slope at this point.

Upon capture, specimens were put into glassine envelopes and cooled to ca. 11°C in a portable fridge. The same day, in an air conditioned room at 22 to 25°C lit with fluorescent lamps, each specimen was taken out of the fridge and placed on a piece of cotton under a 150 W neodynium daylight lamp, giving an irradiance of ca. 280 W/m² at the butterfly level. The thorax temperature of each specimen was measured with a testo®895 IR thermometer with the emissivity set at ε = 0.95, and the recorded temperature was automatically input into a computer database, one datum per second. The behaviour of individuals was reported as they were warming up, especially the basking behaviour, and the position of the wings. The take-off temperature was taken as the last temperature recorded before the butterfly took off. A 4-th degree curve was fitted to the raw data, and its slope was used to estimate the

Table 1. Numbers of individuals tested per species and sex, with measured total wing surfaces and weights.

Family	Subfamily	Species	State of Capture	Females	Males	Total	Mean total Wing surfaces (mm²)		Mean weights (mg)	
							Females	Males	Females	Males
Hesperiidae	Trapezitinae	*Trapezites symmomus* (Hübner, 1823)	Queensland	0	2	2		1086		340
Papilionidae	Papilioninae	*Graphium sarpedon* (Linnaeus, 1758)	Queensland	2	0	2	2200		200	
Papilionidae	Papilioninae	*Papilio aegeus* (Donovan, 1805)	Queensland	0	2	2		4259		399
Pieridae	Coliadinae	*Catopsilia pomona* (Fabricius, 1775)	Queensland	5	4	9	2202	1847	276	208
Pieridae	Coliadinae	*Catopsilia pyranthe* (Linnaeus, 1758)	Queensland	0	1	1		1400		116
Pieridae	Coliadinae	*Eurema brigitta* (Stoll, 1780)	Queensland	1	3	4	513	432	31	31
Pieridae	Coliadinae	*Eurema hecabe* (Linnaeus, 1758)	Queensland	3	3	6	488	581	43	26
Pieridae	Pierinae	*Pieris rapae* (Linnaeus, 1758)	Qld, Sth Austr.	1	20	21	1050	959	70	58
Pieridae	Pierinae	*Belenois java* (Linnaeus, 1758)	South Australia	0	2	2		1373		130
Nymphalidae	Satyrinae	*Melanitis leda* (Linnaeus, 1758)	Queensland	8	8	16	1972	1878	1163,4	113
Nymphalidae	Satyrinae	*Hypocysta adiante* (Hübner, 1831)	Queensland	1	0	1	529		33	
Nymphalidae	Satyrinae	*Hypocysta metirius* (Butler, 1875)	Queensland	1	2	3	355	461	22	16
Nymphalidae	Satyrinae	*Geitoneura klugii* (Guérin-Méneville, 1830)	South Australia	0	1	1		633		52
Nymphalidae	Satyrinae	*Heteronympha merope* (Fabricius, 1775)	South Australia	18	32	50	1935	1402	173	111
Nymphalidae	Nymphalinae	*Hypolimnas bolina* (Linnaeus, 1758)	Queensland	1	2	3	2791	2181	255	208
Nymphalidae	Nymphalinae	*Junonia villida* (Fabricius, 1798)	South Australia	11	3	14	908	777	103,6	83
Nymphalidae	Nymphalinae	*Vanessa kershawi* (McCoy, 1868)	South Australia	1	0	1	1086		127	
Nymphalidae	Biblidinae	*Phaedyma shepherdi* (Moore, 1858)	Queensland	1	0	1	1464		154	
Nymphalidae	Danainae	*Euploea core* (Cramer, 1780)	Queensland	3	3	6	2351	2158	268	237
Nymphalidae	Danainae	*Euploea tulliolus* (Fabricius, 1793)	Queensland	0	7	7		2076		173
Lycaenidae	Polyommatini	*Nacaduba kurava* (Moore, 1858)	Queensland	0	1	1		361		25

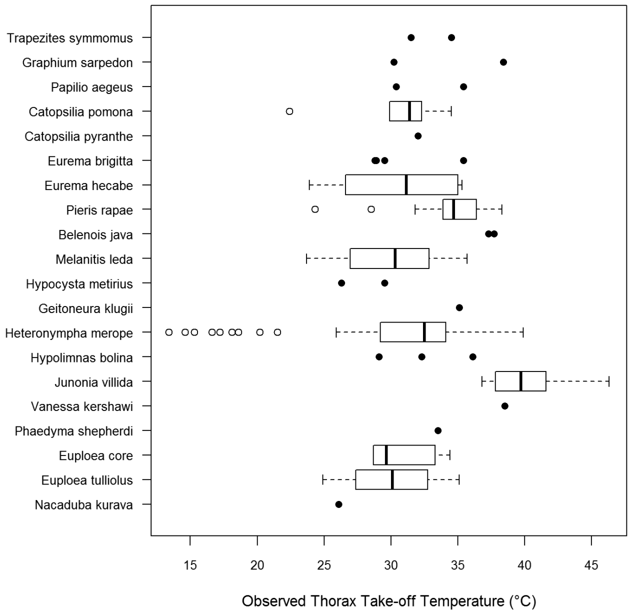

Fig. 2. Thorax take-off temperatures of twenty Australian butterfly species. The vertical lines indicate the median values, and the boxes the range from first to third quartile; dotted lines indicate extreme values if they fall within 1.5 times the interquartile difference, otherwise open circles indicates outliers. All data points are shown in filled circles for species with less than five data points.

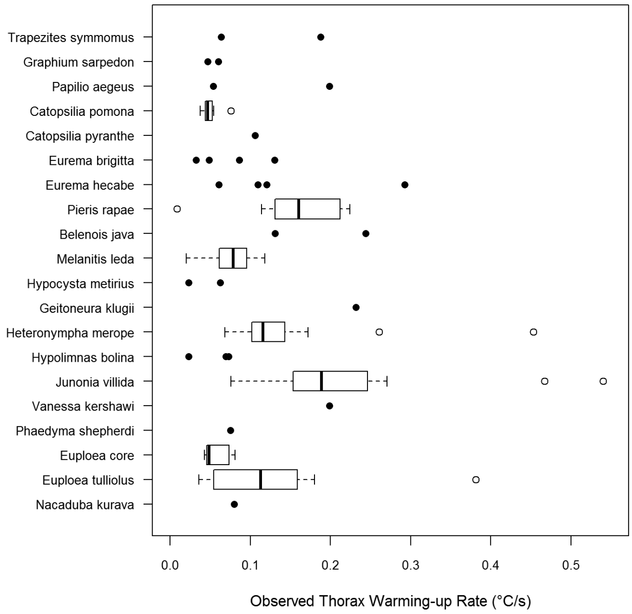

Fig. 3. Warming up speeds at 26°C for all tested Australian butterfly species. Symbols as in Fig. 2.

rate of warming, in °C/s (Fig. 1). The warming up rate at 26°C was used as an index of warming-up speed. This estimate was not possible on 21 specimens for which too few temperature points could be recorded during the warming-up process, hence the lower numbers of available warming-up rate estimates compared with available take-off temperatures.

As a control, three *Heteronympha merope* specimens were tested with a testo® thermocouple sensor set inside their thorax, at the same time as the outside thorax temperature was monitored. The temperature inside the thorax had a mean of 1.3°C warmer than the one measured on the surface, with a low variance of this difference (variance = 0.31).

After the warming-up experiment, each individual was weighed. Twenty-eight of the specimens were released after the experiment, while the rest were retained for references and measurements. These specimens were set with the trailing edge of the hind wing perpendicular to the thorax (Upton & Mantle, 2010). All 125 set specimens were photographed in a standard way with a 60 mm Macro Canon Lens, together with a scale, and the total wing surface was estimated for each specimen using ImageJ (Rasband, 2012). The area of overlap between the forewing and the hind wing is taken into account only once, as this area is effective as a single area in the wing loading of flying butterflies (Dudley, 1990). The wing loading for each specimen was estimated as the ratio of the weight divided by the total wing surface.

All statistical analyses were performed using R (R Development Core Team, 2011). Differences in thorax temperatures at take-off between males and were investigated using t-tests, however only two species (*Heteronympha merope* and *Melantis leda*) had adequate numbers of both males and females for testing. Linear regression was used to assess the relationship between warming up rate and take-off temperature both among species and also within four species. Linear regression was also used to assess the relationship between wing loading and take-off temperature among species. Allometry between wing loading p_w (in mm²) and body mass w (in mg) was studied directly using the R function *nls,* a nonlinear regression modelling function, by fitting the power function $p_w = \mathrm{b}\, w^a$ directly rather than through its logarithmic transformation (Zar, 1968).

A total of 126 *Pieris rapae* specimens had been subjected to the same experimental design in France (Nève, 2010), and direct comparisons with that data set were performed where appropriate, using Wilcoxon Signed Rank tests in R.

Voucher specimens have been deposited either in the private collection of Roger Kitching (Griffith University) for Queensland species, or at the South Australian Museum, Adelaide, for South Australian species and *Eurema* species (South Australian Museum Accession numbers 31-015517 to 31-015538). Details of capture locations, take-off temperatures, body weights, and wing surfaces are given in the supplementary Table S1.

RESULTS

The observed take-off temperatures varied widely among species, ranging from 13.4 to 46.3°C, with an among-species median value of 32.4°C (Fig. 2). The minimum temperature (13.4°C) was recorded for a *Heteronympha merope* specimen, and the highest (46.3°C) for a *Junonia villida* specimen. Warming-up rate at 26°C also varied widely among the twenty tested species, with the lowest values found in *Melanitis leda* (0.02°C/s) and *Hypolimnas bolina* (0.02°C/s) specimens. The highest value were found for *Juniona villida* (0.54°C/s) (Fig. 3).

The two Nymphalidae forest understorey species, *Melanitis leda* and *Heteronympha merope*, showed very similar take-off temperatures (*Melanitis leda*: mean = 30.2°C, *Heteronympha merope*: mean = 30.3, $t_{50} = 0.04$, $P = 0.97$; Fig. 4). In *Heteronympha merope*, females display a wider range of possible take-off temperatures, with a lower mean, than the males (females: from 13.4°C to 39.2°C, mean = 25.2°C +/– sd 7.9°C, n = 18; males: from 16.6°C to 39.9°C, mean = 33.2°C +/– sd = 3.9°C, n = 32). The difference in take-off temperatures between males and females of *H. merope* is highly significant ($t_{22} = -4.00$, $P < 0.001$).

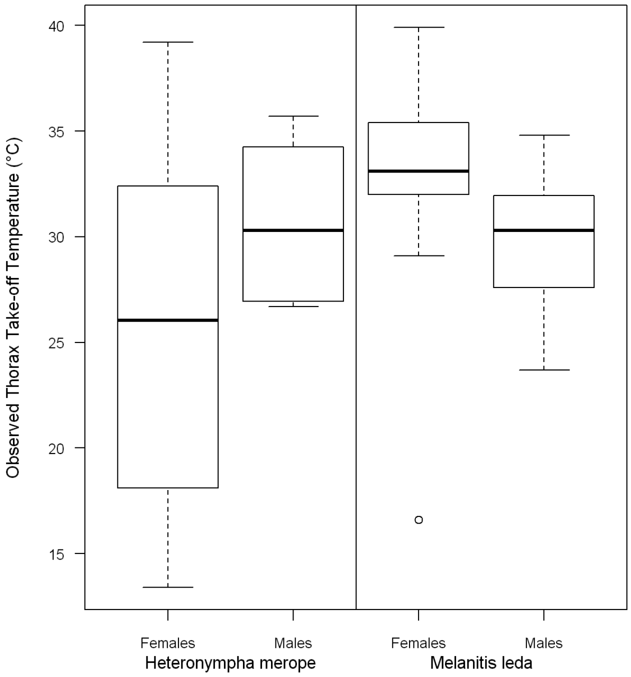

Fig. 4. Thorax temperatures at take-off for males and females of *Heteronympha merope* and *Melanitis leda*. The difference in temperatures between the sexes is highly significant in *H. merope*, but not in *M. leda*.

However, in *Melanitis leda*, there is no significant difference in take-off temperatures between the sexes (t_{14} = 0.49, P = 0.63).

The species with the highest recorded thorax temperature was *Juniona villida*, with temperatures ranging from 36.8°C to 46.3°C, implying an excess temperature (thorax temperature minus ambient temperature) of up to ca. 20°C. The three species with the highest recorded take-off thorax temperatures were *Juniona villida* (median = 39.7°C.), *Belenois java* (median = 37.5°C) and *Pieris rapae* (median = 34.7°C). The latter species is the only non-native species in our data set, having been introduced from Europe (Lokkers & Jones, 1999), allowing comparison with data from its natal range. Australian specimens of *Pieris rapae* flew with a temperature on average one degree warmer (median = 34.7°C, n = 20) than the ones from France (median = 33.7°C, n = 126; W = 821, $P < 0.05$, Fig. 5). Australian specimens also warm up more quickly (median = 0.16°C/s, n = 19) than French specimens (median = 0.12°C/s, n = 126, W = 618, $P < 0.001$).

The among species variation of warming-up rate did correlate significantly with take-off temperature (t_{18} = 4.89, $P < 0.001$, Fig. 6). When the species were looked at individually for the same relationship, among the ones for which at least ten individuals had been tested, only *Melanitis leda* showed such a positive relationship (t_{12} = 2.66, P = 0.02, Fig. 7).

With a value of 0.524 mg/mm^2, *Trapezites symmomus* had a much larger wing loading than all other species, which had values ranging from 0.035 mg/mm^2 (for *Hypocysta metirius* males) to 0.127 mg/mm^2 (for *Catopsilia pomona* females). *Trapezites symmomus* is also the only species in the data set to have a linear relationship between thorax temperature and time during the warming-up process, suggesting a different warming-up process than in other Lepidoptera lineages. *Trapezites symmomus* was then discarded from the data set for the analysis of the effect of wing load on take-off temperature. Among species variation showed that temperature at take-off was positively correlated with wing-loading (t_{17} = 2.28, P = 0.036, Fig. 8).

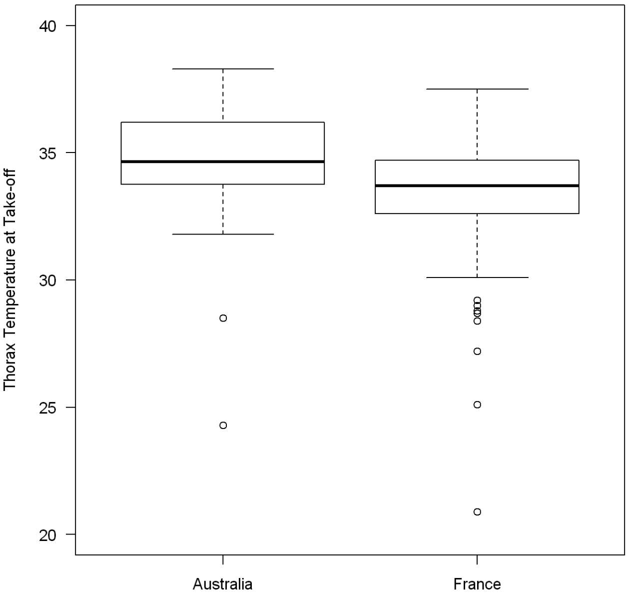

Fig. 5. Thorax take-off temperature for Cabbage White butterflies *Pieris rapae*, from Australia and France. Australian specimens have a take-off temperature on average 1°C warmer than the French specimens.

Wing loading p_w and body mass w were correlated by the allometric function $p_w = 0.030\ w^{0.225}$ (t = 3.86 and t = 4.31, P = 0.001 and $P < 0.001$; Fig. 9).

DISCUSSION

Very few temperature data on the studied species has been published before. Notable exceptions are *Papilio aegeus*, *Melanitis leda* and *Pieris rapae.* Heinrich (1986) gave thorax temperatures of 35°C for *Papilio aegeus* flying in the shade and 42.8°C when flying in the sunshine. Our data on this species shows lower values (30.4 and 35.4°C). Studies by Kemp (2002) gave inside thoracic temperatures of *Melanitis leda* males ranging from 28.2 to 38.9°C. Our data on the same species are lower, ranging from 23.7 to 34.8°C. Even allowing for the difference between inside and outside (about 1.3°C), our estimates in both cases remain lower, probably as a result of our laboratory conditions testing the thorax temperature from which flight is possible, rather the thorax temperature at which individuals usually fly or, in the latter case, perform their courtship.

Pieris rapae was the only species tested from European and Australian populations, and as such provides an interesting comparison between its native and introduced range. The 1°C higher thorax take-off temperature, and the quicker warming-up in Australia may be due its adaptation to the higher temperatures experienced in Australia compared with the native range. Such physiological plasticity

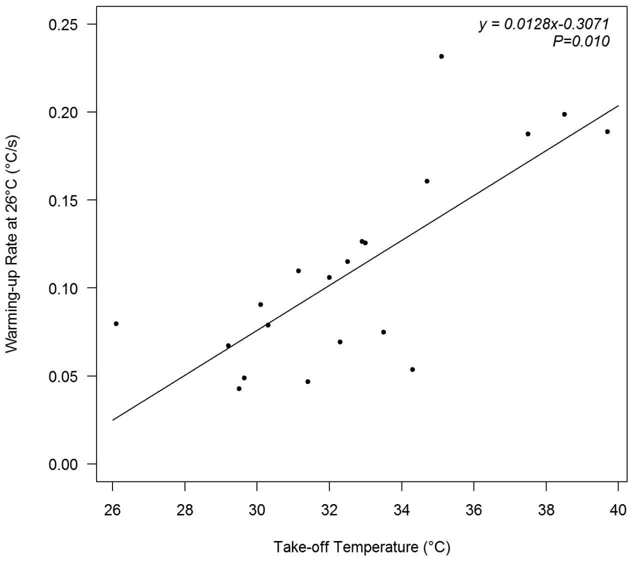

Fig. 6. Warming-up rate at 26°C as a function of take-off temperature, for median values observed in twenty Australian species.

in thermal requirements for flight may impact not only on the invasive ability of a species but also on its ability to adapt to climate change (Matilla, 2015).

As in European butterflies (Nève, 2010), understory specialists require a lower thorax temperature for flight activities when compared to open habitat species. *Melanitis leda* and *Heteronympha merope* are two butterflies active in the forest understorey; as such they experience cooler temperatures than species found in more open habitats (Wickman, 2009), and they have fewer opportunities to bask to gain higher temperatures. In *Heteronympha merope*, females may fly at a much lower thorax temperature than males. This sex difference is unusual, as females need a higher thorax temperature for flight than males in *Colias philodice* (Kingsolver, 1983) and in *Thymelicus lineola* (Pivnick & McNeil, 1986), whereas no difference were found in *Heodes virgaureae* (Douwes, 1976). Unlike these species, *H. merope* shows mainly disjunct population peaks for males and females, with males most abundant in spring and females in autumn (pers. observ., Braby, 2000). The difference in flight thorax temperatures between males and females of *H. merope* may reflect the different climatic conditions experienced by each, and hence an adaptation to minimize excess temperature, thus reducing metabolic costs. Alternatively, differences between sexes may also reflect difference in wing shape and flight behaviour, as in the case of *Pararge aegeria* (Berwaerts et al., 2006).

Melanitis leda, unlike *H. merope*, flies mainly at dusk and dawn (Braby, 1995; Kemp, 2002, 2003), and may be present throughout the year in many localities (Braby, 2000). Their habit of flying during the coolest parts of the day may be linked to their low temperature requirements for flight activities.

The case of *Juniona villida*, with the highest recorded thorax temperatures, half of which close to or above 40°C, raises interesting questions. Previous studies (Kingsolver & Watt, 1983; Matilla, 2015) suggest that *J. villida* may be

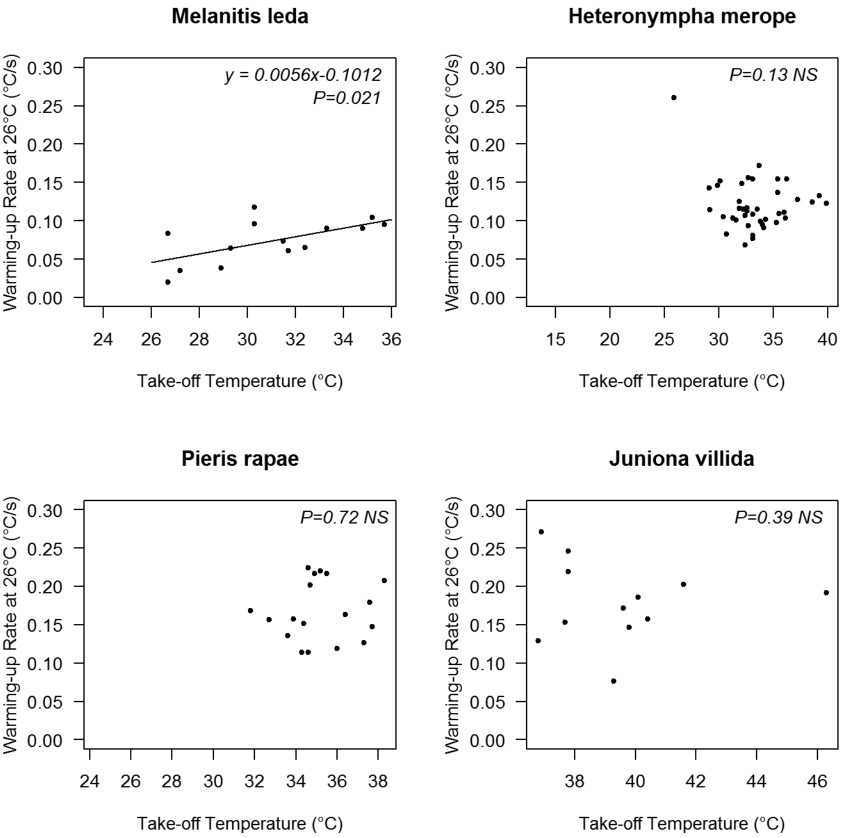

Fig. 7. Warming-up rate at 26°C as a function of take-off temperature, for four Australian species in which at least ten specimens had been tested. NS – not significant. The relationship is significant only in *Melanitis leda*.

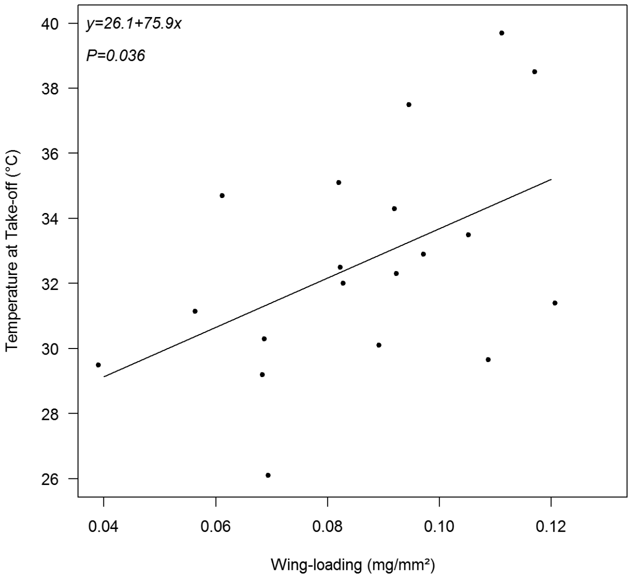

Fig. 8. Temperature at take-off according to wing loading for the 19 tested species (*Trapezites symmomus* is omitted; its wing loading is 0.524 mg/mm^2 and median thorax temperature at take-off was 33°C); dots indicate median values per species. The line indicates the linear correlation for all depicted points.

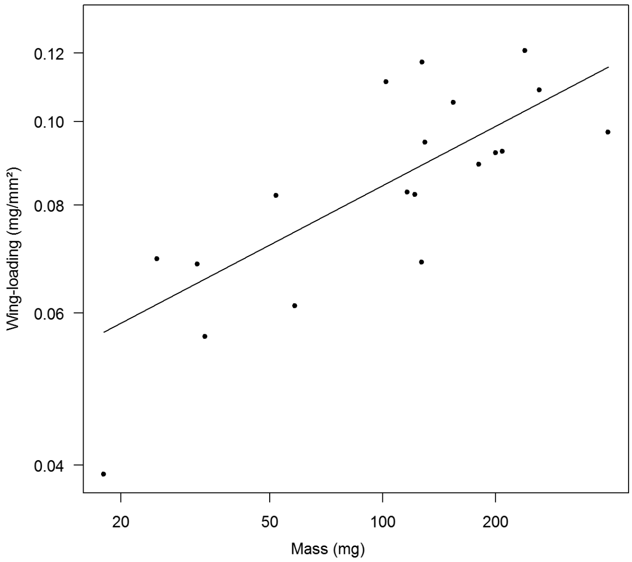

Fig. 9. Wing loading according to mass in 19 Australian butterflies. *Trapezites symmomus* is omitted; its wing loading is 0.524 mg/mm^2 and its mass is 340 mg. Both scales are logarithmic. The fitted allometric function is $y = 0.030x^{0.225}$.

at risk of heat shock at such high temperatures. This species, however, has a flight pattern with quick reactions and fast flights (Braby, 2000; pers. observ.), suggesting a high amount of muscular activity and thus may have adapted to cope with higher than normal flight temperatures. *Juniona villida* may then be an extreme case of high wingbeat frequency related to a high temperature (Pivnick & McNeil, 1986). This species seems thus to have evolved a fast escape strategy against insectivorous bird predators, which also requires high thorax flight temperature, as palatable species often do (Chai & Srygley, 1990).

It should be noted that *Trapezites symmomus*, which has a wing loading ca. five times higher than all other tested butterflies only showed take off temperatures of 31.5°C and 34.5°C, well within the range of the observed butterflies (Fig. 3). It has been shown that the genotypes of the individuals was a major determinant of the ambient temperature at which individual fly (*Colias* butterflies: Watt et al., 1983, Monarch *Danaus plexippus*: Hughes & Zalucki, 1993). Whether *Juniona villida* holds special variants of the loci adapted to high temperature remains an open question.

In our data set, wing loading p_w showed an allometric relationship with body mass w ($p_w = 0.030\ w^{0.225}$). As the exponent is <1, this is a negative allometric relationship (Huxley & Teissier, 1936; Gould, 1966) between wing loading and body mass. Dudley (1990) also obtained a negative allometry (which he erroneously called "positive allometry"), but with a much higher exponent (0.474); only a small part of the difference between our result and Dudley's are due to the use of the direct function for the estimate: an estimate using the classical log-log relationship gives an exponent factor of 0.238 with our data. It should be noted here that for comparative purposes, the exponent does not depend on the units being used, only the multiplying factor does; this was the cause of some debate between Huxley and Teissier (Gayon, 2000). Despite the fact that wing loading and body mass are correlated variables, our analysis clearly shows a correlation between thorax temperature at take-off and wing loading, but not with body mass. Heinrich (1986) could not differentiate these two effects, due to insufficient data. Our data shows that wing loading is more relevant than body mass in respect to thorax flight temperature.

In many insect species, the rate of warming-up is related to muscular activity and body mass, whereas heat loss depends of their external body surface, smaller individuals and species taking longer to warm up than large ones (May, 1976), although in within species comparison, the reverse has occasionally been found (*Thymelicus lineola*: Pivnick & McNeil, 1986; *Hypolimnas bolina*: Kemp & Krockenberger, 2004). In our experimental setting, when looking at multiple species comparison, large butterflies warm up more quickly than small ones. The lack of significance for tests in individual species may be due to the small sample sizes or lower variance of intra-specific variation compared with inter-specific variation.

In the present data set, there is also a trend for the butterflies to warm up faster if they have to reach a higher temperature for flight activity. In this way, the time spent to reach the needed temperature does not increase in proportion with the required flight temperature. However, the energy required to reach a high temperature must be accounted for. While solar energy pays a role in increasing body temperature in butterflies (Wasserthal, 1975), muscular activity is also needed to reach this temperature, especially, as in the case of *Juniona villida*, it is well above the ambient air temperature.

Species showing a low wing loading generally fly with lower wing beat frequencies than the ones with higher wing loadings (Casey & Joos, 1983), and low wing beat frequencies require a lower thorax temperature for flight activity. It is thus no surprise that the species with the lowest take-off temperature, *Eurema brigitta*, *Nacaduba kurava* and *Hypocysta adiante* were also among the ones with the lowest wing loading and total weight. On the other hand, wing loading per se is not sufficient to infer the thorax temperature necessary for flight, as microhabitat and behaviour also have an influence.

The increase of thorax temperature of *Trapezites symmomus* in a linear fashion is similar to the sphinx moth *Manduca sexta* (Heinrich & Bartholomew, 1971), while other butterflies generally show a curve with decreasing rates until the take-off temperature is reached in a plateau fashion (Nève, 2010). This may be related to the shape of these two species, which have a lower surface to volume ratio than butterflies of the families others than Hesperiidae; they have thus a lower heat loss rate (Heinrich, 2003). The increase in temperature is therefore mainly the result of the muscular activity, heat loss being negligible in comparison.

CONCLUSIONS

Temperature needs of butterflies are an important component of their ecological requirements (Wickman, 2009), both at the adult and larval stages (Serratore et al., 2013). The thorax temperature needed for flight activity is the result of multiple effects. Individual weight and total wing surface are physical factors influencing wing beat frequency, but individual differences in behaviour also plays a role. The optimal flight thorax temperature is adaptively determined through selection of enzymes variants active at specific temperatures. Species living in cooler environments, such as the forest understorey, may be adapted to fly at lower temperature as they would mainly rely on their muscular activity for thorax heating. The temperature needed for flight activity is thus the result of trade-off between environmental variables and internal physical and biochemical processes. The results highlight the importance of understanding this dynamic in the face of environmental change. While butterflies can behaviourally adapt to changes in temperature, factors such as body size and wing loading may impose physiological limits to future climate adaptation.

ACKNOWLEDGEMENTS. We wish to thank A. Thiéry, E. Meglécz and D. Mackay for help during the field work and for numerous discussions. R. Kitching introduced the two authors to each other, and he and B. Kitching allowed the field and laboratory work in Queensland to take place. Field work in South Australia was carried out with permit number A26006-1. No such permit was necessary in Queensland, as research there was not undertaken on protected grounds.

REFERENCES

Bartholomew G.A. 1981: A matter of size: an examination of endothermy in insects and terrestrial vertebrates. In Heinrich B. (ed.): *Insect Thermoregulation*. Wiley, New York, pp. 45–78.

Bartholomew G.A. & Casey T.M. 1978: Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. — *J. Exp. Biol.* **76**: 11–25.

Bartholomew G.A. & Heinrich B. 1973: A field study of flight temperatures in moths in relation to body weight and wing loading. — *J. Exp. Biol.* **58**: 123–135.

Berwaerts K., Aerts P. & Van Dyck H. 2006: On the sex-specific mechanisms of butterfly flight: flight performance relative to flight morphology, wing kinematics, and sex in *Pararge aegeria*. — *Biol. J. Linn. Soc.* **89**: 675–687.

Braby M.F. 1995: Seasonal changes in relative abundance and spatial distribution of Australian lowland tropical Satyrine butterflies. — *Austr. J. Zool.* **43**: 209–229.

Braby M.F. 2000: *Butterflies of Australia, their Identification, Biology and Distribution*. CSIRO, Canberra, 976 pp.

Casey T.M. & Joos B.A. 1983: Morphometrics, conductance, thoracic temperature, and flight energetics of noctuid and geometric moths. — *Physiol. Zool.* **56**: 160–173.

Chai P. & Srygley R.B. 1990: Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. — *Am. Nat.* **135**: 748–765.

Douwes P. 1976: Activity in *Heodes virgaureae* (Lep., Lycaenidae) in relation to air temperature, solar radiation, and time of day. — *Oecologia* **22**: 287–298.

Dudley R. 1990: Biomechanics of flight in neotropical butterflies: morphometrics and kinematics. — *J. Exp. Biol.* **150**: 37–53.

Dudley R. 1991: Comparative biomechanics and the evolutionary diversification of flying insect morphology. In Dudley E.C. (ed.): *The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology*. Dioscorides Press, Portland, pp. 503–514.

Gayon J. 2000: History of the concept of allometry. — *Am. Zool.* **40**: 748–758.

Gould S.J. 1966: Allometry and size in ontogeny and phylogeny. — *Biol. Rev.* **41**: 587–638.

Guppy C.S. 1986: The adaptative significance of alpine melanism in the butterfly *Parnassius phoebus* (Lepidoptera: Papilionidae). — *Oecologia* **70**: 205–213.

Heinrich B. 1986: Comparative thermoregulation of four montane butterflies of different mass. — *Physiol. Zool.* **59**: 616–626.

Heinrich B. 2003: Thermoregulation. In Resh V.H. & Cardé R.T. (eds): *Encyclopedia of Insects*. Academic Press, San Diego, pp. 1119–1126.

Heinrich B. & Bartholomew G.A. 1971: An analysis of pre-flight warm-up in the sphinx moth, *Manduca sexta*. — *J. Exp. Biol.* **55**: 223–239.

Hughes J.M. & Zalucki M.P. 1993: The relationship between the pgi locus and the ability to fly at low-temperatures in the monarch butterfly *Danaus plexippus*. — *Biochem. Genet.* **31**: 521–532.

Huxley J.S. & Teissier G. 1936: Terminology of relative growth. — *Nature* **137**: 780–781.

Kemp D.J. 2002: Visual mate-searching behaviour in the evening brown butterfly, *Melanitis leda* (L.) (Lepidoptera: Nymphalidae). — *Austr. J. Entomol.* **41**: 300–305.

Kemp D.J. 2003: Twilight fighting in the evening brown butterfly, *Melanitis leda* (L.) (Nymphalidae): age and residency effects. — *Behav. Ecol. Sociobiol.* **54**: 7–13.

Kemp D.J. & Krockenberger A.K. 2004: Behavioural thermoregulation in butterflies: the interacting effects of body size and basking posture in *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae). — *Austr. J. Zool.* **52**: 229–236.

Kingsolver J.G. 1983: Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. — *Ecology* **64**: 534–545.

Kingsolver J.G. 1985a: Butterfly engineering. — *Scient. Am.* **253**: 106–113.

Kingsolver J.G. 1985b: Butterfly thermoregulation, organismic mechanisms and population consequences. — *J. Res. Lepidopt.* **24**: 1–20.

Kingsolver J.G. & Watt W. 1983: Thermoregulatory strategies in *Colias* butterflies – thermal stress and the limits to adaptation in temporally varying environments. — *Am. Nat.* **121**: 32–55.

Lokkers C. & Jones R.E. 1999: The cabbage white, *Pieris rapae* (Pieridae). In Kitching R.L., Scheermeyer E., Jones R.E. & Pierce N.E. (eds): *Biology of Australian Butterflies, Monographs on Australian Lepidoptera, Vol. 6.* CSIRO, Collingwood, pp. 153–172.

Matilla A.L. 2015: Thermal biology of flight in a butterfly: genotype, flight metabolism, and environmental conditions. — *Ecol. Evol.* **5**: 5539–5551.

May M.L. 1976: Warming rates as a function of body size in periodic endotherms. — *J. Comp. Physiol. (B)* **111**: 55–70.

Nève G. 2010: Variation of flight thorax temperature among Mediterranean butterflies. — *Entomol. Rom.* **15**: 21–24.

Pivnick K.A & McNeil J.N. 1986: Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). — *Ecology* **67**: 1024–1035.

Rasband W.S. 2012: *ImageJ.* U.S. National Institutes of Health, Bethesda, Maryland, URL: http://imagej.nih.gov/ij/.

R Development Core Team 2011: *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, URL: http://www.R-project.org/.

Serratore V.R., Zalucki M.P. & Carter P.A. 2013: Thermoregulation in moulting and feeding *Danaus plexippus* L. (Lepidoptera: Nymphalidae) caterpillars. — *Austr. J. Entomol.* **52**: 8–13

Srygley R.B. & Chai P. 1990: Predation and the elevation of thoracic temperature in brightly colored neotropical butterflies. — *Am. Nat.* **135**: 766–787.

Upton M.S. & Mantle B. 2010: *Methods for Collecting, Preserving and Studying Insects and Other Terrestrial Arthropods. 5th ed.* Australian Entomological Society, Canberra, 81 pp.

Wasserthal L.T. 1975: The rôle of butterfly wings in regulation of body temperature. — *J. Insect Physiol.* **21**: 1921–1930.

Watt W.B., Cassin R.C. & Swan M.S. 1983: Adaptation at specific loci. III. Field behaviour and survivorship differences among *Colias* Pgi genotypes are predictable from in vitro biochemistry. — *Genetics* **103**: 725–739.

Wickman P.O. 2009: Thermoregulation and habitat use in butterflies. In Settele J., Shreeve T., Konvicka M. & Van Dyck H. (eds): *Ecology of Butterflies in Europe.* Cambridge University Press, Cambridge, pp. 55–61.

Zar J.H. 1968: Calculation and miscalculation of the allometric equation as a model in biological data. — *BioScience* **18**: 1118–1120.

Received August 31, 2016; revised and accepted November 14, 2016

Supplementary file:

S1 (http://www.eje.cz/2016/077/S1.pdf). Data set with details for every specimen tested: Identifier, species, sex, wing surface, weight, date and location of capture, body temperature at take-off, warming-up rate at 26°C and, if applicable, South Australian Museum voucher number. NA indicates missing data.