

Tropical butterflies use thermal buffering and thermal tolerance as alternative strategies to cope with temperature increase

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Abstract

Climate change poses a severe threat to many taxa, with increased mean temperatures and frequency of extreme weather events predicted. Insects respond to non-optimal temperatures using behaviours or local microclimates to thermoregulate (thermal buffering ability), or through physiological tolerance. We studied the thermal buffering ability and thermal tolerance of a community of 54 butterfly species in Panama. Thermal buffering ability and tolerance were influenced by family, size, and colour, with Pieridae, large, and dark butterflies having the strongest thermal buffering ability, and with Hesperidae, small, and dark butterflies tolerating the highest temperatures. We identified an interaction between thermal buffering ability and physiological tolerance, where species with stronger thermal buffering abilities had lower thermal tolerance, and vice versa. This interaction implies that most species will be vulnerable to climate change to an extent, considering that species appear to adapt to one strategy at the expense of the other.

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Statement of authorship

Study was designed by EAJ, AJB, BLH, GPAL, ECT, and YB. Data were collected by EAJ, ACZ, AM, AFS, BLH, and SAC. EAJ conducted analyses and wrote the first draft of the manuscript. All authors contributed substantially to revisions.

Data accessibility statement

Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository, and the data DOI will be included at the end of the article.

Conflict of interest

None to declare.

Article title

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Running title

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47 **Abstract**

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49 frequency of extreme weather events predicted. Insects respond to non-optimal
50 temperatures using behaviours or local microclimates to thermoregulate (thermal buffering
51 ability), or through physiological tolerance. We studied the thermal buffering ability and
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55 dark butterflies tolerating the highest temperatures. We identified an interaction between
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57 buffering abilities had lower thermal tolerance, and vice versa. This interaction implies that

58 most species will be vulnerable to climate change to an extent, considering that species

59 appear to adapt to one strategy at the expense of the other.

60

61 **Introduction**

62 Climate change poses a severe threat to many taxa, with increases in mean temperature in
63 many regions disrupting growth (Maino *et al.* 2016), behaviour and survival of individuals
64 (Kingsolver *et al.* 2013), and synchronicity of ecological relationships (Cornelissen 2011).
65 Climate change is also increasing the frequency and intensity of extreme weather events
66 (Jentsch *et al.* 2007). This puts stress on species unable to cope with short periods of
67 extremely high temperatures or extended droughts, and has knock-on effects on populations
68 and wider ecological interactions. It is therefore important to understand both the impacts of
69 changes in mean temperature and temperature extremes on animals.

70

71 Species that are unable to move to track temperature change, must overcome changes in
72 mean and extreme temperature within their local area, especially where conditions are
73 altered outside of their climate niche. Species can maintain their body temperature within
74 tolerable ranges in a changing climate by using behavioural mechanisms, such as movement
75 between suitable microhabitats (Duffy *et al.* 2015). However, such strategies may not be
76 sufficient to maintain body temperatures within tolerable limits during extreme high
77 temperature events (De Palma *et al.* 2017). In such cases, species must rely on physiological
78 processes to tolerate high temperatures, if only for relatively short periods of time (Angilletta
79 Jr 2009). These aspects of thermal adaptation, temperature avoidance or tolerance, may
80 interact; behavioural adaptations can help species overcome changes in ambient conditions
81 by enabling them to seek suitable microclimates, but may have a detrimental effect on the
82 evolution of thermal tolerance by reducing exposure to non-optimal temperatures, and
83 therefore weakening the selective pressure needed to adapt (Huey, Hertz, & Sinervo, 2003;
84 Buckley, Ehrenberger, & Angilletta, 2015). Similarly, species that have high tolerance to high

temperatures may not evolve an ability to behaviourally avoid high temperatures due to weak selective pressure, ultimately leaving them vulnerable to temperature extremes outside of their tolerance. This implies there may be an interaction between these two types of thermal adaptation. If this is the case, many species could be vulnerable to climate change, where species are unable to adapt to cope with both increases in mean temperatures and extreme temperatures. This trade-off may be particularly severe in the tropics, where species tend to have narrower climate niches and lower ability to disperse to climatically suitable areas than their temperate counterparts (Grinder & Wiens 2022), and where habitat degradation, and therefore loss of suitable microclimates, is severe (Senior *et al.* 2019).

Butterflies are an ecologically sensitive group, showing marked responses to environmental change. Body temperature is a key determinant for many processes relevant for butterfly fitness, including flight ability (Merckx *et al.* 2006), reproductive behaviour (McDonald & Nijhout 2000), and fecundity (Karlsson & Wiklund 2005). However, excessive heat above thermal tolerances can cause irreversible protein denaturation, disturb ionic regulation, and result in death (Heath *et al.* 1971; González-Tokman *et al.* 2020). There is, therefore, a strong selective pressure for butterflies to maintain their body temperature within tolerable ranges under variable ambient conditions (thermal buffering ability, see Bladon *et al.* 2020). This can be done in a variety of ways, including behavioural thermoregulation (such as altering wing position relative to the sun) or by selecting favourable microclimates (Clench 1966; Montejo-Kovacevich *et al.* 2020). We hypothesise that a species with strong thermal buffering ability would be at a selective advantage over species with poor thermal buffering ability, as they would be able to maintain their body temperature within a tolerable range across a variety of air temperatures, meaning they are able to efficiently warm up in cool air temperatures

and cool down in warm air temperatures. However, where extreme temperatures rise beyond behavioural thermoregulation capacity, and there are not suitable cool microclimates for butterflies to exploit, species must instead rely on physiological mechanisms, such as the production of heat shock proteins, to tolerate high temperature (González-Tokman *et al.* 2020).

The thermal buffering ability of temperate butterflies is influenced by traits such as taxonomic family and wing length, with Pieridae and larger species tending to have better thermal buffering ability than other families and small species (Bladon *et al.* 2020). Similar aspects of butterfly morphology that affect thermal buffering have also been found to influence thermal tolerance (e.g., a positive relationship between thermal tolerance and body mass in *Bicyclus anynana* (Klockmann, Günter, & Fischer, 2017)). Should similar traits influence both thermal buffering ability and tolerance, there is a possibility that traits may have synergistic or antagonistic effects on these two forms of thermal adaptation. There is currently limited information on how tropical butterflies may be responding to changes in ambient temperatures, and no work has been carried out to quantify thermal buffering ability and compare this to thermal tolerance for tropical butterflies (Fischer, Klockmann, & Reim, 2014; Dongmo *et al.* 2021), despite the majority of butterfly species being found in the tropics (Bonebrake *et al.* 2010).

In this paper, we address the following questions.

1. What is the range of species-specific thermal buffering abilities across a community of tropical butterfly species, and is this influenced by family, size, or colour?

We hypothesise that thermal buffering ability will be influenced by taxonomy and size, as found by Bladon *et al.* (2020), with Pieridae and larger butterflies having stronger thermal buffering abilities than other families and smaller butterflies. We predict that dark wing colour will positively influence thermal buffering ability, following previous studies (Berthier 2005).

2. What is the range of species-specific thermal tolerance (critical thermal maxima) across a community of tropical butterfly species, and is this influenced by family, size, or colour?

We predict that thermal tolerance will be influenced by taxonomy, as previous studies have found that physiological responses to high temperatures (e.g. heat shock proteins) are phylogenetically conserved (González-Tokman *et al.* 2020). We also predict that thermal tolerance will be higher in larger species, as in other insects (Baudier *et al.* 2015). We predict that thermal tolerance will be independent of colour, as we could find limited evidence of a link between colour and temperature tolerance in the literature.

3. Is there a relationship between species-specific thermal buffering ability and thermal tolerance across a community of tropical butterfly species?

We predict that thermal buffering ability will have a negative relationship with thermal tolerance, either due to species with strong thermal buffering abilities rarely experiencing high body temperatures and therefore experiencing weak selection to increase thermal tolerance, or because species with high thermal tolerance experience weak selection to improve thermal buffering ability.

Methods

Study sites

Butterflies were sampled from multiple habitats in Panama from February 2020 to March 2022, across both wet and dry seasons. Data were collected in multiple locations: Gamboa (lowland managed urban green spaces) [9°6'59.13"N, 79°41'47.41"W] (elevation = 28 m), "Pipeline road" in Soberanía National Park (secondary semi-deciduous lowland tropical wet forest) [9° 7'39.04"N, 79°42'17.80"W] (elevation = 92 m), Campana in the Capira District (premontane wet encroaching scrub and secondary forest) [8°40'54.97"N, 79°55'25.08"W] (elevation = 327 m), Sajalices in the Chame District (lowland tropical wet encroaching scrub and secondary forest) [8°40'53.55"N, 79°51'57.90"W] (elevation = 150 m), El Valle (lowland tropical wet encroaching scrub) [8°37'04.7"N, 80°06'56.5"W] (elevation = 674 m), Mount Totumas (lower mountain rainforest and management agroforestry) [8°52'58.6"N, 82°41'01.3"W] (elevation = 1877 m), and San Lorenzo National Park (secondary lowland tropical wet forest) [9°14'49.2"N, 79°58'44.2"W] (elevation = 185 m). This range of sites allowed the collection of a wide variety of species across a range of air temperatures.

Thermal buffering ability

Surveys were undertaken in all weather conditions except rain, between 07:30 and 16:00 hours, and we attempted to capture any butterflies seen. Butterflies were caught in hand nets without chasing (to avoid raising butterfly body temperature). Immediately after capture, butterfly body temperature was recorded using a thermocouple with a handheld indicator (Tecpel Thermometer 305B, Tecpel Co. Ltd., Taiwan), by gently pressing the probe through the net against the butterfly's thorax, without handling or touching the butterfly. Body temperature was recorded within 10 seconds of capture, followed by air temperature, taken with the thermocouple held at waist height in the shade. We then identified individual butterflies to species, and recorded wing length (with callipers from the joint in the thorax to

the tip of the forewing), and wing colour (ranked from 1, almost white, 2, yellow-green, 3, orange, 4, orange-brown or blue, 5, brown, to 6, almost black; as established by Bladon *et al.* 2020). Butterflies were retained in a small cage until the end of the survey (up to a maximum of 6 hours) to prevent re-recording the same individuals, before being released.

Thermal tolerance

From January to March 2022, a subset of butterflies, captured to record their thermal buffering ability, were used for thermal tolerance experiments. Species were chosen based on abundance. The selected individuals were retained in glassine envelopes with moistened cotton and kept outdoors in the shade at ambient temperature before measurement of thermal tolerance (within six hours of capture). To measure critical thermal maximum (CT_{max}), butterflies were placed individually into six glass jars with moistened filter paper (to prevent dehydration) in a water bath (Huber CC-K20 with Pilot ONE, Huber Kältemaschinenbau AG, Germany) at 28°C for five minutes to acclimatise. This starting temperature was chosen as it was the average air temperature recorded during capture of the butterflies. A thermocouple with a hand-held indicator (Tecpel Thermometer 305B, Tecpel Co. Ltd., Taiwan) was placed into a control jar to monitor and record in-jar temperatures. After acclimatisation, the water bath was set to ramp up temperature steadily, at a rate of 0.5°C/min to a maximum of 70°C. By maintaining high humidity throughout the experiment and ramping temperature at an ecologically relevant rate (Terblanche *et al.* 2007), we aimed to simulate features of climate change in the tropics, for example a high temperature weather event, where temperature increases and humidity remains high. During the experiment, water bath internal temperatures (recorded using the water bath internal thermometer) and actual in-jar temperatures (recorded using the thermocouple) were recorded every five minutes to ensure

the set ramping rate was achieved. To prevent inter-run differences affecting results, no more than three individuals of a single species were placed into a single run. The temperature at which each butterfly lost motor control (“knockdown”, assessed as the temperature at which the butterfly fell down and, after being poked, did not right itself) and time to knockdown were recorded (Huey, Crill, Kingsolver, & Weber, 1992). Ambient laboratory temperatures during the experiments ranged from 23-25°C. Before being placed in the water bath, wing length (measured with callipers) (Ribeiro *et al.* 2012) and condition (on a scale of 1-5, following Bladon *et al.* 2020, where 1 is perfect and 5 is significant damage on every wing) of each butterfly was recorded again. Only butterflies of conditions 1-3 were used (assessed beforehand) to prevent senescence or poor condition affecting the results. Exposure duration (including starting temperature and rate of temperature change) is known to influence critical thermal limits recorded (Terblanche *et al.* 2007). As the butterflies were wild-caught, temperature variation experienced throughout the life cycle, and therefore their thermal history, may have influenced our results (Kellermann *et al.* 2017).

Data processing and statistical analyses

Data analyses: Thermal buffering ability

All analyses were conducted in R version 3.6.1 (R Core Development Team, <http://www.r-project.org>). Plots were produced with the ‘interactions’ (Long 2019) and ‘ggplot2’ R packages (Wickham 2016). A total of 54 species from six butterfly families were used for analysis; HesperIIDae (11 species, 219 individuals), LycaenIDae (3 species, 67 individuals), NymphalIDae (26 species, 727 individuals), PapilionIDae (3 species, 53 individuals), PierIDae (8 species, 209

individuals), and Riodinidae (3 species, 59 individuals). We excluded species which had fewer than 10 recordings over less than 5°C air temperature range, as we assessed these as insufficient to determine thermal buffering ability (Bladon *et al.* 2020). To test whether species' traits (family, wing length, colour) were associated with each other, pairwise one-way ANOVA tests were used. Where relationships were detected, model structure was not changed, but we considered these relationships during interpretation.

We fitted linear regression models of body temperature against air temperature for each species. The slope of this regression was used to estimate the ability of each species to "buffer" its body temperature in response to varying air temperature. To aid interpretation, slopes were subtracted from one (hereafter referred to as buffering estimate), so that a small value indicated a weak thermal buffering ability, and a large value indicated a strong thermal buffering ability (Bladon *et al.* 2020). A strong thermal buffering ability (high buffering estimate) occurs when individuals are able to raise their body temperature above air temperature in cool weather, and lower their body temperature towards air temperature in hot weather.

To investigate which traits affected the thermal buffering ability of individual species, we fitted a mixed effect model with body temperature as the response variable, and air temperature, butterfly family, wing length, wing colour, and two-way interactions between air temperature and each of the other variables as explanatory variables. Species was included as a random effect to control for inter-specific differences. The assumptions of mixed effect models were checked and met before fitting using the 'sjPlot' package (Lüdecke 2021). Model selection was conducted through backwards stepwise selection, to avoid suppressor

effects, using the ‘lmerTest’ package (Kuznetsova *et al.* 2017), where non-significant terms were removed until a minimal model was achieved in which all remaining terms were significant. The retention of a two-way interaction between air temperature and a trait in the optimal model indicates that the trait is important in explaining thermal buffering ability. Conditional R^2 values (R^2_c) were compared between the optimal model and models with each significant fixed effect removed, to determine the relative importance of each trait in describing thermal buffering ability using the ‘MuMIn’ package (Bartoń 2022) (where high ΔR^2_c indicates that a trait describes a higher relative proportion of the variance explained by both the fixed and random factors).

Data analyses: Thermal tolerance

We included 23 species, for which sample sizes ranged from 18 to 24 individuals, in the analyses. Five of the six butterfly families were represented, HesperIIDae (7 species, 143 individuals), LycaenIDae (2 species, 41 individuals), NymphalIDae (9 species, 186 individuals), PierIDae (3 species, 67 individuals), RiodinIDae (2 species, 42 individuals). There were no species of PapilionIDae with sufficient numbers to include. The temperature at which 50% of individuals of each species were knocked down (hereafter, LD50) was calculated using the ‘survival’ package (Therneau 2022). Differences between water bath runs were checked before analyses and found to not differ (Appendix 1 Supplementary Methods).

To investigate whether family, wing length, colour, or thermal buffering ability affected thermal tolerance, survival probabilities were assessed with the Kaplan-Meier method using the ‘survival’ package (Therneau 2022). Differences between survival curves were tested with log-rank tests between families, wing lengths, colours (using the same 1-6 scale described

above), and species-specific buffering estimates in individual models. Plots were produced using the 'survminer' package (Kassambara *et al.* 2021).

Results

Thermal buffering ability

A total of 1,334 butterflies were included in analyses, covering 54 species from six butterfly families. Air temperatures at which butterflies were captured ranged from 17.4 to 39.7°C. Species-specific buffering estimates ranged from -0.32 (*Hemiargus hanno*) to 1.00 (*Phoebis argante*) (Appendix 1, Table S1, Figs. S1-S6).

Across species, traits were related to each other. Families differed in the frequency of different colour categories, with Pieridae being paler than the other families ($F_{1,5} = 517.30$, $p < 0.001$). Families also differed in wing length, with Papilionidae being larger than the other families ($F_{1,5} = 145.30$, $p < 0.001$). Wing length and colour were not correlated ($F = 0.02$, $p = 0.896$).

Family, wing length, and colour all affected thermal buffering ability, with family having the largest effect ($\Delta R^2c = 0.007$), followed by colour ($\Delta R^2c = 0.006$), and finally size ($\Delta R^2c = 0.001$). The low ΔR^2c is due to the majority of variation in body temperature being explained by air temperature. The families differed in thermal buffering abilities ($\chi^2_{1,5} = 12.87$, $p = 0.025$), with Pieridae having the highest buffering estimate (mean = 0.41, range: 0.07–1.00), followed by Riodinidae (mean = 0.38, range: 0.31–0.45), Papilionidae (mean = 0.37, range: 0.18–0.55), Nymphalidae (mean = 0.33, range: -0.15–0.79), Hesperidae (mean = 0.19, range: -0.26–0.69)

and Lycaenidae (mean = -0.1, range: -0.32–0.16) (Fig. 1A). Larger species had higher buffering estimates than smaller species ($\chi^2_1 = 16.52$, $p < 0.001$) (Fig. 1B). Dark species had higher buffering estimates than pale species ($\chi^2_1 = 4.41$, $p = 0.036$) (Fig. 1C).

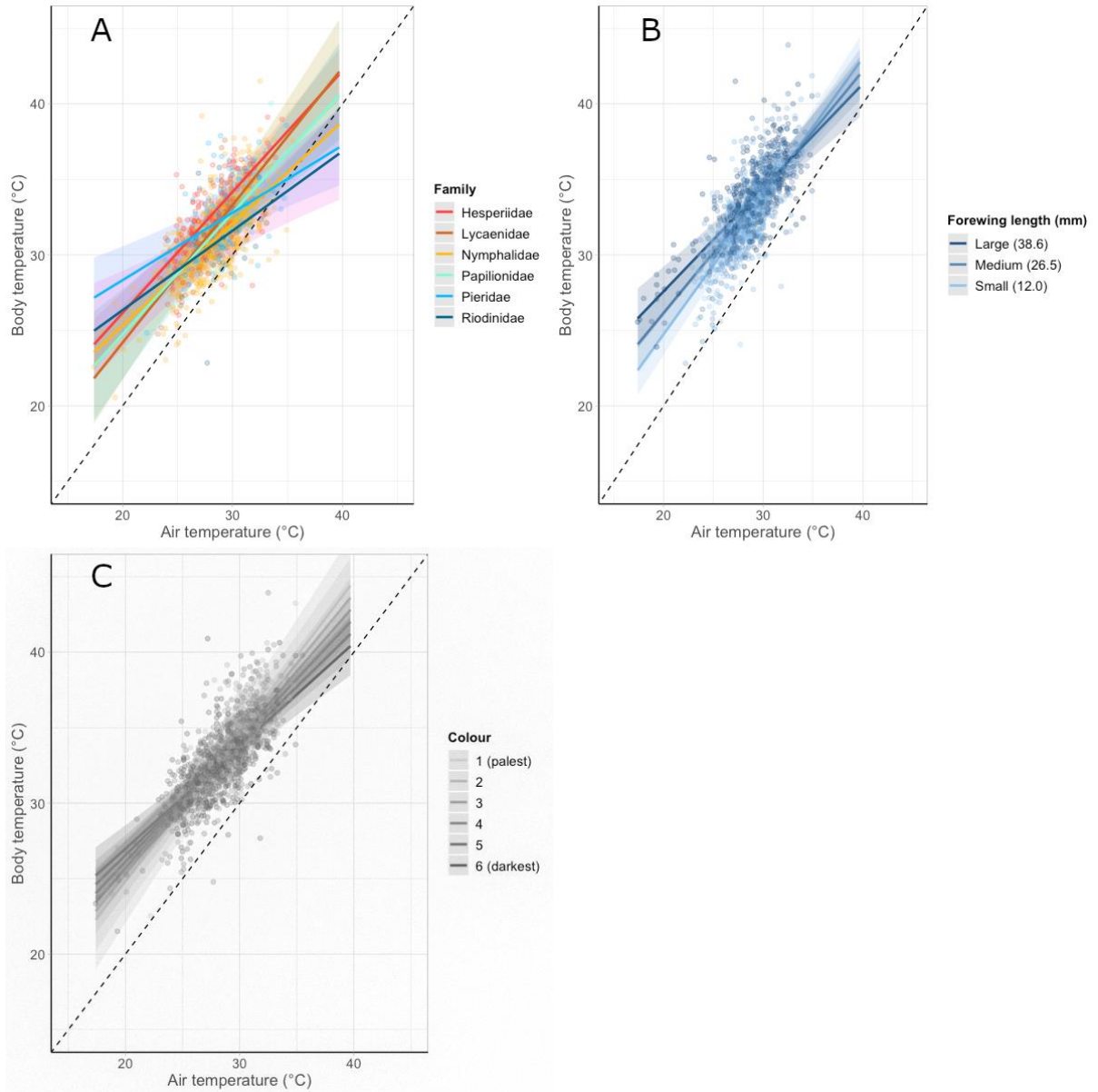


Figure 1: Differences in the relationship between air temperature (°C) and body temperature (°C) (thermal buffering ability) between (A) families, (B) forewing lengths (in mm, modelled as a continuous variable but split into three groups for plotting: medium (mean wing length), large (one standard deviation above the mean) and small (one standard deviation below the

mean)), and (C) colours (on a scale from 1 (almost completely white) to 6 (almost completely black), assessed using established methods (Bladon *et al.* 2020). Lines represent predicted values restricted to the range of temperatures observed. Shaded areas show 95% confidence intervals. Points represent partial residuals (observed data points of individual butterflies with the effects of the other variables accounted for). Dashed lines show a 1:1 relationship between air and butterfly temperature to aid interpretation.

Thermal tolerance

Across 23 species and five butterfly families, temperatures at which 50% of individuals fell (LD50) ranged from 45.05°C (*Itaballia demophile*) to 56.80°C (*Junonia zonalis*). The range of temperatures between which 10% and 90% of individuals fell varied from 2.5°C (*Dione juno*) to 15.1°C (*Urbanus procne*) between species (Appendix 1, Table S3, Fig. S7).

Survival curves differed between butterfly families ($\chi^2_{1,4} = 13.9$, $p = 0.007$): HesperIIDae tolerated the highest temperatures before falling (LD50: 51.3°C, fall range: 11.4°C), followed by Pieridae (LD50: 48.7, fall range: 10.6), Nymphalidae (LD50: 48.5, fall range: 11.8), and Lycaenidae (LD50: 48.4, fall range: 11.6), while Riodinidae had the lowest LD50 and narrowest fall range (LD50: 47.1, fall range: 8.4) (Fig. 2A).

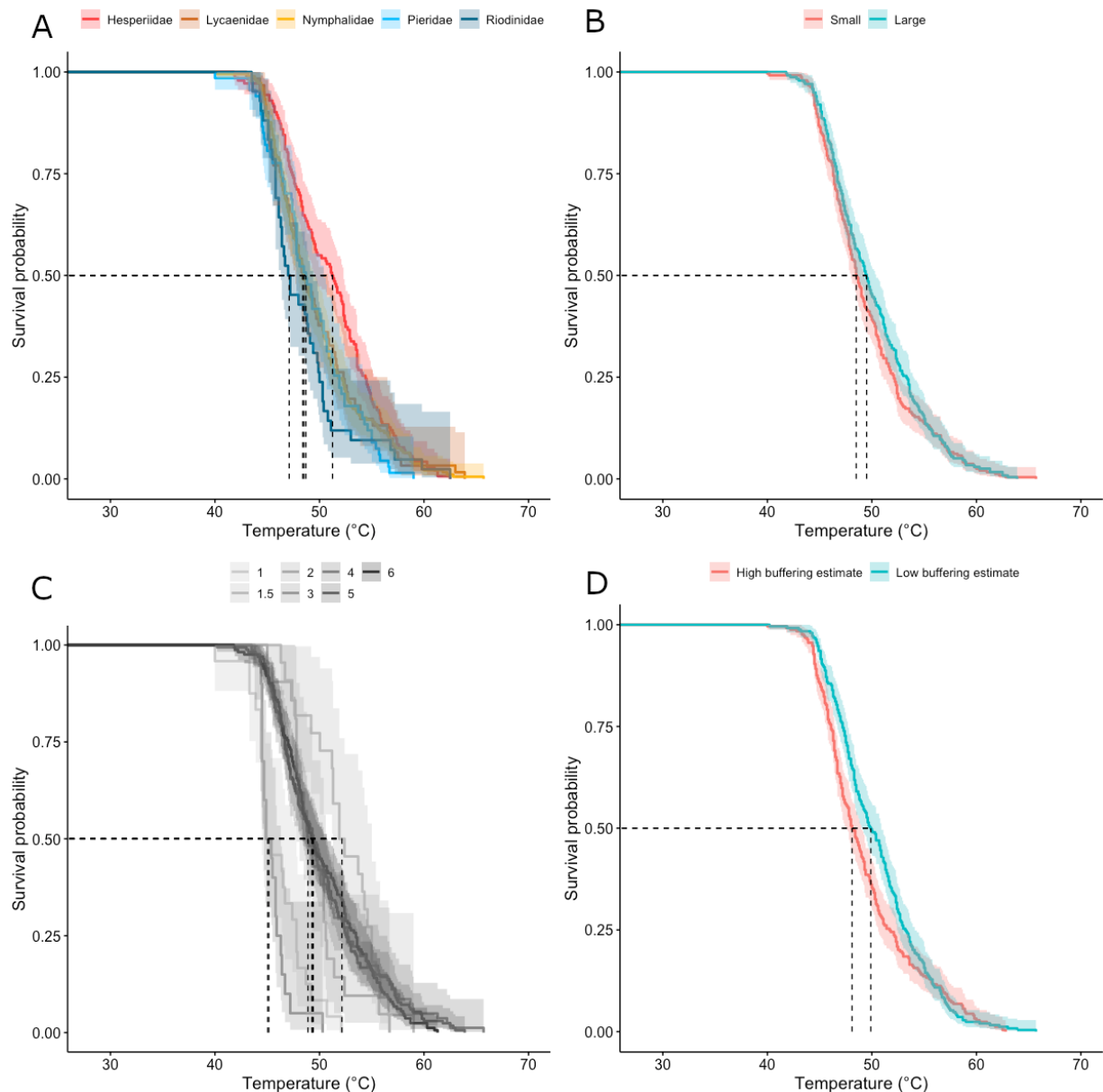
Survival curves differed between species with different wing lengths ($\chi^2_{1,212} = 718.0$, $p < 0.001$). Small species tolerated higher temperatures than large species (small, forewing length \leq median (17.9 mm), LD50: 49.5°C, fall range: 11.3°C; large, forewing length > 17.9 mm, LD50: 48.5°C, fall range: 11.9°C) (Fig. 2B).

333 Survival curves differed between species with different coloured wings ($\chi^2_{1,6} = 116.0$, $p <$
334 0.001). Dark species tolerated higher temperatures than pale species (dark species (colour
335 value > 3), LD50: 49.3°C, fall range: 11.7°C; pale species (colour value ≤ 3), LD50: 48.7°C, fall
336 range: 10.6°C) (Fig. 2C).

337

338 Survival curves differed between species with different thermal buffering abilities ($\chi^2_{1,23} =$
339 263.0 , $p < 0.001$). Species with low buffering estimates were able to tolerate higher
340 temperatures than species with high buffering estimates (weak thermal buffering ability \leq
341 median (0.21), LD50: 49.9°C, fall range: 11.3°C; strong thermal buffering ability > 0.21 LD50:
342 48.6°C, fall range: 12.3°C) (Fig. 2D, Appendix 1, Fig. S8).

343



344

345 Figure S8: Average thermal survival curves across (A) families, (B) forewing length (modelled
 346 as a continuous variable but split into two groups for plotting: small (below median) and
 347 large (above median) (median = 17.8 mm)), (C) colours (on a scale from 1 (almost
 348 completely white) to 6 (almost completely black), assessed using established methods
 349 (Bladon *et al.* 2020), and (D) buffering abilities (modelled as a continuous variable but split
 350 into two groups for plotting: weak buffering ability (below median) and strong buffering
 351 ability (above median) (median = 0.211)). During the experiment, temperature was
 352 increased from 28°C to 70°C at 0.5°C per minute until the butterfly was knocked down (see

Methods). Solid lines represent mean survival, ribbons represent 95% confidence intervals. Dashed lines show the temperature at which 50% of individuals were knocked down (LD50) per group.

Discussion

Our study identified widely-differing thermal buffering abilities and tolerances, which varied systematically with family, size and colour, and interacted with each other, across a large community of tropical butterflies. We found that thermal buffering ability was influenced by taxonomic family, size, and colour. Pieridae had the strongest thermal buffering ability and were able to behaviourally avoid high body temperatures. Contrastingly, Lycaenidae had the weakest thermal buffering ability, being particularly poor at heating up in cool weather and cooling down in hot weather. Small, pale species were particularly poor at buffering against changes in air temperature, whereas large, dark species had stronger buffering abilities, being more able to maintain a relatively stable body temperature across variable air temperatures. Thermal tolerance also differed between families, species, sizes, and colours. Generally, Hesperidae tolerated the highest temperatures, whereas Riodinidae had the lowest thermal tolerance. Small, dark species had the highest thermal tolerance. We also found a negative relationship between thermal buffering ability and thermal tolerance for the first time, indicating an interaction between these strategies. Our findings have important implications for predicting species at greater risk from increases in mean and extreme temperature events in the biodiverse tropics.

Taxonomic group

We found that butterfly families differed in their sensitivities to changing temperatures. This is in-line with studies that have found that heat tolerance mechanisms, such as heat shock proteins, are highly phylogenetically conserved (Nguyen *et al.* 2016), and suggests that thermal tolerance has heritable components that can be exploited by selection. Pieridae had the highest thermal buffering ability of the six butterfly families, as well as relatively high thermal tolerance. Pieridae were also found to have the highest thermal buffering abilities in a similar study on temperate butterflies (Bladon *et al.* 2020). Pieridae generally bask to raise their body temperature (Watt 1968), sometimes using their pale wings to reflect solar energy onto their body (Shanks *et al.* 2015). Their generally large wings and pale colouration may allow Pieridae to reflect light more efficiently than other families, and avoid absorbing excess solar energy when not basking.

Conversely, Lycaenidae had the poorest thermal buffering ability, and low thermal tolerance. Lycaenidae is one of the most speciose butterfly families (Robbins 1982), and commonly consists of small butterflies with bright, often iridescent, colouration. A study on a small temperate Lycaenidae found that wing colour did not strongly impact heating rates, although weight was important (De Keyser *et al.* 2015). This indicates that it may not be the colouration of Lycaenids that makes them poor at buffering temperature, but rather their small size.

Hesperiidae tolerated the highest temperatures of the five families tested, but had relatively weak thermal buffering abilities. This implies that tropical hesperiids may compensate for their poor thermal buffering ability with higher thermal tolerance. Alternatively, their high thermal tolerance may reduce selective pressure to maintain their body temperature within a narrow range. Hesperiidae include butterflies that have short wings and large stout bodies

and rapid wing beats (Betts & Wootton 1988). As wingbeat frequency is temperature dependent (Kammer 1970), the characteristic rapid wingbeats of hesperiids may require higher thoracic temperatures for flight, and so they are more likely to experience high body temperatures and undergo selection to increase thermal tolerance, however there is limited evidence to support this (Nève & Hall 2016). Alternatively, being large-bodied, hesperiids may retain heat more than small-bodied butterflies. This would result in hesperiids being less able to lose heat in warm weather, possibly resulting in them commonly experiencing high body temperatures, and therefore evolving higher temperature tolerance.

Riodinidae had the lowest thermal tolerance across the five families, but had relatively strong thermal buffering abilities. This result suggests they are able to behaviourally avoid high body temperatures, which may compensate for their low thermal tolerance. Alternatively, having low thermal tolerance, Riodinidae may be under strong selective pressure to develop mechanisms to maintain their body temperature within a relatively narrow tolerable range. Many Riodinidae frequently rest on the ventral surface of leaves which tend to be cooler than the dorsal surface (Pallas *et al.* 1967), and as such are rarely seen in direct sunlight. This behaviour may allow the most thermally-sensitive species to persist in high temperature environments, and also offers an explanation for their strong thermal buffering abilities. This implies that tropical riodinids may be able to cope with changes in mean temperatures, but it is unclear to what extent they will be able to cope with extreme high temperature events.

Wing length

Large winged species had stronger thermal buffering abilities than small species, possibly due to a combination of behavioural and morphological adaptations. This result aligns with a

similar study on temperate butterflies (Bladon *et al.* 2020), implying that this is a consistent trend across regions. Previous studies have found that large insects raise and lower their body temperature at a slower rate than small insects (Kemp & Krockenberger 2004), and tend to have more stable body temperatures (Gilchrist 1990). This relative stability, as well as their larger wings, could enable large species to travel further and faster to find suitable microclimates, further buffering their body temperature. Large butterflies could also use their large wings to absorb solar energy more quickly, or reflect more solar radiation onto their bodies (Shanks *et al.* 2015), and so increase their body temperature faster when basking than small species.

We also found that large species tended to have lower thermal tolerance than small species. As butterfly body mass correlates with wing length (Peixoto & Benson 2008), our finding differs from previous studies on tropical butterflies, which found thermal tolerance increased with mass (Luo *et al.* 2014; Klockmann, Günter, & Fischer, 2017). However, these studies tended to be based on single species, and may not reflect patterns across a community. It is possible that the negative relationship we found between wing length and thermal tolerance is related to the higher metabolic rate and oxygen demand in larger insects (Lachenicht *et al.* 2010). This would make large butterflies particularly sensitive to further increases in temperature whereby their metabolism increases beyond oxygen delivery.

Wing colour

Darker butterflies had stronger thermal buffering abilities and could tolerate higher temperatures than paler species. This is in-line with previous evidence that dark butterflies heat up and cool down faster than pale butterflies at a given level of solar radiation (Watt

1968), and achieve higher body temperatures than pale individuals (Dufour *et al.* 2018; Khazan *et al.* 2022). This is also in line with comparative temperate studies across latitudes (Zeuss *et al.* 2014), which found a higher incidence of darker species in cooler conditions, possibly also related to the advantage of dark species in being able to warm in cooler conditions. However, this pattern contradicts the strong thermal buffering ability and thermal tolerance of Pieridae, a family of generally pale butterflies. However darker Pierids had stronger thermal buffering abilities than paler Pierids. Dark species may be more likely to experience high body temperatures and be adapted to cope with the predicted increases in ambient temperatures under climate change. Pale butterflies may benefit from rising temperatures in the tropics, by enabling them to gain heat and become active more quickly. However, as well as warming up slower, pale butterflies are also less able to lose more heat at high ambient temperatures compared to dark butterflies, and we found them to have lower thermal tolerance than dark butterflies, putting them at an increased risk of overheating under rising temperatures.

The relationship between thermal buffering ability and thermal tolerance

We found a negative relationship between thermal buffering ability and thermal tolerance, implying that there could be a trade-off between avoiding or tolerating high temperatures, with species favouring one of these strategies at the expense of the other. This finding may partially reflect mechanistic links between factors affecting the two methods for coping with higher temperatures. For example, smaller species were less able to buffer their body temperature, but were more able to tolerate higher temperatures, perhaps contributing to this pattern. However, in the case of colouration, darker species were both better able to buffer temperature and tolerate high temperatures. An alternative interpretation is that

thermal tolerance has evolved as a result of butterflies with poorer thermal buffering ability being more likely to regularly experience high body temperatures. In contrast, species with strong thermal buffering abilities may be under relatively weak selective pressure to evolve high thermal tolerance: their ability to maintain their body temperature within tolerable ranges means they rarely experience high body temperatures. Given that both average temperatures and more extreme temperatures are predicted to increase with climate change, this inverse relationship between buffering and tolerance may have a large negative effect on many butterfly species, as few species are likely to have both an ability to buffer against average increases and an ability to tolerate extremes.

Conclusions

Our findings have identified family, wing length, and colour as factors influencing the ability of species to cope with temperature change. These findings are strikingly similar to a similar study on temperate butterflies, which identified Pieridae and large butterflies as having the strongest thermal buffering abilities (Bladon *et al.* 2020). This implies a consistent pattern across tropical and temperate butterfly species. These findings provide important information to predict which traits, and species with these traits, may be selected for under warming temperatures in the tropics. This implies that species at risk under higher average and extreme temperature events are predictable based on traits. In particular, Lycaenidae represent 'losers' under future climate change, and as a species-rich family, there may be high species losses in the tropics. The trade-off between thermal buffering ability and thermal tolerance implies that most species will be vulnerable to climate change to an extent, considering that both of these changes are predicted to increase in the future, and species

495 appear to adapt to one strategy at the expense of the other. More work is needed to unpick
496 how these two strategies interact with a species' ability to cope with temperature change.

497

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