

1 **Title page**

2

3 *Statement of authorship*

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

7

8 *Data accessibility statement*

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

11

12 *Conflict of interest*

13 None to declare.

14

15 *Article title*

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

18

19 *Running title*

20 Tropical butterflies temperature tolerance

21

22 *Authors*

23

Author name	Affiliation
Esme Ashe-Jepson	Department of Zoology, University of Cambridge, UK
Benita C. Laird-Hopkins	Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic AND Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic AND Smithsonian Tropical Research Institute, Panama
Yves Basset	Smithsonian Tropical Research Institute, Panama AND Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic AND Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic Maestria de Entomologia, University of Panama
Andrew J. Bladon	Department of Zoology, University of Cambridge, UK
Stephany Arizala Cobo	Smithsonian Tropical Research Institute, Panama
Irena Kleckova	Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
Alex Mcfarlane	Smithsonian Tropical Research Institute, Panama
Katerina Sam	Institute of Entomology, Biology Centre of the Czech Academy of Sciences,

	České Budějovice, Czech Republic AND Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
Amanda F. Savage	Smithsonian Tropical Research Institute, Panama
Ana Cecilia Zamora	Smithsonian Tropical Research Institute, Panama
Edgar C. Turner	Department of Zoology, University of Cambridge, UK
Greg P.A. Lamarre	Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic AND Smithsonian Tropical Research Institute, Panama

24

25 Corresponding author details

26 Name: Esme Ashe-Jepson

27 Email: [ea483@cam.ac.uk](mailto:ea483@cam.ac.uk)

28 Phone: +44 (0)1223 336600

29 Address: Department of Zoology, University of Cambridge, Downing Street, Cambridge,

30 Cambridgeshire, UK

31 CB2 3EJ

32

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47 **Abstract**

48 Climate change poses a severe threat to many taxa, with increased mean temperatures and  
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  
52 thermal tolerance of a community of 54 butterfly species in Panama. Thermal buffering ability  
53 and tolerance were influenced by family, size, and colour, with Pieridae, large, and dark  
54 butterflies having the strongest thermal buffering ability, and with Hesperidae, small, and  
55 dark butterflies tolerating the highest temperatures. We identified an interaction between  
56 thermal buffering ability and physiological tolerance, where species with stronger thermal  
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

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

94

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

109 and cool down in warm air temperatures. However, where extreme temperatures rise beyond  
110 behavioural thermoregulation capacity, and there are not suitable cool microclimates for  
111 butterflies to exploit, species must instead rely on physiological mechanisms, such as the  
112 production of heat shock proteins, to tolerate high temperature (González-Tokman *et al.*  
113 2020).

114

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

128

129 In this paper, we address the following questions.

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

132 We hypothesise that thermal buffering ability will be influenced by taxonomy and size, as  
133 found by Bladon *et al.* (2020), with Pieridae and larger butterflies having stronger thermal  
134 buffering abilities than other families and smaller butterflies. We predict that dark wing  
135 colour will positively influence thermal buffering ability, following previous studies (Berthier  
136 2005).

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

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

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

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

153

## 154 **Methods**

155 *Study sites*

156 Butterflies were sampled from multiple habitats in Panama from February 2020 to March  
157 2022, across both wet and dry seasons. Data were collected in multiple locations: Gamboa  
158 (lowland managed urban green spaces) [9°6'59.13"N, 79°41'47.41"W] (elevation = 28 m),  
159 "Pipeline road" in Soberanía National Park (secondary semi-deciduous lowland tropical wet  
160 forest) [9° 7'39.04"N, 79°42'17.80"W] (elevation = 92 m), Campana in the Capira District  
161 (premontane wet encroaching scrub and secondary forest) [8°40'54.97"N, 79°55'25.08"W]  
162 (elevation = 327 m), Sajalices in the Chame District (lowland tropical wet encroaching scrub  
163 and secondary forest) [8°40'53.55"N, 79°51'57.90"W] (elevation = 150 m), El Valle (lowland  
164 tropical wet encroaching scrub) [8°37'04.7"N, 80°06'56.5"W] (elevation = 674 m), Mount  
165 Totumas (lower mountain rainforest and management agroforestry) [8°52'58.6"N,  
166 82°41'01.3"W] (elevation = 1877 m), and San Lorenzo National Park (secondary lowland  
167 tropical wet forest) [9°14'49.2"N, 79°58'44.2"W] (elevation = 185 m). This range of sites  
168 allowed the collection of a wide variety of species across a range of air temperatures.

169

#### 170 *Thermal buffering ability*

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

180 the tip of the forewing), and wing colour (ranked from 1, almost white, 2, yellow-green, 3,  
181 orange, 4, orange-brown or blue, 5, brown, to 6, almost black; as established by Bladon *et al.*  
182 2020). Butterflies were retained in a small cage until the end of the survey (up to a maximum  
183 of 6 hours) to prevent re-recording the same individuals, before being released.

184

#### 185 *Thermal tolerance*

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

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

218

219

## 220 *Data processing and statistical analyses*

221

### 222 *Data analyses: Thermal buffering ability*

223 All analyses were conducted in R version 3.6.1 (R Core Development Team, [http://www.r-](http://www.r-project.org)  
224 [project.org](http://www.r-project.org)). Plots were produced with the ‘interactions’ (Long 2019) and ‘ggplot2’ R packages  
225 (Wickham 2016). A total of 54 species from six butterfly families were used for analysis;  
226 HesperIIDae (11 species, 219 individuals), LycaenIDae (3 species, 67 individuals), NymphalIDae  
227 (26 species, 727 individuals), PapilionIDae (3 species, 53 individuals), PierIDae (8 species, 209

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

234

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

244

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

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

261

#### 262 *Data analyses: Thermal tolerance*

263 We included 23 species, for which sample sizes ranged from 18 to 24 individuals, in the  
264 analyses. Five of the six butterfly families were represented, HesperIIDae (7 species, 143  
265 individuals), Lycaenidae (2 species, 41 individuals), Nymphalidae (9 species, 186 individuals),  
266 Pieridae (3 species, 67 individuals), Riodinidae (2 species, 42 individuals). There were no  
267 species of Papilionidae with sufficient numbers to include. The temperature at which 50% of  
268 individuals of each species were knocked down (hereafter, LD50) was calculated using the  
269 'survival' package (Therneau 2022). Differences between water bath runs were checked  
270 before analyses and found to not differ (Appendix 1 Supplementary Methods).

271

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

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

278

## 279 **Results**

280

### 281 *Thermal buffering ability*

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

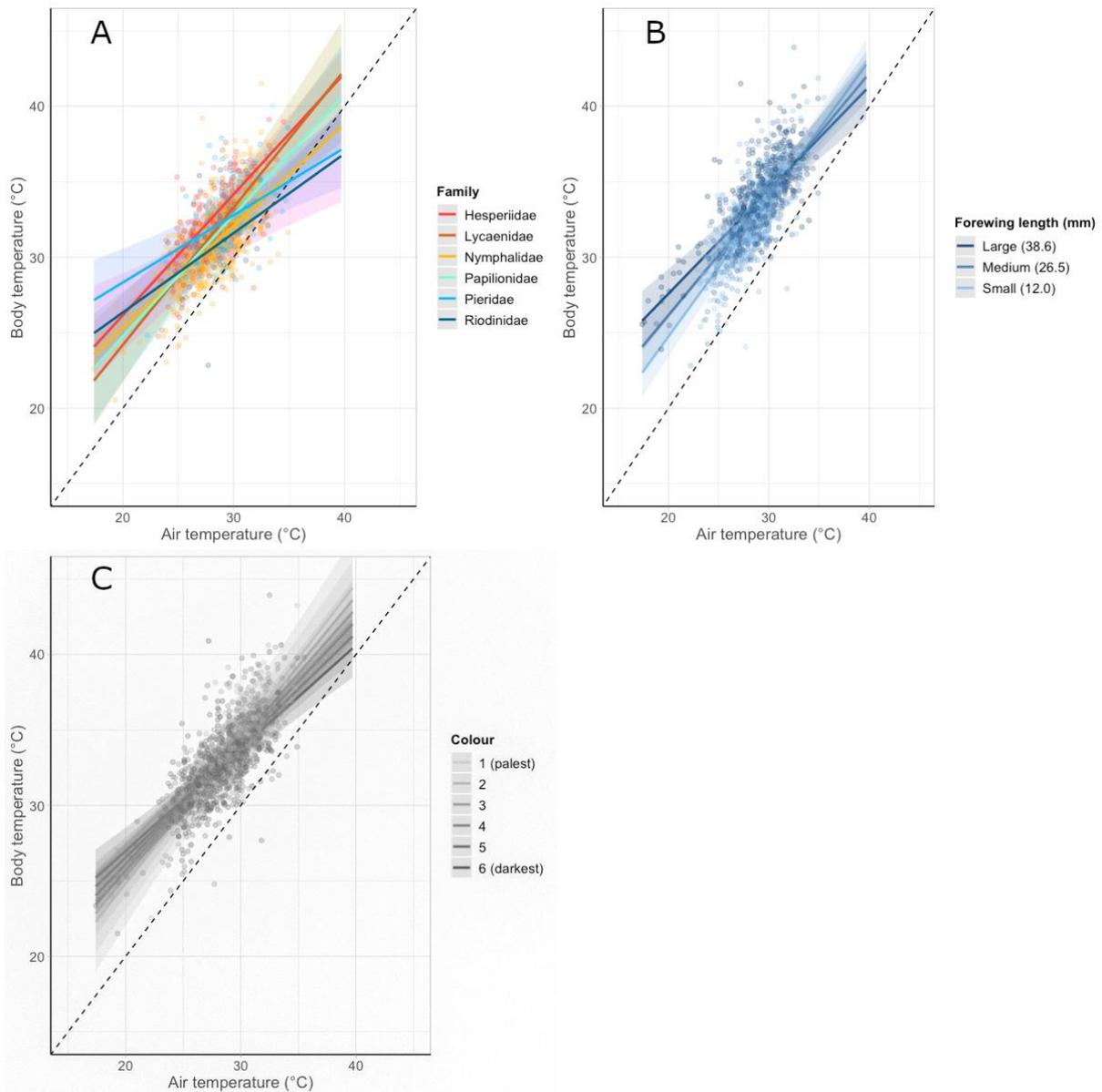
286

287 Across species, traits were related to each other. Families differed in the frequency of  
288 different colour categories, with Pieridae being paler than the other families ( $F_{1,5} = 517.30$ ,  $p$   
289  $< 0.001$ ). Families also differed in wing length, with Papilionidae being larger than the other  
290 families ( $F_{1,5} = 145.30$ ,  $p < 0.001$ ). Wing length and colour were not correlated ( $F = 0.02$ ,  $p =$   
291  $0.896$ ).

292

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

300 and Lycaenidae (mean = -0.1, range: -0.32–0.16) (Fig. 1A). Larger species had higher buffering  
301 estimates than smaller species ( $\chi^2_1 = 16.52$ ,  $p < 0.001$ ) (Fig. 1B). Dark species had higher  
302 buffering estimates than pale species ( $\chi^2_1 = 4.41$ ,  $p = 0.036$ ) (Fig. 1C).  
303



304  
305 Figure 1: Differences in the relationship between air temperature (°C) and body temperature  
306 (°C) (thermal buffering ability) between (A) families, (B) forewing lengths (in mm, modelled  
307 as a continuous variable but split into three groups for plotting: medium (mean wing length),  
308 large (one standard deviation above the mean) and small (one standard deviation below the

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

315

### 316 *Thermal tolerance*

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

321

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

327

328 Survival curves differed between species with different wing lengths ( $\chi^2_{1,212} = 718.0$ ,  $p <$   
329  $0.001$ ). Small species tolerated higher temperatures than large species (small, forewing length  
330  $\leq$  median (17.9 mm), LD50: 49.5°C, fall range: 11.3°C; large, forewing length  $>$  17.9 mm, LD50:  
331 48.5°C, fall range: 11.9°C) (Fig. 2B).

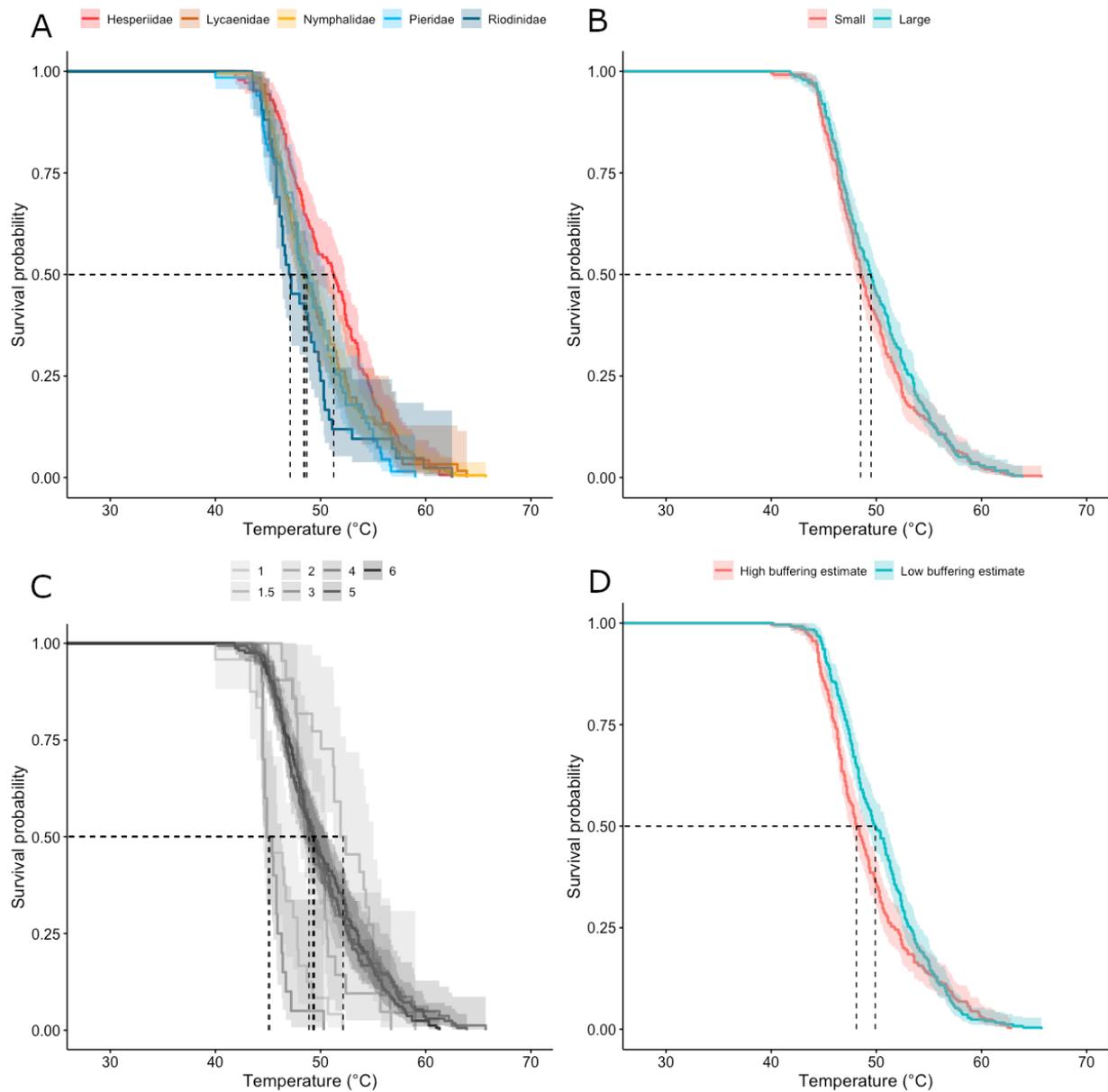
332

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  $\leq 3$ ), 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

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

356

## 357 **Discussion**

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

374

375 *Taxonomic group*

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

387

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

394

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

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

408

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

420

#### 421 *Wing length*

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

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

433

434 We also found that large species tended to have lower thermal tolerance than small species.  
435 As butterfly body mass correlates with wing length (Peixoto & Benson 2008), our finding  
436 differs from previous studies on tropical butterflies, which found thermal tolerance increased  
437 with mass (Luo *et al.* 2014; Klockmann, Günter, & Fischer, 2017). However, these studies  
438 tended to be based on single species, and may not reflect patterns across a community. It is  
439 possible that the negative relationship we found between wing length and thermal tolerance  
440 is related to the higher metabolic rate and oxygen demand in larger insects (Lachenicht *et al.*  
441 2010). This would make large butterflies particularly sensitive to further increases in  
442 temperature whereby their metabolism increases beyond oxygen delivery.

443

#### 444 *Wing colour*

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

448 1968), and achieve higher body temperatures than pale individuals (Dufour *et al.* 2018;  
449 Khazan *et al.* 2022). This is also in line with comparative temperate studies across latitudes  
450 (Zeuss *et al.* 2014), which found a higher incidence of darker species in cooler conditions,  
451 possibly also related to the advantage of dark species in being able to warm in cooler  
452 conditions. However, this pattern contradicts the strong thermal buffering ability and thermal  
453 tolerance of Pieridae, a family of generally pale butterflies. However darker Pierids had  
454 stronger thermal buffering abilities than paler Pierids. Dark species may be more likely to  
455 experience high body temperatures and be adapted to cope with the predicted increases in  
456 ambient temperatures under climate change. Pale butterflies may benefit from rising  
457 temperatures in the tropics, by enabling them to gain heat and become active more quickly.  
458 However, as well as warming up slower, pale butterflies are also less able to lose more heat  
459 at high ambient temperatures compared to dark butterflies, and we found them to have  
460 lower thermal tolerance than dark butterflies, putting them at an increased risk of  
461 overheating under rising temperatures.

462

#### 463 *The relationship between thermal buffering ability and thermal tolerance*

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

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

481

#### 482 *Conclusions*

483 Our findings have identified family, wing length, and colour as factors influencing the ability  
484 of species to cope with temperature change. These findings are strikingly similar to a similar  
485 study on temperate butterflies, which identified Pieridae and large butterflies as having the  
486 strongest thermal buffering abilities (Bladon *et al.* 2020). This implies a consistent pattern  
487 across tropical and temperate butterfly species. These findings provide important  
488 information to predict which traits, and species with these traits, may be selected for under  
489 warming temperatures in the tropics. This implies that species at risk under higher average  
490 and extreme temperature events are predictable based on traits. In particular, Lycaenidae  
491 represent 'losers' under future climate change, and as a species-rich family, there may be high  
492 species losses in the tropics. The trade-off between thermal buffering ability and thermal  
493 tolerance implies that most species will be vulnerable to climate change to an extent,  
494 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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508

509

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