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# Thermal preference and performance in a sub-Antarctic caterpillar: A test of the coadaptation hypothesis and its alternatives.

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1 Thermal preference and performance in a sub-Antarctic caterpillar: a test of the coadaptation  
2 hypothesis and its alternatives

3

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17 ABSTRACT

18 Physiological ecologists have long assumed that thermoregulatory behaviour will evolve to  
19 optimise physiological performance. The coadaptation hypothesis predicts that an animal's  
20 preferred body temperature will correspond to the temperature at which its performance is  
21 optimal. Here we use a strong inference approach to examine the relationship between thermal  
22 preference and locomotor performance in the caterpillars of a wingless sub-Antarctic moth,  
23 *Pringleophaga marioni* Viette (Tineidae). The coadaptation hypothesis and its alternatives  
24 (suboptimal is optimal, thermodynamic effect, trait variation) are tested. Compared to the  
25 optimal movement temperature (22.5°C for field-fresh caterpillars and 25, 20, 22.5, 25 and  
26 20°C following seven day acclimations to 0, 5, 10, 15 and 5-15°C respectively), caterpillar  
27 thermal preference was significantly lower (9.2°C for field-fresh individuals and 9.4, 8.8, 8.1,  
28 5.2 and 4.6°C following acclimation to 0, 5, 10, 15 and 5-15°C, respectively). Together with  
29 the low degree of asymmetry observed in the performance curves, and the finding that  
30 acclimation to high temperatures did not result in maximal performance, all, but one of the  
31 above hypotheses (i.e. 'trait variation') was rejected. The thermal preference of *P. marioni*  
32 caterpillars more closely resembles temperatures at which survival is high (5-10°C), or where  
33 feeding is optimal (10°C), than where locomotion speed is maximal, suggesting that thermal  
34 preference may be optimised for overall fitness rather than for a given trait.

35

36 **Keywords:** Caterpillars, Coadaptation, Fluctuating temperatures, Locomotion, Thermal  
37 performance curves, Thermal preference

38

39 **1. Introduction**

40 Because temperature determines the rate of most biological processes, ectotherms are usually  
41 assumed to select body temperatures that optimise performance. If fitness is positively related  
42 to performance, organisms that prefer optimum temperatures should have an advantage over  
43 those that do not (Huey and Bennett, 1987; Angilletta et al., 2002a; Huey et al., 2003). In  
44 consequence, natural selection should result in similarity between the optimum and preferred  
45 temperatures because this should maximise Darwinian fitness (Martin and Huey, 2008;  
46 Anderson et al., 2011), resulting in thermal coadaptation.

47 Thermal coadaptation has been reported in many ectotherm taxa, including reptiles  
48 (Van Berkum, 1986; Huey and Bennett, 1987; Garland et al., 1991; Kubisch et al., 2011),  
49 insects (Sanford and Tschinkel, 1993; Forsman, 1999; Calabria et al., 2012), nematodes  
50 (Anderson et al., 2011), and fish (Khan and Herbert, 2012). Nonetheless, mismatches between  
51 thermoregulatory behaviour and thermal physiology are common. For example, in lizards,  
52 optimal locomotor performance is achieved at a higher temperature than preferred body  
53 temperatures (reviewed in Martin and Huey, 2008; Fernandez et al., 2011). Similar findings  
54 for population growth have been reported for insects (Smith, 1965; Langer and Young, 1976;  
55 Allsopp et al., 1980; Allsopp, 1981; White, 1987; Jian et al., 2002) and other ectotherms  
56 (Åkesson, 1976; Zhang and Lefcort, 1991; Prevedelli and Simonini, 2001; Jia et al., 2002;  
57 Tepler et al., 2011).

58 Several hypotheses have been proposed to explain this departure from coadaptation,  
59 particularly when optimum temperatures are higher than preferred temperatures. First, Martin  
60 and Huey (2008) suggested that preferred temperatures should be lower than optimum  
61 temperatures (hereafter the ‘suboptimal is optimal’ hypothesis) because asymmetric  
62 performance curves mean that performance decreases rapidly above the optimum temperature  
63 (Huey and Stevenson, 1979; Huey and Kingsolver, 1989). Thus, preference should be for lower

64 temperatures to minimise the risk of reduced performance (and possibly death) when  
65 thermoregulation is imperfect (Martin and Huey, 2008).

66         Second, Asbury and Angilletta (2010) hypothesised that the thermodynamic effect (i.e.  
67 poorer performance at low temperatures because biochemical reactions proceed more slowly,  
68 (Frazier *et al.*, 2006; Angilletta *et al.*, 2010)) means that natural selection should favour a  
69 thermal optimum that is higher than body temperature. On the basis of this thermodynamic  
70 effect, it is argued that adaptation or acclimation to warm environments should therefore confer  
71 greater performance compared to colder environments (i.e. “hotter is better”) (Angilletta 2009;  
72 Angilletta *et al.* 2010). Asbury and Angilletta (2010) argued that selection driven by a  
73 thermodynamic effect could explain differences between thermoregulatory behaviour and  
74 thermal physiology. This is particularly true for the large differences between preferred  
75 temperature and the thermal optimum found in some studies (e.g. *c.* 8°C for geckos (Angilletta  
76 *et al.* 1999) and 17°C for marine invertebrates (Tepler *et al.* 2011)). We term this the  
77 ‘thermodynamic effect’ hypothesis.

78         We term a third hypothesis as the ‘trait variation’ hypothesis. According to this  
79 hypothesis, if optimum temperatures vary among physiological processes, then no single  
80 thermal preference will be optimal for all systems (Huey and Stevenson, 1979). In  
81 consequence, thermal preference may depend on where the major constraints for fitness lie  
82 under a given set of conditions. For example, when nutrients are plentiful, preference for high  
83 temperature in migratory locusts favours maximal growth rather than efficient utilization of  
84 nutrients, but when nutrients are limited, the preferred temperature is lowered to maximize  
85 efficiency (Miller *et al.*, 2009; Coggan *et al.*, 2011; Clissold *et al.*, 2013). This hypothesis  
86 reflects the more general one that there may be differential effects of temperature on individual  
87 traits and on overall fitness, and that understanding the relationships between the adaptive value  
88 of particular traits and overall fitness is important (Kingsolver and Woods, 1997; Woods and

89 Harrison, 2002). Moreover, these effects may take different forms depending on whether  
90 environmental temperatures are relatively constant or variable (Williams et al., 2012; Colinet  
91 et al., 2015; Kingsolver et al., 2015).

92         Although all of these hypotheses enjoy some empirical support, they have rarely been  
93 examined simultaneously. The strong inference approach (Platt, 1964) adopts joint exploration  
94 of alternative explanations for variations in thermal performance (Huey et al., 1999). Here, we  
95 apply this approach to caterpillars of the flightless sub-Antarctic moth, *Pringleophaga marioni*,  
96 for which the thermal biology is well-known (Klok and Chown, 1997; Sinclair and Chown,  
97 2003; Sinclair *et al.*, 2004; Sinclair and Chown, 2005, 2006; Haupt et al., 2014a,b, 2016;  
98 Chown et al., 2016). Specifically, we examine the relationship between thermal preference  
99 ( $T_{\text{pref}}$ ) and the thermal optimum ( $T_{\text{opt}}$ ) for locomotion. First, we compare  $T_{\text{opt}}$  and  $T_{\text{pref}}$ . If these  
100 traits are similar, the coadaptation hypothesis cannot be rejected. If they are different, and the  
101 magnitude of this difference is relatively small and the performance curve asymmetric, the  
102 ‘suboptimal is optimal’ hypothesis cannot be rejected. Alternatively, we determine whether  
103 variation in performance curves following exposure to different acclimation regimes accords  
104 with the expectations of a thermodynamic effect (i.e. is hotter better?), thus testing the  
105 ‘thermodynamic effect’ hypothesis. Finally, we determine whether or not thermal preference  
106 aligns with performance measures other than locomotion, and specifically those that may be  
107 significant for a relatively long-lived (ca 1 year) detritivorous caterpillar (Haupt et al., 2014a).  
108 If so, and all other hypotheses are rejected, the ‘trait variation’ hypothesis cannot be rejected.

109

## 110 **2. Materials and methods**

### 111 *2.1. Study site and species*

112 *Pringleophaga marioni* Viette (Tineidae) is a flightless moth, the caterpillars of which occur  
113 in virtually all habitats on the sub-Antarctic Marion and Prince Edward islands (46.9°S, 36.7°E)

114 (Crafford et al., 1986; Haupt et al., 2014a, 2016). The caterpillars are detritivores and take  
115 nearly a year to progress through this stage (Haupt et al. 2014a). Field collected caterpillars  
116 have a critical thermal minimum ( $CT_{\min}$ ) between -1.6 and 0.1°C, and a critical thermal  
117 maximum ( $CT_{\max}$ ) range of 37.7 to 38.7°C (Klok and Chown, 1997).

118 Marion Island has a low mean annual air temperature of 6.5°C with relatively stable  
119 average ambient air temperatures ranging from 2°C in winter to 7°C in summer and a total  
120 annual precipitation of 1900 mm (Le Roux and McGeoch, 2008). At low altitudes (4-6 m a.s.l),  
121 soil microhabitat temperatures are  $6.1 \pm 2.7$  (°C) (mean  $\pm$  s.d.; range: -1 to 22.5°C). At higher  
122 altitudes (400 m a.s.l) where *P. marioni* are also found (Crafford et al., 1986), mean soil  
123 temperatures are  $3.8 \pm 3.4$  (°C) (mean  $\pm$  s.d.; range: -8.0 to 20.0°C; Lee et al., 2009; Haupt et  
124 al., 2016).

125

## 126 2.2. Collection and acclimation

127 This study was undertaken in the laboratory on Marion Island during relief voyages in 2010,  
128 2011, and 2012 (each voyage included 4-6 weeks at the station). Caterpillars were collected  
129 from abandoned wandering albatross nests (*Diomedea exulans*), where they occur in high  
130 numbers (Haupt et al., 2016), and returned to the laboratory within six hours of collection.  
131 Individuals were placed in petri dishes filled with albatross nest material, which served as both  
132 refuge and food (Haupt et al., 2014a). Maintaining individuals separately was necessary to  
133 avoid cannibalism (French and Smith, 1983).

134 To determine if exposure to different acclimation regimes results in demonstration of a  
135 thermodynamic effect, caterpillars were held for seven days in incubators (MIR 154, Sanyo,  
136 Osaka, Japan, accurate to  $\pm 0.5^\circ\text{C}$ ) set at 0°C, 5°C, 10°C and 15°C. The timing of acclimation  
137 period was based on previous trials showing acclimation responses within a week for this  
138 species (Sinclair and Chown, 2003) and for insects generally (Weldon et al., 2011). The effects

139 of variable temperature regimes were also examined by acclimating caterpillars to a fluctuating  
140 temperature of 5-15°C (see also Chown et al., 2016 who found that this treatment results in a  
141 lower metabolic rate relative to a constant mean temperature of 10°C). Constant temperatures  
142 fall within the soil microhabitat temperature range for this species on Marion Island (Chown  
143 and Crafford, 1992; Lee et al., 2009) and the fluctuating temperature simulates conditions  
144 within wandering albatross nests where caterpillars are abundant (Haupt et al. 2016). A group  
145 of caterpillars were also kept at 5°C for only three days, and these field-fresh individuals served  
146 as a control (Deere and Chown, 2006).

147

### 148 *2.3. Thermal preference trials*

149 An important consideration during measures of thermal preference is the likelihood that low  
150 thermal preference values may be a result of animals effectively trapped at the lower end of the  
151 thermal gradient. Body temperatures and therefore locomotor speed of small ectotherms match  
152 temperatures at a given location, thus making it difficult to distinguish between thermal  
153 preference (selecting a specific temperature) and thermal dependence of movement (which  
154 means the animal cannot move away from low temperatures), thus biasing estimates of thermal  
155 preference downward (Dillon et al., 2012). Here we not only considered this possibility *a*  
156 *priori*, and designed our experiments to avoid it, but we also analysed the data adopting both a  
157 more liberal approach to this effect and a more conservative one. Specifically, the low  
158 temperature end of the preference gradient was set at 0°C, a temperature 0.6°C higher than  
159 average critical thermal minimum ( $CT_{min}$ ) for the species (Klok and Chown 1997). Then,  
160 because the maximum recorded value of  $CT_{min}$  is 0.1°C, we also undertook analyses excluding  
161 all individuals which showed preference temperatures lower than 0.2°C (see below).

162 Thermal preference was first determined along a gradient from *c.* 0-15°C, reflecting the  
163 microclimate temperatures on Marion Island (Chown and Crafford 1992). Because caterpillars



164 showed no defined preference on this gradient (Table S1), this experiment was then repeated  
165 on a gradient of *c.* 0-30°C using a different group of individuals. Experiments were conducted  
166 on a 75-cm temperature gradient (see Fig. S1) with temperatures controlled at each end using  
167 a refrigerated circulator (LTC 12, Grant Instruments Ltd., Cambridge, UK). Temperatures  
168 along the gradient were measured and recorded every 5 s using eight evenly-spaced 40-gauge  
169 Type T thermocouples connected to an eight channel SQ800 Grant Squirrel data logger (Grant  
170 Instruments Ltd, Cambridge, U.K.). Dark walk-through “tunnels” constructed from plastic  
171 tubing served as refuges along the gradient and were placed at intervals corresponding with the  
172 thermocouple positions following Marais and Chown (2008) (See “B” in Fig. S1).

173         At the beginning of each experiment, individuals were weighed ( $\pm$  0.5 mg; AE163  
174 balance, Mettler-Toledo, EngNet, South Africa). An individual was then placed in the centre  
175 of the gradient and the apparatus was covered with black plastic to allow caterpillars to choose  
176 temperatures in the dark. After one hour, the temperature corresponding to the position of the  
177 caterpillar was recorded. In another experiment (using a different group of individuals), the  
178 temperature of the entire gradient was set to *c.* 10°C. This constant temperature gradient  
179 provided a control to confirm that caterpillars show a thermal preference as opposed to  
180 favouring particular ends of the apparatus (Anderson et al., 2007).

181

#### 182 *2.4. Locomotor performance trials*

183 The locomotion speed of individual caterpillars ( $n = 28$  per acclimation temperature) was  
184 measured using a temperature-controlled walking stage with a hardboard interior surface (see  
185 Fig S2). A refrigerated circulator (LTC 12, Grant Instruments, Cambridge, UK) controlled the  
186 temperature of the stage which was measured via a Type-K thermocouple connected to a digital  
187 thermometer (CHY 507, Firemate, Taiwan). To avoid heat shock affecting performance at  
188 lower temperatures (Lachenicht et al., 2010), individuals were examined first at randomized

189 test temperatures of 0°C, 5°C, 10°C, 15°C and 20°C, and then at high temperatures of 25°C,  
190 30°C and 35°C as these temperatures approach the  $CT_{\max}$  of 38°C for *P. marioni*.

191 At the start of each experiment, an individual caterpillar was weighed (as above) and  
192 then placed in the centre of the walking stage and held under a plastic container for four minutes  
193 to equilibrate to the temperature being tested. The caterpillar was then released and when it  
194 moved without faltering, the distance that the head capsule moved over a 20 s period was  
195 recorded. The trial was repeated three times in succession. The longest distance recorded was  
196 used in the analyses, because lower values may reflect an individual's unwillingness to move,  
197 rather than its inability to move faster (Huey and Bennett, 1987; Angilletta et al., 2002b).  
198 Between different temperatures, individual caterpillars were returned to the petri dish they were  
199 taken from and held at their acclimation temperature for a minimum of one hour before the  
200 next temperature trial.

201 From these measurements, the key performance traits of optimum temperature ( $T_{\text{opt}}$ ),  
202 maximum speed at the optimum temperature ( $U_{\text{max}}$ ), and performance breadth (the index of the  
203 breadth of the curve,  $T_{\text{br}}$ ) were obtained. The optimum temperature and maximum speed were  
204 chosen from the experimental data (i.e. the test temperature with the greatest speed) (Gilchrist,  
205 1996), and these values were used to calculate  $T_{\text{br}}$  for each individual using Gilchrist's (1996)  
206 formula:

$$T_{\text{br}} = \sqrt{\frac{\sum [U_i (T_i - T_{\text{opt}})]^2}{U_{\text{max}}^2}}$$

207

208

(Equation 1)

209 where  $T_{\text{opt}}$  is the temperature at which an individual moved the fastest,  $U_{\text{max}}$  is the maximum  
210 speed at  $T_{\text{opt}}$ , and  $U_i$  is the speed at  $T_i$ , i.e. speed at a given test temperature.

211

212 *2.5. Data analyses*

213 Regression analyses revealed no relationships between body mass and each performance trait  
214 or thermal preference ( $p > 0.05$  in all cases, results not shown), indicating that mass was not  
215 responsible for any variation observed and it was therefore not included as a covariate in any  
216 of the analyses. In consequence, analyses proceeded as follows. First, we determined whether  
217  $T_{\text{pref}}$  was influenced by experimental design. The median thermal preference for each  
218 acclimation temperature on the *c.* 0-30°C gradient was calculated using all individuals. To be  
219 certain that individuals were not trapped at their  $CT_{\text{min}}$  temperatures, the median thermal  
220 preference was also calculated after excluding individuals that preferred temperatures below  
221 0.2°C, since the  $CT_{\text{min}}$  range for *P. marioni* lies between -1.6°C and 0.1°C (Klok & Chown,  
222 1997). For each treatment group (i.e. each acclimation temperature and field fresh individuals),  
223 a Wilcoxon rank-sum test (because of non-normal data), as implemented in R.3.0.0 was used  
224 to test whether there were any significant differences in thermal preference when individuals  
225 with preferences close to or within the range of  $CT_{\text{min}}$  values were excluded. We found small,  
226 but significant, differences with these two approaches. Thus, we present results from both the  
227 conservative data set (preferred temperatures below 0.2°C excluded) and the full data set  
228 (preferred temperatures below 0.2°C included) as a comparison for all further analyses (see  
229 Results).

230 Next, to determine if  $T_{\text{opt}}$  and  $T_{\text{pref}}$  are indistinguishable (i.e. coadapted), the medians of  
231  $T_{\text{opt}}$  and  $T_{\text{pref}}$  for each treatment group were compared using Wilcoxon rank-sum tests (because  
232 of non-normal data). In addition, to determine how asymmetric the performance curves were,  
233 the degree of asymmetry was calculated for each individual using the following equation from  
234 Martin and Huey (2008):

235

$$\text{asymmetry} = \frac{2T_{\text{opt}} - T_{\text{max}} - T_{\text{min}}}{T_{\text{max}} - T_{\text{min}}}$$

236

237

(Equation 2)

238

239 where  $T_{\text{opt}}$  is the temperature at which an individual moved the fastest, and  $T_{\text{max}}$  and  $T_{\text{min}}$  are  
240 the upper and lower limiting temperatures for performance respectively (Martin and Huey  
241 2008). We used data from our trials to represent  $T_{\text{max}}$  and  $T_{\text{min}}$ , and then also included data from  
242 Klok & Chown (1997) on  $CT_{\text{min}}$  and  $CT_{\text{max}}$  to estimate the degree of asymmetry. In the latter  
243 case we included combinations of data that used the minimum values for any individual of  
244  $CT_{\text{min}}$  and  $CT_{\text{max}}$  to those which used the maximum values, and applied these to optimum  
245 temperature estimates for all acclimations examined in the current study.

246 To test the ‘thermodynamic effect’ hypothesis, ordered factorial ANOVAs (analysis of  
247 variance) with orthogonal polynomial contrasts as in Huey et al. (1999) were used to  
248 distinguish ‘warmer is better’ from the alternative acclimation hypotheses (see Deere and  
249 Chown, 2006). These analyses compared  $T_{\text{opt}}$ ,  $U_{\text{max}}$  and  $T_{\text{br}}$  between the constant acclimation  
250 temperatures of 0°C, 5°C, 10°C, and 15°C, as these temperatures were ordered. Orthogonal  
251 polynomial contrast analyses require strict adherence to the assumptions of ANOVA, which  
252 are: normally distributed residuals, homogeneity of variance and a balanced design (Huey et  
253 al., 1999). Shapiro-Wilk’s tests showed instances of non-normality, but Levene’s tests and  
254 plots of the residuals indicated normality and homogeneity of variances (Table S2; Fig S3).

255 Finally, to examine the effects of the fluctuating acclimation temperature on  
256 performance, an ANOVA was used to compare performance traits between 5-15°C and 10°C  
257 (i.e. the closest comparable constant temperature). Similarly, field-fresh individuals were  
258 compared with those that were subjected to acclimation. The effect of acclimation on thermal  
259 preference was also examined, and this was done using a Kruskal-Wallis test of significance  
260 (because of non-normal data (Fig. S4)). Analyses were implemented in R3.0.0 (R core team,  
261 2013).

262

### 263 3. Results

264 The distribution of caterpillars under a constant temperature of *c.* 10°C showed that caterpillars  
265 were unlikely to favour a particular end of the gradient because a similar number of individuals  
266 were found at either end, compared to the distribution of caterpillars on the *c.* 0-30°C gradient  
267 where more individuals were found at one end (Fig. S5).

268 Excluding preferred temperatures below the upper bound we set (0.2°C), increased the  
269 median  $T_{\text{pref}}$  slightly, significantly so in 0°C acclimated individuals (from 4.8°C to 9.4°C;  
270 Wilcoxon rank-sum test:  $W = 264.5$ ,  $p = 0.028$ ; Fig. 1). Thus, we used both the full data set  
271 (individuals with preferences below 0.2°C included), as well as the conservative data set  
272 (individuals with preferences below 0.2°C excluded) for further analyses, the latter to account  
273 for the possibility that caterpillars became trapped at low temperatures (cf. Dillon et al., 2012).  
274 Overall, median thermal preference ranged from 4.2-4.6°C (at 5-15°C) to 4.8-9.4°C (at 0°C)  
275 (Fig. 2; Table 1). Acclimation at 15°C and 5-15°C yielded the lowest  $T_{\text{pref}}$  (Table 1).

276 By contrast, median values for  $T_{\text{opt}}$  of locomotor performance were significantly higher  
277 than the preferred temperatures, and ranged between 20°C and 25°C (Table 1, Fig. 3). Mean  
278 optimum temperature ( $T_{\text{opt}}$ ), maximum speed ( $U_{\text{max}}$ ) and performance breadth ( $T_{\text{br}}$ ) ranged  
279 between 21.4-24.1°C, 4.7-5.4 mm.sec<sup>-1</sup>, and 16.1-19.8°C, respectively (Table 2). Acclimation  
280 to different temperatures did not have a significant effect on locomotor performance (Table 3).  
281 Neither  $T_{\text{opt}}$  nor  $U_{\text{max}}$  differed significantly between the fluctuating temperature regime of 5-  
282 15°C and the constant acclimation temperature of 10°C ( $T_{\text{opt}}$ :  $F = 0.26$ ,  $p = 0.61$ ;  $U_{\text{max}}$ :  $F =$   
283  $2.60$ ,  $p = 0.113$ ), but  $T_{\text{br}}$  was significantly narrower after the 5-15°C acclimation compared to  
284 the 10°C treatment ( $F = 5.36$ ,  $p = 0.024$ ; Table 2). Acclimation also had no significant influence  
285 on thermal preference both when data including preferences below 0.2°C were included ( $H =$   
286  $4.381$ , d.f. = 5,  $p = 0.496$ ) and excluded ( $H = 10.925$ , d.f. = 5,  $p = 0.053$ ). The performance of  
287 field fresh individuals also did not differ significantly from those acclimated ( $T_{\text{opt}}$ :  $F = 0.87$ ,  $p$

288 = 0.503;  $U_{\max}$ : F = 1.56, p = 0.174;  $T_{br}$ : F = 2.21, p = 0.056). Locomotor performance curves of  
289 *P. marioni* were not strongly asymmetric (Figure 4), and this is supported by the low symmetry  
290 values for the curves (Table 2), which remained low when including the critical thermal limits  
291 data from Klok & Chown (1997) (varying between 0.08 and 0.30 among acclimations and  
292 using data either on minimum or maximum critical thermal limit values).

293

#### 294 **4. Discussion**

295 In this study, we simultaneously tested the hypothesis of coadaptation of optimal and preferred  
296 body temperatures (Huey and Bennett, 1987; Angilletta et al., 2002a; Huey et al., 2003;  
297 Angilletta 2009), and several of its alternatives (Huey and Stevenson, 1979; Kingsolver and  
298 Woods, 1997; Martin and Huey, 2008; Asbury and Angilletta, 2010). Before doing so, we first  
299 took into account the possibility that animals may have been trapped at the low temperature  
300 end of the thermal gradient, resulting in a misinterpretation of the actual preferred temperatures  
301 (Dillon et al., 2012). We found some support for immobility at low temperatures influencing  
302 estimates of  $T_{\text{pref}}$ . In consequence, we used a truncated data set, excluding all preference values  
303 below 0.2°C to account for potential bias.

304         Bearing this correction in mind, preferred temperatures of *P. marioni* were substantially  
305 lower than the optimum temperatures, particularly so when data were not adjusted for the  
306 likelihood of individuals being trapped at temperatures below their  $CT_{\text{min}}$ . These differences  
307 were 17.3 for field-fresh caterpillars, and 20.2 following acclimation to 0°C, 14.8 after 5°C,  
308 17.1 after 10°C, 20.6 after 15°C and 15.8 after 5-15°C (Table 1a). Thus, we reject the  
309 coadaptation hypothesis (Huey and Bennett, 1987; Angilletta et al. 2006; Angilletta, 2009):  
310  $T_{\text{pref}}$  does not appear to have evolved to match  $T_{\text{opt}}$  in *P. marioni*. Such large discrepancies  
311 between  $T_{\text{opt}}$  and  $T_{\text{pref}}$  have also been found in other species, e.g. 11°C in *Pterohelaeus*  
312 *darlingensis* beetles (Allsopp et al., 1980; Allsopp, 1981), 17°C in intertidal snails (*Clorostoma*

313 *funnebralis*) (Tepler et al., 2011); and 8°C in house geckos, *Hemidactylus turcicus* (Huey et al.,  
314 1989; Angilletta et al., 1999). Moreover, the locomotor performance curves of *P. marioni* are  
315 more-or-less symmetrical, further suggesting that the ‘suboptimal is optimal’ hypothesis,  
316 which assumes asymmetric performance curves (Martin & Huey 2008), can be rejected as a  
317 possible explanation for the large mismatch between  $T_{\text{opt}}$  and  $T_{\text{pref}}$ .

318 An alternative explanation for the current findings is that selection, driven by a  
319 thermodynamic effect, could explain the large differences between preferred temperature and  
320 the thermal optimum (Asbury and Angilletta 2010). Acclimation had little effect on thermal  
321 performance curves or preferred temperature, however, suggesting that the thermodynamic  
322 effect hypothesis can also be rejected. Previous studies have reported varying, but typically  
323 small effects of phenotypic plasticity in response to temperature in terrestrial arthropods from  
324 Marion Island (Deere and Chown, 2006; Deere et al., 2006; Slabber et al., 2007; Marais &  
325 Chown, 2008). *Pringleophaga marioni* caterpillars show little phenotypic plasticity of  
326 metabolic-rate temperature curves in response to acclimation, under both stable and fluctuating  
327 acclimation conditions (Chown et al. 2016). Here, we found a similar effect for thermal  
328 performance curves based on locomotion speed, and in particular for  $T_{\text{opt}}$  and  $U_{\text{max}}$ . The  
329 unpredictability of thermal cues may explain limited phenotypic plasticity in many species on  
330 Marion Island (Deere et al., 2006), including *P. marioni* caterpillars. Nonetheless, after  
331 exposure to fluctuating conditions, caterpillars had a significantly narrower performance  
332 breadth compared to those held at a constant temperature of 10°C (i.e. closest comparable mean  
333 temperature). Performance breadth is expected to change significantly in fluctuating as  
334 opposed to constant temperatures depending on whether variation is within or among  
335 generations (Huey and Slatkin 1976; Huey and Stevenson 1979; Huey and Kingsolver 1993;  
336 Gilchrist, 1995; Huey et al., 1999). Given that 15°C is detrimental to caterpillars within a  
337 generation (Haupt et al. 2014a), the narrowing in performance breadth may well have been due

338 to this negative effect of prolonged high temperature, in keeping with theoretical considerations  
339 (Gilchrist, 1995; see also discussion in Dowd et al., 2015; Kingsolver et al., 2015). Thus,  
340 further consideration of the effects of stable versus fluctuating temperatures is warranted, even  
341 when these effects may initially appear to be small. Such fluctuating temperatures, in  
342 association with a symmetric performance curve may also mean that selection for preferred  
343 temperatures matching the optimum may not be pronounced.

344 In the absence of support for the coadaptation, suboptimal is optimal and  
345 thermodynamic effect hypotheses, an alternative explanation for the differences we observed  
346 between  $T_{\text{pref}}$  and  $T_{\text{opt}}$  is that  $T_{\text{pref}}$  may align with the thermal optimum for some other measure  
347 of performance that may be more significant for a detritivorous caterpillar (Haupt et al., 2014a).  
348 For example, if the optimum temperature for locomotion is higher than that for growth, then  
349 animals may choose a high preference temperature only when the ability to move faster is of  
350 more immediate importance than the ability to grow quickly (Huey and Stevenson, 1979;  
351 Anderson et al., 2011). Thus,  $T_{\text{pref}}$  will be driven by the  $T_{\text{opt}}$  only of physiological systems that  
352 improve fitness (e.g. Miller et al., 2009; Coggan et al., 2011; Clissold et al., 2013). In the case  
353 of *P. marioni*, caterpillar survival to pupation is higher at 5 to 10°C than at 15°C (Haupt et al.,  
354 2014a). Similarly, 10°C is the optimum temperature for caterpillar feeding, and low thermal  
355 preferences may be linked to nutrient or digestive efficiency of caterpillars feeding on a diet of  
356 detritus (Crafford 1990). Compared to the optimum temperature for locomotion (*c.* 23°C),  
357 these temperatures more closely match the preferred temperatures of 4.6°C to 9.2°C. Thus, for  
358 *P. marioni* caterpillars on Marion Island, although locomotion may be important for locating  
359 food resources and suitable microhabitats that minimise predation (Haupt et al., 2014a, b;  
360 2016), caterpillars may prefer lower temperatures where survival and assimilation efficiency is  
361 maximal (Haupt et al., 2014a), or where costs associated with high temperatures are minimized.  
362 Thus, the trait variation hypothesis may explain the large mismatch observed between  $T_{\text{pref}}$  and



363  $T_{opt}$ . We suggest that this hypothesis, and the more general idea of differential effects of  
364 temperature on individual traits and on overall fitness (Kingsolver and Woods, 1997; Darveau  
365 et al., 2002; Woods and Harrison, 2002) deserve further consideration both for this species and  
366 for others.

367

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373

### 374 **Appendix A. Supplementary data**

375 Supplementary data associated with this article can be found, in the online version, at xxx

376

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580

581 **Table 1**

582 Median values for thermal preference ( $T_{\text{pref}}$ ) (c. 0-30°C gradient) ((**a**) = individuals with  
 583 preferences below 0.2°C included, (**b**) = individuals with preferences below 0.2°C excluded),  
 584 optimum temperature ( $T_{\text{opt}}$ ), the difference between  $T_{\text{pref}}$  and  $T_{\text{opt}}$ , and results of the Wilcoxon  
 585 rank-sum test comparing  $T_{\text{pref}}$  and  $T_{\text{opt}}$  are shown for each treatment group.

586 **(a) All individuals**

Group	$T_{\text{pref}}$ (°C)	$T_{\text{opt}}$ (°C)	Difference (°C)	Wilcoxon rank-sum test
field-fresh	5.2 (n = 35)	22.5	17.3	W = 863, p < 0.0001
acclimation				
0°C	4.8 (n = 35)	25	20.2	W = 868, p < 0.0001
5°C	5.2 (n = 35)	20	14.8	W = 853, p < 0.0001
10°C	5.4 (n = 35)	22.5	17.1	W = 887, p < 0.0001
15°C	4.4 (n = 35)	25	20.6	W = 944, p < 0.0001
5-15°C	4.2 (n = 33)	20	15.8	W = 965, p < 0.0001
n = sample size				

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596 **(b) Individuals with preferences below 0.2°C excluded**

Group	$T_{\text{pref}}(^{\circ}\text{C})$	Number of individuals with $T_{\text{pref}} < 0.2^{\circ}\text{C}$	$T_{\text{opt}}(^{\circ}\text{C})$	Difference ( $^{\circ}\text{C}$ )	Wilcoxon rank-sum test
field-fresh	9.2 (n = 25)	10	22.5	13.3	W = 583, p < 0.0001
acclimation					
0°C	9.4 (n = 23)	12	25	15.6	W = 532, p < 0.0001
5°C	8.8 (n = 27)	8	20	11.2	W = 629, p < 0.0001
10°C	8.1 (n = 28)	7	22.5	14.4	W = 691, p < 0.0001
15°C	5.2 (n = 29)	6	25	19.8	W = 776, p < 0.0001
5-15°C	4.6 (n = 24)	11	20	15.4	W = 657, p < 0.0001
n = sample size					

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599 **Table 2**

600 Summary statistics showing means and standard errors for the performance traits: optimum  
 601 temperature ( $T_{opt}$ ), maximum speed ( $U_{max}$ ), and performance breadth ( $T_{br}$ ).

	$T_{opt}$ (°C)		$U_{max}$ (mm·sec <sup>-1</sup> )		$T_{br}$ (°C)		Degree of asymmetry
	Mean	s.e.	Mean	s.e.	Mean	s.e.	
field-fresh	23.8	1.14	4.7	0.18	18.7	0.99	0.31
acclimation							
0°C	23.2	1.04	5	0.24	19.8	0.78	0.36
5°C	21.4	1.09	4.8	0.20	18.6	0.89	0.25
10°C	23.6	1.15	4.9	0.25	18.9	0.92	0.35
15°C	24.1	0.86	5.2	0.26	17.8	0.71	0.38
5-15°C	22.9	0.79	5.4	0.22	16.1	0.77	0.37
s.e. = standard error							

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607 **Table 3**

608 Outcome of the orthogonal polynomial contrasts on the effects of acclimation on the optimum  
 609 temperature ( $T_{opt}$ ), maximum speed ( $U_{max}$ ), and the performance breadth ( $T_{br}$ ). In each case, the  
 610 main effects of acclimation, as well as the orthogonal polynomial contrasts (i.e. linear and  
 611 quadratic), together with the sign and value of their estimates are shown.

$T_{opt}$					
Source	d.f.	SS	MS	$F$	$P$
Acc	3	113	37.72	1.25	0.294
Contrast					
acc linear	1	33	32.54	1.08	0.301
acc quadratic	1	38	37.72	1.25	0.265
Parameter		Estimate	s.e.	$t$	$P$
acc linear		1.08	1.04	1.04	0.301
acc quadratic		1.16	1.04	1.12	0.265
$U_{max}$					
Source	d.f.	SS	MS	$F$	$P$
Acc	3	2.63	0.88	0.55	0.647
Contrast					
acc linear	1	0.89	0.89	0.56	0.455
acc quadratic	1	1.64	1.64	1.03	0.312
Parameter		Estimate	s.e.	$t$	$P$
acc linear		0.18	0.24	0.75	0.455
acc quadratic		0.24	0.24	1.02	0.312
$T_{br}$					

Source	d.f.	SS	MS	<i>F</i>	<i>P</i>
Acc	3	58	19.34	1.01	0.391
Contrast					
acc linear	1	24.6	45.57	2.38	0.126
acc quadratic	1	0.3	0.26	0.01	0.908
Parameter		Estimate	s.e.	<i>t</i>	<i>P</i>
acc linear		-1.28	0.83	-1.54	0.126
acc quadratic		0.10	0.83	0.12	0.908
acc = acclimation temperature					
SS = sums of squares; MS = mean squares; s.e. = standard error					

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615 **Figure legends**

616

617 **Fig. 1.** Difference in the thermal preference (median) of *Pringleophaga marioni* caterpillars  
618 when  $CT_{\min}$  values are excluded (excl. $CT_{\min}$ ) and not (incl. $CT_{\min}$ ). This is shown for caterpillars  
619 acclimated at 0°C, 5°C, 10°C, 15°C, and 5-15°C, as well as field-fresh individuals. Box plots  
620 show the median and interquartile range of thermal preference, and boxes in which notches  
621 (i.e. narrowing of the box around the median) overlap are unlikely to have significantly  
622 different medians under an appropriate test (Crawley 2007).

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624 **Fig. 2.** The thermal preference of *Pringleophaga marioni* caterpillars. In **(a)**, individuals with  
625 preferences below 0.2°C are included. In **(b)** they are excluded. Individuals acclimated at 0°C  
626 (blue), 5°C (green), 10°C (orange), 15°C (red), and 5-15°C (grey), and field-fresh individuals  
627 (black), as the number of counts on a gradient ranging from *c.* 0-30°C.

628

629 **Fig. 3.** The difference between the optimum temperature ( $T_{\text{opt}}$ ) and thermal preference ( $T_{\text{pref}}$ )  
630 of *Pringleophaga marioni* caterpillars. In **(a)**, individuals with preferences below 0.2°C are  
631 included. In **(b)** they are excluded. Caterpillars acclimated at 0°C, 5°C, 10°C, 15°C, and 5-  
632 15°C, as well as field-fresh individuals are shown. Box plots show the median and interquartile  
633 range for both  $T_{\text{opt}}$  and  $T_{\text{pref}}$  and boxes in which notches (i.e. narrowing of the box around the  
634 median) do not overlap are likely to have significantly different medians under an appropriate  
635 test (Crawley 2007).

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637 **Fig. 4.** The locomotor performance of *Pringleophaga marioni* caterpillars, i.e. speed (mm.sec<sup>-1</sup>)  
638 <sup>1</sup>) over test temperatures at 0°C to 35°C, at five acclimation treatments: 0°C (blue), 5°C (green),



639 10°C (purple), 15°C (red) and 5-15°C (grey dashed), and field-fresh (FF) individuals (black)  
640 (Mean  $\pm$  SE)

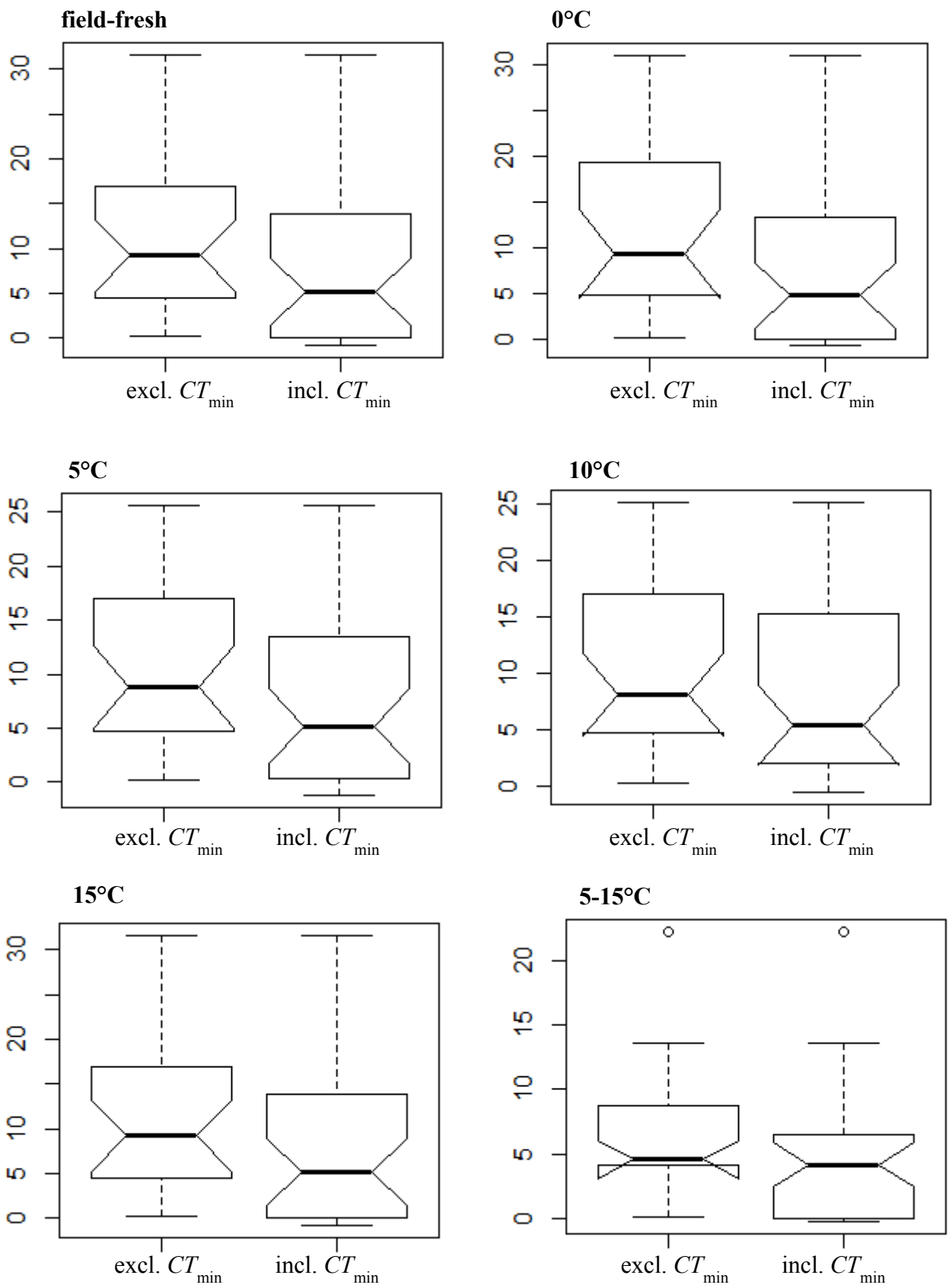
641 Figure 1

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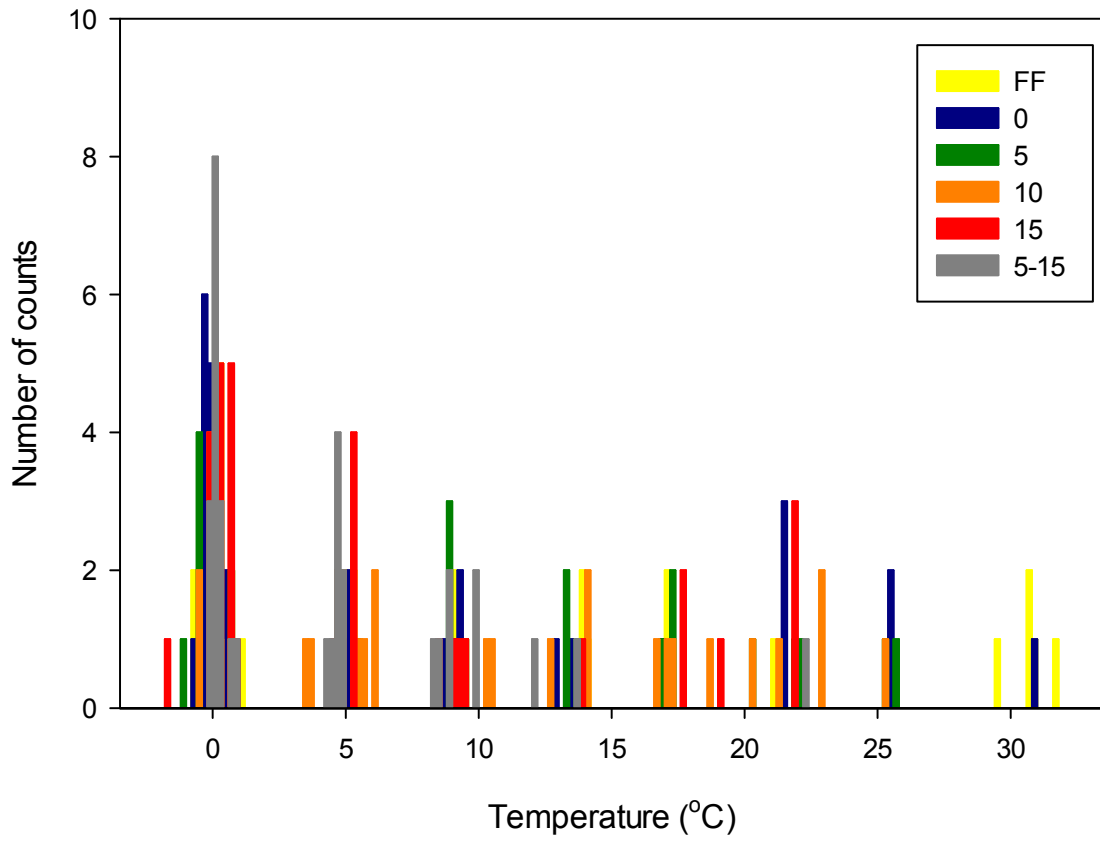
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646 Figure 2a

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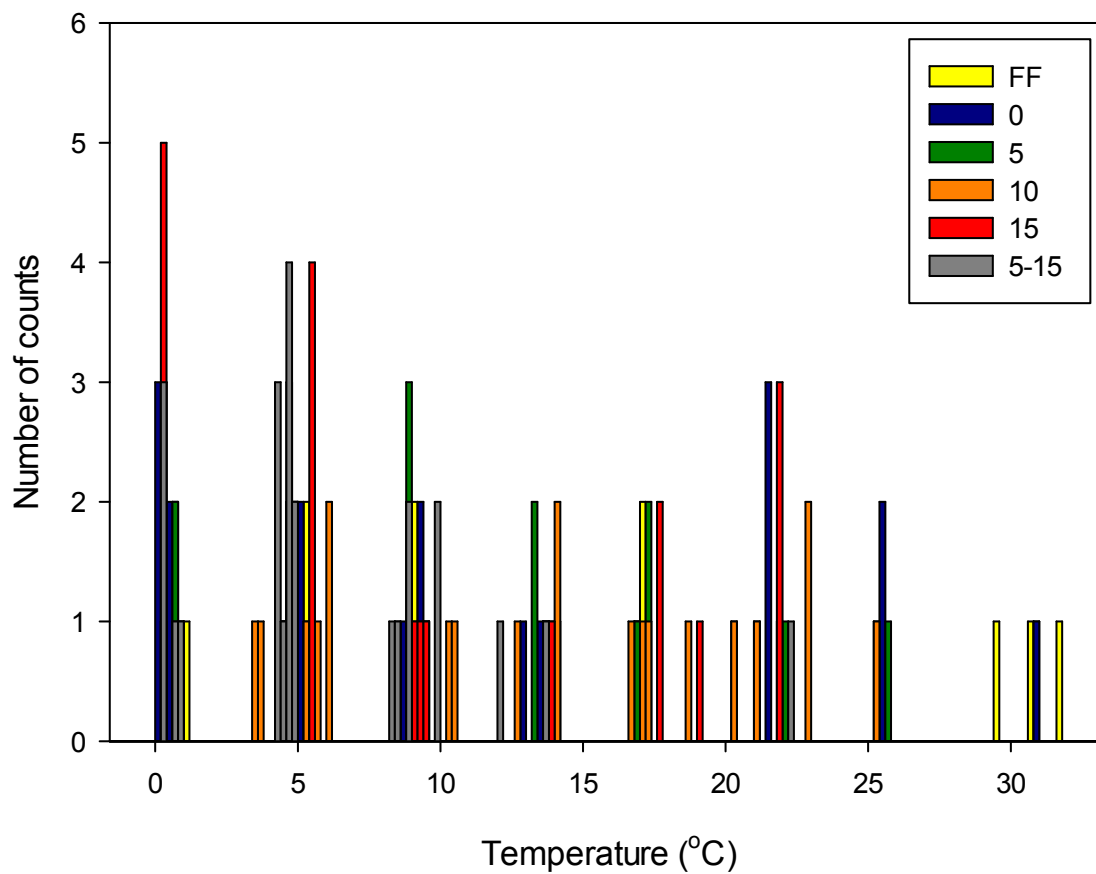
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664 Figure 2b

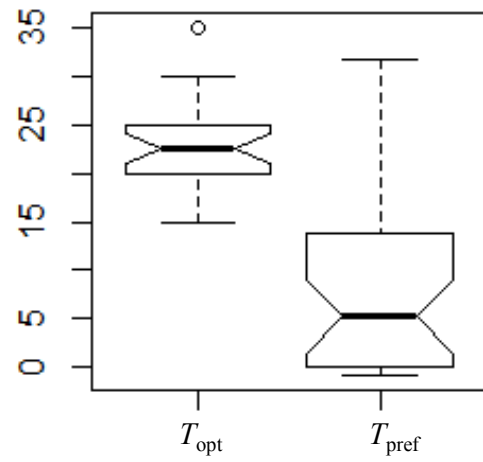
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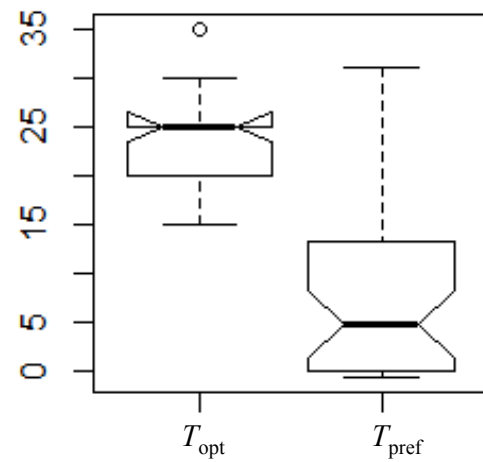
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667 Figure 3a

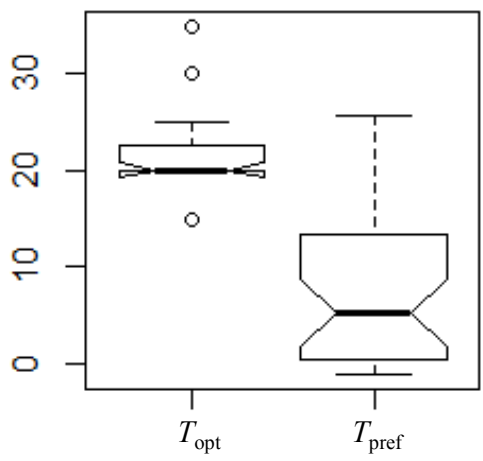
field-fresh



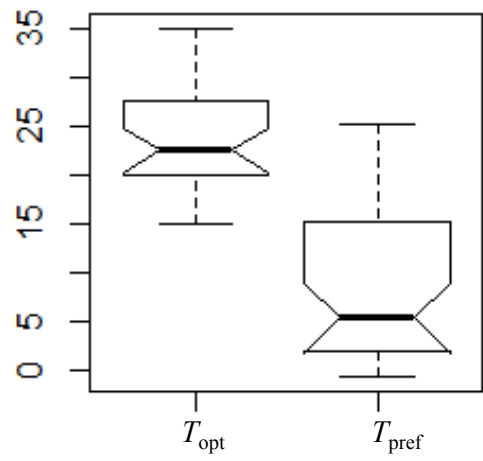
0°C



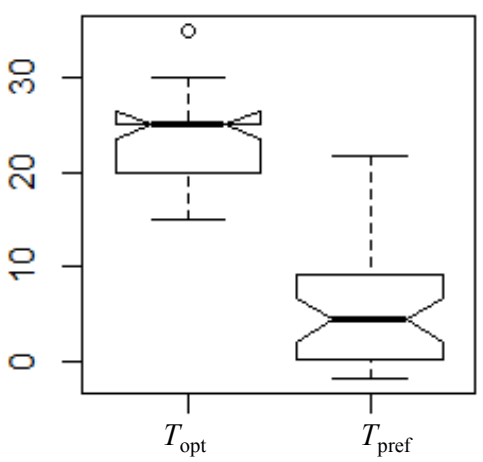
5°C



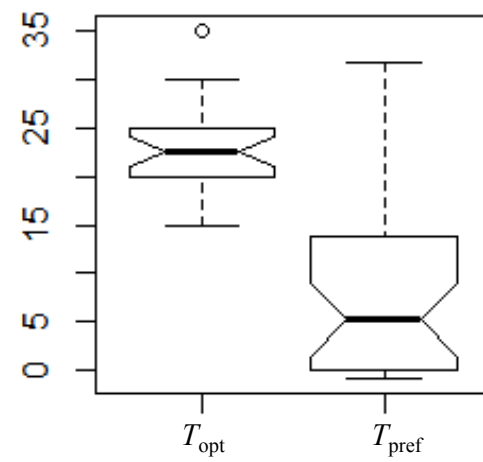
10°C



15°C



5-15°C



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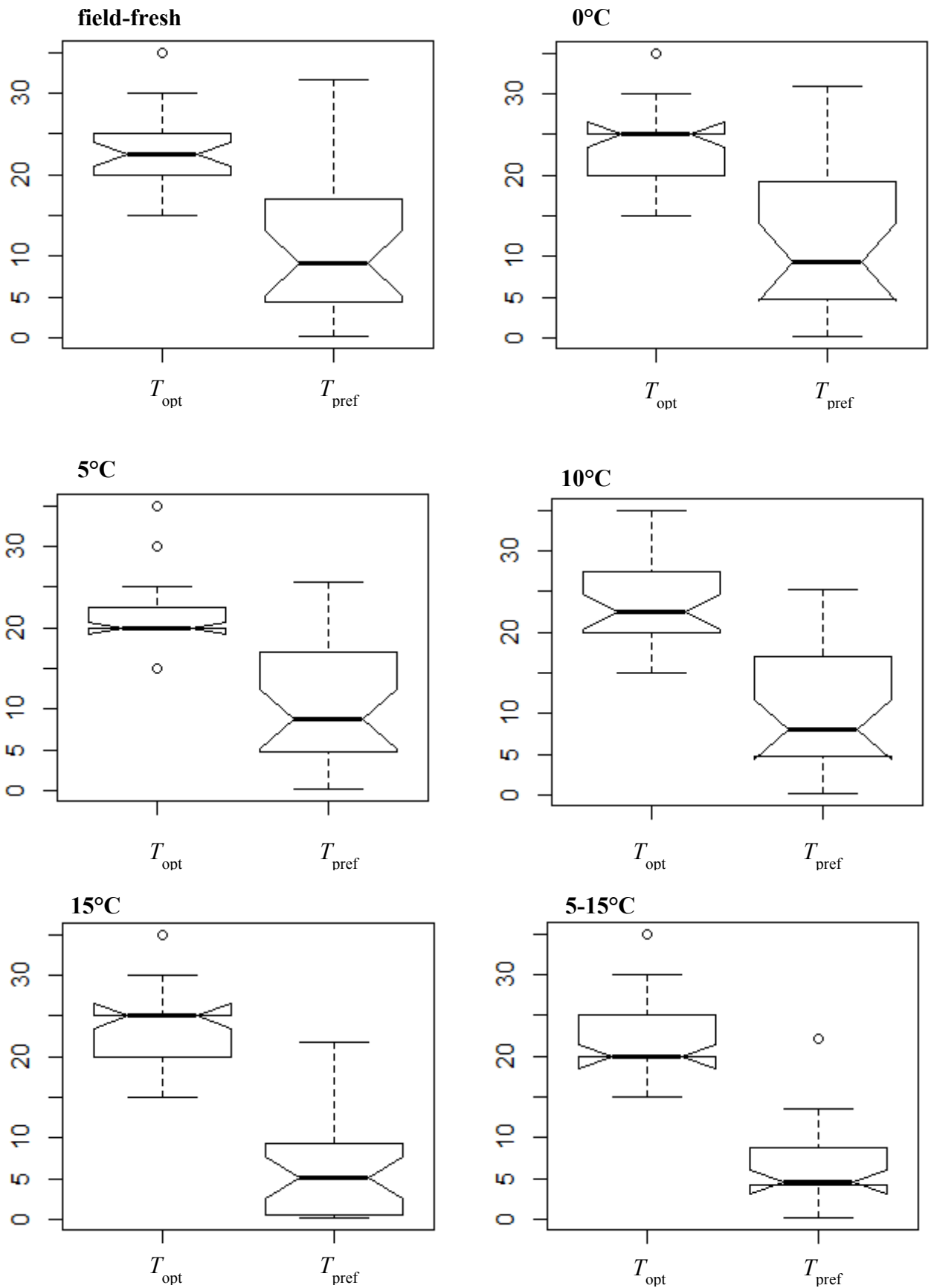
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672 Figure 3b

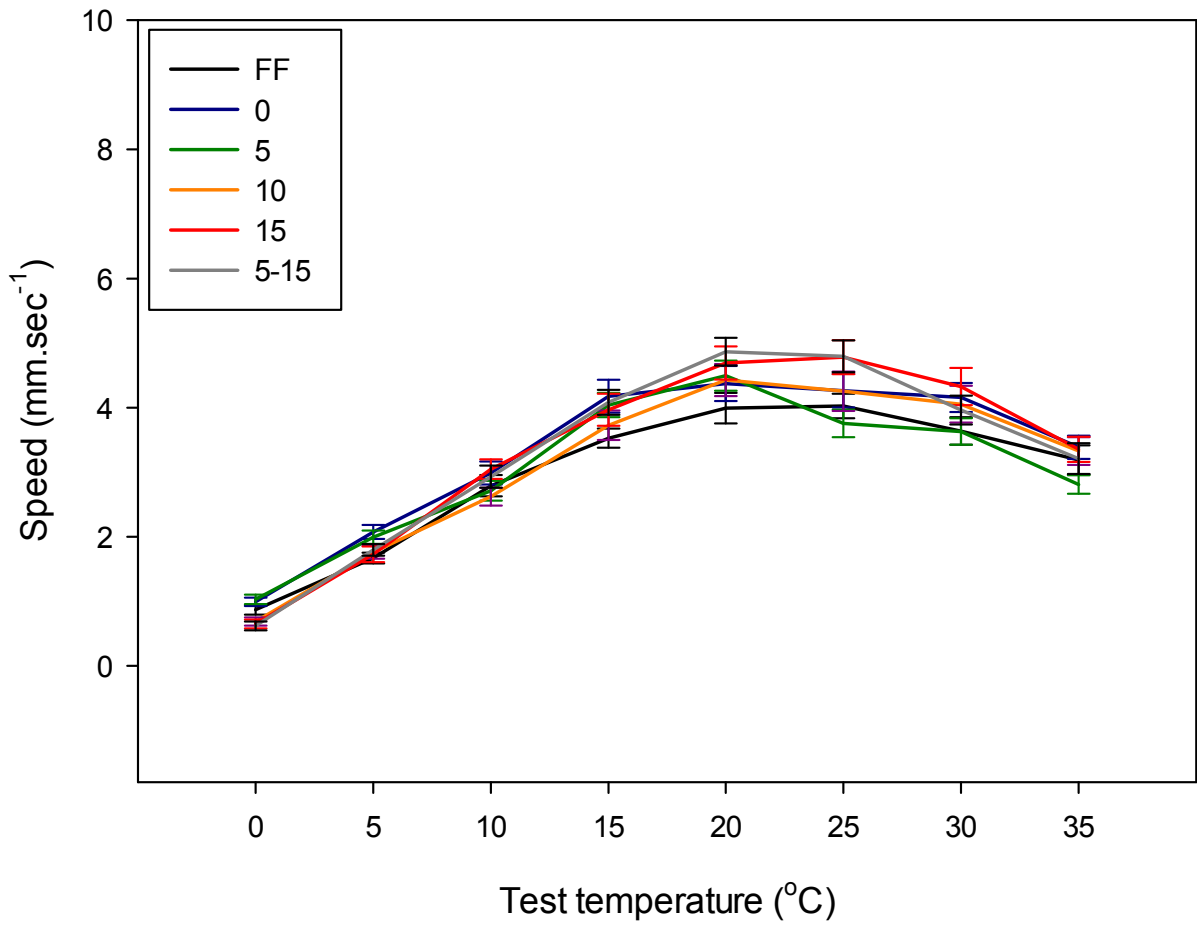
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676 Figure 4  
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692 **Appendix A. Supplementary data**

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694 **Table S1**

695 Thermal preference of *Pringleophaga marioni* caterpillars on a gradient of *c.* 0-15°C (medians  
696 of thermal preference are shown for each acclimation temperature).

Acclimation temperature (°C)	Thermal preference (°C)	
	$CT_{min}$ values included	$CT_{min}$ values excluded
0°C	0.6	6.7
5°C	7	9.5
10°C	3.4	6.6
15°C	3.6	7
5-15°C	2.8	7.3

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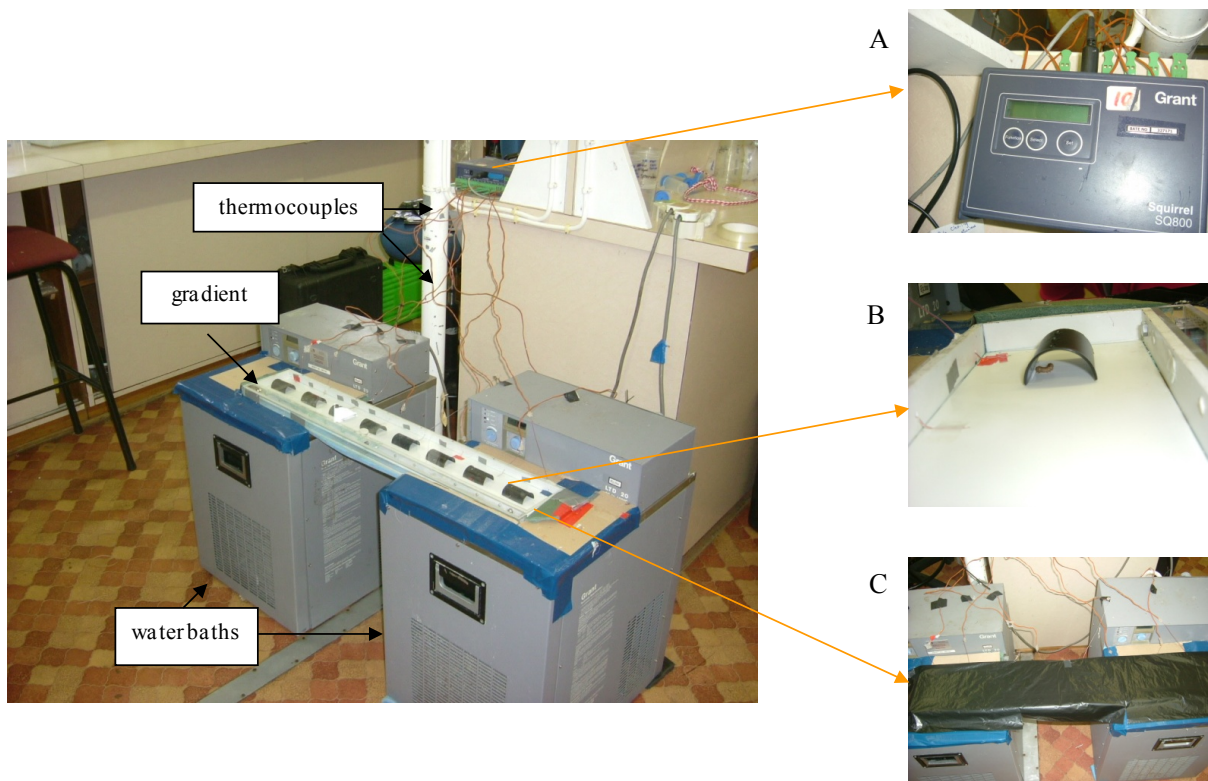


712 **Table S2**

713 Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of  
 714 variances for each test conducted on  $T_{opt}$ ,  $U_{max}$  and  $T_{br}$ . These were: i) an ANOVA comparing  
 715 all groups together, i.e. field fresh, 0°C, 5°C, 10°C, 15°C and 5-15°C, ii) orthogonal polynomial  
 716 contrast analyses, which compared the equally spaced acclimation temperatures of 0°C, 5°C,  
 717 10°C and 15°C, and iii) an ANOVA in which the 5-15°C and 10°C acclimation temperatures  
 718 were compared to examine the effects of fluctuating versus constant acclimation temperatures.

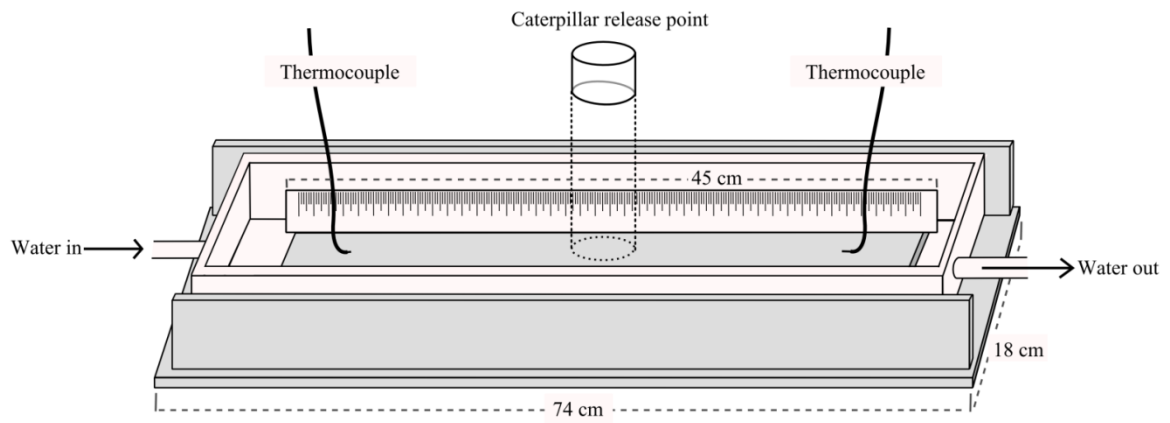
Test	Shapiro-Wilk's	Levene's
All groups		
$T_{opt}$	W = 0.89, p < 0.0000	F = 1.21, d.f. = 5,162, p = 0.3044
$U_{max}$	W = 0.99, p = 0.5262	F = 0.64, d.f. = 5,166, p = 0.5664
$T_{br}$	W = 0.93, p < 0.0000	F = 0.78, d.f. = 5,166, p = 0.5664
0, 5, 10, and 15°C		
$T_{opt}$	W = 0.89, p < 0.0000	F = 0.96, d.f. = 3,108, p = 0.4127
$U_{max}$	W = 0.99, p = 0.5262	F = 0.29, d.f. = 3,108, p = 0.8348
$T_{br}$	W = 0.94, p < 0.0000	F = 0.61, d.f. = 3,108, p = 0.6120
5-15 and 10°C		
$T_{opt}$	W = 0.89, p < 0.0000	F = 4.33, d.f. = 1,54, p = 0.0423
$U_{max}$	W = 0.99, p = 0.5262	F = 0.61, d.f. = 1,54, p = 0.4393
$T_{br}$	W = 0.93, p < 0.0000	F = 1.37, d.f. = 1,54, p = 0.2467

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**Fig. S1.** The experimental set-up of the thermal preference gradient with inserts of the squirrel data logger (A), plastic refuges (B), and the stage covered with a black plastic bag to eliminate light (C).

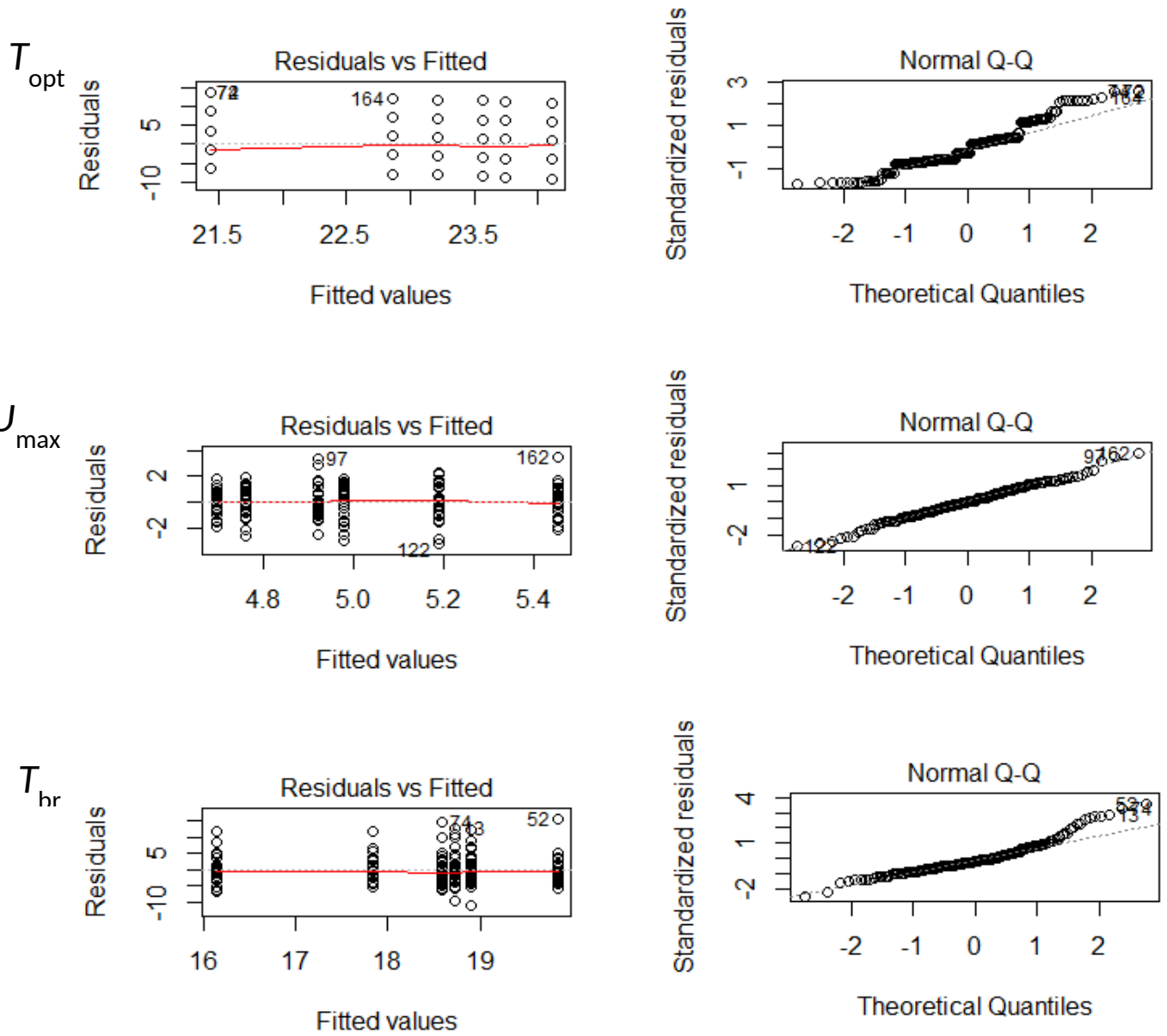


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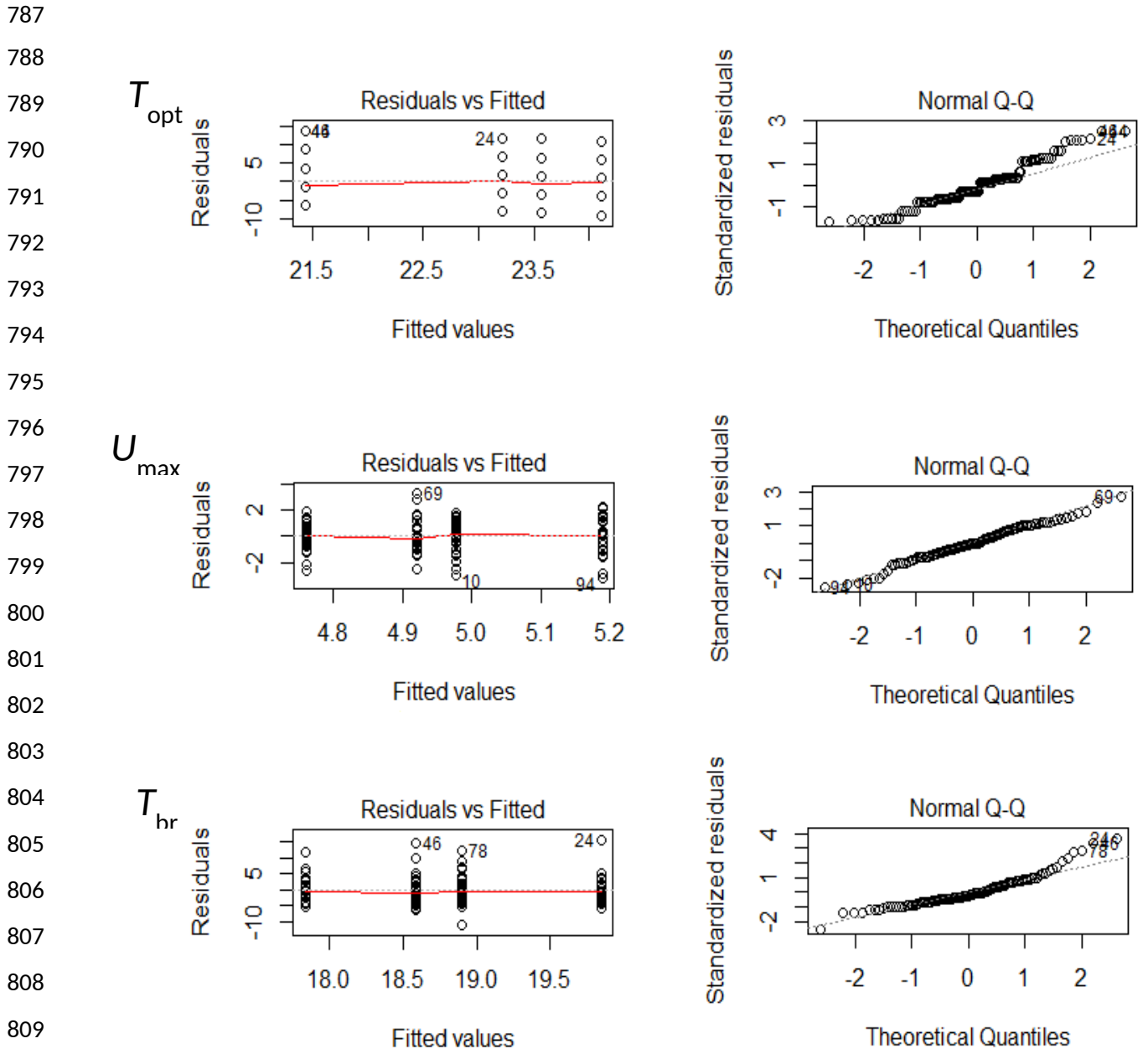
752 **Fig. S2.** The walking stage used in locomotor performance trials (details are given in the text).

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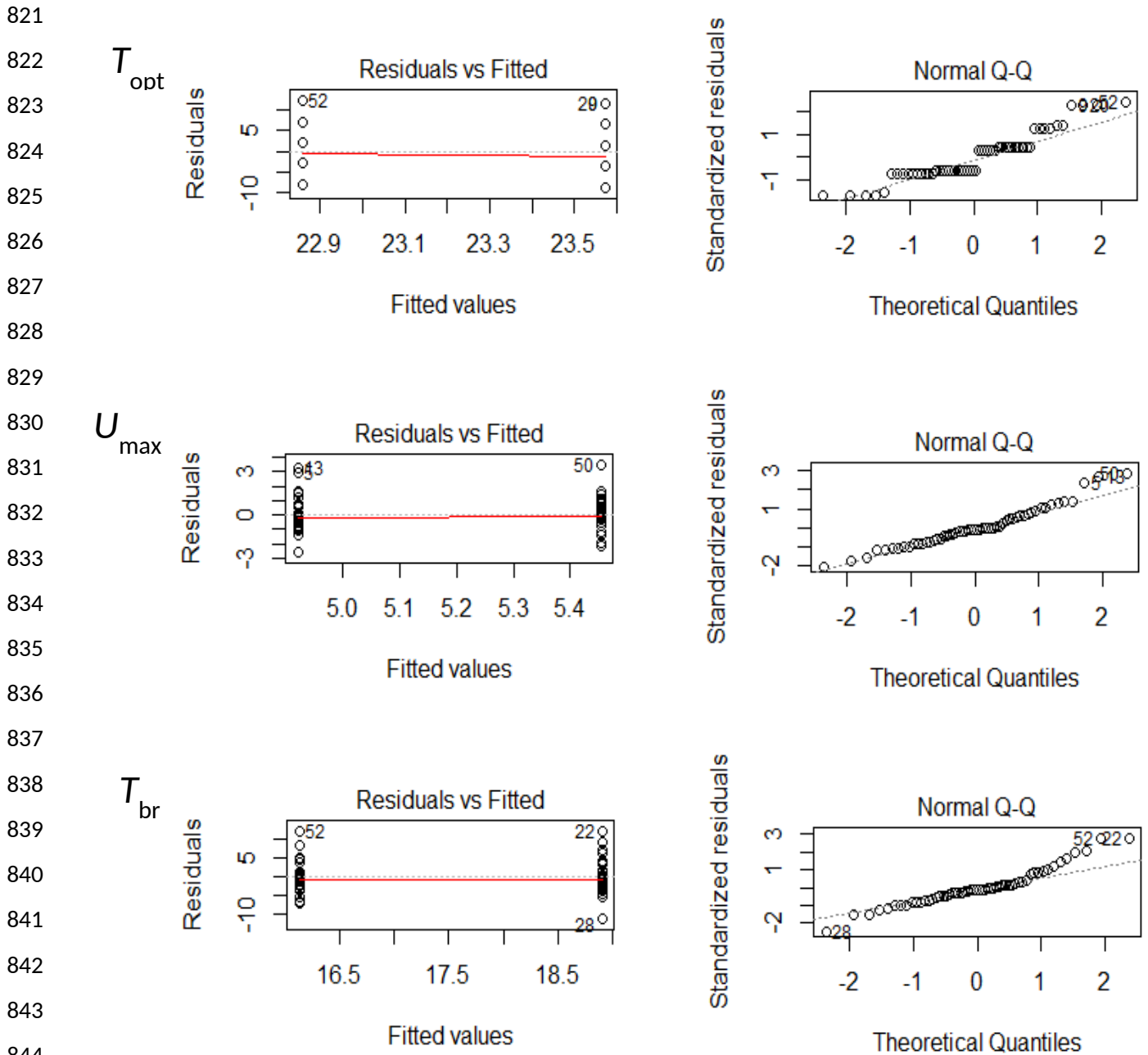
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**Fig. S3a.** Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the ANOVA in which performance measures (i.e.  $T_{opt}$ ,  $U_{max}$  and  $T_{hr}$ ) between all groups (i.e. field fresh, 0°C, 5°C, 10°C, 15°C and 5-15°C acclimation temperatures) were compared.

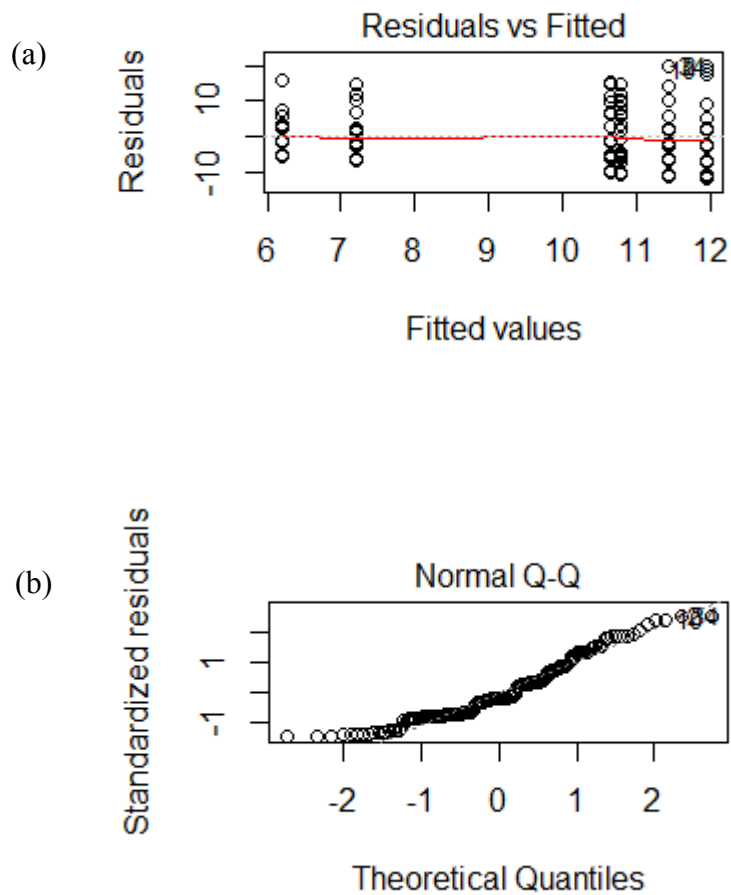


**Fig. S3b.** Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the orthogonal polynomial contrast analyses in which performance measures (i.e.  $T_{opt}$ ,  $U_{max}$  and  $T_{br}$ ) between the 0°C, 5°C, 10°C and 15°C acclimation temperatures were compared.



847 **Fig. S3c.** Model assumption plots (i.e. normal probability plots and residual versus fitted value  
 848 plots) to test normality and equal variances for the anova in which performance measures (i.e.  
 849  $T_{opt}$ ,  $U_{max}$  and  $T_{br}$ ) between the fluctuating temperature of 5-15°C and the constant temperature  
 850 of 10°C were compared.

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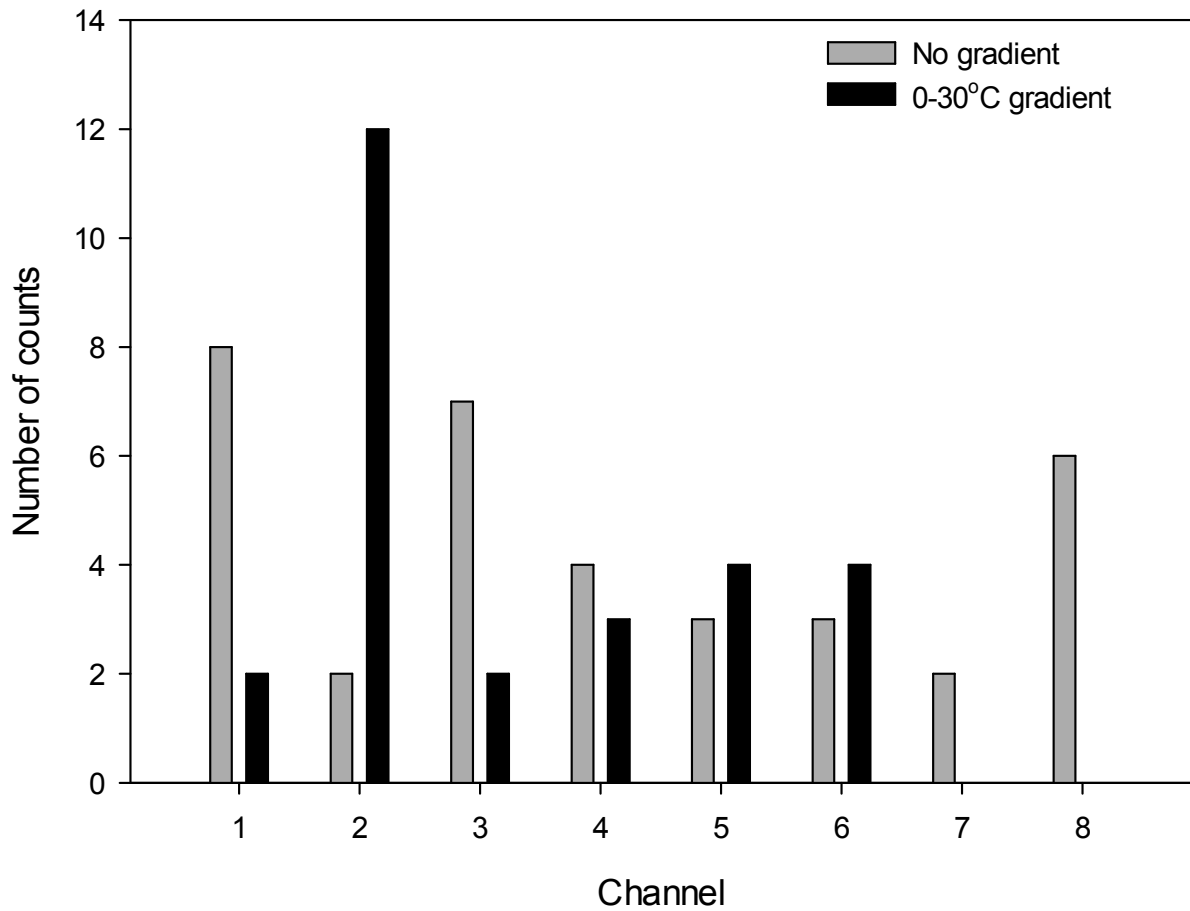


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857 **Fig. S4.** Residual plots of thermal preference at all acclimation temperatures (0°C, 5°C, 10°C,  
 858 15°C and 5-15°C), as well as field fresh individuals on a gradient of *c.* 0-30°C showing (a)  
 859 the residuals versus fitted values, and (b) a normal probability plot. A Shapiro-Wilk's test  
 860 indicated that data were not normally distributed ( $W = 0.91$ ,  $p < 0.0001$ ), and a Levene's test  
 861 showed that variances were not equal ( $F = 3.26$ , d.f. = 5,150,  $p = 0.008$ ).

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865 **Fig. S5.** The distribution of 10°C acclimated individuals on the gradient at a constant  
866 temperature of *c.* 10°C (n = 35, grey bars), compared to temperatures ranging from *c.* 0-30°C  
867 (n = 28, black bars).

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