4-1-2017

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

Tanya M Haupt

Brent J Sinclair

Steven L Chown

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

Tanya M. Haupt[a,1,*], Brent J. Sinclair[b], Steven L. Chown[c]

[a] *Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*

[b] *Department of Biology, The University of Western Ontario, London, Ontario N6A 5B7, Canada*

[c] *School of Biological Sciences, Monash University, Victoria 3800, Australia*

* Corresponding author.

E-mail address: THaupt-Schuter@environment.gov.za (T.M. Haupt).

[1] Current address: Department of Environmental Affairs, Oceans and Coastal Research, Private Bag X2, Roggebaai 8012, South Africa.

ABSTRACT

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

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

1. Introduction

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

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

Several hypotheses have been proposed to explain this departure from coadaptation, particularly when optimum temperatures are higher than preferred temperatures. First, Martin and Huey (2008) suggested that preferred temperatures should be lower than optimum temperatures (hereafter the 'suboptimal is optimal' hypothesis) because asymmetric performance curves mean that performance decreases rapidly above the optimum temperature (Huey and Stevenson, 1979; Huey and Kingsolver, 1989). Thus, preference should be for lower temperatures to minimise the risk of reduced performance (and possibly death) when thermoregulation is imperfect (Martin and Huey, 2008).

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

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

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

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

2. Materials and methods

2.1. Study site and species

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

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

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

2.2. Collection and acclimation

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

To determine if exposure to different acclimation regimes results in demonstration of a thermodynamic effect, caterpillars were held for seven days in incubators (MIR 154, Sanyo, Osaka, Japan, accurate to ± 0.5°C) set at 0°C, 5°C, 10°C and 15°C. The timing of acclimation period was based on previous trials showing acclimation responses within a week for this species (Sinclair and Chown, 2003) and for insects generally (Weldon et al., 2011). The effects of variable temperature regimes were also examined by acclimating caterpillars to a fluctuating temperature of 5-15°C (see also Chown et al., 2016 who found that this treatment results in a lower metabolic rate relative to a constant mean temperature of 10°C). Constant temperatures fall within the soil microhabitat temperature range for this species on Marion Island (Chown and Crafford, 1992; Lee et al., 2009) and the fluctuating temperature simulates conditions within wandering albatross nests where caterpillars are abundant (Haupt et al. 2016). A group of caterpillars were also kept at 5°C for only three days, and these field-fresh individuals served as a control (Deere and Chown, 2006).

2.3. Thermal preference trials

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

Thermal preference was first determined along a gradient from *c*. 0-15°C, reflecting the microclimate temperatures on Marion Island (Chown and Crafford 1992). Because caterpillars showed no defined preference on this gradient (Table S1), this experiment was then repeated on a gradient of *c*. 0-30°C using a different group of individuals. Experiments were conducted on a 75-cm temperature gradient (see Fig. S1) with temperatures controlled at each end using a refrigerated circulator (LTC 12, Grant Instruments Ltd., Cambridge, UK). Temperatures along the gradient were measured and recorded every 5 s using eight evenly-spaced 40-gauge Type T thermocouples connected to an eight channel SQ800 Grant Squirrel data logger (Grant Instruments Ltd, Cambridge, U.K.). Dark walk-through "tunnels" constructed from plastic tubing served as refuges along the gradient and were placed at intervals corresponding with the thermocouple positions following Marais and Chown (2008) (See "B" in Fig. S1).

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

2.4. Locomotor performance trials

The locomotion speed of individual caterpillars (n = 28 per acclimation temperature) was measured using a temperature-controlled walking stage with a hardboard interior surface (see Fig S2). A refrigerated circulator (LTC 12, Grant Instruments, Cambridge, UK) controlled the temperature of the stage which was measured via a Type-K thermocouple connected to a digital thermometer (CHY 507, Firemate, Taiwan). To avoid heat shock affecting performance at lower temperatures (Lachenicht et al., 2010), individuals were examined first at randomized test temperatures of 0°C, 5°C, 10°C, 15°C and 20°C, and then at high temperatures of 25°C, 30°C and 35°C as these temperatures approach the CT_{max} of 38°C for *P. marioni*.

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

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

$$T\mathrm{br} = \sqrt{\Sigma[\frac{Ui\ (Ti - T\mathrm{opt})}{U\mathrm{max}}]2}$$

(Equation 1)

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

2.5. Data analyses

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

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

$$\text{asymmetry} = \frac{2Topt - T\max - T\min}{T\max - T\min}$$

(Equation 2)

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

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

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

3. Results

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

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

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

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

4. Discussion

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

Bearing this correction in mind, preferred temperatures of *P. marioni* were substantially lower than the optimum temperatures, particularly so when data were not adjusted for the likelihood of individuals being trapped at temperatures below their CT_{min}. These differences were 17.3 for field-fresh caterpillars, and 20.2 following acclimation to 0°C, 14.8 after 5°C, 17.1 after 10°C, 20.6 after 15°C and 15.8 after 5-15°C (Table 1a). Thus, we reject the coadaptation hypothesis (Huey and Bennett, 1987; Angilletta et al. 2006; Angilletta, 2009): T_{pref} does not appear to have evolved to match T_{opt} in *P. marioni.* Such large discrepancies between T_{opt} and T_{pref} have also been found in other species, e.g. 11°C in *Pterohelaeus darlingensis* beetles (Allsopp et al., 1980; Allsopp, 1981), 17°C in intertidal snails (*Clorostoma* *funebralis*) (Tepler et al., 2011); and 8°C in house geckos, *Hemidactylus turcicus* (Huey et al., 1989; Angilletta et al., 1999). Moreover, the locomotor performance curves of *P. marioni* are more-or-less symmetrical, further suggesting that the 'suboptimal is optimal' hypothesis, which assumes asymmetric performance curves (Martin & Huey 2008), can be rejected as a possible explanation for the large mismatch between T_{opt} and T_{pref}.

An alternative explanation for the current findings is that selection, driven by a thermodynamic effect, could explain the large differences between preferred temperature and the thermal optimum (Asbury and Angilletta 2010). Acclimation had little effect on thermal performance curves or preferred temperature, however, suggesting that the thermodynamic effect hypothesis can also be rejected. Previous studies have reported varying, but typically small effects of phenotypic plasticity in response to temperature in terrestrial arthropods from Marion Island (Deere and Chown, 2006; Deere et al., 2006; Slabber et al., 2007; Marais & Chown, 2008). *Pringleophaga marioni* caterpillars show little phenotypic plasticity of metabolic-rate temperature curves in response to acclimation, under both stable and fluctuating acclimation conditions (Chown et al. 2016). Here, we found a similar effect for thermal performance curves based on locomotion speed, and in particular for T_{opt} and U_{max}. The unpredictability of thermal cues may explain limited phenotypic plasticity in many species on Marion Island (Deere et al., 2006), including *P. marioni* caterpillars. Nonetheless, after exposure to fluctuating conditions, caterpillars had a significantly narrower performance breadth compared to those held at a constant temperature of 10°C (i.e. closest comparable mean temperature). Performance breadth is expected to change significantly in fluctuating as opposed to constant temperatures depending on whether variation is within or among generations (Huey and Slatkin 1976; Huey and Stevenson 1979; Huey and Kingsolver 1993; Gilchrist, 1995; Huey et al., 1999). Given that 15°C is detrimental to caterpillars within a generation (Haupt et al. 2014a), the narrowing in performance breadth may well have been due to this negative effect of prolonged high temperature, in keeping with theoretical considerations (Gilchrist, 1995; see also discussion in Dowd et al., 2015; Kingsolver et al., 2015). Thus, further consideration of the effects of stable versus fluctuating temperatures is warranted, even when these effects may initially appear to be small. Such fluctuating temperatures, in association with a symmetric performance curve may also mean that selection for preferred temperatures matching the optimum may not be pronounced.

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

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

Acknowledgments

Jennifer Lee, Justine Shaw, Asanda Phiri, Kersti Hickley and Mashudu Mashau assisted with field work. Two reviewers provided helpful comments on a previous version of the manuscript. This study was supported by National Research Foundation of South Africa Grant SNA14071475789 and the South African National Antarctic Programme.

Appendix A. Supplementary data

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

References

Åkesson, B. 1976. Temperature and life cycle in *Ophryotrocha labronica* (Polychaeta, Dorvilleidae). Ophelia, 15, 37-47.

Allsopp, P.G. 1981. Development, longevity and fecundity of the false wireworms *Pterohelaeus darlingensis* and *P. alternatus* (Coleoptera: Tenebrionidae). I. Effect of constant temperature. Australian Journal of Zoology, 29, 605-619.

Allsopp, P.G., Adams, G.D. & Butler, D.G. 1980. Response of larvae of *Pterohelaeus darlingensis* Carter (Coleoptera: Tenebrionidae) to moisture, temperature and gravity. Australian Journal of Entomology, 19, 281-285.

Anderson, J.L., Albergotti, L., Ellebracht, B., Huey, R.B. & Phillips, P.C. 2011. Does thermoregulatory behavior maximize reproductive fitness of natural isolates of *Caenorhabditis elegans*? BMC Evolutionary Biology, 11, 157.

Anderson, J.L., Albergotti, L., Proulx, S., Peden, C., Huey, R.B. & Phillips, P.C. 2007. Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. Journal of Experimental Biology, 210, 3107-3116.

Angilletta Jr., M. J. 2009. Thermal adaptation: A theoretical and empirical synthesis. Oxford University Press, Oxford.

Angilletta Jr, M.J., Bennett, A., Guderley, H., Navas, C.A., Seebacher, F. & Wilson, R.S. 2006. Coadaptation: a unifying principle in evolutionary thermal biology. Physiological and Biochemical Zoology, 79, 282-294.

Angilletta Jr, M.J., Hill, T. & Robson, M.A. 2002b. Is physiological performance optimized by thermoregulatory behaviour?: a case study of the eastern fence lizard, *Sceloporus undulates*. Journal of Thermal Biology, 27, 199-204.

Angilletta Jr, M.J., Huey, R.B. & Frazier, M.R. 2010. Thermodynamic effects on organismal performance: is hotter better? Physiological and Biochemical Zoology, 83, 197-206.

Angilletta Jr, M. J., Montgomery, L.G. & Werner, Y.L. 1999. Temperature preference in geckos: diel variation in juveniles and adults. Herpetologica, 55, 212-222.

Angilletta Jr, M.J., Niewiarowski, P. H. & Navas, C.A. 2002a. The evolution of thermal physiology in ectotherms. Journal of Thermal Biology, 27, 249-268.

Asbury, D.A. & Angilletta Jr, M.J. 2010. Thermodynamic effects on the evolution of performance curves. American Naturalist, 176, 40-49.

Calabria, G., Dolgova, O., Rego, C., Castañeda, L.E., Rezende, E.L., Balanya, J., Pascual, M., Sørensen, J.G., Loeschcke, V. & Santos, M. 2012. Hsp70 protein levels and thermotolerance in Drosophila subobscura: a reassessment of the thermal co-adaptation hypothesis. Journal of Evolutionary Biology, 25, 691-700.

Chown, S.L. & Crafford, J.E. 1992. Microhabitat temperatures at Marion Island (46°54'S 37°45'E). South African Journal of Antarctic Research, 22, 51-58.

Chown, S.L., Haupt, T.M. & Sinclair, B.J. 2016. Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth. Journal of Insect Physiology, 85, 10-16.

Clissold, F.J., Coggan, N. & Simpson, S.J. 2013. Insect herbivores can choose microclimates to achieve nutritional homeostatis. Journal of Experimental Biology, 216, 2089-2096.

Coggan, N., Clissold, F.J. & Simpson, S.J. 2011. Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. Proceedings of the Royal Society B, 278, 2745-2752.

Colinet, H., Renault, D., Hance, T. & Vernon, P. 2006. The impact of fluctuating thermal regimes on the survival of a cold-exposed parasitic wasp, *Aphidius colemani*. Physiological Entomology, 31, 234-240.

Crafford, J.E. 1990. Patterns of energy flow in populations of the dominant insect consumers on Marion Island. PhD thesis, University of Pretoria, South Africa.

Crafford, J.E., Scholtz, C.H. & Chown, S.L. 1986. The insects of sub-Antarctic Marion and Prince Edward Islands, with a bibliography of entomology of the Kerguelen Biogeographical Province. South African Journal of Antarctic Research, 16, 42-84.

Darveau, C.A., Suarez, R.K., Andrews, R.D. & Hochachka, P.W. 2002. Allometric cascade as a unifying principle of body mass effects on metabolism. Nature. 417, 166-170.

Deere, J.A. & Chown, S.L. 2006. Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. American Naturalist, 168, 630-644.

Deere, J.A., Sinclair, B.J., Marshall, D.J. & Chown, S.L. 2006. Phenotypic plasticity of thermal tolerances in five oribatid mite species from sub-Antarctic Marion Island. Journal of Insect Physiology, 52, 693-700.

Dillon, M.E., Liu, R., Wang, G. & Huey, R. 2012. Disentangling thermal preference and the thermal dependence of movement in ectotherms. Journal of Thermal Biology, 37, 631-639.

Dowd, W.W., King, F.A. & Denny, M.W. 2015. Thermal variation, thermal extremes and the physiological performance of individuals. Journal of Experimental Biology, 218, 1956-1967.

Fernández, J.B., Smith Jr, J., Scolaro, A. & Ibargüengoytía, N.R. 2011. Performance and thermal sensitivity of the southern lizards in the world, *Liolaemus sarmientoi* and *Liolaemus magellanicus*. Journal of Thermal Biology, 36, 15-22.

Forsman, A. 1999. Variation in thermal sensitivity of performance among colour morphs of a pygmy grasshopper. Journal of Evolutionary Biology, 12, 869-878.

Frazier, M.R., Huey, R.B. & Berrigan, D. 2006. Thermodynamics constraints the evolution of insect population growth rates: “Warmer is Better”. American Naturalist, 168, 512-520.

French, D.D. & Smith, V.R. 1983. A note on the feeding of *Pringleophaga marioni* Viette larvae at Marion Island. South African Journal of Antarctic Research, 13, 45-46.

Garland Jr, T., Huey, R.B. & Bennett, A.F. 1991. Phylogeny and coadaptation of thermal physiology in lizards: a reanalysis. Evolution, 45, 1969-1975.

Gilchrist, G.W. 1995 Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. American Naturalist, 146, 252-270.

Gilchrist, G.W. 1996. A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. Evolution, 50, 1560-1572.

Gvoždík, L., Puky, M. & Šugerková, M. 2007. Acclimation is beneficial at extreme test temperatures in the Danube crested newt, *Triturus dobrogicus* (Caudata, Salamandridae). Biological Journal of the Linnean Society, 90, 627-636.

Hadamová, M. & Gvoždík, L. 2011. Seasonal acclimation of preferred body temperature improves the opportunity for thermoregulation in newts. Physiological and Biochemical Zoology, 84, 166-174.

Haupt, T.M., Crafford, J.E., Chown, S.L. 2014a. Solving the puzzle of *Pringleophaga* – threatened, keystone detritivores in the sub-Antarctic. Insect Conservation and Diversity 7, 308-313.

Haupt, T.M., Sinclair, B.J., Chown, S.L., 2014b. Chemosensory and thermal cue responses in the sub-Antarctic moth *Pringleophaga marioni*: Do caterpillars choose Wandering Albatross nest proxies? Polar Biology 37, 555-563.

Haupt, T.M., Sinclair, B.J., Shaw, J., Chown, S.L., 2016. Further support for thermal ecosystem engineering by wandering albatross on Marion Island. Antarctic Science, 28, 35-43.

Huey, R.B. & Bennett, A.F. 1987. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. Evolution, 41, 1098-1115.

Huey, R.B., Berrigan, D., Gilchrist, G.W. & Herron, J.C. 1999. Testing the adaptive significance of acclimation: a strong inference approach. American Zoologist, 39, 323-336.

Huey, R.B., Hertz, P.E. & Sinervo, B. 2003. Behavioral drive versus behavioral inertia in evolution: a null model approach. American Naturalist, 161, 357-366.

Huey, R.B. & Kingsolver, J.G. 1989. Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology and Evolution 4, 131-135.

Huey, R.B. & Kingsolver, J.G. 1993. Evolution of resistance to high temperature in ectotherms. American Naturalist, 142, 21-46.

Huey, R.B., Niewiarowski, P.H., Kaufmann, J. & Herron, J.C. 1989. Thermal biology of nocturnal ectotherms: is sprint performance of geckos maximal at low body temperatures? Physiological Zoology, 62, 488-504.

Huey, R.B. & Slatkin, M. 1976. Costs and benefits of lizard thermoregulation. Quarterly Review of Biology, 51, 363-384.

Huey, R.B. & Stevenson, R.D. 1979. Integrating thermal physiology of ectotherms: a discussion of approaches. American Zoologist, 19, 357-366.

Hutchison, V.H. & Hill, L.G. 1976. Thermal selection in the Hellbender, *Cryptobranchus alleganiensis*, and the mudpuppy, *Necturus maculosus*. Herpetologica, 32, 327-331.

Jia, Q.X., Xu, N., Zhu, J. & Cao, H. 2002. Effects of temperature on the development and population growth of brine shrimp (*Artemia parthenogenetica gahai*). Acta Zoologica Sinica, 48, 175-182.

Jian, F., Jayas, D.S., White, N.D.G. & Muir, W.E. 2002. Temperature and geotaxis preference by *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) adults in response to 5°C/m temperature gradients at optimum and hot temperatures in stored wheat and their mortality at high temperature. Environmental Entomology, 31, 816-826.

Khan, J.R. & Herbert, N.A. 2012. The behavioural thermal preference of the common triplefin (*Forsterygion lapillum*) tracks aerobic scope optima at the upper limit of its distribution. Journal of Thermal Biology, 37, 118-124.

Kingsolver, J.G., Higgins, J.K., Augustine, K.E., 2015. Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time dependent effects. Journal of Experimental Biology 218, 2218-2225.

Kingsolver, J.G., Higgins, J.K., Augustine, K.E., 2015. Fluctuating temperatures and ectotherm growth: distinguishing non-linear and time dependent effects. Journal of Experimental Biology, 218, 2218-2225.

Kingsolver, J.G. & Woods, H.A. 1997. Thermal sensitivity of feeding and digestion in Manduca caterpillars. Physiological Zoology, 70, 631-638.

Klok, C.J. & Chown, S.L. 1997. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). Journal of Insect Physiology, 43, 685-694.

Kubisch, E.L., Fernández, J.B. & Ibargüengoytía, N.R. 2011. Is locomotor performance optimised at preferred body temperature? A study of *Liolaemus pictus argentinus* from northern Patagonia, Argentina. Journal of Thermal Biology, 36, 328-333.

Lachenicht, M.W., Clusella-Trullas, S., Boardman, L., Le Roux, C. & Terblanche, J.S. 2010. Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). Journal of Insect Physiology, 56, 822-830.

Langer, G.S. & Young, A.M. 1976. Habitat selection in two species of flour beetles (Tribolium) on a temperature gradient. American Midland Naturalist, 95, 131-143.

Lee, J.E., Janion, C., Marais, E., Jansen van Vuuren, B. & Chown, S.L. 2009. Physiological tolerances account for range limits and abundance structure in an invasive slug. Proceedings of the Royal Society B, 276, 1459-1468.

Le Roux, P.C. & McGeoch, M.A. 2008. Changes in climatic extremes, variability and signature on sub-Antarctic Marion Island. Climatic Change, 86, 309-329.

Marais, E. & Chown, S.L. 2008. Beneficial acclimation and the Bogert effect. Ecology Letters, 11, 1027-1036.

Martin, T.L. & Huey, R.B. 2008. Why "suboptimal" is optimal: Jensen's Inequality and ectotherm thermal preferences. American Naturalist, 171, 102-118.

Miller, G.A., Clissold, F.J., Mayntz, D. & Simpson, S.J. 2009. Speed over efficiency: locusts select body temperatures that favour growth rate over efficient nutrient utilization. Proceedings of the Royal Society B, 276, 3581-3589.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. Global Change Biology, 19, 2373-2380.

Platt, J.R. 1964. Strong inference: certain systematic methods of scientific thinking may produce much more rapid progress than others. Science, 146, 347-353.

Prevedelli, D. & Simonini, R. 2001. Effect of temperature on demography of *Ophryotrocha labronica* (Polychaeta: Dorvilleidae). Vie Milieu, 51, 173-180.

R Development Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org, accessed on 03.04.2013.

Sanford, D.P. & Tschinkel, W.R. 1993. Fire ant thermal preferences: behavioral control of growth and metabolism. Behavioral Ecology and Sociobiology, 32, 321-329.

Sinclair, B.J. & Chown, S.L. 2003. Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). Journal of Insect Physiology, 49, 45-52.

Sinclair, B.J. & Chown, S.L. 2005. Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. Journal of Experimental Biology, 208, 869-879.

Sinclair, B.J. & Chown, S.L. 2006. Caterpillars benefit from thermal ecosystem engineering by wandering albatrosses on sub-Antarctic Marion Island. Biology Letters, 2, 51-54.

Sinclair, B.J., Klok, C.J. & Chown, S.L. 2004. Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. Journal of Experimental Biology, 207, 1287-1294.

Slabber, S., Worland, M.R., Leinaas, H.P. & Chown, S.L. 2007. Acclimation effects on the thermal tolerances of springtails from sub-Antarctic Marion Island: indigenous and invasive species. Journal of Insect Physiology, 53, 113-125.

Smith, L.B. 1965. The intrinsic rate of natural increase of *Cryptolestes ferrugineus* (Stephens) (Coleoptera, Cucujidae). Journal of Stored Product Research, 1, 35-49.

Tepler, S., Mach, K. & Denny, M. 2011. Preference versus performance: body temperature of the intertidal snail *Chlorostoma funebralis*. Biological Bulletin, 220, 107-117.

Van Berkum, F.H. 1986. Evolutionary patterns of the thermal sensitivity of sprint speed in Anolis lizards. Evolution, 40, 594-604.

Weldon, C.W., Terblanche, J.S. & Chown, S.L. 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. Journal of Thermal Biology, 36, 479-485.

White, G.G. 1987. Effects of temperature and humidity on the rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), in wheat grain. Australian Journal of Zoology, 35, 43-59.

Williams, C.M., Marshall, K.E., MacMillan, H.A., Dzurisin, J.D.K., Hellmann, J.J., Sinclair, B.J., 2012. Thermal variability increases the impact of autumnal warming and drives metabolic depression in an overwintering butterfly. PLoS One, 7, 34470.

Woods, H.A. & Harrison, J.F. 2002. Interpreting rejections of the Beneficial Acclimation Hypothesis: when is physiological plasticity adaptive? Evolution, 56, 1863-1866.

Zhang, L. & Lefcort, H. 1991. The effects of ploidy level on the thermal distributions of brine shrimp *Artemia parthenogenetica* and its ecological implications. Heredity, 66, 445-452.

Table 1

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

(a) All individuals

Group	T_{pref} (°C)	T_{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

(b) Individuals with preferences below 0.2°C excluded

Group	T_{pref} (°C)	Number of individuals with $T_{pref} < 0.2$°C	T_{opt} (°C)	Difference (°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

Table 2

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

	T_{opt} (°C)		U_{max} (mm·sec^{-1})		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

Table 3

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

$\boldsymbol{T_{opt}}$					
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
$\boldsymbol{U_{max}}$					
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
$\boldsymbol{T_{br}}$					

Source	d.f.	SS	MS	*F*	*P*
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.	*t*	*P*
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

Figure legends

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

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

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

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

10°C (purple), 15°C (red) and 5-15°C (grey dashed), and field-fresh (FF) individuals (black) (Mean ± SE)

Figure 1

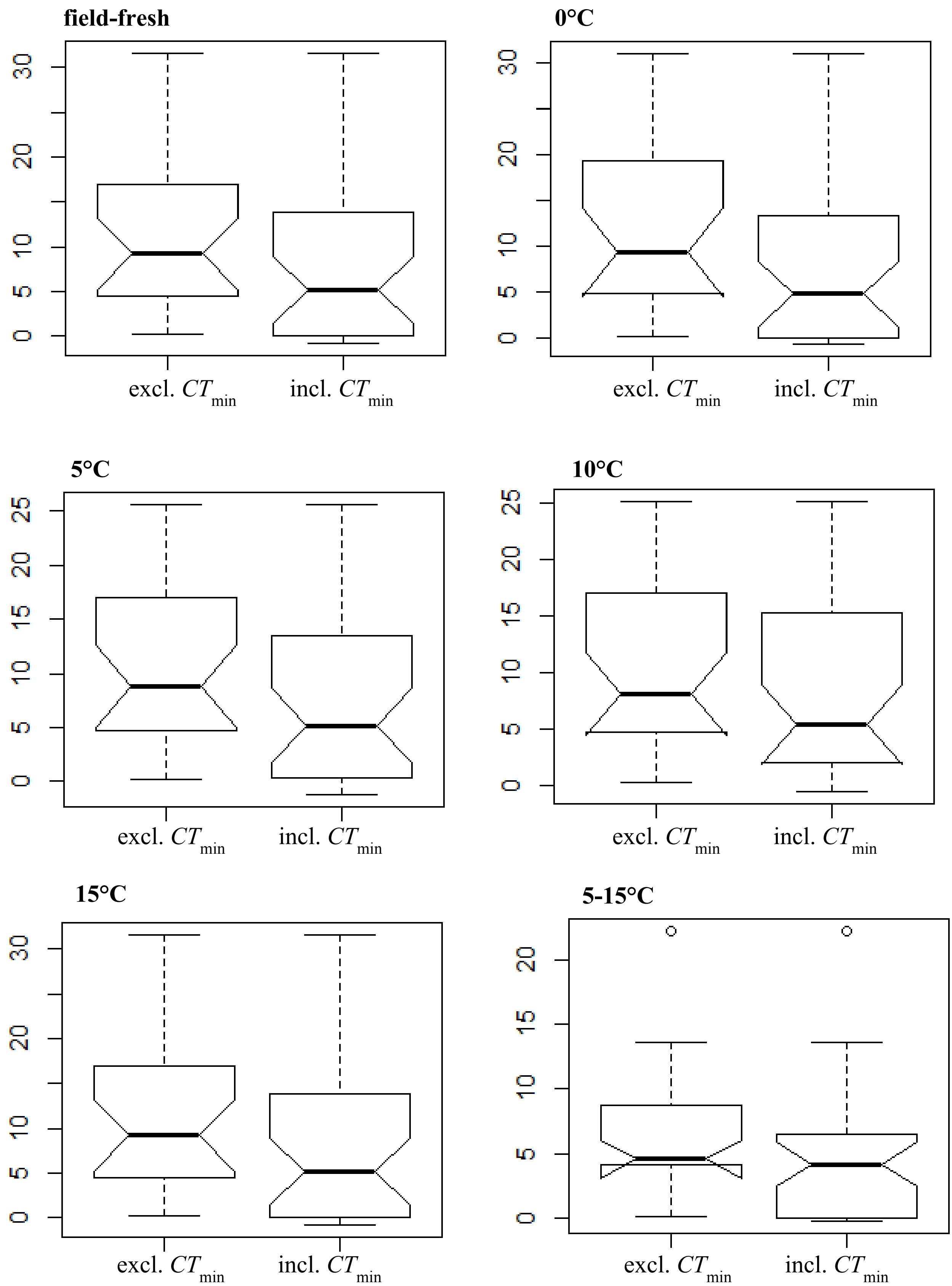
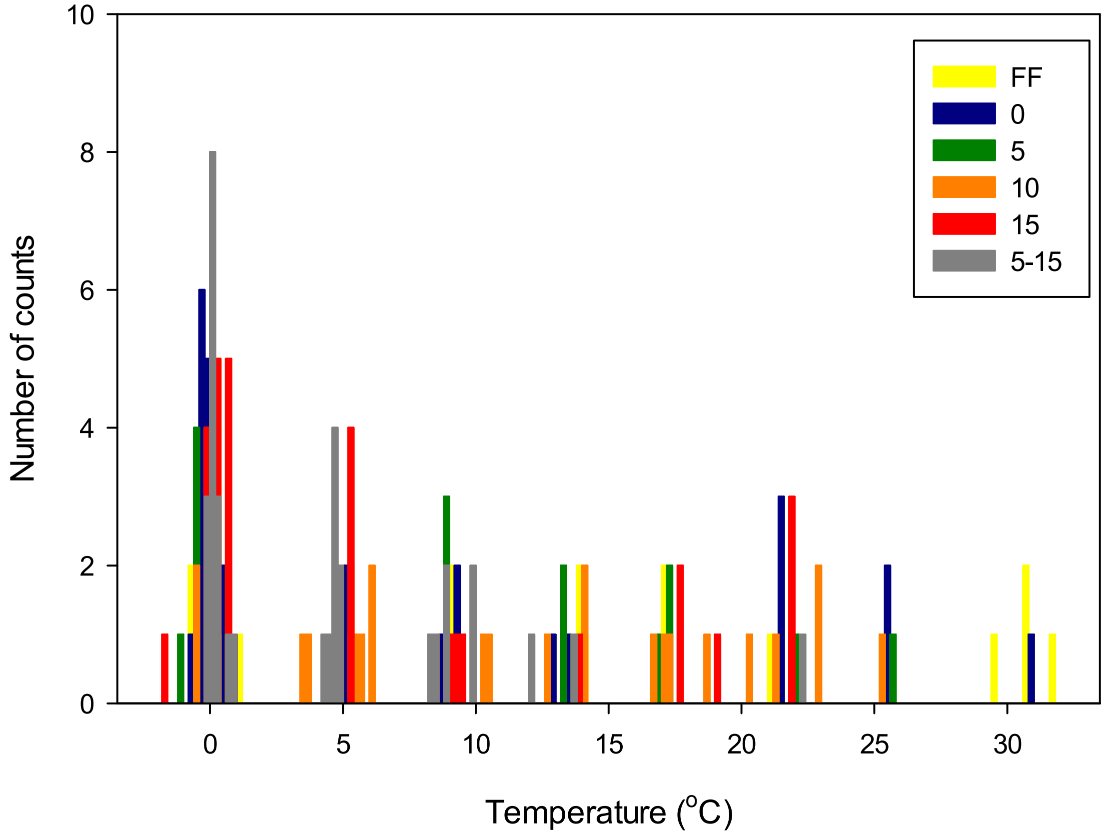

field-fresh
0°C
5°C
10°C
15°C
5-15°C
excl. CTmin
incl. CTmin
0
5
10
15
20
25
30

Figure 2a



10
8
6
4
2
0
Number of counts
0
5
10
15
20
25
30
Temperature (^{o}C)
FF
0
5
10
15
20
25
5-15

Figure 2b

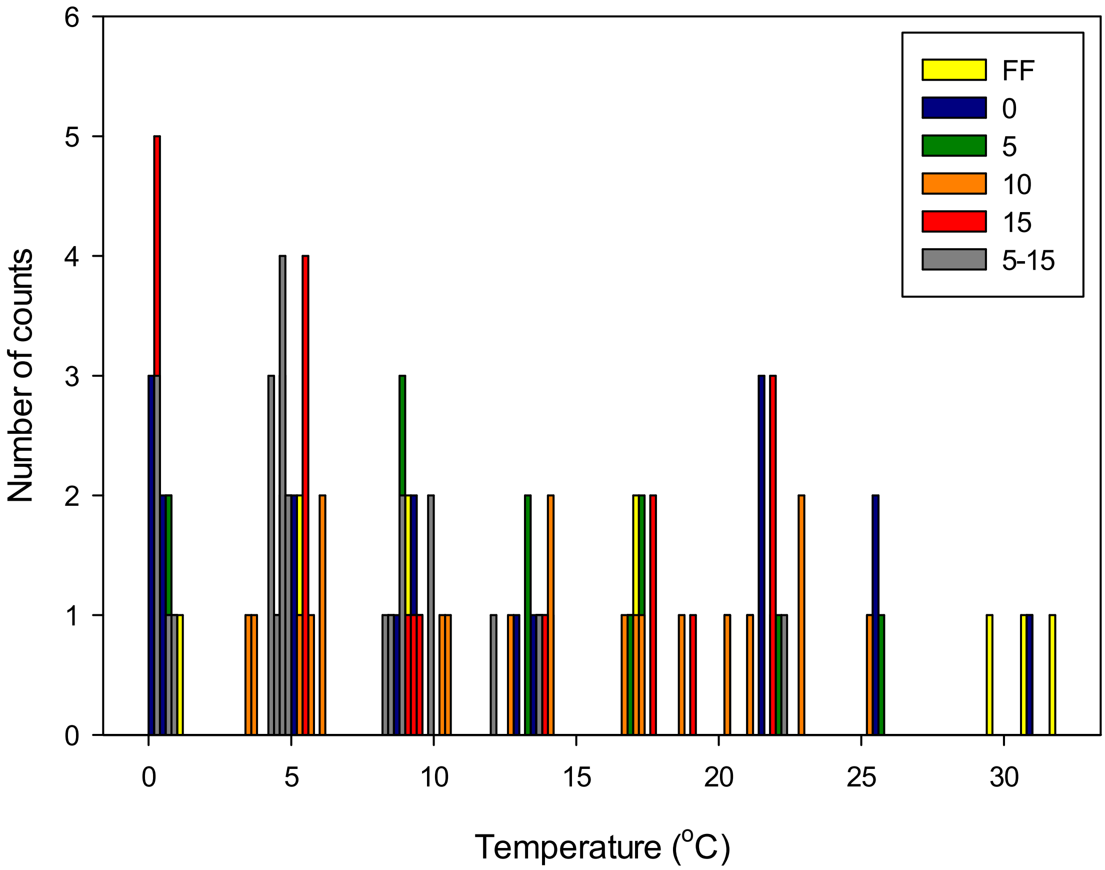

Number of counts
Temperature (°C)
FF
0
5
10
15
5-15
0
1
2
3
4
5
6
0
5
10
15
20
25
30

Figure 3a

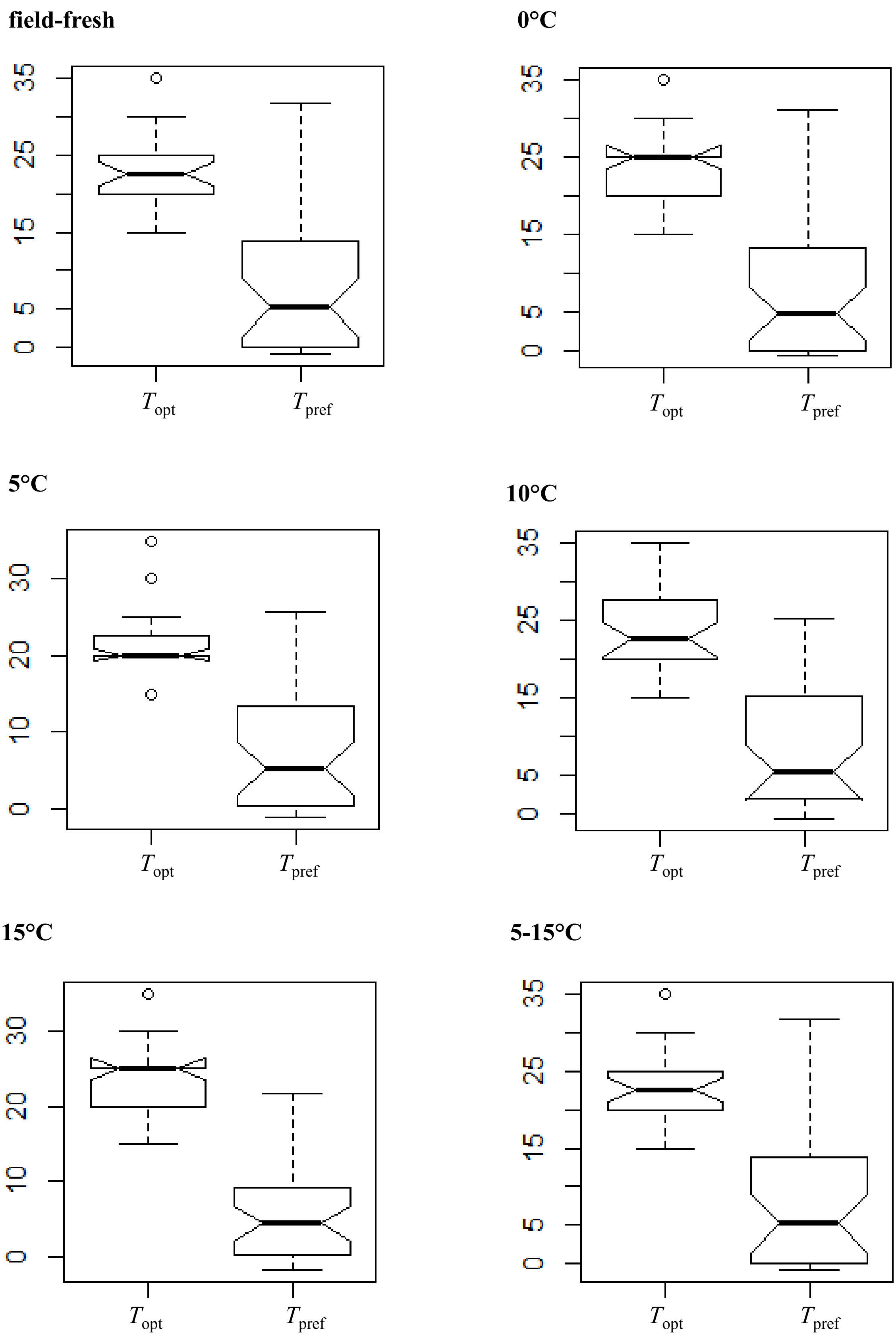


Figure 3b

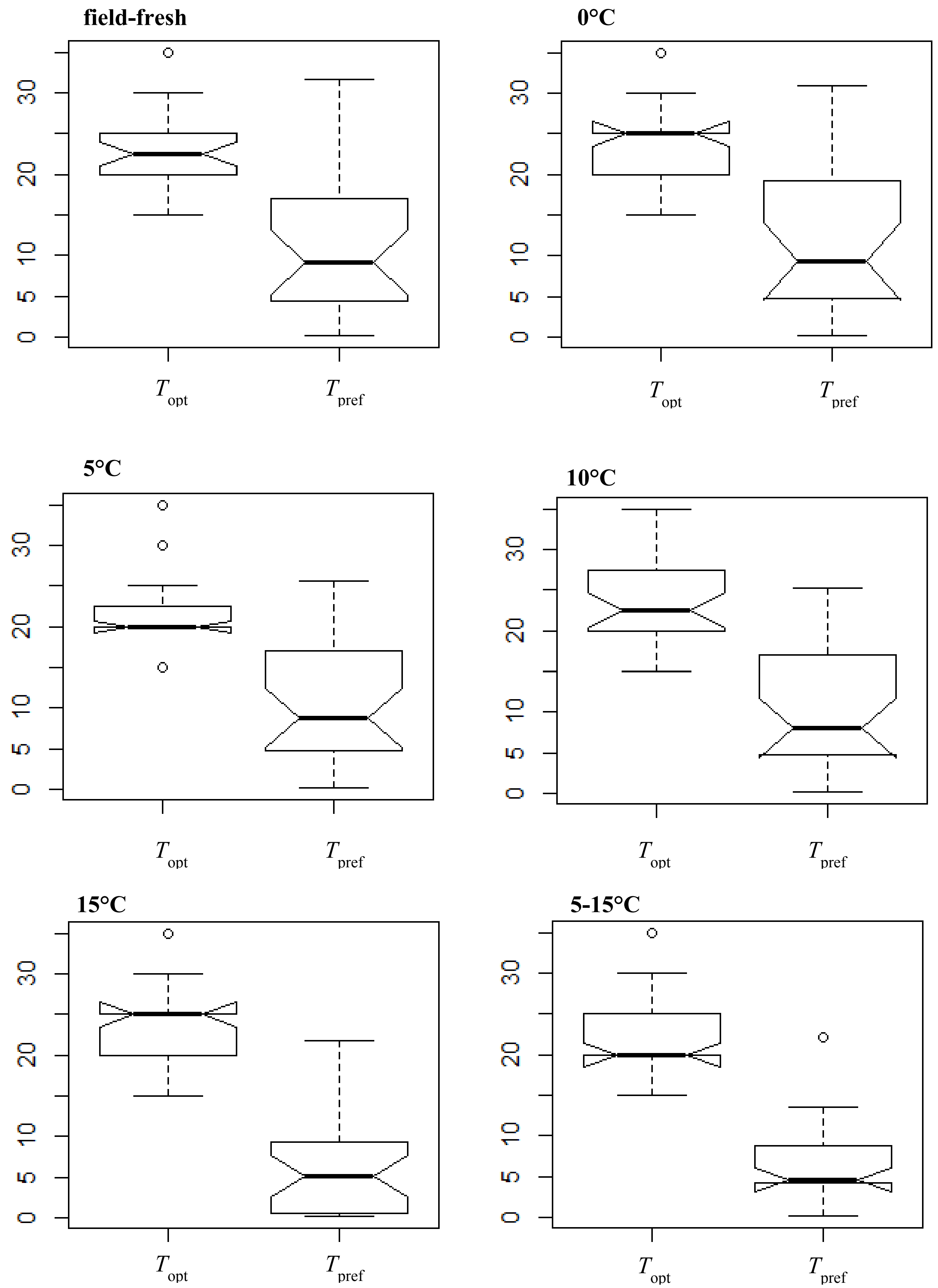
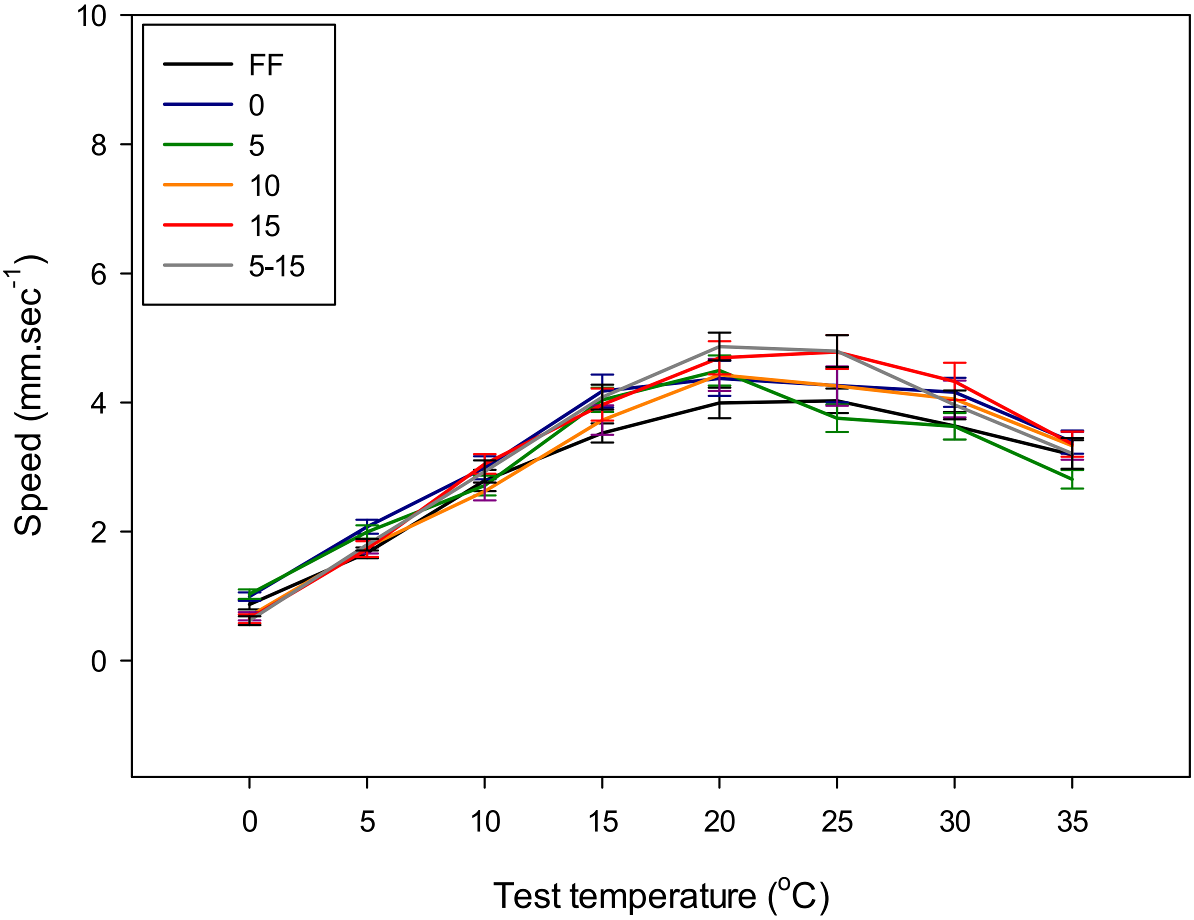


Figure 4



FF
0
5
10
15
5-15
Speed (mm.sec^{-1})
Test temperature (^{o}C)
0
2
4
6
8
10
0
5
10
15
20
25
30
35

Appendix A. Supplementary data

Table S1

Thermal preference of *Pringleophaga marioni* caterpillars on a gradient of *c*. 0-15°C (medians 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

Table S2

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

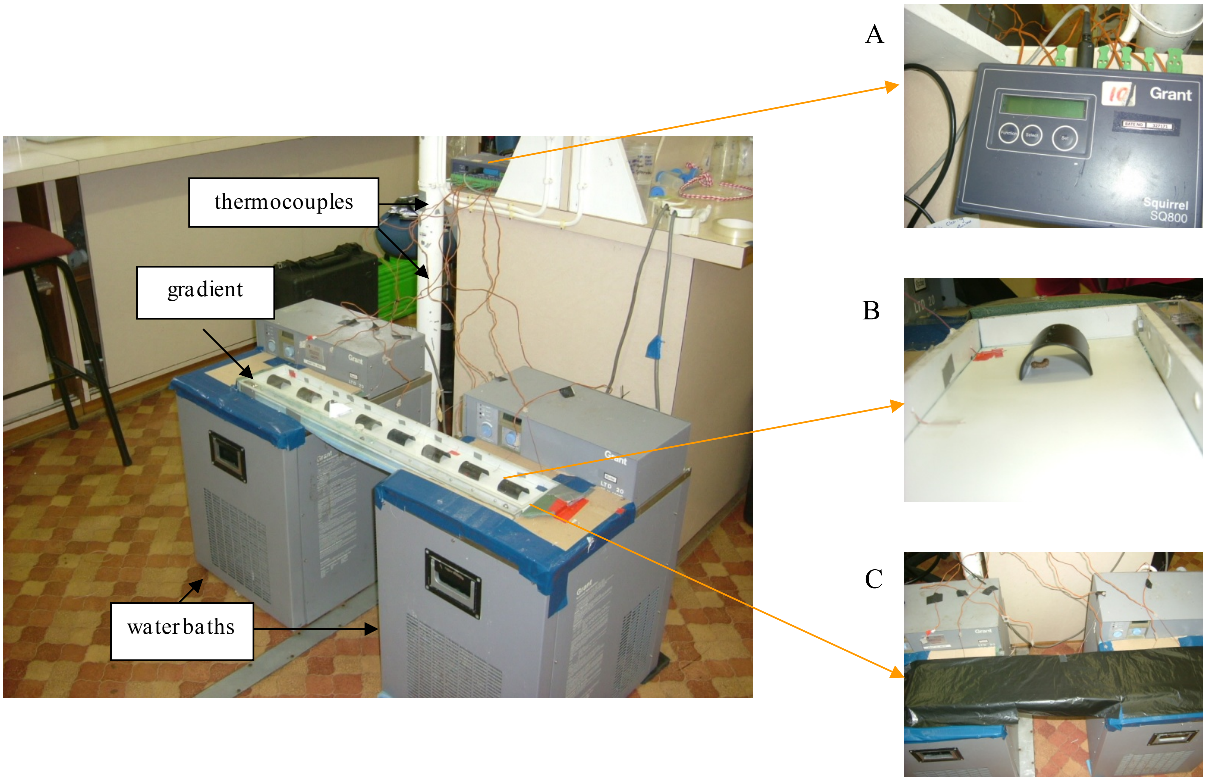


Fig. S1. The experimental set-up of the thermal preference gradient with inserts of the squirrel data logger (A), plastic refuges (B), and the stage covered with a black plastic bag to eliminate light (C).

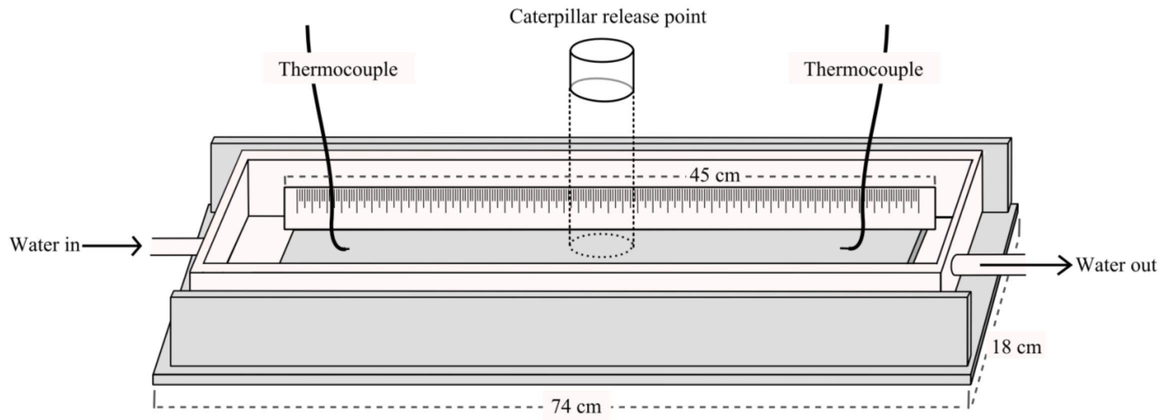


Fig. S2. The walking stage used in locomotor performance trials (details are given in the text).

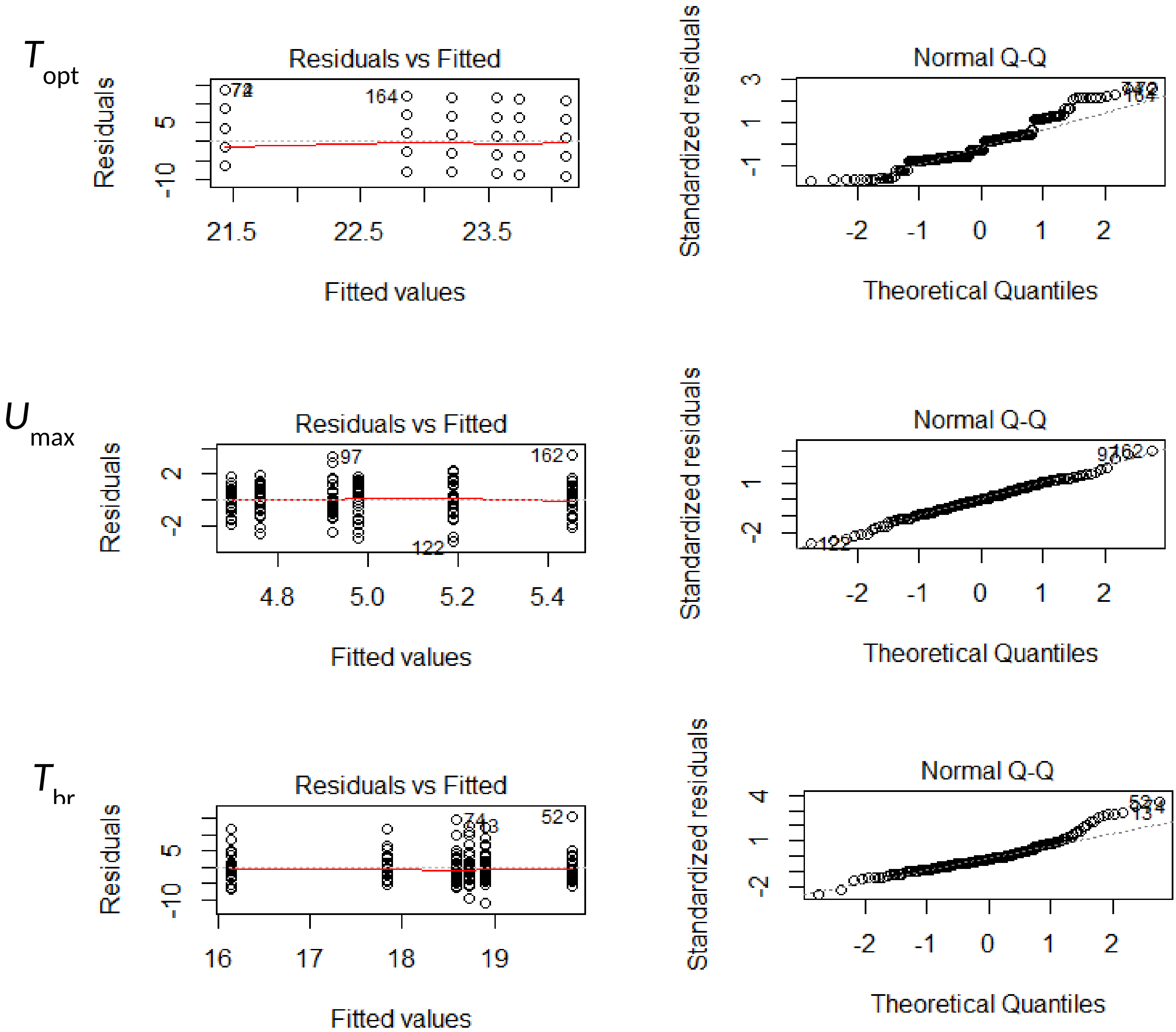


Fig. S3a. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the ANOVA in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between all groups (i.e. field fresh, 0°C, 5°C, 10°C, 15°C and 5-15°C acclimation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

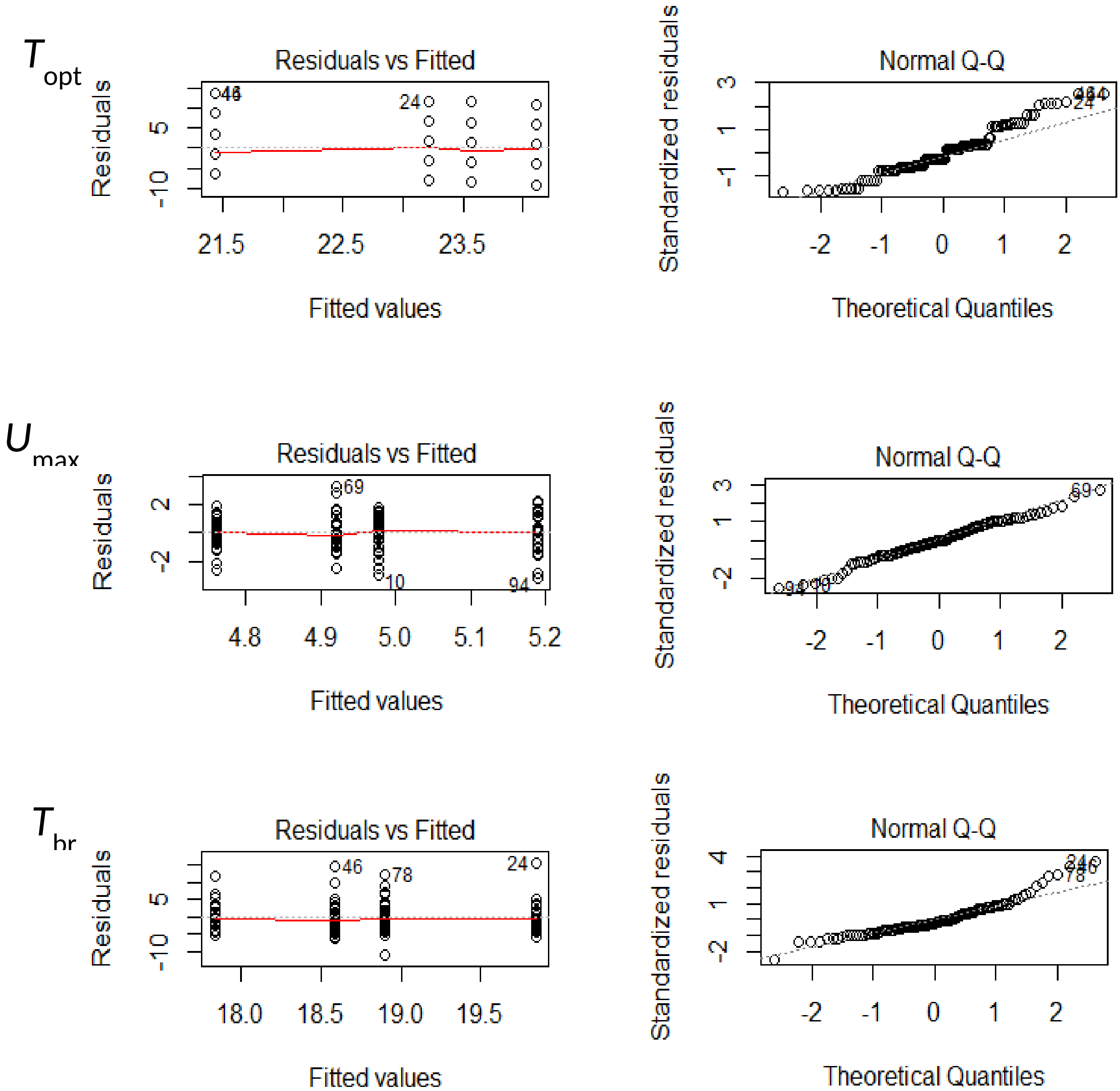


Fig. S3b. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the orthogonal polynomial contrast analyses in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between the 0°C, 5°C, 10°C and 15°C acclimation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

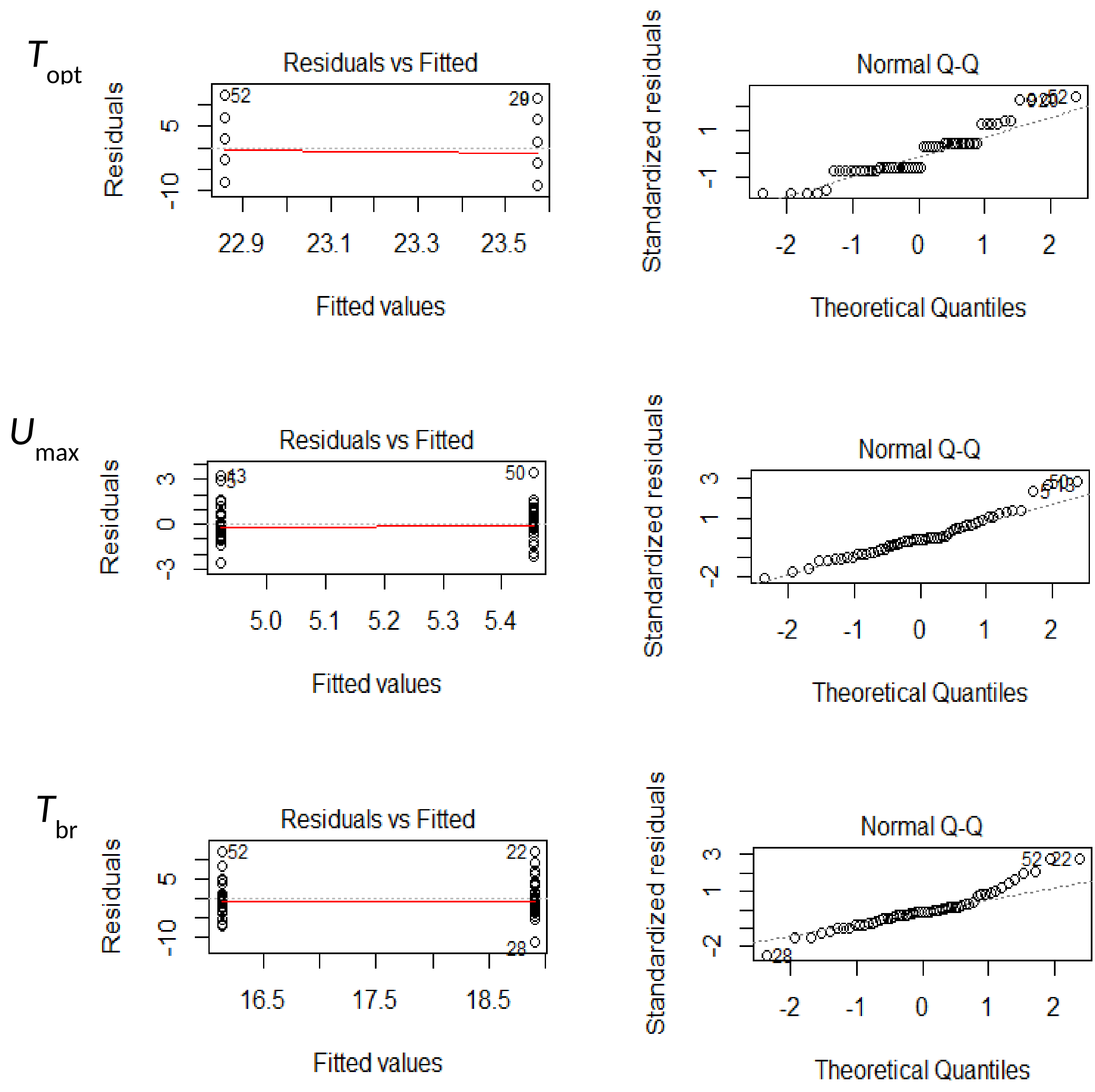
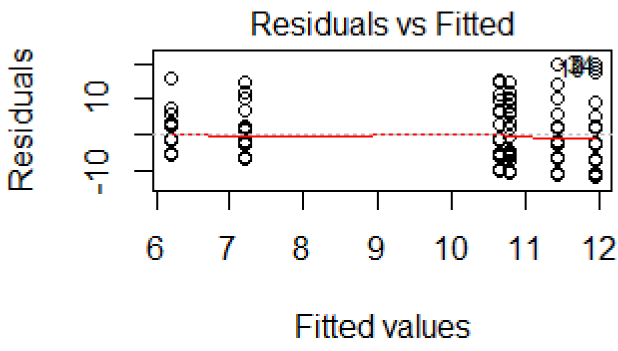


Fig. S3c. Model assumption plots (i.e. normal probability plots and residual versus fitted value plots) to test normality and equal variances for the anova in which performance measures (i.e. T_{opt}, U_{max} and T_{br}) between the fluctuating temperature of 5-15°C and the constant temperature of 10°C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

(a)



(b)

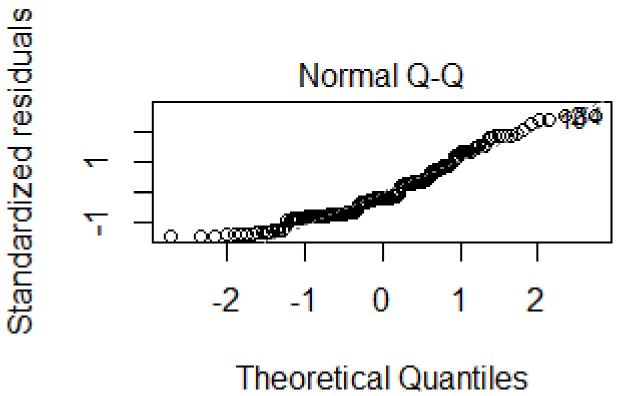


Fig. S4. Residual plots of thermal preference at all acclimation temperatures (0°C, 5°C, 10°C, 15°C and 5-15°C), as well as field fresh individuals on a gradient of *c*. 0-30°C showing (a) the residuals versus fitted values, and (b) a normal probability plot. A Shapiro-Wilk's test indicated that data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

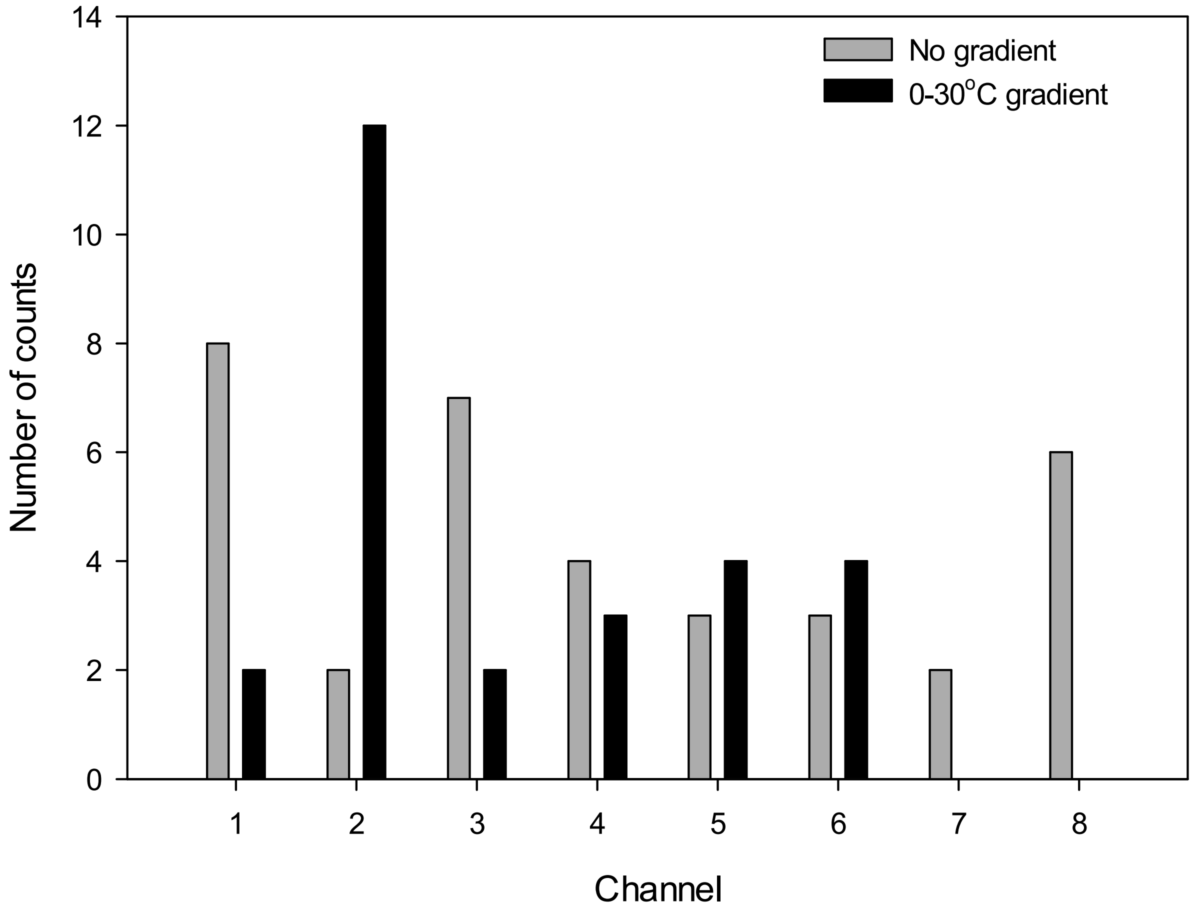


Fig. S5. The distribution of 10°C acclimated individuals on the gradient at a constant temperature of *c.* 10°C (n = 35, grey bars), compared to temperatures ranging from *c*. 0-30°C (n = 28, black bars).