

2-1-2016

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Citation of this paper:

Chown, Steven L; Haupt, Tanya M; and Sinclair, Brent J, "Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth." (2016). *Biology Publications*. 90.
<https://ir.lib.uwo.ca/biologypub/90>

Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth

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ABSTRACT

Temperature compensation in whole-animal metabolic rate is one of the most controversial of the responses thought to characterize insects from low temperature environments. Temperature compensation may either involve a change in absolute values of metabolic rates or a change in the slope of the metabolic rate – temperature relationship. Moreover, assessments of compensation may be complicated by animal responses to fluctuating temperatures. Here we examined whole animal metabolic rates, at 0°C, 5°C, 10°C and 15°C, in the caterpillars of the sub-Antarctic moth, *Pringleophaga marioni* Viette (Tineidae), following one week acclimations to 5°C, 10°C and 15°C, and fluctuating temperatures of 0°C to 10°C, 5°C to 15°C, and 10°C to 20°C. Over the short term, temperature compensation was found following acclimation to 5°C, but the effect size was small (3-14%). By comparison with the caterpillars of 14 other lepidopteran species, no effect of temperature compensation was present, with the relationship between metabolic rate and temperature having a Q_{10} of 2 among species, and no effect of latitude on temperature-corrected metabolic rate. Fluctuating temperature acclimations for the most part had little effect compared with constant temperatures of the same mean value. Nonetheless, fluctuating temperatures of 5°C to 15°C resulted in lower metabolic rates at all test temperatures compared with constant 10°C acclimation, in keeping with expectations from the literature. Absence of significant responses, or those of large effect, in metabolic rates in response to acclimation, may be a consequence of the unpredictable temperature variation over the short-term on sub-Antarctic Marion Island, to which *P. marioni* is endemic.

Keywords:

Fluctuating temperatures

Metabolic rate

Polar environments

Temperature compensation

1. Introduction

Because most ectotherms are unable to use metabolically produced heat to regulate their body temperatures, they have developed strategies to cope with environmental temperature variation. Among the most controversial is temperature compensation or metabolic cold adaptation (Clarke, 2003). To compensate for persistent exposure to low temperature conditions, populations or species from low temperature environments are thought to maintain metabolic rates at low temperatures equivalent to those of their counterparts from warmer environments measured at higher temperatures (that is – they show complete or partial temperature compensation – Cossins and Bowler, 1987). Metabolic cold adaptation may also take the form of reduced sensitivity (i.e. lower slope) of the metabolic rate temperature (MR-T) curve in species or populations from low temperature environments compared to their counterparts from warmer areas (Addo-Bediako et al., 2002). Reduced slopes mean that metabolic rates will not decline at low temperatures as much as they might have done had the slope of the MR-T been steeper. Maintenance of metabolic rate in low temperature conditions is thought to provide a fitness advantage because it enables growth and development to be completed (Sømme and Block, 1991). For insects, evidence for metabolic cold adaptation has been found in a range of studies, though contrary findings also exist (Chown and Gaston, 1999).

Alternatively, it has been suggested that because maintenance metabolism represents a cost to organisms, little fitness benefit exists to metabolic cold adaptation (Clarke, 1991, 1993, 2003). In consequence, controversy has swirled about the theoretical basis for metabolic cold adaptation as well as the extent to which it is supported by empirical data across a range of organisms (e.g., Clarke, 1993; Chown and Gaston, 1999; Lardies et al., 2004; White et al., 2012; Gaitán-España and Nespolo, 2014). For terrestrial ectotherms, notably insects, the suggestion has been made that differences in metabolic rate or rate-

temperature curves among populations from low and high temperature environments might not always represent metabolic cold adaptation, but rather responses to warm, dry conditions. In this case, metabolic rate at high temperatures is reduced to decrease water loss from the respiratory system, or to conserve resources more generally (Clarke, 1993; Chown and Gaston, 1999; Terblanche et al., 2010; Schimpf et al., 2012).

Several factors may complicate interpretation of experiments designed to test the metabolic cold adaptation hypothesis. Among these, two are most significant. First, responses may differ seasonally because of the substantial differences to both day length and temperature cues experienced by individuals at different times during the growing season, which influence physiological rates (Gotthard 2004). Second, many of the experiments that examine acclimation effects on rate-temperature curves use only constant temperatures in their acclimation treatments. By contrast, fluctuating temperatures are a characteristic feature of the natural environment, differing both in their extent and predictability (Angilletta et al., 2006; Deere and Chown, 2006; Folguera et al., 2009; Colinet et al., 2015; Marshall and Burgess, 2015). Performance curves and their components may take very different forms following exposures to constant versus fluctuating temperatures (Lalouette et al., 2011; Niehaus et al., 2012; Williams et al., 2012; Paaajmans et al., 2013; Colinet et al., 2015; Kingsolver et al., 2015). In consequence, recent overviews have argued cogently for the inclusion of fluctuating regimes in the investigation of the way in which physiological and other traits respond to temperature, especially in the context of the thermal environments experienced by the organisms (e.g., Angilletta et al., 2006; Martin and Huey, 2008; Ragland and Kingsolver, 2008; Dowd et al., 2015; Woods et al., 2015). In the case of metabolic rate-temperature relationships, their overall form is typically associated with substantial increases in metabolism at high temperatures (Ruel and Ayres, 1999). Thus, in fluctuating environments, metabolic rate will be disproportionately higher during the warm periods than

it is lower during the cool periods, leading to an elevation of the overall mean (Ruel and Ayres, 1999; Dowd et al., 2015). Ectotherms may thus respond to these relationships by altering the thermal sensitivity of metabolic rate, depending on the conditions they experience (Pásztor et al., 2000; Niehaus et al., 2011; Williams et al., 2012). Thus, thermal variability and its context and consequences are an essential component of any assessment of metabolic rate and rate-temperature variation, such as metabolic cold adaptation vs. water conservation.

Metabolic cold adaptation therefore needs to be explored not only in a species that occupies an environment where it might be evolutionarily advantageous, such as polar environments (Sømme & Block 1991; Addo-Bediako et al. 2002), but using an experimental design that includes both constant and fluctuating temperatures, to account for the importance of thermal variability. Sub-Antarctic Marion Island is an especially appropriate setting for such work because it has low thermal seasonality, reducing seasonal differences in characteristics, and has been extensively characterized (Chown and Froneman, 2008). In particular, the dominant detritivore, and a keystone species in the island's terrestrial ecosystems is the larva of a flightless moth, *Pringleophaga marioni*, which spends the majority of its life cycle as a caterpillar (Smith and Steenkamp, 1992; Haupt et al., 2014a), and has well-characterized thermal biology (Klok and Chown, 1997; Sinclair et al., 2004; Sinclair and Chown, 2005; Haupt et al., 2014a, 2014b, 2015). Moreover, caterpillars are unlikely to be subject to substantial desiccation stress because of the perpetually moist litter and soil habitats they occupy, along with the consumption of moist detritus as a major food source (Crafford et al., 1986; Klok and Chown, 1997). In consequence, they have continual access to water, making water conservation unlikely a factor influencing metabolic rate (see also Klok and Chown, 1998).

Here we therefore explore temperature compensation in the metabolic rate-temperature relationship of *P. marioni* after acclimation at both constant and fluctuating

temperatures reflective of the thermal conditions of its habitat (Haupt et al., 2015). We hypothesise that sub-Antarctic species should show temperature compensation and reduced sensitivity of the rate-temperature relationship as a form of metabolic cold adaptation in response to low temperature exposures (Addo-Bediako et al., 2002). Because this species is negatively affected by prolonged exposure to even 15 °C (Haupt et al., 2014a), we expect that acclimation under fluctuating thermal regimes that approach or exceed this temperature should reduce the slope of the temperature-metabolic rate relationship to mitigate the negative metabolic consequences of these temperatures. Conversely, at low temperatures, reduced thermal sensitivity should allow the animal to maintain performance at lower temperatures (consistent with MCA).

2. Materials and methods

2.1 Study site and species collection

Marion Island (46°54'S, 37°45'E) is a small (*ca.* 290 km², 1280 m maximum elevation), volcanic island which forms part of the Prince Edward Island group. The island's environment is characterized by persistently low temperatures (mean annual ambient temperature of 6.5°C), high precipitation (1900 mm total precipitation per year) (Chown & Froneman, 2008), and two major biomes: tundra in the low to mid elevations and polar desert at the highest elevations. *Pringleophaga marioni* (Lepidoptera: Tineidae) is found in most habitats across the full elevational extent of the island (Crafford et al., 1986). Although broadly distributed, the species' preferred habitats include lowland mire vegetation and wandering albatross nests, for which microclimate variation has been characterised (Haupt et al., 2015). In these habitats the species has a life cycle length of approximately one year, the majority of which is taken up by the larval stage, whereas the brachypterous adults are short-lived (Haupt et al. 2014a).

Caterpillars were collected from abandoned wandering albatross nests in the vicinity of the research station in early May 2011 and returned to the laboratory within six hours of collection. Individuals were placed separately (see French and Smith, 1983 for records of intraspecific predation) in Petri dishes with albatross nest material, which served as both refuge and food (Haupt et al., 2014a), and were transferred under refrigeration at *ca.* 5°C (12L: 12 D light cycle) to the laboratory in Stellenbosch within six days.

In the laboratory, caterpillars were housed in controlled temperature chambers at 5°C (MIR 154, Sanyo, Osaka, Japan, accurate to $\pm 0.5^\circ\text{C}$, verified using Thermchron DS1921 i-button dataloggers, Dallas Semiconductors, Huston, Dallas, USA) for a further three days before being transferred to constant temperatures of 5°C, 10°C, and 15°C, or fluctuating temperatures of 0-10°C, 5-15°C, and 10-20°C (12L:12D light regime; n = 18 caterpillars per acclimation treatment). These temperatures represent the soil microhabitat temperatures recorded in both albatross nests and adjacent to them in the soil on Marion Island (Haupt et al., 2015). The acclimation treatments were maintained for seven days (see Sinclair and Chown, 2003 for rationale). The overall design follows previous investigations of phenotypic plasticity in metabolic rate variation of insects (e.g., Schimpf et al., 2009; Terblanche et al., 2010).

2.2 Experimental procedure

The rate of CO₂ production ($V\text{CO}_2$) as an indicator of metabolic rate (MR) was measured using flow-through respirometry. Trials were undertaken on individual caterpillars at 0°C, 5°C, 10°C and 15°C, randomised among acclimation treatments and test temperatures, except for the 15°C test temperature, which was always examined last to avoid any negative interaction between high test and high acclimation temperatures.

Each individual was weighed (FA324T balance, Avery Berkel, South Africa, 0.1 mg resolution) before and after each trial, and the mean mass (used in analyses) then calculated for the individual. Cylinder air (21% O₂, balance N) was scrubbed of residual CO₂ and H₂O using soda lime, silica gel and drierite. This dry, CO₂-free air flowed through Bev-A-Line plastic tubing (Cole-Parmer, USA) at 200 ml min⁻¹ maintained using a Side-track (Sierra Instruments, Monterey, CA, USA) mass flow controller, into a 10 ml cuvette containing an individual caterpillar in darkness. The cuvette was placed into an activity detector (AD-2, Sable Systems International, Las Vegas, NV, USA [SSI]), and the activity detector plus the cuvette were enclosed inside a Ziploc bag and placed into a Grant LTC 12 water bath (Grant Instruments, Cambridge, UK) set at the desired test temperature. The temperature within the cuvette was measured with a Type-T 40 AWG thermocouple and logged either using a TC-1000 thermocouple thermometer (SSI), or a digital thermometer (CHY 507, Taiwan). Only when the temperature in the cuvette matched that of the test temperature, did trial runs begin. Concentrations of CO₂ were measured in excurrent air from the cuvette with a LiCor 7000 CO₂/H₂O analyser (LiCor, Lincoln, NE, USA). All electronic units in the respirometry system were connected to a desktop computer and controlled by DATACAN V software (SSI). For each trial, a baseline measurement was taken before the caterpillar was placed into the cuvette, as well as at the end after the caterpillar had been removed. Trial runs lasted for 60 min, during which CO₂ concentration was recorded every second.

2.3 Data extraction and analysis

The data for each individual were imported into Expedata (SSI), baseline-corrected, and VCO₂ calculated from flow rates and instantaneous concentration measurements (Lighton 2008) across a 5-10 min period where VCO₂ production was low and stable at each

temperature. Measurements of $V\text{CO}_2$ were converted to microwatts (μW) assuming a joule equivalence of 24.65 kJ L^{-1} .

Metabolic rate (MR) and body mass were \log_{10} -transformed prior to analyses. To assess the temperature compensation hypothesis, the effects of constant acclimation temperatures (5°C , 10°C and 15°C) on MR were first compared using an Analysis of Covariance (ANCOVA) which tested for differences in slopes and intercepts. This model required that the 15°C test temperature be removed to keep the comparison among the linear parts of rate-temperature relationships are investigated (Irlich et al., 2009). This approach was used in all subsequent ANCOVA analyses. To obtain the minimum adequate model, model simplification by stepwise deletion of non-significant interaction terms was used (Crawley, 2007). Using this approach, the final ANCOVA model for MR included acclimation as a categorical variable, test temperature as a continuous variable, and mass as a covariate.

The effects of acclimation and test temperature on MR were further examined using orthogonal polynomial contrast analysis (Huey et al., 1999). Here, the linear and quadratic effects of acclimation and test temperature were examined to determine whether temperature compensation or an alternative acclimation hypothesis was supported (see Huey et al., 1999; Deere and Chown, 2006 for rationale). In these analyses, the 15°C test temperature was retained, and test temperature and acclimation were included as ordered factors in the model, with mass as a covariate.

Analyses of covariance were used to compare the effects of acclimation on MR at each fluctuating acclimation temperature with its comparable arithmetic mean constant temperature (i.e. 5°C vs. $0\text{-}10^\circ\text{C}$, 10°C vs. $5\text{-}15^\circ\text{C}$, 15°C and $10\text{-}20^\circ\text{C}$). For all comparisons, the 15°C test temperature was removed, and the best-fit model via model simplification included test temperature as a continuous variable, mass as a covariate, and acclimation as a categorical variable.

ANCOVA and orthogonal polynomial contrast analyses require strict adherence to the assumptions of ANOVA, which include normally distributed residuals, homogeneity of variance, and a balanced design (Huey et al., 1999). Formal tests showed instances of non-normality (Shapiro-Wilk's tests), but variances were homogenous (Levene's tests) (see Supplementary Material Table S1), and after log transformation of MR, plots of the residuals (i.e. residual vs fitted values, histogram and probability plots) indicated normally distributed residuals with homogenous variances (see Supplementary Material Figs S1-S4). All analyses were implemented in R3.0.0 (R Development Core Team, 2013).

2.4 Comparisons with other larval *Lepidoptera*

To compare metabolic rate of *P. marioni* with other species, metabolic rate, mass and temperature data were extracted from 15 other investigations of metabolic rates in caterpillars. These studies were identified based on searches in ISI Web of Science and Google Scholar using the Boolean combinations of the search terms “metabolic rate*”, “respirat*”, “respiration rate*”, “caterpillar*”, “larva*” and “Lepidoptera*”. All data (Supplementary Table S2) were converted from the original units to μW , assuming an RQ of 0.84 unless it was provided by the original study. Closed versus open system studies (Addo-Bediako et al., 2002) were not distinguished, and the lowest metabolic rates were used, bearing in mind that some effect of specific dynamic action (Chown and Nicolson, 2004) is likely unavoidable in caterpillars. The data were mass corrected assuming a mass scaling coefficient of 0.76, calculated from the data using a linear model in R (see also Gillooly et al., 2006). The logged, mass-corrected metabolic rate data were then plotted against $1/kT$, where k is the Boltzmann constant and T the temperature in Kelvin, including data from *P. marioni* recorded at 10°C after 5°C acclimation. The slope of the ordinary least squares (OLS) relationship between metabolic rate and $1/kT$ was used to calculate activation energy E_a and

the Q_{10} (Gillooly et al., 2006). Here SigmaPlot v. 13.0 (Systat Software, San Jose, U.S.A.) was used throughout, with the OLS relationship checked in R. Because suggestions have been made that temperature-corrected metabolic rate data should be examined against environmental temperature or latitude as an assessment of metabolic cold adaptation (White et al., 2012), this was done here too. Metabolic rate data were temperature corrected to 15°C assuming a Q_{10} of 2.4. The relationship between log metabolic rate and experimental temperature, or log metabolic rate and latitude of the collection site for each species (obtained from the original studies or by extraction from Google Earth based on locality name), in each case including log body mass as a covariate, was examined using a linear model in R.

3. Results

Metabolic rate-temperature relationships were curvilinear in all cases, with similar forms (Fig. 1; Table 1). Constant acclimation temperatures of 5°C, 10°C and 15°C had no effect on VCO_2 in the initial ANCOVA analyses, whereas both test temperature and mass had a significant effect (Table 2). In addition, no interaction terms were significant, indicating no variation in slopes for VCO_2 . By contrast, the orthogonal polynomial contrast analysis revealed significant negative linear and quadratic effects of acclimation on VCO_2 (Table 3). Following the 5°C acclimation, VCO_2 was typically higher across the test temperatures than following acclimation to 15°C, and between the 10°C and 15°C test temperatures VCO_2 did not increase in individuals acclimated to 10°C and 15°C (Fig. 1).

Relative to constant temperatures, fluctuating acclimation temperatures had no significant effects (Table 4), except in the 10°C vs. 5-15°C comparison, where VCO_2 was on average 30% lower following the fluctuating temperature acclimation (Fig. 1), while in none of the cases were significant interactions found between test and acclimation temperatures, indicating equivalent metabolic rate temperature slopes.

A significant relationship was found between $1/kT$ and \ln mass-corrected metabolic rate, though with considerable variation about it (Fig. 2). E_a was estimated as 0.56, and from that value Q_{10} estimated as 2.09. Little change to the relationship was found if *P. marioni* was excluded from the assessment (see the legend to Fig. 2). Using the temperature corrected data revealed a strong relationship between \log metabolic rate and \log body mass, but no significant effect of temperature or of latitude (Table 5).

4. Discussion

In the caterpillars of this sub-Antarctic moth, response to a short-term (7 day) acclimation treatment involving temperatures likely to be encountered in its microhabitats (Haupt et al., 2015), metabolic rate (measured as VCO_2) tended to be higher following acclimation to low temperature than to higher temperatures. Although statistically significant, the effect size was typically modest (3-14% on average). By contrast, no response in the slopes of the metabolic rate-temperature relationships was found. In consequence, these results provide evidence for short-term temperature compensation, or metabolic cold adaptation in its broadest sense (i.e. not implying genotypic adaptation) (Precht et al., 1973; Sømme and Block, 1991; Clarke, 1993), though not involving any change in rate sensitivity to temperature. Little variation in the slope of the rate-temperature relationship following acclimation is in keeping with general findings for terrestrial ectotherms (Seebacher et al., 2015, although the outcome may be variable from group to group, e.g. Mackay, 1982; Terblanche et al., 2009). By contrast, a small increase in rates generally has been found in a range of other species following low temperature acclimation (see discussions in Sømme and Block, 1991; Chown and Gaston, 1999).

An alternative explanation for the short-term acclimation findings is that metabolic rates are reduced at higher temperatures to conserve water (Chown and Gaston, 1999;

Terblanche et al., 2010). Such an explanation seems unlikely given the moist habitats inhabited by the caterpillars (Crafford et al., 1986; Haupt et al., 2015), their almost continual feeding on moist detritus, and their water balance characteristics which are similar to those of mesic species (Chown and Klok, 1997, 1998). A formal test of this idea would require water loss to be separated into its cuticular and respiratory components (Terblanche et al., 2010). The caterpillars show continuous gas exchange (Sinclair et al., 2004), precluding the use of the closed phase of discontinuous gas exchange to distinguish the respiratory from the cuticular contributions to water loss (see Quinlan and Hadley, 1993). During the course of the experimental trials we measured H_2O concentration to calculate V_{H_2O} with a view to using the intercept method to estimate the relative contributions of cuticular and respiratory water loss to total water loss (Gibbs and Johnson, 2004). Unfortunately, the method proved unreliable under our experimental conditions, with negative cuticular water loss rates being returned in more than half of the estimates (Supplementary Material Table S3). In consequence, we were not able to test this idea formally, though we consider it an unlikely explanation for the patterns found.

In the comparison of the metabolic rates of *P. marioni* caterpillars with those of other lepidopterans, the assumption here has been made that latitude is a reasonably proxy, at global scales, for environmental temperature variation (e.g., Irlich et al., 2009; White et al., 2012). From either the perspective of latitudinal or experimental temperature-related variation in temperature-corrected metabolic rates, no evidence was found here for temperature compensation. Indeed, the relationship between log metabolic rate (without temperature correction) and $1/kT$ provides an estimate of activation energy of of 0.68 eV and an estimate of Q_{10} of 2 across all of the species. The former is well within the typical range of 0.6 to 0.7 eV (Gillooly et al., 2001, 2006), although interpretation of this value has proven controversial among the insects (Irlich et al., 2009). The latter is also fairly standard for

whole-animal metabolic rates, though perhaps towards the low end (Cossins and Bowler, 1987; Sømme and Block, 1991; White et al., 2012). Both approaches indicate, therefore, that at least among the species investigated here, temperature compensation is not present. In other words, the metabolic rate value measured for *P. marioni* is what would be expected from the general relationship between metabolic rate and temperature. Thus, metabolic rates tend to be lower in species from colder environments, in keeping with a thermodynamic effect (Frazier et al., 2006; Angilletta et al., 2010). While this finding appears to contradict previous assessments of the likelihood of temperature compensation in high latitude species (Addo-Bediako et al., 2002), the effects in the Addo-Bediako et al. (2002) analysis were quite small and the extent of the data here may well not be sufficient to detect them. Much variation was found among the relationships here, to be expected also when the effects of specific dynamic action (Chown & Nicolson, 2004) cannot be fully accounted for and sample sizes relatively small. Clearly, scope exists for determining to what extent temperature compensation may be characteristic of adult versus juvenile insects, given the preponderance of the former in most databases on insect metabolic rates (e.g. Irlich et al., 2009)

We also failed to find strong evidence of an influence of constant vs. fluctuating temperatures on the form of the metabolic rate-temperature relationships and the absolute values of metabolic rate, as might have been expected (Ruel and Ayers, 1999; Pásztor et al., 2000; Williams et al., 2012; Colinet et al., 2015; Kingsolver et al., 2015). Only in the case of the 10°C vs 5-15°C was VCO_2 reduced, by about 30% on average, in the fluctuating conditions relative to the more constant ones. Such a reduction is in keeping with predictions that reductions in rate might be a means to deal with metabolic costs of high temperatures (e.g. Ruel and Ayers., 1999; Williams et al., 2012). Nonetheless, the overall lack of an influence of fluctuating temperatures on metabolic rates is similar to that found for growth rate in this species over the entire duration of the larval stage, where growth rates at 10°C and

in a fluctuating temperature regime of 5-15°C are equivalent (Haupt et al. 2014a). In part, these responses, as well as the very limited acclimation effects generally, may be a consequence of the nature of environmental variation at the Prince Edward Islands, to which the caterpillars are endemic (Crafford et al., 1986). In particular, not only is thermal variability small in overall magnitude at all timescales (Haupt et al., 2015), but it is also unpredictable over the short-term (Deere and Chown, 2006). Indeed, significant, positive temporal autocorrelation of temperatures rarely extends beyond 24 h, and temperatures are almost entirely unpredictable over periods of a week, unlike the situation in other regions (see Chown and Terblanche, 2007). Under such conditions, phenotypic plasticity, of which acclimation responses are one form, is not to be expected because of the mismatch between the animal response and the changing environment (e.g. Tufto, 2000). Such a lack of plasticity has been detected for several other species on Marion Island (e.g. Deere and Chown, 2006).

In conclusion, this study has shown that acclimation at constant and fluctuating temperatures has small or insignificant effects on metabolic rate or metabolic-rate temperature relationships in the caterpillars of *P. marioni*. Such reduced phenotypic plasticity is expected where environmental variation is unpredictable. Nonetheless, some indication of temperature compensation over the short-term was found, though by comparison with species from other, typically warmer, environments, evidence for compensation was absent. The current work supersedes data from a previous study (Crafford and Chown, 1993) which provided much higher estimates for metabolic rate in the species.

Acknowledgments

Jennifer Lee, Justine Shaw, Asanda Phiri and Mashudu Mashau assisted with field work. This study was supported by National Research Foundation of South Africa Grant SNA14071475789 and the South African National Antarctic Programme.

Supplementary material

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

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Table 1

Summary statistics showing means and standard errors of standard metabolic rate (μW) and body mass (mg) of *Pringleophaga marioni* caterpillars.

Acc ($^{\circ}\text{C}$)	TT ($^{\circ}\text{C}$)	Body mass (mg)		Metabolic rate (μW)	
5	0	130.0	\pm 8.6	16.21	\pm 2.06
5	5	128.0	\pm 7.4	38.49	\pm 5.75
5	10	128.6	\pm 7.5	61.72	\pm 7.40
5	15	113.5	\pm 7.4	74.23	\pm 7.33
10	0	111.0	\pm 8.4	16.64	\pm 3.55
10	5	111.1	\pm 8.8	34.54	\pm 4.55
10	10	111.5	\pm 8.9	66.52	\pm 8.26
10	15	107.9	\pm 14.8	66.53	\pm 6.99
15	0	128.1	\pm 9.7	10.99	\pm 1.19
15	5	127.2	\pm 8.4	31.81	\pm 4.52
15	10	126.5	\pm 9.1	62.50	\pm 8.90
15	15	108.2	\pm 7.8	67.74	\pm 10.67
0~10	0	128.8	\pm 10.9	12.67	\pm 1.22
0~10	5	127.8	\pm 12.1	35.31	\pm 4.35
0~10	10	128.5	\pm 10.9	60.69	\pm 6.12
0~10	15	106.1	\pm 9.4	61.80	\pm 6.52
5~15	0	120.5	\pm 9.8	9.05	\pm 1.10
5~15	5	119.1	\pm 11.3	25.19	\pm 3.51
5~15	10	118.4	\pm 10.4	44.39	\pm 5.29
5~15	15	102.7	\pm 9.7	58.27	\pm 8.53
10~20	0	139.3	\pm 10.2	13.52	\pm 2.14
10~20	5	141.0	\pm 11.6	31.65	\pm 4.35
10~20	10	143.3	\pm 12.3	58.95	\pm 5.96
10~20	15	122.7	\pm 9.6	61.22	\pm 7.87

Table 2

Outcome of the analysis of covariance of metabolic rate ($\log_{10} \mu\text{W}$) of *Pringleophaga marioni* caterpillars at constant acclimation temperatures (5°C, 10°C, 15°C).

VCO₂	Estimate	s.e.	<i>t</i>	<i>P</i>
Intercept	1.913	0.125	15.338	< 0.0001
Acclimation 10	0.052	0.043	1.210	0.228
Acclimation 15	-0.078	0.042	-1.875	0.0626
Test temperature	0.067	0.004	16.118	< 0.0001
Mass	0.881	0.131	6.741	< 0.0001

Table 3

Outcomes of the orthogonal polynomial contrast analysis of metabolic rate ($\log_{10} \mu\text{W}$) of *Pringleophaga marioni* caterpillars at constant acclimation temperatures (5°C, 10°C, 15°C).

VCO₂	d.f.	SS	MS	F-value	P
Acclimation	2	0.269	0.135	3.099	0.0472
Test temperature	3	17.182	5.727	131.866	< 0.0001
Mass	1	2.522	2.522	58.073	< 0.0001
Contrast	Estimate		s.e.	t	P
Acclimation linear	-0.057		0.025	-2.314	0.0217
Acclimation quadratic	-0.062		0.025	-2.472	0.0142
Test temperature linear	0.573		0.029	19.880	< 0.0001
Test temperature quadratic	-0.153		0.029	-5.354	< 0.0001
Mass	0.798		0.105	7.621	< 0.0001

Table 4

Outcomes of the analyses of covariance comparing metabolic rate ($\log_{10} \mu\text{W}$) of *Pringleophaga marioni* caterpillars following acclimation at constant and fluctuating temperatures.

Group		Estimate	s.e.	<i>t</i>	<i>P</i>
5°C vs. 0-10°C	Intercept	1.749	0.146	11.941	<0.0001
	Acclimation	0.001	0.041	0.031	0.9750
	Test temperature	0.063	0.005	12.484	<0.0001
	Mass	0.679	0.152	4.462	<0.0001
10°C vs. 5-15°C	Intercept	1.950	0.137	14.213	<0.0001
	Acclimation	-0.204	0.043	-4.775	<0.0001
	Test temperature	0.069	0.005	13.306	<0.0001
	Mass	0.875	0.134	6.515	<0.0001
15°C vs. 10-20°C	Intercept	2.035	0.118	17.190	<0.0001
	Acclimation	0.006	0.037	0.170	0.8650
	Test temperature	0.070	0.004	15.789	<0.0001
	Mass	1.120	0.131	8.555	<0.0001

Table 5

Outcome of the linear models examining the effects of log body mass and experimental temperature, or log body mass and latitudinal position of collection sites, on temperature-corrected metabolic rate (MR) among 14 species of lepidopteran caterpillars.

MR	Estimate	s.e.	<i>t</i>	<i>P</i>
Intercept	2.785	0.236	11.825	< 0.0001
Log mass	0.761	0.102	7.478	< 0.0001
Temperature	0.007	0.011	0.627	0.544
MR	Estimate	s.e.	<i>t</i>	<i>P</i>
Intercept	3.002	0.179	16.762	< 0.0001
Log mass	0.723	0.100	7.201	< 0.0001
Latitude	-0.002	0.004	-0.673	0.515

Figure legends

Fig. 1. Effect of temperature on metabolic rate following acclimation either to constant or to fluctuating temperatures in *Pringleophaga marioni* caterpillars. (a) Constant acclimation temperatures of 5°C, 10°C and 15°C, (b) 5°C vs. 0-10°C acclimation, (c) 10°C vs. 5-15°C acclimation, (d) 15°C vs. 10-20°C acclimation. In each case, means \pm SE are shown.

Fig. 2. The relationship between \ln mass-corrected metabolic rate and $1/kT$, where k is the Boltzmann constant and T is temperature in K, for 14 lepidopteran caterpillars. The fitted line has the form $y = -0.68 (\pm 0.15) * x + 34.21 (\pm 6.13)$, $R^2 = 0.62$, $F_{(1,12)} = 19.32$, $p = 0.0009$. If *P. marioni* is excluded, the relationship takes the form, $y = -0.68 (\pm 0.20) * x + 34.18 (\pm 7.89)$, $R^2 = 0.51$, $F_{(1,11)} = 11.57$, $p = 0.006$, suggesting little influence of the species, and therefore similar conclusions about the interspecific rate-temperature relationship.

Fig. 1

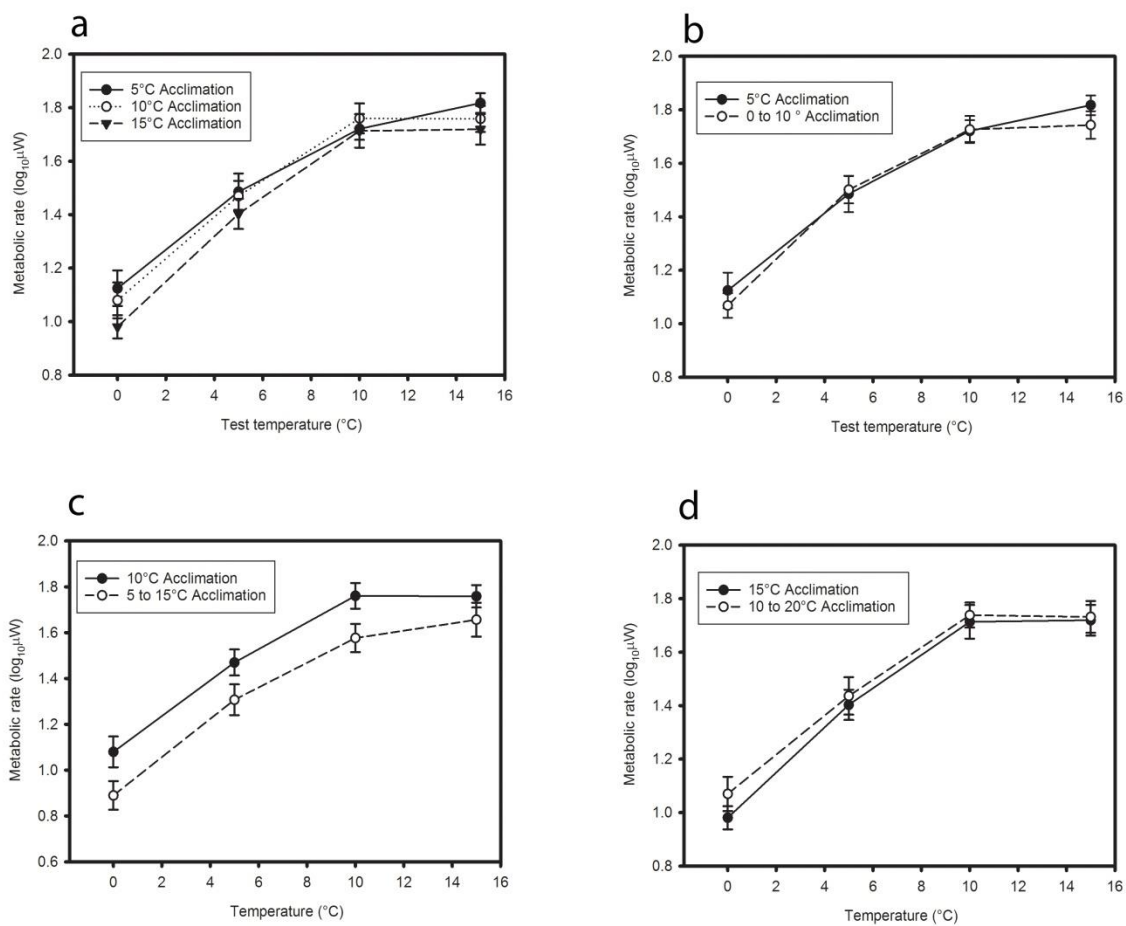
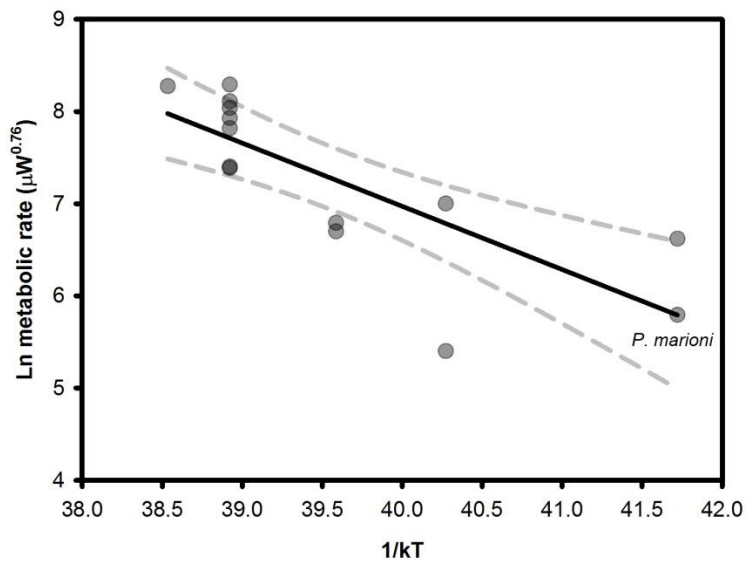


Fig. 2



Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth

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Supplementary material

Table S1. Results from a Shapiro-Wilk's test for normality and Levene's test for homogeneity of variances for metabolic rate ($\log_{10} \mu\text{W}$) of *Pringleophaga marioni* at constant (const.) acclimation temperatures of 5°C, 10°C and 15°C, and fluctuating (fluc.) acclimation temperatures of 0-10°C, 5-15°C and 10-20°C.

acclimation temperature	Shapiro-Wilk's	Levene's
5°C	W = 0.9543, p = 0.0107	
10°C	W = 0.9738, p = 0.1371	
15°C	W = 0.9794, p = 0.2847	
const. (5, 10 & 15°C)	W = 0.9801, p = 0.1045	F = 0.001, d.f. = 1,106, p = 0.9818
0-10°C	W = 0.9636, p = 0.0353	
5-15°C	W = 0.9821, p = 0.3986	
10-20°C	W = 0.9667, p = 0.0537	
const. vs. fluc. (5 & 0-10°C)	W = 0.9801, p = 0.1045	F = 0.001, d.f. = 1,142, p = 0.9767
const. vs. fluc. (10 & 5-15°C)	W = 0.9870, p = 0.3816	F = 0.08, d.f. = 1,106, p = 0.7843
const. vs. fluc. (15 & 10-20°C)	W = 0.9836, p = 0.2054	F = 0.03, d.f. = 1,106, p = 0.8532

Table S2. Data extracted from studies of the metabolic rates (MR) of caterpillars of 14 lepidopteran species from across the globe.

Species	Family	Latitude	Longitude	Test temp (°C)	Mass (g)	MR (μ W)	Reference
<i>Spodoptera exempta</i>	Noctuidae	-1.29	36.83	25	0.328	1059.275	Aidley 1976
<i>Gynaephora groenlandica</i>	Lymantriidae	81.82	-71.37	15	0.0517	112.8209	Bennett et al. 1999
<i>Spodoptera exigua</i>	Noctuidae	32.61	-85.45	28	0.26	1396.83	Dingha et al. 2004
<i>Lymantria dispar</i>	Lymantriidae	46.13	-90.86	25	0.0183	74.54237	Foss et al. 2013
<i>Manduca sexta</i>	Sphingidae	36.10	-79.44	25	0.1	680.9027	Greenlee & Harrison 2005
<i>Spodoptera eridania</i>	Noctuidae	9.74	-81.65	25	0.1	528.0018	Karowe & Martin 1989
<i>Cymbalophora pudica</i>	Arctiidae	43.31	16.65	20	0.245	276.4112	Kostal et al. 1998
<i>Pyrrarctica isabella</i>	Arctiidae	43.00	81.25	5	0.541	470.087	Marshall & Sinclair 2011
<i>Tyria jacobea</i>	Arctiidae	44.56	-123.28	25	0.2125	1013.696	McEvoy 1984
<i>Phyllonorycter blancardella</i>	Gracillariidae	47.40	0.68	25	0.00061	9.339958	Pincebourde & Casas 2006
<i>Eutrichia capensis</i>	Lasiocampidae	-33.94	18.86	20	0.6	604.3976	Schoombie et al. 2013
<i>Thaumatotibia leucotreta</i>	Tortricidae	-32.59	19.01	25	0.0515	167.8882	Terblanche et al. 2014
<i>Erynnis propertius</i>	Hesperiidae	49.65	-125.45	15	0.2105	67.15529	Williams et al. 2012
<i>Pringleophaga marioni</i>	Tineidae	-46.88	37.87	10	0.1135	61.72	This study

Table S3. Calculations of respiratory water loss rate (RWLR) and cuticular water loss rate (CWLR) from total water loss rate (TWLR) using the $VH_2O - VCO_2$ y intercept method. The example given below is of 12 out of 19 individuals at the 5°C acclimation temperature for which negative values were obtained for respiratory water loss at one or more test temperatures (TT) using the above method. These numbers were 15 and 10 individuals for 10°C and 15°C respectively, proving this method ineffective for calculating RWLR.

Individual	TT (°C)	TWLR (mg h ⁻¹)	slope	intercept/CWLR (mg h ⁻¹)	r ²	F	P	RWLR (TWLR - CWLR) (mg h ⁻¹)
5.19	0	1.123	-15.655	1.232	0.026	7.050	0.008	-0.109
5.19	5	2.422	10.453	2.313	0.051	15.925	< 0.0001	0.110
5.19	10	3.496	5.932	3.430	0.097	27.145	< 0.0001	0.066
5.19	15	5.147	8.045	4.998	0.059	30.957	< 0.0001	0.149
5.20.	0	0.569	-0.184	0.569	0.0003	0.109	0.4	-0.0002
5.20.	5	1.631	13.139	1.560	0.017	7.093	0.008	0.071
5.20.	10	2.393	0.817	2.386	0.007	4.228	0.040	0.007
5.20.	15	3.465	-1.202	3.480	0.013	6.529	0.011	-0.015
5.21	0	1.728	-3.091	1.740	0.001	0.359	0.450	-0.012
5.21	5	1.824	169.803	0.373	0.820	1021.258	< 0.0001	1.451
5.21	10	4.654	23.852	3.941	0.106	35.511	< 0.0001	0.713
5.21	15	4.848	21.487	4.567	0.414	242.163	< 0.0001	0.281
5.23	0	1.221	-1.790	1.275	0.001	0.540	0.463	-0.054
5.23	5	3.693	29951.900	1.660	0.690	685.634	< 0.0001	2.033
5.23	10	6.126	-232.418	8.407	0.239	151.160	< 0.0001	-2.281
5.23	15	7.435	46.831	7.013	0.273	225.320	< 0.0001	0.422

table S3 cont.

5.24	0	1.956	-7.900	1.974	0.012	4.171	0.004	-0.018
5.24	5	2.723	8.755	2.686	0.193	61.558	< 0.0001	0.037
5.24	10	3.666	6.246	3.456	0.142	50.208	< 0.0001	0.210
5.24	15	5.711	71.478	4.867	0.109	73.411	< 0.0001	0.844
5.25	0	0.098	-34.691	0.133	0.036	10.795	0.001	-0.035
5.25	5	2.713	44.437	2.487	0.039	17.217	< 0.0001	0.226
5.25	10	4.134	534.378	0.824	0.526	351.819	< 0.0001	3.310
5.25	15	5.717	23.897	5.449	0.049	21.001	< 0.0001	0.268
5.29	0	2.308	11.324	2.245	0.005	1.749	0.187	0.063
5.29	5	1.928	8.540	1.844	0.109	63.429	< 0.0001	0.084
5.29	10	2.772	18.394	2.159	0.579	422.291	< 0.0001	0.613
5.29	15	4.271	-7.154	4.505	0.049	16.559	< 0.0001	-0.235
5.31	0	1.382	-23.669	1.425	0.059	26.730	< 0.0001	-0.044
5.31	5	2.490	-5.233	2.504	0.004	1.774	0.184	-0.013
5.31	10	2.859	-17.760	2.940	0.051	16.475	< 0.0001	-0.081
5.31	15	4.016	6.069	3.961	0.193	88.973	< 0.0001	0.055
5.34	0	4.825	-2.152	5.871	0.026	8.127	0.0047	-1.046
5.34	5	3.673	24.970	3.615	0.022	13.164	0.0003	0.058
5.34	10	10.243	25.898	9.254	0.008	3.001	0.0840	0.989
5.34	15	15.447	789.688	5.264	0.560	563.220	< 0.0001	10.183
5.37	0	3.213	8.307	3.204	0.052	18.703	< 0.0001	0.009
5.37	5	4.452	27.655	4.355	0.014	4.197	0.041	0.097
5.37	10	10.687	-512.444	15.390	0.359	137.356	< 0.0001	-4.703

table S3 cont.

5.37	15	7.843	444.763	5.111	0.433	270.062	< 0.0001	2.732
5.38	0	3.901	55.476	3.696	0.025	8.267	< 0.0001	0.206
5.38	5	4.929	173.857	3.312	0.194	60.067	< 0.0001	1.617
5.38	10	7.959	153.257	5.858	0.255	205.204	< 0.0001	2.100
5.38	15	10.835	-35.379	11.401	0.068	33.769	< 0.0001	-0.566
5.40.	0	6.462	73.567	6.367	0.093	31.013	< 0.0001	0.095
5.40.	5	6.241	30.626	6.131	0.005	2.363	0.125	0.110
5.40.	10	5.795	8.629	5.729	0.022	12.550	0.000	0.067
5.40.	15	7.319	-8.480	7.382	0.003	1.417	0.234	-0.063

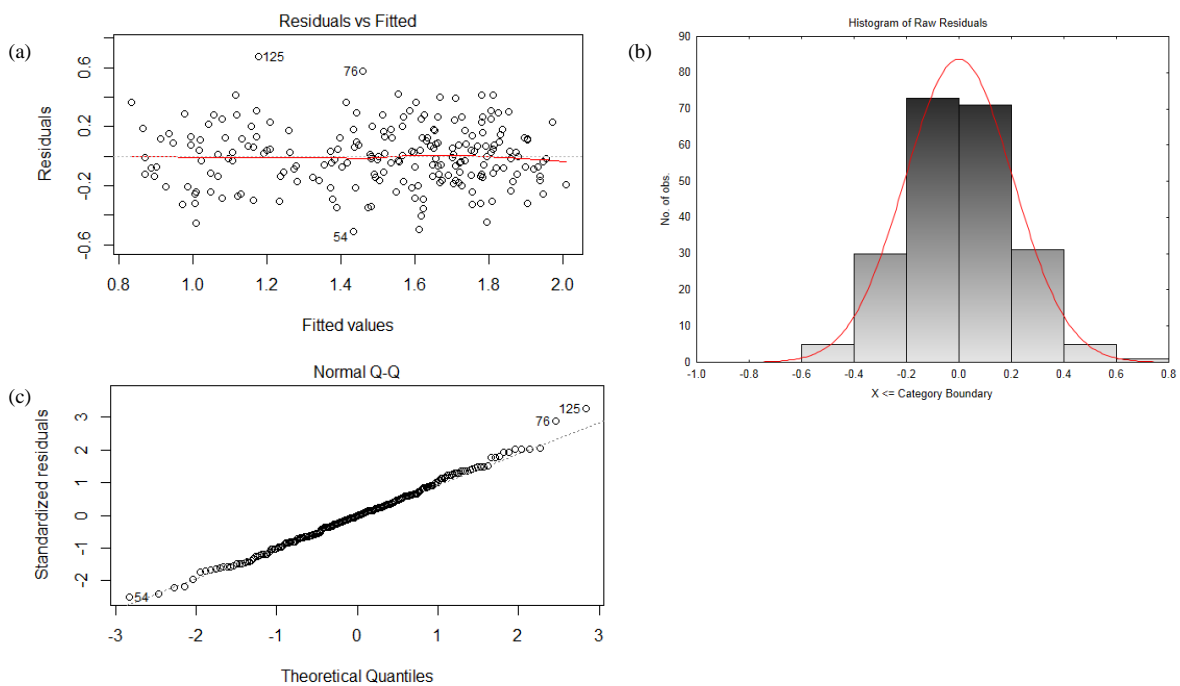


Fig. S1. Metabolic rate ($\log_{10} \mu\text{W}$) at constant acclimation temperatures (5°C , 10°C and 15°C). (a) Residuals versus fitted values, (b) histogram of residuals, and (c) normal probability plot of residuals..

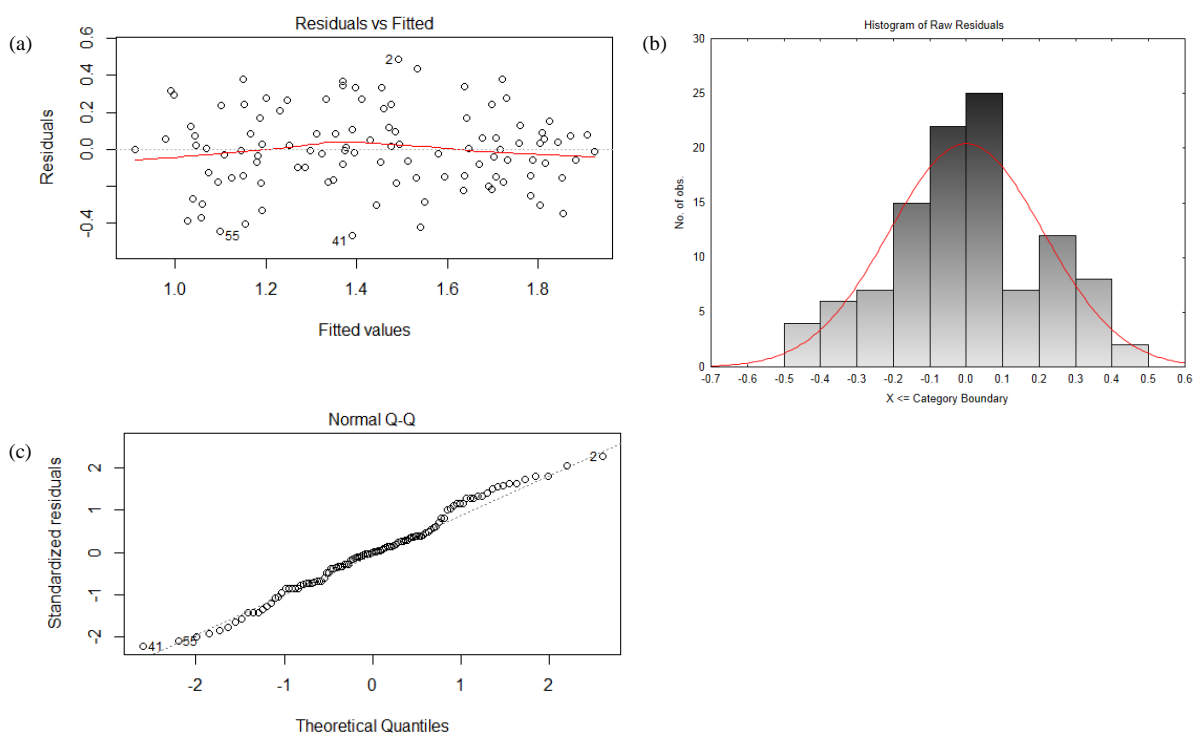


Fig. S2. Metabolic rate ($\log_{10} \mu\text{W}$) at 5°C and $0\text{-}10^{\circ}\text{C}$. (a) Residuals versus fitted values, (b) histogram of residuals, and (c) normal probability plot of residuals.

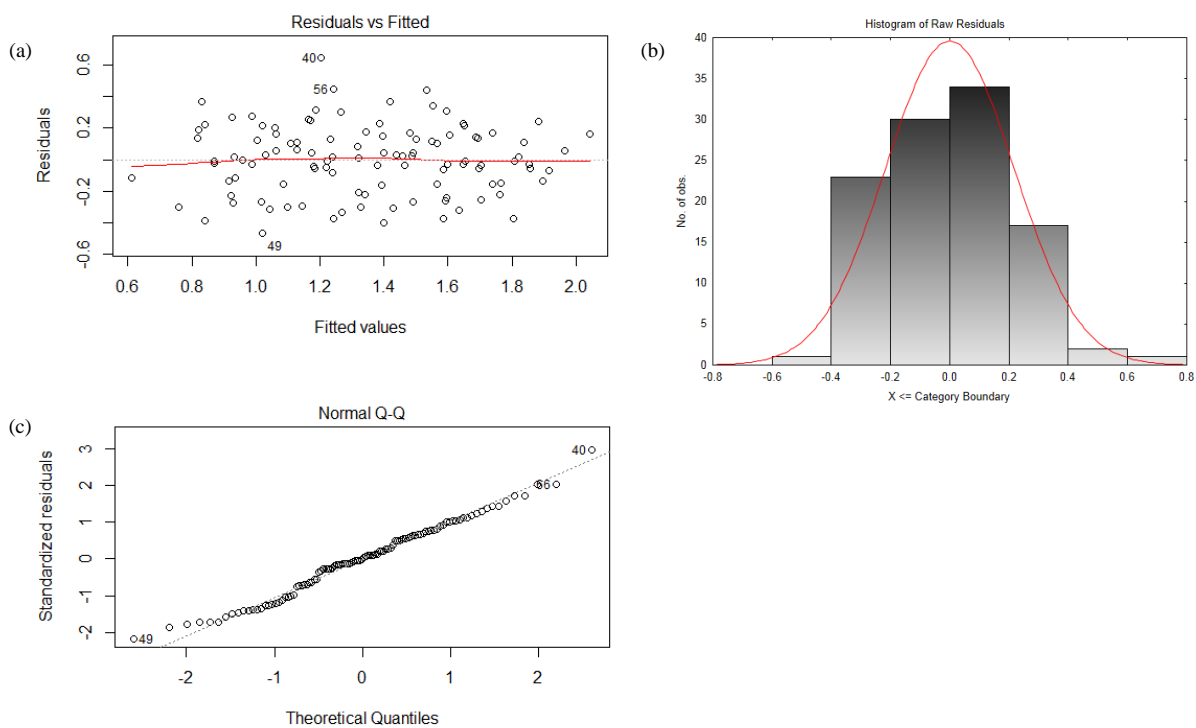


Fig. S3. Metabolic rate ($\log_{10} \mu\text{W}$) at 10°C and $5\text{-}15^\circ\text{C}$. (a) Residuals versus fitted values, (b) histogram of residuals, and (c) normal probability plot of residuals.

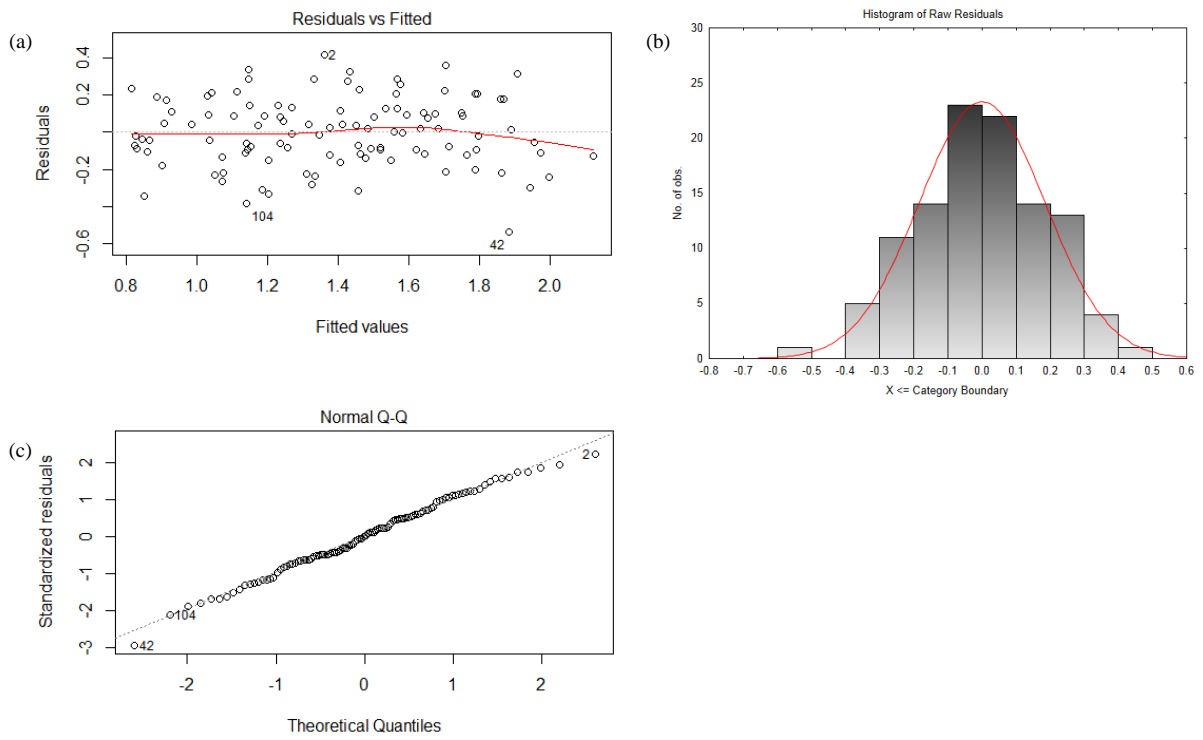


Fig. S4. (a) Metabolic rate ($\log_{10} \mu\text{W}$) at 15°C and $10\text{-}20^\circ\text{C}$. (a) Residuals versus fitted values, (b) histogram of residuals, and (c) normal probability plot of residuals.

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