Intraspecific and interspecific variation in metabolic rate across an elevational gradient in Australian mountain grasshoppers

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Abstract

Metabolic rate provides a fundamental linkage between individual energetics and population ecology. Exploring variation in metabolic rate and other traits across environmental gradients such as elevation provides insights into the selective pressures operating in different habitats. Moreover, this variation reveals evolutionary and ecological consequences of individual energetics. Metabolic rate is influenced by temperature and body mass of organisms. Numerous studies have suggested different mechanisms by which body mass and metabolic rate vary with elevational gradients. The metabolic cold adaptation hypothesis proposes that metabolic rate is higher for populations from colder climates. The temperature-size rule indicates that body size should increase with decreasing temperature. Yet, these generalised observations remain controversial. Therefore, we investigate the intraspecific variation in body mass and metabolic rate in *Monistria concinna* (Walker, 1871) and then use *Kosciuscola cognatus* Rehn, 1957 for interspecific comparison of metabolic rates. We found that *M. concinna* reverses the temperature-size rule and displays sexual dimorphism. Elevation and sex are significant determinants of body mass and metabolic rate in *M. concinna*. Our data suggest that *M. concinna* and *K. cognatus* do not show metabolic cold adaptation. Our data also support previous suggestions of an asymmetry in insect cold physiology between the northern and southern hemispheres.

Key Words

Kosciuscola cognatus, metabolic cold adaptation, metabolic intensity, *Monistria concinna*, temperature

Introduction

Metabolism is the biological processing of energy and materials. Thus, metabolic rate, the rate at which organisms acquire, transform and expend energy, provides a fundamental linkage between the biology of individuals and the ecology of populations through energy (Brown *et al.* 2004). Investigating variation in metabolic rate and other traits across environmental gradients can reveal insights into the selective pressures operating in different habitats (Addo-Bediako *et al.* 2002). Moreover, studies of clinal variation have also shown the ecological and evolutionary ramifications of individual energetics (Buckley *et al.* 2014).

One well-documented case of clinal variation in energetics is the temperature-size rule, wherein insects from higher elevations experience colder temperatures, which delay their development and result in larger body sizes (Whitman 2008). Kleiber's law suggests that metabolic rate scales to the ¾ power of body mass in an organism (Brown *et al.* 2004). Hence, the temperature-size rule relates to variation in metabolic rate across temperature and elevation gradients. Interestingly, grasshoppers typically reverse the temperature-size rule, perhaps due to constraints on food availability at higher elevations (Whitman 2008).

Another case of clinal variation in energetics is the controversial metabolic cold adaptation (MCA) hypothesis. The MCA hypothesis argues that populations from cold climates will have elevated metabolic rates compared to those from warm regions (Clarke 1993). From a life-history perspective, higher metabolic rates should allow populations from colder climates to meet the elevated energy cost of growth and development in the relatively short, cold growing seasons. However, despite the numerous results in favour of the MCA hypothesis, many other studies have questioned the validity and

reliability of such findings (Gaston and Chown 1999). Therefore, the question of elucidating variations in metabolic rate across elevation and temperature gradients remains unanswered.

Intraspecific and interspecific variation in metabolic rate can be modelled with mountain grasshoppers since populations and species are distributed across comparable spatial distances (Slatyer *et al.* 2016). *Monistria concinna* and *Kosciuscola cognatus* are two flightless mountain grasshopper species from the Australian Alps (Key 1985; Slatyer *et al.* 2016). Flightless species have limited dispersal capacity and, thus, show greater genetic differences among geographical populations. Hence, they possess more potential for local adaptation (Knowles 2000; Knowles and Otte 2000). In this study, we employ *M. concinna* and *K. cognatus* as model organisms to study both intraspecific and interspecific variations in metabolic rate across an elevational gradient in the Australian Alps.

First, we examine whether ambient temperatures are lower at higher elevations. Next, we test the hypothesis that *M. concinna*, like other grasshoppers, will reverse the temperature-size rule (Whitman 2008). Then, we investigate the variation in metabolic rate across an elevational gradient for *M. concinna* and examine the influence of sex. In doing so, we test the hypothesis that metabolic rate in *M. concinna* is higher at higher elevations (Addo-Bediako *et al.* 2002). Then, we compare the metabolic intensities of *M. concinna* and *K. cognatus* in order to determine if MCA is characteristic of this group.

This research could contribute to our understanding of the energetics and ecology of mountain grasshoppers from Australia since insects from the southern hemisphere are under-represented in studies on insect metabolism (Addo-Bediako *et al.* 2002). The results of this study can also aid in predicting the ramifications of climate change on alpine grasshoppers and their distribution (Buckley *et al.* 2014).

Methods

We examined variation among grasshopper populations across three sites along an elevation gradient in Kosciuszko National Park, NSW, Australia: Charlotte Pass (CP; 1,868 m a.s.l.), Rainbow Lake (RL; 1,735 m) and Island Bend (IB; 1,276 m). The dominant vegetation at these sites are closed heathlands (CP), open heathland near wetland communities (RL) and subalpine grassland with some shrubs (IB).

Microclimate data

At each collection site, three iButton data loggers were installed adjacent to shrubs at roughly 5 cm above the ground. iButtons were attached with tape to wooden skewers and shielded from the sun with aluminium foil. The iButtons were set to record ambient temperature every hour. We used these temperature data to estimate the ambient temperature experienced by the grasshoppers at the respective sites.

Identification

Monistria concinna individuals were collected from each site whereas *K. cognatus* individuals were collected only from Island Bend. Grasshoppers were classified into male, female and juvenile, with juvenile grasshoppers not sexed as sex is more difficult to distinguish at this stage (Table 1). Most grasshoppers were left overnight without food prior to measurements of metabolic rate, except for a few that were fasted for only ~2 to 5 hours.

Table 1: Summary of grasshoppers collected at Island Bend (IB; 1,276 m), Rainbow Lake (RL; 1,735 m) and Charlotte Pass (CP; 1,868 m).

Species	Population	N female	N male	N juvenile	Total
Monistria concinna	IB	1	1	13	15
	RL	3	5	7	15
	CP	5	6	6	17
Kosciuscola cognatus	IB			8	8

Respirometry

We assessed the metabolic rate for each grasshopper using a closed respirometry system as described below. Between 8 and 21 individuals were measured for each population categorised by site and species (mean n = 15 individuals per population; Table 1).

Two heat mats were set to 21.5°C ($\pm$ 0.5°C) and were put into foil trays covered with sand. Two 20 mL respirometer vials were placed inside each tray and one grasshopper was put into each of the four vials. Grasshoppers were allowed to acclimate to the new surroundings for 10 minutes, then oxygen (O_2) concentrations within the chamber vials were recorded for ~15 minutes using a Pyro Logger V3.213 and FireStingO$_2$. Rates of O_2 consumption were calculated as given below:

$Starting\ O_2\ concentration\ (\%\ O_2) =$
$mean\ of\ first\ 10\ respirometer\ measurements\ for\ \%\ O_2\ in\ the\ vial$ $\qquad\qquad$ *(1)*

$Final\ O_2\ concentration\ (\%\ O_2) =$
$mean\ of\ final\ 10\ respirometer\ measurements\ for\ \%\ O_2\ in\ the\ vial\ (time = \ 831 \pm 1s)$ $\qquad$ *(2)*

$O_2\ consumption\ (\%) = Starting\ \%\ O_2 - Final\ \%\ O_2$ $\qquad\qquad\qquad\qquad\qquad\qquad\qquad$ *(3)*

Metabolic rate is represented by the rate of O_2 consumption of individual grasshoppers. The volume of space occupied by each grasshopper (mL) in the chamber vial is estimated to be equivalent to its body mass (g).

Since O_2 consumption was measured for 831 s, the amount of O_2 (ml) consumed over 831 s is multiplied by 4.332 to obtain the rate of O_2 consumption per hour (as $831\ s \times 4.332 \approx 3600s = 1\ hour$).

$Metabolic\ rate\ (ml.\,h^{-1}) = 4.332 \times [O_2\ consumption/100] \times [Chamber\ Volume\ (ml) -$

$Mass\ of\ grasshopper(ml)]$ $\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad$ *(4)*

Metabolic intensity (MI) was calculated to find the metabolic rate per unit mass.

$MI\ (ml.\,g^{-1}h^{-1}) = \dfrac{4.332 \times [O_2\ consumption/100] \times [Chamber\ Volume\ (ml) - Mass\ of\ grasshopper(ml)]}{Mass\ of\ grasshopper(g)}$ $\qquad$ *(5)*

Following metabolic rate measurements, each grasshopper was weighed and checked for the presence of any physical anomalies, e.g. missing leg. Eleven out of 52 recordings were excluded from the analysis because a negative metabolic rate was recorded. This can happen if, for example, the temperature within the respirometry vial changes during the recording.

Statistical analysis

All statistical analyses were performed in JMP 13.0 software. Graphs were replotted in Microsoft Excel 15.30.

Ambient temperature at different elevations was compared by fitting a linear trend line in Excel and evaluating the corresponding coefficient of determination (R^2).

To test the effects of sex and elevation on the body mass of *M. concinna* (i.e. to test if the temperature–size rule can be applied to *M. concinna*), we used a two-way analysis of variance (ANOVA) with sex

(male/female/juvenile), elevation (as a categorical variable), and a sex–elevation interaction included as factors.

A two-way ANOVA was also used to test the effects of sex and elevation on the metabolic rate of *M. concinna.* An independent samples *t*-test was performed to compare metabolic rates for each pair of sex categories (male, female, juvenile).

Finally, we used an unpaired *t*-test to compare the metabolic intensities of the two species, using data only from juveniles as no adult *K. cognatus* were found.

Results

Elevation and ambient temperature

Ambient temperature was lower at higher elevations in the period 7–15 December 2016 ($R^2 = 1.00$) at Kosciuszko National Park. The fluctuation of temperature at the sites is represented by the magnitude of error bars indicating a 95% confidence interval (Figure 1).
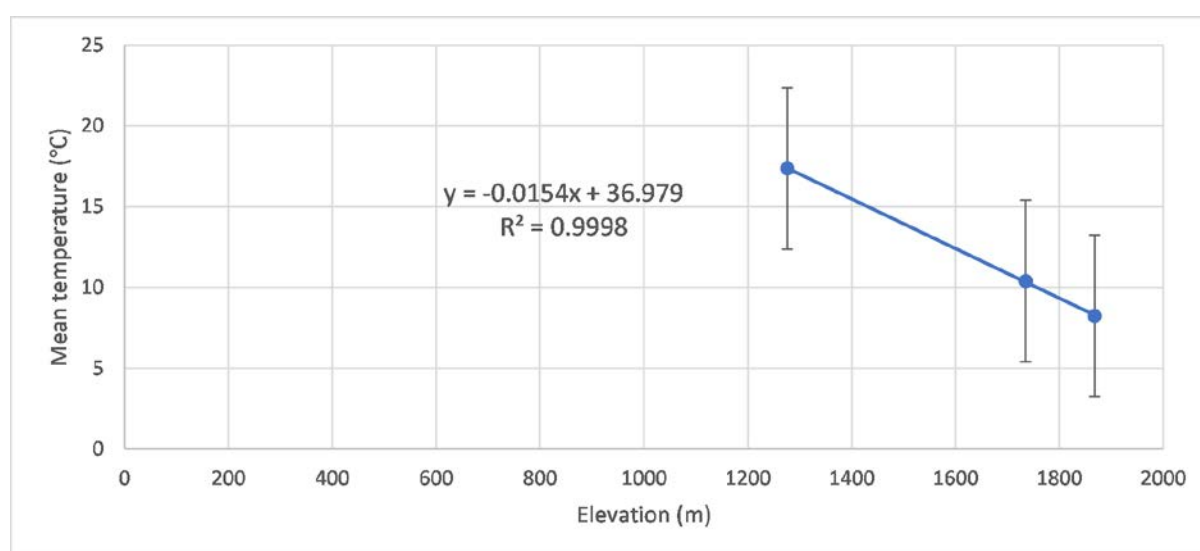
Figure 1: Ambient temperatures (mean ± 95 per cent CI) at different elevations in Kosciuszko National Park. Measurements were taken every hour in periods between 7 and 15 December 2016 with three iButtons per site except at Island Bend (1,276 m). Two out of the three iButtons at Island Bend were found to have broken in the field.

Variation in mass

Body mass in *M. concinna* was related to both elevation (ANOVA: $F_{2,36} = 4.61$, $p = 0.017$) and sex (ANOVA: $F_{2,36} = 182.87$, $p < 0.0001$). Body mass was generally lower at higher elevations and females were significantly larger than both males and juveniles (Figure 2). However, the decline in body mass with elevation was sex/age-specific, i.e. the interaction between sex and elevation is significant ($F_{4,36} = 24.37$, $p < 0.0001$). Although adults of both sexes were smallest at the highest elevation, juveniles were largest in the highest-elevation population (Figure 2).
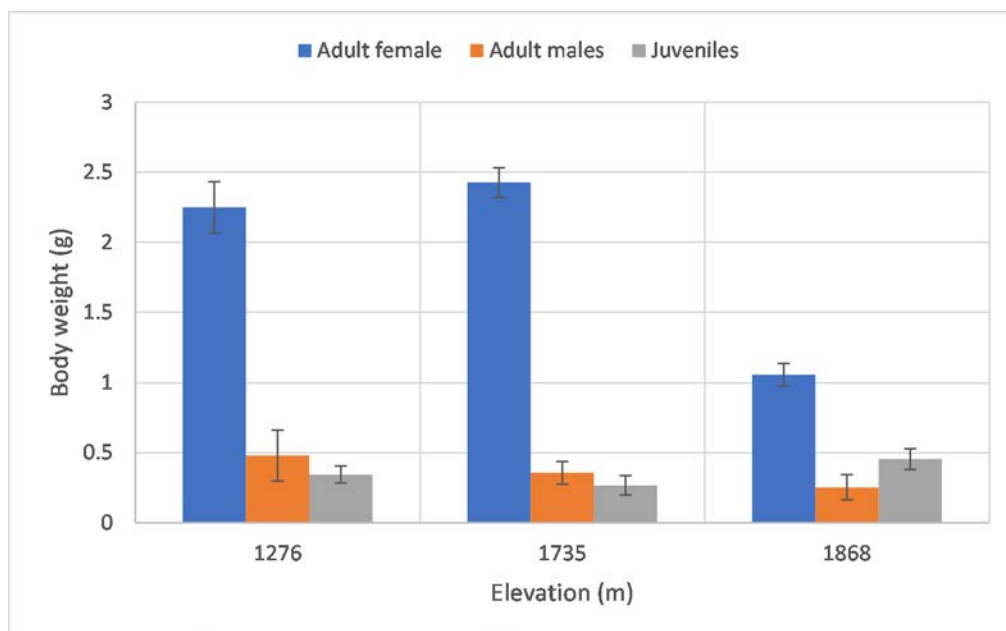
Figure 2: Variation in body mass (mean ± SE) of male, female and juvenile populations of *M. concinna* from different elevations. Measurements were taken from 8 to 9 December 2016 in Kosciuszko National Park.

Variation in metabolic rate

Metabolic rate varied significantly with sex (ANOVA: $F_{2,36}$ =111.44, $p < 0.0001$) and elevation ($F_{2,36}$ = 5.47, $p < 0.0084$) for *M. concinna* and, on average, was lower at higher elevations (Figure 3). However, the interaction between sex and elevation was highly significant ($F_{4,36} = 15.199$, $p < 0.0001$), with a much stronger decline in metabolic rate with elevation among females than among males or juveniles (Figure 3).

Females had significantly higher metabolic rate than males (*t*-test: $t = 4.90$, $p = 0.0009$) and juveniles (*t*-test: $t = 5.19$, $p = 0.0007$), but the metabolic rates of males and juveniles were not significantly different (*t*-test: $t = -0.88$, $p = 0.3881$).
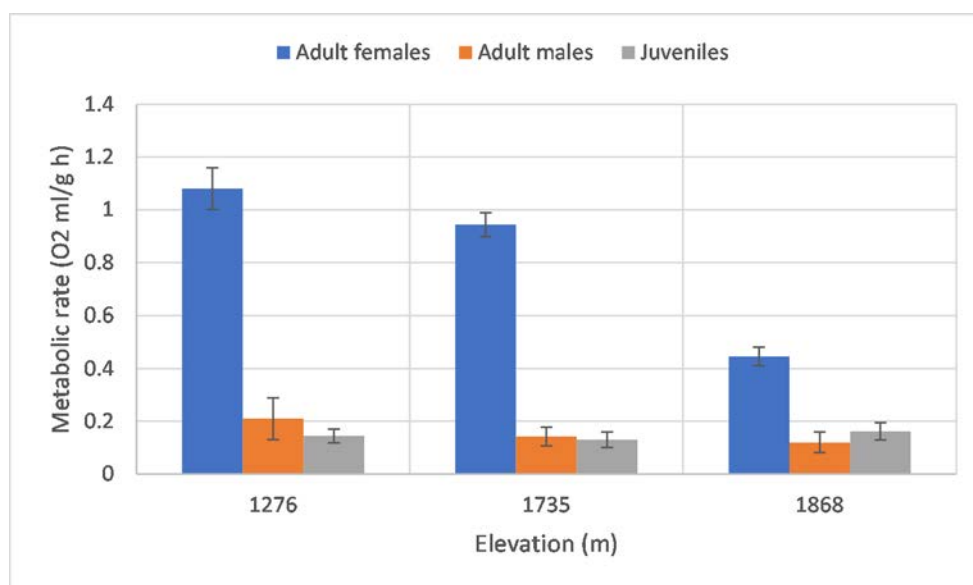

Figure 3: Variation in metabolic rate (mean ± SE) of populations of *M. concinna* from different elevations. Measurements were taken on 8 and 9 December 2016 in Kosciuszko National Park. Eleven out of 52 individuals were excluded from the analysis due to the occurrence of faulty and/or misleading data from instrumental errors and anomalies.

Interspecific variation in metabolic intensity

Student's *T*-test comparison between mean metabolic intensities of juveniles of the two species revealed that juveniles of *M. concinna* had significantly lower metabolic intensity than juvenile *K. cognatus* from the same site (1,276 m, Island Bend) (t = -2.26, p = 0.0200; Figures 4 and 5).
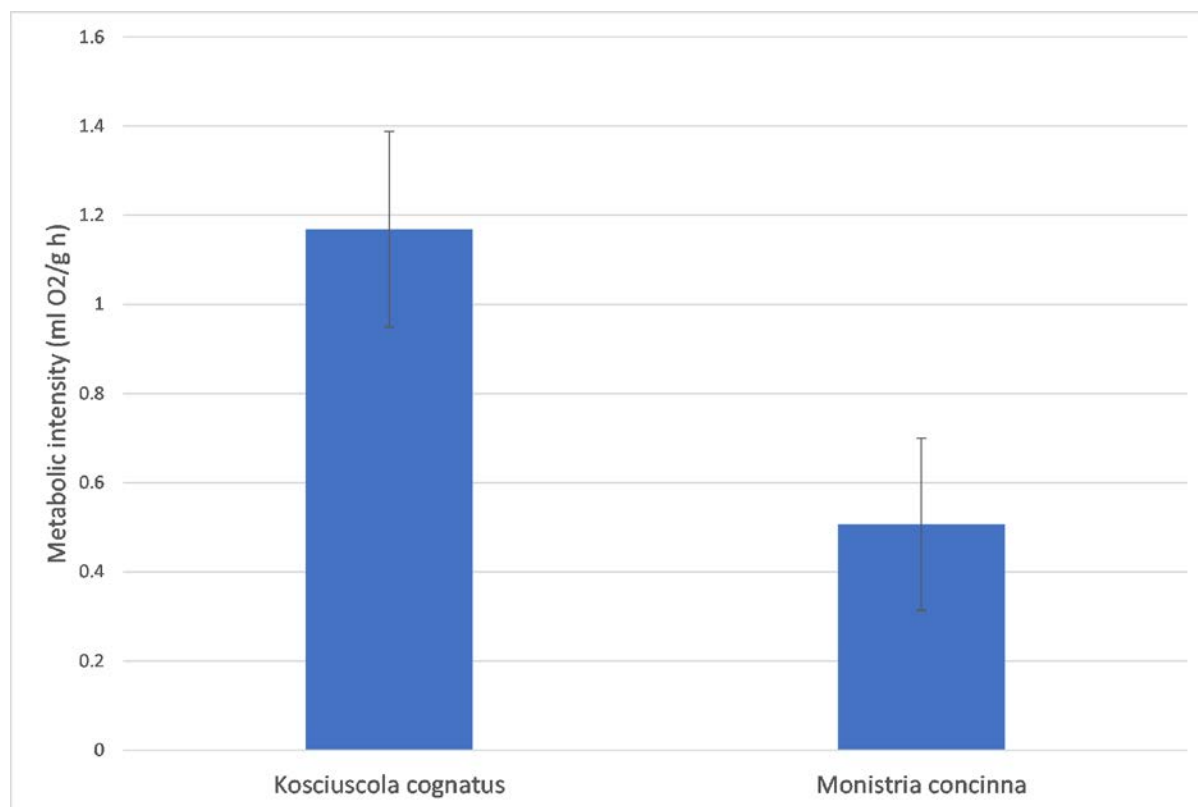

Figure 4: Comparison of metabolic intensity (mean ± SE) between juvenile populations of *M. concinna* and *K. cognatus* from Island Bend (1,276 m). Data from seven juvenile *K. cognatus* and nine juvenile *M. concinna* were employed for this analysis. Measurements were taken on 8 and 9 December 2016 at Kosciuszko National Park.
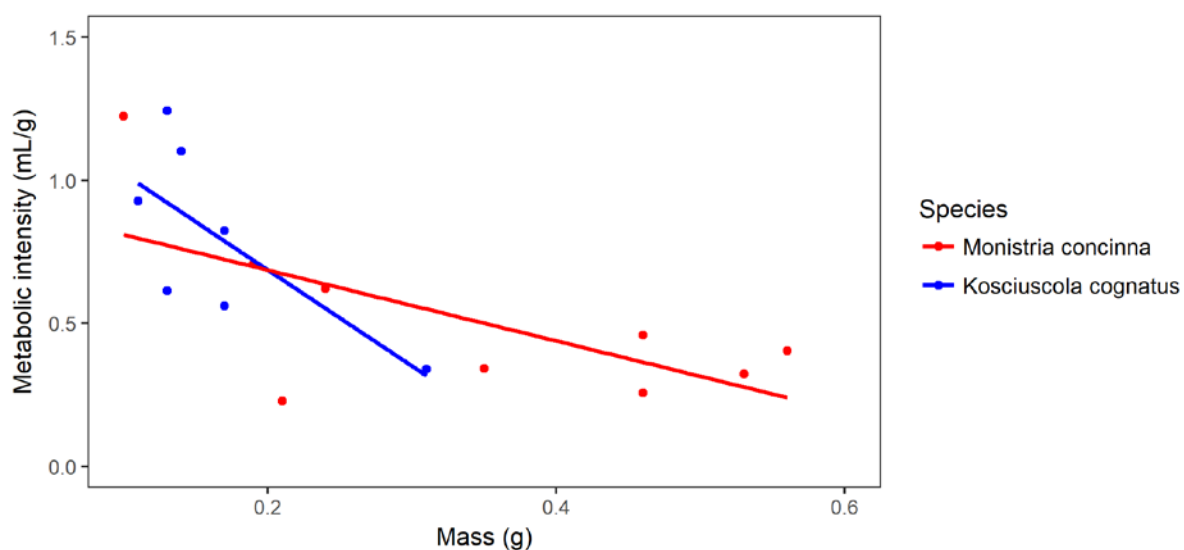

Figure 5: Least squares regression plot of metabolic intensity vs body weight for juvenile *K. cognatus* and *M. concinna* populations at Island Bend.

Discussion

We observed a linear relationship between elevation and ambient temperatures at our three collection sites, where higher elevations had lower average ambient temperatures within the period of our experimentation, i.e. early summer in December 2016 (Figure 1). Therefore, we assume that higher elevations have lower environmental temperatures in the Kosciuszko region, NSW. Note though, that it might also be argued that the use of mean summer temperatures as an indication of environmental temperature is inappropriate because *M. concinna* is active throughout the year (Key 1985).

Significant variation in physiology and size of insects along elevational gradients are regularly observed (Buckley *et al.* 2014) and, in this study, we detected a decline in body mass and metabolic rate in *M. concinna* populations with elevation (Figure 2). The observed decline in body mass with elevation supports the hypothesis that grasshoppers reverse the temperature-size rule (Whitman 2008). This variation in mass has been broadly observed to influence grasshopper physiology, behaviour and fitness (Whitman 2008). In addition, *M. concinna* females are significantly heavier than their male and juvenile counterparts, confirming that *M. concinna* exhibits sexual dimorphism.

The similarity of trends in body mass and metabolic rate across elevational gradients (Figures 2 and 3) is in accordance with the general predictions of Kleiber's law, which states that metabolic rate scales positively with body mass (Brown *et al.* 2004). Further analysis is required to discern if the scaling of metabolic rate with body mass in *M. concinna* is in the exact proportion suggested by Kleiber's law since variations in body compositions can influence the scaling factor.

Contrary to our expectations, metabolic rate in *M. concinna* did not increase with elevation. Instead, metabolic rates in females were significantly lower at higher elevations. For males and juveniles, metabolic rate did not vary with elevation to the same extent as was seen in females (Figure 3). This could mean that metabolic cold adaptation does not apply across populations of *M. concinna*. Rather, this research is more in keeping with the recent suggestions of a large-scale asymmetry in the physiology of insects associated with the hemisphere they occupy. Most data in favour of MCA in insects are from the northern hemisphere (Addo-Bediako *et al.* 2002). Therefore, MCA may be more generally observed in insects of the northern hemisphere.

On the other hand, we must consider the fact that our populations were less geographically separated than populations elsewhere that exhibited local adaptation (Buckley *et al.* 2014). Thus, gene flow may have hindered the extent of local adaptation in our populations. In addition, plasticity may have obscured some of the differences between our field-collected populations. Prior to our respirometry measurements, the grasshoppers from different elevations may have acclimatised and adjusted their metabolic rates to suit the new laboratory environment.

Investigations of interspecific variation in metabolic intensities of *M. concinna* and *K. cognatus* revealed that juveniles of *K. cognatus* had significantly higher metabolic intensities than *M. concinna* juveniles from the same elevation. Since *M. concinna* is also found at elevations above the range of elevations at which *K. cognatus* is distributed (Slatyer *et al.* 2016), we expected to observe higher metabolic intensities in *M. concinna* based on the predictions of the MCA hypothesis. The possible reasons explained above for the absence of empirical evidence for intraspecific MCA in this research can also be applied here for our results on interspecific variation in metabolic intensities. The degree to which metabolic rate increases with elevation may differ between the two species, which could also lead to results such as ours that seem to suggest that these two species do not follow the expectations of MCA. Note that this result is based on only juvenile populations from one site and is not as comprehensive as we would have liked. Therefore, further evidence is needed to confidently denounce the relevance of MCA in our two focal species.

We recommend future researchers to explore the metabolic rates and thermal responses of *M. concinna* and *K. cognatus* across a wider range of elevations over a year (Addo-Bediako *et al.* 2002). Thus, temporal variation in developmental stages can also be considered. We would also have liked to explore differences between species in rates at which their respective metabolic rates vary over elevation gradient. This will allow interspecies variation in metabolic rates across elevational gradients to be better explained.

Conclusions

In sum, our results provide significant support for the reversal of the temperature-size rule in grasshoppers and for the dependence of metabolic rate on body mass as suggested by the metabolic theory of ecology. Sexual dimorphism has been observed in *M. concinna.* The significance of elevation and sex in variations in body mass and metabolic rates in insects is highlighted by our research. Our data suggest that *M. concinna* and *K. cognatus* are not characterised by metabolic cold adaptation and thus, lends another argument against the MCA hypothesis. It is also consistent with the presence of a large-scale asymmetry in insect physiology between the northern and southern hemispheres.

Acknowledgements

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