Journal of Insect Physiology 75 (2015) 30–38

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Elevational variation in adult body size and growth rate but not in metabolic rate in the tree weta *Hemideina crassidens*

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ARTICLE INFO

Article history:
Received 23 February 2015
Accepted 28 February 2015
Available online 6 March 2015

Keywords:
Growth rates
Hemideina
Insects
Metabolic cold adaptation
Morphological variation
Plasticity
New Zealand
Standard metabolic rates

ABSTRACT

Populations of the same species inhabiting distinct localities experience different ecological and climatic pressures that might result in differentiation in traits, particularly those related to temperature. We compared metabolic rate (and its thermal sensitivity), growth rate, and body size among nine high- and low-elevation populations of the Wellington tree weta, *Hemideina crassidens*, distributed from 9 to 1171 m a.s.l across New Zealand. Our results did not indicate elevational compensation in metabolic rates (metabolic cold adaptation). Cold acclimation decreased metabolic rate compared to warm-acclimated individuals from both high- and low-elevation populations. However, we did find countergradient variation in growth rates, with individuals from high-elevation populations growing faster and to a larger final size than individuals from low-elevation populations. Females grew faster to a larger size than males, although as adults their metabolic rates did not differ significantly. The combined physiological and morphological data suggest that high-elevation individuals grow quickly and achieve larger size while maintaining metabolic rates at levels not significantly different from low-elevation individuals. Thus, morphological differentiation among tree weta populations, in concert with genetic variation, might provide the material required for adaptation to changing conditions.

1. Introduction

Abiotic variables can drive spatial and/or temporal variation in physiological traits of organisms (Gaston et al., 2009). Populations of the same species inhabiting distinct localities can experience different ecological and climatic conditions giving rise to differentiation in one or more traits (Mayr, 1963; Endler, 1977). Many species are distributed along elevation gradients, with high-elevation populations experiencing lower temperatures, decreased partial pressure of gases, and increased radiation input compared to those at low elevations (Rezende et al., 2005; Hodkinson, 2005). These same ecological pressures acting on high-elevation populations are useful to test the generality of intraspecific responses to abiotic variation at contrasting elevations.

Insects are ectotherms, and generally have limited ability to regulate their resting body temperature, particularly at night (Chown and Nicolson, 2004). The rate of biochemical processes is temperature-dependent, thus ambient temperature directly affects physiological rates (Tattersall et al., 2012). Ectotherms from cold environments often maintain metabolic rates at low temperatures that are equivalent to those of species from warmer environments at much higher temperatures, a phenomenon known as metabolic cold adaptation (MCA; Clarke, 1991, 1993; Chown and Gaston, 1999). These elevated metabolic rates are thought to be adaptive, allowing accelerated physiological processes in environments with shorter periods of optimal conditions, and so counteracting the environmental effect of depressed temperatures on growth rates (Clarke, 1993; Conover and Schultz, 1995; Chown and Gaston, 1999; Addo-Bediako et al., 2002; Gaitán-Espitía and Nespolo, 2014). At the intraspecific level, there is support for MCA in the form of elevated metabolic rates in individuals from high-elevation/latitude, compared with low-elevation/latitude populations, in several insect species (Kennington, 1957; Hadley and Massion, 1985; Chown et al., 1997). By contrast, other studies found no evidence for MCA (Nylund, 1991; Ashby, 1997). Most of

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http://dx.doi.org/10.1016/j.jinsphys.2015.02.012
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these studies are conducted across a single elevational or latitudinal gradient (reviewed by Chown and Gaston, 1999). Here, we explore MCA in the context of multiple, independent, high-elevation populations of the same species, predicting a consistent replicated response between contrasting elevations.

Evidence suggests that a larger body and slower growth is adaptive in colder environments (see below, Angilletta et al., 2004), and might be coupled with elevated metabolic rates to sustain activity and allow for completion of life cycles in the shorter growing seasons characteristic of high-elevation environments. Intraspecific variation in body size has been reported for elevational gradients with a strong relationship between individual size and environment (Angilletta and Dunham, 2003). In general, animals found in colder climates tend to be larger as adults than their conspecifics in warmer climates. This pattern holds when either altitude or latitude is a proxy for environmental temperature (Ashton, 2002; Ashton and Feldman, 2003).

Models of ectotherm life-history evolution predict larger adult size in cold environments that retard growth (Atkinson, 1994; Berrigan and Charnov, 1994). Furthermore, individuals from colder environments are often larger at maturity than those from warmer environments when reared in common garden conditions; e.g., *Drosophila melanogaster* reared at lower temperatures were larger than high-temperature lines (reviewed by Partridge and French, 1996). To reach larger body sizes in cold environments, individuals must prolong growth and delay reproduction relative to those in warm environments. Such delayed maturation is adaptive when a colder environment enables an increase in fecundity or higher survival rates (Stearns, 1992; Angilletta et al., 2004).

The Wellington tree weta, *Hemideina crassidens* (Orthoptera: Anostostomatidae), is a large, flightless, nocturnal insect endemic to New Zealand. It is found in the southern third of the North Island and on the west coast of the South Island (Bulgarella et al., 2014). Some populations are isolated on mountaintops or in low-elevation areas while others are continuously distributed from sea level to 1500 m a.s.l. (Trewick and Morgan-Richards, 1995, 2014). Tree weta hide in tree holes during the daytime, emerging at night to feed mainly on the leaves of trees and shrubs (Wehi et al., 2013). Adult *H. crassidens* are sexually dimorphic: males have enlarged heads and mandibles that they use to compete for harems of 1–13 females (Asher, 1977; Field and Deans, 2001; Kelly, 2005). Individuals of this species from one high-elevation population had longer tibiae and larger body mass than individuals from a low-elevation site, whether collected as adults, or raised from the 3rd or 4th instar in captivity (Minards et al., 2014).

Studies of the physiology of New Zealand montane insects have centred on the alpine weta *Hemideina maori* and the alpine cockroach *Celatoblatta quinquemaculata* (reviewed by Wharton, 2011). In contrast to *H. maori*, *H. crassidens* features in only two thermal biology studies so far (Sinclair et al., 1999; Minards et al., 2014). New Zealand sits on the continental boundary of the Pacific and Indo-Australian plates the activity of which resulted in the formation of the Southern Alps and the first widespread alpine habitats (about 5 Ma; Trewick and Bland, 2012). North Island mountains are of volcanic origin and more recent (<1 Ma). About 75% of New Zealand is >200 m a.s.l. with 19 peaks exceeding 3000 m a.s.l. (Wallis and Trewick, 2009). Consequently, there are records of repeated invasions of alpine zones in multiple taxa (reviewed by Buckley et al., 2014) and therefore New Zealand provides an excellent opportunity to explore the consequences of adaptation to the alpine zone.

We hypothesise that local selection at high elevations leads to MCA. Thus we expect to find consistently elevated metabolic rates in high-elevation populations when compared to conspecific weta from low elevations. Another hypothesis holds that larger adult body size is an advantage at higher elevations, leading to selection for faster growth rates (perhaps because of increased growth efficiency; Angilletta and Dunham, 2003). If this phenotypic pattern is controlled by genetically-determined differences we predict that high-elevation individuals will grow faster when reared under common-garden conditions at a constant temperature (reviewed by Arendt, 1997; Angilletta et al., 2002).

2. Materials and methods

2.1. Animal collection and care

We collected weta by searching tree holes during daytime between March and December 2011 (New Zealand Department of Conservation collection permits WE-31465-FAU, NM-32444-FAU, TW-32116-FAU and WA-22197-RES, where appropriate). Adult weta ($n = 55$, 33 female and 22 male) from seven populations were included in the metabolic rate trials (Fig. 1). Juvenile weta ($n = 138$, 60 female and 78 male) from nine localities were included in the growth trials, and their adult size compared after their final moult. Our sampling represented populations with different elevational (9–1171 m a.s.l) distributions (Fig. 1). Two pairs of weta populations in our study are continuously distributed from low to high elevation. On the North Island of New Zealand, the weta from Nga Manu (low elevation) and Mt. Hector (high elevation) are part of a contiguous population, as are those from Graham river (low elevation) and Flora (high elevation) in the South Island. All other populations studied are located on the North Island of New Zealand (Fig. 1).

We used previously-published 480 bp mitochondrial DNA (mtDNA) cytochrome b sequences (Bulgarella et al., 2014) to determine the extent of haplotype sharing among 92 individuals from the nine sampled populations by constructing a haplotype network using the median-joining algorithm in the software Network 4.1 (Bandelt et al., 1999; Fig. 1).

Weta were transported to the laboratory where they were kept in individual plastic containers ($17 \times 17 \times 9$ cm) with wire mesh in the lids in a temperature-controlled room at 14 ± 1 °C with a 14:10-h light:dark cycle. The weta were provided with a roost hole made from hollowed flax stalk (*Phormium tenax*), and moisture via damp paper-towel. Food was leaves of mahoe (*Melicytus ramiflorus*), taupata (*Coprosma repens*) and miro (*Prumnopitys ferruginea*) provided *ad libitum*. These three plants species are browsed by tree weta in the wild (Griffin et al., 2011; Dewhurst, 2012; Wehi et al., 2013). We supplemented this diet weekly with soy pellets for protein (Horleys, Naturalac Nutrition Ltd., Auckland, New Zealand).

2.2. Respirometry

We estimated standard metabolic rate (SMR) of adult weta from the rate of oxygen consumption in a closed system (Lighton, 2008). Weta were fasted for three days prior to measurements to avoid specific dynamic action and to standardise fuel sources (Sinclair et al., 2011). This fasting time was chosen because these large insects feed intermittently in the wild (Wehi et al., 2013). All metabolic trials were performed in the dark during the day, which corresponds to resting conditions for this nocturnal species; weta remained inactive for the period of respirometry, as the experimental chamber closely resembled their typical roost environment. Each weta was weighed (±0.1 g; Avery Berkel FB711 balance, UK) and placed in an open-ended, 30.4 cm^3 glass cylinder (2.3 cm diameter, 7.6 cm height) sealed with an airtight rubber bung through which an oxygen optode (Ocean Optics Inc., USA) passed. The O_2 optode was connected to a fibre-optic O_2 sensing system (Tau Theta Instruments LLC, Fort Collins, Colorado) that recorded O_2 partial pressure at 2 s intervals over each 3 h run.

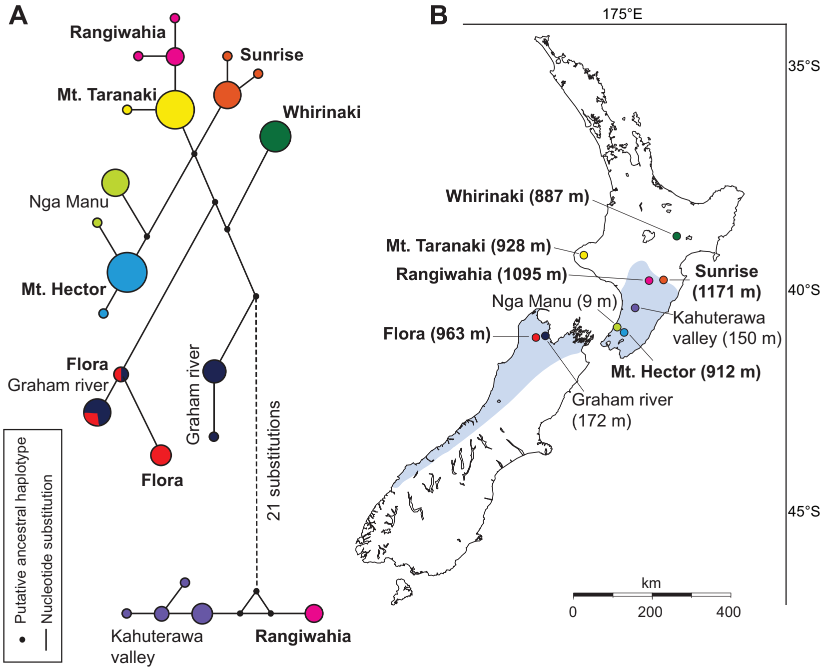

Fig. 1. (A) Genetic differentiation of populations of *Hemideina crassidens* tree weta presented by an unrooted parsimony network ($n = 92$) showing the relationships of mitochondrial DNA cytochrome *b* haplotypes. The areas of the circles are proportional to the number of haplotypes sampled (resampled from Bulgarella et al., 2014). Colours correspond to locations indicated on map. (B) New Zealand localities where *Hemideina crassidens* tree weta individuals were collected for this study. The shaded area indicates the reported range of this species (redrawn from Gibbs, 1998). High-elevation populations are shown in bold. As elevation increases, the mean annual temperature decreases. Individuals from Kahuterawa Valley and Mt. Taranaki were included in the morphological but not metabolic analyses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The oxygen levels in the testing cylinder did not drop below 19 kPa. The O_2 sensing system was calibrated for multiple temperatures and O_2 percentages by the manufacturer, and recalibrated after each reading using a double point calibration (0 and 21 vol% O_2). The environmental temperature was monitored continuously to ± 0.5 °C accuracy with a DS1921K Thermochron iButton (Maxim, San Jose, California). We did not observe condensation in the chamber after any run, suggesting that there was relatively little respiratory water loss under these conditions. All experiments were performed in Palmerston North, at 179 m a.s.l.

Partial pressure profiles from each completed run were used to calculate O_2 consumption rates for each individual. We discarded the first 15 min of each run as equilibration time (McGaughran et al., 2009). Rates of O_2 consumption (ml O_2/h) were calculated using a modification from Vleck's (1987) equation: $V(F_i - F_f)/(1 - F_f)t$ where V is the volume of air in the chamber, F_i and F_f are the initial and final O_2 lifetime, respectively, and t is the duration of the measurement period, expressed in hours (Nespolo et al., 2003).

To determine the thermal sensitivity of metabolic rate, as well as the effect of thermal acclimation on both metabolic rate and thermal sensitivity, we acclimated weta to 4 °C or 14 °C for a minimum of four and a maximum of eight weeks, and measured metabolic rates at both those temperatures after the acclimation period. We selected these temperatures based on current knowledge of the range of night temperatures in forest habitats at high and low elevation within the spatial range of this species (climate data from WorldClim database, Hijmans et al., 2005; Supplementary material, Table S1). After measurement, each weta was switched to the other acclimation temperature for at least four weeks and O_2 consumption again measured at 4 °C and 14 °C. The order of the acclimation temperatures was randomized for each individual.

We log-transformed the absolute oxygen consumption data prior to analysis. We used a mixed model analysis of covariance with body mass as the covariate. This analysis included 55 weta (43 acclimated to both temperatures, 11 weta that were only acclimated to 14 °C, and 1 weta acclimated to 4 °C only). For comparative purposes, the populations were pooled into high-elevation habitat (five locations; elevation range: 887–1171 m a.s.l.) and low-elevation habitat (two locations; elevation 9 and 172 m a.s.l.). For illustrative purposes we show oxygen consumption in graphs standardised to body mass, expressed in ml O_2/h/g live mass (mass-specific O_2 consumption).

To test if low temperature in high-elevation environments led to metabolic cold adaptation in those populations, we asked whether high-elevation populations had increased metabolic rates and increased sensitivity to temperature compared to low-elevation populations. We compared the metabolic rate-temperature dependence interaction plot of high-elevation weta with that of low-elevation weta at two different acclimation and test temperatures. Under MCA, we would predict that the SMR of high-elevation weta should be consistently higher than the SMR of low-elevation weta, regardless of acclimation conditions, and this pattern would result from genetic adaptation not plasticity.

We calculated the index of thermal sensitivity (Q_{10}) for each individual at the two different acclimation temperatures using the equation: $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$ (Schmidt-Nielsen, 1997), where R_1 is the rate at the lower temperature (T_1), and R_2 the rate at the higher temperature (T_2). We compared Q_{10} calculated after 4 °C acclimation versus the Q_{10} calculated after 14 °C acclimation and among sexes and elevations with matched-pair t tests ($n = 43$). Analyses were performed with R v.3.0.2 (R Development Core Team, 2014) and JMP v.10 (SAS Institute Inc., Cary, NC, USA) with a significance level of $p < 0.05$.

2.3. Morphological measurements

A male weta was considered adult when the cerci were curved and long enough for the tips to almost touch. In mature females, the ovipositor was strongly curved and the tip pointed with the upper valves distinctly longer than the lower valves (Gibbs, 1998). Juvenile individuals were those that had not yet reached their last (adult) instar. After collection, we measured tibia length and body mass weekly until weta reached adulthood. The right tibia was measured with digital callipers (model DSE, Dick Smith Electronics, New Zealand; accuracy ± 0.03 mm), except when only the left tibia was intact, when that was measured instead. We identified the occurrence of each moult by finding the shed cuticle in the container or by the pale soft appearance of the cuticle of a recently moulted individual.

To determine if high-elevation weta grew faster and reached a larger body size, we compared growth rates, adult body mass and tibia length data between high- and low-elevation populations and between sexes with ANOVA, after accounting for the data non-independence. We calculated the mean and standard deviation of tibia length for each instar. We also modelled the actual change in tibia length for each moult (in mm) and the rate of change in tibia length for each moult (after moult mean divided by pre-moult mean). Once weta had completed their final moult to adulthood, we compared tibia length and adult body mass in the high- versus the low-elevation populations using ANOVA. Mean ± standard errors are presented throughout.

3. Results

3.1. Genetics

The network of mtDNA haplotypes (Fig. 1) for the nine sampled populations in this study confirms that neither low- nor high-elevation populations comprised individuals from a single origin but each were genetically similar to geographically adjacent populations, independent of their elevation. Only the connected high- and low-elevation sites, Flora and Graham River, shared identical haplotypes. This pattern may have resulted from either remaining variation from an earlier more panmictic *H. crassidens* population that have not yet completed lineage sorting by geographic isolation, or from recent gene flow. In contrast, all other localities had uniquely derived (non-shared) haplotypes consistent with restricted gene flow among most populations.

3.2. Metabolic rates

Standard metabolic rate of tree weta was higher when measured at 14 °C than when measured at 4 °C, and increased with increasing body mass (Fig. 2). Oxygen consumption was significantly affected by test temperature, weta body mass, acclimation temperature and the interaction of body mass with acclimation temperature (Table 1). Mean metabolic rates did not differ statistically for males and females, and elevation did not have a significant effect on metabolic rate when accounting for body mass (Table 1), however, high-elevation weta were significantly larger than low-elevation weta (see below).

In both high-and low-elevation populations, cold acclimation (4 °C) decreased metabolic rate compared to warm-acclimated individuals (14 °C). Similarly, mean oxygen consumption was influenced by test temperature, with low oxygen consumption at low temperature and high oxygen consumption at higher temperature, for both acclimation temperatures (Figs. 3 and 4). Although not significant, males had slightly higher mean oxygen consumption than females (Fig. 3). Mean mass-specific metabolic rate for both sexes from each population is illustrated in Fig. S1. Contrary to the predictions of MCA, the SMR of high- and low-elevation weta did not differ significantly at either of the two test temperatures (Table 1 and Fig. 4).

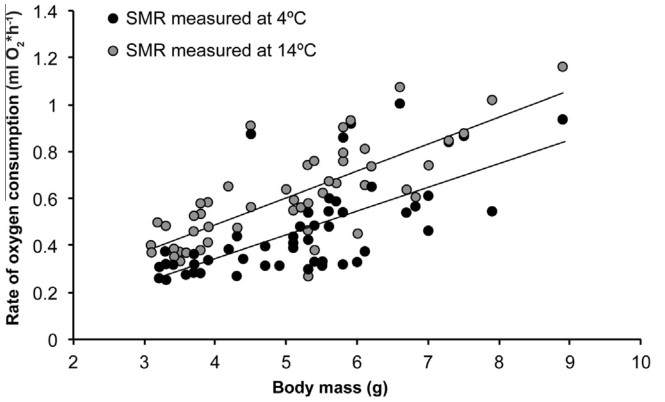

Fig. 2. There is a positive relationship between rate of oxygen consumption, measured at either 4 °C or 14 °C, and body mass in adult *Hemideina crassidens* tree weta, $n = 43$. This graph was obtained after 14 °C acclimation, but the relationship holds after acclimation to 4 °C.

Table 1
Mixed model analysis of variance of standard metabolic rate with body mass as the covariate for *Hemideina crassidens* tree weta comparing standard metabolic rate between sexes, elevations, acclimation and test temperatures. Values in bold are significant at the $p < 0.05$ level.

	Degrees of freedom	Sum of squares	*F*-ratio	Pr (>*F*)
Intercept	**1**	**134**	**436.83**	**<0.001**
Sex	1	51	0.40	0.534
Elevation (high or low)	1	51	1.48	0.229
Body mass (BM)	**1**	**134**	**24.53**	**<0.001**
Test temperature (TT)	**1**	**134**	**47.12**	**<0.001**
Temperature of acclimation (TA)	**1**	**134**	**25.53**	**<0.001**
Sex × elevation	1	51	0.28	0.594
BM × TT	1	134	0.07	0.784
BM × TA	**1**	**134**	**6.33**	**0.013**
TA × TT	1	134	0.61	0.435
BM × TT × TA	1	134	0.01	0.910

3.3. Thermal sensitivity of metabolic rate

Q_{10} was calculated only for those individuals that survived both acclimation periods ($n = 43$, 27 females, 16 males). The mean Q_{10} for weta after acclimation to 4 °C was 1.53 ± 0.06, significantly higher than the mean Q_{10} after acclimation to 14 °C (1.32 ± 0.05;

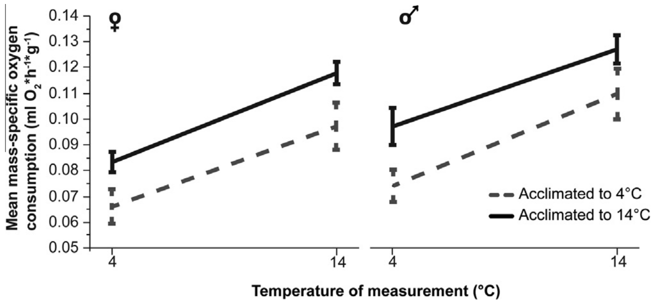

Fig. 3. Mean mass-specific oxygen consumption for adult male and female *Hemideina crassidens* tree weta after acclimation to 4 °C and 14 °C and when measured at 4 °C and 14 °C, respectively.

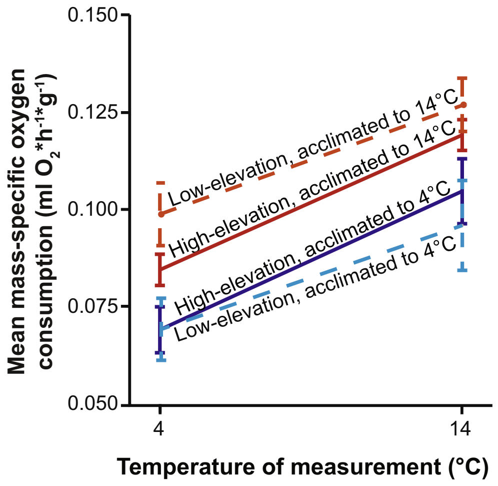

Fig. 4. Schematic representation of the metabolic rate-temperature dependence for *Hemideina crassidens* tree weta collected from high and low elevation, following acclimation to two temperatures (4 °C and 14 °C).

$t_{42} = 2.77$, $p < 0.01$). Q_{10} did not differ significantly between the sexes ($t_{42} = 0.1$, $p = 0.74$) or between high- and low-elevation populations ($t_{42} = 0.001$, $p = 0.98$).

3.4. Growth rate

We reared and measured a total of 138 weta (60 female and 78 male) collected as immature from six high-elevation sites and three low-elevation sites in common-garden conditions. Weta underwent 1–8 moults (mode = 2, Fig. S2) in the laboratory. Younger weta, in their earlier instars, grew faster and moulted more often than older weta, which had slower growth rates and longer intermoult intervals, evident from the duration of the periods between moults in the raw data (Fig. 5).

The number of weeks spent at each instar can differ from weta to weta and from instar to instar, as shown in the two examples in Fig. 5. Our modelling approach needed to reduce the impact that these differences have on the pattern of growth over time so that while measurements were taken each week, the analysis was applied on an instar-by-instar basis (see Supplementary material for whole model explanation). Our approach has the advantage of allowing for inclusion of additional variables and the discrete jump nature of the phenomenon being modelled; it also builds on the standard exponential growth pattern common in nature.

Tibia length increases from one moult to the next depend on the size of the weta previous to the moult. Moulting takes longer in later (larger) instars. As the weta grows in size, the amount by which tibia length changes increases as well. In each moult, tree weta get bigger (overall body mass and tibia length) by approximately 10–20%. Our analysis of growth rate (Table 2) included weta size as a variable to incorporate the variation in growth rate among instars. Growth rate also depended on the sex and the elevation at which weta were collected; females grew faster than males, following different trajectories. High-elevation weta grew faster than low-elevation individuals (Table 2 and Fig. 6).

3.5. Adult size

A total of 98 individuals grew to adulthood. Adult body mass differed significantly between high- and low-elevation populations and between the sexes (Table 3). Similarly, final tibia length was significantly different between high and low-elevation localities and between the sexes (Table 3). Male and female high-elevation weta had longer tibia measurements than low-elevation male and female weta, respectively, when reared under common environmental conditions (Fig. 7).

In summary, high-elevation weta had larger mean adult body mass and longer mean final tibia length than low-elevation individuals, and females grew faster to a larger body size than males (Fig. S3). On average, male weta from the Kahuterawa valley (150 m a.s.l) had the lowest mean adult body mass (2.80 g) and tibia length (19.66 mm) whereas females from Mt. Hector (912 m a.s.l) had the highest mean adult body mass (7.18 g) and females from Rangiwahia (1095 m a.s.l) the longest mean adult tibia (24.37 mm).

4. Discussion

We compared multiple low- and high-elevation populations of *H. crassidens* to test whether high-elevation weta were metabolically adapted to colder conditions, and if constant rearing temperatures eliminated body size variation. We found no support for Metabolic Cold Adaptation, but weta collected from high-elevation populations grew faster to larger sizes than low-elevation weta when reared under common-garden conditions, supporting population differentiation in growth rates.

We found a significant positive effect of adult acclimation on metabolic rate following low- and high-temperature exposure. Mass-specific oxygen consumption was lower following low-temperature acclimation than after higher-temperature acclimation, and in all cases, it was lower when measured at 4 °C than when measured at 14 °C. Standard metabolic rate also increased with test temperature and body size but it did not vary significantly among high- and low-elevation populations.

To compare our results to published values for insects, we converted the metabolic rate estimates to microwatts, assuming a RQ = 0.84 (mixed diet), and corrected to 25 °C using our calculated Q_{10} values and a scaling exponent of 0.75. *H. crassidens* had a mean SMR of 593.65 (± 65.5) μW/g, lower than the average value reported for Gryllidae (753 μW/g, measured using closed-system respirometry; Chown et al., 2007) and for Acrididae (1768 μW/g, Chown et al., 2007). For example, *Gryllus bimaculatus* crickets had a mean SMR of 899.5 μW/g (Sinclair et al., 2011) and 2200 μW/g for *Gryllus lineaticeps* crickets (Hoback and Wagner, 1997). The relatively low SMR of *H. crassidens* might be explained by their suite of life history traits; they are among the largest Orthoptera, long-lived, wingless, nocturnal and herbivorous. Flying, in particular, is associated with elevated SMR (Reinhold, 1999; Addo-Bediako et al., 2002). Whether the long apterous history of this group (Pratt et al., 2008) might have led to selection for low metabolic rates or the long-term selection for low-energy lifestyles led to the evolution of aptery is not known.

Tree weta have a relatively long lifespan as they take one year to reach maturity and live at least one year as adults (Morgan-Richards, 2000; Kelly, 2006). According to the 'rate of living theory', high mass-specific metabolic rate is associated with short lifespan (Rubner, 1908; Pearl, 1928). Although an appealing generalisation, recent research demonstrates that the relationship between metabolic rate, oxidative damage and lifespan is a highly complex one (Niitepõld and Hanski, 2013). We used closed-system respirometry, which often results in higher metabolic rate estimates than open-flow respirometry (Lighton and Fielden, 1995; Addo-Bediako et al., 2002), so this does not explain our results. Thus, the low SMR of tree weta is in keeping with our understanding of the metabolic requirements of a large, flightless, plant-eating orthopteran.

The metabolic cold adaptation hypothesis states that individuals from cold climates have elevated metabolism compared to individuals from warmer climates, when measured at the same

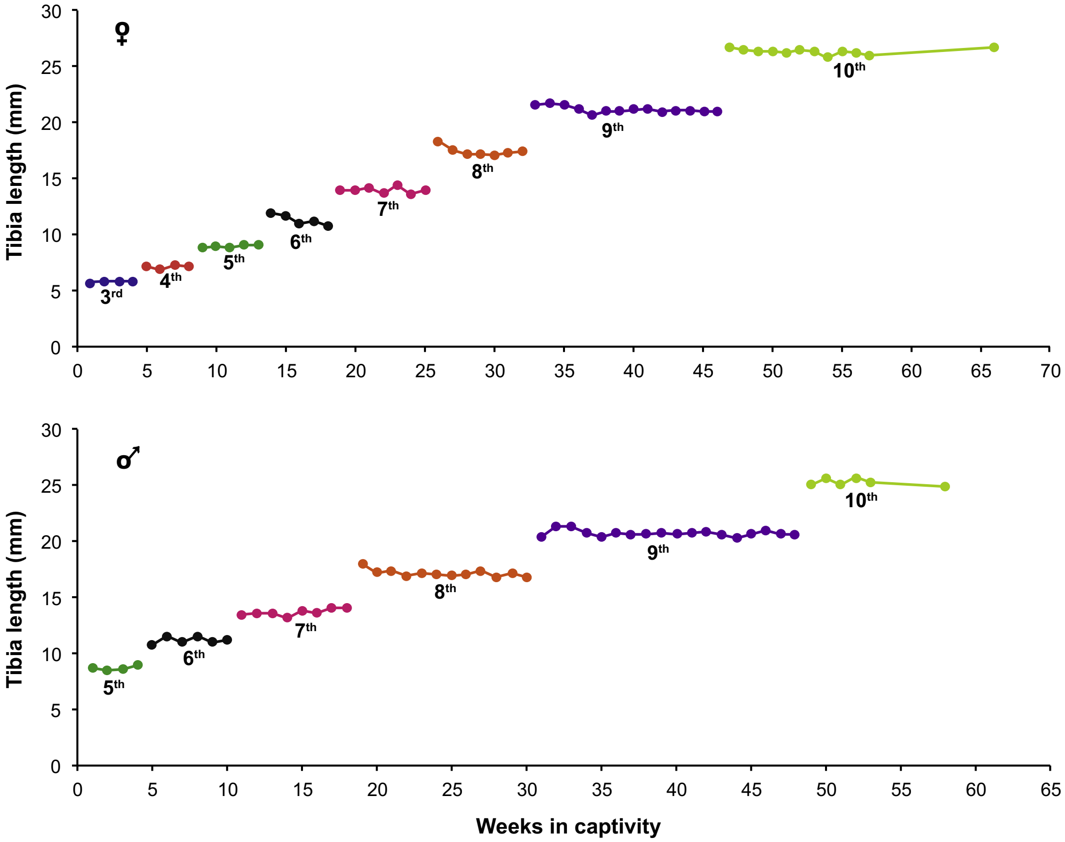

Fig. 5. Growth of *Hemideina crassidens* tree weta recorded by hind tibia length measured weekly, female (top) and male (bottom), each collected from a high-elevation location and maintained under constant conditions. There is variability in time spent within each instar with the measurement error being minor compared to the 'jumps' between moults. The numbers under the lines indicate the instar.

Table 2
Analysis of variance to explain variation in growth rate of *Hemideina crassidens* tree weta in captivity (hind-tibia length). Values in bold are significant at the $p < 0.05$ level.

	Degrees of freedom	Sum of squares	*F*-ratio	Pr (>*F*)
Last mean	**1**	**0.78**	**27.78**	**<0.001**
Sex	**1**	**0.40**	**14.32**	**<0.001**
Elevation	**1**	**0.13**	**4.53**	**0.034**
Last mean ∗ sex	**1**	**0.31**	**10.87**	**0.001**
Last mean ∗ elevation	1	0.00	0.00	0.972
Sex ∗ elevation	**1**	**0.22**	**7.65**	**<0.001**
Last Mean ∗ sex ∗ elevation	1	0.02	0.58	0.446

trial temperature. Most intraspecific studies of MCA lack information about population differentiation (it might indicate the direction of evolved trait changes), and fail to examine whether phenotypic plasticity could explain the observed patterns (Clarke, 1991, 1993; Terblanche et al., 2009). In our study, mitochondrial DNA showed that gene flow is low enough to allow for population differentiation with the possible exception of one pair of populations continuously distributed from low to high elevation (Graham river with Flora; Fig. 1) that shared haplotypes. However, our results demonstrated a lack of differences in the energetic costs of maintenance (measured as SMR) between high- and low-elevation populations of the Wellington tree weta. Phenotypic plasticity seems to be playing a more important role in determining the metabolic functioning of high-elevation tree weta than genetic adaptation does. In fact, our results are in agreement with the inverse cold acclimation phenomenon, in which animals acclimated to low temperatures gradually decrease metabolism further than the initial Q_{10} effect (Hazel and Prosser, 1970). The absence of differences in SMR among tree weta populations could indicate that they all need similar amounts of

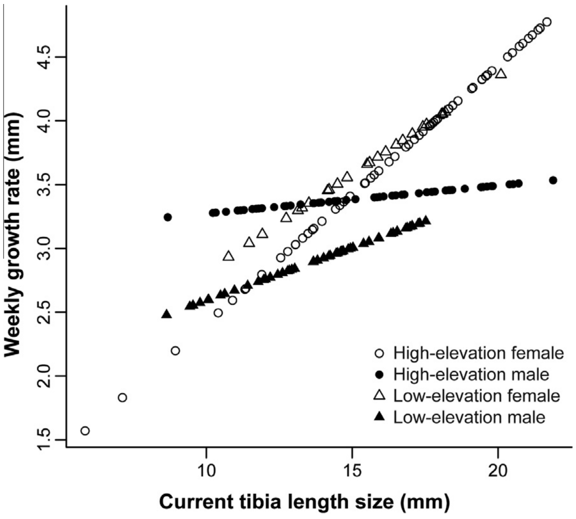

Fig. 6. Growth rate variation of tree weta *Hemideina crassidens* as measured by the weekly change in hind tibia length (predicted change in tibia length over the coming week; mm) versus the mean current size of the tibia length (mm) for female and male weta from high- and low-elevations raised in captivity at constant temperatures. Data are derived from the model explained in the Supplementary material.

Table 3
Analysis of variance for mean adult body mass and mean adult tibia length measurements shows significant differences between high and low-elevation and between male and female *Hemideina crassidens* tree weta reared at 14 °C. Values in bold are significant at the $p < 0.05$ level.

Source	Degrees of freedom	Sum of squares	*F*-ratio	*p* Values
Adult body mass				
Elevation (high vs. low)	**1**	**31.13**	**19.39**	**<0.001**
Sex	**1**	**14.15**	**8.81**	**0.003**
Adult tibia length				
Elevation (high vs. low)	**1**	**104.30**	**37.47**	**<0.001**
Sex	**1**	**34.01**	**12.22**	**<0.001**

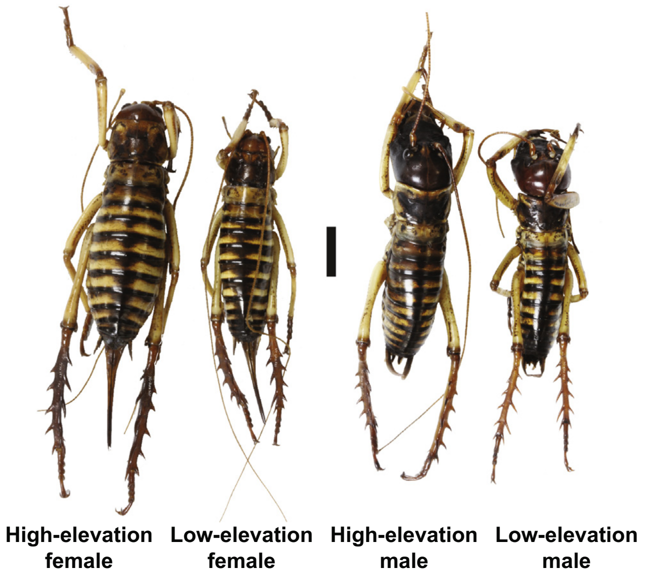

Fig. 7. Adult tree weta *Hemideina crassidens* from high-elevation are larger than conspecifics from low-elevation (raised in captivity at a constant temperature). Scale bar = 10 mm.

energy for maintenance during physical inactivity (Lindgren and Laurila, 2009; Gaitán-Espitía and Nespolo, 2014).

H. crassidens presented low Q_{10} values that might be related to their nocturnal habits. Tree weta are active and forage at night when microclimate temperatures are cooler and more homogeneous than during the day. Thus, there may have been selection for reduced thermal sensitivity to preserve a relatively high nocturnal metabolic rate (cf. Chappell, 1983).

Body size and mass influence nearly all aspects of biology. They determine fitness and are targets of natural selection (Whitman, 2008). We found strong differentiation in body size and growth rate between *H. crassidens* from high- and low-elevations and between the sexes. Growth rate was faster in females than males and also faster in high-elevation than low-elevation populations, at constant temperature. Because we collected weta as juveniles, we were unable to control for maternal effects, such as egg size (high-elevation weta might be larger because high-elevation females lay larger eggs). However, five high-elevation tree weta populations are genetically isolated from each other and from the low-elevation populations, yet we saw a consistent pattern of increased growth rate and size at high elevations. This increase in growth rate and size with increasing elevations is predicted by theory (Chown and Gaston, 2010), but because we cannot disentangle genetic adaptation from the plastic and epigenetic effects of early development in the different environments, we cannot currently conclude that these differences are a result of local adaptation to the elevational environment. This is a common problem when working with field-collected animals (Kawecki and Ebert, 2004; Williams et al., 2012).

Although we detected significant variation in growth rate between males and females, resting metabolic rate of adult weta did not differ significantly between sexes. Growth is determined by rates of nutrient acquisition, assimilation and allocation. Ectotherms can alter their rates of growth using diverse mechanisms involving particular trade-offs (e.g., Bayne, 2004; Angilletta et al., 2004). Female *H. crassidens* have larger bodies (tibia length and mass) whereas male have bigger heads (Kelly, 2005). Males might be investing more energy in mandible growth rather than in rapid tibia growth. Also, sexual dimorphism in size might reflect the need for females to produce and store large numbers of eggs as documented in grasshoppers (Uvarov, 1966).

In a similar pattern, high-elevation weta grew faster than low-elevation weta but when adult their resting metabolic rates did not differ significantly. This suggests that low-elevation weta may trade-off growth rate for other aspects of behaviour or development. We found that the weta collected from high-elevation locations grew into larger individuals than the low-elevation weta, when raised at identical temperatures. Thus, tree weta might thrive in the harsher high-elevation environment if, for example, they had a higher capacity to convert ingested energy into biomass than low-elevation tree weta (adaptive variation in growth efficiency). If this is the case, then high-elevation populations would not exhibit elevated metabolism, just as observed in this study. Instead, in order to maintain high growth rate, high-elevation weta may consume more food, have a higher capacity for conversion to biomass or a combination of both mechanisms (Present and Conover, 1992; Billerbeck et al., 2000; Lindgren and Laurila, 2005, 2009; Fielding and Defoliart, 2007). However, preliminary analysis has found no evidence for variation in bulk feeding rate between two of these populations (McKean and Morgan-Richards, unpubl. obs.). Alternatively, absorption efficiency or population-diet interactions may modify growth rate, and this would be an interesting topic for further investigation in this and other comparisons of body size at contrasting elevations.

Climate warming has profound implications for the future distribution and abundance of insects (Hodkinson, 2005). Population differentiation within a species' range can provide genetic diversity that could be useful in responding to changing conditions (Bridle and Vines, 2007). *H. crassidens* populations are genetically diverse (at least at the mtDNA level) and conspecific patterns of genetic variation have been used to infer range shifts due to past climate change (Bulgarella et al., 2014). We have shown here that this weta also has considerable intraspecific variation in size and growth rates, that in concert with range shifts, might allow the species to keep pace with climate change.

5. Conclusion

The energetic maintenance cost of an organism determines the amount of energy available towards activity, growth, and reproduction. Our results suggest that high- and low-elevation populations of the Wellington tree weta have equal maintenance metabolism but high-elevation weta have increased growth rates and larger final size. There is differentiation between tree weta populations and, in concert with genetic diversity; this variation might facilitate adaptation to changing conditions.

Acknowledgements

Thanks to the weta hunters: Dylan Anderson, Robyn Dewhurst, Natasha McKean, Alejandra Oyanedel and Briar Smith. The helpful

IAE technicians: Paul Barrett, Tracy Harris, Shaun Nielsen and Cleland Wallace. Karen Smillie from Naturalac Nutrition Ltd (Auckland, New Zealand) donated the protein we used to supplement the diet of our captive weta. Dr. Gabe Redding helped set up the respirometry experiments. Gareth Boyt and Pete Shaw allowed and helped us to collect weta at Pohokura, and Rhys Mills allowed us to sample in the Nga Manu Nature Reserve. Milan Plećaš helped obtain the climate data. The following offices and staff of the New Zealand Department of Conservation helped us: Gareth Boyt (Rangataiki), Jeanine Bishop (Wellington), Beverley Freer and Ian Millar (Nelson Malborough), and Robyn Ellis (Tongariro Wanganui Taranaki). We are grateful to several anonymous referees for critical comments on an earlier version of the manuscript. This work was supported by a Massey University Research Grant (MURF–2010: What limits a weta?) and the Royal Society of New Zealand (Marsden PVT–601).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2015.02.012.

References

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. Funct. Ecol. 16, 332–338.

Angilletta Jr., M.J., Dunham, A.E., 2003. The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. Am. Nat. 162, 332–342.

Angilletta Jr., M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249–268.

Angilletta Jr., M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate and body size in ectotherms: fitting pieces of a life history puzzle. Integr. Comp. Biol. 44, 498–509.

Arendt, J.D., 1997. Adaptive growth rates: an integration across taxa. Quart. Rev. Biol. 72, 149–177.

Ashby, P.D., 1997. Conservation of mass-specific metabolic rate among high- and low-elevation populations of the acridid grasshopper *Xanthippus corallipes*. Phys. Zool. 70, 701–711.

Asher, G.W. 1977. Ecological aspects of the common tree weta (*Hemideina thoracica*) in native vegetation. Ecological Division Report, Lower Hutt, New Zealand.

Ashton, K.G., 2002. Do amphibians follow Bergmann's rule? Can. J. Zool. 80, 708–716.

Ashton, K.G., Feldman, C.R., 2003. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57, 1151–1163.

Atkinson, D., 1994. Temperature and organism size – a biological law for ectotherms? In: Begon, M., Fitter, A.H. (Eds.), Advances in Ecological Research, vol. 25. Academic Press, Oxford, pp. 1–58.

Bandelt, H.-J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Bayne, B.L., 2004. Phenotypic flexibility and physiological trade-offs in the feeding and growth of marine bivalve molluscs. Integr. Comp. Biol. 44, 425–432.

Berrigan, D., Charnov, E.L., 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70, 474–478.

Billerbeck, J.M., Schultz, E.T., Conover, D.O., 2000. Adaptive variation in energy acquisition and allocation among latitudinal populations of the Atlantic silverside. Oecologia 122, 210–219.

Bridle, J., Vines, T.H., 2007. Limits to evolution at range margins: when and why does adaptation fail? Trends Ecol. Evol. 22, 140–147.

Buckley, T.R., Krosch, M., Leschen, R.A.B., 2014. Evolution of New Zealand insects: summary and prospectus for future research. Aust. Entomol. 54, 1–27.

Bulgarella, M., Trewick, S.A., Minards, N.A., Jacobson, M.J., Morgan-Richards, M., 2014. Shifting ranges of two tree weta species (*Hemideina* spp.): competitive exclusion and changing climate. J. Biogeogr. 41, 524–535.

Chappell, M.A., 1983. Metabolism and thermoregulation in desert and montane grasshoppers. Oecologia 56, 126–131.

Chown, S.L., Gaston, K.J., 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. Biol. Rev. 74, 87–120.

Chown, S.L., Gaston, K.J., 2010. Body size variation in insects: a macroecological perspective. Biol. Rev. 85, 139–169.

Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press, Oxford.

Chown, S.L., van der Merwe, M., Smith, V.R., 1997. The influence of habitat and altitude on oxygen uptake in sub-Antarctic weevils. Phys. Zool. 70, 116–124.

Chown, S.L., Marais, E., Terblanche, J.S., Klok, C.J., Lighton, J.R.B., Blackburn, T.M., 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Funct. Ecol. 21, 282–290.

Clarke, A., 1991. What is cold adaptation and how should we measure it? Am. Zool. 31, 81–92.

Clarke, A., 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? Funct. Ecol. 7, 139–149.

Conover, D.O., Schultz, E.T., 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol. Evol. 10, 248–252.

Dewhurst, R. 2012. The diet of tree weta: natural and captive folivory preferences of *Hemideina crassidens* and *Hemideina thoracica*. MSc Thesis, Massey University, Palmerston North, New Zealand.

Endler, J.A., 1977. Geographic Variation, Speciation, and Clines. Princeton University Press, p. 246.

Field, L.H., Deans, N.A., 2001. Sexual selection and secondary sexual characters of weta and king crickets. In: Field, L.H. (Ed.), The Biology of Wetas, King Crickets and Their Allies. CAB International, Wallingford, pp. 179–204.

Fielding, D.J., Defoliart, L.S., 2007. Growth, development, and nutritional physiology of grasshoppers from Subantarctic and temperate regions. Physiol. Biochem. Zool. 80, 607–618.

Gaitán-Espitía, J.D., Nespolo, R., 2014. Is there metabolic cold adaptation in terrestrial ectotherms? Exploring latitudinal compensation in the invasive snail *Cornu aspersum*. J. Exp. Biol. 217, 2261–2267.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., et al., 2009. Macrophysiology: a conceptual reunification. Am. Nat. 174, 595–612.

Gibbs, G., 1998. New Zealand Weta. The Reed Species Guide. Raupo Publishing, Auckland, New Zealand.

Griffin, M., Morgan-Richards, M., Trewick, S.A., 2011. Is the tree weta *Hemideina crassidens* an obligate herbivore? N. Z. Nat. Sci. 36, 11–19.

Hadley, N.F., Massion, D.D., 1985. Oxygen consumption, water loss and cuticular lipids of high and low elevation populations of the grasshopper *Aeropedellus clavatus* (Orthoptera: Acrididae). Comp. Biochem. Physiol. Part A 80, 307–311.

Hazel, J., Prosser, C.L., 1970. Interpretation of inverse acclimation to temperature. Z. Vgl. Physiol. 67, 217–228.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25, 1965–1978.

Hoback, W.W., Wagner, W.E., 1997. The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. Physiol. Entomol. 22, 286–290.

Hodkinson, I.D., 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. Biol. Rev. 80, 489–513.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7, 1225–1241.

Kelly, C.D., 2005. Allometry and sexual selection of male weaponry in Wellington tree weta, *Hemideina crassidens*. Behav. Ecol. 16, 145–152.

Kelly, C.D., 2006. Resource quality or harem size: what influences male tenure at refuge sites in tree weta (Orthoptera: Anostostomatidae)? Behav. Ecol. Sociobiol. 60, 175–183.

Kennington, G.S., 1957. Influence of altitude and temperature upon rate of oxygen consumption of *Tribolium confusum* Duval and *Camponotus pennsylvanicus modoc* Wheeler. Physiol. Zool. 30, 305–314.

Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, New York.

Lighton, J.R.B., Fielden, L.J., 1995. Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. Physiol. Zool. 68, 43–62.

Lindgren, B., Laurila, A., 2005. Proximate causes of adaptive growth rates: growth efficiency variation among latitudinal populations of *Rana temporaria*. J. Evol. Biol. 18, 820–828.

Lindgren, B., Laurila, A., 2009. Physiological variation along a geographical gradient: is growth rate correlated with routine metabolic rate in *Rana temporaria* tadpoles? Biol. J. Linn. Soc. 98, 217–224.

Mayr, E., 1963. Animal Species and Evolution. Belknap Press, Cambridge, Massachusetts.

McGaughran, A., Redding, G.P., Stevens, M.I., Convey, P., 2009. Temporal metabolic rate variation in a continental Antarctic springtail. J. Insect Physiol. 55, 130–135.

Minards, N.A., Trewick, S.A., Godfrey, A.J.R., Morgan-Richards, M., 2014. Convergent local adaptation in size and growth rate but not metabolic rate in a pair of parapatric Orthoptera species. Biol. J. Linn. Soc. 113, 123–135.

Morgan-Richards, M., 2000. Robertsonian translocations and B chromosomes in the Wellington tree weta, *Hemideina crassidens* (Orthoptera: Anostostomatidae). Hereditas 132, 49–54.

Nespolo, R.F., Lardies, M.A., Bozinovic, F., 2003. Intrapopulational variation in the standard metabolic rate of insects: repeatability, thermal dependence and sensitivity (Q_{10}) of oxygen consumption in a cricket. J. Exp. Biol. 206, 4309–4315.

Niitepõld, K., Hanski, I., 2013. A long life in the fast lane: positive association between peak metabolic rate and lifespan in a butterfly. J. Exp. Biol. 216, 1388–1397.

Nylund, L., 1991. Metabolic rates of *Calathus melanocephalus* (L.) (Coleoptera: Carabidae) from alpine and lowland habitats, Jeloy and Fines, Norway and Drenthe, the Netherlands. Comp. Biochem. Physiol. Part A 100, 853–862.

Partridge, L., French, V., 1996. Thermal evolution of ectotherm body size: why get big in the cold? In: Johnston, I.A., Bennett, A.F. (Eds.), Animals and Temperature. Cambridge University Press, Phenotypic and Evolutionary Adaptation, pp. 265–292.

Pearl, R., 1928. The Rate of Living. Knopf, New York.

Pratt, R.C., Morgan-Richards, M., Trewick, S.A., 2008. Diversification of New Zealand weta (Orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia. Philos. Trans. R. Soc. Lond. 363, 3427–3438.

Present, T.M.C., Conover, D.O., 1992. Physiological basis of latitudinal growth differences in *Menidia menidia*: variation in consumption or efficiency? Funct. Ecol. 6, 23–31.

R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2014. URL <http://www.R-project.org>.

Reinhold, K., 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. Funct. Ecol. 13, 217–224.

Rezende, E.L., Gomes, F.R., Ghalambor, C.K., Russell, G.A., Chappell, M.A., 2005. An evolutionary frame of work to study physiological adaptation to high altitudes. Rev. Chil. Hist. Nat. 78, 323–336.

Rubner, M., 1908. Das Problem der Lebensdauer und Seine Beziehungen zu Wachstum und Ernährung. R. Oldenbourg, Munich.

Schmidt-Nielsen, K., 1997. Animal Physiology: Adaptation and Environment, fifth ed. Cambridge University Press.

Sinclair, B.J., Worland, M.R., Wharton, D.A., 1999. Ice nucleation and freezing tolerance in New Zealand alpine and lowland weta, *Hemideina* spp. (Orthoptera; Stenopelmatidae). Physiol. Entomol. 24, 56–63.

Sinclair, B.J., Bretman, A., Tregenza, T., Tomkins, J.L., Hosken, D.J., 2011. Metabolic rate does not decrease with starvation in *Gryllus bimaculatus* when changing fuel use is taken into account. Physiol. Entomol. 36, 84–89.

Stearns, S.C., 1992. The Evolution of Life Histories. Oxford University Press, Oxford.

Tattersall, G.J., Sinclair, B.J., Withers, P.C., Fields, P.A., Seebacher, F., Cooper, C.E., Maloney, S.K., 2012. Coping with thermal challenges: physiological adaptations to environmental temperatures. Comp. Physiol. 2, 2151–2202.

Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Van Vuuren, B.J., Chown, S.L., 2009. Directional evolution of the slope of the metabolic rate-temperature relationship is correlated with climate. Physiol. Biochem. Zool. 82, 495–503.

Trewick, S.A., Bland, K., 2012. Fire and slice: palaeogeography for biogeography at New Zealand's North Island/South Island juncture. J. R. Soc. N. Z. 42, 153–183.

Trewick, S.A., Morgan-Richards, M., 1995. On the distribution of tree weta in the North Island, New Zealand. J. R. Soc. N. Z. 25, 485–493.

Trewick, S.A., Morgan-Richards, M., 2014. New Zealand Wild Life. Penguin, Auckland.

Uvarov, B., 1966. Grasshoppers and Locusts, vols. 1–2. Cambridge University Press, London.

Vleck, D., 1987. Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. J. Appl. Physiol. 62, 2103–2106.

Wallis, G.P., Trewick, S.A., 2009. New Zealand phylogeography: evolution on a small continent. Mol. Ecol. 18, 3548–3580.

Wehi, P.M., Raubenheimer, D., Morgan-Richards, M., 2013. Tolerance for nutrient imbalance in an intermittently feeding herbivorous cricket, the Wellington tree weta. PLoS ONE 8, e84641. http://dx.doi.org/10.1371/journal.pone.0084641.

Wharton, D.A., 2011. Cold tolerance of New Zealand alpine insects. J. Insect Physiol. 57, 1090–1095.

Whitman, D.W., 2008. The significance of body size in the Orthoptera: a review. J. Orthoptera Res. 17, 117–134.

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 suppression in an overwintering butterfly. PLoS ONE 7, e34470. http://dx.doi.org/10.1371/journal.pone.0034470.