Elevational clines in the temperature dependence of insect performance and implications for ecological responses to climate change

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To what extent is insect hopping and feeding performance, which constrains the ability to obtain and assimilate resources, thermally adapted along an elevation gradient? Does temperature dependence vary between populations and species and can differences account for individualistic responses to past climate change? We investigate these questions for three species of grasshoppers along a Rocky Mountain elevation gradient. All species and populations exhibit warm adaptation for consumption and digestion, with only modest inter- and intra-specific differences. Species differ substantially in the temperature of peak hopping performance. Low-elevation populations of the warm-adapted species exhibit the highest performance at high temperatures and the lowest performance at low temperatures. Developmental plasticity influences the temperature dependence of performance; grasshoppers reared at higher temperatures perform better at higher temperatures and possess broader thermal tolerance. We fitted thermal performance curves to examine whether performance shifts can account for changes in abundance between initial surveys in 1958–1960 and recent surveys since 2006. All species and populations are able to achieve greater feeding rates now. Estimated shifts in hopping performance vary between species and along the elevation gradient. The cool-adapted species has experienced declines in hopping performance, particularly at the lower elevation sites, while the warm-adapted species has experienced increases in performance concentrated at higher elevations. These estimated performance shifts broadly concur with observed abundance shifts. Performance metrics may have a greater potential to elucidate differential responses to climate change between populations and species than coarser and oft-used proxies, such as thermal tolerance. Assessing performance directly when temperature dependence varies between processes such as the acquisition and assimilation of energy may be essential to understanding population- and species-level impacts.

Key words: Feeding rate, grasshopper, local adaptation, locomotion, plasticity, thermal performance curve

Introduction

Environmental temperature can constrain ectothermic organisms by limiting their ability to locate, gather, consume and assimilate resources. These processes follow a hump-shaped rate function (thermal performance curve, TPC), with performance being optimal at an intermediate body temperature (Huey and Kingsolver, 1989). Local adaptation in TPCs has
been widely observed along elevation and latitudinal gradients (Angilletta, 2009; Kingsolver, 2009), particularly for insects (Hodkinson, 2005). This dependence of performance and fitness on temperature has been used effectively as a basis for understanding and predicting the ecological impacts of climate change on populations and species. For example, Huey et al. (2009) predicted declining performance for a cool-adapted tropical lizard in response to climate warming over recent decades. Related analyses suggest that restrictions in lizard performance and activity duration associated with climate change drive extinctions (Sinervo et al., 2010) and range shifts (Buckley et al., 2010; Kearney, 2012).

Latitudinal and altitudinal patterns of thermal specialization and adaptation shape sensitivity to climate change. Deutsch et al. (2008) predicted that detrimental effects of climate shifts would be most intense in tropical species with narrow thermal specialization, despite the lesser magnitude of climate change experienced. Subsequent analyses suggest that more variable temperate climates may increase the incidence of thermal stress events and equalize the impacts of climate change across latitudes (Kingsolver et al., 2013). Acclimatization and adaptation of temperature-dependent performance traits is important for understanding future responses to warming climates (Hofmann and Todgham, 2010) and identifying species most vulnerable to change (Huey et al., 2012).

Many analyses of the potential impacts of climate change rely on physiological metrics that are easy to measure, such as critical thermal minima and maxima (CT_{min} and CT_{max}); however, the body temperatures of organisms rarely approach these extreme temperatures. Researchers often use CT_{min} and CT_{max} to estimate TPCs (Angilletta, 2009) and thus responses to more ecologically relevant temperatures, but the ecological significance of CT_{min} and CT_{max} remains questionable.

Phenological and abundance shifts by grasshoppers along a 2000 m elevation gradient near Boulder (CO, USA) in response to climate change over the last 50 years have varied in extent and direction among populations and species (Nufio et al., 2010). Thermal tolerances, thermal preferences and metabolic rate responses varied among species, but inter-population differences were insufficient to account for the individualistic responses to past climate change (Buckley et al., 2013b). Grasshoppers, however, exhibit a pronounced temperature dependence in hopping activity (Harrison et al., 1991) and feeding behaviours (Harrison and Fewell, 1995). Thus, in the present study we test whether TPCs for hopping and feeding differ within and among three species along the above elevation gradient and then consider whether these performance measures can account for the individualistic responses to observed climate change. This addresses whether more refined performance metrics offer greater promise for understanding differential responses to climate change than cruder thermal tolerance metrics.

Finally, we examine the influence of developmental plasticity in shaping TPCs by rearing grasshoppers in a range of constant temperatures. Thermal performance curve are more likely to be developmentally plastic when environmental conductions during development are indicative of those experienced later (Kingsolver and Huey, 1998). Plasticity in thermal traits along elevation gradients are likely to be complex, because high-altitude sites, while generally colder, are more variable, with high levels of radiation producing short periods of high body temperatures (Buckley et al., 2013a). Mean environmental conditions and extreme events combine to influence the position, breadth and shape of TPCs (Schulte et al., 2011). We examine developmental plasticity in TPCs, which provides insight into whether acclimatization of TPCs will contribute to responses to thermal stress.

### Materials and methods

#### Study organisms, sites and rearing

We examined three grasshopper species that are expected to differ in their exposure to climate change and in their potential for local adaptation (Table 1). Melanoplus boulderensis (part of the Melanoplus dodgei species complex; Otte, 2012) is a short-winged species with limited dispersal and high potential for local adaptation. Camnula pellucida and Melanoplus sanguinipes are, in contrast, long-winged species with a higher dispersal capacity and a greater potential for gene flow across populations. All species occur from the upper foothills to the subalpine. Melanoplus sanguinipes and C. pellucida are broadly distributed geographically, while

| Species               | Dispersive | Thermal adaptation | Seasonal timing | Phenology (↑ earlier) | Abundance |
|-----------------------|------------|--------------------|-----------------|-----------------------|-----------|
|                       |            |                    |                 | 2195 m 2591 m 3048 m  |           |
| Melanoplus boulderensis | No         | Cool               | Early           | ↓ ↓ ≈               | ↓ ↓ ≈    |
| Camnula pellucida      | Yes        | Warm               | Middle          | ↑ ↓ ↓               | ↓ ↓ ↑    |
| Melanoplus sanguinipes | Yes        | Generalist         | Late            | ↑ ↑ a               | ↓ ↓ a    |

*Low abundance during initial time period prevents comparison.*
M. boulderensis is thought to have evolved in the Rocky Mountains within the last million years (Knowles and Otte, 2000). Melanoplus boulderensis and C. pellucida tend to be cool and warm adapted, respectively, whereas M. sanguinipes is a thermal generalist (Buckley et al., 2013b). We previously delineated cool and warm adaptation based on critical thermal limits on activity and preferred body temperatures using field-collected individuals, so we have been unable to exclude developmental and maternal effects. Although maternal effects have been observed for Melanoplus species via photoperiod influencing the depth of embryonic diapause (Dean, 1982), we are unaware of documented maternal effects in Melanoplus relevant to the thermal metrics we investigated in the present study. Here we present thermal tolerance and performance metrics for laboratory-reared individuals of M. sanguinipes. Similarities in these metrics between field-collected and laboratory-reared individuals support the hypothesis of local adaptation across species, but further work to partition genetic and plastic effects on phenotypes will be required to establish local adaptation. All three species consume a variety of forbes and grasses. 

We examined variation in thermal performance curves among populations and species across five sites along the following elevation gradient: Red Fox (1574 m, 40.05N, 105.19W), A1 (2195 m, 40.01N, 105.37W), B1 (2591 m, 40.02N, 105.43W), C1 (3048 m, 40.03N, 105.55W) and D1 (3739 m, 40.06N, 105.62W; Alexander and Hilliard, 1969; descriptions can be found at http://niwot.colorado.edu/site_info/site_info.html). The sites are grassy clearings associated with upper prairie, foothill, montane, subalpine and alpine life zones, respectively. Adults from populations at each site were kept separately with access to organic romaine lettuce and wheat bran and acclimated overnight in an incubator to an “intermediate” temperature of 25°C. Individuals were then transferred to polyurethane containers for assessment of their performance and feeding rate. When not undergoing tests they were maintained in the same conditions described above.

In order to examine how plasticity influences the temperature dependence of performance, we repeated the performance assessments for M. sanguinipes reared at constant 24 or 27°C and a 14 h–10 h light–dark cycle in Percival I-36VL incubators. We reared the other species and, additionally, M. sanguinipes at 18 and 30°C, but low hatching, development or survival rates prevented analysis. Eggs were collected the previous summer by allowing females to oviposit in damp sand and then sieving the sand to collect the egg pods. Egg pods were then placed in damp vermiculite within condiment containers. The surface was periodically coated with 0.25% methyl-p-hydroxy benzoate to inhibit fungal or microbial growth. The eggs were allowed to develop for 3 weeks at 25–30°C and were then stored at 2°C for ~110 days to enable diapause. The egg containers were subsequently placed in a 24 or 27°C incubator. Upon hatching, the egg containers were placed within rectangular 2.25 l polyurethane containers, and cooled or heated in a water bath. Given that warming rates

**Temperature dependence of performance**

Grasshoppers were fasted prior to each feeding trial for 12 h, a sufficient period to complete digestion and absorption (Harrison and Fewell, 1995), and provided with a damp paper towel for humidity. The feeding trials were conducted at six temperatures (15, 20, 25, 30, 35 and 40°C; temperature range after Harrison and Fewell, 1995). Trials at two temperatures were run concurrently each day, with individuals from each population randomly partitioned between the two temperatures. We assessed performance for an average of 20 individuals from each population (range 14–22). The order of temperature trials was randomized. Grasshoppers were acclimated to the test temperature for 1 h prior to being provided with organic, baby romaine leaves at the start of two consecutive feeding periods each day. The first feeding period lasted for 2 h (reflecting rates of ingestion and of crop and mid-gut filling) and the later feeding period lasted an additional 6 h (reflecting rates of ingestion, crop filling and gut throughput; Harrison and Fewell, 1995). The initial feeding trials commenced between 07.00 and 09.00 h. We used a flatbed scanner (Canon LiDE100) to photograph the leaves before and after each of the feeding trials. We estimated leaf areas using ImageJ software (http://rsbweb.nih.gov/ij/).

In order to assess the temperature dependence of hopping performance, grasshoppers were acclimated for 1 h at one of four possible temperatures (10, 17, 25 or 35°C). Grasshoppers were then removed individually from the incubators and immediately placed in the centre of the experimental arena at room temperature. The arena consisted of a 1.8 m × 1.8 m sheet of fabric with a checkered pattern at an interval of 2.5 cm (methods follow Harrison et al., 1991). Hopping was induced by manual prodding if necessary. We marked the position of the grasshopper after each of five jumps and subsequently recorded the x and y locations to an x and y resolution of 2.5 cm. We used the means of the five jumps for analysis, but checked that results were similar when using the median. Each individual was tested at each of the four temperatures, with the order of temperatures randomized. Performance of C. pellucida was observed to be minimal at the lowest temperature (10°C) and was thus not assessed.

For reared M. sanguinipes individuals, we measured both CTmin and CTmax, which were defined as the lower and upper temperatures at which the grasshoppers were no longer able to right themselves. Grasshoppers were placed individually into 50 ml centrifuge tubes, which were slowly (~0.2°C min−1) cooled or heated in a water bath. Given that warming rates
may influence estimates of critical thermal limits, we chose an intermediate rate of warming (Chown et al., 2009). To minimize stress, we first cooled body temperatures and then began heating body temperatures after an hour.

We used maximum likelihood to fit linear mixed-effects models describing feeding and hopping rates as a function of species, elevation, temperature and their interaction (function lme in R). We accounted for repeated measures of individuals by including an individual identifier as a random variable. For the reared grasshoppers, we additionally included a clutch identification as a random variable in which the individual identification was nested. We subsequently dropped the clutch identification as a random effect because its inclusion did not influence our results qualitatively.

**Thermal performance curves**

We fitted TPCs to our hopping and feeding performance data to determine the degree to which performance may have shifted through recent climate change along the elevation gradient. We fitted TPCs as a product of a Gaussian function and a Gompertz function (Frazier et al., 2006), as follows:

\[ P(T) = P_{\text{max}} e^{-\frac{1}{\sigma}(\rho(T - T_o) - 6) - \sigma(T - T_o)^3} , \]

where \( P(T) \) is the feeding rate at temperature \( T \), \( P_{\text{max}} \) is the maximal performance rate, \( T_o \) is the optimal temperature, and \( \rho \) and \( \sigma \) determine the thermal sensitivity of performance at temperatures above and below \( T_o \), respectively. We estimated parameters using the nls2 function in R. We assumed that \( \rho = 0.7 \), because we had limited data with which to fit the upper portion of the performance curve.

We assumed that the \( T_o \) was within 3°C of each species’ preferred body temperature (Buckley et al., 2013b) because the upper portion of the performance curve was poorly characterized by our data, particularly for *C. pellucida*. Additionally, we accounted for the minimal performance of *C. pellucida* at 10°C by including data points corresponding to zero performance at the \( CT_{\text{min}} \) (species mean ± SD = 9.33 ± 1.05°C; Buckley et al., 2013b). The number of these data points was set at a quarter of the number of individuals included in the performance assay to avoid unduly influencing the TPC fit. Temperature values (x-axis) were drawn from a normal distribution based on the mean and standard deviation of \( CT_{\text{min}} \) for each population of *C. pellucida* (Buckley et al., 2013b).

**Biophysical model and weather data**

We estimated performance shifts over the past 50 years at our study sites based on predictions of grasshopper body temperature. We used a steady-state budget of energy flux to describe the flow of energy between grasshoppers and their environments and predict operative body temperature, \( T_e \), as follows:

\[ Q_e = Q_s + Q_v + Q_{\text{cond}} \]

Here \( Q_s \) is the total input of heat flux due to solar radiation; \( Q_v \) describes the flux of thermal radiative heat due to both incoming thermal radiation (ground and sky) and that emitted by the grasshopper; \( Q_{\text{cond}} \) is the heat flux between the grasshopper and the surrounding fluid (air) via convection; and \( Q_{\text{cond}} \) is the heat flux between the grasshopper’s body and the solid surfaces with which the grasshopper’s body is in contact via conduction. We used this energy flux model to solve for \( T_e \), because rates of thermal radiative heat flux, convection and conduction are functions of \( T_e \). We omitted evaporative heat loss because it is negligible for grasshoppers in most conditions (Anderson et al., 1979). The detailed description and validation of our biophysical model for grasshoppers is given elsewhere (Anderson et al., 1979; Buckley et al., 2013b). We assumed the mean body size among our species to be 21 mm. Preliminary analysis of body size trends over time suggest that any body size shifts have been modest. We also assumed that the absorptivity of the grasshopper’s body is 0.7 and the albedo of the substrate is 0.3 (Kingsolver, 1983). These parameters determine the rate of energy exchange between the grasshopper and its environment.

Many insects, including grasshoppers, use thermoregulatory behaviours to buffer environmental variation along altitudinal gradients (Chappell and Whitman, 1990; Samietz et al., 2005). We bracketed the range of potential body temperatures by assuming that grasshoppers can move between full sun and full shade (no direct radiation). We also assumed that the grasshoppers can move between the ground (25% contact of surface area) and vegetation (0% contact of surface area). This assumption determines rates of conduction between the ground and the grasshopper. We assumed that individuals of each species thermoregulate to the available body temperature closest to the species’ preferred body temperature (Buckley et al., 2013b). We examined sensitivity to the assumption of thermoregulation by additionally considering grasshoppers in vegetation (no conduction with the ground) that are unable to thermoregulate.

We parameterized the biophysical model with daily maximum and minimum temperature data from weather stations at our study sites (McGuire et al., 2012). We estimated hourly air temperatures (\( T_a \)) using a diurnal temperature variation function based on maximum and minimum temperatures and the Julian date as modelled by Parson and Logan (1981). Daytime temperatures were fitted using a sine wave, while night-time temperatures were modelled using an exponential function. We assumed that ground temperatures were 8.4°C above \( T_g \) during daylight hours (Kingsolver, 1983). We predicted global solar radiation at the ground surface (in watts per square metre) as a function of latitude and altitude using an algorithm by Nikolov and Zeller (1992).

**Results**

**Temperature dependence of feeding**

Grasshopper feeding rates increased with increasing temperature over the range of body temperatures chronically realized among our study sites (15–40°C; Fig. 1). Temperature was the primary predictor of (mass-corrected) feeding rates over a
2 h period in an ANOVA also including species, population elevation and all interactions (Table 2). The effect of temperature on feeding rate differed significantly between species and as a function of elevation. The effect of elevation on feeding rate differed significantly between species. The three-way interaction between temperature, species and elevation was also significant. We omitted sex from this and subsequent models because it did not have a significant effect. The significant predictors of feeding rate were similar over an 8 h period, with temperature remaining the primary predictor (Table 2). In ANOVAs with only main effects, the rate of increase in feeding rate with temperature was similar between the 2 and 8 h periods (mean \( \pm \) SEM = 0.037 ± 0.002 vs. 0.030 ± 0.001 h pixel\(^{-1}\) °C\(^{-1}\), respectively, when normalized to a 2 h period). This suggests that rates of consumption and assimilation have similar temperature dependence.

The temperature dependence of feeding varied between species. While populations of *M. sanguinipes* exhibited limited feeding at low temperatures over 8 h, the low-elevation populations tended to capitalize best on warm temperatures (elevation, \( F_{1,63} = 14.1, P = 0.0004 \); temperature, \( F_{1,19} = 58.7, P < 0.0001 \); and temperature \( \times \) elevation, 0.003 ± 0.002, \( F_{1,19} = 6.0, P = 0.02 \), in an ANOVA for temperatures > 25°C). In contrast, high-elevation populations of the cool-adapted *M. boulderensis* exhibited higher feeding rates over 8 h across temperatures (elevation, –0.0006 ± 0.0003, \( F_{1,72} = 11.5, P = 0.001 \); temperature, \( F_{1,130} = 126.7, P < 0.0001 \); and temperature \( \times \) elevation, \( F_{1,130} = 13.8, P = 0.0003 \) in an ANOVA).

### Table 2: Results of ANOVAs examining the determinants of grasshopper feeding rates \((\times 10^6\) pixels\) over a 2 or 8 h period

| Parameters                        | 2 h \( F_{407} \) | 8 h \( F_{407} \) |
|-----------------------------------|-------------------|-------------------|
| Temperature (°C)                  | 254.8***          | 651.6***          |
| Species                           | 4.6*              | 8.4***            |
| Elevation (m)                     | 0.2               | 1.0               |
| Temperature \( \times \) species | 3.9*              | 21.1***           |
| Temperature \( \times \) elevation| 8.4**             | 5.1*              |
| Species \( \times \) elevation   | 16.8***           | 25.1***           |
| Temperature \( \times \) species \( \times \) elevation| 4.2*             | 15.3***           |

Significance levels are depicted (*\( P < 0.05 \), **\( P < 0.01 \) and ***\( P < 0.001 \)).

### Temperaure dependence of hopping

The temperature dependence of hopping performance varied between populations and species (Fig. 2). Temperature was the primary predictor of hopping distance (temperature included as a polynomial, \( F_{419} = 152.5, P < 0.0001 \) in an ANOVA also including species \((F_{2,230} = 44.7, P < 0.0001)\).
source elevation of the population ($F_{1,230} = 0.7, P = 0.4$) and all interactions. The effect of temperature on hopping distance differed significantly between species ($F_{2,619} = 27.5, P < 0.0001$) and as a function of elevation ($F_{1,619} = 18.2, P < 0.0001$). The effect of elevation on hopping distance differed significantly between species ($F_{1,619} = 3.5, P = 0.03$). The three-way interaction between temperature, species and elevation was also significant ($F_{2,619} = 5.5, P = 0.004$). We omitted mass from this and subsequent models because it did not have a significant effect. We additionally omitted sex, which had a weakly significant main effect but did not alter the models qualitatively.

We next used ANOVAs for each species to explore further how the temperature dependence of hopping varied between species. The cool-adapted *M. boulderensis* exhibited peak performance at lower temperatures than the other species (Table 3 and Fig. 2). High-elevation populations exhibited higher performance. Accounting for mass differences between populations in the ANOVA did not account for the elevation differences (mass, $F_{1,72} = 1.2, P = 0.3$). The temperature dependence varied as a function of source elevation for *M. boulderensis*. Populations of the warm-adapted *C. pellucida* also differed in their temperature dependence, with grasshoppers from the low-elevation sites tending to exhibit the strongest temperature dependence (Table 3 and Fig. 2), i.e. the low-elevation populations exhibited the highest performance at high temperatures and the lowest performance at low temperatures. Our trial temperatures were generally lower than the thermal optima for *C. pellucida*. *Melanoplus sanguinipes* tended to be a thermal generalist, with low performance at low temperatures but otherwise limited temperature dependence. The temperature dependence did not vary between populations (Table 3).

**Shifts in performance over time**

Estimated shifts in performance between 1955–1965 and 2000–2010 varied between species and along the elevation gradient (Fig. 3). Parameters for the fitted TPCs are compiled in Supplementary material, Table S1. Climate change in the interim has been more pronounced at higher elevations. We estimated that all species have experienced an increase in feeding rate in response to recent climate change. Predicted increases have been greater at higher elevations for *C. pellucida* and at lower elevations for *M. boulderensis* and *M. sanguinipes* (change in feeding rate for *M. boulderensis*, 2195 m = 10.7%, 2591 m = 5.6% and 3048 m = 4.7%; for *C. pellucida*, 2195 m = 5.4%, 2591 m = 11.5% and 3048 m = 10.7%; and for *M. sanguinipes*, 2195 m = 10.0%, 2591 m = 9.0% and 3048 m = 6.7%). We additionally considered shifts in feeding rate for a grasshopper on vegetation unable to thermoregulate. In this case, augmentation of feeding rates increased with elevation for all species (Supplementary material, Fig. S1). We noted that these increases are likely to be overestimates because we did not detect an upper temperature at which feeding rate declined in our feeding trials.

We estimated that the cool-adapted *M. boulderensis* has experienced hopping performance declines at the lower
elevation sites (change in performance, 2195 m = −3.8%, 2591 m = −7.0% and 3048 m = 0.0%; Fig. 4). We estimated that the warm-adapted C. pellucida has experienced performance increases concentrated at higher elevations (2195 m = 0.5%, 2591 m = 2.2% and 3048 m = 4.3%). We estimated that M. sanguinipes has experienced hopping performance declines at lower elevations and a weak performance increase at the highest elevation (2195 m = −4.5%, 2591 m = −5.3% and 3048 m = 0.6%). In the case of a grasshopper on vegetation unable to thermoregulate, we estimated that performance declines are most pronounced at mid-elevation for the warm-adapted C. pellucida and at mid- to high elevation for the other species (Supplementary material, Fig. S2).

**Importance of plasticity**

We repeated the performance assays for M. sanguinipes reared in constant conditions to examine the extent to which developmental plasticity influences the temperature dependence of performance. Neither the main effect of rearing temperature \((F_{1,179} = 1.43, P = 0.2)\) nor its interaction with temperature was a significant predictor of feeding rate over a 2 h period in an ANOVA including temperature, elevation, rearing temperature and all interactions (Fig. 5). Only the main effect of temperature was significant. However, plasticity did appear to influence feeding rate over an 8 h period. Rearing temperature exhibited a significant main effect \((F_{1,179} = 107.4, P < 0.0001)\), a significant interaction with elevation \((F_{1,179} = 4.7, P = 0.03)\) and a non-significant but suggestive interaction with assessment temperature \((F_{1,179} = 2.9, P = 0.09)\) as a predictor of feeding rate over an 8 h period in an ANOVA including temperature, elevation, rearing temperature and all interactions. No other terms were significant. This suggests that the temperature dependence of assimilation is more plastic than the temperature dependence of consumption.

We found more evidence for plasticity in the temperature dependence of hopping. Across three populations, we found that individuals reared at 27°C exhibited higher hopping performance at high temperatures than did individuals reared at 24°C (Fig. 6). We found strong temperature dependence (with temperature included as a polynomial, \(F_{1,262} = 142.4, P < 0.0001\)) in an ANOVA with elevation \((F_{1,262} = 0.2, P = 0.7)\), rearing temperature \((F_{1,262} = 4.7, P = 0.03)\) and all...
interactions (temperature × elevation, $F_{1,262} = 1.2, \ P = 0.3$; and three-way interaction, $F_{1,262} = 0.0, \ P = 1$). In addition to the significant main effect of rearing temperature, rearing temperature altered the temperature dependence of hopping (interaction, $F_{1,262} = 8.2, \ P = 0.005$).

We measured CTmin and CTmax for the reared grasshoppers to assess further the influence of plasticity on thermal tolerance. We found that CTmin was significantly lower (mean $\pm$ 95% confidence interval for 24°C = 9.38 $\pm$ 0.39 and for 27°C = 8.15 $\pm$ 0.27; $F_{2,64} = 2816, \ P < 0.0001$) and CTmax significantly higher (24°C = 53.9 $\pm$ 0.76 and 27°C = 58.7 $\pm$ 0.54; $F_{2,64} = 32305, \ P < 0.0001$) for the grasshoppers reared at 27°C compared with those reared at 24°C. Thermal tolerances did not differ significantly between populations from different elevations. The CTmin (8.49 $\pm$ 0.35°C) and CTmax (57.52 $\pm$ 0.31°C) of field-collected M. sanguinipes were intermediate between the values for the laboratory-reared grasshoppers (Buckley et al., 2013b).

**Discussion**

Our analyses suggest that performance metrics offer greater promise for understanding differential responses to climate change than cruder thermal tolerance metrics. Our focal grasshoppers have extremely high CTmax (>57°C; Buckley et al., 2013b), presumably due to the need to tolerate high body temperatures associated with radiation spikes at altitude. We did not detect upper thermal limits for feeding performance and did not fully assess those for hopping performance, but our data suggest that upper limits occur at temperatures substantially below CTmax. We previously found only limited differences in critical thermal tolerances and preferred body temperatures between the grasshopper species and populations (Buckley et al., 2013b). These previously observed differences were insufficient to account for differential responses to recent climate change. Although CTmin and CTmax reflect thermal limits for activity as measured in the laboratory, the thermal range for activity tends to be more thermally restricted in the field (Huey, 1982). The CTmax reflects the ability to withstand thermal extremes and is more closely predicted by temperature variation than mean temperature (Clusella-Trullas et al., 2011). The CTmin reflects both thermal means and extremes (Clusella-Trullas et al., 2011). Another limitation is that estimates of critical thermal limits are sensitive to methodology (Chown et al., 2009). The relevance of thermal tolerances to climate-change responses is dependent on whether individual fitness and population dynamics tend to be more limited by acute thermal stress or by chronic energetic conditions (Huey, 1982). The limiting
mechanism is related to the distance between limits on activity and lethal limits and how this distance compares with environmental variability (Woodin et al., 2013). Thermal tolerances are likely to be better predictors for populations near latitudinal and elevational range limits, where individuals may be more likely to encounter stressful temperatures (Bozinovic et al., 2011).

We find that grasshoppers are adapted to feed at warm temperatures even in cool, alpine environments. This is consistent with grasshoppers being effective thermoregulators, able to achieve high body temperatures (Harrison and Fewell, 1995; Lactin and Johnson, 1995) even at altitude. Using field-collected individuals, we found similar temperature dependence for food processing (8 h measurements) and consumption (2 h measurements). Temperature sensitivities ($Q_{10}$) were greater for $C.\ pellucida$ and $M.\ sanguinipes$ (0.8–1.1) than for the cool-adapted $M.\ boulderensis$ (~0.4). In contrast to our results, food processing was found to be more temperature sensitive ($Q_{10}$) than chewing and crop filling rates for a low-elevation population of another Colorado grasshopper species (Harrison and Fewell, 1995).

Grasshopper hopping performance, which corresponds to the ability to locomote for foraging and escape from predators, does not exhibit the same warm adaptation as feeding. These thermal constraints differ from those on vertebrate ectotherms, whose TPCs for assimilation tend to be narrower than those for locomotion (Angilletta, 2009). Due to the broader TPCs for assimilation than those for locomotion for grasshoppers, we estimate that recent climate change has driven declines in hopping performance but an increased capacity for feeding for species other than the warm-adapted $C.\ pellucida$ at most sites. We expect that grasshoppers will face restrictions in activity time available for foraging, but that they will readily be able to consume and assimilate the vegetation obtained. What will these counteracting shifts mean for energetics and population dynamics? Integrating their effects is challenging because grasshoppers are likely to partition these activities temporally; they may forage during the cool mornings and then rapidly digest food during warm afternoons (Harrison and Fewell, 1995).

Do our expectations for performance shifts align with observed abundance changes in the sites between 1959–1960?
and 2006–2008? Abundance has declined for the three focal species at the lower elevation sites. At the highest elevation site, *C. pellucida* abundance has increased and *M. boulderensis* abundance has remained approximately constant since initial surveys. *Melanoplus sanguinipes* was absent or at low abundance at the highest elevation site during the initial surveys (Fig. S1 of Buckley et al., 2013b; Table 1). We estimate hopping performance increases concentrated at high elevation for the warm-adapted *C. pellucida* since the initial surveys. We estimate that *M. boulderensis* experienced hopping performance declines at the lower elevation sites and no change in hopping performance but increased feeding performance at the high-elevation site. We estimate performance declines at lower elevations but a weak increase at the high-elevation site for *M. sanguinipes*. These estimates roughly correspond to the observed abundance shifts in response to recent climate change.

Our findings highlight the potential importance of plasticity to responses to climate change (Chevin et al., 2010). The greater plasticity of hopping relative to feeding may result from all populations and species being selected to take advantage of the warm temperatures available across elevations by basking in sunny areas for feeding and assimilation. However, we find that the assimilation rates of high-elevation populations increase with temperature more strongly at the lower rearing temperature. This suggests the importance of taking advantage of high temperatures for assimilation when they are available in cold environments. The greater plasticity of assimilation compared with consumption may stem from differences in the potential for enzymatic alterations. Likewise, rearing at warmer temperatures increases hopping performance at high temperatures, providing additional evidence that organisms take advantage of warm temperatures. This plasticity is consistent with a hotter-is-better dynamic (Angilletta, 2009).

The observed plasticity in hopping performance may mitigate some of the performance declines predicted to result from climate change. Indeed, plasticity has played a substantial role in recent responses, with most evidence coming from changes in body size resulting from shifts in growing season and survival (Ozgul et al., 2009, 2010) and changes in breeding (Réale et al., 2003; Charmantier et al., 2008) and flowering phenology (Anderson et al., 2012). Plasticity will both enable rapid responses to climate change and buffer the selection potentially required to cope with climate change in the long term (Chevin et al., 2010). Plasticity can substantially enhance the rate of climate change that organisms can pace (Gienapp et al., 2013).

While less is known about how thermal acclimatization has contributed to recent responses to climate change, thermal acclimatization in response to extreme temperatures—such as cold hardening—is well documented (Sinclair et al., 2003; Angilletta, 2009). Both short-term reversible acclimation and developmental acclimatization will be likely to be important to responses to climate change (Angilletta, 2009). Less is known about the potential for developmental acclimatization because few laboratory studies have examined development and fitness in realistically variable environments (Kingsolver et al., 2013). Further examination of developmental plasticity in variable environments would refine our understanding of how plasticity may alleviate performance declines resulting from climate change.

**Supplementary material**

Supplementary material is available at *Conservation Physiology* online.

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