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Ectotherm Thermal Stress and Specialization Across Altitude and Latitude

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Synopsis Gradients of air temperature, radiation, and other climatic factors change systematically but differently with altitude and latitude. We explore how these factors combine to produce altitudinal and latitudinal patterns of body temperature, thermal stress, and seasonal overlap that differ markedly from patterns based solely on air temperature. We use biophysical models to estimate body temperature as a function of an organism’s phenotype and environmental conditions (air and surface temperatures and radiation). Using grasshoppers as a case study, we compare mean body temperatures and the incidence of thermal extremes along altitudinal gradients both under past and current climates. Organisms at high elevation can experience frequent thermal stress despite generally cooler air temperatures due to high levels of solar radiation. Incidences of thermal stress have increased more rapidly than have increases in mean conditions due to recent climate change. Increases in air temperature have coincided with shifts in cloudiness and solar radiation, which can exacerbate shifts in body temperature. We compare altitudinal thermal gradients and their seasonality between tropical and temperate mountains to ask whether mountain passes pose a greater physiological barrier in the tropics (Janzen’s hypothesis). We find that considering body temperature rather than air temperature generally increases the amount of overlap in thermal conditions along gradients in elevation and thus decreases the physiological barrier posed by tropical mountains. Our analysis highlights the limitations of predicting thermal stress based solely on air temperatures, and the importance of considering how phenotypes influence body temperatures.

Introduction

Much of our understanding of the ecological impacts of climate change relies on the generally erroneous assumption that the body temperatures of ectothermic organisms are equal to air temperatures. In reality, air temperatures and surface temperatures, solar radiation, humidity, and wind speed interact with the phenotypes of organisms to produce complex mosaics of body temperatures in space and time. These interactions can drive significant microclimatic variation that departs from broad-scale patterns in air temperature. For example, in the intertidal zone, the incidence of thermal stress depends strongly on the coincidence of high air temperatures, diurnal peaks of solar radiation, and low tides. Consequently, the incidence of stressfully high body temperatures is greater at higher than at lower

latitudes for some intertidal organisms (Helmuth et al. 2002). Furthermore, body temperatures vary substantially among organisms from the same site, but which have different phenotypes (e.g., differential coloration and material properties of seastars and mussels) or which occupy different microclimates at that site (Helmuth 2009). On land, topography can produce substantial variation in the body temperatures achievable by organisms ($\sim 17^\circ\text{C}$ over 100 m^2 for a lizard) (Sears et al. 2011). This variation may enable organisms to effectively buffer environmental variation across space and time by selecting suitable microclimates (Kearney et al. 2009; Sears et al. 2011).

Additionally, many ecological analyses rely on air temperatures averaged over monthly or annual time scales. Yet, survival and fecundity may be more

strongly influenced by acute exposures to extreme temperatures than by chronic environmental conditions. Whether thermal means or extremes more strongly impact organisms can vary spatially. For example, the intertidal distribution of Mediterranean mussels is determined in some regions by exposure to lethal temperatures and in others by chronic exposure to thermal stress that reduces fecundity (Sarà et al. 2011). In some systems, responses to acute and chronic thermal conditions combine to determine demography. For example, in *Colias* butterflies, mean environmental temperatures and key phenotypic traits determine the duration of time available for flight, which corresponds to fecundity as they lay eggs singly on host plants. However, thermal extremes can decrease fecundity by decreasing the viability of eggs (Kingsolver and Watt 1983). A demographic model combining these two determinants of fecundity along an elevational gradient in Colorado suggests that decreases in fecundity associated with overheating have been more than offset by increases in fecundity associated with more time available for flight (Buckley and Kingsolver 2012). Another model based on the temperature-dependence of the use and acquisition of energy demonstrated that temporal data of high-resolution are important for accurately estimating rates of survival and fecundity, particularly for species unable to behaviorally buffer environmental fluctuations (Kearney et al. 2012).

Given these differences between body temperatures and ambient temperatures for many organisms, operative temperatures (T_e) provide a useful metric for ecological and evolutionary studies of ectotherms (Angilletta 2009). T_e is the equilibrium body temperature of an ectotherm with given thermal and radiative properties in a specified location (Bakken 1992). Environmental conditions and their variation over space and time can be translated into T_e values using biophysical models (Porter and Gates 1969; Helmuth et al. 2005). These energy budget models can be validated using measurements of body temperatures both in the field and in physical mimics.

The differences between operative and ambient air temperatures can be highlighted by comparing latitudinal and altitudinal gradients (Fig. 1). Ambient mean temperatures decline with increasing latitude at about $7^\circ\text{C}/10^\circ$ latitude (mean \pm 95% CI = 7.16 ± 0.16 , $F(1,45) = 9.3$, $r^2 = 0.99$, $P < 10^{-15}$; regression for 0.5° IIASA database A03 cells binned by degree latitude). Similarly, ambient temperatures decline with increasing altitude at about $6^\circ\text{C}/\text{km}$ of altitudinal increase (Dillon et al. 2006). However, other physical factors show different latitude and

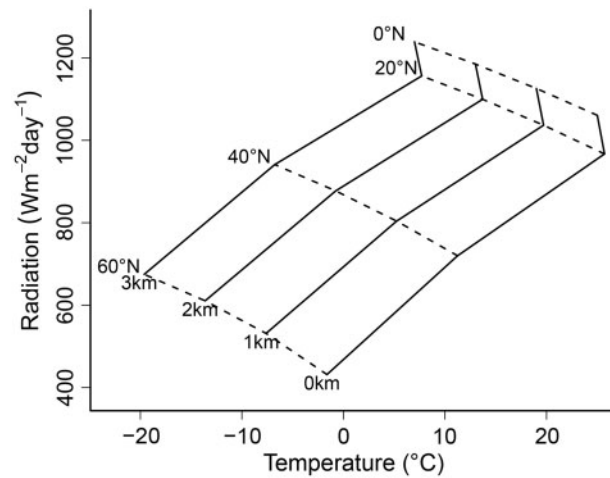


Fig. 1 Temperature ($^\circ\text{C}$) and the mean annual solar radiation ($\text{Wm}^{-2} \text{day}^{-1}$) received on a horizontal surface at the earth's surface both vary systematically across latitude and altitude. Temperatures are calculated at sea level based on 0.5° IIASA database A03 cells binned by degree of latitude. We then assume an adiabatic cooling rate of $6^\circ\text{C}/\text{km}$ of altitude. We estimated daily radiation as a function of latitude and altitude using an existing algorithm (Nikolov and Zeller 1992). For the estimates of radiation, we assumed constant values of temperature (15°C), humidity (50%), and precipitation (50 mm/month) across locations and months.

altitude gradients. Solar radiation received at the earth's surface declines with increasing latitude due to the declining angle of incoming radiation and increased atmospheric absorption. In contrast, solar radiation increases with increasing altitude due to decreased atmospheric absorption. We implement an algorithm for predicting solar radiation as a function of latitude and altitude (Nikolov and Zeller 1992) to depict these gradients. The increase in radiation with altitude is particularly pronounced in summer at high latitudes (Supplementary Fig. S1). As a result, ambient temperature alone can be a poor indicator of thermal conditions (Fig. 1). For example, the mean temperature at sea level at 40°N latitude is similar to that at 2.5 km altitude at the equator, but radiation levels are 80% greater at the higher altitude. Additionally, as altitude increases, reduced humidity increases evaporative heat loss and reduced density of the air limits convective heat loss (Dillon et al. 2006). The implications of these altitudinal gradients for insect physiology have been reviewed elsewhere (Dillon et al. 2006).

Examining organismal responses to recent climatic change is complicated by interdependent shifts in temperature, radiation, and cloudiness. In recent decades, minimum temperatures have generally increased faster than maximum temperatures, resulting in a decrease in diurnal temperature range (DTR).

These decreases in DTR have been coincident with increases in cloud cover over much of the earth in recent decades (Dai et al. 1999). However, feedbacks between clouds and other climatic variables remain a primary source of uncertainty in projections of climate change and prevent attributing directionality to the relationship (Brient and Bony 2012). The key issue is that clouds both reflect incoming solar radiation and block outgoing radiation from the earth's surface. Attributing trends in radiation is hindered by methodological shifts in assessing cloud cover. Automated systems for observing the earth's surface and satellite data both have replaced estimates of cloudiness by visual observation since the early 1990s, and the comparability of the methods is limited (Dai et al. 2006). Given these limitations, increases in cloudiness on the order of 1.4% (of the sky) per decade over most of the United States between 1976 and 2004 have been estimated (Dai et al. 2006). Predictions of future trends in cloudiness and radiation will likely remain elusive. Nevertheless, this uncertainty motivates our examination of how temperature and radiation interact to determine body temperatures and the incidence of thermal stress.

Janzen (1967) posed the question “are mountain passes higher in the tropics?” which has since catalyzed research examining the ecological and evolutionary implications of variability of temperature within days and across seasons. Janzen proposed that temperature differences along elevational gradients must be interpreted relative to organismal physiology and that reduced diurnal and seasonal variation in temperature in the tropics selects for greater physiological specialization. Altitudinal gradients may thus pose a greater physiological barrier for thermally-specialized tropical organisms, resulting in smaller distributions and increased species turnover. Janzen (1967) evaluated his hypothesis by quantifying the amount of temperature space shared between lower and upper montane sites relative to the thermal variability within the sites. This “seasonal thermal overlap” metric characterizes the extent to which an organism could remain within its thermal niche while moving along an elevation gradient. Janzen found that seasonal thermal overlap is reduced and declines more rapidly with greater elevational differences between sites in the tropics compared with temperate areas. Ghalambor et al. (2006) compiled subsequent support for Janzen's hypothesis and suggested that Janzen's analysis of seasonal thermal overlap should be repeated using T_e rather than T_a , as T_e more accurately reflects how organisms experience their environment. A key component of Janzen's hypothesis is that the more constant tropical

temperatures select for narrow thermal tolerances (physiological data compiled by Brattstrom 1968, 1970; Huey et al. 2009; Sunday et al. 2010). The thermal specialization of tropical ectotherms may make them more sensitive to climate change (Deutsch et al. 2008; Tewksbury et al. 2008).

We used grasshoppers as a case study for examining T_e across altitudinal and latitudinal gradients. Grasshoppers are highly sensitive to T_e (Belovsky and Slade 1995), which influences rates of locomotion, feeding, growth, and development, as well as life-history traits such as clutch size and egg size (Dearn 1977; Hassall et al. 2006). In addition, high-elevation insects typically respond to the reduced and variable temperatures and to short growing seasons by reducing the number of developmental stages, generations per year, or developmental thresholds (Hodkinson 2005). Patterns of local adaptation in our focal grasshopper species, *Melanoplus sanguinipes*, are consistent with selection along the elevational gradient associated with environmental uncertainty and season length (Dingle et al. 1990).

Here, we focus on how altitudinal shifts in temperature and radiation interact with grasshoppers' phenotypes to determine altitudinal gradients in body temperature and in the incidence of thermal stress. We first consider altitudinal patterns in body temperatures and the incidence of thermal extremes and how these patterns have shifted over recent decades. We focus our analysis on the Rocky Mountains of Colorado. We then examine patterns of T_e between temperate and tropical mountains in relation to Janzen's hypothesis.

Methods

We first introduce the biophysical model used to translate T_a into T_e . We next describe the weather data used in both our detailed analysis of a single elevational gradient in temperature and a comparison of temperate and tropical gradients in elevation. Finally, we describe the metrics used to compare thermal similarity between low and high sites to assess Janzen's hypothesis.

Biophysical model

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 : $Q_s = Q_t + Q_c + Q_{\text{cond}}$. Here, Q_s is the total input of heat flux due to solar radiation; Q_t describes the flux of thermal radiative heat due to both incoming thermal radiation (ground and sky) and that emitted by the grasshopper; Q_c is the heat

flux between the grasshopper and the surrounding fluid (air) via convection; and Q_{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 use this energy-flux model to solve for T_e , as rates of thermal radiative heat flux, convection, and conduction are functions of T_e . We omitted evaporative heat loss as it is negligible for grasshoppers under most conditions (Anderson et al. 1979). The detailed description and validation of our biophysical model for grasshoppers is given elsewhere (Buckley et al. 2013; see also Anderson et al. 1979). We parameterized the model for a *M. sanguinipes* grasshopper with a length of 21.1 mm (the mean and median for the species along our focal elevational gradient). *Melanoplus* is a widespread genus spanning much of North America, including Hawaii. Although we do not know of *Melanoplus* individuals in Puerto Rico, they are common in Florida and present in Mexico. Moreover, the modeled phenotype is reasonably generic and similar to those of grasshoppers in Puerto Rico (L. Buckley, personal observation). We assumed that 25% of the grasshopper's surface is in contact with the substrate (ground or vegetation) in our determinations of conduction. 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 behaviors to buffer environmental variation along altitudinal gradients (Samietz et al. 2005). We did not evaluate the influence of behavioral thermoregulation in detail here, but were able to bracket the range of potential body temperatures by calculating T_e values for multiple microhabitats.

Weather data

We first considered microclimatic measurements averaged over 3-min intervals in July 2011 along the Niwot Ridge LTER elevational gradient (<http://culter.colorado.edu/NWT/>). We examined four sites along the 40th N parallel in Boulder County, Colorado: Eldorado (1740 m), A1 (2185 m), B1 (2591 m), and C1 (3048 m). We measured (shaded) temperatures of the air and surface (Pace PT907 30 k Ω thermistor, $\pm 0.15^\circ\text{C}$), total global horizontal radiation (Pace SRS-100 Silicon Photodiode, 400–1100 nm, $\pm 5\%$ accuracy), and wind speed (anemometer, 0.9–78 m/s range, $\pm 5\%$ accuracy) using a Pace XR5 datalogger. We used these weather data to estimate grasshoppers'

body temperatures and averaged the values between sunrise and sunset.

We then examined long-term trends along additional elevation gradients on temperate mountains. We analyzed hourly temperature and radiation data from 1991 to 2005 for Rocky Mountain stations in Colorado, New Mexico, and Montana (see [Supplementary Table S1](#)) in the National Solar Radiation Database II (NSRDB, http://rredc.nrel.gov/solar/old_data/nsrdb/). Data on solar radiation in the National Radon Safety Board (NRSB) are generated as a hybrid of model output from the Meteorological–Statistical model, which is based on observations of cloud cover and solar radiation, and a State University of New York model based on the Geostationary Operational Environmental Satellite (GOES). Estimates stemming from meteorological and satellite measurements were found to be robust and the modeled data are intended to preserve statistical properties (e.g., means, standard deviations, and cumulative frequency distributions) of measured solar data over days, months, and years. Although the trend toward increasing solar radiation over the period of analysis is consistent with other observations (Wild et al. 2005; Ohmura 2009), the trend is influenced by shifts in the modeling procedure and underlying inputs. Inconsistencies are introduced as the cloudiness shifted from observations by humans to automated observations in the 1990s; GOES data only date from 1998. We tested for a shift in radiation levels corresponding to the methodological shift by including an offset term in a regression of solar radiation as a function of year and time period (corresponding to before and including 1977 as opposed to later than 1997) (Weatherhead et al. 1998). We found a significant increase in solar radiation after 1997 for three of our four focal sites in the Rocky Mountains of Colorado (Limon mean $\pm 95\%$ CI: $40 \pm 8.8 \text{ W/m}^2$, $P < 0.001$; Eagle: $17.7 \pm 12.6 \text{ W/m}^2$, $P = 0.18$; Alamosa: $68.5 \pm 14.4 \text{ W/m}^2$, $P < 0.001$; Leadville: $119.4 \pm 12.4 \text{ W/m}^2$, $P < 0.001$). Consequently, our analysis illustrates how shifts in solar radiation interact with air temperature to determine body temperatures, but should not be interpreted as a robust time series. We did not correct estimates of solar radiation in our subsequent calculations (e.g., T_e) because of the wide range of estimated correction factors. We investigated additional solar radiation time series, but found them to be unreliable due to inconsistencies in measurement and solar sensors, and we concluded that the NSRDB was more appropriate for our analysis.

We then averaged the hourly data from 2000 to 2005 to examine seasonal patterns and thermal

overlap between sites at different elevations. To compare patterns between temperate areas and the tropics, we analyzed hourly data on temperature and radiation from 2005 to 2010 for Puerto Rican and Hawaiian stations in the USDA National Resources Conservation Services Soil Climate Analysis Network (SCAN, [Supplementary Table S1](http://www.wcc.nrcs.usda.gov/scan/), <http://www.wcc.nrcs.usda.gov/scan/>). As we lack data on surface temperatures, we use air temperatures as proxies.

We calculated seasonal thermal overlap between sites along temperate and tropical elevation gradients, as did [Janzen \(1967\)](#):

$$\text{overlap} = \sum_{i=1}^{12} \frac{d_i}{\sqrt{R_{1i}R_{2i}}},$$

where d_i is the amount of overlap ($^{\circ}\text{C}$) between the monthly minimum temperature of the lower elevation site and the monthly maximum of the higher elevation site for the i th month. The d_i value is negative in the case of no-thermal overlap between the sites. R_{1i} is the difference ($^{\circ}\text{C}$) between the monthly mean maximum and the monthly mean minimum for the i th month at the higher elevation; R_{2i} is the equivalent value for the lower elevation. The metric represents relative overlap as it is estimated relative to the geometric mean between R_{1i} and R_{2i} . A high positive value indicates that the range in temperature shared between the sites at low and high elevations is large relative to the temperature variation within the sites. A high negative value indicates that the separation between the temperature minimum at the low elevation and the temperature maximum at the high elevation is large relative to the temperature variation within the sites. Note that the degree of niche overlap between sites can be asymmetric, which is not reflected in Janzen's metric of overlap. To evaluate this, we also calculated the asymmetry in the amount of non-overlapping temperature space between the low and high elevations as follows:

$$\text{asymmetry} = \sum_{i=1}^{12} \text{abs}\left(\frac{d_i}{R_{1i}} - \frac{d_i}{R_{2i}}\right)$$

A high value of asymmetry indicates that the number of degrees of temperature by which the low-elevation site is warmer than the high-elevation site is greater than the number of degrees by which the high-elevation site is cooler than the low-elevation site or vice versa. Janzen calculated overlap for pairs of sites at low and high elevations. Because our analysis is limited by the availability of data on radiation at sets of sites along several elevational gradients, we calculated pairwise overlap between

all sites along each elevational gradient. Variation in diurnal and seasonal temperature tends to be buffered by the ocean in coastal locations. [Janzen \(1967\)](#) demonstrated the robustness of his analysis to these continental effects. We were unable to conduct an analogous analysis as we were unable to locate coastal, temperate elevational gradients for which there were data available on radiation.

Results

Elevational gradients

Shifts in temperature and radiation along the Niwot Ridge elevational gradient interact with organismal phenotypes to determine patterns of body temperature. Air temperatures decline more steeply with elevation than do surface temperatures ([Fig. 2A](#)). We applied the biophysical model to predict that body temperatures for grasshoppers on the surface will be warmer than those in the vegetation both at low and at high elevations. Peak (90th percentile) soil temperatures are high at altitude despite lower mean temperatures due to high levels of radiation ([Fig. 2B](#)). These high peak surface temperatures result in a high incidence of extreme body temperatures for grasshoppers on the surface ([Fig. 2C](#)). The proportion of predicted extreme body temperatures for grasshoppers in the vegetation declines more steadily along the elevational gradient ([Fig. 2C](#)). Mean radiation is greatest at the lowest site, consistent with lesser cloudiness, but then increases with elevation at the higher elevations ([Fig. 2D](#)). In contrast, peak (90th percentile) radiation levels increase with elevation ([Fig. 2D](#)), with consequences for the incidence of high body temperatures.

We next considered how patterns of body temperature for a grasshopper in the vegetation shifted among Rocky Mountain sites differing in elevation in responses to recent climatic change ([Fig. 3](#) and [Supplementary Fig. S2](#) for additional sites). Minimum, mean, and maximum air temperatures have increased along the elevational gradient since 1991, resulting in predicted increases in grasshoppers' body temperatures ([Fig. 3](#), left). Increases in the proportion of body temperatures exceeding thresholds of 30°C , 35°C , and 40°C exhibit more pronounced temporal trends than do mean body temperatures ([Fig. 3](#), middle). At Leadville, the site at the highest elevation, the predicted grasshopper body temperatures (0.53 ± 0.11 , $F(1,13) = 24.5$, $r^2 = 0.65$, $P = 2.7 \times 10^{-4}$) increased more rapidly since 1991 than did air temperatures (0.065 ± 0.022 , $F(1,13) = 8.8$, $r^2 = 0.40$, $P = 0.01$). The incidence of predicted body temperatures exceeding 30°C

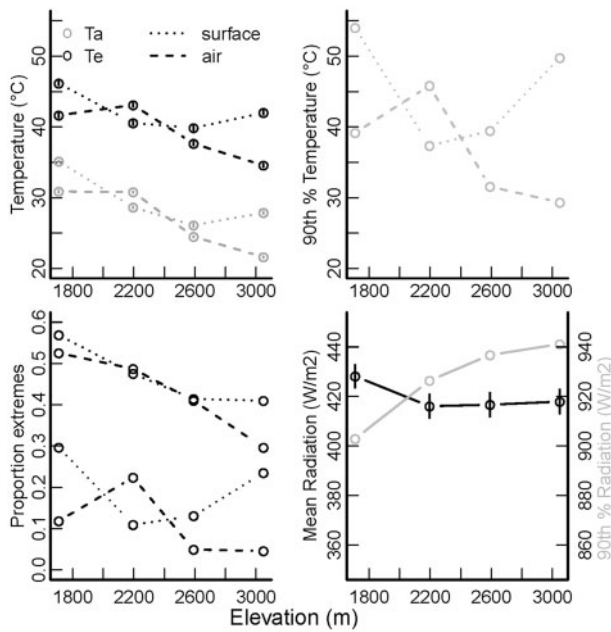


Fig. 2 Weather conditions and their implications for grasshoppers' body temperatures vary along the elevational gradient at Niwot Ridge. Data are means (and 95% confidence intervals) or other metrics during daylight for July 2011. **(A)** The decline in temperature along the gradient is steeper for air (gray dashed line) than the ground surface (gray dotted line). This difference is reflected in predicted body temperatures for grasshoppers both on the ground (black dotted line) and on vegetation (black dashed line). **(B)** Soil temperatures peak (90th percentile) at both low and high elevations, whereas air temperatures decline more steadily along the elevational gradient. **(C)** Grasshoppers on the ground experience a high proportion of extreme body temperatures (40°C and 60°C thresholds). The proportion of extreme body temperatures declines more rapidly with elevation for grasshoppers in the vegetation. **(D)** Mean global horizontal radiation is highest at the lowest elevation consistent with reduced cloudiness. However, intense peak horizontal radiation (90th percentile) at high elevations increases the incidence of acute heat stress.

increased most rapidly (0.0082 ± 0.0020 , $F(1,13) = 17.3$, $r^2 = 0.57$, $P = 0.0011$), followed by increases in temperatures exceeding 35°C (0.0058 ± 0.0013 , $F(1,13) = 19.7$, $r^2 = 0.60$, $P = 6.7 \times 10^{-4}$) and 40°C (0.0029 ± 0.11 , $F(1,13) = 21.2$, $r^2 = 0.62$, $P = 5.0 \times 10^{-4}$). Shifts in solar radiation contributed to the greater incidence of extremes (Fig. 3, right), but we note that temporal trend in the radiation data is questionable (see “Methods” section). The DTR does not shift consistently across the sites (Fig. 3, right).

Comparison of temperate and tropical mountains

We compared patterns of seasonal thermal overlap along elevational gradients between tropical and

temperate areas (Fig. 4; see Supplementary Fig. S3 for additional sites). Janzen (1967) demonstrated that seasonal overlap in T_a is greater and declines more steeply with elevational distance between sites for temperate mountains compared with tropical ones. We depict the monthly means of daily minimum, daily mean, and daily maximum temperatures along temperate (Limon, CO: 1695 m, Telluride, CO: 2769 m) and tropical (Combate, PR: 10 m, Guilarte Forest, PR: 1020 m) elevational gradients. For these examples, seasonal thermal overlap is higher for T_e than for T_a . Janzen's pattern of greater overlap among temperate sites persists when considering T_e (Fig. 3). When extending this comparison of seasonal thermal overlap along temperate (CO, MT, and NM) and tropical (HI and PR) elevational gradients, we confirmed Janzen's finding that T_a overlap declines more steeply with elevational distance between sites on tropical mountains ($-1.40 \times 10^{-2} \pm 1.28 \times 10^{-3}$, $F(1,18) = 119.0$, $r^2 = 0.87$, $P = 2.30 \times 10^{-9}$) than on temperate ones ($-6.54 \times 10^{-3} \pm 6.34 \times 10^{-4}$, $F(1,44) = 106.6$, $r^2 = 0.71$, $P = 2.5 \times 10^{-13}$).

The decline in the overlap of T_e with greater elevational distances between sites is similar between the temperate sites ($-2.57 \times 10^{-3} \pm 3.86 \times 10^{-4}$, $F(1,44) = 44.1$, $r^2 = 0.50$, $P = 3.8 \times 10^{-8}$) and those in Hawaii ($-2.05 \times 10^{-3} \pm 3.30 \times 10^{-4}$, $F(1,8) = 38.8$, $r^2 = 0.83$, $P = 2.5 \times 10^{-4}$). However, the overlap of T_e declines more steeply with elevational distance between sites for Puerto Rico ($-7.59 \times 10^{-3} \pm 1.40 \times 10^{-3}$, $F(1,8) = 29.6$, $r^2 = 0.79$, $P = 6.2 \times 10^{-4}$). This finding can be explained by increases in asymmetry with elevational distance between sites along the Puerto Rican elevational gradient (Fig. 5). The tropical example depicted in Fig. 4 is representative. Considering T_e instead of T_a dramatically increases the extent to which the warmer, low-elevation site reaches warmer maximum temperatures compared with the cooler, higher-elevation site. This asymmetry increases with greater elevational distances between sites in Puerto Rico ($1.82 \times 10^{-3} \pm 4.82 \times 10^{-4}$, $F(1,8) = 14.3$, $r^2 = 0.64$, $P = 5.4 \times 10^{-3}$). In contrast, the extent to which maximum temperatures at the low-elevation are above and minimum temperatures at the high-elevation site are below the thermal overlap is roughly similar for T_a in the tropical sites ($9.04 \times 10^{-5} \pm 2.86 \times 10^{-4}$, $F(1,18) = 0.10$, $r^2 = 0.00$, $P = 0.76$) and T_e both in the temperate sites ($-2.35 \times 10^{-4} \pm 1.96 \times 10^{-4}$, $F(1,44) = 1.4$, $r^2 = 0.48$, $P = 0.24$) and in Hawaii ($-4.48 \times 10^{-5} \pm 3.30 \times 10^{-4}$, $F(1,8) = 0.6$, $r^2 = 0.00$, $P = 0.90$). Thus, asymmetry remains relatively constant as a function of elevational distance between sites in these locations. DTR tends to increase with increasing elevation (Supplementary Fig. S1), resulting in a

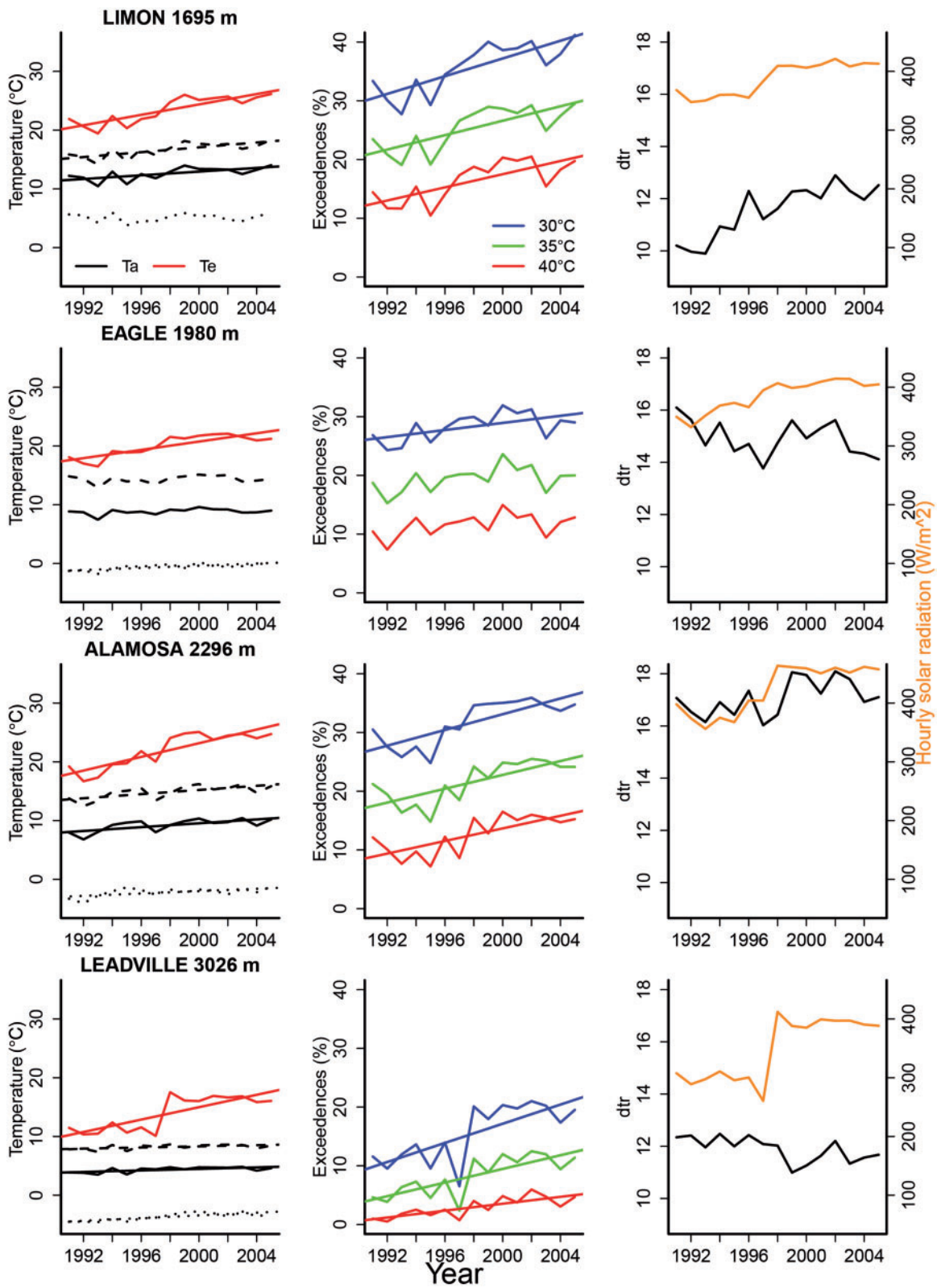


Fig. 3 Air (minimum, dotted black line; mean, solid black line; and maximum, dashed black line) and predicted grasshopper body temperatures (red line) have increased along an elevational gradient in Colorado since 1991 (left). Increases in the proportion of body temperatures exceeding thresholds of 30°C, 35°C, and 40°C exhibit more pronounced temporal trends than do mean body temperatures (middle). Increases in body temperature are more pronounced than are increases in air temperature due to increases in solar radiation (right). Certainty of the trends in solar radiation is low due to methodological inconsistencies (see text for details). The DTR does not shift consistently across the sites. Data are averaged annually across daylight hours. In the left two columns, significant trends ($P < 0.05$) are indicated by lines corresponding to the linear regressions.

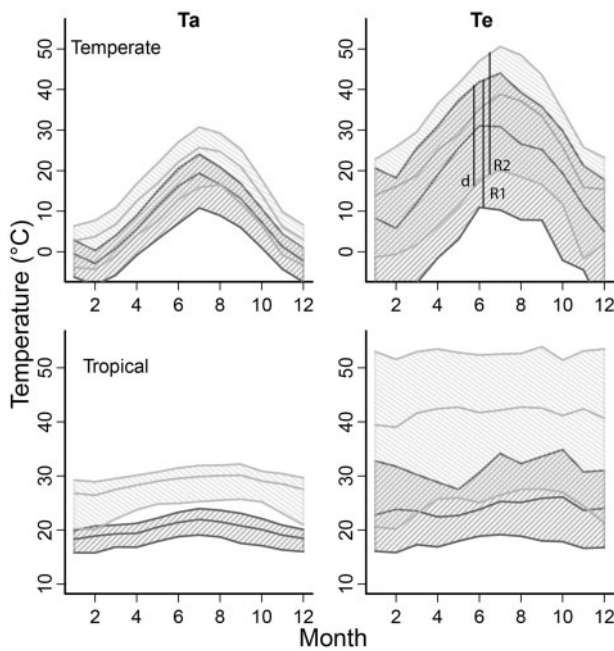


Fig. 4 Seasonal overlap between sites at low and high elevations is greater in temperate mountains than in tropical ones and is greater for T_e than for T_a . The monthly means of daily minimum, mean, and maximum temperatures are depicted for temperate (Limon, CO: 1695 m and Telluride, CO: 2769 m) and tropical (Combate, PR: 10 m, Guilarte Forest, PR: 1020 m) sites. We depict the metrics (d , R_1 , and R_2) used to calculate thermal overlap and asymmetry.

decrease in asymmetry with elevational distance between sites for the T_a of temperate sites ($-1.33 \times 10^{-3} \pm 5.41 \times 10^{-4}$, $F(1,44) = 6.1$, $r^2 = 0.12$, $P = 0.02$).

Discussion

The interaction of temperature, radiation, and other environmental factors may cause altitudinal and latitudinal patterns of thermal stress to depart from those based solely on air temperature. Although mean radiation declines with altitude due to increased cloudiness, peak radiation increases because of decreased atmospheric scattering. Consequently, peak soil temperatures are also high at high altitudes. Decreases in vegetative cover along the gradient may also contribute to high peak soil temperatures. The interaction of temperature and radiation and its implications for altitudinal and latitudinal patterns of acute and chronic thermal stress drives patterns of thermal adaptation (Angilletta 2009) and the ecological impacts of climate change (Smith 2011). Resistance to thermal stress was found to vary for *Drosophila* along an altitudinal gradient based on the metric considered, perhaps consistent with altitudinal

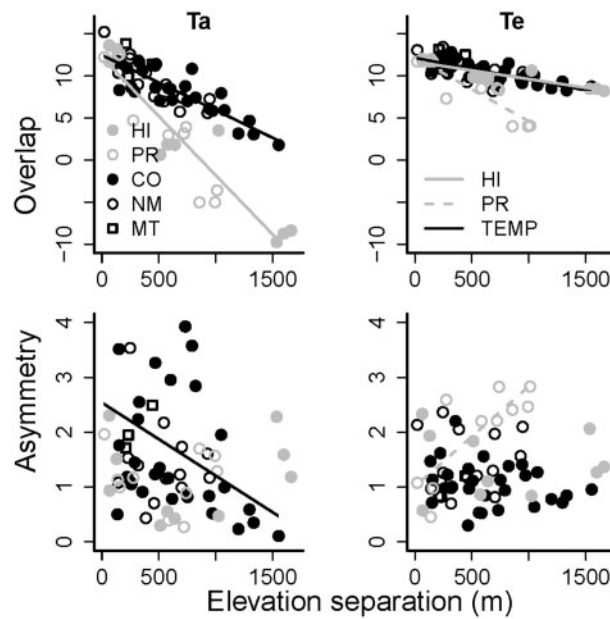


Fig. 5 Seasonal thermal overlap (calculated as in Janzen 1967) declines more rapidly with elevational distance between sites in the tropics (HI: Hawaii, PR: Puerto Rico) than in the temperate zone (Temp; CO: Colorado, NM: New Mexico, and MT: Montana) when considering T_a . A fast decline of overlap in the tropics is observed for an elevation gradient in Puerto Rico but not for one in Hawaii. Asymmetry in T_a declines more steeply with elevational distance between sites for temperate sites than for tropical ones. Asymmetry in T_e varies little as a function of elevational distance between sites in the temperate sites and in Hawaii. Asymmetry in T_a increases with elevational distance between sites for Puerto Rico.

shifts in the incidence of chronic and acute heat stress (Sørensen et al. 2005). Survival in response to a heat shock of 41°C did not vary altitudinally, but the duration of performance at 37°C did decline with altitude. The expression of heat-shock proteins increased with altitude at 38°C, but did not vary clinally at 36°C (Sørensen et al. 2005). These data may suggest that low-altitude populations are warm-adapted, but that the resistance to acute thermal stress is similar across altitudes. However, the degree of heat-shock responses by organisms generally depends on the thermal variability of the organism's environment (Fangue et al. 2006; Tomanek 2010).

An organism's phenotype mediates its sensitivity to shifts in temperature and radiation. For example, increases in the melanism of butterflies' wings at higher elevations bestows the ability to heat up in generally cooler environments, but also conveys sensitivity to high peaks of radiation. Dark phenotypes at higher elevation may thus be more sensitive to environmental variation (Kingsolver and Watt 1983). Seasonal

plasticity of melanism of the wings in response to pupal temperatures enables optimizing this trade-off in response to environmental variability (Kingsolver and Huey 1998). Such plasticity tends to be favored when the spatial scale of environmental variables matches that of a population, when the population can respond to the environmental factor faster than the factor itself changes, and when environmental variation is reasonably predictable (Alpert and Simms 2002). Geographic clines in seasonal plasticity are driven by shifts in seasonality across latitude and altitude (Ghalambor et al. 2007; Bradshaw and Holzapfel 2010). Phenotypes may also be differentially sensitive to shifts in environmental variables. For example, a common garden experiment found that plant ecotypes responded similarly to thermal treatments, but differed in their ability to tolerate extreme drought (Beierkuhnlein et al. 2011). Shifts in thermally relevant phenotypes in response to recent climate change confirm the importance of phenotype in mediating body temperature (Gardner et al. 2011; Karell et al. 2011; Ożgo and Schilthuizen 2011).

Our simulations indicate that since 1991 grasshoppers' body temperatures increased more rapidly along our focal environmental gradient than did air temperatures. This suggests analyses that assume body temperatures = T_a may underestimate the ecological impacts of climate change. Furthermore, the incidence of thermal extremes increases more rapidly than does mean body temperature. One methodological limitation to examining historic trends in T_e is that consistent data on radiation are scarce. Radiation models based on estimates of cloudiness provide data for many stations and regions, but the equivalency of estimates of cloudiness is questionable over the past several decades due to shifts in methodology. A strategy for dealing with this limitation is to examine the implications of recent temporal (diurnal and seasonal) and spatial (altitudinal and latitudinal) variation in radiation on patterns of body temperatures and on organismal thermal stress. Shifts in phenotypes and multiple environmental factors may combine to produce complex shifts in body temperatures and may help explain why responses to recent climate change have differed among populations and locations. For example, a resurvey of grasshoppers along our focal elevational gradient near Boulder, Colorado since 1960 showed that the extent and even the direction of shifts in phenology and abundance have varied among species and along the gradient (Nufio et al. 2010). The shifts in mean and peak temperature and radiation along altitudinal gradients illustrated here may provide insight into these idiosyncratic responses.

Our analysis suggests that the “height” of mountain passes is similar between temperate and some tropical mountains (e.g., in Hawaii) for those organisms that experience elevated body temperatures due to radiation (i.e., a substantial difference between T_e and T_a). On other tropical mountains (e.g., in Puerto Rico), seasonal overlap in T_e decreases more rapidly with elevational distance between sites compared with temperate mountains. Overall, our analysis suggests that considering T_e rather than T_a diminishes the extent to which mountains pose a greater physiological dispersal barrier in the tropics. However, we note that our analysis assumes full sunlight. Radiation may account for the different trends in overlap between Hawaii and Puerto Rico, as Hawaii tends to be more consistently sunny. High elevations in Puerto Rico tend to be densely vegetated, such that body temperatures may be similar to air temperatures because of low levels of radiation. We tested the robustness of our analysis to this vegetational gradient by assuming that $T_e = T_a$ for sites above 200 m in Puerto Rico. This assumption did not qualitatively alter our results.

Body temperatures tend to be closer to air temperatures for tropical ectotherms (at least for lizards) compared with temperate endotherms as vegetative cover tends to be greater (Huey et al. 2009). Additionally, behavioral thermoregulation has the potential to buffer environmental differences and to substantially influence T_e (Kearney et al. 2009). In an examination of range size along elevational gradients, McCain (2009) found that ectotherms exhibit a stronger increase in the amount of elevation spanned by a range with increasing latitude than do endotherms, suggesting the potential importance of thermoregulation. Thermoregulation may be less relevant when explicitly considering a dispersing organism as in Janzen's hypothesis. Greater genetic (Martin and McKay 2004) and phenotypic (Martin and Tewksbury 2008) divergence in tropical organisms provides evidence of reduced dispersal. The greater overlap in T_e along elevational gradients in the tropics and the potential for thermoregulation to influence body temperatures could diminish estimates of the degree to which tropical organisms are likely to be more sensitive to climate change than are temperate organisms (Deutsch et al. 2008; Tewksbury et al. 2008; Huey et al. 2009; Kearney et al. 2009).

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Supplementary data

Supplementary Data available at *ICB* online.

References

- Alpert P, Simms EL. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evol Ecol* 16:285–97.
- Anderson RV, Tracy CR, Abramsky Z. 1979. Habitat selection in two species of short-horned grasshoppers. *Oecologia* 38:359–74.
- Angilletta MJ. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford: Oxford University Press.
- Bakken GS. 1992. Measurement and application of operative and standard operative temperatures in ecology. *Integr Comp Biol* 32:194–216.
- Beierkuhnlein C, Thiel D, Jentsch A, Willner E, Kreyling J. 2011. Ecotypes of European grass species respond differently to warming and extreme drought. *J Ecol* 99:703–13.
- Belovsky GE, Slade JB. 1995. Dynamics of two Montana grasshopper populations: relationships among weather, food abundance and intraspecific competition. *Oecologia* 101:383–96.
- Bradshaw WE, Holzapfel CM. 2010. Light, time, and the physiology of biotic response to rapid climate change in animals. *Ann Rev Physiol* 72:147–66.
- Brattstrom BH. 1968. Thermal acclimation in anuran amphibians as a function of latitude and altitude. *Comp Biochem Phys* 24:93–111.
- Brattstrom BH. 1970. Thermal acclimation in Australian amphibians. *Comp Biochem Phys* 35:69–103.
- Brient F, Bony S. 2012. How may low-cloud radiative properties simulated in the current climate influence low-cloud feedbacks under global warming? *Geophys Res Lett* 39:L20807.
- Buckley LB, Kingsolver JG. 2012. The demographic impacts of shifts in climate means and extremes on alpine butterflies. *Funct Ecol* 26:969–77.
- Buckley LB, Nufio C, Kingsolver JG. Forthcoming 2013. Phenotypic clines, energy balances, and ecological responses to climate change. *J Anim Ecol*.
- Dai A, Karl TR, Sun B, Trenberth KE. 2006. Recent trends in cloudiness over the United States: a tale of monitoring inadequacies. *Bull Am Meteorol Soc* 87:597–606.
- Dai A, Trenberth KE, Karl TR. 1999. Effects of clouds, soil moisture, precipitation, and water vapor on diurnal temperature range. *J Climate* 12:2451–73.
- Dearn JM. 1977. Variable life history characteristics along an altitudinal gradient in three species of Australian grasshopper. *Oecologia* 28:67–85.
- Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc Natl Acad Sci USA* 105:6668–72.
- Dillon ME, Frazier MR, Dudley R. 2006. Into thin air: physiology and evolution of alpine insects. *Integr Compar Biol* 46:49–61.
- Dingle H, Mousseau TA, Scott SM. 1990. Altitudinal variation in life cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* (F.). *Oecologia* 84:199–206.
- Fangue NA, Hofmeister M, Schulte PM. 2006. Intraspecific variation in thermal tolerance and heat shock protein gene expression in common killifish, *Fundulus heteroclitus*. *J Exp Biol* 209:2859–72.
- Gardner JL, Peters A, Kearney MR, Joseph L, Heinsohn R. 2011. Declining body size: a third universal response to warming? *Trends Ecol Evol* 26:285–91.
- Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr Compar Biol* 46:5–17.
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407.
- Hassall M, Walters RJ, Telfer M, Hassall MRJ. 2006. Why does a grasshopper have fewer, larger offspring at its range limits? *J Evol Biol* 19:267–76.
- Helmuth B. 2009. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *J Exp Biol* 212:753–60.
- Helmuth B, Harley CDG, Halpin PM, O'Donnell M, Hofmann GE, Blanchette CA. 2002. Climate change and latitudinal patterns of intertidal thermal stress. *Science* 298:1015–7.
- Helmuth B, Kingsolver JG, Carrington E. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Ann Rev Physiol* 67:177–201.
- Hodkinson ID. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol Rev* 80:489–513.
- Huey RB, Deutsch CA, Tewksbury JJ, Vitt LJ, Hertz PE, Álvarez Pérez HJ, Garland T. 2009. Why tropical forest lizards are vulnerable to climate warming. *Proc R Soc B* 276:1939–48.
- Janzen DH. 1967. Why mountain passes are higher in the tropics. *Amer Nat* 101:233–49.
- Karell P, Ahola K, Karstinen T, Valkama J, Brommer JE. 2011. Climate change drives microevolution in a wild bird. *Nat Commun* 2:208.
- Kearney M, Shine R, Porter WP. 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proc Natl Acad Sci USA* 106:3835–40.
- Kearney MR, Matzelle A, Helmuth B. 2012. Biomechanics meets the ecological niche: the importance of temporal data resolution. *J Exp Biol* 215:922–33.
- Kingsolver JG. 1983. Thermoregulation and flight in *Colias* butterflies: elevational patterns and mechanistic limitations. *Ecology* 64:534–45.
- Kingsolver JG, Huey RB. 1998. Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Amer Zool* 38:545–60.

- Kingsolver JG, Watt WB. 1983. Thermoregulatory strategies in *Colias* butterflies—thermal-stress and the limits to adaptation in temporally varying environments. *Amer Nat* 121:32–55.
- Martin PR, McKay JK. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. *Evolution* 58:938–45.
- Martin PR, Tewksbury JJ. 2008. Latitudinal variation in sub-specific diversification of birds. *Evolution* 62:2775–88.
- McCain CM. 2009. Vertebrate range sizes indicate that mountains may be “higher” in the tropics. *Ecol Lett* 12:550–60.
- Nikolov NT, Zeller KF. 1992. A solar radiation algorithm for ecosystem dynamic models. *Ecol Model* 61:149–68.
- Nufio CR, McGuire CR, Bowers MD, Guralnick RP, Moen J. 2010. Grasshopper community response to climatic change: variation along an elevational gradient. *PLoS One* 5:1969–76.
- Ohmura A. 2009. Observed decadal variations in surface solar radiation and their causes. *J Geophys Res* 114:D00D05.
- Ozgo M, Schilthuizen M. 2011. Evolutionary change in *Cepaea nemoralis* shell colour over 43 years. *Glob Change Biol* 18:74–81.
- Porter WP, Gates DM. 1969. Thermodynamic equilibria of animals with environment. *Ecol Monogr* 39:227–44.
- Samietz J, Salser MA, Dingle H. 2005. Altitudinal variation in behavioural thermoregulation: local adaptation vs. plasticity in California grasshoppers. *J Evol Biol* 18:1087–96.
- Sarà G, Kearney M, Helmuth B. 2011. Combining heat-transfer and energy budget models to predict thermal stress in Mediterranean intertidal mussels. *Chem Ecol* 27:135–45.
- Sears MW, Raskin E, Angilletta MJ Jr. 2011. The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integr Comp Biol* 51:666–75.
- Smith MD. 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *J Ecol* 99:656–63.
- Sørensen JG, Norry FM, Scannapieco AC, Loeschcke V. 2005. Altitudinal variation for stress resistance traits and thermal adaptation in adult *Drosophila buzzatii* from the New World. *J Evol Biol* 18:829–37.
- Sunday JM, Bates AE, Dulvy NK. 2010. Global analysis of thermal tolerance and latitude in ectotherms. *Proc R Soc B: Biol Sci* 278:1823–30.
- Tewksbury JJ, Huey RB, Deutsch CA. 2008. Putting the heat on tropical animals. *Science* 320:1296–7.
- Tomanek L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species’ biogeographical distribution ranges and metabolic costs. *J Exp Biol* 213:971–9.
- Weatherhead EC, Reinsel GC, Tiao GC, Meng X-L, Choi D, Cheang W-K, Keller T, DeLuisi J, Wuebbles DJ, Kerr JB. 1998. Factors affecting the detection of trends: statistical considerations and applications to environmental data. *J Geophys Res* 103:17, 149–17, 161.
- Wild M, Gilgen H, Roesch A, Ohmura A, Long CN, Dutton EG, Forgan B, Kallis A, Russak V, Tsvetkov A. 2005. From dimming to brightening: decadal changes in solar radiation at Earth’s surface. *Science* 308:847–50.