Journal of Thermal Biology 59 (2016) 52–57

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Altitudinal variation in bumble bee (*Bombus*) critical thermal limits

K. Jeannet Oyen*, Susma Giri, Michael E. Dillon

Department of Zoology and Physiology & Program in Ecology, University of Wyoming, Laramie, WY 82071, USA

ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form
29 April 2016
Accepted 30 April 2016
Available online 4 May 2016

Keywords:
Bumble bees
Critical thermal limits
Elevation
CT_{min}
CT_{max}
T_{rec}

ABSTRACT

Organism critical thermal limits are often tightly linked to current geographic distribution and can therefore help predict future range shifts driven by changing environmental temperatures. Thermal tolerance of diverse organisms often varies predictably with latitude, with upper thermal limits changing little and lower thermal limits decreasing with latitude. Despite similarly steep gradients in environmental temperatures across altitude, few studies have investigated altitudinal variation in critical thermal limits. We estimated critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}) and recovery temperature (T_{rec}) by tracking righting response of three bumble bee species during thermal ramps: *Bombus huntii* collected from 2180 m asl, and *Bombus bifarius* and *Bombus sylvicola* collected from 3290 m asl in Wyoming, USA. Overall, larger bees could tolerate more extreme temperatures, likely due to a thermal inertia driven lag between core body temperatures and air temperatures. Despite their smaller size, high altitude bumble bees tolerated colder air temperatures: they had ~1 °C lower CT_{min} and recovered from cold exposure at ~3–4 °C lower air temperatures. Conversely, low altitude bees tolerated ~5 °C hotter air temperatures. These altitudinal differences in thermal tolerance parallel differences in average daily minimum (1.2 °C) and maximum (7.5 °C) temperatures between these sites. These results provide one of the few measurements of organism thermal tolerance across altitude and the first evidence for geographical differences in tolerance of temperature extremes in heterothermic bumble bees.

1. Introduction

Geographic distributions of diverse organisms have shifted in concert with recent changes in global temperatures (Walther et al., 2002; Parmesan, 2006; Chen et al., 2011). These shifting distributions likely reflect, in part, a mismatch between local temperatures and the temperatures that organisms can tolerate (Calosi et al., 2010; Sunday et al., 2012). As such, studies of thermal tolerance have provided key mechanistic links between organism physiology and species distributions (Kellermann et al., 2012; Andersen et al., 2015). In particular, differences in thermal tolerance can help explain which species must move to track climate warming and which species can tolerate increasing temperatures (Deutsch et al., 2008; Hoffmann et al., 2013).

The thermal tolerance of diverse organisms often matches the temperature extremes they are likely to encounter. Organisms at high latitudes and altitudes experience large seasonal and diurnal temperature fluctuations (Wang and Dillon, 2014) and therefore should be able to tolerate hotter maximum temperatures and colder minimum temperatures than organisms from lower latitudes and altitudes. Indeed, a large body of work documents the relationship between the organism thermal limits and latitude, revealing strong increases in critical thermal maxima (CT_{max}) and decreases in critical thermal minima (CT_{min}) at higher latitudes (Addo-Bediako et al., 2000; Overgaard et al., 2011; Sunday et al., 2014). Altitudinal gradients are characterized by strong temperature gradients across even shorter geographic distances (Dillon et al., 2006; Fig. 1), but comparatively few studies have measured altitudinal variation in thermal tolerance (Gaston and Chown, 1999; Huang et al., 2006; Sheldon and Tewksbury, 2014).

Shifts in elevational and latitudinal ranges have recently been documented for bumble bees across Europe and North America (Kerr et al., 2015). Shifts to higher elevations and range compressions among southern bumble bee species appear unrelated to changes in land or pesticide use, and are unlikely to reflect shifts in resources, but strongly correlate with changes in climate (Kerr et al., 2015). The thermal physiology of bumble bees may play a critical role in these recently observed responses to climate warming, but thermal tolerance of these vital pollinators has rarely been measured (Goller and Esch, 1990; Owen et al., 2013; Martinet et al., 2015). Perhaps because bumble bees are heterothermic and can regulate body temperature independent of environmental temperature both during foraging trips and while in

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* Correspondence to: Dept. Zoology and Physiology, University of Wyoming, 1000 East University Avenue - Dept 3166, Laramie, WY 82071, USA.
E-mail address: koyen@uwyo.edu (K.J. Oyen).

http://dx.doi.org/10.1016/j.jtherbio.2016.04.015
0306-4565/

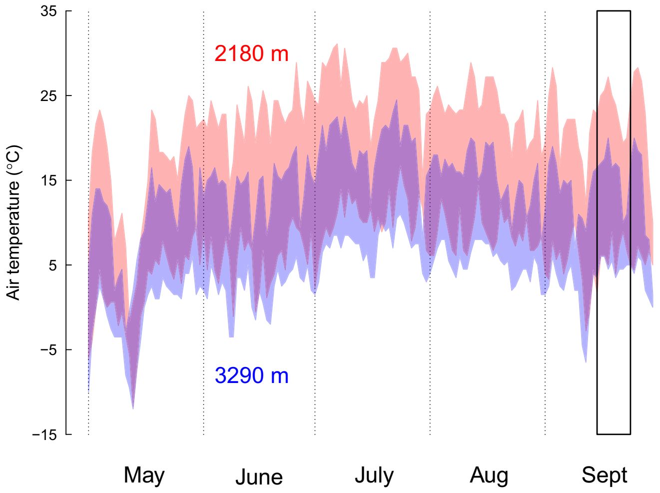

Fig. 1. Air temperatures recorded near collection localities. Shaded regions show daily maximum and minimum air temperatures from weather stations near the low (2180 m, red) and high (3290 m, blue) elevation sites throughout the growing season in 2014. The sampling period of September 15–24 is indicated by the black rectangle. Dotted vertical lines indicate the first day of every month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the hive (Heinrich, 1974), tolerance of extreme temperatures in these organisms has been understudied. However, even their exceptional thermoregulatory abilities cannot protect them from periodic exposures to extreme high and low temperatures (Heinrich, 1976).

Tolerance of insects to extreme temperatures has been measured in diverse ways (Terblanche et al., 2011), including measurements of lower lethal limits (Lee and Denlinger, 1991), of changes in metabolic rate (Lighton and Turner, 2004), and of knockdown temperatures (Huey et al., 1992). Behavioral estimates of lower and upper critical temperatures, though sometimes less clearly tied to physiological thresholds (Hazell and Bale, 2011) can be measured as the minimum (CT_{min}) and maximum (CT_{max}) temperatures at which an organism loses critical motor function, failing to right itself (Fry, 1967), thereby losing the ability to escape from conditions that could lead to its death (Lowe and Vance, 1955; Hutchison, 1961). Thus, although these behavioral thermal limits provide limited information about the underlying physiological mechanisms, they are nevertheless ecologically relevant because they reveal the ambient temperature extremes at which fitness is likely to be severely reduced (Cowles and Bogert, 1944). We measured behavioral critical thermal limits and recovery temperatures (T_{rec}, the temperature at which bees recovered from chill-coma) of bumble bees collected across a 1000 m altitudinal gradient. As expected given the biophysics of heat transfer, larger bees failed at more extreme (hotter and colder) air temperatures. After accounting for mass effects, we found reduced cold and heat tolerance for bumble bees collected from high altitudes.

2. Materials and methods

2.1. Study sites and collections

We collected male and female (worker) bumble bees from two sites in southeast Wyoming, selected for their proximity and range of elevations: the University of Wyoming campus (2180 m; N 41° 18.767′, W 105° 34.912′), and near Libby Flats in Medicine Bow National Forest (3290 m; N 41° 20.527′, W 106° 17.840′). Because growing seasons are not synchronous across altitude, species composition varied between the two sites. *Bombus huntii* dominated the low-elevation site and *Bombus bifarius* and *Bombus sylvicola* were the most common species at the high-elevation site during the collection period (September 15–24, 2014). Bees were collected by hand net and immediately transported to the lab in a cooler without ice (to standardize temperatures experienced just prior to testing) for subsequent determination of critical thermal limits within 2 h of collection.

These sites differed in the maximum and minimum air temperatures potentially experienced by bees at these altitudes throughout the growing season (Fig. 1; average daily maximum temperatures of 22.1 and 14.6 °C and average minimum daily temperatures of 6.4 and 3.4 °C for low and high altitude sites, respectively). Air temperature differences between sites were similarly pronounced during the field collection period (Fig. 1, black rectangle; average maximum daily temperatures of 23.5 and 15.9 °C and average minimum daily temperatures of 6.0 and 4.8 °C for low and high altitude sites, respectively). Although operative temperatures (Bakken, 1992) were not available, these air temperature data point to the differences in operative thermal environments commonly experienced by organisms at different altitudes. Between foraging bouts and at night, bumble bees return to nests in which temperature is regulated at relatively high set points (~30 °C) that do not appear to vary geographically (reviewed by Seeley and Heinrich 1981). Bumble bee workers therefore likely experience at least nightly acclimatization to high nest temperatures but have to contend with site-specific air temperatures during daily foraging bouts. Thermal tolerance metrics could therefore reflect combined effects of evolutionary history, population differentiation and, to a lesser extent given nest temperature regulation, acclimatization to local conditions.

2.2. Measurement of thermal tolerance

After removing pollen loads, bees were weighed to the nearest mg (Acculab ALC 210.4, Sartorius, NY, USA) and then placed in individual chambers (2.5 cm diameter) milled in a solid aluminum block (14 × 15 cm). Each chamber had an acrylic lid with a ~2 mm hole, where a pin could be inserted to flip bees without affecting chamber temperatures. The block was mounted on a thermoelectric plate (TEC1-12709, 40 × 40 cm, 90 W, $\Delta T = 67$ °C), with the active side of the TEC and the block insulated from room air within a foam cooler (30 × 25 × 35 cm and 4 cm thick). A K-type thermocouple placed within a chamber on the block measured air temperatures used by a proportional integral derivative controller (Auber Instruments, GA, USA) to regulate chamber temperature. A separate T-type thermocouple placed within a central chamber on the block tracked air temperatures at which bees reached thermal limits. Preliminary experiments revealed little variation in temperature among chambers (temperatures at the four corners of the block differed from the center of the block by < 0.2 °C on average) or vertically within individual chambers (at 1 cm above the chamber floor, a distance higher than all bees tested, temperature differed by a maximum of 0.4 °C relative to the chamber floor).

For each experimental run, eight bees were each placed in individual chambers on the block and held at 22 °C for 15 min before temperature was ramped to 0 °C at a rate of 0.25 °C min^{-1}, a rate commonly used in the literature (Chidawanyika and Terblanche, 2011; Sformo et al., 2011; Boardman et al., 2012). As temperatures dropped, bees gradually became sluggish, at which point they were flipped onto their backs to induce a righting response. CT_{min} was taken as the highest chamber air temperature at which bees were unable to right themselves within 30 s after being flipped. The block temperature was allowed to drop 0.5 °C below the last CT_{min} value measured and bees were held at that temperature for two minutes to ensure that all individuals spent a minimum