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RESEARCH ARTICLE

Critical thermal limits of bumblebees (*Bombus impatiens*) are marked by stereotypical behaviors and are unchanged by acclimation, age or feeding status

K. Jeannet Oyen* and Michael E. Dillon

ABSTRACT

Critical thermal limits often determine species distributions for diverse ectotherms and have become a useful tool for understanding past and predicting future range shifts in response to changing climates. Despite recently documented population declines and range shifts of bumblebees (genus *Bombus*), the few measurements of thermal tolerance available for the group have relied on disparate measurement approaches. We describe a novel stereotypical behavior expressed by bumblebee individuals during entry into chill coma. This behavioral indicator of minimum critical temperature (CT_{min}) occurred at ambient temperatures of 3–5°C (approximately 7–9°C core temperatures) and was accompanied by a pronounced CO_2 pulse, indicative of loss of spiracle function. Maximum critical temperature (CT_{max}) was indicated by the onset of muscular spasms prior to entering an unresponsive state and occurred at ambient temperatures of approximately 52–55°C (42–44°C core temperatures). Measurements of CT_{min} and CT_{max} were largely unaffected by acclimation, age or feeding status, but faster ramping rates significantly increased CT_{max} and decreased CT_{min}. This high-throughput approach allows rapid measurement of critical thermal limits for large numbers of individuals, facilitating large-scale comparisons among bumblebee populations and species – a key step in determining current and future effects of climate on these critical pollinators.

KEY WORDS: Thermal tolerance, Ramping rate, Chill coma, Metabolism, CT_{min}, CT_{max}

INTRODUCTION

At extreme cold and hot temperatures organisms lose neuromuscular function (Robertson et al., 2017) making them unable to feed or escape from predators (Cowles and Bogert, 1944; Huey and Kingsolver, 1989). The coldest and hottest temperatures at which organisms can maintain muscle control (CT_{min} and CT_{max}, respectively) may therefore delineate climates where populations can persist (Calosi et al., 2010; Ayrinhac et al., 2004; Overgaard et al., 2014) and vary predictably across latitude and altitude for diverse ectotherms (Gaston and Chown, 1999; Addo-Bediako et al., 2000; Sheldon and Tewksbury, 2014; Oyen et al., 2016). Furthermore, thermal tolerance and its plasticity are key traits for predicting distributions of diverse organisms in response to changing climates (Ayrinhac et al., 2004; Kellermann et al., 2009; Rezende et al., 2011).

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Received 30 June 2017; Accepted 7 March 2018

Shifts in elevational and latitudinal ranges have been recently documented for bumblebees across Europe and North America (Kerr et al., 2015). Shifts to higher elevations and range compressions among southern bumblebee 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). Differences among bumblebee populations and species in tolerance of temperature extremes may in part underlie these recently observed responses to climate warming (Hamblin et al., 2017). However, despite their ecological (Goulson et al., 2008) and economic (Morandin et al., 2001; Velthuis and van Doorn, 2006) importance and broad geographic distributions (Goulson, 2010), thermal tolerance of bumblebees (genus *Bombus*) has rarely been measured (but see Goller and Esch, 1990; Owen et al., 2013; Martinet et al., 2015; Oyen et al., 2016; Hamblin et al., 2017), a surprising gap given a long history of study of bumblebee thermal biology (Heinrich, 1975). Bumblebees are heterothermic, capable of regulating body temperatures across a large range of ambient temperatures (Heinrich, 1976). Nevertheless, like other organisms, they lose physiological function at extreme low and high temperatures. By directly measuring muscle potentials, Goller and Esch (1990) found that three bumblebee species lost flight muscle activity (i.e. entered chill coma; MacMillan and Sinclair, 2011) when thorax temperatures were below approximately 7–8°C. More recently, Oyen et al. (2016) used a righting response assay to measure CT_{min} and CT_{max} of three bumblebee species. Both CT_{min} (approximately 9–10°C) and CT_{max} (40–45°C) declined with altitude, suggesting that alpine bumblebees are more tolerant of cold extremes and less tolerant of extreme heat. CT_{max} of three urban bumblebee species was measured as the temperature (44–46°C) at which they lost postural control, and was correlated with population responses to urban warming (Hamblin et al., 2017).

These limited estimates of bumblebee thermal tolerance have been measured by different approaches, potentially limiting their utility in broader-scale comparative work, which requires standardized, repeatable methods (Terblanche et al., 2007; Sinclair et al., 2015). Although changes in muscle potentials (Goller and Esch, 1990; Findsen et al., 2014; Andersen et al., 2015) and in nervous system function (Anderson and Mutchmor, 1968; Bradfisch et al., 1982; Robertson, 2004; Robertson et al., 2017) can provide direct physiological evidence of thermal limits, the difficulty of these experimental approaches make them less attractive for large-scale comparative studies. Conversely, the simplicity of measuring righting response (Fry, 1967) has led to its prodigious use as a metric of thermal tolerance, but righting response may be affected by differences in motivation (bees may choose not to right even when they are able to; Hazell and Bale, 2011), so it is unclear whether these behavioral differences

Journal of Experimental Biology (2018) 221, jeb165589. doi:10.1242/jeb.165589

accurately represent physiological thresholds (Lutterschmidt and Hutchison, 1997a; Sinclair et al., 2015). Bumblebees may fail to right at non-stressful room temperatures and remain on their backs for minutes to hours, occasionally righting at much lower temperatures (Oyen et al., 2016).

In addition, thermal tolerance may vary in response to many intrinsic factors including nutritional status, age and previous temperature exposure. Cold tolerance depends strongly on maintenance of ion homeostasis for chill-susceptible insects (Coello Alvarado et al., 2015; MacMillan et al., 2015). Hemolymph ion balance can be altered by food intake (Shreve et al., 2007; Coleman et al., 2015; Koštál et al., 2016), so cold tolerance can change in response to uptake of dietary salts and sugars. For example, in *Drosophila*, increased dietary salts such as KCl and NaCl led to faster recovery from chill coma (Yerushalmi et al., 2016), whereas increased dietary sugars reduced cold tolerance (Colinet et al., 2013b). Whether dietary sugars alter thermal tolerance of bumblebees, which feed primarily on floral nectar, is unknown.

Thermal tolerance can also vary with age (Bowler and Terblanche, 2008). Many studies have shown that thermal tolerance traits vary among and within life stages (Davison, 1969; Bale et al., 1989; Crill et al., 1996; Nyamukondiwa and Terblanche, 2009; Chidawanyika et al., 2017). Variation in thermal tolerance within life stages (e.g. larvae or adults) may be due to age-related morphological and physiological re-organization or to senescence (Bowler and Hollingsworth, 1966; Bowler, 1967; Bowler and Terblanche, 2008; Colinet et al., 2013a). High thermal tolerance in pre-adult stages is often followed by marked declines in thermal tolerance after eclosion to the adult stage (Bowler, 1967; Pappas et al., 2007; Colinet et al., 2013a). To our knowledge, thermal tolerance of larval and pupal bumblebees has not been measured; for adult bumblebees, muscle physiology and metabolism can change markedly with age (Skandalis et al., 2011), so bumblebees may show age-related shifts in thermal tolerance.

Previous temperature exposure can also alter thermal tolerance in insects. Over short time scales, differences in ramping rates often alter thermal tolerance (Overgaard et al., 2006; Terblanche et al., 2007). For example, *Drosophila* up-regulate heat shock protein (HSP) expression more at slower ramping rates such that they can tolerate hotter temperatures (higher CT_{max}) and suffer less cellular damage after heat exposure (Sørensen et al., 2013). Conversely, slower ramping rates may allow core temperatures to more closely track external temperatures, potentially resulting in more conservative estimates of thermal limits than suggested by faster ramping rates. Compared with other insects, bumblebees are large heterotherms (Heinrich, 1976), and can generally maintain high thoracic temperatures at ambient temperatures between ∼9 and 30°C (Heinrich, 1972). Therefore, even at more extreme temperatures associated with CT_{min} and CT_{max}, thoracic temperatures may be offset from ambient temperatures, potentially with a lag dependent on body size and ramping rate (Gates, 1980).

The ability to quickly mount a physiological or biochemical response to stressful environmental temperatures may facilitate persistence in changing climates (Somero, 2010). Therefore, acclimation capacity is a potentially important factor not only for determining plasticity in thermal tolerance traits but also persistence under current and future climate change (Stillman, 2003; Gunderson et al., 2017). The mechanisms allowing insects to increase thermal tolerance in response to stressful temperatures include changes in membrane composition (Overgaard et al., 2008), up-regulation of HSPs (Joplin et al., 1990; Colinet et al., 2010) and extensive changes in the transcriptome and metabolome (Teets et al., 2012). Little is known about the response of bumblebee thermal tolerance to acclimation. Queen bumblebees show tissue-specific changes in HSPs during diapause (Kim et al., 2008) and both queens and workers have increased survival at low temperatures following a cold exposure (Owen et al., 2013). These limited lines of evidence suggest that bumblebee critical thermal limits could also change in response to thermal history.

A better understanding of the potential role of thermal tolerance in past and future responses of bumblebees to changing climates requires an easily implemented approach to measuring thermal tolerance that is also clearly tied to organism physiology and knowledge of plasticity of thermal tolerance over short timescales (Allen et al., 2016). Here, we validate a new high-throughput method for measurement of CT_{min} and CT_{max} in bumblebees. We show that stereotypical behaviors (previously undescribed in bumblebees) are tightly linked to a final release of CO_2 due to loss of spiracle control, clearly marking entry into chill coma (CT_{min}) (Lighton and Turner, 2004; Sinclair et al., 2004; MacMillan et al., 2012) and are likely to be indicative of loss of neuromuscular function (Robertson and Money, 2012; Robertson et al., 2017). We further show that bumblebee CT_{max} is indicated by the onset of muscular spasms and the measurement of CT_{max} is not influenced by previous measurement of CT_{min}. Using this high-throughput method, we find that estimates of CT_{min} and, to a lesser extent, CT_{max} are generally consistent among individuals within a nest. Thermal limits are largely unaffected by acclimation temperature, feeding status, age or body mass, but are influenced by temperature ramping rate.

MATERIALS AND METHODS

Animal rearing

All experimental animals came from three commercially reared *Bombus impatiens* colonies (Koppert Biological Systems, Howell, MI, USA), which each contained ∼250 female workers, the natal queen, and a bag of proprietary sucrose solution. One colony was used for initial measurements of critical thermal limits, for determining the effect of CT_{min} on CT_{max}, and also for respirometry and core temperature measurements. A second colony was used for acclimation treatments, and a third colony was used to determine the effects of ramping rate, age and feeding status on critical thermal limits. All colonies were kept in the laboratory at ∼22°C under a 12 h:12 h day:night cycle. Colonies were provided with ∼10 g of ground fresh-frozen pollen (Brushy Mountain Bee Farm, Moravian Falls, NC, USA) every other day. Female workers were taken directly from colonies immediately prior to experiments, except where otherwise noted.

Determination of CT_{min} and CT_{max}

After removing pollen loads, bees were weighed to the nearest milligram (Acculab ALC 210.4, Sartorius, NY, USA) and then placed in individual 2-dram clear glass vials (2 cm width×5 cm height) with acrylic lids and two ∼2 mm air holes. The inside of vials was first coated with INSECT-a-SLIP (BioQuip, Rancho Dominguez, CA, USA) to prevent bees from climbing the walls and then placed in wells (16 total, 20 mm diameter, 3 mm deep) milled in a solid aluminium block. A slot within each well housed a T-type thermocouple (30-gauge) in contact with both the aluminium well and the side wall of each vial. These 'vial' temperatures were individually tracked using two TC-08 thermocouple data loggers (Pico Technology, Tyler, TX, USA). The aluminium block was mounted on two thermoelectric plates (TEC1-12706, 40×40 cm, 12 V, 92 W, ΔT=63°C), with the active side of the TEC and the

Journal of Experimental Biology (2018) 221, jeb165589. doi:10.1242/jeb.165589

block insulated from room air within a foam cooler (40×30×15 cm and 5 cm thick, rigid foam insulation). A K-type thermocouple mounted on the block as described above measured vial and block temperatures used by a proportional integral derivative controller (Auber Instruments, Alpharetta, GA, USA) to regulate temperature.

For each experimental run, 16 bees were placed in individual vials on the block and held at 22°C for 10 min before vial temperature was ramped to −5°C at a rate of ∼0.25°C min^{-1} (realized ramping rates were within 0.02°C min^{-1} across runs). As temperature decreased, bees were continuously monitored for signs of curling (see Movie 1 and Results section for a full description of CT_{min} behavior). Bees were immediately removed from the block following CT_{min} and allowed to warm to room temperature (approximately 20–22°C) on the bench top at a rate of ∼0.15°C min^{-1}. After the aluminium block had equilibrated to room temperature (∼20 min), we immediately started CT_{max} trials. Bees were returned to the block and held at 22°C for 10 min followed by ramping vial temperature to 65°C at a rate of 0.25±0.02°C min^{-1}. As temperatures rose, bees became agitated, lost muscular coordination, and began to spasm, at which point CT_{max} was recorded (see Movie 2 and Results section for full description of CT_{max} behavior).

Determination of bumblebee core thoracic temperatures

Tracking of vial temperatures allows for high-throughput measurement of bee responses to ambient temperatures, facilitating characterization of ecologically relevant thermal limits for populations of bees (Table 1). However, both to confirm that core temperatures track vial temperatures and to estimate core temperatures associated with CT_{min} and CT_{max}, we measured core temperatures in a second set of ramping experiments. Fine 37-gauge thermocouple wire (Omega Engineering, Stamford, CT, USA) was implanted at 3 mm depth (typical thorax depth is ∼7 mm) into a small hole near the midline of the thorax between the wing bases created with an insect pin and subsequently sealed with beeswax (bees lived up to 2 weeks after the implant was removed, suggesting limited long-term effects of the approach). Bees with implanted thermocouples were placed in vials on the aluminium block (as described above) and cooled or heated to CT_{min} or CT_{max}, respectively, at nominal rates of 0.1, 0.25 and 1°C min^{-1} (vial temperatures were simultaneously monitored as described above). Realized rates of heating and cooling for both core thoracic and vial temperatures are reported in Table 2. For clarity, we have used the labels 0.1, 0.25 and 1°C min^{-1} throughout. Unless otherwise noted, we report vial temperatures throughout the manuscript; however, the summary values in Table 2 allow estimation of associated core temperatures.

Table 1. Thermal tolerance of *Bombus impatiens* was largely unaffected by diverse experimental conditions

Experiment (*N*)	CT_{min} (°C)	CT_{max} (°C)	TTB (°C)	Mass (mg)
$CT_{min/max}$ (15)	3.7±1.6	52.7±4.4	48.9±4.1	148±37
CT_{max} (15)	–	53.1±3.0	–	165±38
Acclimation				
Nest (48)	4.7±1.3	53.7±4.8	49.0±5.0	139±43
4°C (12 h) (16)	4.6±1.6	53.9±4.3	49.3±4.9	144±31
15°C (72 h) (16)	4.6±1.1	51.5±3.4	46.9±3.9	124±33
32°C (72 h) (16)	4.8±1.6	52.8±5.9	48.0±6.3	167±48
Ramping rate (°C min^{-1})				
0.1 (14)	3.4±1.2^{b}	48.7±3.5^{b}	45.3±3.9	158±27
1.0 (15)	1.4±1.0^{a}	58.5±4.5^{a}	57.1±4.6	163±31
Age (days)				
3 (8)	3.5±1.2	56.6±2.4^{a}	53.1±2.7	190±37
4 (10)	3.4±1.4	51.1±3.2^{b}	47.7±3.2	169±26
7 (14)	3.0±1.2	55.6±2.5^{a}	52.6±2.8	177±51
Feeding status				
Fed (16)	3.6±1.0	52.9±4.0	49.2±4.2	185±62
Unfed (16)	3.0±1.4	53.6±3.4	50.5±3.8	175±41

Critical thermal minimum and maximum (CT_{min} and CT_{max}, respectively) and thermal tolerance breadth (TTB) across experiments. Values are means±s.d. of vial temperatures, with sample sizes given in parentheses. Lowercase letters (a,b) indicate statistical differences within experiments. See Results section, 'Ramping rate, age and feeding status' for statistical analyses.

Respirometry

We measured CO_2 production of bumblebees during cold ramps using a flow-through respirometry system with data acquisition software (ExpeData, Sable Systems International, Las Vegas, NV, USA). For each experimental run, a single bee was placed in a glass chamber (75 mm length×20 mm diameter, 53 ml volume) with aluminium end-caps sealed with rubber O-rings. Dry, CO_2-free air was pumped at a flow rate of 100±2 ml min^{-1} through the chamber containing the bee as well through an identical but empty 'baseline' chamber using regulated pumps (SS4, Sable Systems International). The respirometry chambers rested on a temperature-controlled aluminium block, attached to a thermoelectric cooler and were controlled using a proportional integral derivative controller (see above) to ramp at 0.25°C min^{-1}. A 36-gauge (∼0.5 mm diameter, 2 mm long) T-type thermocouple inserted through one end of the baseline chamber was attached to a digital thermocouple reader (Omega HH23A), to monitor air temperature throughout experiments (recorded approximately every 4 min or ∼1°C, with intervening temperatures linearly interpolated). A BL-2 baselining unit (Sable Systems International) controlled by the data acquisition software allowed for automatic switching between the baseline and experimental (with bee) chambers. Excurrent air was subsampled at a rate of 50±3 ml min^{-1} (SS4, Sable Systems International) through a LI-COR LI-7000 (LI-COR, Lincoln, NE, USA) which measured CO_2 (p.p.m.) and water vapor pressure (kPa). The LI-7000 was zeroed and spanned daily, using a column of magnesium perchlorate and ascarite and primary standard 1020 p.p.m. CO_2, respectively. Both the BL-2 and LI-7000 were connected to a desktop computer via a 16-bit data acquisition interface (Sable Systems International UI2, basic accuracy 0.03%). The temperature profile during metabolic experiments mirrored the steps described above for CT_{min}: 22°C to −5°C at 0.25°C min^{-1}. CO_2 measurements continued for 10 min after observation of curling behavior to verify the lack of subsequent CO_2 pulses. A minimum of 60 s of baseline data at the beginning and end of each experiment allowed for lag and drift correction of traces prior to analyses.

Acclimation

To test for effects of acclimation on CT_{min} and CT_{max}, worker bees were removed from a single nest and placed in separate feeding containers (19×14×9 cm, with fifteen 2 mm air holes) for 12 h at 4°C, or 72 h at each of 15 and 32°C. Pilot experiments demonstrated that bees were unable to feed below 13°C and therefore could not be held below this threshold for longer than 12 h (normal day–night cycle), and that bees held above 34°C for any length of time suffered high mortality. We therefore selected 15°C as an intermediate cool temperature at which bees foraged normally and could therefore be held for up to 72 h without high mortality and 32°C as the highest temperature at which bees survived and maintained normal feeding behaviors. The feeding containers were placed in a 1280 oz PowerChill Thermoelectric Cooler (Coleman Outdoor Company, Golden, CO, USA) modified with heat lamps and timed lights (12 h:12 h light:

Table 2. Bumblebee core temperatures lag behind vial temperatures during temperature ramps

Nominal cooling/heating rate (°C min^{-1}) (*N*)	Vial cooling/heating rate (°C min^{-1})	Core cooling/heating rate (°C min^{-1})	Core–vial slope	Offset range	Mass (mg)
Cooling					
0.10 (7)	−0.11±0.002	−0.09±0.01	0.80±0.03[a]	4.0, 5.3	151.5±47
0.25 (8)	−0.24±0.03	−0.20±0.02	0.88±0.09[b]	3.5, 4.3	152.2±42
1.0 (8)	−0.96±0.05	−0.74±0.03	0.77±0.05[a]	5.2, 6.7	145.3±47
Heating					
0.10 (8)	0.12±0.01	0.07±0.01	0.57±0.08[a]	−7.8, −17.5	154.3±37
0.25 (8)	0.29±0.02	0.18±0.01	0.61±0.05[a]	−6.4, −15.2	144.2±26
1.0 (11)	0.98±0.05	0.67±0.04	0.67±0.04[b]	−6.4, −13.7	145.3±47

Heating and cooling rates of bee core temperatures were less than vial heating and cooling rates, such that the slope of the relationship between core and vial temperature was always less than 1 (Figs 2 and 3; a slope of 1 would indicate core temperatures perfectly tracked vial temperatures), resulting in increasingly larger offsets at more extreme temperatures. The offsets (differences between core and vial temperatures) are given for the extreme ranges of vial temperatures at which bees failed across all experiments (1.4–8.0°C for CT_{min} and 42.0–64.6°C for CT_{max}; see Figs S1–S6). Values are means±s.d. with sample sizes indicated by numbers in parentheses. Statistically significant differences between ramping rates in core–vial slopes are indicated by lowercase letters a and b (see Results section, 'Differences between thoracic and vial temperatures' for statistical analyses).

dark). A K-type thermocouple mounted within the cooler measured air temperatures used by a PID controller (Auber Instruments) to regulate temperature. Air temperatures were verified using HOBO Pendant Loggers (Onset Computer Corporation, Pocasset, MA, USA). Bees were fed nectar (50% sucrose–water solution) *ad libitum* (for those kept at 32 or 15°C) or only once (for those held for 12 h at 4°C). Following acclimation, bees were weighed and then tested for CT_{min} and CT_{max} as described above. To control for run effects and for direct comparison with acclimated bees, eight additional bees were taken directly from the hive and tested with acclimated bees.

Temperature ramping rate

Because ramping rates may alter estimates of thermal limits (Terblanche et al., 2007), we additionally measured critical thermal limits with temperatures ramped at nominal rates of 1°C min^{-1} and 0.1°C min^{-1}. Realized cooling rates were 0.90±0.03°C min^{-1} and 0.095±0.004°C min^{-1}, respectively, and realized heating rates were 0.99±0.13°C min^{-1} and 0.10±0.01°C min^{-1}, respectively. For clarity, we have used the labels 1°C min^{-1} and 0.1°C min^{-1} throughout.

Age

To determine whether critical thermal limits change with age (bumblebee physiology can vary with age; Skandalis et al., 2011), newly emerged individuals from a single nest (clearly indicated by gray pile and curled wings) were marked with unique colors indicating emergence date. We measured CT_{min} and CT_{max} for 3-, 4- and 7-day-old bees to span the range of ages included in previous experiments.

Feeding

Feeding status may affect critical thermal limits due to resource availability or mass differences. We therefore measured CT_{min} and CT_{max} of bees removed from a single nest, placed in separate containers and provided with either water or nectar for 5 h immediately following the 12 h night cycle. Pilot experiments revealed that bees did not survive the 4–5 h experiment if previously deprived of nectar for more than 5 h.

Analyses

We used ANOVA to compare thermal tolerance metrics among treatment groups with mass as a covariate and *post hoc* comparisons by Tukey's honest significant difference (HSD) test. We compared variance in thermal tolerance using *F*-tests. We used Pearson's *r* to evaluate correlations between core and vial temperatures. Unless otherwise noted, means are reported with standard deviations (s.d.).

RESULTS

Measurements of CT_{min} and CT_{max} in bumblebees

Critical thermal limits of bumblebees were indicated by stereotypical behaviors, which occurred spontaneously, without stimulus (see Movie 1 of CT_{min} and Movie 2 of CT_{max} behavior). As bumblebees approached CT_{min}, they were largely motionless due to cold temperatures but still responsive to stimulation with a metal probe. At CT_{min}, the bees spontaneously began moving, typically rocking back and forth. Wings would then flutter vigorously as legs adducted beneath the abdomen. Lastly, the abdomen, head and antennae would curl ventrally, often causing the bee to fall over. At this stage bees were completely unresponsive when stimulated. After measurement of CT_{min}, over 95% of bees survived longer than 24 h and those placed back in the nest survived for up to 2 weeks.

Critical thermal maxima of *B. impatiens* was determined as the onset of muscular spasms, a metric often used to determine upper critical thresholds of ectotherms (reviewed by Lutterschmidt and Hutchison, 1997a,b). As bees approached this limit, the wings fluttered as the head and antennae, normally held erect, curled ventrally. Subsequently, the abdomen adducted, the wings unfolded and spread laterally, and the stinger extended before the bee became still. Bees typically survived 2–10 h after measurement of CT_{max} with fewer than 30% of bees surviving 24 h or longer.

CT_{min} of sister bumblebees taken from the same nest occurred at vial temperatures of 3.7±1.6°C (range 1.4–7.2°C) and did not vary significantly with mass ($F_{1,13}$<0.001, P=0.989; Fig. 1, open blue symbols; Table 1). CT_{max} measured immediately after measurement of CT_{min} (52.7±4.4°C; Fig. 1, red symbols filled with blue; Table 1) did not differ significantly from measurements of CT_{max} taken independently (53.1±3.0°C; Fig. 1, open red symbols; Table 1; $F_{1,28}$=0.122, P=0.730). CT_{max} (range 45.0–61.0°C vial temperatures in this experiment) was more variable than CT_{min} ($F_{14,14}$=7.2, P<0.001), but increased variance did not appear to be caused by measuring CT_{max} immediately after CT_{min} because variance of CT_{max} was similarly high when CT_{max} was measured independently ($F_{14,14}$=2.1, P=0.183). As with CT_{min}, in this experiment mass did not affect CT_{max} ($F_{1,28}$=0.4, P=0.533).

Differences between thoracic and vial temperatures

Across 23 bees varying in body mass from 96 to 243 mg (mean 149±44 mg), core temperatures cooled more slowly than vial temperatures (Fig. 2; Table 2). The slope of core relative to vial temperature varied with cooling rate (ANOVA, $F_{2,20}$=6.35, P=0.007), with bees ramped at 0.25°C min^{-1} having a significantly

Journal of Experimental Biology (2018) 221, jeb165589. doi:10.1242/jeb.165589

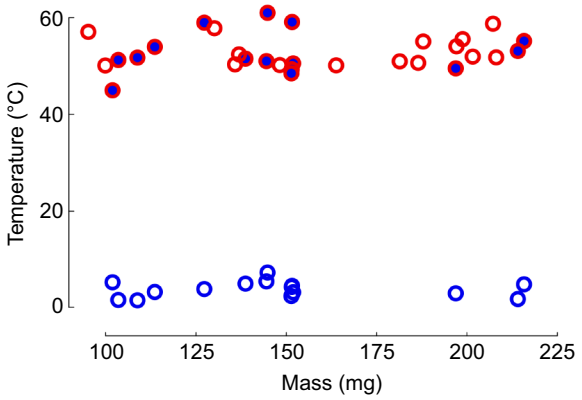

Fig. 1. Critical thermal limits of *Bombus impatiens* vary with body mass. CT_{min} (open blue symbols) and CT_{max} of bumblebees with CT_{max} measured after CT_{min} (blue-filled red symbols) and independently (open red symbols). There was no effect of CT_{min} on subsequent measurements of CT_{max} ($F_{1,28}$=0.4, P=0.533), and no effect of mass on CT_{min} or CT_{max} ($F_{1,13}$<0.001, P=0.989). See Results section, 'Measurement of CT_{min} and CT_{max} in bumble bees' for statistical analyses.

steeper slope (more closely tracking vial temperatures) than bees ramped at 0.1 or 1.0°C min^{-1} (Tukey's HSD, P=0.025 and P=0.011, respectively), which were indistinguishable (P=0.959). Slopes did not vary significantly with mass for any of the ramping rate treatments (ANOVA, all P>0.255). Because slopes were shallower than 1, the difference between core and vial temperature increased as bees were cooled (and varied with ramping rate, Table 2), ranging from 3.5–5.2°C at vial temperatures of 8°C to 4–6.7°C at vial temperatures of 1.4°C (Table 2; these vial temperatures encompass the extreme values recorded across all CT_{min} experiments).

Across 30 bees varying in body mass from 101 to 231 mg (mean 151±37 mg), core temperatures increased more slowly than vials (Fig. 3; Table 2). The slope of core relative to vial temperature depended on heating rate (ANOVA, $F_{2,27}$=9.81, P<0.001), with bees ramped at 1.0°C min^{-1} having significantly steeper slopes than

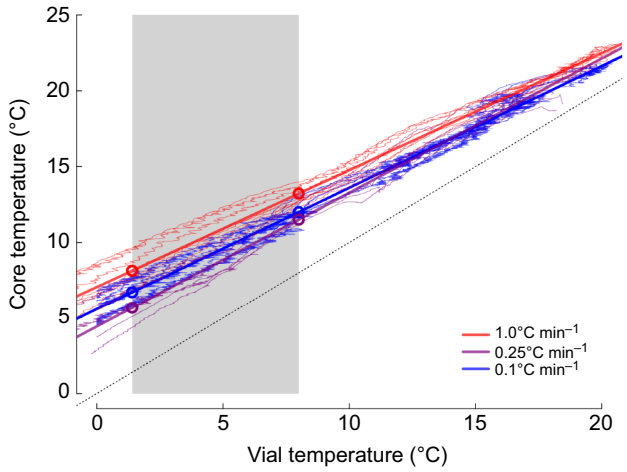

Fig. 2. Bumblebee core temperatures decrease linearly with vial temperatures during cooling ramps. Thick lines indicate average response of core temperatures for 23 bees (thin lines) held in vials ramped at 0.1, 0.25 and 1.0°C min^{-1} (blue, purple and red, respectively). The gray shaded area indicates the range of vial temperatures at which bees reached CT_{min} across all experiments (see Table 1). Note that experiments proceed from right to left as vials were cooled. Circles indicate offset values reported in Table 2. See Results section, 'Differences between thoracic and vial temperatures' and Table 2 for statistical analyses.

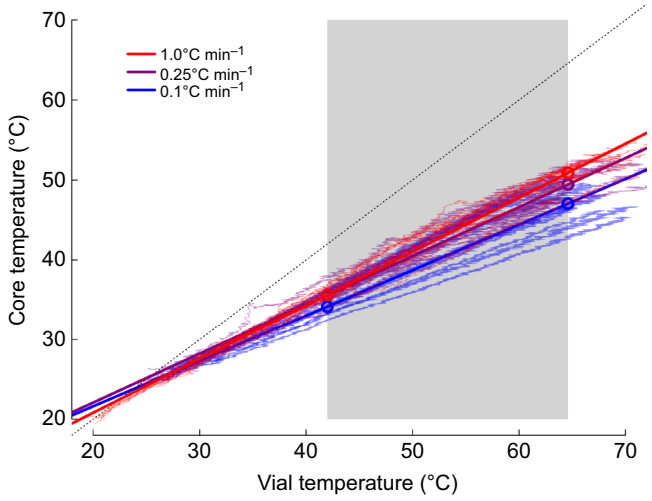

Fig. 3. Bumblebee core temperatures increase linearly with vial temperatures during heating ramps. Thick lines indicate average response of core temperatures for 27 bees (thin lines) held in vials ramped at 0.1, 0.25 and 1.0°C min^{-1} (blue, purple and red, respectively). The gray shaded area indicates the range of vial temperatures at which bees reached CT_{max} across all experiments (see Table 1). Circles indicate offset values reported in Table 2. See Results section, 'Differences between thoracic and vial temperatures' and Table 2 for statistical analyses.

those ramped at 0.1 or 0.25°C min^{-1} (Tukey's HSD, P<0.001 and P=0.018, respectively), which were indistinguishable (P=0.518; Table 2). Slopes did not vary significantly with mass for any of the ramping treatments (all P>0.190). Because slopes were shallower than 1, the difference between core and vial temperature increased as bees were heated (and varied with ramping rate; Table 2). Core temperatures ranged from 6.4–7.8°C cooler than vials at a vial temperature of 42°C to 13.7–17.5°C cooler at vial temperatures of 64°C (Table 2; these vial temperatures encompass the extreme CT_{max} values recorded across all experiments).

Respirometry

We measured CO_2 production during cold ramps of seven bumblebees ranging in size from 143 to 236 mg. Bees stayed active with mass-specific metabolic rates exceeding 13 ml CO_2 g^{-1} h^{-1} at temperatures above 12°C. At lower temperatures, metabolic traces were characterized by steady, low CO_2 release with occasional CO_2 pulses (Fig. 4), probably corresponding to periods when spiracles were closed and open, respectively (Lighton, 1996). We saw strong correspondence between CT_{min} and a final, isolated CO_2 pulse (Fig. 4). For all seven bees, a final CO_2 pulse began 51±33 s prior to observation of curling behavior and peaked 60±28 s after observation of curling behavior, resulting in a release of 2.3 µl CO_2 mg^{-1} body mass on average (Fig. 4; Table 3). Neither total CO_2 released during the CT_{min} CO_2 pulse ($F_{1,5}$=0.77, P=0.419), nor the duration of the CO_2 pulse ($F_{1,5}$=0.73, P=0.433) were related to body mass. For the three bees taken down to their freezing point (−6.6, −4.9 and −4.3°C), we saw no further metabolic peaks after the pulse associated with CT_{min}.

Acclimation

We found no effect of acclimation treatment ($F_{3,88}$=0.10, P=0.960), mass ($F_{1,88}$=2.4, P=0.126), or their interaction ($F_{3,88}$=0.9, P=0.423) on CT_{min} (Fig. 5; Table 1). We found a marginally non-significant difference in CT_{max} between acclimation treatments ($F_{3,91}$=2.4, P=0.069) driven by the tendency for bees in the 15°C acclimation

Journal of Experimental Biology (2018) 221, jeb165589. doi:10.1242/jeb.165589

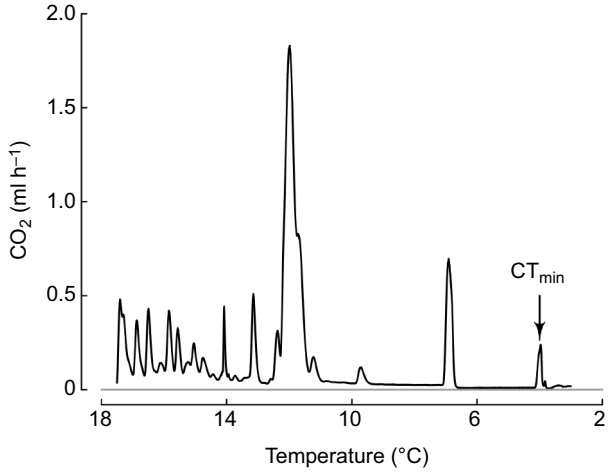

Fig. 4. Bumblebee CT_{min} corresponds to a final CO_2 pulse indicative of loss of spiracle control. Representative respirometry trace for a bumblebee ramped at 0.25°C min^{-1} from 18°C (room temperature) to 2°C (below CT_{min}). The point at which CT_{min} was recorded based on curling behavior (see Movie 1) is indicated by the arrow, corresponding to a final peak in CO_2. There were no further peaks following CT_{min} until the bee froze (not shown). See Table 3 for summary data and Results section.

treatment to fail at slightly (~3.3°C) cooler temperatures than bees taken directly from the nest (P=0.083; Fig. 5). CT_{max} increased by ~4°C for every 100 mg increase in body mass ($F_{1,91}$=12.5, P<0.001).

Ramping rate, age and feeding status

Overall, CT_{min} varied with ramping rate ($F_{2,41}$=15.8, P<0.001), with bees ramped at 1°C min^{-1} having ~2°C colder CT_{min} than bees ramped at rates of 0.1 or 0.25°C min^{-1} (Tukey's HSD, both P<0.001), which did not differ in CT_{min} (P=0.879; Fig. 5, Table 1). CT_{max} increased significantly with ramping rate ($F_{2,38}$=32.3, P<0.001): ramping at 1°C min^{-1} yielded CT_{max} estimates 3.1°C warmer than estimates obtained from ramping at 0.25°C min^{-1} (Tukey's HSD, P=0.062), which were 7.0°C warmer than estimates obtained from ramping at 0.1°C min^{-1} (Fig. 5; Table 1). There was no effect of mass or the interaction between mass and ramping rate on either CT_{min} or CT_{max} (all P>0.119).

Neither age ($F_{2,26}$=0.39, P=0.682), mass ($F_{1,26}$=1.8, P>0.192), nor the interaction between age and mass ($F_{2,26}$=0.3, P=0.749) significantly altered CT_{min} (Fig. 5; Table 1). CT_{max} varied significantly with bumblebee age ($F_{2,26}$=12.0, P<0.001; Fig. 5; Table 1). The 4-day-old bees had significantly lower CT_{max} compared with 3- and 7-day-old bees (Tukey's HSD, both P<0.002), which were indistinguishable (P=0.717).

We found no difference in CT_{min} ($F_{1,28}$=2.2, P>0.149) or CT_{max} ($F_{1,28}$=0.4, P>0.509) between fed and unfed bumblebees (Fig. 5; Table 1). CT_{max} decreased with mass for fed bees ($F_{1,28}$=16.9, P<0.001) but not unfed bees.

DISCUSSION

Critical thermal limits of bumblebees

Laboratory-reared *B. impatiens* reached CT_{min} at vial temperatures of ~4°C corresponding to core temperatures of ~8°C (for all bees ramped at 0.25°C min^{-1}). Wild-caught bumblebees lost the ability to right themselves at ambient temperatures of 7–10°C (Oyen et al., 2016). Differences in these estimates of CT_{min} could reflect differences in methodology as bees probably lose righting response prior to reaching chill coma (i.e. at warmer temperatures; we did not disturb bees to measure righting response in the current study). In addition, these (wild and laboratory-reared) species could differ in lower critical thermal limits, as has been documented for diverse insects (Sunday et al., 2011; Overgaard and MacMillan, 2017). Application of the methodology described here can facilitate future comparisons among bumblebee species and populations using a standardized approach. The only other estimates of bumblebee cold tolerance are lower lethal limits of *B. terrestris*, which ranged from −5 to −9°C (Owen et al., 2013). However, we do not expect chill coma and lower lethal temperatures to occur at the same temperatures as they reflect different physiological mechanisms: reversible loss of muscle coordination at CT_{min} is probably driven by nervous system failure and depolarization of muscle potentials (Goller and Esch, 1990; Andersen et al., 2015; Robertson et al., 2017), whereas death at the lower lethal limit is probably due to irreversible loss of ion homeostasis (Bale, 1993; Hazell and Bale, 2011; Overgaard and MacMillan, 2017).

Bees reached CT_{max} at vial temperatures of ~53°C (corresponding to ~43°C core temperatures), much higher than previous estimates of bumblebee CT_{max}, which range from ~30 to 46°C (ambient temperature) when measured using righting response (Oyen et al., 2016). Hamblin et al. (2017) found CT_{max} indicated by loss of postural control for three species of *Bombus* (including *B. impatiens*) varied between 43 and 52°C, when bees were heated at 0.5°C min^{-1}. The muscular spasms we relied on to indicate CT_{max} happened after loss of postural control and probably after loss of righting response (although we did not interfere with bees, so we lack estimates of righting response for these laboratory-reared *B. impatiens*). Martinet et al. (2015) used a static approach to estimate how long bees held at 40°C could maintain postural control. Although their static approach cannot be directly compared with the

Table 3. CT_{min} assessed by behavior corresponded to a final CO_2 pulse

		CO_2 pulse							
		Start		Peak		End		Total	
Mass (g)	CT_{min} (°C)	Temperature (°C)	Time before CT_{min} (s)	Temperature (°C)	Time after CT_{min} (s)	Temperature (°C)	Time after CT_{min} (s)	CO_2 (µl)	Time (min)
0.143	5.5	5.9	94	5.3	62	4.6	246	0.5	5.7
0.167	4.2	4.7	140	4.1	73	3.4	310	0.55	7.5
0.188	3.3	3.4	81	3.2	43	3.2	421	0.41	8.3
0.198	3.9	4.1	89	4.0	34	3.8	381	0.68	7.8
0.209	3.7	3.7	83	3.7	42	3.2	567	0.4	10.8
0.214	4.9	4.9	12	4.7	53	3.9	343	1.01	5.9
0.236	1.9	2.8	190	1.7	16	−1.0	580	0.91	12.8

Bee mass and CT_{min} for individual bumblebees in relation to characteristics of the CO_2 pulse (Fig. 4). There was no relationship between bumblebee mass and the total amount of CO_2 released or the duration of the CO_2 peak. See Results section, 'Respirometry' for statistical analyses.

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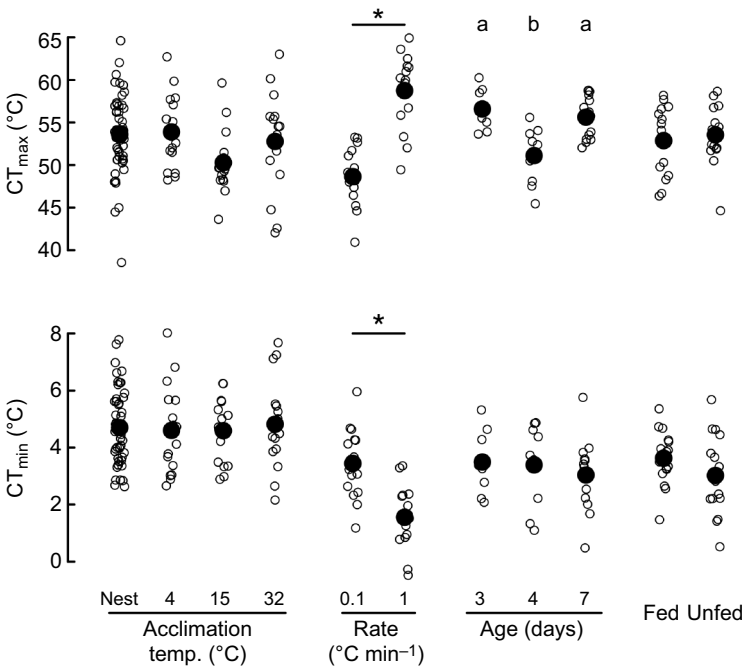

Fig. 5. Critical thermal limits were generally invariant across experimental conditions. CT_{max} (upper panel) and CT_{min} (lower panel) in relation to acclimation treatments, ramping rates, age and feeding. Open symbols indicate values for individual bees, and large filled symbols indicate treatment means. CT_{max} was significantly higher and CT_{min} was significantly lower (indicated by *) in the bees ramped at 1°C min^{-1} relative to those ramped at 0.1°C min^{-1}. Four-day-old bees had significantly lower CT_{max} than either 3- or 7-day-old bees (indicated by lower case letters: a and b). See Table 1 for summary data and Results section, 'Ramping rate, age and feeding status' for statistical analyses.

present study (as they report times rather than temperatures), static approaches will probably give lower estimates of CT_{max} relative to the ramping approaches (increased at 0.25°C min^{-1}) used here (Santos et al., 2011; Nguyen et al., 2014).

High CT_{max} and low CT_{min} resulted in ∼50°C thermal tolerance breadth to ambient temperatures for *B. impatiens*, greatly exceeding estimates of thermal tolerance breadth (TTB) for most ectothermic organisms (also usually based on ambient temperatures; Sunday et al., 2011). This corresponded to tolerance of ∼35°C range in core temperatures. The difference between thoracic and ambient thermal tolerance limits in bumblebees may arise from the ability of heterothermic bumblebees to modulate internal temperatures at both cold and hot ambient temperatures (Heinrich, 1976). Despite their prodigious thermoregulatory ability, extremely cold and hot temperatures have marked effects on their behavior, probably reflecting a loss of neuromuscular function (see supplemental Movies 1 and 2; Fig. 4).

Previous work on bumblebees suggested strong effects of mass on thermal tolerance limits (Oyen et al., 2016). Here, we found that CT_{min} was consistent across bees varying in mass from 53 to 285 mg, whereas CT_{max} increased approximately 1°C for every 25 mg increase in body mass. Contrary to our expectations, this effect was not explained by the difference between core and vial temperature: for neither CT_{min} nor CT_{max} did the slope of the relationship between core and vial temperature depend on mass (Table 2). Alternatively, the increase in CT_{max} with mass could be due to larger bees escaping hot temperatures by climbing the walls of the vials (despite the application of INSECT-a-SLIP) more effectively than their smaller counterparts. Regardless, these results suggest that CT_{max} may vary by ∼8°C within a population given the typical range in mass of bumblebee workers (50–300 mg).

In all experiments, CT_{min} was generally less variable, ranging from 1.4 to 8.0°C, than CT_{max} which varied from 42 to 65.0°C across all experiments (vial temperatures; Table 1). Ranges in estimated core temperatures at failure were smaller: approximately 6–11°C for CT_{min} and approximately 36–50°C for CT_{max} (Table 2; Figs 2 and 3). This pattern is the opposite of many other measurements of critical thermal limits where CT_{max} tends to be less variable than CT_{min} (Mitchell et al., 1993; Klok and Chown, 2001; Jumbam et al., 2007). Our measurements of CT_{min} were less variable (s.d. of 1.6°C across all experiments; Table 1) than CT_{min} measurements for other insects (Gaston and Chown, 1999; Slabber and Chown, 2005; Klok and Chown, 2001; Sheldon and Tewksbury, 2014). This limited variability in CT_{min} is in part methodological as bees show clearly visible, stereotyped and short-lived behaviors (Movie 1) at the onset of chill coma (Fig. 4) but may also reflect strong genetic and developmental similarity between workers within colonies. Bees failed over a narrower range of estimated core temperatures (approximately 6–11°C for CT_{min}), with the larger variation in vial temperatures at failure in part due to differences among bees in how core temperatures tracked vial temperatures (particularly in response to different ramping rates; Fig. 2; Table 2). Aside from these differences in core–vial offsets, variability in CT_{min} may reflect innate individual variation in cold tolerance, given that acclimation, feeding status and age did not influence CT_{min}.

Variation in our estimates of CT_{max} are within the range of reported values for other insects (Sunday et al., 2011). Comparable studies using loss of postural control (Hamblin et al., 2017) and righting response (Oyen et al., 2016) to indicate CT_{max}, also resulted in high levels of variation with CT_{max} ranging from ∼45 to 52°C and from ∼30 to 46°C, respectively. Here we show that variability in the offset between thoracic temperature and vial temperature could explain as much as 9°C of variation in our estimates of CT_{max}, given that offsets of bees heated at 0.25°C min^{-1} were between 6 and 15°C. Higher variability in CT_{max} may also reflect the length of the behavior (onset of muscular spasms, which may last for minutes) and the difficulty distinguishing the onset of muscle spasms from the erratic behavior of bumblebees in hot temperatures.

Respirometry

Metabolic traces of all measured individuals followed a similar pattern with high levels of CO_2 output above 12°C followed by lower overall CO_2 production, which typically corresponded to lower activity levels. Differences among individuals in the duration and total CO_2 released during the CT_{min} CO_2 pulse were not related

to body mass but might reflect time elapsed since the previous CO_2 pulse. The clear behavioral indication of CT_{min} (see Movie 1) always corresponded to a final pulse and subsequent decrease in CO_2 release, matching similar patterns in CO_2 production observed in other insects as they enter chill coma (Sinclair et al., 2004; Stevens et al., 2010; MacMillan et al., 2012). The CO_2 pulse probably indicates a loss of muscular control at CT_{min} and resulting inability to close spiracles, leading to an efflux of CO_2 without subsequent periodic pulses (Goller and Esch, 1990; Hosler et al., 2000). Relaxation of spiracles typically, but not always, results in opening rather than closing (Chapman, 1998) and therefore may lead to a slow release of CO_2 after muscular failure (Stevens et al., 2010). The loss of muscle control following CT_{min} could represent a localized failure at the muscular level, systemic failure within the central nervous system, or both (Overgaard and MacMillan, 2017). Because this physiological threshold is marked by clear behavior, bumblebees provide a compelling system for studying the mechanisms underlying effects of extreme temperatures on insects.

Acclimation

Acclimation in critical thermal limits has been documented in many insects (Fields et al., 1998; Overgaard et al., 2008; Chidawanyika and Terblanche, 2011) and may represent a key physiological mechanism allowing species to cope with environmental change (Overgaard et al., 2011; Seebacher et al., 2015; but see Gunderson et al., 2017). However, we found little evidence for effects of temperature acclimation on either CT_{min} or CT_{max} of *B. impatiens*. Ants show a similarly weak response of thermal limits to acclimation, with more pronounced effects of acclimation on CT_{max} than on CT_{min} (Jumbam et al., 2008). Few data are available for acclimation capacity in bees, but rapid cold hardening, a form of plasticity probably driven by up-regulation of molecular chaperones and changes in cell membrane structure, has been documented in *B. terrestris* (Owen et al., 2013). Although rapid cold hardening, the heat shock response and acclimation are potentially physiologically distinct responses (Bowler, 2005; Sinclair and Roberts, 2005), the minimal response of CT_{min} and CT_{max} to acclimation temperatures reported here suggests that adult bumblebees must behaviorally compensate for environmental heat waves or cold snaps.

Ramping rate

Ramping rates may alter estimates of critical thermal limits by increasing or decreasing the lag between environmental temperature and organism core temperature equilibration or by inducing different physiological responses associated with the duration of exposure (Terblanche et al., 2007). Bumblebees ramped at 1°C min^{-1} had significantly (~2°C) lower CT_{min} and (~10°C) higher CT_{max} (Fig. 5), suggesting that either the offset between core and vial temperatures was greater at faster ramping rates or that tolerance increased because duration of exposure to stressful temperatures decreased. Given that thoracic temperatures of bumblebees cooled at 1.0°C min^{-1} were not significantly different from those ramped at 0.1°C min^{-1}, the difference in CT_{min} at faster cooling rates may be driven by decreased exposure time to physiologically stressful temperatures (Terblanche et al., 2007).

Bumblebees failed at thoracic temperatures between ~32 and 46°C when heated at 0.25 or 0.1°C min^{-1}, but failed at thoracic temperatures between approximately 48 and 58°C when heated at 1.0°C min^{-1}. This increase in CT_{max} estimates at faster ramping rates was not due to larger offsets between core and vial temperatures because the offset between thoracic and vial temperatures decreased at faster ramping rates (Fig. 3; Table 2). Rather, faster ramping rates decreased the time bees were exposed to physiologically stressful conditions, such that those ramped more quickly reach higher temperatures before failure. Increased thoracic temperatures at faster heating rates may represent a breakdown in thermoregulatory ability. Bumblebees actively shunt heat from the thorax to the abdomen via blood flow to prevent overheating (Heinrich, 1976), but if temperatures rise too quickly, they may not be able to effectively regulate body temperature via blood flow.

Critical thermal limits of cockroaches (Cocking, 1959) and fruit flies (Overgaard et al., 2006) also depend on ramping rate. Slower ramping rates may provide sufficient time for hardening, a form of phenotypic plasticity (Hoffmann et al., 2003), which involves changes in cellular membrane structure that protect cells from injury (Anneli Korhonen and Lagerspetz, 1996; Kelty and Lee, 2001). Tsetse flies have lower CT_{min} and CT_{max} when ramped more slowly, possibly due to rapid cold hardening prior to CT_{min} and increased duration of exposure to stressful hot temperatures near CT_{max} (Terblanche et al., 2007). Rapid cold hardening has been documented in *B. terrestris* (Owen et al., 2013), but is unlikely to explain lower CT_{min} of *B. impatiens* at faster ramping rates (Tables 1 and 2), because time for cold hardening was reduced. For the same reason, elevated CT_{max} at fast ramping rates is unlikely to reflect up-regulation of stress compounds, such as heat shock proteins, or thermoprotective metabolites, e.g. sorbitol (Wolfe et al., 1998) or glucose (Sformo et al., 2010). Broader thermal tolerance measures (higher CT_{max} and lower CT_{min}) at faster ramping rates may instead reflect a shorter duration of exposure to stressful temperatures (Terblanche et al., 2007).

Age and feeding

Age and feeding status can affect physiological and biochemical processes and therefore may alter critical thermal limits. Several studies have shown variation in critical thermal limits with age (Bowler and Hollingsworth, 1966; Bowler, 1967; Nyamukondiwa and Terblanche, 2009; Chidawanyika et al., 2017). Age did not alter CT_{min} in *B. impatiens*, but CT_{max} was significantly lower in 4-day-old bees relative to either 3- or 7-day-old bees. The reason for this pattern is an open question. In fruit flies, CT_{min} decreased with age and CT_{max} increased with age up to 14 days old (Nyamukondiwa and Terblanche, 2009). We found little variation in thermal tolerance of bumblebees up to 7 days old (Table 1). However, bumblebees may sometimes live for more than 14 days (Goulson, 2010) and whether these older bumblebees show shifts in thermal tolerance remains to be tested.

Maintenance of ion homeostasis at low temperatures probably underlies cold tolerance in many organisms. Feeding can therefore alter lower thermal limits through effects on hemolymph ion concentrations. In both fruit flies and beetles, feeding led to higher CT_{max}, perhaps by increasing the overall biomass of the organism or by improving nutritional status (Nyamukondiwa and Terblanche, 2009; Chidawanyika et al., 2017). In bumblebees, feeding had no effect on thermal tolerance, but starvation longer than 5 h led to higher mortality at moderate temperatures (between 26 and 31°C), emphasizing the importance of constant feeding for these animals. Bumblebee workers have only minimal glycogen stores. Queens, however, may significantly increase energy stores before overwintering (Röseler and Röseler, 1986). Although we saw no effect of feeding versus starvation on the thermal limits of bumblebees, wild bees may regularly experience differences in nutritional quality of nectar (Nicolson and Thornburg, 2007), which alters foraging activity (Pankiw et al., 2004) and influences

physiological condition (Stabler et al., 2015). Investigating the effects of differences in nutritional properties of nectar on thermal limits of bees may therefore be a fruitful avenue for future research and reveal differences in thermal tolerance traits related to bumblebee diet.

Acclimation, age and feeding status had little influence on critical thermal limits of bees. However, CT_{min} and CT_{max} varied significantly among nests, when all bees from a nest were considered together, regardless of experimental treatment ($F_{2,203}$=33.7, P<0.001). Bees for experiments came from three distinct nests, with bees used in the acclimation experiments having CT_{min} ∼1°C higher overall than bees used in the initial measurements of CT_{min} (Tukey's HSD, P=0.034), and ∼1.7°C higher than bees used in ramping rate, age and feeding experiments (Tukey's HSD, P<0.001), with bees from the latter two nests indistinguishable in terms of CT_{min} (Tukey's HSD, P=0.184). CT_{max} did not differ significantly among nests ($F_{2,217}$=0.90, P=0.407). These analyses group bees from different experimental treatments, so must be interpreted with caution. However, they do suggest that thermal limits may differ between colonies, perhaps due to genetic or maternal effects or to differences in developmental conditions; but we know little about the history of the commercially reared nests. Future work on among-colony differences in thermal tolerance will be particularly revealing if the source of queens and developmental conditions of the colonies are known.

Critical thermal limits of bumblebees described here are repeatable and largely unaffected by acclimation, feeding status or age, and are clearly associated with physiological thresholds. This strong link between an easily observable behavior and the underlying physiological limit makes bumblebees a compelling system for studying the cellular mechanisms leading to loss of muscular control at CT_{min} and CT_{max}. Furthermore, measurements of critical thermal limits of bumblebees across populations and species may provide valuable insights relating to recent population declines and range shifts (Grixti et al., 2009; Cameron et al., 2011; Kerr et al., 2015), as well as facilitating mechanistic predictions (Kearney and Porter, 2009) of the effects of climate change on future distributions of these vital pollinators.

Acknowledgements
We thank Steve DeVries for extensive help with designing and building the equipment used in these experiments.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.J.O., M.E.D.; Methodology: K.J.O., M.E.D.; Validation: K.J.O., M.E.D.; Formal analysis: K.J.O., M.E.D.; Investigation: K.J.O.; Writing - original draft: K.J.O., M.E.D.; Writing - review & editing: K.J.O., M.E.D.; Visualization: K.J.O.; Supervision: M.E.D.; Project administration: M.E.D.; Funding acquisition: M.E.D.

Funding
This work was funded by National Science Foundation – Division of Environmental Biology grant number 1457659 to M.E.D.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.165589.supplemental

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