1 ORIGINAL ARTICLE

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

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12

13 Abstract

14 Organism critical thermal limits are often tightly linked to current geographic distribution and
15 can therefore help predict future range shifts driven by shifting environmental temperatures.
16 Thermal tolerance of diverse organisms often varies predictably with latitude, with upper thermal
17 limits changing little and lower thermal limits decreasing with latitude. Despite similarly steep
18 gradients in environmental temperatures across altitude, few studies have investigated altitudinal
19 variation in critical thermal limits. We estimated critical thermal minimum (CT_{min}), critical
20 thermal maximum (CT_{max}) and recovery temperature (T_{rec}) by tracking righting response of three
21 bumble bee species during thermal ramps: *Bombus huntii* collected from 2,180 m asl, and *B.*
22 *bifarius* and *B. sylvicola* collected from 3,290 m asl in Wyoming, USA. Overall, larger bees
23 could tolerate more extreme temperatures, likely due to a thermal inertia driven lag between core
24 body temperatures and air temperatures. Despite their smaller size, high altitude bumble bees
25 tolerated colder air temperatures: they had ~ 1 °C lower CT_{min} and recovered from cold exposure
26 at ~ 3 -4 °C lower air temperatures. Conversely, low altitude bees tolerated ~ 5 °C hotter air
27 temperatures. These altitudinal differences in thermal tolerance parallel differences in average
28 daily minimum (1.2 °C) and maximum (7.5 °C) temperatures between these sites. These results
29 provide one of the few measurements of organism thermal tolerance across altitude and the first
30 evidence for geographical differences in tolerance of temperature extremes in heterothermic
31 bumble bees.

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33 **Keywords.** Bumble bees, critical thermal limits, elevation, CT_{min} , CT_{max} , T_{rec} .

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35

36 1. Introduction

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

45

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

57

58 Shifts in elevational and latitudinal ranges have been recently documented for bumble bees
59 across Europe and North America (Kerr et al., 2015). Shifts to higher elevations and range
60 compressions among southern bumble bee species appear unrelated to changes in land or
61 pesticide use, and are unlikely to reflect shifts in resources, but strongly correlate with changes in
62 climate (Kerr et al., 2015). The thermal physiology of bumble bees may play a critical role in
63 these recently observed responses to climate warming, but thermal tolerance of these vital
64 pollinators has rarely been measured (Goller and Esch, 1990; Owen et al., 2013; Martinet et al.,
65 2015). Perhaps because bumble bees are heterothermic and can regulate body temperature
66 independent of environmental temperature both during foraging trips and while in the hive
67 (Heinrich, 1974), tolerance of extreme temperatures in these organisms have been understudied.
68 However, even their exceptional thermoregulatory abilities cannot protect them from periodic
69 exposures to extreme high and low temperatures (Heinrich, 1976).

70

71 Tolerance of insects to extreme temperatures has been measured in diverse ways (Terblanche et
72 al., 2011), including measurements of lower lethal limits (Lee and Denlinger, 1991), of changes
73 in metabolic rate (Lighton and Turner, 2004), and of knockdown temperatures (Huey et al.,
74 1992). Behavioral estimates of lower and upper critical temperatures, though sometimes less
75 clearly tied to physiological thresholds (Hazell and Bale, 2011) can be measured as the minimum
76 (CT_{min}) and maximum (CT_{max}) temperatures at which an organism loses critical motor function,
77 failing to right itself (Fry, 1967), thereby losing the ability to escape from conditions that could
78 lead to its death (Lowe and Vance, 1955; Hutchison, 1961). Thus, although these behavioral
79 thermal limits provide limited information about the underlying physiological mechanisms, they
80 are nevertheless ecologically-relevant because they reveal the ambient temperature extremes at

81 which fitness is likely to be severely reduced (Cowles and Bogert, 1944). We measured
82 behavioral critical thermal limits and recovery temperatures (T_{rec} , the temperature at which bees
83 recovered from chill-coma) of bumble bees collected across a 1,000 m altitudinal gradient. As
84 expected given the biophysics of heat transfer, larger bees failed at more extreme (hotter and
85 colder) air temperatures. After accounting for mass effects, we found reduced cold and heat
86 tolerance for bumble bees collected from high altitudes.

87

88 **2. Materials and methods**

89 *2.1 Study sites and collections*

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

100

101 These sites differed in the maximum and minimum air temperatures potentially experienced by
102 bees at these altitudes throughout the growing season (Fig. 1; average daily maximum
103 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 X 15 cm). Each chamber had an acrylic lid with a ~2mm 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 x 40 cm, 90W, $\Delta T=67$ °C), with the active side of the TEC and the block insulated from room air within a foam cooler (30 X 25 X 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

127 chamber temperature. A separate T-type thermocouple placed within a central chamber on the
128 block tracked air temperatures at which bees reached thermal limits. Preliminary experiments
129 revealed little variation in temperature among chambers (temperatures at the four corners of the
130 block differed from the center of the block by $<0.2\text{ }^{\circ}\text{C}$ on average) or vertically within individual
131 chambers (at 1 cm above the chamber floor, a distance higher than all bees tested, temperature
132 was a maximum of $0.4\text{ }^{\circ}\text{C}$ higher than at the chamber floor).

133

134 For each experimental run, eight bees were each placed in individual chambers on the block and
135 held at $22\text{ }^{\circ}\text{C}$ for 15 minutes before temperature was ramped to $0\text{ }^{\circ}\text{C}$ at a rate of $0.25\text{ }^{\circ}\text{C min}^{-1}$, a
136 rate commonly used in the literature (Chidawanyika and Terblanche, 2011; Sformo et al., 2011;
137 Boardman et al., 2012). As temperatures dropped, bees gradually became sluggish, at which
138 point they were flipped onto their backs to induce a righting response. $\text{CT}_{\min}$ was taken as the
139 highest chamber air temperature at which bees were unable to right themselves within 30 s after
140 being flipped. The block temperature was allowed to drop $0.5\text{ }^{\circ}\text{C}$ below the last $\text{CT}_{\min}$ value
141 measured and bees were held at that temperature for two minutes to ensure that all individuals
142 spent a minimum amount of time below $\text{CT}_{\min}$ prior to testing recovery time. The block was then
143 removed from the cooler and allowed to warm to room temperature on the bench top (~ 0.15
144 $^{\circ}\text{C/min}$). The recovery temperature (T_{rec}) was taken as the chamber air temperature at which an
145 individual bee regained motor function and righted. When the aluminum block had equilibrated
146 with room temperature, we immediately started the $\text{CT}_{\max}$ trials. Bees were held at $22\text{ }^{\circ}\text{C}$ for 10
147 minutes followed by ramping temperature to $55\text{ }^{\circ}\text{C}$ at a rate of $0.50\text{ }^{\circ}\text{C min}^{-1}$, a rate commonly
148 used in the literature (Piyaphongkul et al., 2012; Vorhees and Bradley, 2012). As temperatures
149 rose, bees became more agitated before beginning to lose muscle coordination, at which point

150 they were flipped onto their backs to test for righting response. $CT_{\max}$ was recorded as the lowest
151 chamber air temperature at which an individual could not right itself (if bees did not right
152 immediately, they would not subsequently right themselves). Measurement of $CT_{\max}$
153 immediately after $CT_{\min}$ may alter estimates of the former, but is common practice (Gaston and
154 Chown, 1999; Gvoždík and Castilla, 2001)

155

156 2.3 Analyses

157 ANOVA was used to compare mass among species and sexes. Linear regression revealed strong
158 effects of mass on thermal tolerance metrics and mass varied significantly among species, so
159 residuals from the regression of thermal tolerance metrics on mass were compared among
160 species by ANOVA followed by Tukey HSD post-hoc tests. All analyses and figures were done
161 in R version 3.1.3 (R Core Team, 2015).

162

163 3. Results

164 $CT_{\min}$, $CT_{\max}$, and T_{rec} were measured for 72 bumble bees representing three species (*B. huntii*,
165 *B. bifarius*, and *B. sylvicola*) collected from the two sites (2180, 3290 m). Mass may affect
166 metrics of thermal tolerance (Baudier et al., 2015), so we first assessed whether body mass
167 (measured prior to the start of experiments) varied with species and sex. *B. huntii* was
168 significantly larger than the other two species (both $P < 0.001$; Table 1), which did not differ
169 significantly in mass ($P = 0.862$, Table 1). *B. sylvicola* males were significantly larger than
170 females of this species ($P = 0.046$; Table 1), whereas there were no significant differences in mass
171 between males and females for the other two species (both $P > 0.120$; Table 1).

172

173 Across all bees, CT_{min} decreased significantly with increasing body mass (Fig. 2B, dashed gray
174 regression line; Table 2, $P=0.014$), but did not differ between males and females (Table 2,
175 $P=0.244$). Within species, CT_{min} decreased significantly with mass for *B. huntii* (Fig. 2B, dashed
176 red line; Table 2, $P<0.001$), did not change significantly with mass for *B. sylvicola* or *B. bifarius*,
177 and was not significantly different between males and females for *B. huntii* or *B. sylvicola* (Table
178 2; low sample sizes precluded analysis of sex differences for *B. bifarius*). To account for the
179 strong overall effect of mass on CT_{min} and for covariance between mass and species (see above),
180 we analyzed the residuals of the regression of CT_{min} on mass among species (Table 2, All bees;
181 Figure 2B, dashed gray line). After accounting for mass effects, the three species differed slightly
182 but significantly in CT_{min} ($F_{2,68}=3.31$, $P=0.042$). Both *B. bifarius* and *B. sylvicola* tended to have
183 lower mass-corrected CT_{min} than did *B. huntii* (Fig. 4), but this effect was small for both species
184 (~ 1.8 and ~ 1 °C, respectively), marginally significant for *B. bifarius* (Tukey HSD, $P=0.062$), and
185 not significant for *B. sylvicola* (Tukey HSD, $P=0.155$).

186

187 CT_{max} increased significantly with body mass among all bees (Fig. 2A, dashed gray regression
188 line; Table 2, $P<0.001$) and within *B. huntii* (Fig. 2A, red regression line; Table 2, $P=0.040$), but
189 did not change significantly with mass for either *B. bifarius* or *B. sylvicola* (Table 2, both
190 $P>0.454$). Further, CT_{max} did not differ significantly between males and females among all bees
191 or within species (Table 2). After accounting for mass effects, CT_{max} differed significantly among
192 species (analysis of residuals from regression of CT_{max} on mass, $F_{2,68}=4.66$, $P=0.013$; Fig. 4).
193 Mass-corrected CT_{max} for the high-elevation species *B. sylvicola* was significantly lower than for
194 *B. huntii* by ~ 2.9 °C (Tukey HSD, $P=0.009$; Fig. 4), but was otherwise indistinguishable among
195 species.

196

197 T_{rec} did not vary significantly with either body mass or sex among or within species (Table 2;
198 Fig. 3). Species differed significantly in T_{rec} ($F_{2,61}=10.98$, $P < 0.001$), with the high-elevation
199 species, *B. bifarius* and *B. sylvicola*, having recovery temperatures 2.8 and 2.1 °C colder,
200 respectively, than recovery temperatures of *B. huntii* (Figs 3, 4). As with CT_{min} and CT_{max} , to
201 account for covariation between mass and species, we also analyzed variation among species in
202 the residuals from the regression of T_{rec} on mass. Mass-corrected T_{rec} varied significantly among
203 species ($F_{2,61}=10.98$, $P<0.001$), with both high-elevation species (*B. bifarius* and *B. sylvicola*)
204 recovering at temperatures 2.8 and 2.1 °C, lower than the low-elevation species, *B. huntii* (Tukey
205 HSD, $P<0.001$ and $P=0.001$, respectively). The similar patterns of variation in CT_{min} and T_{rec}
206 among species may in part reflect that the two measurements were positively correlated
207 (Pearson's $r=0.53$, $P<0.001$).

208

209 4. Discussion

210 Few studies have reported critical thermal limits of bees. This may in part be due to assumption
211 that heterothermic bees, which are able to regulate heat loss from highly active flight muscles
212 (Heinrich, 1976), are freed from the constraints of extreme temperatures. However, bumble bees
213 may regularly experience temperature extremes when foraging outside the nest at cooler
214 temperatures or when their prodigious ability to generate heat (Heinrich, 1974) and evolutionary
215 origins in cool climates (Hines, 2008) make them susceptible to overheating even in moderately
216 warm conditions. Overall, differences in thermal tolerance among bumble bees matched
217 expectations based on environmental conditions: the high altitude species were more cold
218 tolerant and less heat tolerant than the low altitude species. The effect of mass on critical thermal

limits (Figs 2, 3) is likely due to the biophysics of heat transfer: cooling and heating rates of bees (Heinrich and Heinrich, 1983) and other insects (Stevenson, 1985) depend strongly on mass. We measured *air* temperatures at which bees reached thermal limits, and the thermal inertia of larger bees will cause their core body temperatures to lag behind air temperatures and therefore fail at warmer and cooler air temperatures. However, if the differences in critical thermal limits we found were driven solely by this effect of body size on cooling rates, we would expect *higher* CT_{min} for the smaller-bodied high-altitude species (*B. bifarius* and *B. sylvicola*). We saw the opposite: despite their small size, *B. bifarius* could tolerate lower air temperatures than *B. huntii* (Figs 2B, 4). This provides strong evidence that *B. bifarius* has greater physiological tolerance to cold temperatures than does its low-elevation relative, *B. huntii*.

Across a 1000 m altitude gradient, CT_{min} was approximately 1 °C lower in *B. bifarius* relative to *B. huntii*, despite short geographic distance (~60 km), and little change in latitude (0.03°). This difference in cold tolerance was similar to the difference between sites in daily minimum temperatures during the collection period (1.2 °C), but less than these temperature differences over the growing season (3.1 °C). These difference in CT_{min} appears to be relatively small compared to ~3 °C decrease in CT_{min} of dung beetles across a 1000 m altitudinal gradient (Gaston and Chown, 1999). However, a global analysis of latitudinal variation in CT_{min} of ectotherms found that critical thermal minima decreased by only 2.4 °C per 10° of latitude (Sunday et al., 2012), suggesting that the differences we document here could be ecologically relevant. Further, CT_{min} for *B. sylvicola* and *B. bifarius* were ~2 and 3 °C lower, respectively, than CT_{min} of comparably-sized *B. huntii* (Fig. 2B). These differences in CT_{min} could be attributed to evolutionary differences between *B. bifarius* and *B. sylvicola*, which are

242 predominantly high-altitude species, and *B. huntii*, which is typically more abundant at lower
243 elevations (Williams et al., 2014; Lozier et al., 2016). These differences in critical thermal limits
244 could also be due to acclimatization, because bees were tested within two hours of capture.

245

246 Differences in T_{rec} paralleled those in CT_{min} : bees from high altitude recovered at colder
247 temperatures than did bees from low altitude (Figs 3, 4; Table 2). This finding suggests that high-
248 altitude bumble bees may be better able to recover from short-term cold snaps. We know little
249 about variation in T_{rec} across altitude, but previous studies have found decreased recovery time in
250 *Drosophila* from higher latitudes (Hoffmann et al., 2002; David et al., 2003; Ayrinhac et al.,
251 2004). The ability to recover from and remain active at low temperatures may in part be
252 attributed to rapid cold hardening, which affords subtle benefits during acute cold exposure, such
253 as allowing *D. melanogaster* to remain active at lower temperatures than they otherwise could
254 (Kelty and Lee, 2001). Rapid cold hardening has been demonstrated in another bumble bee
255 species (*B. terrestris audax*; Owen et al., 2013), and may be particularly relevant to high altitude
256 specialists, which may need to forage widely from patchy resources and at unpredictably low
257 temperatures.

258

259 The strong correlation between CT_{min} and T_{rec} may indicate that they are driven by similar
260 mechanisms such as loss (CT_{min}) and re-establishment (T_{rec}) of ion homeostasis (MacMillan et
261 al., 2012). Alternatively, individuals with the lowest CT_{min} spent the least amount of time below a
262 physiological threshold and therefore were able to recover sooner. We cannot distinguish
263 between these possibilities because bumble bees were not held below CT_{min} for the same amount
264 of time; some minimum amount of time below a cold threshold is likely necessary to induce

265 similar physiological responses from all individuals (i.e., chill coma recovery time; Sinclair et
266 al., 2015).

267

268 The low elevation species (*B. huntii*) tolerated temperatures nearly 5 °C hotter than did the two
269 high elevation species (*B. bifarius* and *B. sylvicola*; Fig. 2A, Table 1), even after accounting for
270 the generally larger body size of *B. huntii*. These differences in heat tolerance were similar to
271 differences between these sites in average daily maximum temperature throughout the growing
272 season (7.5 °C) and during the sampling period (7.6 °C). Again, these differences may reflect
273 evolutionary patterns (species differences) or acclimation effects (bumble bees were tested
274 straight from the field). In another study, two high-elevation species (*B. balteatus* and *B. alpinus*)
275 were less resistant to heat stress (measured as survival after exposure to 40 °C) than species
276 typically found at low elevations and latitudes (*B. monticola*; Martinet et al., 2015). Several
277 studies found more subtle geographical variation in upper thermal tolerance: CT_{max} of dung
278 beetles decreased by only ~1 °C over a 1000 m altitudinal gradient (Gaston and Chown, 1999),
279 and CT_{max} of diverse ectotherms decreased by an average of only 0.3 °C for every 10° of latitude
280 (Sunday et al., 2011). However, the more pronounced altitudinal shifts in CT_{max} than in CT_{min}
281 that we document for bumble bees are consistent with shifts in thermal limits of dung beetles
282 across multiple elevational gradients (Sheldon and Tewksbury, 2014). The limited available data
283 suggest that thermal tolerance limits may not vary with altitude in the same way they vary with
284 latitude (Sunday et al., 2011, 2012).

285

286 Geographic differences in thermal tolerance may play an important role in climate-driven
287 poleward and upslope range shifts of bumble bees (Kerr et al., 2015) and other pollinators

288 (Parmesan, 2006). Even heterothermic bumble bees show clear geographic variation in thermal
289 tolerance, suggesting the importance of these traits for surviving in diverse environmental
290 conditions. In contrast to the plethora of studies of latitudinal variation in thermal tolerance
291 (reviewed by Sunday et al., 2011), very few studies have investigated altitudinal variation in
292 these key physiological traits. More work characterizing thermal tolerance across altitude for
293 diverse ectotherms will be critical in the context of ongoing rapid climate change.

294

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302

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Table 1. Variation in body mass and thermal tolerance metrics among species and sexes for three
bumble bee species collected from two different altitudes. Values are mean $\pm$ SD. Numbers next
to species indicate significant differences in body mass (see text for details). Letters next to mass
measurements indicate significant differences between the sexes within a species. See text and
Table 2 for analyses of thermal tolerance metrics.

species (N)	mass (mg)	CT _{min} (°C)	CT _{max} (°C)	T _{rec} (°C)
2,180 m				
<i>B. huntii</i> ¹ ♀ (21)	127.5 $\pm$ 20.2	9.8 $\pm$ 2.4	44.8 $\pm$ 2.6	14.4 $\pm$ 1.3
♂ (10)	111.8 $\pm$ 26.8	10.7 $\pm$ 2.1	45.0 $\pm$ 1.4	15.7 $\pm$ 2.0
3,290 m				
<i>B. bifarius</i> ² ♀ (6)	90.2 $\pm$ 23.4	9.9 $\pm$ 1.4	39.6 $\pm$ 5.8	11.7 $\pm$ 0.9
♂ (3)	102.0 $\pm$ 11.0	7.1 $\pm$ 0.8	43.8 $\pm$ 0.0	11.2 $\pm$ 2.5
<i>B. sylvicola</i> ² ♀ (17)	84.4 $\pm$ 11.5 ^a	10.5 $\pm$ 2.0	38.2 $\pm$ 4.4	12.3 $\pm$ 2.3
♂ (15)	97.0 $\pm$ 20.5 ^b	9.3 $\pm$ 2.4	39.8 $\pm$ 4.3	12.0 $\pm$ 1.5

Table 2. Effects of body mass and sex on CT_{min}, CT_{max}, and T_{rec} for the three bumble bee species. Results of ANOVAs among (“all bees”) and within species without the mass:sex interaction, which was not significant for any of the analyses. The effect of sex was not assessed for *B. bifarius* due to low sample sizes of males (Table 1). Significant effects are in bold.

		CT _{min}		CT _{max}		T _{rec}	
		<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>	<i>F</i> (d.f.)	<i>P</i>
All bees							
mass		6.34 (1,68)	0.014	23.91 (1,68)	<0.001	2.74 (1,61)	0.103
sex		1.38 (1,68)	0.244	0.53 (1,68)	0.467	0.03 (1,61)	0.874
<i>B. huntii</i>							
mass		16.16 (1,27)	<0.001	4.65 (1,27)	0.040	3.10 (1,20)	0.094
sex		<0.01 (1,27)	0.983	0.82 (1,27)	0.374	1.28 (1,20)	0.272
<i>B. bifarius</i>							
mass		0.30 (1,4)	0.613	0.22 (1,4)	0.663	0.35 (1,4)	0.588
<i>B. sylvicola</i>							
mass		1.57 (1,29)	0.220	0.58 (1,29)	0.454	0.16 (1,29)	0.697
sex		1.09 (1,29)	0.306	0.63 (1,29)	0.434	0.08 (1,29)	0.774

313

314

315 **Figure legends**

316

317 **Figure 1.** Air temperatures recorded near collection localities. Shaded regions show daily
318 maximum and minimum air temperatures from weather stations near the low (2180 m, red) and
319 high (3290 m) elevation sites throughout the growing season in 2014. The sampling period of
320 September 15-24 is indicated by the black rectangle. Dotted vertical lines indicate the first day of
321 every month.

322

323 **Figure 2.** Bumble bee critical thermal limits varied with body mass and species. Small points
324 show estimates of CT_{max} (A) and CT_{min} (B) for individual *B. huntii* collected at 2180 m (red),
325 and for *B. bifarius* (purple) and *B. sylvicola* (blue) both collected at 3290 m. Large solid points
326 indicate species means and associated horizontal and vertical bars indicate 1 SD for mass and
327 critical thermal limits, respectively. Within-species regressions were only significant for *B.*
328 *huntii* (dashed red lines; see Table 2 for statistical details). Dashed black lines indicate significant
329 regressions across all individuals (see Table 2).

330

331 **Figure 3.** Recovery temperatures varied strongly among species but were not affected by body
332 mass. Small points indicate individual estimates of T_{rec} with colors as in Fig. 2. Large solid
333 points and associated lines indicate species means $\pm$ 1 SD, respectively. See Table 2 for statistical
334 details.

335

336 **Figure 4.** Mass-corrected critical thermal limits varied among Bumble bee species. Individual
337 values (open points) and species means (solid points) of the residuals from the regression of

338 critical thermal limits on mass (CT_{min} , T_{rec} , and CT_{max}) plotted for *B. huntii* (red), *B. bifarius*

339 (purple) and *B. sylvicola* (blue). Vertical lines indicate ± 1 SD. Significant pairwise comparisons

340 are shown by black bars ($\sim P \leq 0.1$, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$; see text for statistical
341 details).

342

343

344

Figure 1

Figure 2

Figure 3

Figure 4