Thermal stability and muscle efficiency in hovering orchid bees (Apidae: Euglossini)

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Summary

To test whether variation in muscle efficiency contributes to thermal stability during flight in the orchid bee, *Euglossa imperialis*, we measured CO_2 production, heat loss and flight kinematics at different air temperatures (T_a). We also examined the relationship between wingbeat frequency (WBF) and T_a in five additional species of orchid bees. Mean thoracic temperature (T_{th}) for *Eg. imperialis* hovering in a screened insectary and in the field was $39.3 \pm 0.77°C$ (mean ± 95% C.I.), and the slope of T_{th} on T_a was 0.57. Head and abdominal temperature excess ratios declined with T_a, indicating that *Eg. imperialis* were not increasing heat dissipation from the thorax at high T_a. Elevation of T_{th} above T_a was correlated with WBF, but T_{th} alone was not. Estimates of heat production from both respirometry and heat loss experiments decreased 33% as T_a rose from 24 to 34°C. Mean muscle efficiency over this temperature range was 18% assuming perfect elastic energy storage and 22% assuming zero elastic energy storage. Both efficiency estimates increased significantly as T_a rose from 24 to 34°C. In all six species examined, WBF declined significantly with T_a. These data indicate that hovering orchid bees regulate heat production through changes in wingbeat kinematics and consequent changes in energy conversion by the flight motor. Temperature-dependent variation in elastic energy storage or muscle contraction efficiency or both may contribute to the observed trends.

Key words: flight energetics, thermoregulation, muscle, power, efficiency, bee, *Euglossa imperialis*, Euglossini.

Introduction

Muscles convert chemical energy into mechanical work, and a byproduct of this process is the release of heat energy (Hill, 1938). Although heat represents inefficiency of muscle contraction, this inefficiency proves adaptive for endothermic organisms that regulate myogenic heat to raise body temperatures at low air temperatures. Shivering is the most familiar and widespread example of this phenomenon, whereby contractions of muscles elevate body temperatures in animals ranging from honeybees to humans (Heinrich, 1993).

Because numerous flying insects activate their thoracic muscles at high frequency, they produce a substantial amount of heat during flight (Dudley, 2000). For the past 25 years, the prevailing view has been that flying insects are capable of regulating heat dissipation but not heat production (Casey, 1989; Heinrich, 1980). However, this perspective has been called into question by recent studies with honeybees (Harrison et al., 1996), centridine bees (Roberts et al., 1998) and dragonflies (May, 1995a,b) that suggest control of heat production *via* modulation of wingbeat kinematics. More specifically, these authors have hypothesized that insects increase wingbeat frequency to elevate body temperatures at low air temperatures.

Although support for in-flight regulation of heat production is growing, a number of issues remain unresolved (Harrison

and Fewell, 2002). To draw closure to this controversy, researchers have identified three lines for future investigation: complete analyses of wingbeat kinematics and heat production during flight (Roberts and Harrison, 1999), comparative investigations demonstrating the generality of the phenomenon (May, 1995a,b) and field-based tests representative of natural flight behavior (May, 1995a; Stevenson and Woods, 1997). Accordingly, we incorporated these suggestions in determining whether orchid bees (Apidae: Euglossini) regulate metabolic heat production during flight.

The flight energetics of orchid bees have been studied extensively in the past (Casey and May, 1985; May and Casey, 1983), and a recent re-analysis of data from these studies suggested that they regulate heat production during flight (May, 1995a). As a neotropical group, orchid bees may not encounter as great a range of air temperatures as do temperate-zone species but they are an ideal organism for flight studies because they will readily hover over floral fragrances located in the field of view of a video camera (Evoy and Jones, 1971). In the present study, we recorded wingbeat kinematics, carbon dioxide production and heat loss for *Euglossa imperialis* hovering in a screened insectary and wingbeat frequency alone for *Eg. imperialis* and five other orchid bee species hovering

in the field. These data support the hypothesis that orchid bees regulate metabolic heat production during flight *via* changes in wingbeat frequency and muscle efficiency.

Materials and methods
Study site and animal

Euglossa imperialis Cockerell is an abundant and widespread species of orchid bee from Central America (Roubik and Ackerman, 1987). It ranges from sea level up to 1500 m and can be captured throughout the year in a variety of habitats ranging from intact forest to open fields. During the early wet season of 2002, *Eg. imperialis* was captured at cineole fragrance baits from a lowland population in the Laboratory Clearing and Central Tower on Barro Colorado Island, Panama (BCI). *Eg. imperialis* is active at fragrance baits on dry days between 07.00 h and 14.00 h (Armbruster and Berg, 1994), making these times the primary period of data collection.

Wingbeat kinematics and mechanical power output

Bees hovering at fragrance baits in the Laboratory Clearing were lured into a screened insectary and induced to hover in front of a handheld digital camcorder (Canon ZR10; 60 fields s^{-1}; shutter speed 1/60 s), which was positioned to record wingbeat amplitude from the dorsal view of the animal. Bees hovered over a translucent plastic sheet, lit from behind to enhance contrast of images. A front surface mirror oriented at 45° with respect to the field of view provided a simultaneous image of stroke plane angle and body angle. After transferring suitable sequences to NIH Image, projected amplitude, stroke plane angle (β) and body angle (χ) were measured at five frames during the sequence. Wingbeat amplitude (Φ) was obtained by dividing projected amplitude by the cosine of the stroke plane angle to correct for foreshortening. Wingbeat frequency (*WBF*) was recorded on the camcorder's audio track using an optical tachometer (Unwin and Ellington, 1979). A virtual instrument programmed in LabView (National Instruments, Austin, TX, USA) was used to filter out noise and estimate the fundamental frequency from 0.5 s determinations, which were then averaged over the duration of the flight sequence.

Following measurements of heat loss (see below), standard morphological parameters were determined for all insects (Ellington, 1984). Body mass (m_b) and wing mass (m_w) were measured to the nearest 0.0001 g using an analytical balance. Wing length (r) was measured to the nearest 0.01 mm using digital calipers. Wing area (S), wing loading (p_w), wing aspect ratio (*AR*) and non-dimensional radii for moments of wing area and virtual mass were determined from digital photos of wings. Non-dimensional moments of wing mass were taken from Dudley (1995).

Mass-specific mechanical power output was estimated from inertial (P^*_{acc}), induced (P^*_{ind}) and profile powers (P^*_{pro}) using the hovering aerodynamic model of Ellington (1984) for the cases of perfect elastic energy storage (P^*_{per}) and zero elastic energy storage (P^*_{zero}). The mean lift coefficient (C_L) was calculated using the mean force balance during hovering (Ellington, 1984), and the profile drag coefficient (C_D) was estimated assuming a lift-to-drag ratio of 0.55 (Feuerbacher et al., 2003; Sane and Dickinson, 2001).

We used least-squares regressions to test if flight kinematics or mechanical power or both changed with temperature.

Metabolic power

Following the filming of wingbeat kinematics, metabolic power was estimated using closed-system respirometry. Bees were placed in a 1 or 2 liter glass bottle equipped with an infrared carbon dioxide sensor (Vernier Software and Technology, Beaverton, OR, USA) accurate to 10% of the reading between 0 and 5000 p.p.m. CO_2. Carbon dioxide concentration was sampled at a rate of 1 Hz using a Vernier LabPro 12-bit A-D converter connected *via* USB cable to an Apple Macintosh G3 Powerbook running Vernier LoggerPro software. Mass-specific metabolic rate (P^*_{met}) was calculated from the slope of carbon dioxide concentration as a function of time, assuming a respiratory quotient of one and an energy equivalent of 21.4 J ml^{-1} O_2.

In spite of hovering abilities exhibited during kinematic trials, bees were reluctant to engage in continuous flight in the respirometry chamber. We categorize flight behavior during these experiments as 'agitated' because it was necessary to shake the chamber periodically to prolong flight periods (Harrison et al., 1996; Suarez et al., 1996). Only respirometry trials in which bees flew continuously for at least 2 min were included in our final data set. The purpose of these measurements was not to replicate the comprehensive gas-exchange studies of Roberts et al. (1998) or Roberts and Harrison (1999) but to complement our measurements of heat loss and wingbeat kinematics from freely hovering bees. Consequently, we derived independent estimates of heat production by subtracting mechanical power output from metabolic power input.

Power for heat production

At equilibrium, metabolic heat production will equal the sum of radiative, convective and evaporative heat loss. Radiative and convective heat loss can be inferred from body segment temperatures following flight and the Newtonian cooling constants of freshly killed specimens (Heath and Adams, 1969; May, 1976; May and Casey, 1983). After at least 1 min of hovering, bees were captured in a hand net. An Omega mini-hypodermic copper–constantan thermocouple probe (outer diameter 0.2 mm) was sequentially inserted into the thorax, abdomen and head of the insect within 10 s following capture. Temperatures were recorded using an Omega 450 ATT temperature meter (Stamford, CT, USA) with 0.1°C resolution. The insect was killed in acetone fumes and brought to the lab for morphological measurements and determinations of cooling constants.

Body segment temperatures and *WBF* were also recorded from an additional 20 bees at the Central Tower and Laboratory clearings on BCI. Variation in heat transfer

between the thorax and the head or abdomen was investigated using the head temperature excess ratio:

$$R_h = (T_h - T_a)/(T_{th} - T_a) , \qquad (1)$$

where T_h is head temperature, T_a is air temperature and T_{th} is thoracic temperature, and the abdominal temperature excess ratio:

$$R_{ab} = (T_{ab} - T_a)/(T_{th} - T_a) , \qquad (2)$$

where T_{ab} is the abdominal temperature. According to the three-compartment model outlined by Stavenga et al. (1993), R_h or R_{ab} will be independent of T_a if heat transfer does not vary between the thorax and the respective segment. If insects actively dissipate heat from their thoraces at high T_a, then R_h or R_{ab} will increase with T_a. Conversely, if insects regulate their heads or abdomens above T_a at low T_a then R_h or R_{ab} should decrease with T_a.

The induced flow field around a hovering insect provides convective cooling in the absence of winds or forward flight speed. The magnitude of this downwash was calculated using the aerodynamic model of Ellington (1984), assuming uniform flow across all body segments. The primary orientation of flow with respect to the body was approximated as the sum of the body angle and the stroke plane angle. Preliminary experiments with two individuals oriented at 0, 30, 60 and 90° indicated that flow orientation exhibits the greatest influence on head and abdominal conductance measurements. Thoracic conductance did not appear to be sensitive to flow orientation. Based on kinematic analyses of 16 individuals, the angle between the body and the stroke plane was 31.24±2.16° (mean ± 95% C.l.). Insects filmed in flight were thus mounted on a thin metal rod and placed in the test section of a desktop wind tunnel (TSl Model 8392 Certifier; TSl Incorporated, St Paul, MN, USA; turbulence intensity 0.25%) at an angle of 30° with respect to oncoming flow. A mini-hypodermic thermocouple probe was inserted into the thorax, abdomen or head of the insect, and a fiber optic microscope lamp was used to warm the insect to 50°C. After cooling at least 5°C, temperatures were logged by hand every 20 s until segment temperature was within 1°C of air temperature. This procedure was repeated on each body segment at air speeds of 0, 1 and 2 m s^{-1}. Air speed was measured using a calibrated hot-wire anemometer (TSl Incorporated). Following trials, head, abdomen and thorax (without wings or legs) were weighed using an analytical balance (±1 mg). Body segment masses were multiplied by a correction factor based on the ratio of total mass before cooling trials to the sum of the segment masses. The cooling constant, k, for each segment was calculated from the natural logarithm of the slope of the linear regression of temperature elevation *versus* time. Using data specific to each individual, we employed a regression model to estimate the cooling constants at the appropriate induced velocity. Non-evaporative heat loss was calculated as:

$$P_{heat} = k_{th}hm_{th}(T_{th} - T_a) + k_{ab}hm_{ab}(T_{ab} - T_a) + k_hhm_h(T_h - T_a) , \qquad (3)$$

where h is the specific heat of insect tissue (3.48), m_{th} is the thoracic mass, m_{ab} is the abdominal mass and m_h is the head mass (May and Casey, 1983). This simple model is useful as a first approximation but it disregards internal conductance due to hemolymph flow and assumes that heat is generated independently in the head, thorax and abdomen. More detailed approaches can be found in May (1995b) and Stavenga et al. (1993).

Mass-specific evaporative heat loss (P^*_{evap}) was estimated using regression equations for another member of the family Apidae, *Apis mellifera* (Roberts and Harrison, 1999). Although *A. mellifera* is known to modulate evaporative cooling at extremely high temperatures (Heinrich, 1979; Roberts and Harrison, 1999), at temperatures below 35°C evaporative heat loss is consistent with data reported for the anthophorid bees *Centris pallida* (Roberts et al., 1998) and *Xylocopa capitata* (Nicolson and Louw, 1982). Based on our estimates, evaporative heat loss accounts for 6–7% of the total heat loss from a hovering *Eg. imperialis*, and errors in our extrapolations are unlikely to influence our major results.

We used a least-squares regression to test whether metabolic heat production declined with T_a.

Muscle efficiency

Muscle efficiency (η) is the ratio of mechanical power output (P^*_{mech}) divided by metabolic power input (Josephson et al., 2001):

$$\eta_{met} = P^*_{mech}/P^*_{met} . \qquad (4)$$

Alternatively, efficiency can be calculated using P^*_{mech} in conjunction with heat loss measurements (Wakeling and Ellington, 1997):

$$\eta_{heat} = P^*_{mech}/(P^*_{heat} + P^*_{evap} + P^*_{mech}) . \qquad (5)$$

We calculated η using equations 2 and 3 for the cases of perfect and zero elastic energy storage and used these values to test whether η changes with T_a.

In the above calculations of muscle efficiency, we have assumed elastic energy storage to be constant but we will also consider the possibility of variable elastic energy storage in our Discussion. It is possible to calculate efficiency *via* subtraction using only respirometric and heat loss data:

$$\eta_{subtraction} = (P^*_{met} - P^*_{heat} - P^*_{evap})/P^*_{met} . \qquad (6)$$

This efficiency estimate will be largely independent of Ellington's (1984) model and assumptions regarding both elastic energy storage and the lift-to-drag ratio. In effect, wingbeat kinematics are only used in estimating the induced flow velocity. Theoretically, the numerator of equation 6 should fall between P^*_{per} and P^*_{zero}, providing an indication of the degree of elastic energy storage. Unfortunately, because variance in the numerator was over two orders of magnitude greater than variance in P^*_{per} or P^*_{zero}, low statistical power compromised our ability to estimate elastic energy storage or test temperature trends using this efficiency estimate.

Field measurement of wingbeat frequency

Wingbeat frequencies for six species of orchid bees hovering at fragrance baits were recorded at field sites throughout Costa Rica (October 2002–January 2003) and at the Central Tower on Barro Colorado Island (June 2002): *Euglossa championi* Cheesman, *Euglossa imperialis*, *Euglossa purpurea* Friese, *Euglossa sapphirina* Moure, *Eulaema meriana* Olivier and *Eulaema cingulata* Fabricius. The genus *Euglossa* consists of smaller (<300 mg) glabrous bees whereas *Eulaema* tend to be larger (400–1000 mg), pubescent bees. An optical tachometer was used to record *WBF* on a portable cassette recorder (Sony TCM-20DV). Air temperature was measured using a handheld thermocouple shielded from the sun and, when bees were hovering in direct sunlight, operative temperature (T_{op}) was also obtained from a thermocouple implanted in the corpse of a freshly killed bee (Armbruster and Berg, 1994). Operative temperature provides a rough metric for the convective and radiative heat environment experienced by a hovering bee although it does not take into account the contribution of air movements produced by the flapping wings. In addition, evaporative heat loss from our bee corpses may have led to an underestimate of T_{op}, but we feel these measurements are more useful than T_a because of variability in solar radiation at our field sites. After netting bees, body mass was measured using a portable microbalance (±1 mg).

Audio clips were transferred to a Macintosh G3 Powerbook with 16-bit resolution at a sampling rate of 44.1 kHz. A custom virtual instrument in LabView was used to electronically filter out noise and determine the fundamental frequency from a distribution of 0.1 s determinations (Fig. 1A,B). Least-squares regression was used to test whether *WBF* declined with T_a and T_{op}.
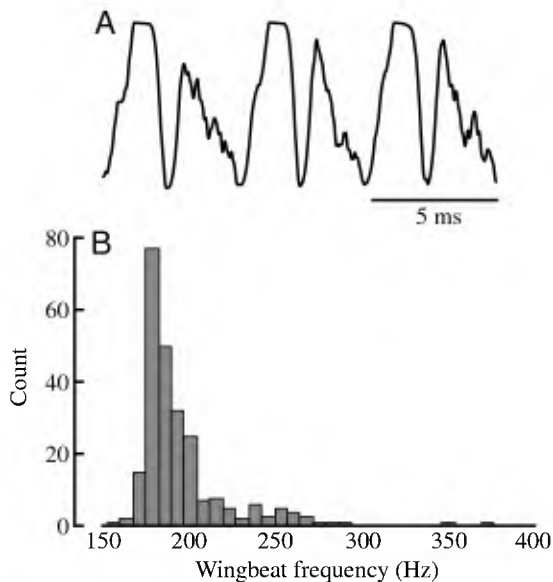

Fig. 1. (A) Filtered optical tachometer signal of three wingbeats from a *Euglossa imperialis* hovering over a chemical bait. (B) Histogram of wingbeat frequency determinations from the complete record sampled in A.

Results

Body temperature and morphology

Air temperatures during these experiments ranged from 24.9 to 33.2°C. T_{th}, T_h and T_{ab} of *Eg. imperialis* increased with T_a (Fig. 2). The slope of T_{th} on T_a was significantly less than one, but T_h exhibited even greater stability than T_{th} (Fig. 2). The significant negative correlation between R_h and T_a suggests that the head is being actively thermoregulated *via* supplemental heat transfer at low T_a (Fig. 3). R_{ab} also showed a slight, but significant, decline with T_a, a finding that indicates that additional heat is also being transferred to the abdomen at low T_a (Fig. 3).

Mean body mass for *Eg. imperialis* on BCI was 160.8±6.2 mg (mean ± 95% C.I.). Mean wing loading was 21.7±1.3 N m^{-2}. Neither body mass nor wing loading were significantly correlated with T_a ($P>0.1$). Cooling constants for all body segments increased with airspeed (Table 1).

Kinematics and mechanical power output

Out of 28 trials, complete respirometric, thermal and kinematic measurements were obtained for 13 *Eg. imperialis* individuals. For these 13 individuals filmed in the insectary, *WBF* declined with T_a, but this trend was not statistically significant (Fig. 4; Table 2). Further investigation using data
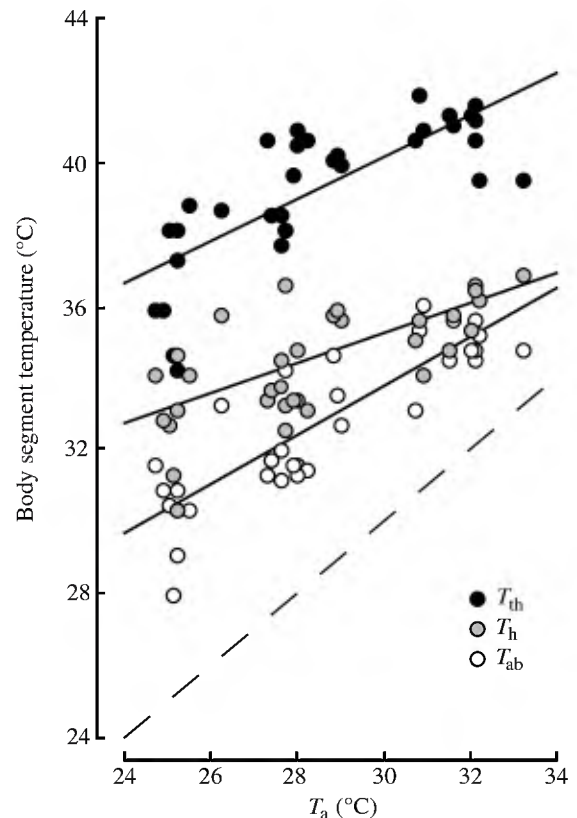

Fig. 2. Thorax (T_{th}), head (T_h) and abdomen (T_{ab}) temperatures plotted against air temperature (T_a) in hovering *Euglossa imperialis*. Least-squares regressions: $T_{th}=0.574T_a+22.9$, $r^2=0.596$, $P<0.0001$; $T_h=0.418T_a+22.6$, $r^2=0.497$, $P<0.0001$; $T_{ab}=0.675T_a+13.5$, $r^2=0.745$, $P<0.0001$.
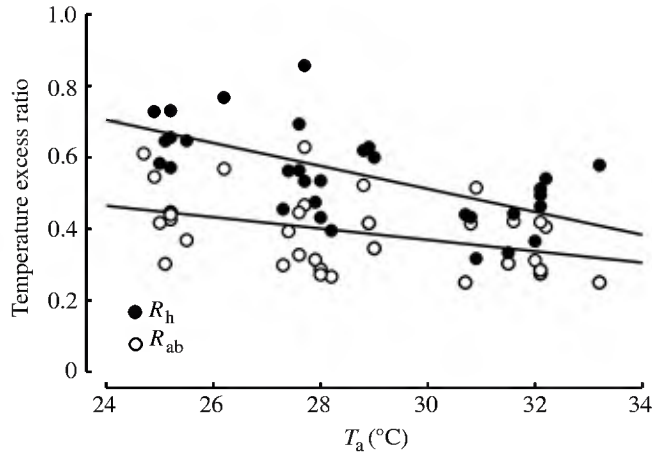
Fig. 3. Head temperature excess ratio (R_h) and abdominal temperature excess ratio (R_{ab}) decline with air temperature (T_a) in hovering *Euglossa imperialis*. Least-squares regressions: $R_{ab}=-0.016T_a+0.846$, $r^2=0.16$, $P=0.0213$; $R_h=-0.032T_a+1.475$, $r^2=0.395$, $P<0.0001$.
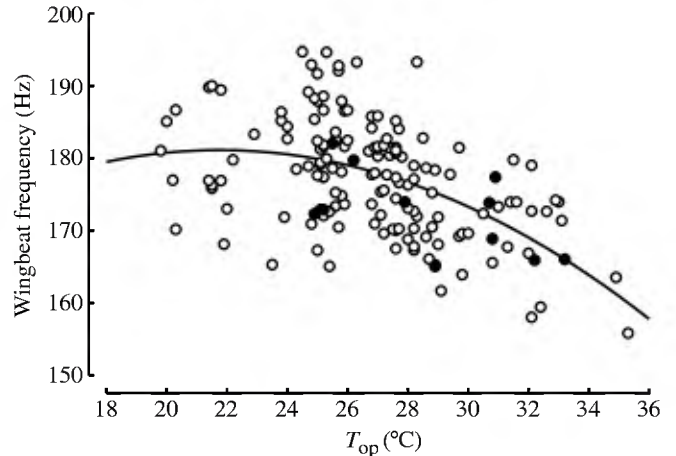

Fig. 4. Wingbeat frequency (*WBF*) declines at higher operative temperatures (T_{op}) in hovering *Euglossa imperialis* (13 experimental bees shown as filled circles; 139 additional bees from Costa Rica and Panama shown as open circles). Least-squares regression: $WBF=-0.115T_{op}^2+5.00T_{op}+127$.

from 33 individuals from BCI and 126 individuals from Costa Rica provided statistical support for this trend (Fig. 4; BCI alone, $P=0.0024$; Costa Rica alone, $P<0.0001$). *WBF* declined from approximately 180 Hz at 20°C to 170 Hz at 34°C. A quadratic regression provided the best fit to the pooled data (Table 3). T_{th} was not significantly correlated with *WBF* ($F_{1,32}=2.380$, $P=0.1331$). However, the elevation of T_{th} over T_a did increase significantly with *WBF* (Fig. 5). No other kinematic parameters were significantly correlated with T_a (Table 3).

Mean induced velocity was 1.85 ± 0.08 m s^{-1}. Mean mechanical power output was 152 W kg^{-1} assuming perfect elastic energy storage or 197 W kg^{-1} assuming zero elastic energy storage (Table 2). Neither estimate was correlated with T_a (Fig. 6; Table 2).

Flight metabolic rate, heat production and muscle efficiency

Mass-specific metabolic rate, P^*_{met}, and non-evaporative heat loss, P^*_{heat}, declined linearly with T_a (Fig. 6; Table 2). Metabolic heat production declined by 33% as T_a increased from 24 to 34°C. Subtracting mechanical power output from P^*_{met}, we calculated metabolic heat production to be 705 ± 85.5 W kg^{-1} assuming zero elastic energy storage or 751 ± 85.7 W kg^{-1} with perfect elastic energy storage. Neither of these values was significantly different from

677 ± 80.3 W kg^{-1}, calculated by adding P^*_{evap} to P^*_{heat} ($P>0.1$). All three of these estimates declined significantly with T_a ($P<0.05$). We note that P^*_{heat} for individual #5 was greater than P^*_{met}, indicating an error in either one or both measurements. However, removal of this data point does not alter our primary conclusions. Mechanical power output estimated by subtraction ($P^*_{met}-P^*_{heat}-P^*_{evap}$) was 225 ± 98.5 W kg^{-1}, which was not significantly greater than either P^*_{per} or P^*_{zero} ($P>0.1$).

Muscle efficiency increased linearly with T_a (Fig. 7; Table 2). Mean muscle efficiency was 18% assuming perfect elastic energy storage and 22% assuming zero elastic energy storage (Table 2). Individual values for efficiency estimated by subtraction (equation 6) varied widely (–17 to 47%), but the mean value, 24% (28% without the negative value calculated for individual #5), was only slightly higher than our other efficiency estimates (Table 2).

Comparative wingbeat frequency

In the six species of orchid bees studied, *WBF* scaled as $m_b^{-0.29}$ ($r^2=0.959$). In all six species, *WBF* declined significantly with T_{op} (Table 3; Figs 4, 8, 9) and T_a (results not shown). In the two species with the greatest sample size, *Eg. imperialis* and *Eg. purpurea*, a quadratic fit provided the best

Table 1. *Newtonian cooling constants,* k, *for body segments of* Euglossa imperialis *at three airspeeds*

Airspeed (m s^{-1})	k (s^{-1})		
	Thorax ($N=13$)	Abdomen ($N=12$)	Head ($N=10$)
0	0.009 (0.008–0.010)	0.017 (0.016–0.019)	0.022 (0.019–0.025)
1	0.015 (0.014–0.017)	0.031 (0.025–0.037)	0.042 (0.036–0.047)
2	0.019 (0.017–0.020)	0.039 (0.031–0.048)	0.068 (0.062–0.075)

Values in parentheses are 95% confidence intervals for the means.

Table 2. *Mean kinematics, aerodynamic coefficients, body mass-specific powers and efficiencies for 13* Euglossa imperialis *individuals hovering in a screened insectary on Barro Colorado Island, Panama*

	Mean	95% C.I.	$F_{1,12}$	P
WBF (Hz)	173	169–176	4.37	0.061
Φ (deg.)	124	121–126	1.59	0.233
β (deg.)	9.94	7.50–12.39	0.093	0.766
χ (deg.)	21.5	19.9–23.1	0.990	0.350
Re	1650	1540–1750	0.421	0.530
C_L	1.49	1.32–1.66	0.331	0.577
C_D	2.71	2.40–3.03	0.335	0.574
V_{ind} (m s^{-1})	1.85	1.77–1.93	2.12	0.173
$P*_{ind}$ (W kg^{-1})	18.2	17.4–19.1	2.64	0.135
$P*_{pro}$ (W kg^{-1})	133	128–138	0.068	0.800
$P*_{acc}$ (W kg^{-1})	82.1	69.8–94.5	0.188	0.673
$P*_{zero}$ (W kg^{-1})	197	191–203	0.002	0.963
$P*_{per}$ (W kg^{-1})	152	148–156	0.108	0.749
$P*_{met}$ (W kg^{-1})	903	819–986	10.5	0.012
$P*_{heat}$ (W kg^{-1})	634	553–716	9.06	0.012
$P*_{evap}$ (W kg^{-1})	44.7	35.8–53.5	n/a	n/a
$\eta_{heat, zero}$ (%)	22.9	21.1–24.7	7.70	0.018
$\eta_{met, zero}$ (%)	22.2	20.3–24.2	8.56	0.014
$\eta_{heat, per}$ (%)	18.6	16.9–20.4	6.85	0.024
$\eta_{met, per}$ (%)	17.1	15.6–18.6	10.5	0.008
$\eta_{subtraction}$ (%)	24.1	14.8–33.4	0.16	0.696

F-statistic and *P*-values are the results of a linear regression for a given parameter on air temperature. Profile drag coefficients were estimated using a lift-to-drag ratio of 0.55 (Sane and Dickinson, 2001). For definitions, see text and List of symbols.
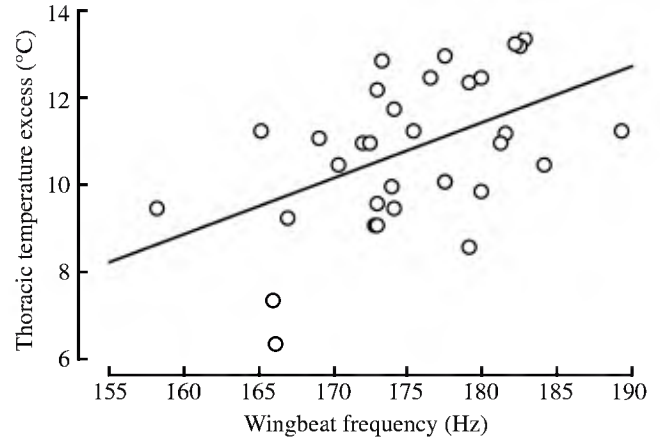

Fig. 5. Thoracic temperature excess increases linearly with wingbeat frequency (*WBF*) in hovering *Euglossa imperialis*. Least-squares regression: $T_{excess}=0.128WBF-11.6$, $r^2=0.240$, $P=0.0038$.

fit to the observed data (Figs 4, 9), with *WBF* remaining relatively constant at T_{op} between 20 and 25°C and declining above 25°C.

WBF declined between 4 and 9% (mean 6%) as T_{op} increased from 24 to 34°C. Percent change in *WBF* was not correlated with body mass ($P>0.1$).

Discussion

Results from this study support the hypothesis that hovering orchid bees regulate metabolic heat production *via* modulation of wingbeat frequency. Our conclusions are based on kinematic, thermal and respirometric measurements from a single species, *Euglossa imperialis*, and wingbeat frequency recordings from five additional species. Both metabolic and heat loss measurements indicate that heat production in *Eg. imperialis* increases by 33% as air temperature decreases by 10°C. These results are close to values measured for *Centris pallida* (Roberts et al., 1998) but higher than the 20–25% increase estimated for *Apis mellifera* (Harrison et al., 1996; Roberts and Harrison, 1999). Moffatt (2001) found that foraging metabolic rate (flight and feeding) in *A. mellifera* varies with both air temperature and sucrose reward rate, recording up to a 50% drop in metabolic rate with an increase of 10°C. Previous data from *Eg. imperialis* suggested a decline between 20 and 30% (May and Casey, 1983), values that fall within the 95% confidence intervals for our regression estimates. We suggest that the ability to regulate metabolic heat production is a widespread feature of the corbiculate bees but caution that no study to date has actually demonstrated a metabolic response of an individual insect subjected to a range of thermal environments as has been shown in hummingbirds (Chai et al., 1998).
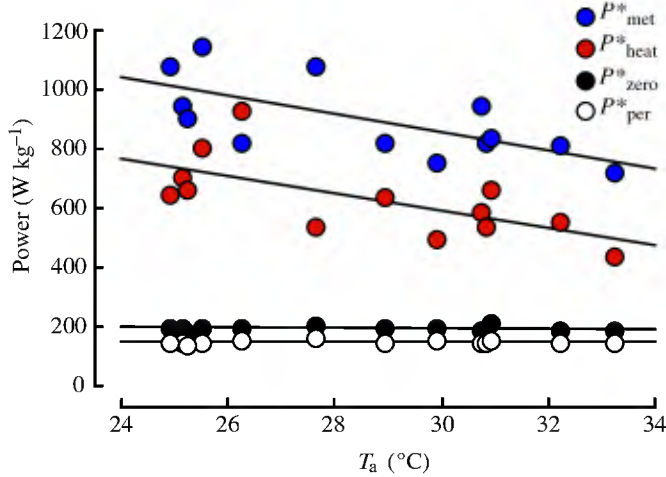
Table 3. *Mean body mass, mean wingbeat frequency (range), sample size and least-squares regression statistics for equations describing the relationship between wingbeat frequency and operative temperature for six species of euglossine bees hovering over chemical baits in Costa Rica and Panama*

Species	m_b (mg)	*WBF* (Hz)	N	r^2	F	P
Eg. sapphirina	65	211 (190–224)	60	0.140	9.41	0.033
Eg. purpurea	97	223 (199–236)	103	0.228*	14.8	<0.0001
Eg. championi	106	191 (178–204)	16	0.306	6.18	0.0261
Eg. imperialis	158	177 (156–195)	159	0.272*	29.1	<0.0001
El. cingulata	545	128 (118–135)	19	0.274	6.41	0.0215
El. meriana	962	99 (90–108)	33	0.248	10.2	0.0032

*Quadratic equation.

Fig. 6. Metabolic power ($P*_{met}$), non-evaporative heat loss ($P*_{heat}$) and mechanical power output ($P*_{zero}$ and $P*_{per}$) plotted against air temperature (T_a) in hovering *Euglossa imperialis*. The equation for metabolic rate is: $P*_{met}=-30.6T_a+1780$. The equation for heat loss is: $P*_{heat}=-29.9T_a+1490$.

In comparison with pubescent bees (e.g. *Bombus, Eulaema*), *Euglossa* is a relatively poor thermoregulator (Roberts and Harrison, 1998). The slope of T_{th} on T_a for *Eg. imperialis* is closer to 1 than to 0, which led previous authors to classify tropical bees as thermoconformers rather than thermoregulators (Heinrich, 1993). The present findings demonstrate that *Eg. imperialis* regulates heat production at low air temperatures, and we suggest that its poor thermoregulatory ability is largely a consequence of its small size and lack of pubescent insulation. In *Bombus* and *Eulaema*, pubescence substantially lowers the convective cooling constant (Heinrich, 1993; May and Casey, 1983), but when these bees encounter high temperatures their thick pile
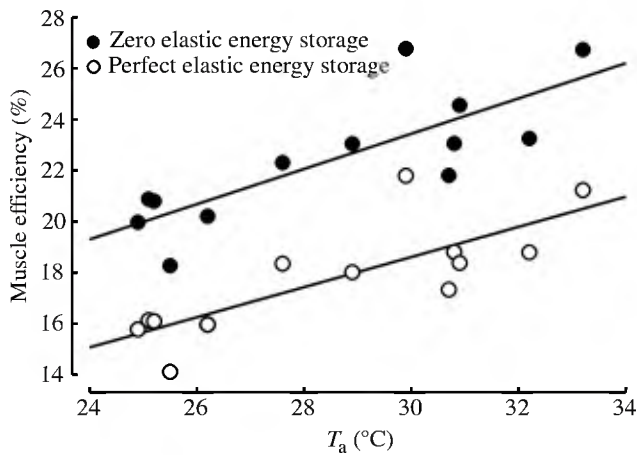

Fig. 7. Muscle efficiency (η) increases with air temperature (T_a) in hovering *Euglossa imperialis*. Data points are mean values for each individual from respirometry and heat loss experiments. The equation for the least-squares regression line assuming perfect elastic energy storage is $\eta=0.006T_a+0.009$ and for zero elastic energy storage is $\eta=0.007T_a+0.027$.
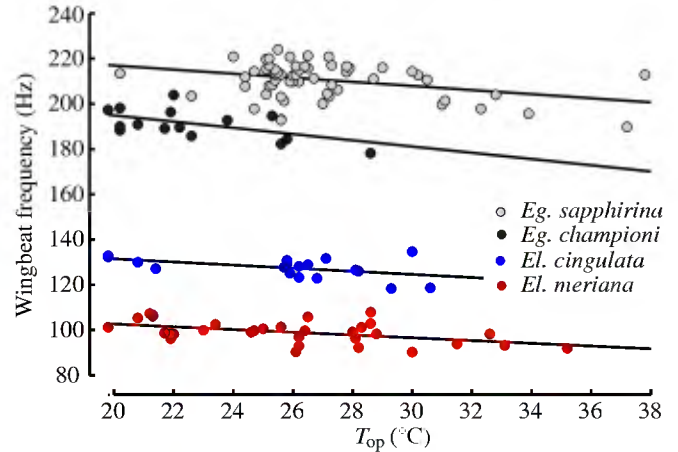

Fig. 8. Wingbeat frequency declines linearly with operative temperature in four species of orchid bees hovering in the field.

becomes a liability and they must shunt excess heat to their abdomens (Roberts and Harrison, 1998). By contrast, *Apis, Centris* and *Euglossa* all possess a countercurrent exchange mechanism limiting the dissipation of thoracic heat to the abdomen (Wille, 1958). This anatomical constraint suggests that the modulation of heat production will play a critical role in thoracic temperature regulation by these bees. Curiously, our data indicated that the abdominal excess ratio declined with ambient temperature, but this appears to be a rather anomalous finding in light of previous research on orchid bees (May and Casey, 1983) and other bees (Roberts et al., 1998; Roberts and Harrison 1999).

An additional reason why the slope of T_{th} on T_a may be so steep in *Euglossa* is that these bees warm their heads at the expense of their flight muscles. We found that *Eg. imperialis* regulates T_h by augmenting heat transfer to the head at low T_a. Modulation of heat transfer between the head and thorax has also been demonstrated in the bee *Centris pallida* and the
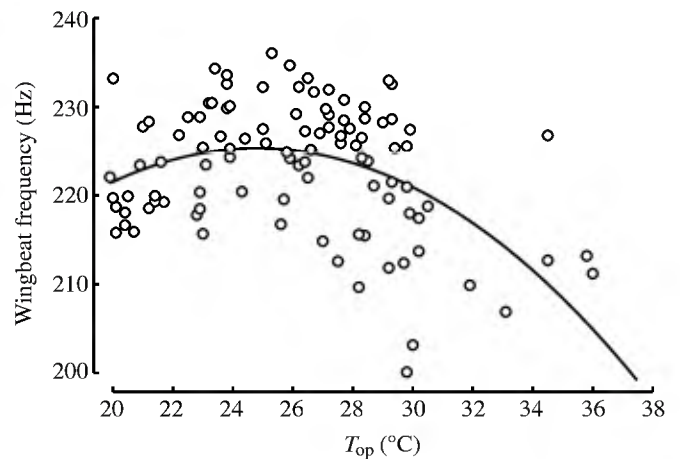

Fig. 9. Wingbeat frequency (*WBF*) declines at higher operative temperatures (T_{op}) in hovering *Euglossa purpurea*. Least-squares regression: $WBF=-0.163T_{op}^2+8.09T_{op}+125$.

dragonfly *Anax junius*, but the hypothesized function of this ability is not to dissipate excess heat from the thorax but to optimize cephalic neural functioning (Roberts et al., 1998; May, 1995b). We conclude from our measurements of body segment temperatures that the stability of both T_h and T_{th} during flight at low T_a is achieved by variation in heat production not heat dissipation.

Wingbeat frequency in orchid bees declines with air temperature while muscle efficiency increases with air temperature. Unfortunately, kinematic data from our 13 experimental bees proved inadequate for identifying compensatory mechanisms by which bees maintain constant body weight support across a range of wingbeat frequencies. Ruby-throated hummingbirds compensate for declining frequency at high temperatures by increasing the amplitude of wing motions (Chai et al., 1998). In the present study, we found no evidence for compensatory changes in wingbeat amplitude, which is not surprising given the variety of lift-generating mechanisms available to flying insects (Dickinson et al., 1999; Srygley and Thomas, 2002).

The modulation of wingbeat frequency observed in orchid bees was consistent with recent data from other insects that regulate heat production during flight. For *Eg. imperialis*, an increase of 10°C results in a 6% decline in wingbeat frequency. *A. mellifera* shows an 8% drop over the same temperature range, and *C. pallida* declines by almost 14% (Harrison et al., 1996; Roberts and Harrison, 1998). Wingbeat frequency of two species of dragonflies declined by an average of 18% as heat production dropped by almost 60% (May, 1995a). Interestingly, in many of the bees examined to date (*Euglossa*, *Centris* and *Apis*), wingbeat frequency appears to exhibit a two-phase relationship with air temperature, suggesting that wingbeat frequency may be maximized at air temperatures below 25°C.

In contrast to flying bees and dragonflies, most insects show a positive relationship between wingbeat frequency and air temperature (Oertli, 1989). In beetles, this phenomenon is manifested as a positive relationship between frequency and thoracic temperature (Oertli, 1989). Oertli (1989) argues convincingly that the temperature sensitivity of wingbeat frequency is a consequence of the resonant properties of the thorax. The present data demonstrate that thoracic temperature does not influence wingbeat frequency in orchid bees, rather modulation of frequency influences thoracic temperature *elevation*. Indeed, work loop experiments with asynchronous flight muscle indicate that heat production increases when muscles are activated above their usual contraction frequencies (Josephson et al., 2001). With our experimental methods, we were unable to distinguish whether changes in muscle efficiency represent a shift in the biochemical efficiency of muscle contraction (Baker and Thomas, 2000; Maughan and Vigoreaux, 1999) or in the amount of elastic energy stored by the thorax during the deceleration phase of the wing stroke (Dickinson and Lighton, 1995; Ettema, 2001; Roberts et al., 1998). Metabolic heat production increased by over 200 W kg^{-1} as temperature decreased by 10°C. Assuming a

constant efficiency of 20%, a complete shift from perfect elastic energy storage to zero elastic energy storage would result in a 45 W kg^{-1} increase in mechanical power requirements and a >200 W kg^{-1} increase in metabolic power. Alternatively, if elastic energy storage remains constant, then efficiency must decrease by approximately 6% to account for this 200 W kg^{-1} increase. Further work is needed to identify the physiological and biochemical mechanisms by which heat production may be decoupled from useful aerodynamic work.

Because enzyme function is highly temperature dependent, physiological and biochemical processes may be optimized through temperature specialization (Hochachka and Somero, 1984). One hypothesis for specializing at high temperatures is that it may reduce the threat of overheating during strenuous activities such as locomotion (Heinrich, 1977). Animals that are adapted to operate at high body temperatures must possess a mechanism to warm themselves at cooler temperatures. Flying bees accomplish this task by beating their wings at elevated frequencies.

List of symbols

Φ	stroke amplitude
β	stroke plane angle
χ	body angle
η	muscle efficiency
AR	wing aspect ratio
C_D	coefficient of drag
C_L	coefficient of lift
h	specific heat of insect tissue
k	Newtonian cooling constant of the thorax, head or abdomen
m_b	body mass
m_{th}	thoracic mass
m_h	head mass
m_{ab}	abdominal mass
m_w	wing mass
P^*_{acc}	body mass-specific inertial power
P^*_{evap}	body mass-specific evaporative heat loss
P^*_{heat}	body mass-specific non-evaporative heat loss
P^*_{ind}	body mass-specific induced power
P^*_{mech}	body mass-specific mechanical power output
P^*_{met}	body mass-specific metabolic power
P^*_{per}	body mass-specific mechanical power output assuming perfect elastic energy storage
P^*_{pro}	body mass-specific profile power
P^*_{zero}	body mass-specific mechanical power output assuming zero elastic energy storage
P_{heat}	non-evaporative heat loss
p_w	wing loading
r	wing length
R_{ab}	abdominal temperature excess ratio
Re	Reynolds number of the wing chord
R_h	head temperature excess ratio
S	wing area
T_a	air temperature

T_{ab} abdominal temperature
T_h head temperature
T_{op} operative temperature
T_{th} thoracic temperature
V_{ind} induced velocity
WBF   wingbeat frequency

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