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Thermoregulation in four species of tropical solitary bees: the roles of size, sex and altitude

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Abstract. Body temperatures during free flight in the field, warm-up rates during pre-flight warm-up, and temperatures during tethered flight are measured for four tropical solitary bee species at three sites of differing altitude in Papua New Guinea. All four species are capable of endothermic preflight warm-up; three species give slopes of thoracic temperature on ambient temperature of significantly less than 1, indicating regulation of thoracic temperature. In the kleptoparasitic *Coelioxys* spp. (Megachilidae) and *Thyreus quadrimaculatus* (Anthophoridae), warm-up rates and thoracic temperatures in flight are low by comparison with the two provisioning species *Creightonella frontalis* (Megachilidae) and *Amegilla sapiens* (Anthophoridae). In both *C. frontalis* and *A. sapiens* thoracic temperatures correlate positively and significantly with both ambient temperature and body mass. In *A. sapiens*, body mass increases with altitude; this can be interpreted as a response to lower ambient temperatures at higher altitude, an example of Bergmann's rule. In both *A. sapiens* and *C. frontalis* populations at higher altitude have higher thoracic temperatures independent of differences of body mass, suggestive of additional morphological or physiological adaptation to lower ambient temperatures. In *A. sapiens* there is no qualitative difference in body temperatures between males and females after controlling for body mass, while male *C. frontalis* have significantly lower thoracic temperatures than females of the species. This difference between *A. sapiens* and *C. frontalis* is discussed with reference to variation in mating systems found in the Apoidea.

Key words: Thermoregulation – Body temperature – Apoidea – Anthophoridae – Megachilidae

Introduction

Most studies on endothermy in bees have concentrated on temperate species, with many studies on bumblebees in the genus *Bombus* (e.g. Heinrich 1974, 1975) and on the honeybee, *Apis mellifera* [reviewed by Schmaranzer (1991), Southwick (1991) and Stabentheiner (1991)]. This may be at least partly because it is in cold climates that elevation of body temperatures, allowing continued activity in the face of poor climatic conditions, has particularly obvious advantages. Endothermy is also known from a number of solitary bees from arid environments, particularly members of the anthophorid genera *Xylocopa* (e.g. Chappell 1982; Nicolson and Louw 1982; Louw and Nicolson 1983; Gerling et al. 1983; Willmer 1988), *Centris* (Chappell 1984), *Anthophora* (Stone 1993) and the megachilid *Chalicodoma sicula* (Willmer 1986). Among bees of the wet tropics the most extensive studies have been of neotropical euglossine bees (May and Casey 1983; Casey et al. 1985; Inouye 1985). Detailed thermophysiological studies of individual tropical solitary bee species are rare. This is of particular importance because we now know that pre-flight thermogenesis in the Apoidea is restricted neither to large species nor to temperate species, but is widespread in the taxon (Stone and Willmer 1989a). Warm-up rates and T_{th}s under a given set of conditions are lowest in small species active in warm climates (Stone and Willmer 1989a). This study investigates whether small tropical solitary bees are capable of rates of preflight thermogenesis and levels of thermoregulation before and during flight compatible with their temperate counterparts.

This study extends the work on tropical bees to four species from P.N.G. For each species the study involves measurement of body temperatures during free flight in the field, and of body temperatures during pre-flight warm-up and tethered flight in the laboratory. *Creightonella frontalis* Fab. (Megachilidae) and *Amegilla sapiens* Cockerell (Anthophoridae) are two solitary bee species of applied interest through their roles as pollinators of introduced coffee (Willmer and Stone 1988) and carda-

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Abbreviations: C.R.I., Christensen Research Institute; P.N.G., Papua New Guinea; SFT, stable flight temperature; T_a, ambient air temperature; T_{ab}, abdominal temperature; T_{dif}, the temperature difference between thorax and abdomen; T_{ex}, thoracic temperature excess; VFT, voluntary flight temperature

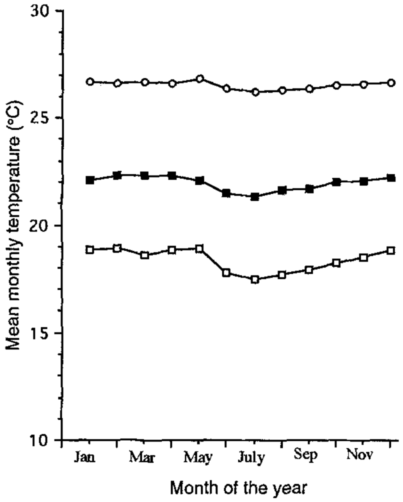

Fig. 1. Mean monthly temperatures over the year for Madang (○), Baiyer River (■) and Mount Hagen (□) (From Anon, 1974)

mom crops (Stone and Willmer 1989b) in P.N.G., and are known to be capable of endothermic preflight warm-up (Stone and Willmer 1989c). *Thyreus quadrimaculatus* Rad (Anthophoridae) is a nest parasite of *Amegilla sapiens*, and *Coelioxys* spp. (Megachilidae) a nest parasite of *C. frontalis*. Kleptoparasitic bees develop in the nest cells constructed by their host bee species, and have no need to provision cells with pollen or nectar. They are thus not dependent on continued pollen foraging for reproductive success, unlike the females of their hosts. All four species are also found in a wide range of environments, from hot coastal plains (Willmer and Stone 1988) to lower montane rainforest (Stone et al. 1988; Stone and Willmer 1989b). *Thyreus quadrimaculatus* was studied at two sites, and *A. sapiens* and *C. frontalis* at three sites, differing in altitude and climate.

Unlike female solitary bees, all of which (barring kleptoparasitic species) share the common need to provision cells, male bees vary considerably in their reproductive strategies (Thornhill and Alcock 1983; Chappell 1984; Larsson 1989). While males of some species actively patrol female nest locations or foraging sites, males of others pursue less energetically costly strategies. This study examines intersexual differences in body temperatures in two bee species with very different male mating strategies – active territoriality in *A. sapiens* (Stone et al. 1988) and scramble competition for emerging females in *C. frontalis*.

Study sites

Baiyer River Sanctuary is situated in an area of low montane rainforest (altitude 1160 m) in the Baiyer River valley near Mount Hagen, Western Highlands Province. This location was, until recently, a reserve operated by the P.N.G. Department for the Environment and Conservation. This site was first visited in July, August and September 1986 (Stone et al. 1988), and again in October 1987.

Kuk Agricultural Research Station (altitude 1550–1600 m), located just outside Mount Hagen, is operated by the P.N.G. Department of Primary Industry [for a fuller description see Stone and Willmer (1989b)].

Madang is a major town on the northern coast of P.N.G. Physiological work was carried out in the laboratories of the C.R.I., located 10 km to the north of Madang town. Field work on *Creightonella frontalis* was carried out at Siar coffee plantation (Willmer and Stone 1988). All work at Madang and Kuk was carried out in September and October 1987. *Creightonella frontalis* and *Amegilla sapiens* were studied at all three sites, and *Thyreus quadrimaculatus* at Madang and Baiyer River. The three study sites differ significantly in climate over the year (Anon. 1974). Kuk, which is the site at greatest altitude, is also the coolest site (Fig. 1), while Madang, at sea level, is considerably warmer at all times of the year (Fig. 1).

Materials and methods

Measurement of body temperatures

Field measurements of body temperature in flight were made by inserting a thermocouple mounted inside a fine syringe needle into the thoracic flight musculature (T_{th}) or abdominal cavity (T_{ab}). The bee was restrained in the net against a piece of styrofoam in the shade. The delay between capture of the bee and insertion of the thermocouples was approximately 5 s (Stone and Willmer 1989c). The masses of bees sampled in this way were determined with a portable battery-powered balance (Unwin 1980).

Continuous laboratory measurements of T_{th} were obtained using a fine (0.2 mm diameter) copper-steel thermocouple inserted mid-dorsally in the bee's thorax to a depth of less than 1 mm (Stone and Willmer 1989a). The bee was restrained in a cooled, padded clamp (ca. 5 °C) and the thermocouple held in place with flexible adhesive (Copydex, Unibond U.K.). When released from the clamp, the bees were supported by the thermocouple, and given a small piece of spherical styrofoam to hold in their tarsi. The bee usually warmed passively to T_a. Only rises in body temperature above T_a are considered. Preflight warm-up was either initiated spontaneously by the bee, or induced by gentle tapping of the antennae with fine forceps. Tethered flight was initiated when the bee released the styrofoam and initiated powerful wing movements of full amplitude with the legs held against the body, as in free flight. Other forms of tethered flight were ignored. The thermocouple proved to be sufficient support during tethered flight. Bees were released after each experiment, and were apparently unharmed.

The possible errors involved in this method of T_{th} measurement are discussed in Stone and Willmer (1989a). Readings from a thermocouple thermometer (P.I. 8013, Portec Instruments, U.K.) were dictated every few seconds to a continuously running tape recorder. Time intervals between readings were later measured and used to reconstruct patterns of body temperature over time. Warm-up and tethered flight were examined in still air in rooms maintained at T_a of 21–22, 23, 26.5 and 31.5 °C. Tethered flight was examined at 35 and 38 °C for a single female *C. frontalis*. Warm-up and thoracic temperatures during tethered flight were quantified using the following terms (see Stone and Willmer 1989a, c). Examples of changes in T_{th} over time for *C. frontalis* and *A. sapiens* are given in Fig. 2.

1. Warm-up rate. Warm-up rates were calculated over 3 °C intervals of the warm-up curve between T_a and T_{th} at which tethered flight was initiated. Means of these values were taken to obtain a mean individual warm-up rate for each bee at a given T_a.

2. *Voluntary flight temperature* (VFT). This is the T_{th} at which the bee initiated tethered flight. Again, means were obtained for each individual at each T_a.

3. *Stable flight temperature*. This is the T_{th} which is reached and maintained during tethered flight for at least 90 s. In a continuous record of T_{th} this value is shown as an asymptote approached from VFT over time.

For *A. sapiens* and *C. frontalis* field body temperature data were obtained for populations at all three study sites, whereas laboratory examinations of warm-up rates, SFT and VFT were undertaken only for females in the Madang populations of each species. Field measurements of body temperature were obtained for female *T. quadrimaculatus* at Baiyer River and Madang. Laboratory data for *Thyreus* and *Coelioxys* were obtained for Madang populations.

T_as during field measurement of body temperatures were determined using the thermocouple used to obtain field measurements of body temperature, dried and shaded, as close as possible to the site of capture.

Statistical methods

Rather than examining different individual bees at each T_a, in some cases the warm-up of the same individual *A. sapiens* or *C. frontalis* at different T_a was examined by moving the experimental apparatus between rooms maintained at different temperatures. This was necessitated by the small populations of both these species available for examination. In calculating the mean figures given in Figs. 4, 5 and 8 a mean has been calculated for each individual at each T_a, and the mean of all these individual means obtained taken to give the mean value for the species at that T_a. These values serve to illustrate the form of the relationships between VFT, SFT and T_a, but have not been used in statistical analyses.

A multiple regression was used to examine the statistical relationships between variables. A fundamental assumption of regression techniques is that the data points involved are independent. This situation clearly does not hold if individuals contribute differing quantities of data to the analysis. For multiple regression analyses I have generated a mean warm-up rate, VFT and SFT for each individual over all the T_as at which that individual was tested. These means are referred to as overall individual means. The corresponding T_a is the mean of the T_as at which the data contributing to the overall individual mean were taken. Multiple regression analyses of laboratory data only involve individual means. Each individual therefore contributes one value to the analysis, and regression techniques can be used. Errors stated are ± 1 standard error.

Table 1. Body masses in *Amegilla sapiens*

Category	Mean body mass (mg) ± 1 SE	Range	Sample size
All females	132.0 ± 2.1	85–183	100
All males	91.8 ± 4.6	68–120	16
Madang females	119.9 ± 3.0	85–160	39
Baiyer River females	127.8 ± 2.5	112–162	22
Kuk females	147.1 ± 2.8	105–183	39

Results

Physiological thermoregulation in Amegilla sapiens

Morphological variation in A. sapiens. At all three sites there is a sexual dimorphism in body mass in *A. sapiens*, males being of significantly lower mass than females (Table 1). There is also a significant difference in the mean masses of females between sites, with the heaviest *A. sapiens* at the highest altitude (Anova: Sex $F_{1,114} = 41.0$, $P < 0.001$; Site $F_{2,114} = 8.10$, $P < 0.001$; Table 1).

Field measurements of body temperatures. A summary of the results of analyses of field body temperature data for *A. sapiens* is given in Table 2. T_{th} and T_{ab} correlated strongly with T_a, T_{th}s during flight ranging from 32 to 33 °C at $T_a = 20$–21 °C to 40 °C at $T_a = 30$–33 °C. When data for *A. sapiens* from all three field sites are included, the slope of T_{th} on T_a for all *A. sapiens* at all three sites is 0.54 ± 0.03 (95% confidence limits 0.47–0.61), while the slope of T_{ab} on T_a is 0.84 ± 0.04 (95% confidence limits 0.76–0.92), suggesting that T_{th} is regulated (T_{th}: $n = 115$, $r^2 = 0.671$, $P < 0.001$; T_{ab}: $n = 115$, $r^2 = 0.791$, $P < 0.001$). Within the species there is considerable variation in T_{th} at a given T_a, particularly at high T_a. Multiple regression shows that body mass, T_a and site all have significant effects on T_{th} (Table 2). There is no significant effect of sex when these other variables have been controlled for.

Having controlled for the effect of T_a there were significant differences in T_{th} between the Kuk and Baiyer River populations of *A. sapiens* ($n = 75$, $r^2 = 0.8$: T_a $P < 0.001$; site $P < 0.001$) and even more pronounced differences between the Kuk (highest altitude) and Madang (lowest altitude) populations (Fig. 3a). In each comparison, bees at a greater altitude have higher T_{th}s at a given

Table 2. Summary of multiple regression analysis of field body temperature data for *A. sapiens* for males and females at Kuk, Baiyer River and Madang. Each analysis involves regression of the *y* variable on sex, site, air temperature and body mass

y variable	*P* values for *x* variable having controlled for other *x* variables						
	Site	Sex	Body mass	Mass × site	T_a	*n*	r^2
Thoracic temperature	0.040	0.520	<0.001		<0.001	115	0.795
Abdominal temperature	0.001	0.900	<0.001		<0.001	115	0.894
Thoracic temperature excess	0.040	0.520	<0.001		<0.001	115	0.550
Thoracic temperature excess	0.930	0.300	0.020	0.058	<0.001	115	0.575
T_{dif}	<0.001	0.610	0.190		<0.001	115	0.750

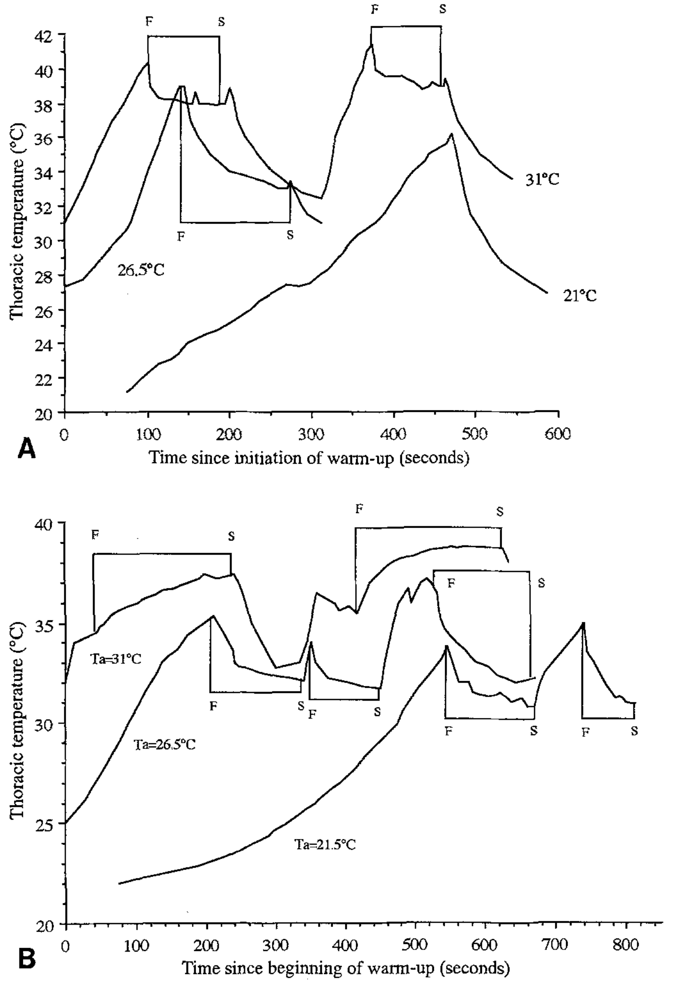

Fig. 2A, B. Typical changes in thoracic temperature over time during warm-up and tethered flight at three ambient temperatures for *A. sapiens* (**A**) and *C. frontalis* (**B**). All six data sets are for females. *Horizontal bars* indicate tethered flight, in which the voluntary flight temperature is indicated by *F* and the stable flight temperature by *S*

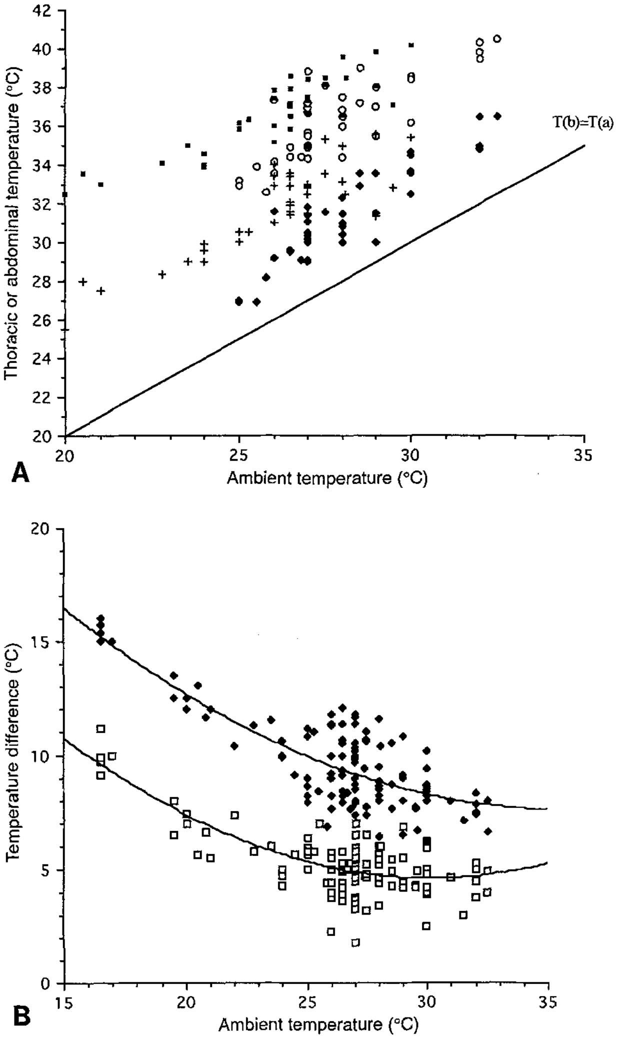

Fig. 3. **A** Thoracic and abdominal temperatures of *A. sapiens* as functions of ambient temperature, divided between Kuk and Madang populations: Kuk T_{th} (■), Kuk T_{ab} (+), Madang T_{th} (○), Madang T_{ab} (◆). **B** T_{ex} (◆) and T_{dif} (□) as functions of ambient temperature for *A. sapiens* from all three sites (T_{ex}: $y = 34.1 - 1.49x + 0.021x^2$, $r^2 = 0.624$; T_{dif} $y = 29 - 1.62x + 0.027x^2$, $r^2 = 0.578$)

T_a. Because at a given T_a, T_{th} increases with body mass (Table 2), and mean body mass increases with altitude (Table 1), these between-site differences are at least partly explained by the significant differences in body mass between the populations compared (Table 1). However, site (analysed as a continuous variable in order of ascending altitude) remains a significant correlate of all the body temperature variables when the effects of mass and T_a are controlled for (Table 2): T_{th}, T_{ab}, and T_{th} excess ($T_{th} - T_a$, or T_{ex}) for a bee of given mass at a given T_a increase with altitude. T_{dif} decreases significantly with increasing altitude, having controlled for other variables (Table 2).

The differences between populations in T_{th} are responsible for an artificially low slope of T_{th} on T_a when data for all three sites are analysed together. A single regression through three such sets of data points generates a lower slope than that existing within each separate data set. The slope for the Madang population (0.89, 95% confidence limits 0.69–1.10) is significantly higher than the slope for the higher altitude Baiyer River population (0.54, 95% confidence limits 0.45–0.63). The slope for Kuk, the other high altitude population, is also low (0.63, 95% confidence limits 0.53–0.74). The regressions of T_{th} on T_a for the three sites individually are all highly significant ($P < 0.001$).

T_{ex} and T_{dif} as functions of T_a for all *A. sapiens* are shown in Fig. 3B. From T_{th} approaching ambient at high T_a the T_{ex} generated increased to 15–16 °C at $T_a = 17$ °C, indicating active elevation of T_{th} at low T_a. Multiple regression reveals that both T_{ex} and T_{dif} correlate negatively with T_a, and positively with mass, when sex and site have been controlled for (Table 2).

Laboratory investigation of body temperatures. Warm-up rates were obtained from 11 female *A. sapiens*, with a

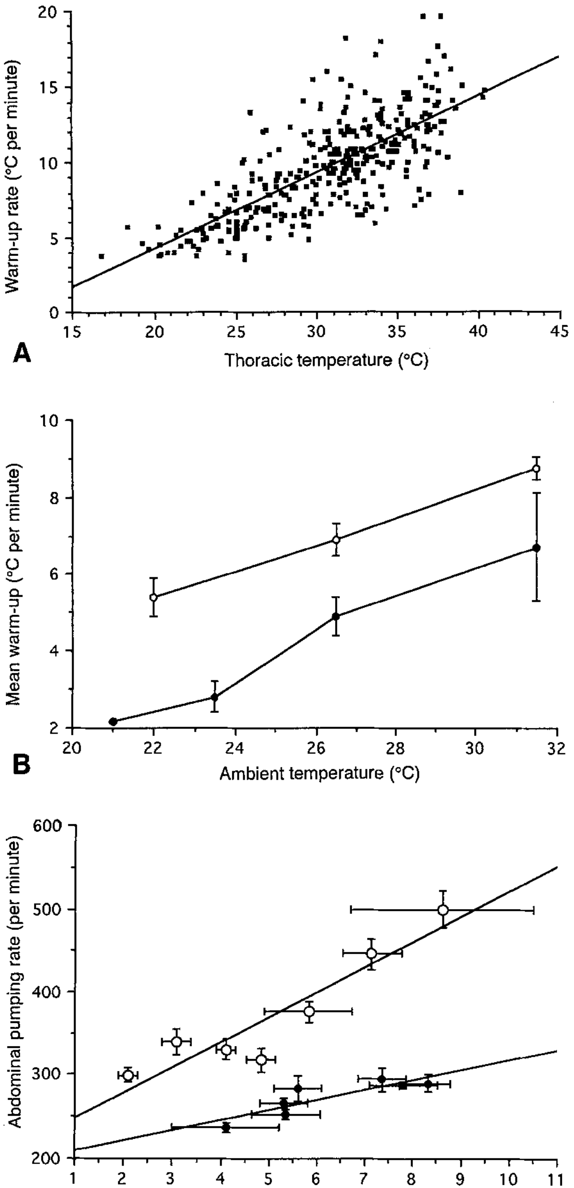

Fig. 4. **A** Warm-up rate as a function of thoracic temperature in an individual *A. sapiens* ($y = -11.9 + 0.49\,x$, $r^2 = 0.672$). **B** Mean warm-up rate as a function of ambient temperature for *Amegilla sapiens* (●) and *Creightonella frontalis* (○). Error bars are ±1 standard error. **C** The relationship between warm-up rate and abdominal pumping rate and warm-up rate for *A. sapiens* (○) and *C. frontalis* (●). Errors are ±1 standard error

maximum pre-flight warm-up rate of 13.8 °C·min^{-1} being obtained for a 96-mg female at $T_a = 32$ °C. Typical changes in T_{th} during preflight warm-up and tethered flight are shown for three females at three T_as in Fig. 2A. Warm-up rate for an individual *A. sapiens* increases with T_{th} (Fig. 4A), and mean warm-up rate also increases with T_a (Fig. 4B). During warm-up an indication of the increased metabolic rate necessary for maintainance of increasing warm-up rates is given by the increase in rates of abdominal pumping, and hence of oxygen supply to the tissues. From 300·min^{-1} for a warm-up rate of 2 °C·min^{-1}, the pumping rate increased to almost 500 min^{-1} at a warm-up rate of 9 °C·min^{-1} (Fig. 4C). The maximum T_{th} excess obtained in the lab for *A. sapiens* (15.2 °C at a T_a of 21 °C) was close to that obtained in the field (16 °C at a T_a of 16.5 °C).

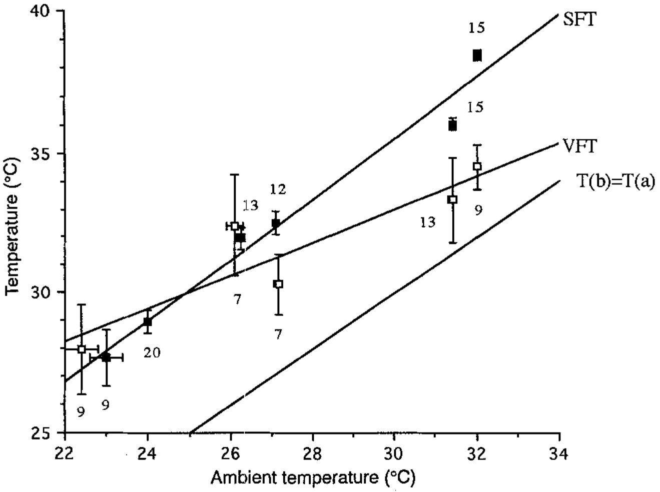

Fig. 5. Mean SFT (■) and mean VFT (□) as functions of ambient temperature for *A. sapiens* (least squares regressions have been fitted to give an indication of the gradients of these two variables on T_a. SFT: $y = 6.3 + 0.98\,x$, $r^2 = 0.924$; VFT: $y = 14.6 + 0.61\,x$, $r^2 = 0.997$). The number of individuals contributing to the mean at each ambient temperature is shown by the data point. Errors are ±1 standard error

Voluntary flight temperatures and stable flight temperatures both increase with T_a (Fig. 5). At temperatures below 25 °C, VFT exceeds SFT, and flight leads to cooling of the thorax. At T_as greater than approximately 25 °C, this situation is reversed (Fig. 5). Having controlled for the relationship with T_a, there is no significant correlation between VFT and body mass. SFT gives highly significant positive correlations with both body mass and T_a ($n = 11$ individual means, $r^2 = 0.964$, T_a $P < 0.001$, body mass $P < 0.001$) (Fig. 6A, B).

Physiological thermoregulation in Creightonella frontalis

Morphological variation in C. frontalis. *Creightonella frontalis* shows considerable sexual dimorphism in body mass: females collected at the same three sites had a mean mass of 252±7 mg ($n = 59$, range 135–350 mg), and males 115±4 mg ($n = 25$, range 80–160 mg). Unlike *A. sapiens, C. frontalis* showed no significant difference in mass between sites (Anova, 83 *df*: Sex $P < 0.001$; Site $P = 0.09$).

Field measurements of body temperatures. A summary of the results of analyses of 'grab-and-stab' data for *C. frontalis* is given in Table 3. T_{th} and T_{ab} correlated strongly with T_a, with T_{th} ranging in females from 35 °C at a T_a of 25 °C to 40–42 °C at a T_a of 33 °C. T_{th} and T_{ab} also correlated strongly and positively with body mass, and males had significantly lower T_{th} than females when mass, site and T_a had been controlled for (Table 3). The slopes of the best fit regressions for T_{th} on T_a are less than one for both males (0.79 ± 0.07; 95% confidence limits 0.65–0.92) and females (0.68 ± 0.05; 95% confidence limits 0.58–0.77), indicating that both are capable of some degree of thoracic thermoregulation (females: $n = 58$, $r^2 = 0.782$, $P < 0.001$; males: $n = 26$, $r^2 = 0.851$, $P < 0.001$). The slope of the best-fit regression of female T_{ab} on T_a (1.00 ± 0.05) indicates that T_{ab} was not regulated. The slope of the regression of T_{th} on T_a for all females or all males is not significantly different from those obtained separately for males and for females within each population. The slopes for the high altitude populations are lower than that for Madang, as found for *A. sapiens*. In no cases, however, are the differences in slope significant (Madang: slope = 0.79 ± 0.08, 95% confidence limits 0.63–0.95. Baiyer River: slope = 0.48 ± 0.13, 95% confidence limits 0.20–0.76. Kuk: slope = 0.69 ± 0.10, 95% confidence limits 0.48–0.90. All regressions are significant at the 1% level).

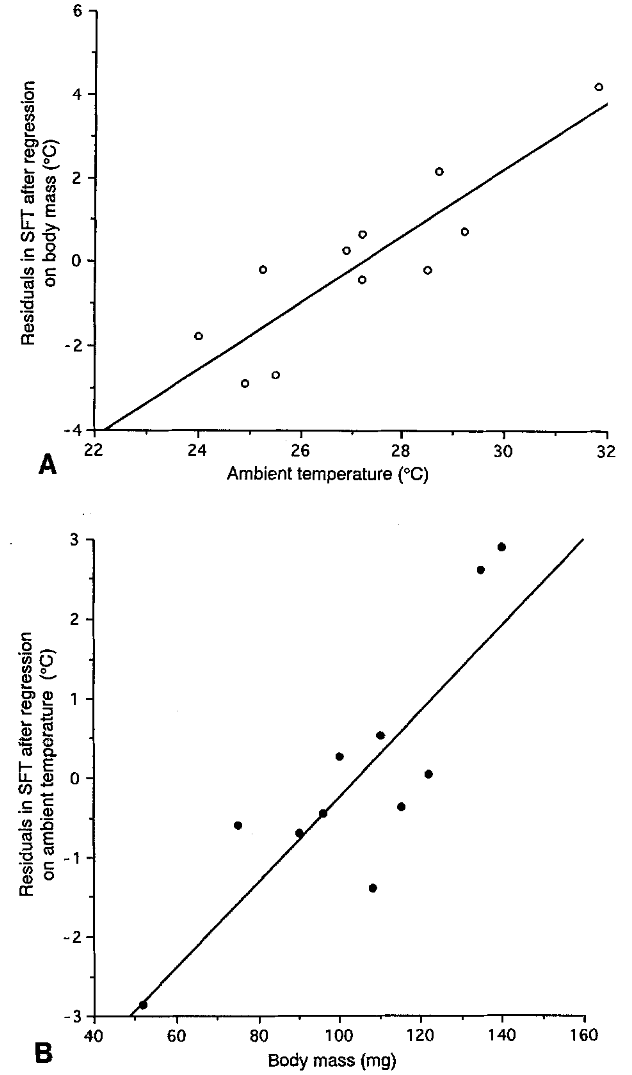

Fig. 6. All the data used in this figure are overall individual means. **A** Residuals in SFT after regression on body mass as a function of ambient temperature for *Amegilla sapiens*. **B** Residuals in SFT after regression on ambient temperature as a function of body mass for *Amegilla sapiens*

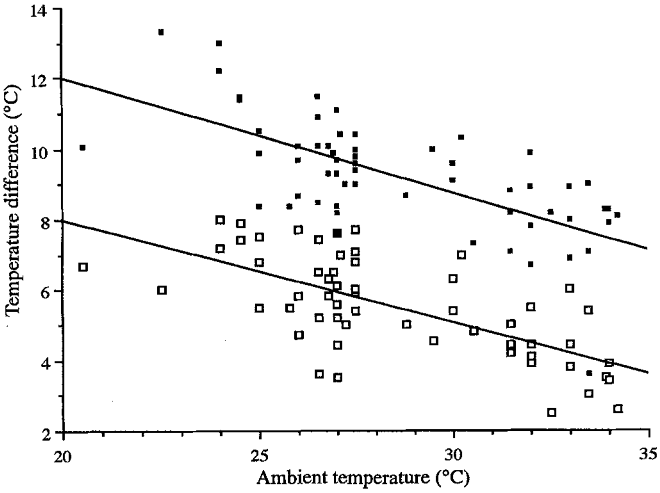

Fig. 7. T_{ex} (■) and T_{dif} (□) as functions of ambient temperature for ♀ *C. frontalis* (T_{ex}: $y = 18.5 - 0.32\,x$, $r^2 = 0.449$; T_{dif}: $y = 13.8 - 0.29\,x$, $r^2 = 0.462$

Table 3. Summary of multiple regression analysis of 'grab-and-stab' data for *Creightonella frontalis* males and females at Kuk, Baiyer River and Madang. Each analysis involves regression of the y variable on sex, site in order of increasing altitude, air temperature and body mass

y variable	P values for x variable having controlled for other x variables					
	Site	Sex	Body mass	T_a	n	r^2
Thoracic temperature	0.011	0.047	<0.001	<0.001	84	0.850
Abdominal temperature	0.210	0.580	0.034	<0.001	79	0.860
Thoracic temperature excess	0.011	0.047	<0.001	<0.001	84	0.800
T_{dif}	0.500	0.100	0.120	<0.001	79	0.530

Table 4. Summary of multiple regression analyses of field body temperature data for *Thyreus quadrimaculatus*

y variable	P values for x variable having controlled for other x variables			
	Body mass	T_a	n	r^2
Thoracic temperature	0.640	<0.001	14	0.813
Abdominal temperature	0.700	<0.001	14	0.771
Thoracic temperature excess	0.640	0.150	14	0.060
T_{dif}	0.900	0.430	14	0.183

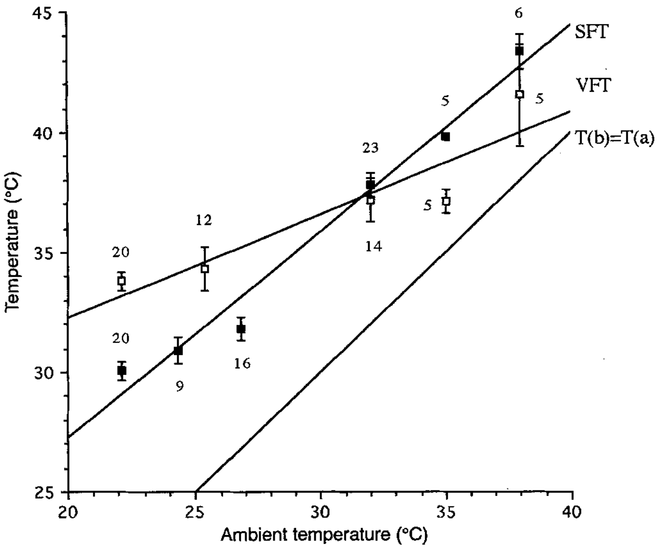

Fig. 8. Mean SFT (■) and mean VFT (□) as functions of ambient temperature for ♀ *C. frontalis* (SFT: $y = 9.7 + 0.87x$, $r^2 = 0.988$; VFT: $y = 22.5 + 0.46x$, $r^2 = 0.878$)

As for *A. sapiens*, both T_{ex} and T_{dif} correlated negatively with T_a (Fig. 7), and T_{ex} also correlated positively with mass (Table 4). T_{th} and T_{ex} increased significantly with altitude when other effects had been controlled for, but there were no other significant effects of site (Table 3). This suggests that there were some qualitative differences between the Kuk and Madang populations not explained by T_a or body mass differences, and in the same direction as those found in *A. sapiens*.

Laboratory investigation of body temperatures. Measurements of warm-up rate were obtained from 12 female *C. frontalis* over a range of T_a. Maximum warm-up rates of 10.2 °C·min^{-1} were obtained at $T_a = 32$ °C from two females weighing 255 and 310 mg. Typical changes in T_{th} during preflight warm-up and tethered flight are shown for three females at three T_as in Fig. 2B. Mean warm-up rate in female *C. frontalis* increases with T_a (Fig. 4B; multiple regression: $n = 12$: T_a $P = 0.008$; body mass $P = 0.100$; T_{th} $P = 0.500$). As for *A. sapiens*, rates of abdominal pumping increased with warm-up rate (Fig. 4C). The highest rates of pumping in *C. frontalis* were substantially lower than those recorded for *A. sapiens*, reaching a maximum of 300·min^{-1} at a warm-up rate of 8.5 °C·min^{-1}. Again, maximum T_{th} excess obtained during warm-up in the laboratory (14 °C for a female at $T_a = 22$ °C) was close to the maximum obtained in the field (16 °C at $T_a = 31.5$ °C, also for a female).

VFT and SFT both increase with T_a (Fig. 8). At T_as below approximately 32 °C, VFT is greater than SFT, and initiation of tethered flight results in cooling of the thorax. This situation is reversed for $T_a > 32$ °C. As for *A. sapiens*, overall individual mean SFT correlated positively with both mass and T_a ($n = 11$, $r^2 = 0.974$: body mass $P < 0.001$; T_a $P < 0.001$; Fig. 9A, B).

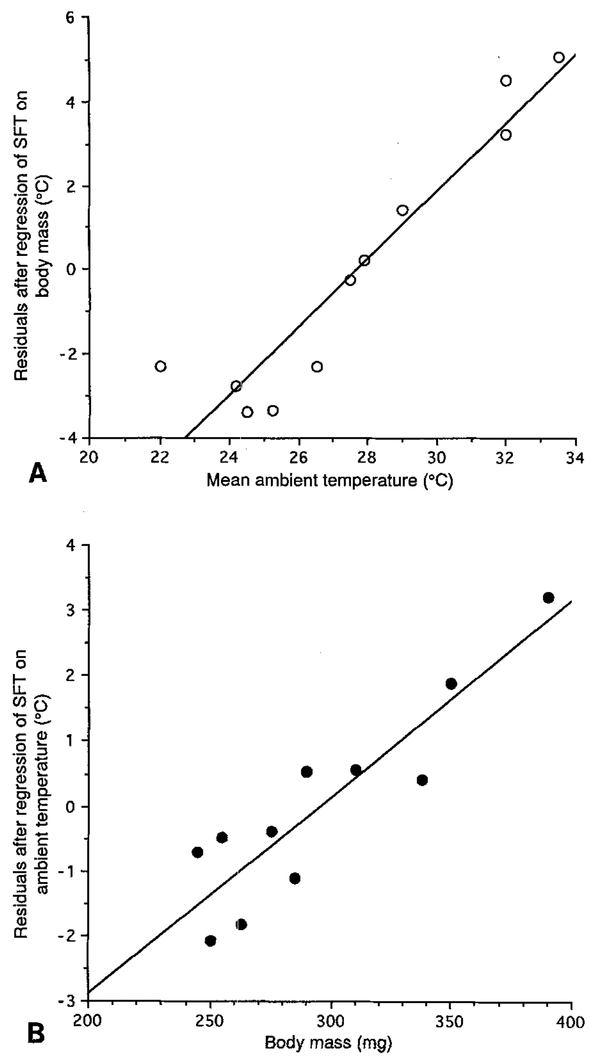

Fig. 9. All the data used in this figure are overall individual means: **A** Residuals in SFT after regression on body mass as a function of ambient temperature for *Creightonella frontalis*. **B** Residuals in SFT after regression on ambient temperature as a function of body mass for *Creightonella frontalis*

Warm-up rates and body temperatures in Thyreus quadrimaculatus

Although in the same family as *A. sapiens*, and with a similar body mass (100.0 ± 4.7 mg, $n = 14$), the behaviour of this bee is radically different. Its flight is typically slow, and has none of the fast, precise darting and hovering shown by *A. sapiens*. The minimum T_a at which this species was observed to fly was 25 °C at both Madang and Baiyer River, and flight activity ceased very soon after clouding over or shading of a foraging site at which this species had previously been active. The maximum T_{th} excess recorded for *T. quadrimaculatus* was 6.6 °C at $T_a = 26$ °C, and at T_as higher than 29–30 °C, T_{th} was only 1–2 °C more than T_a.

The relationships between T_{th}, T_{ab} and T_a for *T. quadrimaculatus* are shown in Fig. 10. The slopes of the best-

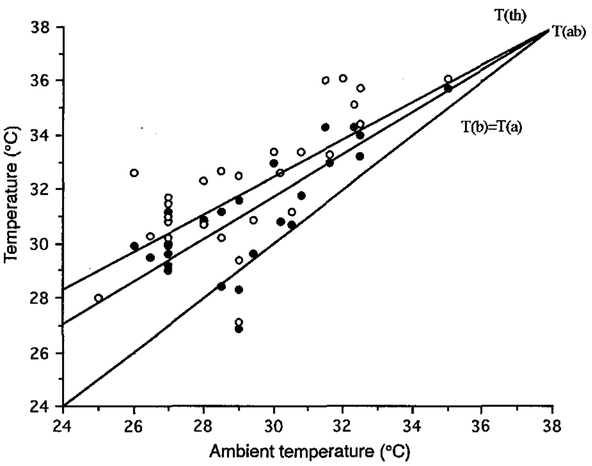

Fig. 10. T_{th} (○) and T_{ab} (●) as functions of ambient temperature for *Thyreus quadrimaculatus* (T_{th}: $y = 10.7 + 0.73\,x$, $r = 0.76$; T_{ab}: $y = 8.43 + 0.78\,x$, $r = 0.78$)

fit regressions of these variables on T_a are 0.74 ± 0.12 (95% confidence limits 0.48–0.99) and 0.78 ± 0.12 (95% confidence limits 0.52–1.04); both regressions are significant (T_{th} $n = 28$, $r^2 = 0.571$, $P < 0.001$; T_{ab} $n = 27$, $r^2 = 0.610$, $P < 0.001$), indicating some ability to regulate thoracic temperature. A summary of the results of analyses of 'grab-and-stab' data for *T. quadrimaculatus* is given in Table 4. Having controlled for the effect of T_a, there is no significant correlation between mass and any of T_{th}, T_{ab}, T_{ex} or T_{dif} for *T. quadrimaculatus*. The mean warm-up rate of this species in the laboratory at $T_a = 22$ °C was 1.75 °C·min^{-1}, with a maximum recorded warm-up rate of only 2.5 °C·min^{-1}.

Warm-up rates and body temperatures in Coelioxys

Only a limited amount of data was obtained for *Coelioxys*. In flight this species closely resembles *Thyreus*, although taxonomically the two are widely separated. Females of this species could be seen flying slowly along the front of the *Creightonella* nest site, frequently landing and walking from one host nest entrance to the next. In two females caught in the field at $T_a = 33$–34 °C (body masses 85 and 115 mg), T_{th} excesses of 5–6 °C were recorded, slightly higher than values obtained for *Thyreus* at the same T_a. It was difficult to obtain detectable warm-up from this species in the laboratory, and warm-up rates were low, with a mean of 1 °C·min^{-1} at $T_a = 22$ °C and a maximum recorded rate of 2.0 °C·min^{-1}.

Discussion

Warm-up rates in A. sapiens *and* C. frontalis

Amegilla sapiens and *Creightonella frontalis* both show evidence of active thermoregulation of T_{th} through raising T_{th} at low T_a. Laboratory investigation of warm-up demonstrates that both species are capable of endothermic pre-flight warm-up. The warm-up rates these species show at a given T_a, and the T_a range over which they show warm-up behaviour, are rather lower than those obtained at the same T_a for closely related bee species active in temperate climates (Stone and Willmer 1989a; Stone 1993). Demonstration of endothermic abilities in these tropical species depends critically on T_a. Although both *Amegilla sapiens* and *Creightonella frontalis* have high maximum warm-up rates, these were achieved only at very high T_as of 31–32 °C. *Anthophora plumipes* is a solitary species active during the spring in Britain, and a taxonomically close relative of *A. sapiens*. In contrast to *A. sapiens*, *Anthophora plumipes* will not demonstrate warm-up and cannot be induced to commence tethered flight at a T_a of 31–32 °C, although capable of considerable pre-flight thermogenesis at lower T_as (Stone 1993). At $T_a = 20$–22 °C, the mean warm-up rates for females of both P.N.G. species are modest: 3.75 °C·min^{-1} ($n = 19$) for *A. sapiens*, and 5.8 °C·min^{-1} ($n = 29$) for *C. frontalis*, compared to those obtained for *A. plumipes* females (12.3 °C·min^{-1}; Stone 1993). At the lowest temperatures at which warm-up was observed in *A. plumipes*, both of the tropical species were relatively unresponsive to tactile stimuli and did not exhibit pre-flight warm-up behaviour. The maximum T_{th}s tolerated by flying *C. frontalis* (up to 48 °C), similar to those recorded by Chappell (1984) for *Centris pallida* (Anthophoridae), are lethal to *Anthophora plumipes* (Stone 1989).

Body temperatures in A. sapiens *and* C. frontalis: *body mass and altitude*

Both *A. sapiens* and *C. frontalis* give positive correlations between T_{th}s in flight and body mass. This is true whether the data are T_{th}s obtained during free flight in the field or during tethered flight in the laboratory. This implies that if a minimum T_{th} for flight is required in *C. frontalis* and *A. sapiens*, then larger individuals will be able to maintain this T_{th}, and keep flying, at a lower T_a. This has been demonstrated both for *Anthophora plumipes* in Britain (Stone 1993) and *Colletes cunicularius* (Colletidae) in Sweden (Larsson 1989). If the upper tolerated T_{th} for flight in *C. frontalis* of 48 °C given above is relatively constant within a population, then bees of lower mass should be able to remain below this value, and so keep flying, at higher T_a than heavier bees. This has been demonstrated for *Anthophora plumipes* (Stone 1989, 1993).

While a number of factors may generate intraspecific, interpopulation differences in body mass (such as differences in the availability of food during development), the mass-differences between populations of *A. sapiens* may also be interpreted as selective responses to the differing thermal regimes of their habitats. The higher mean body mass of the Kuk population is compatible with warm-up and maintenance of T_{th} at low T_a, while the lower mean mass of the Madang population is compatible with sustained flight at the high T_a common in their habitat. Although data for only three populations of *A. sapiens* are discussed here, if this situation were true for this species in general it would constitute an intraspecific insect example of Bergmann's Rule, i.e. within an endothermic lineage, body size tends to be greater in cold climates than in warm climates (Bergmann 1847). This is not the first observation of such a relationship in bees. Geographic variation has been studied in considerable detail in the honeybee, *Apis mellifera*, and Ruttner (1988) cites four morphological rules which apply to this species. With increasing or decreasing latitude from the equator, body size increases, pubescence becomes longer, limbs become shorter relative to body size and body colour becomes darker. Similar relationships exist in *A. mellifera* for increasing altitude at a single latitude in Tanzania (Smith 1961). Although the thermal physiology of the honeybee populations contributing to these relationships remains largely unstudied, the thermal effects of qualitative changes in pubescence (Church 1960; Bartholomew and Epting 1975), body colour (Willmer and Unwin 1981; Willmer 1983) and body size (Stone and Willmer 1989a) can all be predicted: all changes would tend to allow continued thermoregulation in response to lower mean T_as.

The significant differences in body temperatures between different populations of *A. sapiens* and *C. frontalis* that are independent of body mass suggest that there are physiological and/or morphological differences between populations that have evolved as a result of differences between the sites. Both *C. frontalis* and *A. sapiens* had higher T_{th} at a given T_a at higher altitude than would be predicted on the basis of differences in body mass alone, suggesting either differences in the thermogenic abilities per unit mass between populations, or differences in factors affecting rates of heat loss, such as the thermal conductivity of the cuticle, or both.

Thermoregulation during flight

In both of these species the slope of the relationship between VFT and T_a is lower than that between SFT and T_a. This means that thermoregulation before flight is more effective than it is during flight, as found for almost all of the endothermic insects studied to date. Possible advantages of pre-flight thermoregulation are considered by Stone (1993). Field measures of body temperature also showed that in *A. sapiens* (and, less conclusively, in *C. frontalis*) populations at higher altitude had lower slopes of T_{th} on T_a, indicating greater thermoregulatory ability. The populations at altitude achieve this by maintaining greater T_{th} excesses at low T_a. In *A. sapiens* factors contributing to this ability are both the larger size and the higher T_{th} independent of size of individuals in the higher altitude populations.

Sex-related differences in body temperatures

The sex-related differences in T_{th} in *C. frontalis* (Megachilidae) were not found in *A. sapiens* (Anthophoridae). In *C. frontalis*, all other things being equal, males had lower T_{th} than females, while in *A. sapiens* males and females were qualitatively similar. It is tempting to suggest that these differences in the endothermy of males relative to females are due to the different selective pressures which have acted on male bees employing different mating strategies. Males of *A. sapiens* are active patrollers of foraging sites, and are able to fly at minimum T_as quite close to those of females (Stone et al. 1988). In comparison, male *C. frontalis* wait for emerging females at nest sites and are very sluggish fliers. Courtship and mating activity in male *C. frontalis* is severely effected by changes in T_a (Stone 1989). It is possible that the differences in male strategies and levels of endothermy between the two species described here are associated with differences in the levels of flight activity involved in each species. However, it is also possible that these differences are due to evolutionary history and not to any difference in selective pressures. A test of this hypothesis would be to examine the endothermic ability of the males of megachilid species that demonstrate an *Amegilla*-like mating strategy. An absence of any significant difference in the endothermic abilities of males and females, or higher levels of endothermy in males than in females, would support the suggestion that mating systems have coevolved with endothermic abilities in male bees. Suitable megachilids for study would be members of the genus *Anthidium*, the males of which are larger than the females, and defend nectar resources aggressively against other bees in order to attract females (Severinghaus et al. 1981). Although females of *Anthidium manicatum* only fly at relatively warm T_as (with a minimum T_a for flight of approximately 20 °C), if males are to guard a nectar supply effectively they must be capable of vigorous flight activity at T_as at which their nectar competitors are active (Stone 1989). Male *A. manicatum* were active at air temperatures above 14–15 °C, and in this type of mating system one might predict higher levels of endothermy in males than in females. This prediction remains untested.

Body temperatures and warm-up rates in Thyreus *and* Coelioxys

The behaviour of *T. quadrimaculatus* and *Coelioxys* spp. is consistent with that of insects dependent to a large degree on behavioural thermoregulation, and without great endothermic ability. These species have low T_{th}s in flight in comparison to their provisioning hosts; for example, at a T_a of 25 °C *Thyreus quadrimaculatus* in flight have a T_{th} of near 29 °C, while female *C. frontalis* and *A. sapiens* have T_{th} of ca. 32 and 35 °C, respectively. The mean warm-up rates for these species are also low for bees of their body mass (Stone and Willmer 1989a). It is probable that behavioural thermoregulation dominates in the thermal ecology of both *Thyreus quadrimaculatus* and *Coelioxys*. By limiting flight activity to locations and periods of time for which suitable T_as allow maintenance of required T_{th}s, these kleptoparasites are able to complete their lifecycles without need for extensive endothermy or reduction of T_{th} at higher T_a. It is noteworthy that *Melecta albifrons* (Anthophoridae), a kleptoparasite of *Anthophora plumipes* active in unpredictable spring weather, has considerably greater powers of endothermy for its body mass than these tropical kleptoparasites. Whether low levels of endothermy are characteristic of kleptoparasitic species requires confirmation from a larger set of data.

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