Behav Ecol Sociobiol (2007) 61:887–897
DOI 10.1007/s00265-006-0317-7

ORIGINAL PAPER

The effect of ambient temperature on forager sound production and thoracic temperature in the stingless bee, *Melipona panamica*

Felipe A. L. Contrera · James C. Nieh

Received: 6 April 2006 / Revised: 7 November 2006 / Accepted: 14 November 2006 / Published online: 19 December 2006

Abstract Foragers of the stingless bees genus *Melipona* may produce intranidal sounds that are correlated with food location and quality. In this study, we provide the first detailed analysis of pulsed sounds produced by *Melipona panamica* foragers while feeding on a carbohydrate food source. We trained foragers to a 2.5-M sucrose feeder under normal, ambient temperature (23–33°C) and lower temperature (11–25°C) conditions. We recorded forager sounds under both conditions and tested the effect of temperature of the thorax, feeder plate, and air on sound temporal characteristics. Forager energetic expenditure and the number of pulses per visit were significantly higher in the cold condition than in the normal condition. Foragers spent a longer time at the feeder under the cold condition than during the normal condition. Interpulse durations were significantly shorter in the cold condition than in the normal condition and became progressively and significantly shorter at the end of each performance. Thus, pulse production increased before departure. Foragers increased their thoracic temperatures above ambient at all experimental air temperatures. Under chilled conditions, foragers had a significantly greater difference between thorax temperature and ambient air temperature than under normal conditions. Foragers must achieve a minimum flight muscle temperature before take-off, and thus forager sounds may be linked to muscle warm-up.

Communicated by J. Traniello

F. A. L. Contrera (✉) · J. C. Nieh
Section of Ecology, Behavior, and Evolution,
Division of Biological Sciences,
University of California San Diego,
Mail Code 0116, 9500 Gilman Drive, La Jolla,
San Diego, CA 92093-0116, USA
e-mail: fcontrera@ucsd.edu

Keywords Thoracic temperature · Stingless bees · Sound production · Foraging · Flight warm-up

Introduction

The ability to generate metabolic heat is widespread among insects (Heinrich 1993) and has been studied particularly in bumblebees and honeybees (Seeley and Heinrich 1981; Heinrich 1985). For example, individual honeybees can generate heat through shivering thermogenesis (Stabentheiner et al. 2003), a tetanic contraction of antagonistic flight muscles against a skeletal stop (Esch and Goller 1991; Esch et al. 1991). Honeybees can also contribute to nest thermoregulation by remaining motionless in the cap of the brood and generating endothermic heat, or by entering into empty cells adjacent to sealed cells to increase their temperature (Kleinhenz et al. 2003). Stingless bees, a monophyletic sister group of the honeybees (Michener 2000; Cameron and Mardulyn 2001), are also able to regulate nest temperature (Kerr and Laidlaw 1956; Kerr et al. 1967; Zucchi and Sakagami 1972; Darchen 1973; Michener 1974; Roubik 1989) primarily to maintain the brood area at a temperature optimal for larval development. They can achieve this through nest architecture, modifying their nest entrances or pores to keep intranidal temperature relatively constant (Darchen 1973; Sakagami 1982; Engels et al. 1995), or through active thermoregulation.

Little is known about thermoregulation in individual meliponine bees. Pacheco and Kerr (1989) reported that *Melipona compressipes fasciculata* workers had elevated thorax temperatures (1.0–3.4°C) while working as compared to resting, and *Trigona* (*Plebeina*) *denoiti* workers

increased brood temperature when external temperature dropped from 31 to 15.4°C (Fletcher and Crewe 1981). These results raised interesting possibilities about stingless bee foragers being able to regulate body temperature according to net food profitability, as has been shown in other Hymenoptera (e.g., *Apis mellifera*, *Paravespula vulgaris*, Kovac and Stabentheiner 1999; Bujok et al. 2002).

Nieh and Sánchez (2005) recently tested this possibility in the stingless bee, *Melipona panamica*. Using infrared thermography, they showed that *M. panamica* foragers can elevate their thoracic temperature in response to profitable food sources (higher sucrose concentration, location closer to the nest) on the feeder and inside the nest. Inside the nest, the difference between thoracic temperature and ambient air temperature (ΔT_A) was weakly correlated with a 0.4°C decrease with each 100 m increase in feeder-to-nest distance and strongly correlated with a 0.1°C per 1 mol l^{-1} increase with increasing sucrose concentration (Nieh and Sánchez 2005). In stingless bees, the ability to adjust body temperature according to food quality could be linked to flight because honeybee and bumblebee flight muscles must reach a minimal temperature before take-off (Coelho 1991; Esch and Goller 1991; Heinrich 1993; Dudley 2000; Harrison and Fewell 2002; Woods et al. 2005). In bumblebees, the minimal temperature of the flight muscles required to achieve flight is approximately 30°C (Heinrich 1979). Schulze-Motel and Lamprecht (1994) found that metabolic heat generation was highly correlated with sounds produced by bumblebee workers (*Bombus lapidarius*) inside flight cages.

Sound production also occurs during stingless bee foraging (Nieh 2004). *Melipona panamica* foragers, as well as foragers of other *Melipona* species, can produce intranidal recruitment sounds that are correlated with the distance and quality of food sources (Esch et al. 1965; Nieh and Roubik 1998; Aguilar and Briceño 2002; Nieh et al. 2003b; Hrncir et al. 2004b), although there is debate about the use of such information by recruits and the meaning of these sounds (Hrncir et al. 2004a, 2006). Nieh (1998) observed that departing *M. panamica* foragers sometimes produced a buzzing sound before leaving the feeder. Based upon our preliminary observations of *M. panamica* and other *Melipona* species (*M. bicolor*, *M. mandacaia*, *M. marginata*, and *M. quadrifasciata*) we suspected that foragers might also produce sounds while feeding perched upon the food source. In this study, we provide the first detailed description of meliponine feeding sounds. We used *M. panamica* because this is the best-studied meliponine species in regard to foraging body temperatures (Nieh and Sánchez 2005) and because this species does particularly well at imbibing viscous sucrose solutions (Roubik and Buchmann 1984), as induced by our experimental manipulations of feeder temperature. In *M. panamica*, perched foraging sounds probably are not related to food source communication because foragers may produce sounds when they are alone on the food source and no other bees are in the vicinity. Thus, we performed experiments with feeders at different temperatures to determine if the feeder sounds produced by *M. panamica* are correlated with body temperature before flight.

Materials and methods

Study site and colonies

This study was performed on Barro Colorado Island at the Smithsonian Tropical Research Institute (STRI), Republic of Panama, from October 24 to November 11, 2005. We used two colonies of *M. panamica* Cockerell 1918 (previously known as *Melipona eburnea* and *Melipona fasciata*, David Roubik, personal communication) for our experiments. Colonies of *M. panamica* usually contain 500 to 800 adult individuals (Roubik 1992; Nieh and Roubik 1995) and occur from northern Colombia to southern Costa Rica (David W. Roubik, personal communication). *Melipona panamica* colonies (M1 and M2) were placed inside observation colonies (description in Nieh and Roubik 1995); one (M1) inside the bee laboratory (9°9.923′N, 79°50.193′W) and the other (M2) on a balcony outside the bee laboratory. Colony M1 corresponds to colony D (approximately 2,000 adult workers) and colony M2 to colony G (approximately 800–1,000 adult workers) studied by F. A. L. Contrera and J. C. Nieh (unpublished data). Colony M1 was connected to the outside with a 1-cm inner diameter, 10-cm long clear vinyl tube. Observations were made between 0800 and 1600. We closed the entrances of *M. panamica* colonies not under study during our experiment to avoid feeder visitation by nonsubject colonies.

Feeders and training

The feeder consisted of a clear glass bottle (40 ml) filled with an unscented 2.5 M sucrose solution (Ultra Pure, ICN Biomedicals, cat# 821721). We used a 2.5-M sucrose solution because Roubik and Buchmann (1984) reported that sucrose concentrations of nectar collected by four species of *Melipona* in central Panama during the dry season (including *M. panamica* colonies) ranged from 0.6 M (19%) to 2.8 M (72%), and noted that *M. panamica* colonies did particularly well in imbibing viscous, high concentration sucrose solutions and because of competition from natural food sources, relatively high sucrose concentrations are required to elicit consistent foraging in artificial feeders, even during periods of relative food dearth (Nieh

2004). The feeder with the solution was inverted over a 10-cm diameter circular oxidized metal plate (modified from von Frisch 1967; Nieh et al. 2003a). We used a metal plate to facilitate heat transfer. This plate was placed on top of a 1-m high tripod, allowing us to move the feeder. In all experiments, we trained bees by placing the feeder next to the nest entrance and then moving it to progressively greater distances once bees began to visit (von Frisch 1967). All newcomers visiting the feeder were individually marked on the thorax with different combinations of colored latex paints (Binney and Smith, code #54-0125), and experienced foragers that were captured during the experiments were marked by gluing colored, numbered plastic tags (Bee Queen Marking Kit) to their thoraces. Thus, experienced foragers could be distinguished from naïve foragers (newcomers who had never previously experienced the feeder in any location in any time; Nieh 2004).

Experiment: effect of feeder temperature on thoracic temperature and forager sound production

We trained six foragers from each colony to visit a feeder located 25 m south of the colony. At the final feeder position, we captured all foragers, except one, which served as the focal bee for the experiment. We censused the number of foragers during each experiment and used insect aspirators to capture excess foragers and newcomers (Nieh et al. 2003c). In total, 64 foragers (from both colonies) visited our feeder.

For each forager visit during each experimental condition (see below), we measured the performance time (the total amount of time that a bee spent on the feeder during each visit), the thorax temperature (T_{TH}), the ambient air temperature at the feeder (T_A), and the feeder plate temperature (T_{PLATE}). We also calculated the difference between the thorax temperature and the ambient air temperature at the feeder (ΔT_A; Stone 1993) and the difference between the thorax temperature and the feeder plate temperature (ΔT_{PLATE}).

To determine the forager's thoracic temperature and the feeder plate temperature, we used a Radio Shack IR thermometer (catalog #22-325). For the ambient air temperature we used a Kestrel™ 4000 weather meter, importing data into a Dell PC laptop. To measure forager thoracic temperature, we allowed the forager to land on the feeder and feed for approximately 5 s. Then, we placed the infrared thermometer (4 mm measurement aperture diameter) 1 to 2 mm away from her thorax (approximately 4–5 mm in diameter) to measure temperature. We only measured T_{TH} from painted-thorax foragers. The measurement of painted surfaces to determine accurate substrate temperatures is a standard thermographic practice (Wolfe and Zissis 1985), and the thin layer of paint does not interfere with meliponine temperature measurements (Nieh and Sánchez 2005). The feeder plate temperature was collected at a point at the side of the place the forager was feeding with the device also held 1 to 2 mm away from the surface (plate oxidization allowed accurate IR temperature measurement, Wolfe and Zissis 1985).

Using a Canon XL-1 Digital Video camcorder and a Radio Shack electret condenser microphone (catalog #33-1052) connected to a Teflon tube (4.5 cm long, 1.7 mm inner diameter), we video recorded the behavior of the forager at the feeder and the sounds she produced during her visit. To record forager sounds at the feeder, we placed the microphone 1–2 mm from the junction of the wings and thorax and recorded from the moment the forager landed on the feeder until she departed. *Melipona panamica* workers produce sounds by vibrating the wing muscles (sound and high speed video analysis, Nieh and Schofield, in preparation). The video and sound data were imported into a Macintosh iBook computer and analyzed with iMovie v 4.0.1 and Canary v1.2.4 software.

Condition 1: normal temperature. We allowed the forager to visit the feeder 20 times, and in each visit we collected the data described above. The feeder was always placed in the shade to allow accurate ambient temperature measurements. After the 20 visits of the focal bee, we shifted to the second experimental condition.

Condition 2*:* cold temperature. After the end of condition 1, we moved the feeder from the top of the tripod, while the focal bee was still feeding, to the top of a Styrofoam box (50×30×50 cm) filled with ice cubes. Approximately 1 cm above (and not in contact with) the ice, we placed a Kestrel™ 4000 weather meter temperature sensor to record the air temperature close to the feeder. If the focal bee avoided the feeder under the cold conditions, we placed the feeder back on the top of the tripod and, when the forager resumed feeding, we moved the feeder back to the ice. Generally, foragers became acclimated to the colder conditions after 5 min. We performed 20 observations per bee per condition, and captured the focal bee with an aspirator after these observations. Then, we released one of the previously captured foragers to substitute for her.

For each colony, we collected thoracic and feeder temperature, as described above, from six bees under normal temperatures and under experimentally reduced temperatures. We reversed the order of the conditions after each trial, to avoid any effect of choice order on forager behavior. Thus, in six trials (three per colony), we began the experiment with the feeder under a normal temperature and then changed to a reduced temperature, and in six trials (three per colony) we began the experiment with the feeder in a reduced temperature and then changed to a normal temperature. At the end of each day of study, we released

Table 1 Effect of experimental condition upon recruitment and feeder sounds

	Normal phase	Cold phase
Recruits	48**	4**
Pulse duration (s)	0.15±0.45*** (N=265)	0.34±0.30*** (N=428)
Pulses per visit	1.09±3.10*	1.80±4.02*
Interpulse duration (s)	2.57±4.92*** (N=200)	0.89±3.78*** (N=342)

Values are given in mean ± SD. N=12 focal bees. Pooled data from both colonies.
*P=0.02
**P=0.01
***P<0.0001

the marked foragers and the recruits after individually marking them with paint. If a previously observed forager visited the feeder, we captured her and waited for a new forager to arrive at the feeder or released a previously captured bee.

Statistical analyses

We used JMP IN v4.0.4 software for linear and multiple regression, ANOVA, and Wilcoxon tests (test-statistic reported as Z) as appropriate for the data (Zar 1999). We pooled data from both colonies for all analyses because we found no significant colony effects. Performances lasted for different durations and thus we calculated standardized percentage performance times by making the total duration of each performance equal to 100%. This allowed us to analyze the distribution of sound pulses for performances of different durations. Averages are presented as mean ± 1 SD.

Results

Recruitment

Recruitment was significantly higher (Z=2.58, P=0.01) under normal condition (N=48 recruits) than in the cold condition (N=4 recruits, Table 1).

Pulses and interpulses

Foragers produced pulsed sounds at the feeder with a fundamental frequency of 401.68±18.33 Hz (Fig. 1a,b). These sounds are fairly loud and audible to human observers without amplification. Foragers also produced

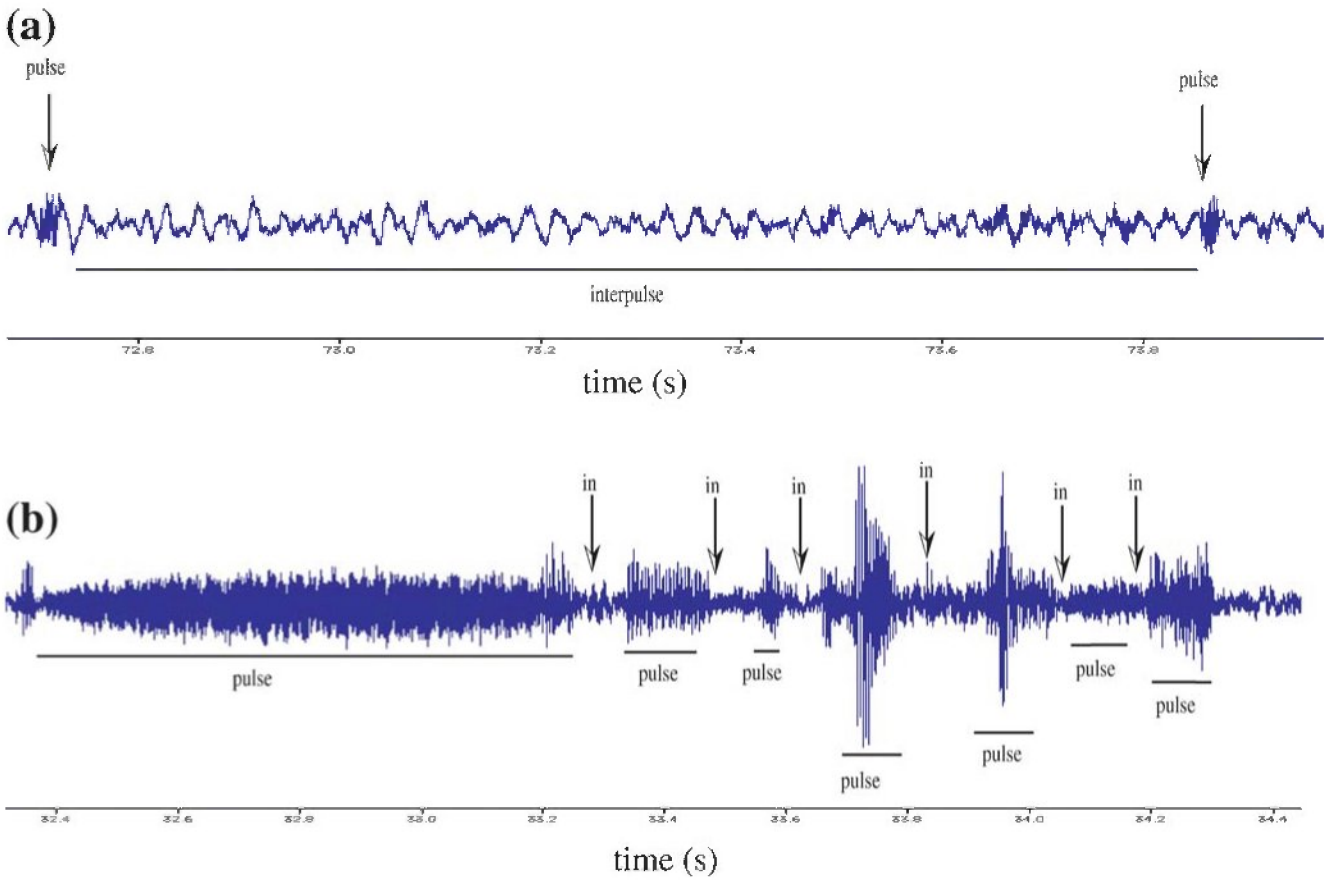

Fig. 1 Sample oscillographs of feeder sounds produced by foragers during **a** normal (n=240) and **b** cold conditions (n=240). *in* Interpulse

significantly longer sound pulses in the cold condition than in the normal condition (N=693, Z=−13.66, P<0.0001, Table 1, Fig. 2a). There was a significant difference in the number of pulses per visit between conditions. Foragers produced more pulses per visit in the cold condition (normal: 1.09±3.10 pulses, cold: 1.80±4.02 pulses; Z=−2.26, P=0.02, Table 1, Fig. 2b).

There was no significant relationship between the number of pulses bees produced on the feeder and T_{PLATE}, T_A, T_{TH}, and between ΔT_A and ΔT_{PLATE} ($P \geq 0.18$, Table 2). There was a weak but significant effect of ΔT_A on the number of pulses in the normal condition (R^2=0.02, $F_{1,230}$=4.93, P=0.03, Table 2).

The distribution of pulses by performance time was different in both conditions. In the cold condition, the majority of pulses were concentrated at the end of the performance whereas in the normal condition the pulses were more evenly distributed (Z=−5.42, P<0.0001). In the normal condition, pulses were longer, though not significantly, at the end of the performance (after approximately 80% of the total performance time, Fig. 3, R^2=0.01, $F_{1,263}$=4.07, P=0.04). In the cold condition, pulse durations were constant throughout the whole performance ($F_{1,431}$=0.10, P=0.75, Fig. 3).

Interpulse duration was significantly longer (Z=9.67, P<0.0001) in the normal condition than in the cold condition (N=545, Table 1, Fig 4a). For both conditions, normal (R^2=0.42, $F_{1,198}$=148.05, P<0.0001) and cold (R^2=0.16, $F_{1,339}$=64.69, P<0.0001), there was a similar and significant pattern for the interpulse duration to be shorter at the end of the performance (Fig. 4b). A sample of pulses and interpulses for both conditions is shown in Fig. 1a (normal condition) and Fig. 1b (cold condition).

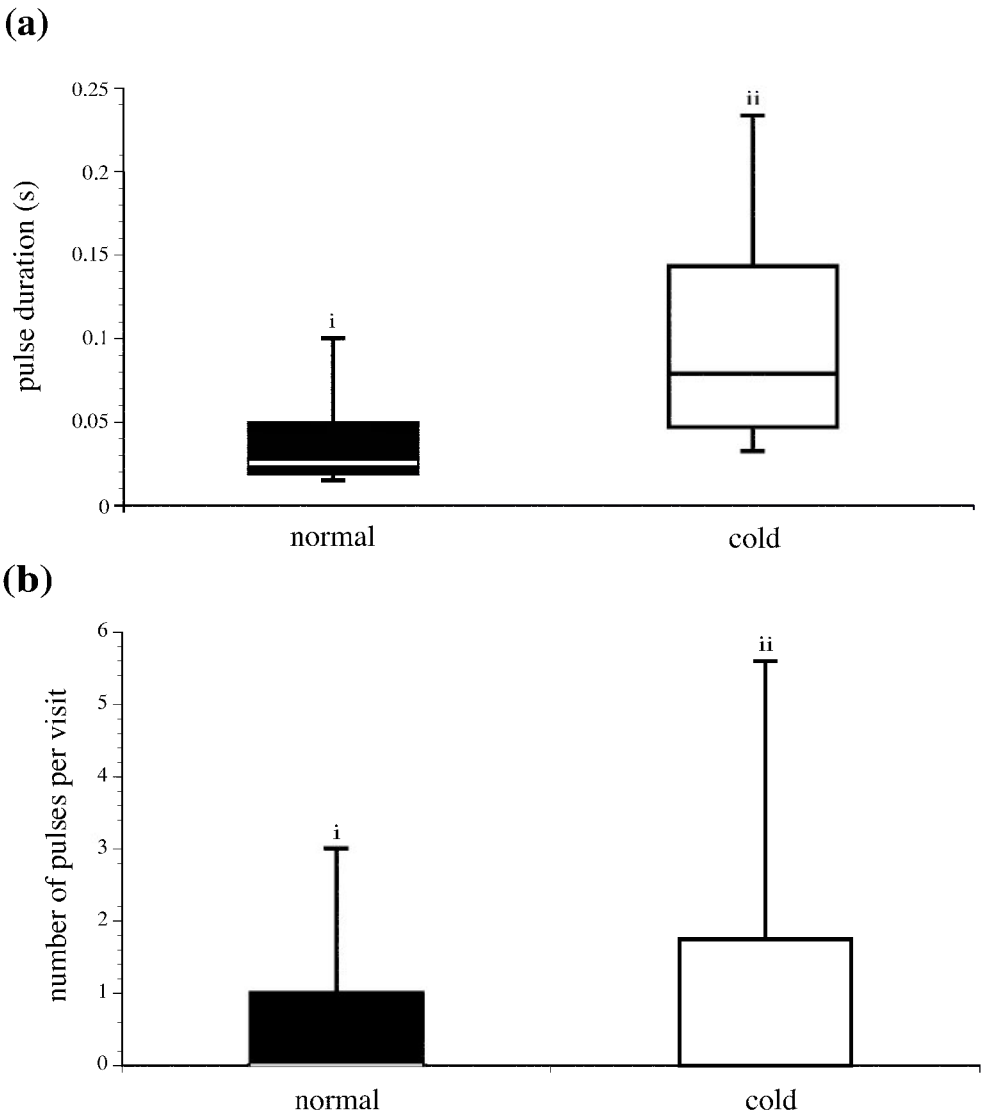

Fig. 2 *Box plots* showing the distribution percentiles (10th, 25th, 50th, 75th, and 90th) of **a** pulse duration and **b** number of pulses per visit produced on the feeder during normal and cold conditions (left-skewed distributions and thus lower percentiles compressed in plot). *Letters above each box plot* denote significant differences (Wilcoxon test)

Table 2 Ambient and forager thoracic temperatures under different experimental conditions

Variable	Condition (n=240)	ANOVA	P-value
T_A (°C)	Normal	$F_{1,230}$=1.82	0.18
	Cold	$F_{1,237}$=1.01	0.31
T_{TH} (°C)	Normal	$F_{1,230}$=1.01	0.31
	Cold	$F_{1,237}$=0.17	0.68
T_{PLATE} (°C)	Normal	$F_{1,230}$=0.07	0.78
	Cold	$F_{1,237}$=1.55	0.21
ΔT_A (°C)	Normal	$F_{1,230}$=4.93	0.03[a]
	Cold	$F_{1,237}$=1.10	0.29
ΔT_{PLATE} (°C)	Normal	$F_{1,230}$=1.28	0.26
	Cold	$F_{1,237}$=0.91	0.34

[a] R^2 =0.02

Relationships between performance time and T_{TH}, T_{PLATE}, and T_A

Foragers spent significantly more time on the feeder in the cold condition (88.46±46.67 s) than during the normal condition (39.61±15.88 s, Z=−15.40, P<0.00001, Fig. 5a). In addition, there was a significant positive correlation between performance time and T_{PLATE} during the cold condition (R^2<0.13, $F_{1,237}$<35.80, P<0.0001), but not during the normal condition ($F_{1,230}$=0.85, P=0.36; Fig. 5b).

Air temperature did not affect performance time in the cold condition ($F_{1,237}$=0.06, P=0.81). However, in the normal condition, performance time increased with decreasing T_A (R^2=0.08, $F_{1,230}$=19.51, P<0.0001). In the cold condition, performance time decreased with increasing T_{TH} (R^2=0.09, $F_{1,237}$=25.10, P<0.0001), but not in the normal condition ($F_{1,237}$=1.37, P=0.24).

Relations between T_{TH}, T_{PLATE}, and T_A

Treatment significantly affected T_{TH} because foragers maintained a greater thoracic temperature excess relative to ambient temperature (ΔT_A) at low ambient temperatures than at higher ambient temperatures (ΔT_A cold: 13.30°C± 2.39°C, ΔT_A normal: 6.47°C±1.40°C, Z=−18.48; P< 0.0001). In the cold condition (multiple regression: R^2= 0.11), T_{PLATE} accounted for approximately 48 times more of the variance in T_{TH} than T_A did (T_{TH}: $F_{1,235}$=29.14, SS_{PLATE}=50.21, P<0.0001; T_A: $F_{1,235}$=0.60, SS_A=1.04, P=0.44; Fig. 6). Feeder plate temperature and T_A were highly and significantly correlated in the normal condition (R^2=0.34, $F_{1,230}$=116.69, P<0.0001), but only very weakly correlated in the cold condition (R^2=0.02, $F_{1,237}$=4.54, P=0.03).

Discussion

Nieh (1998) made a preliminary observation of buzzing departure sounds produced by *M. panamica* foragers feeding on a carbohydrate reward, and we have now shown that these pulsed sounds (401.68±18.33 Hz fundamental

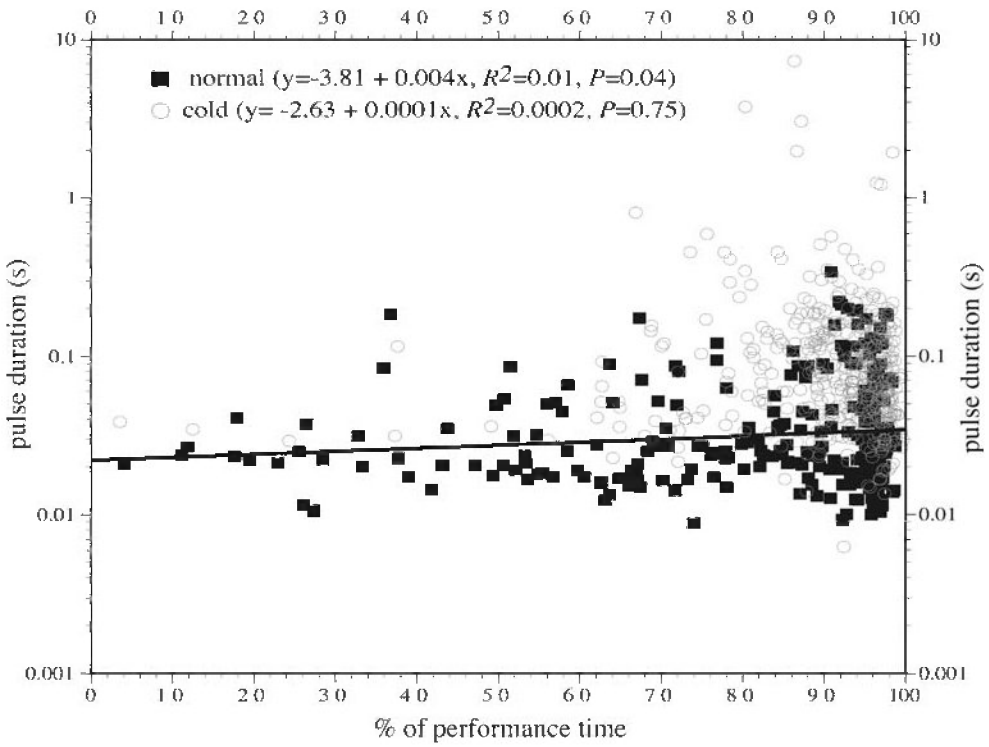

Fig. 3 Pulse duration (*log scale*) vs scaled performance times per visit (%) during normal and cold conditions. The significant linear regression line is shown

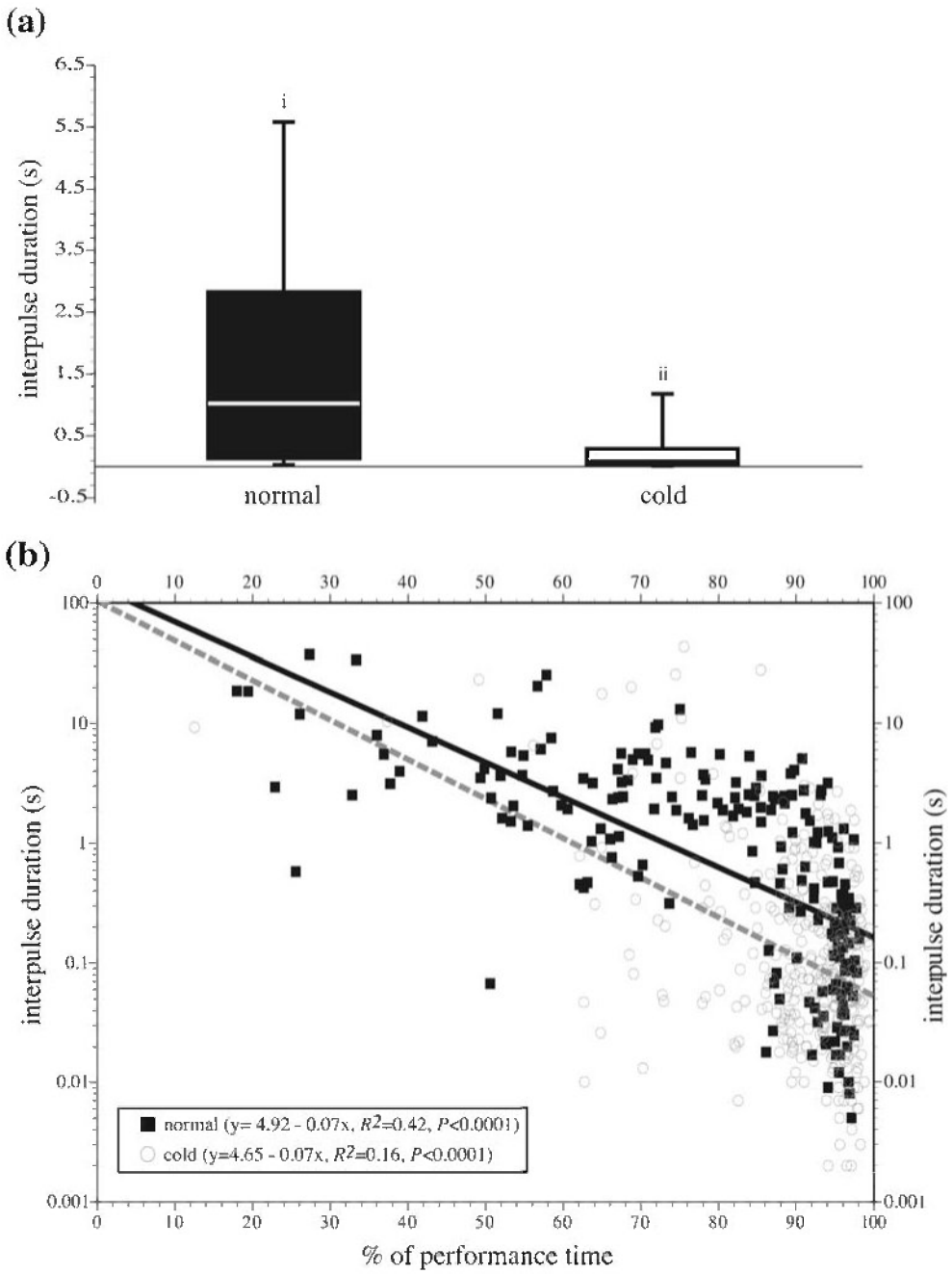

Fig. 4 **a** *Box plots* showing the distribution percentiles of interpulse duration (*different letters above each box plot* denote significant differences, Wilcoxon test) and **b** interpulse duration (*log scale*) vs scaled performance times per visit (%) during normal and cold conditions (the significant linear regression lines are shown)

frequency) can be produced throughout the entire time a forager is on the feeder. This is the first detailed analysis of the sounds produced by meliponine bees feeding on a carbohydrate reward, although it was already known that *Melipona* may vibrate flowers with poricidal anthers without floral damage (Buchmann 1983; Guibu et al. 1988; Roubik 1989), producing audible sounds during this process.

We tested the hypothesis that forager sound production increases with decreasing temperature by offering a feeder at normal (23–33°C) or chilled ambient temperature (11–25°C). The pulse duration (Fig. 2a) and number of pulses per visit (Table 1) were higher in the cold condition than in the normal condition. Performance time (the total time that a forager spent on the feeder in a single visit) was also higher in the cold condition than in the normal condition (Fig. 5a,b). Interpulse duration (the time interval between two pulses) was lower in the cold condition than in the normal condition (Fig. 4a). Thus, the amount of time (and consequently energy) spent producing sound increased as ambient feeder temperature decreased. Under both normal and cold conditions, interpulse duration was significantly shorter at the end of the performance (Fig. 4b). Thus, foragers increased the rate of pulse production towards the

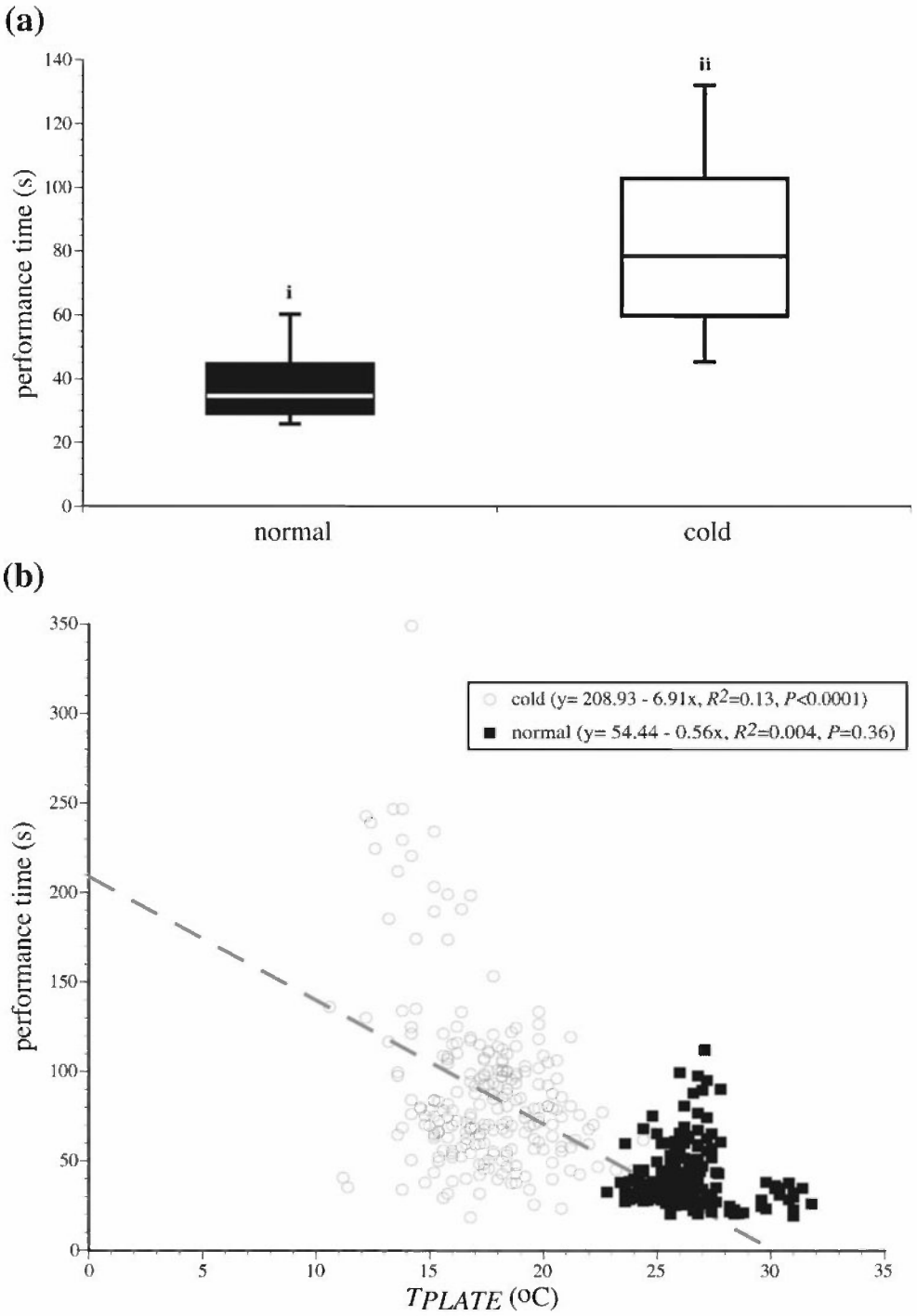

Fig. 5 *Box plots* showing the distribution percentiles of total performance time **a** under different conditions (*different letters above each box plot* denote significant differences, Wilcoxon test) and **b** at different feeder temperatures (the significant linear regression line is shown)

end of each performance, as expected if sound pulses play a role in thoracic warm-up before flight departure.

Performance time

When we compared the total performance time that foragers spent at the feeder in both treatments (normal and cold), we found that foragers spent significantly longer times at the feeder in the cold condition than in the normal condition (Fig. 5a,b). Feeder plate temperature may have affected sucrose solution viscosity and thus influenced the total performance time of bees at the feeder because sucrose solution viscosity is higher at colder temperatures (viscosity of a 65% sucrose solution at 20°C is 147 mPa s^{-1} and at 30°C is 77.3 mPa s^{-1}, Bubnik et al. 1995). Foragers could experience these viscosities at natural food sources because the lower ambient temperatures in our study can occur at altitudes (up to 1400 m) within the range of this species (Nieh and Roubik 1995).

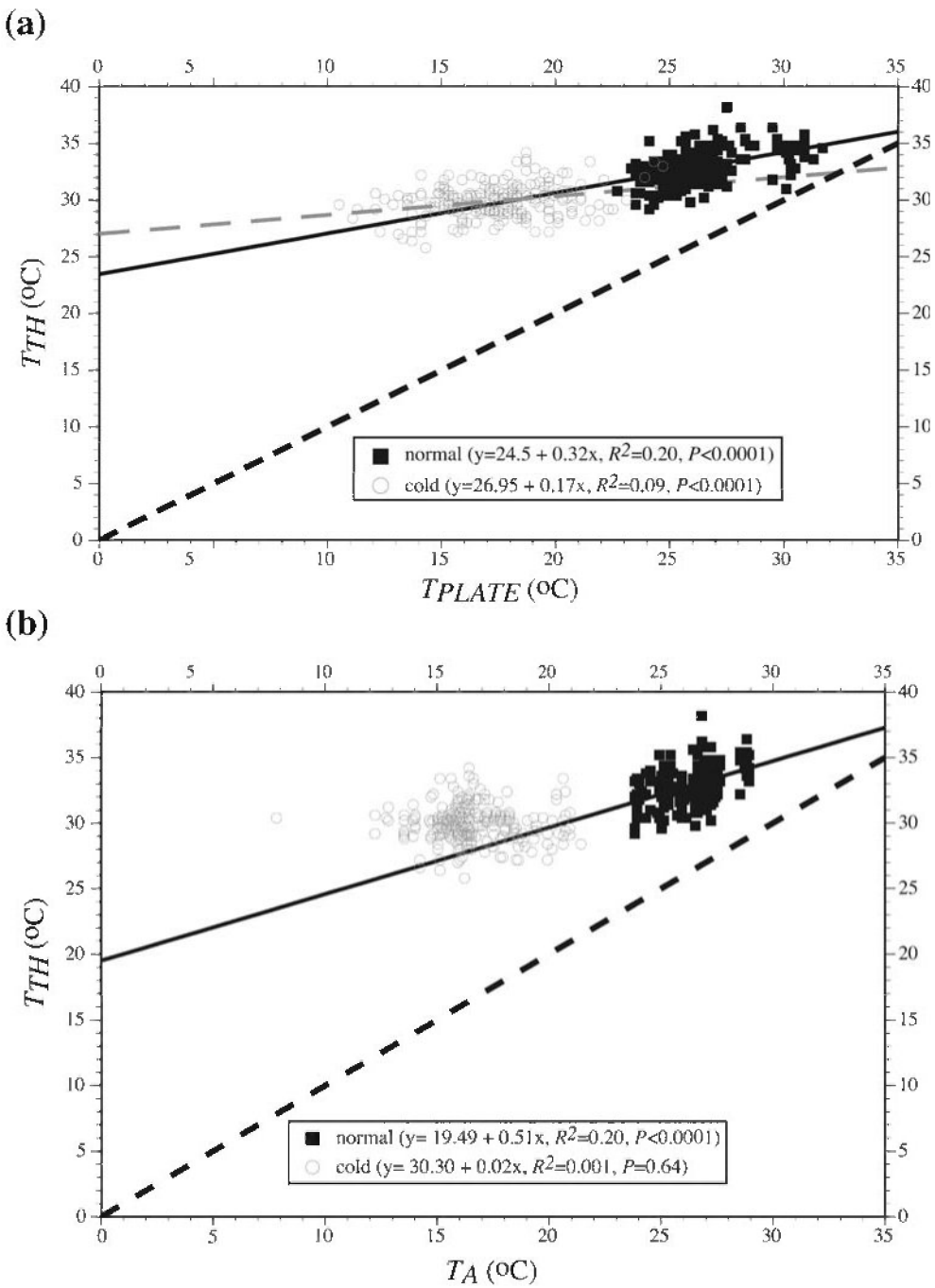

Fig. 6 Effect of **a** feeder plate temperature and **b** air temperature on forager thoracic temperature under the two experimental conditions. The isothermal lines for equivalent T_{TH} vs different measures of ambient temperature are given (significant linear regression lines shown)

Pulses and interpulses

Bee thoracic muscles need to reach a minimal temperature before take-off (Dudley 2000). If sound production plays a role in warming up thoracic temperature before flight, a greater number of pulses (and thus shorter interpulses) and longer pulses would be expected under colder ambient conditions if the sound production is correlated with heat generation, as in *B. lapidarius* (Schulze-Motel and Lamprecht 1994).

As expected, the average duration of pulses was significantly shorter in the normal condition (0.15± 0.45 s) than in the cold condition (0.34±0.30 s, Table 1, Fig. 1a,b). Moreover, the average duration of interpulses (the time between two consecutive pulses) was significantly shorter in the cold condition (0.89±3.78 s) than in the normal condition (2.57±4.92 s, Table 1, Fig 4a). Thus, the total number of pulses per visit was significantly higher in the cold condition than in the normal condition (Fig. 2b).

The distribution of pulses across time (Fig. 3) supports the hypothesis that bees produce feeder sounds to warm up before flight departure. In the cold condition, pulses were concentrated in the end of the performance; approximately 50% of pulses occurred after 80–90% of the total performance time, whereas they were more evenly distributed in the normal condition (Fig. 3). In both conditions, but with a stronger pattern in the cold condition, the interval between pulses was significantly shorter at the end of each performance. Thus, pulses were more concentrated at the end of each performance (Fig. 4b).

Under cold and normal conditions, there were no significant correlations between the number of pulses and T_{TH}, T_A, T_{PLATE}, ΔT_A, and ΔT_{PLATE} (Table 2). This suggests that the primary effect of lower temperature is to increase feeder sound pulse duration. It is possible that experimenting with a wider range of temperatures within each condition or change the timing of temperature measurement (we measured T_{TH} from 5 to 10 s after the forager began feeding) may allow the discernment of a significant effect of T_{TH}, T_A, T_{PLATE}, ΔT_A, or ΔT_{PLATE} upon the number of pulses.

Finally, foragers may not necessarily produce sounds when they heat up their wing muscles (bumblebees sometimes do not produce noticeable sounds during thermoregulation, Heinrich 1972, 1975; Esch and Goller 1991), although sound production in *M. panamica* is highly correlated with wing muscle contraction (high speed video analysis, Nieh and Schofield, unpublished data). Continuous measurements of thoracic temperature or measurements immediately before departure may be revealing (as in *Bombus*, Schulze-Motel and Lamprecht 1994) and are planned for future studies using IR image scanning.

Effect of feeder and air temperature on T_{TH}

In our experiments, T_{PLATE} and T_A significantly influenced T_{TH}, and their effect was different between the treatments (Fig. 6a,b). In the normal condition, T_{PLATE} and T_A influenced T_{TH}. In the cold condition, only T_{PLATE} significantly influenced bee's thoracic temperature. This may be due to the high correlation between T_{PLATE} and T_A in the normal condition (R^2=0.34, P<0.0001) but not in the cold condition (R^2=0.02, P=0.03). Under normal conditions, we would not expect any difference between the plate temperature and the ambient temperature, and we did not find any difference. Under cold conditions, T_{TH} of foragers was 29.93±1.38°C and under normal condition it was 32.89±1.41°C (P<0.0001). Also, foragers maintained a higher thoracic temperature excess (ΔT_{TH}) relative to ambient temperature and feeder temperature at low ambient temperatures (ΔT_A cold: 13.30°C±2.39°C, ΔT_A normal: 6.47°C±1.40°C, P<0.0001; Fig. 6a,b), as Nieh and Sánchez (2005) predicted for *M. panamica*.

Thermoregulation is a significant phenomenon with ecological implications (Corbet et al. 1993) in insects. Internal heat generation allows solitary (Stone 1994) and social bees (Heinrich 1993) to forage and pollinate under colder ambient temperatures compared to animals without the ability to actively thermoregulate. In *M. panamica*, several factors influence sound production inside the nest, such as food source distance, and height (Nieh and Roubik 1998). With regards to feeder sounds, this is the first study that evaluates the influence of ambient temperature on feeder sounds in stingless bees, and our results strongly suggest that feeder sounds are not correlated with food source location communication, but that are correlated with body warm-up before flight. We are therefore currently investigating the effects of other factors, such as sucrose concentration, that may also influence sound pulse production at the food source.

Acknowledgments We would to like David W. Roubik for his kind support, providing us with the *M. panamica* colonies, and for his suggestions that improved our article. Jennifer Schofield provided invaluable fieldwork assistance. We also would like to thank David Holway for statistical advice and the Smithsonian Tropical Research Institute in the Barro Colorado Field Station, especially Oris Acevedo and Mélida Ruiz, for facilities and administrative help. This research was funded by a grant from the National Science Foundation, NSF IBN-0316697 and supported by the Opportunities for Research in the Behavioral Sciences (ORBS) Program at UCSD.

References

Aguilar I, Briceño D (2002) Sounds in *Melipona costaricensis* (Apidae: Meliponini): effect of sugar concentration and nectar source distance. Apidologie 33:375–388

Bubnik Z, Kadlec P, Urban D, Bruhns M (1995) Sugar technologists manual, 8th edn. Bartens, Berlin

Buchmann SL (1983) Buzz pollination in angiosperms. In: Little RJ (ed) Handbook of Experimental Pollination Biology. Van Nostrand Reinhold Publishing, New York, pp 73–113

Bujok B, Kleinhenz M, Fuchs S, Tautz J (2002) Hot spots in the bee hive. Naturwissenschaften 89:299–301

Cameron SA, Mardulyn P (2001) Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). Syst Biol 50:194–214

Coelho JR (1991) The effect of thorax temperature on force production during tethered flight in honeybee (*Apis mellifera*) drones, workers, and queen. Physiol Zool 64:823–835

Corbet SA, Fussell M, Ake R, Fraser A, Gunson C, Savage A, Smith K (1993) Temperature and the pollinating activity of social bees. Ecol Entomol 18:17–30

Darchen R (1973) La thermoregulation et l'écologie de quelques espèces d'abeilles sociales d'Afrique (Apidae, Trigonini et *Apis mellifica* Var. *adansonii*). Apidologie 4:341–370

Dudley R (2000) The biomechanics of insect flight. Princeton University Press, Princeton, NJ

Engels W, Rosenkranz P, Engels E (1995) Thermoregulation in the nest of the Neotropical stingless bee *Scaptotrigona postica* and a

hypothesis on the evolution of temperature homeostasis in highly eusocial bees. Stud Neotrop Fauna Environ 30:193–205
Esch H, Goller F (1991) Neural control of fibrillar muscles in bees during shivering and flight. J Exp Biol 159:419–431
Esch H, Esch I, Kerr WE (1965) Sound: an element common to communication of stingless bees and to dances of the honey bee. Science 149:320–321
Esch H, Goller F, Heinrich B (1991) How do bees shiver. Naturwissenschaften 78:325–328
Fletcher DJC, Crewe RM (1981) Nest structure and thermoregulation in the stingless bee, *Trigona* (*Plebeina*) *denoiti* Vachal (Hymenoptera: Apidae). J Entomol Soc South Afr 44:183–196
Guibu LS, Ramalho M, Kleinert-Giovannini A, Imperatriz-Fonseca VL (1988) Exploração dos recursos florais por colônias de *Melipona quadrifasciata* (Apidae, Meliponinae). Rev Bras Biol 48:299–305
Harrison JF, Fewell JH (2002) Environmental and genetic influences on flight metabolic rate in the honey bee, *Apis mellifera*. Comp Biochem Physiol, Part A Mol Integr Physiol 133A:323–333
Heinrich B (1972) Energetics of temperature regulation and foraging in a bumblebee, *Bombus terricola* Kirby. J Compar Physiol 77:49–64
Heinrich B (1975) Thermoregulation by bumblebees. II. Energetics of warm-up and free flight. J Comp Physiol 96:155–166
Heinrich B (1979) Bumble-bee economics. Harvard University Press, Cambridge
Heinrich B (1985) The social physiology of temperature regulation in honeybees. In: Lindauer M (ed) Experimental behavioral ecology and sociobiology; International Symposium, Mainz, West Germany, Oct. 17–19, 1983. Sinauer Associates, Sunderland, pp 393–406
Heinrich B (1993) The hot-blooded insects: strategies and mechanisms of thermoregulation. Springer, Berlin Heidelberg New York
Hrncir M, Jarau S, Zucchi R, Barth FG (2004a) Thorax vibrations of a stingless bee (*Melipona seminigra*). I. No influence of visual flow. J Comp Physiol A 190:539–548
Hrncir M, Jarau S, Zucchi R, Barth FG (2004b) Thorax vibrations of a stingless bee (*Melipona seminigra*). II. Dependence on sugar concentration. J Comp Physiol A 190:549–560
Hrncir M, Barth FG, Tautz J (2006) Vibratory and airborne-sound signals in bee communication (Hymenoptera). In: Claridge MF (ed) Insect sounds and communication. Taylor & Francis Group, New York, pp 421–436
Kerr WE, Laidlaw HH (1956) General genetics of bees. Adv Genet 8:109–153
Kerr WE, Sakagami SF, Zucchi R, Portugal-Araújo Vd, Camargo JMF (1967) Observações sobre a arquitectura dos ninhos e comportamento de alumas espécies de abelhas sem ferrão das vizinhanças de Manaus, Amazonas (Hymenoptera, Apoidea). Atlas do Simpósio sobre a Biota Amazônica 5:255–309
Kleinhenz M, Bujok B, Fuchs S, Tautz J (2003) Hot bees in empty broodnest cells: heating from within. J Exp Biol 206:4217–4231
Kovac H, Stabentheiner A (1999) Effect of food quality on the body temperature of wasps (*Paravespula vulgaris*). J Insect Physiol 45:183–190
Michener CD (1974) The social behavior of the bees. The Belknap Press of Harvard University Press, Cambridge, Massachusetts
Michener CD (2000) The bees of the world. Johns Hopkins University Press, Baltimore and London, UK
Nieh JC (1998) The role of a scent beacon in the communication of food location in the stingless bee, *Melipona panamica*. Behav Ecol Sociobiol 43:47–58
Nieh JC (2004) Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). Apidologie 35:159–182
Nieh JC, Roubik DW (1995) A stingless bee (*Melipona panamica*) indicates food location without using a scent trail. Behav Ecol Sociobiol 37:63–70
Nieh JC, Roubik DW (1998) Potential mechanisms for the communication of height and distance by a stingless bee, *Melipona panamica*. Behav Ecol Sociobiol 43:387–399
Nieh JC, Sánchez D (2005) Effect of food quality, distance and height on thoracic temperature in the stingless bee Melipona panamica. J Exper Biol 28:3933–3943
Nieh JC, Contrera FAL, Ramírez S, Imperatriz-Fonseca VL (2003a) Variation in the ability to communicate three-dimensional resource location by stingless bees from different habitats. Anim Behav 66:1129–1139
Nieh JC, Contrera FAL, Rangel J, Imperatriz-Fonseca VL (2003b) Effect of food location and quality on recruitment sounds and success in two stingless bees, *Melipona mandacaia* and *Melipona bicolor*. Behav Ecol Sociobiol 55:87–94
Nieh JC, Ramírez S, Nogueira-Neto P (2003c) Multi-source odor-marking of food by a stingless bee, *Melipona mandacaia*. Behav Ecol Sociobiol 54:578–586
Pacheco RdLF, Kerr WE (1989) Temperatura em abelhas da espécie *Melipona compressipes fasciculata*. Ciênc Cult 41: 490–495
Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge University Press, Cambridge
Roubik DW (1992) Stingless bees: a guide to Panamanian and Mesoamerican species and their nests (Hymenoptera: Apidae: Meliponinae). In: Aiello A (ed) Insects of Panama and Mesoamerica: selected studies. Oxford University Press, Oxford, pp 495–524, 653–663
Roubik DW, Buchmann SL (1984) Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. Oecologia 61: 1–10
Sakagami SF (1982) Stingless bees. In: Herrmann HR (ed) Social insects, vol III. Academic Press, Hakushinka, pp 361–423
Schulze-Motel P, Lamprecht I (1994) Correlation of sound generation and metabolic heat flux in the bumblebee *Bombus lapidarius*. J Exp Biol 187:315–318
Seeley TD, Heinrich B (1981) Regulation of temperature in the nests of social insects. In: Heinrich B (ed) Insect thermoregulation. Wiley, New York, pp 159–234
Stabentheiner A, Pressl H, Papst T, Hrassnigg N, Crailsheim K (2003) Endothermic heat production in honeybee winter clusters. J Exp Biol 206:353–358
Stone GN (1993) Thermoregulation in four species of tropical solitary bees: the roles of size, sex and altitude. J Comp Physiol B, Biochem Syst Environ Physiol 163:317–326
Stone GN (1994) Patterns of evolution of warm-up rates and body temperatures in flight in solitary bees of the genus *Anthophora*. Funct Ecol 8:324–335
von Frisch K (1967) The dance language and orientation of bees, 2nd printing, 1993 edn. Belknap Press, Cambridge, Massachusetts
Wolfe WL, Zissis GL (1985) The infrared handbook. The Office, Washington, pp 1700
Woods WAJ, Heinrich B, Stevenson RD (2005) Honeybee flight metabolic rate. J Exp Biol 208:1161–1173
Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, New Jersey, USA
Zucchi R, Sakagami SF (1972) Capacidade termoreguladora em *Trigona spinipes* e em algumas outras espécies de abelhas sem ferrão (Hymenoptera: Apidae: Meliponinae). In: Zucchi R (ed) Homenagem à W. E. Kerr. UNESP, Rio Claro, pp 301–309