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Original article

Food profitability affects intranidal recruitment behaviour in the stingless bee *Nannotrigona testaceicornis**

Veronika M. SCHMIDT[1], Michael HRNCIR[2], Dirk Louis P. SCHORKOPF[1], Sidnei MATEUS[2], Ronaldo ZUCCHI[2], Friedrich G. BARTH[1]

[1] Department of Neurobiology and Cognition Research, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
[2] Department of Biology, FFCLRP, University of São Paulo, Av. Bandeirantes 3900, Ribeirão Preto, SP, 14040-901, Brazil

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Abstract – Does the food's sugar concentration affect recruitment behaviour in the stingless bee *Nannotrigona testaceicornis*? We recorded intranidal forager behaviour while offering sugar water of constant, increasing, or decreasing concentrations. Running speed was not correlated with sugar concentration but the jostling contacts/sec were. Food profitability also affected the recruiter's thorax vibrations: Pulse duration and duty cycle followed both concentration increases and decreases. Sugar concentration also influenced the number of recruited bees. In comparison to the phylogenetically closely related *Scaptotrigona*, *Nannotrigona's* intranidal recruitment behaviour showed a more elaborate association with food profitability. This is likely to reflect differences in ecology and foraging strategies as *Nannotrigona* – in contrast to *Scaptotrigona* – does not lay scent trails to guide recruits to a food source.

stingless bees / communication / recruitment / foraging / thorax vibrations

1. INTRODUCTION

One of the main challenges in the study of recruitment mechanisms in stingless bees (Meliponini) is the diversity found in this subfamily of the Apidae (Hymenoptera) (Michener, 2000). Aiming at a comparative and evolutionary understanding of the various clues used in recruitment communication, an obvious first task is to gather data on more species representing different phylogenetic groups.

Present knowledge of intranidal recruitment behaviour of stingless bees mainly comes from work on the genus *Melipona* and focuses on airborne sound and vibrational signals produced by the recruiters (Esch et al., 1965; Esch, 1967; Nieh, 1998; Nieh and Roubik, 1998; Hrncir et al., 2000, 2004a,b, 2006; Aguilar and Briceño, 2002; Nieh et al., 2003). In addition, a zigzag run and the jostling of nestmates inside the nest were observed (Lindauer and Kerr, 1958; Kerr and Esch, 1965; Esch, 1967; Pereboom and Sommeijer, 1993; Nieh, 1998; Hrncir et al., 2000). Both, the vibrations and the jostling contacts by the recruiting bee stimulate the nestmates to leave the nest in search for food (Lindauer and Kerr, 1958; Esch, 1967; Hrncir et al., 2000). So far, we know that *Melipona* bees adjust their intranidal activity both to increases and decreases of the sugar water concentration (Aguilar and Briceño, 2002; Nieh et al., 2003; Hrncir et al., 2004b) and that they recruit more nestmates to more profitable food sources (Biesmeijer and Ermers, 1999).

Only recent work took a close look at the intranidal recruitment behaviour of another genus of stingless bees, *Scaptotrigona*

Corresponding author: F.G. Barth,
friedrich.g.barth@univie.ac.at
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(Schmidt et al., 2006b). In contrast to *Melipona*, *Scaptotrigona* bees use an eye-catching behaviour outside the nest in addition to jostling and vibrating inside the nest: They lay a scent trail between the food source and the nest and thus guide recruits to valuable feeding sites (Lindauer and Kerr, 1958; Kerr et al., 1963; Schmidt et al., 2003; Sánchez et al., 2004). Unlike *Melipona* the intranidal recruitment of *Scaptotrigona* remained rather unaffected by increases of food quality (energy gain) and foragers reduced their intranidal recruitment activity only after a drastic decrease of food profitability (Schmidt et al., 2006b).

The presently studied genus *Nannotrigona* is closely related to the genus *Scaptotrigona* regarding its phylogeny (Michener, 2000; Camargo and Roubik, 2005). However, the genera differ greatly in their ecology, foraging strategies, and choice of food plants (Hubbel and Johnson, 1978; Jarau et al., 2003; Biesmeijer and Slaa, 2006). This circumstance raises the question how in fact *Nannotrigona* recruits its nestmates. One might expect a more elaborate intranidal signalling in a species not using a scent trail like *Nannotrigona* than in one using it (*Scaptotrigona*).

The present work examined the effectiveness of recruitment in *Nannotrigona testaceicornis* in conjunction with its intranidal recruitment behaviour and the dependence of both on food profitability. Precisely we asked (i) whether the parameters of intranidal recruitment follow changes of the food's sugar concentration and (ii) how the number of recruited nestmates changes with increases and decreases of food profitability.

2. METHODS

2.1. Study site and bees

All experiments were carried out on the Ribeirão Preto campus of the University of São Paulo, Brazil, between January and April 2005. We used three colonies of *Nannotrigona testaceicornis* Lepeletier 1836. The body size of this species is only 3–5 mm. Colonies are large with up to several 1000 individuals. They were kept inside the laboratory in wooden boxes with a plastic tube inserted through the wall of the building which served the bees as the entrance and exit to their nest. For recording the intranidal recruitment behaviour we placed a plastic box ($5 \times 10 \times 2$ cm^3) between the nest and the tube. The box was either covered with glass for video recordings or with a thin transparent foil for laser-vibrometry. Similar recording boxes were already successfully used in other species of stingless bees (e.g. Hrncir et al., 2004a,b; Schmidt et al., 2006b). Before starting the present study we observed that *Nannotrigona* foragers readily accepted the box and spent most of their intranidal time in it performing normal recruitment behaviour.

2.2. Measuring food intake

In order to measure the actual profitability of the different sugar water concentrations chosen from the biologically relevant range (Roubik et al., 1995), we recorded the feeding behaviour of *Nannotrigona testaceicornis*. We trained several bees to feed from a drop of sugar water offered on a Plexiglas® plate 35 m away from the nest. There we marked every bee with an individual combination of two coloured dots on its thorax. The bees were captured at the feeder and subsequently released again, one at a time. When the released bee returned to the feeding site it was offered 8 μL of sugar water (Eppendorf pipette) with a concentration of either 20%, 30%, or 40% w/w. We measured the imbibing time with a digital stop watch. We took up the volume of the left-over sugar water with a 5 μL microcapillary (Sigma Microcaps) immediately after the bee's departure in order to reduce inaccuracies due to evaporative losses. From the imbibing time (s) and the imbibed crop load (μL) (crop load = 8 μL – x $\mu L_{\text{remaining sugar water}}$) we calculated the sugar intake rate (mg/s), and the total amount of sugar per crop load (mg). Upon her second return to the feeder the same bee was captured again and kept in a separate container. Subsequently, another one of the originally captured bees was released and the procedure repeated. We observed 45 bees in this way, i.e. 15 bees for each concentration (ambient temperature 29.9 ± 1.3 °C). The sugar water contained unrefined cane sugar (99.8% sucrose, 0.1% glucose and fructose, 0.1% mineral salts). We measured its concentration with a field refractometer (Krüss Optronic HR 25/800). In all cases we added 10 μL rosewood essence per 100 mL sugar solution. Honey bees gauge the energy gains at the food source and thus its profitability by way of the sugar uptake rate (mg/s) and the amount of sugar (mg)

carried by the forager (Farina and Núñez, 1991). The same is likely to apply to stingless bees.

2.3. Experimental design

We used 13% w/w sugar water to train eight foragers to a feeding site 35 m away from the nest. For each trial we used new bees, which had not previously participated in any experiment. The bees fed from an inverted glass dish on a grooved plate which was mounted on a tripod 1 m above the ground. They did not recruit nestmates to a feeder with a solution of such a low concentration. We marked each forager individually on its thorax with two coloured dots. When the bees visited the site regularly we put the training feeder away in an airtight plastic container and offered fresh sugar water in an alcohol cleaned feeder. The time of the first uptake of the new sugar water by one of the marked foragers was defined as the start of the trial. Each trial lasted for three hours and was part of one of three different series: (a) *control series* offering 30% sugar water throughout the entire observation time, (b) *increasing concentrations* with hourly changes from 20% to 30% and to 40% w/w sugar water, and (c) *decreasing concentrations* with hourly changes from 40% to 30% and to 20% sugar water. Immediately before and after each trial we verified the sugar concentrations with a field refractometer (Krüss Optronic HR 25/800). Again, the sugar solutions were scented in all trials (10 μL rosewood essence per 100 mL). We used a fresh alcohol-cleaned feeder whenever the sugar water concentration was changed in order to exclude the accumulation of attractive scent marks (Schmidt et al., 2005). During the control series we changed the feeding dish hourly as well but not the sugar concentration. Each series consisted of six trials performed on different days in a random order. Trials were conducted in the afternoon starting between 12:30 h and 13:45 h were only included into the analysis if all of the eight foragers were collecting at the feeder until the end of the trial. Every bee newly arriving at the feeder was captured and its time of arrival recorded. The number of recruited bees per hour was taken as a measure of the recruitment activity of the eight foragers. After each trial all captured bees were marked with a coloured dot before their release. Marked bees later arriving at the feeding site and already familiar with it were not included in the analysis. We used different colours to mark the foragers of the three experimental colonies. All nests of *Nannotrigona testaceicornis* except the one used for an experiment were closed in the morning of the day of an experiment. Bees marked with the colour of one colony were never found at the nest entrance of any other colony.

2.4. Data collection

We recorded the behaviour of the recruiting bees in one of two ways for the entire duration of an experiment: (i) We videotaped the behaviour of the individually marked recruiting bees inside the glass covered recording box with a digital video camera (Panasonic NV-GS400GE). (ii) We recorded the thorax vibrations of the recruiters inside the foil covered recording box with a portable laser vibrometer (Polytec, PDV 100). Distortions of the laser signal when passing through this foil can be excluded for frequencies lower than 4 kHz (Hrncir and Schmidt, unpublished data). The laser-vibrometer was mounted on a tripod. An easily manoeuvrable mirror fixed above the recording box was used to direct the laser beam onto the thorax of the recruiter (Hrncir et al., 2006). The vibrometer output was recorded with a notebook (sampling rate 22.05 kHz; 32-bit sound card; Soundforge 7.0, Sonic Foundry Inc., Madison WI, USA).

(i) The video material was digitised and subsequently analysed using the software Videopoint 2.5 (LENOX Softworks, Lenox MA, USA). We counted the jostling contacts of each recruiter with her nestmates. They were defined as contacts if the recruiter bumped into a nestmate and displaced it or changed its running direction (Hrncir et al., 2000; Schmidt et al., 2006b). We divided the number of jostling contacts by the time spent inside the recording box to calculate jostling contacts per second. The average running speed (cm/s) of the recruiter during the time taken by ten video frames upon entering the recording box was used as another measure of intranidal activity. However, running events were only used for analysis if the bee did not stop for trophallaxis during or immediately after the time of the measurement. For each of the three test series we analysed the behaviour of eight bees (four bees from two different trials; $N_{total} = 24$).

Although information transfer during trophallaxis is known to be important in honeybees, we did not include its analysis in our study. There are not enough data about trophallaxis in stingless published yet, to be suited for comparison between the species.

(ii) The thorax vibrations of the recruiting bees were analysed using the software SpectraPro 3.32 (Sound Technology Inc., USA). We measured the pulse duration (PD) and interpulse duration (IP), the duration of the pulse sequence (PS = PD + subsequent IP), the duty cycle (DC = PD/PS), the main frequency contained in every pulse (Hz), and the velocity amplitude of every pulse (mm/s). Again, eight bees were analysed for each test series (four bees from two trials per series; $N_{total} = 24$).

2.5. Statistics

SigmaStat 3.1 (Systat Software Inc., Erkrath, Germany) and SPSS 11.0 (SPSS Inc., Chicago, USA) were used to analyse the data. All given P values in this work are two tailed. Each test series (control series, increasing concentrations, and decreasing concentrations) consisted of six individual trials. When the Kolmogorov-Smirnov-test had confirmed a normal distribution ($P > 0.05$) and the Levene-median test an equal variance ($P > 0.05$), we applied parametrical tests (one-way ANOVA). When the Kolmogorov-Smirnov-test or the Levene-median test was below the significance level ($P < 0.05$), non-parametric tests were applied (Kruskal-Wallis test; Sokal and Rohlf, 1995). In order to find out which parameters changed with the changing sugar concentrations we calculated the relative changes of the median value of a parameter between the different experimental steps for each individual bee ($N_{total} = 48$). The average of the median changes of the eight bees per test series should differ significantly from 0% in cases of a significant influence of the changed sugar concentration (one-sample t test) (Sokal and Rohlf, 1995; Hrncir et al., 2004a).

3. RESULTS

3.1. Food and sugar uptake

The crop load (μL) of *Nannotrigona testaceicornis* did not change significantly when the bees took up 20%, 30%, or 40% sugar water (ANOVA: $F_{2,43} = 0.3$, $P = 0.74$) (Fig. 1a). The imbibing time was significantly longer for the 40% than for the 20% concentration but the time for the 30% concentration did not differ significantly from one or the other (ANOVA: $F_{2,43} = 6.65$, $P = 0.03$, Tukey comparison: $P < 0.05$) (Fig. 1b). The parameters which did increase significantly with the sugar water concentration were the sugar intake rate (mg/s) and the amount of sugar (mg) per crop load (Kruskal Wallis test: mg/s: $H_2 = 38.1$, $P < 0.001$, Dunn's test: $P < 0.05$; mg: $H_2 = 38.9$, $P < 0.001$, Dunn's test: $P < 0.05$) (Fig. 1c,d). Thus, with statistical significance, the 20% solution was the least profitable one, the 30% solution was more profitable than the 20% solution, and the 40% solution was the most profitable one among the three.

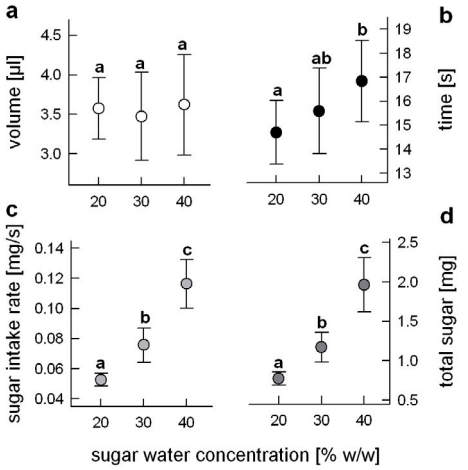

Figure 1. Food intake at three different sugar water concentrations offered subsequently in random order. (a) The imbibed volume did not differ significantly between the concentrations. (b) The imbibing time was significantly longer when 40% w/w sugar water was offered than when bees drank 20% w/w. Both (c) the sugar intake rate (mg/s) and (d) the total amount of sugar per crop load (mg) significantly increased with sugar concentration. Data represent mean ± SD. Different letters mark significant differences between the groups ($P < 0.05$).

3.2. Intranidal recruitment activity

When a successful forager returns into the nest she runs around bumping into her nestmates and often stops for trophallaxis emitting thorax vibrations and airborne sound which can even be heard by the human ear. Most of the behavioural parameters vary greatly between different trials and between individuals.

Table I. Pooled data of eight foragers measured during the first hour of control tests (30% w/w sugar water). From each forager we calculated the mean value and give here the mean ± SD of these (N = 8) for each parameter of intranidal recruitment behaviour.

		mean ± SD
Jostling	(contacts/s)	1.72 ± 0.09
Running speed	(cm/s)	3.7 ± 0.2
Pulse duration	(s)	0.645 ± 0.49
Interpulse duration	(s)	0.145 ± 0.09
Pulse sequence	(s)	0.603 ± 0.23
Duty cycle	(/)	0.68 ± 0.15
Main frequency	(Hz)	399.7 ± 6
Velocity amplitude	(mm/s)	89.8 ± 20.6

We therefore calculated for each bee the median value of the signal parameters during each hour of the trial (Hrncir et al., 2004a). The changes of the median value (%) of each individual bee between the different hours of the trials indicated whether and how the individuals changed their behaviour after having experienced increasing or decreasing changes of the sugar concentration (number of analysed bees per test series = 8). Hence positive values in Figures 2 and 4 stand for increases of the signal parameter by the bees whereas negative values indicate corresponding decreases. In the case of control experiments any changes of the median value would indicate that the signal parameter did not stay constant during the entire trial although a constant sugar concentration was offered. The mean and standard deviation of the analysed data are summarised in Table I.

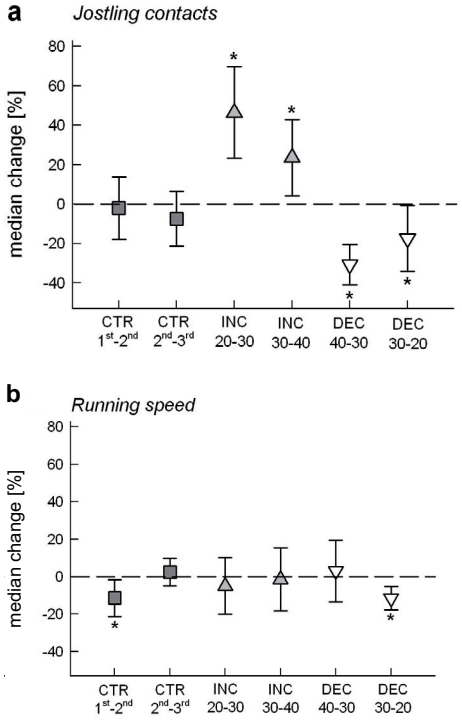

Figure 2. Median changes of jostling contacts (a) and running speed (b) of individual foragers. Control tests: Differences between first and second hour (CTR 1st–2nd) and between second and third hour (CTR 2nd–3rd) are shown. Experiments: changes of median values after each change of sugar concentration (from 20% to 30% [INC 20–30], from 30% to 40% [INC 30–40], from 40% to 30% [DEC 40–30], from 30% to 20% [DEC 30–20]). Changes significantly differing from 0% (dashed line) are indicated by asterisks.

3.2.1. Jostling contacts

The bees' jostling activity remained constant for three hours when the sugar water concentration was kept constant (Fig. 2a). Neither the changes of the foragers' median values between the first and the second hour of the trial (CTR 1st–2nd) nor between the second and the third hour (CTR 2nd–3rd) differed significantly from zero (one sample t test: $t_7 = -0.36$, $P = 0.73$ $_{[\text{CTR 1st–2nd}]}$, $t_7 = -1.5$, $P = 0.17$ $_{[\text{CTR 2nd–3rd}]}$). In contrast, all foragers increased their jostling contacts/second after changes of the sugar water concentration from 20% to 30% w/w (INC 20–30) and from 30% to 40% w/w (INC 30–40) (one sample t test: $t_7 = 5.6$, $P = 0.001$ $_{[\text{INC 20–30}]}$, $t_7 = 3.5$, $P = 0.01$ $_{[\text{INC 30–40}]}$) (Fig. 2a). Likewise, both the decrease of the sugar concentration from 40% to 30% w/w (DEC 40–30) and that from 30% to 20% (DEC 30–20) caused the individual foragers to reduce jostling activity (one sample t test: $t_7 = -8.5$, $P < 0.001$ $_{[\text{DEC 40–30}]}$, $t_7 = -2.9$, $P = 0.02$ $_{[\text{DEC 30–20}]}$) (Fig. 2a).

3.2.2. Running speed

The running speed of the foragers inside the nest was not significantly influenced by the sugar water concentration of the food source (Fig. 2b). During control experiments the bees reduced their running speed inside the nest after the first hour (one sample *t* test: $t_7 = -3.3$, $P = 0.013$ $_{[CTR\ 1st-2nd]}$). Thereafter it remained on a constant level (one sample *t* test: $t_7 = 0.9$, $P = 0.38$ $_{[CTR\ 2nd-3rd]}$). Increasing the sugar concentration had no effect on the intranidal running speed of the foragers (one sample *t* test: $t_7 = -0.9$, $P = 0.39$ $_{[INC\ 20-30]}$, $t_7 = -0.23$, $P = 0.82$ $_{[INC\ 30-40]}$) (Fig. 2b). Accordingly, the first decrease of the sugar concentration did not affect the recruiters' running velocity, whereas they ran significantly slower after the change from 30% to 20% (one sample *t* test: $t_7 = 0.53$, $P = 0.61$ $_{[DEC\ 40-30]}$, $t_7 = -5.2$, $P = 0.001$ $_{[DEC\ 30-20]}$) (Fig. 2b).

3.2.3. Thorax vibrations

Although the variability of the thoracic vibrations was high (Fig. 3), there was a striking influence of the sugar water concentration on their temporal structure.

When a constant sugar water concentration was offered for three hours in control tests the bees did neither change pulse duration (PD) or pulse sequence (PS), nor the duty cycle (DC) of their vibrations (one sample *t* tests: PD: $t_7 = -1.3$, $P = 0.2$ $_{[CTR\ 1st-2nd]}$, $t_7 = -0.85$, $P = 0.4$ $_{[CTR\ 2nd-3rd]}$; PS: $t_7 = -0.7$, $P = 0.5$ $_{[CTR\ 1st-2nd]}$, $t_7 = 1.3$, $P = 0.9$ $_{[CTR\ 2nd-3rd]}$; DC: $t_7 = -1.2$, $P = 0.3$ $_{[CTR\ 1st-2nd]}$, $t_7 = -2.05$, $P = 0.08$ $_{[CTR\ 2nd-3rd]}$) (Fig. 4). The only significant change found in the temporal pattern of the vibrations during the control series was the longer duration of the interpulses (IP) during the 3rd hour (one sample *t* test: $t_7 = 2.9$, $P = 0.02$ $_{[CTR\ 2nd-3rd]}$).

Increasing the sugar water concentration hourly was accompanied by an increase of both the pulse duration and the duty cycle (one sample *t* test: PD: $t_7 = 3.5$, $P = 0.01$ $_{[INC\ 20-30]}$, $t_7 = 4.1$, $P = 0.005$ $_{[INC\ 30-40]}$; DC: $t_7 = 3.2$, $P = 0.02$ $_{[INC\ 20-30]}$, $t_7 = 3.8$, $P < 0.01$ $_{[INC\ 30-40]}$) (Fig. 4). The interpulse duration declined after the first increase of the concentration only. It remained unaffected by the second change whereas the pulse sequence stayed constant after the first change and increased after the second change of the sugar concentration (one sample *t* test: IP: $t_7 = -4.4$, $P = 0.003$ $_{[INC\ 20-30]}$, $t_7 = -0.7$, $P = 0.5$ $_{[INC\ 30-40]}$; PS: $t_7 = -1.8$, $P = 0.12$ $_{[INC\ 20-30]}$, $t_7 = 3.6$, $P = 0.008$ $_{[INC\ 30-40]}$).

Decreasing the concentration hourly affected almost all parameters defining the temporal pattern of the vibrations. Each reduction of the concentration was followed by a decrease of pulse duration and duty cycle and an increase of the interpulse durations (one sample *t* test: PD: $t_7 = -7.2$, $P < 0.001$ $_{[DEC\ 40-30]}$, $t_7 = -5.5$, $P = 0.001$ $_{[DEC\ 30-20]}$; DC: $t_7 = -10.4$, $P < 0.001$ $_{[DEC\ 40-30]}$, $t_7 = -12.1$, $P < 0.001$ $_{[DEC\ 30-20]}$; IP: $t_7 = 5.6$, $P = 0.001$ $_{[DEC\ 40-30]}$, $t_7 = 6.3$, $P < 0.001$ $_{[DEC\ 30-20]}$) (Fig. 4). The pulse sequence remained constant for the first two hours of the experiment but increased after the sugar concentration had finally been lowered from 30% to 20% w/w (one sample *t* test: $t_7 = -1.1$, $P = 0.3$ $_{[DEC\ 40-30]}$, $t_7 = 5.7$, $P = 0.001$ $_{[DEC\ 30-20]}$).

The main frequency component contained in the pulses varied least among the analysed parameters (Fig. 4). Due to the significant changes over time during the control series, the changes during the increasing and decreasing series can not be accredited to corresponding changes of the sugar concentration. During control experiments the bees first increased the main frequency and then reduced it again during the third hour (one sample *t* test: $t_7 = 7.5$, $P < 0.001$ $_{[CTR\ 1st-2nd]}$, $t_7 = -8.1$, $P < 0.001$ $_{[CTR\ 2nd-3rd]}$). Every increase of the sugar concentration led to higher main frequencies (one sample *t* test: $t_7 = 8.3$, $P < 0.001$ $_{[INC\ 20-30]}$, $t_7 = 3.4$) whereas they decreased significantly after the reduction from 30% to 20% w/w (one sample *t* test: $t_7 = 1.6$, $P = 0.16$ $_{[DEC\ 40-30]}$, $t_7 = -12.4$, $P < 0.001$ $_{[DEC\ 30-20]}$).

Regarding the velocity amplitude of the vibrations we found hardly any changes during the experiments (Fig. 4). There was a slight decrease of the amplitude after the second hour of the control experiments (one sample *t* test: $t_7 = -1.0$, $P = 0.35$ $_{[CTR\ 1st-2nd]}$, $t_7 = -3.8$,

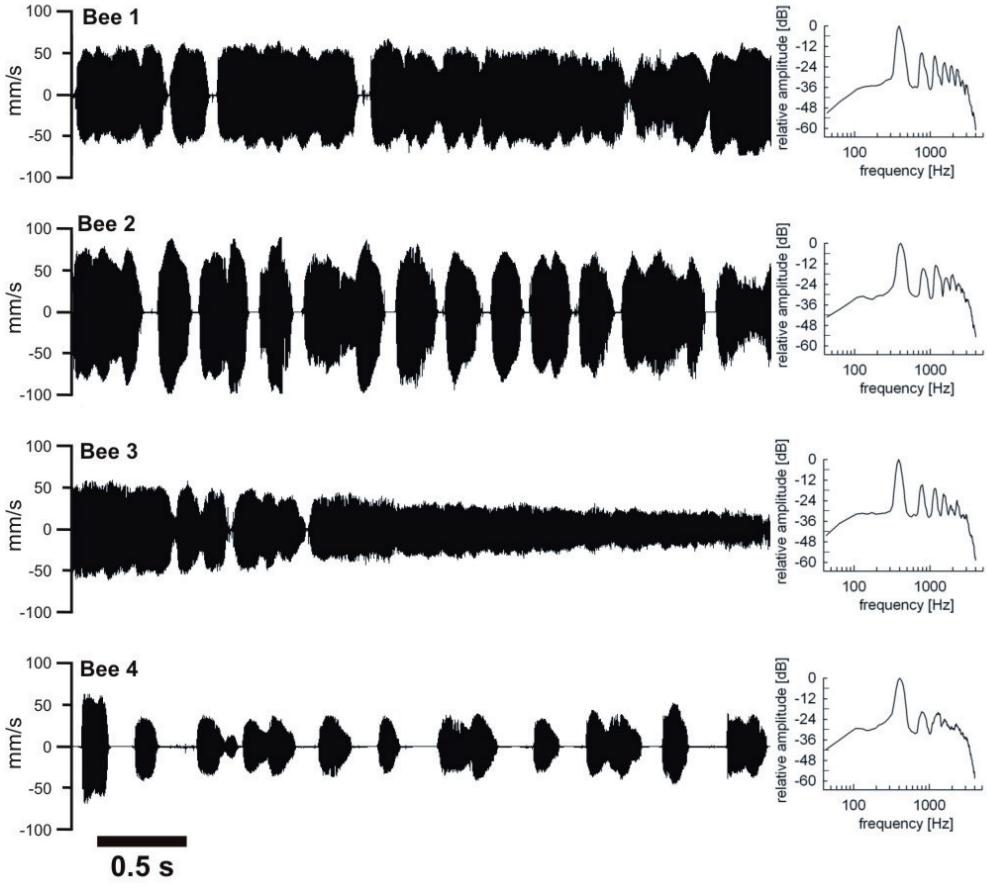

Figure 3. Variability of thorax vibrations of *Nannotrigona testaceicornis*. Sections of typical vibrational signals produced by four different individuals during the first hour of the same control experiment with 30% w/w sugar water offered. Panels on right: each signal's frequency power spectrum (FFT, 1024 pts) with its main frequency component (0 dB) and harmonics.

$P < 0.01$ $_{[CTR\,2nd-3rd]}$). None of the increases of the sugar concentration significantly modified the velocity amplitude (one sample *t* test: $t_7 = 2.2$, $P = 0.07$ $_{[INC\,20-30]}$, $t_7 = 1.3$, $P = 0.24$ $_{[INC\,30-40]}$). Only the decrease of the sugar water concentration from 30% to 20% was paralleled by a significant decrease of the vibration amplitudes (one sample *t* test: $t_7 = -1.7$, $P = 0.14$ $_{[DEC\,40-30]}$, $t_7 = -7.3$, $P < 0.001$ $_{[DEC\,30-20]}$).

3.3. Recruitment effectiveness

The number of bees recruited by the eight foragers varied considerably between the different days of the trials (min = 9, max = 43). Therefore the total number of recruits at the end of each trial was taken as 100% and the recruits per hour as percentage of this value (Jarau et al., 2003; Schmidt et al., 2006b).

Control series: When the bees were offered 30% sugar water for three hours there was a significant peak of recruitment activity during the second hour (Fig. 5). The percentage of recruits during the first and during the third hour did not differ significantly from each other (ANOVA: $F_{2,16} = 21.7$, $P < 0.001$, Tukey's test, $P < 0.001$).

Increasing concentrations: The foragers recruited hardly any bee when they fed on 20%

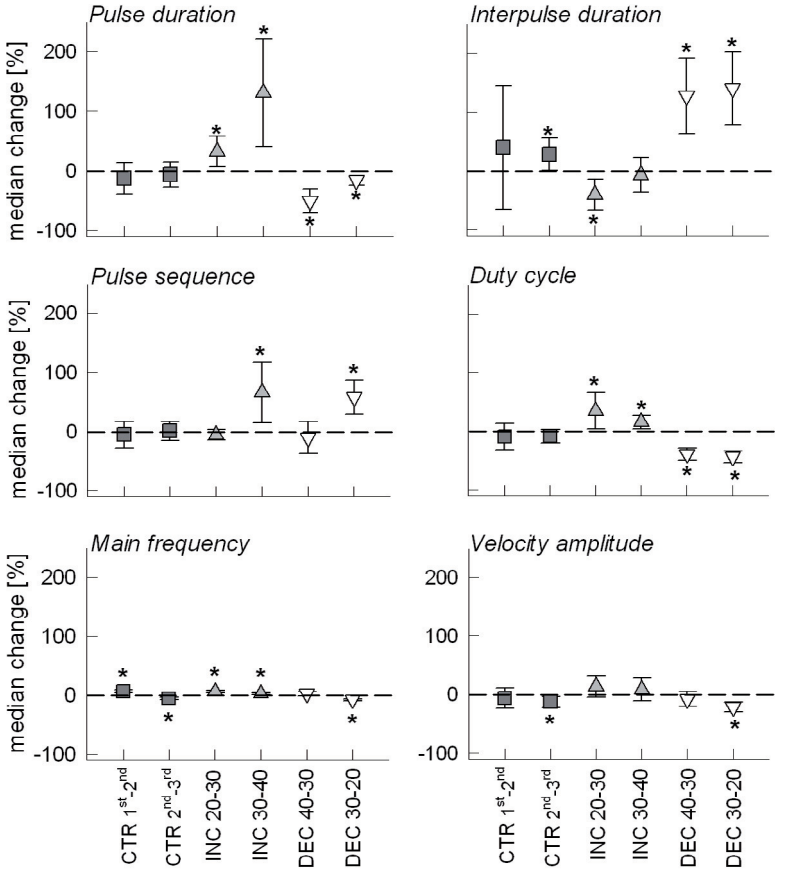

Figure 4. Changes of median values of various vibration parameters of individual foragers during control tests (CTR 1st–2nd hour, CTR 2nd–3rd hour) and after changing the sugar water concentration (increasing concentrations INC 20–30 and INC 30–40, decreasing concentrations DEC 40–30 and DEC 30–20). Values significantly differing from 0% (dashed line) are marked with asterisks.

sugar water. Only 0–2 recruits arrived at the feeding site during the first hour of a series of increasing concentrations. The percentage of recruited nestmates increased significantly with an hourly increase of the sugar water concentration (Fig. 5) (Kruskal Wallis test: H_2 = 15.1, $P < 0.001$, Student-Newman-Keul's test, $P < 0.05$).

Decreasing concentrations: Although the sugar concentration was decreased from 40% to 30%, the bees recruited significantly more nestmates during the second hour (Fig. 5) (Kruskal Wallis test: H_2 = 13.7, P = 0.001, Student-Newman-Keul's test, $P < 0.05$). Following the subsequent drop of the sugar concentration to 20% the recruitment almost stopped and only 0–4 recruits landed at the feeding site.

Taking a close look, these results reveal an association of the number of recruits with the food's sugar concentration. The increase of the number of recruits during the second hour of decreasing concentrations can be explained by the process of recruitment taking more than one hour to reach its peak (see control series). Comparing the percentages of recruits that arrived in a particular hour of the trials shows, that in the first hour significantly fewest recruits arrived when 20% sugar water was offered (increasing series), a medium number arrived at 30% (control series), and significantly most newcomers were counted when foragers

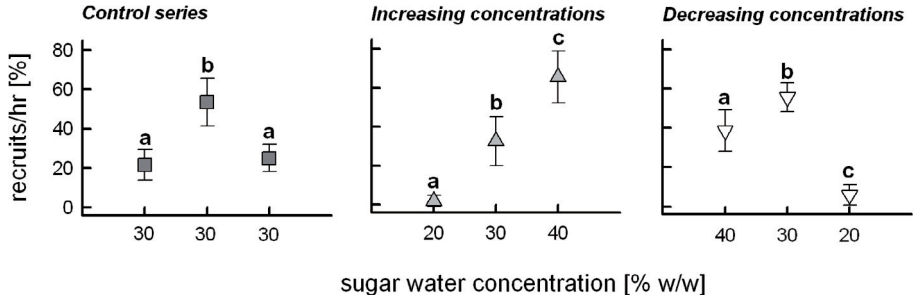

Figure 5. Recruitment to food sources of either constant (*control series*) or varying profitability (*increasing* and *decreasing concentrations*). 100% represents the total number of recruits per trial (3 h). Data are mean (± SD) percentages per hour; different letters mark significant differences ($P < 0.05$).

fed at 40% concentration (decreasing series) (Kruskal Wallis test: $H_2 = 13.8$, $P = 0.001$, Student-Newman-Keul's test, $P < 0.05$). During the second hour 30% sugar water was offered in all series and recruitment peaked in the control series and the series of decreasing concentration. During the third hour of trials almost no bee arrived at the feeder with 20% sugar water (decreasing series), significantly more bees at 30% (control series), and significantly most recruits found the 40% feeder (increasing series) (Kruskal Wallis test: $H_2 = 15.2$, $P = 0.001$, Student-Newman-Keul's test, $P < 0.05$).

4. DISCUSSION

(i) In our experiments with *Nannotrigona testaceicornis* the intranidal recruitment behaviour was closely associated with food profitability. The recruiting bees modified jostling contacts and most parameters of their thorax vibrations according to increasing and decreasing sugar concentrations. From this we conclude, that the intranidal communication in *N. testaceicornis* depends on the energy gains at the food source. (ii) The number of recruits arriving at the feeding site did not follow the exact pattern of the offered food profitability, but it was always largest when 40% and least when 20% sugar water was offered.

4.1. Intranidal recruitment behaviour

In *N. testaceicornis* intranidal recruitment activity correlated with food profitability. This matches our expectation for bees which do not use an extranidal scent trail to guide their recruits and therefore should more strongly depend on intranidal communication.

Jostling. Inside the nest, the jostling activity and thus also the probability of an unemployed forager to be jostled followed the concentration of the sugar water offered.

The only other species analysed in the same way as *N. testaceicornis* is *Scaptotrigona* aff. *depilis* (Schmidt et al., 2006b). Its jostling activity and running speed inside the nest did not change after increases but only after a drastic decrease of the sugar concentration. Thus, a closely related species using a scent trail exhibited less elaborate intranidal signalling than *Nannotrigona.* Unfortunately, a comparison with other non scent trail laying bees like *Melipona* is not possible yet. Their jostling activity has been reported a long time ago (Lindauer and Kerr, 1958) and according to Hrncir et al. (2000) it is correlated with successful recruitment (number of recruits at the feeder). So far, there are no studies in *Melipona* on the jostling activity's dependence on food profitability.

Thorax vibrations. Like jostling the "liveliness" of the thoracic vibrations of *N. testaceicornis* was closely linked to food profitability, reminiscent of *Melipona* bees (*M. costaricensis*: Aguilar and Briceño, 2002; *M. mandacaia*, *M. bicolor*: Nieh et al., 2003; *M. seminigra*: Hrncir et al., 2004b) and suggesting that the foraging strategy (e.g. no scent trail, random search for food) had a strong impact on the evolution of intranidal recruitment communication. In contrast *Scaptotrigona*, a scent

trail laying bee, produced less lively vibrations after decreases of the energy intake, but did not increase intensity with increases of the sugar concentration (Schmidt et al., 2006b). Apparently, for species not using a scent trail, promptly available information about the energetic value of different food sources is important: Their colonies can quickly switch their focus of food collection from one resource to another. Therefore, richer food sources are exploited by more recruits than the less valuable food sources (Seeley, 1995; Biesmeijer and Ermers, 1999). Scent trail laying stingless bees, however, stick to the food source discovered first until it is depleted (Hubbel and Johnson, 1978; Schmidt et al., 2006a). There the vibrational communication about food profitability seems less important because it is the autocatalytic nature of the scent trail which leads to an increasing number of recruits at the most profitable source (Schmidt et al., 2006a).

Throughout all studies in the literature and the present study we find a high variation of the duration of the vibration pulses (Nieh and Roubik, 1998; Hrncir et al., 2000, 2004b; Aguilar and Briceño, 2002; Nieh et al., 2003; Schmidt et al. 2006b), which considerably lowers the probability of the contained message to be understood by the receiver. Therefore, the communication with pulse duration will be a very crude one at best. We still lack comprehensive studies directly demonstrating the influence of vibrations inside the nest on the behaviour of the receivers. Recently, the transmission of forager vibrations onto the hive bees has been analysed in detail by Hrncir et al. (2006) in *Melipona* bees. Future studies will have to concentrate on the behavioural response of the hive bees to the vibrations.

4.2. Recruitment of newcomers

Our control series showed, that the process of recruitment in *Nannotrigona testaceicornis* takes a while until it reaches its peak (Fig. 5). Only in the second hour a maximum of recruited bees arrived at the feeing site. Taking this into account allows us to interpret the results of decreasing and increasing series: the percentages of recruits that arrived during the first hour of experiments depended on the offered sugar concentration. They were largest for 40% sugar water (decreasing series), midway for 30% (control series), and smallest for 20% (increasing series). During the second hour of all trials 30% sugar water was offered. Nevertheless the same relation is again apparent in the last hour of the trials. Most recruits arrived at the feeding site with 40% sugar water (increasing series), fewer in the control series with 30% sugar water and almost none landed at the feeder with 20% sugar water (decreasing series).

The delayed recruitment peak, however, was previously observed when *N. testaceicornis* was allowed to feed and recruit ad libitum at a single food source (sugar water concentration constant at ~ 50% w/w) (Jarau et al., 2003). Likewise Lindauer and Kerr (1958) already reported a slow onset of recruitment to an artificial food source in *N. testaceicornis*. In contrast, whenever several food sources were offered simultaneously, the number of recruits quickly increased at all of the feeding stations (Hubbel and Johnson, 1978; Allerstorfer, 2004). The difference between the studies using one or many food sources, respectively, strongly suggests that *Nannotrigona* adopts random search: It takes its foragers a long time to find one particular feeding site but they arrive more promptly at the food whenever many sources are available. This conjecture should be considered in all experiments working with a single food source and with bees potentially using random search to detect food sources.

Further evidence for random search of the recruits of *N. testaceicornis* comes from other studies examining the distribution of newcomers among many feeding sites. When offered several food sources there was no preference for the feeder already visited by their nestmates. Instead an equal number of recruits arrived at nearly all feeders (Hubbel and Johnson, 1978; Allerstorfer, 2004). When offered more than one feeder simultaneously, *Nannotrigona* brought only 45–53% of the recruits to the feeder used by their nestmates (Allerstorfer, 2004), whereas the value for its relative *Scaptotrigona* spp. was 90−100%, where recruits use the scent trail to find

the food source (Lindauer and Kerr, 1958; Schmidt et al., 2003; Sánchez et al., 2004).

To understand the recruitment communication in the genus *Nannotrigona* is particularly relevant in view of its abundance in the neotropics (Michener, 2000) and thus its agricultural importance. Bees of this genus feed on a wide spectrum of flowering plants (Sommeijer et al., 1983; Biesmeijer and Slaa, 2006) and are important vectors in crop pollination (Maeta et al., 1992; Heard, 1999; Cauich et al., 2004) significantly increasing fruit yield (Bego et al., 1989; Roselino et al., 2004; Santos et al., 2004).

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La qualité de la nourriture agit sur le comportement de recrutement à l'intérieur du nid chez l'abeille sans aiguillon *Nannotrigona testaceicornis*.

Abeille sans aiguillon / communication / recrutement / butinage / vibration du thorax / Apidae

Zusammenfassung – Futterqualität beeinflusst intranidales Rekrutierungsverhalten bei der Stachellosen Biene *Nannotrigona testaceicornis*. In der vorliegenden Studie wurde das Rekrutierungsverhalten der stachellosen Biene *Nannotrigona testaceicornis* untersucht. Phylogenetisch ist diese Art nahe mit der Gattung *Scaptotrigona* verwandt, jedoch unterscheidet sie sich im Verhalten während der Futtersuche: *Nannotrigona* legt im Gegensatz zu *Scaptotrigona* keine Duftpfade als Wegweiser zur Futterquelle. Obgleich mit *Nannotrigona* nicht nahe verwandt, legen auch Bienen der Gattung *Melipona* keinen Duftpfad bei der Nahrungssuche an. Frühere Arbeiten (z.B. Nieh et al., 2003; Hrncir et al., 2004a,b; Schmidt et al., 2006b) beschrieben das Rekrutierungsverhalten von *Melipona* und *Scaptotrigona* innerhalb des Nestes. Dabei zeigte sich, dass *Melipona* (kein Duftpfad) auf Veränderungen von Futterqualität mit einer Veränderung des Verhaltens (Thoraxvibrationen) innerhalb des Nestes reagiert, während *Scaptotrigona* (Duftpfad) nach Veränderungen der Futterqualität im Nest ihr Verhalten kaum änderte. Die vorliegende Studie fragt nach dem Einfluss der Futterqualität auf das intranidale Rekrutierungsverhalten einer Art, die trotz naher taxonomischer Verwandtschaft mit Duftpfad-Bienen keinen Duftpfad auslegt. Im Experiment wurde *Nannotrigona* 35 m vom Nest entfernt eine Futterquelle geboten, deren Zuckerwasserkonzentration konstant gehalten (Kontrollversuche) oder erhöht bzw. erniedrigt wurde. Zugleich wurde innerhalb des Nestes gemessen, mit welcher Geschwindigkeit die Sammelbienen im Nest laufen, wie oft sie ihre Nestgenossinnen pro Sekunde rempeln und welche Thoraxvibrationen sie abgeben. Die Kontrollversuche zeigten, dass die Rempelaktivität gegensätzlich zur Laufgeschwindigkeit bei konstanter Zuckerkonzentration über die Versuchszeit konstant blieb. Mit veränderten Zuckerkonzentrationen änderten die Bienen ihre Rempelaktivität: Während deren Intensität mit zunehmendem Zuckergehalt ebenfalls zunahm, verminderte sie sich infolge abnehmender Zuckerkonzentration. Ebenso hingen die Eigenschaften der Thoraxvibrationen stark von der Konzentration des Futterwassers ab: Bei ansteigender Zuckerkonzentration erhöhten die Bienen die Pulslänge der Vibrationen und auch der Duty Cycle (Aktivitätsmaß) stieg signifikant an. Bei stündlich abnehmender Zuckerkonzentration wurden die Vibrationspulse signifikant kürzer, die Pausen zwischen den Pulsen länger und damit auch der Duty Cycle kleiner. Diese Befunde erfüllen die Erwartungen, dass Bienen ohne Duftpfad (*Nannotrigona*) eine raffiniertere Kommunikation innerhalb des Nestes anwenden als nahe verwandte Bienen, die Duftpfade außerhalb des Nestes als Wegweiser nutzen.

Stachellose Bienen / Kommunikation / Rekrutierung / Nahrungssuche / Thorax-Vibrationen

REFERENCES

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

Allerstorfer S. (2004) Rekrutierung und Kommunikation bei *Nannotrigona testaceicornis* Lep. (1836) (Hymenoptera; Apidae; Meliponini), diploma thesis, University of Vienna.

Bego L.R., Maeta Y., Tezuka T., Ishida K. (1989) Floral preference and flower constancy of a Brazilian stingless bee, *Nannotrigona testaceicornis* kept in a greenhouse (Hymenoptera, Apidae), Bull. Fac. Agric. Shimane Univ. 23, 46–54.

Biesmeijer J.C., Ermers C.W. (1999) Social foraging in stingless bees: how colonies of *Melipona*

fasciata choose among nectar sources, Behav. Ecol. Sociobiol. 46, 129–140.

Biesmeijer J.C., Slaa J.E. (2006) The structure of bee assemblages in Brazil, Apidologie 37, 240–258.

Camargo J.M.F., Roubik D.W. (2005) Neotropical Meliponini: *Paratrigonoides mayri*, new genus and species from western Colombia (Hymenoptera, Apidae, Apinae) and phylogeny of related genera, Zootaxa 1081, 33–45.

Cauich O., Quezada-Euán J.J.G., Macias-Macias J.O., Reyes-Oregel V., Medina-Peralta S., Parra-Tabla V. (2004) Behavior and pollination efficiency of *Nannotrigona perilampoides* (Hymenoptera: Meliponini) on greenhouse tomatoes (*Lycopersicon esculentum*) in Subtropical México, J. Econ. Entomol. 97, 475–481.

Esch H. (1967) Die Bedeutung der Lauterzeugung für die Verständigung der stachellosen Bienen, Z. Vgl. Physiol. 56, 199–220.

Esch H., Esch I., Kerr W.E. (1965) Sound: an element common to communication of stingless bees and to dances of honey bees, Science 149, 320–321.

Farina W.M., Núñez J.A. (1991) Trophallaxis in the honeybee, *Apis mellifera* (L.) as related to the profitability of food sources, Anim. Behav. 42, 389–394.

Heard T. (1999) The role of stingless bees in crop pollination, Annu. Rev. Entomol. 44, 183–206.

Hrncir M., Jarau, S., Zucchi R., Barth F.G. (2000) Recruitment behavior in stingless bees, *Melipona scutellaris* and *M. quadrifasciata*. II. Possible mechanisms of communication, Apidologie 31, 93–113.

Hrncir M., Jarau S., Zucchi R., Barth F.G. (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 F.G. (2004b) Thorax vibrations of a stingless bee (*Melipona seminigra*): II. Dependence on sugar concentration, J. Comp. Physiol. A 190, 549–560.

Hrncir M., Schmidt V.M., Schorkopf D.L.P., Jarau S., Zucchi R., Barth F.G. (2006) Vibrating the food receivers: a direct way of signal transmission in stingless bees (*Melipona seminigra*), J. Comp. Physiol. A 192, 879–887.

Hubbel S.P., Johnson L.K. (1978) Comparative foraging behavior of six stingless bee species exploiting a standardized resource, Ecology 59, 1123–1136.

Jarau S., Hrncir M., Schmidt V.M., Zucchi R., Barth F.G. (2003) Effectiveness of recruitment behavior in stingless bees (Apidae, Meliponini), Insectes Soc. 50, 365–374.

Kerr W.E., Esch H. (1965) Comunicação entre as abelhas sociais brasileiras e sua contribuição para o entendimento da sua evoluçã, Ciência e Cultura 17, 529–538.

Kerr W.E., Ferreira A., de Mattos N.S. (1963) Communication among stingless bees: additional data (Hymenoptera: Apidae), J.N.Y. Entomol. Soc. 71, 80–90.

Lindauer M., Kerr W.E. (1958) Die gegenseitige Verständigung bei den stachellosen Bienen, Z. Vgl. Physiol. 41, 405–434.

Maeta Y., Tezuka T., Nadano H., Suzuki K. (1992) Utilization of the Brazilian stingless bee, *Nannotrigona testaceicornis* as a pollinator of strawberry, Honey Bee Sci. 13, 71–78.

Michener C.D. (2000) The Bees of the World, Baltimore, John Hopkins University Press, London.

Nieh J.C. (1998) The food recruitment dance of the stingless bee, *Melipona panamica,* Behav. Ecol. Sociobiol. 43, 133–145.

Nieh J.C., Roubik D.W. (1998) Potential mechanisms for the communication of height and distance by a stingless bee, *Melipona panamica,* Behav. Ecol. Sociobiol. 43, 387–399.

Nieh J.C., Contrera F.A.L., Rangel J., Imperatriz-Fonseca V.L. (2003) Effect of food location and quality on recruitment sounds and success in two stingless bees, *Melipona mandacaia* and *Melipona bicolour,* Behav. Ecol. Sociobiol. 55, 87–94.

Pereboom J.J.M., Sommeijer M.J. (1993) Recruitment and flight activity of *Melipona favosa*, foraging on an artificial food source, Proc. Exp. Appl. Entomol. N.E.V. 4, 73–78.

Roselino A.C., Bego L.R., Santos S.A.B. (2004) Pollination of strawberry *Fragariax ananassa* var. Carmarosa, by *Scaptotrigona* aff. *depilis* and *Nannotrigona testaceicornis,* Proc. 8th IBRA Int. Conf. on Tropical Bees, p. 670.

Roubik D.W., Yanega D., Aluja M., Buchmann S.L., Inouye D.W. (1995) On optimal nectar foraging by some tropical bees (Hymenoptera: Apidae), Apidologie 26, 197–211.

Sánchez D., Nieh J.C., Hénaut Y., Cruz L., Vandame R. (2004) High precision during food recruitment of experienced (reactivated) foragers in the stingless bee *Scaptotrigona mexicana* (Apidae, Meliponini), Naturwissenschaften 91, 346–349.

Santos S.A.B., Bego L.R., Roselino A.C. (2004) Pollination of cucumber – *Cucumis sativus* – by stingless bees (Hymenoptera, Meliponini), Proc. 8th IBRA Int. Conf. on Tropical Bees, p. 689.

Schmidt V.M., Zucchi R., Barth F.G. (2003) A stingless bee marks the feeding site in addition to the scent path (*Scaptotrigona* aff. *depilis* Moure 1942), Apidologie 34, 237–248.

Schmidt V.M., Zucchi R., Barth F.G. (2005) Scent marks left by *Nannotrigona testaceicornis* at the

feeding site: cues rather than signals, Apidologie 36, 285–291.

Schmidt V.M., Schorkopf D.L.P., Hrncir M., Zucchi R., Barth F.G. (2006a) Collective foraging in a scent trail laying stingless bee (*Trigona recursa*): dependence on food profitability and sequence of discovery, Anim. Behav. 72, 1309–1317.

Schmidt V.M., Zucchi R., Barth F.G. (2006b) Recruitment in a scent trail laying stingless bee: changes with reduction but not with increase of the energy gain (*Scaptotrigona* aff. *depilis*), Apidologie 37, 487–500.

Seeley T.D. (1995) The Wisdom of the Hive, Cambridge, Harvard University Press, Massachusetts.

Sokal R.R., Rohlf F.J. (1995) Biometry, 3rd ed., W.H. Freeman, New York.

Sommeijer M.J., de Rooy G.A., Punt W., de Bruijn L.L.M. (1983) A comparative study of foraging behavior and pollen resources of various stingless bees (Hym. Meliponinae) and honeybees (Hym. Apinae) in Trinidad, West-Indies, Apidologie 14, 205–224.