

Thoracic vibrations in stingless bees (*Melipona seminigra*): resonances of the thorax influence vibrations associated with flight but not those associated with sound production

Michael Hrnčir^{1,2,*}, Anne-Isabelle Gravel³, Dirk Louis P. Schorkopf², Veronika M. Schmidt², Ronaldo Zucchi¹ and Friedrich G. Barth²

¹Department of Biology, University of São Paulo, FFCLRP, Av. Bandeirantes 3900, 14040–901 Ribeirão Preto, SP, Brazil,

²Department of Neurobiology and Cognition Research, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria and

³Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, M3J 1P3, Canada

*Author for correspondence (e-mail: michael.hrnčir@gmx.at)

Accepted 14 November 2007

SUMMARY

Bees generate thoracic vibrations with their indirect flight muscles in various behavioural contexts. The main frequency component of non-flight vibrations, during which the wings are usually folded over the abdomen, is higher than that of thoracic vibrations that drive the wing movements for flight. So far, this has been concluded from an increase in natural frequency of the oscillating system in association with the wing adduction. In the present study, we measured the thoracic oscillations in stingless bees during stationary flight and during two types of non-flight behaviour, annoyance buzzing and forager communication, using laser vibrometry. As expected, the flight vibrations met all tested assumptions for resonant oscillations: slow build-up and decay of amplitude; increased frequency following reduction of the inertial load; and decreased frequency following an increase of the mass of the oscillating system. Resonances, however, do not play a significant role in the generation of non-flight vibrations. The strong decrease in main frequency at the end of the pulses indicates that these were driven at a frequency higher than the natural frequency of the system. Despite significant differences regarding the main frequency components and their oscillation amplitudes, the mechanism of generation is apparently similar in annoyance buzzing and forager vibrations. Both types of non-flight vibration induced oscillations of the wings and the legs in a similar way. Since these body parts transform thoracic oscillations into airborne sounds and substrate vibrations, annoyance buzzing can also be used to study mechanisms of signal generation and transmission potentially relevant in forager communication under controlled conditions.

Key words: stingless bees, thoracic vibration, forager vibration, annoyance buzzing, flight vibration, resonance, natural frequency, laser vibrometry.

INTRODUCTION

In many insects, the wing movements of flight are generated in an indirect way. Here, a rhythmic oscillation of the thorax, which is maintained through stretch activation of the antagonistic flight muscles, induces the up- and downstrokes of the forewings (Snodgrass, 1956; Nachtigall, 2003). During flight, the stretch activation occurs at the natural frequency of the oscillating system. The natural frequency of the thorax is determined by a variety of parameters such as the elasticity of the thoracic capsule, the muscle tension and the moment of inertia due to the wing loading (Roeder, 1951; Soltavala, 1952; Nachtigall, 2003; Darveau et al., 2005). As in other resonant mechanical systems, the frequency of the thorax oscillations, and thus that of the wingbeats, changes when the natural frequency of the system is modified. Experimentally, such modifications can be achieved through partly clipping or complete removal of the wings. The reduction in wing area reduces the mass and inertial load on the oscillating system and, therefore, increases the oscillation frequency (Chadwick and Williams, 1949; Roeder, 1951; Soltavala, 1952).

In addition to the most prominent purpose, which is to move the wings for flight, thoracic vibrations are produced by eusocial bees (bumble bees, stingless bees and honey bees) in the contexts of recruitment communication (Esch, 1961; Wenner, 1962; Michelsen et al., 1986; Hrnčir et al., 2006a), pollen collection (Michener, 1962;

Wille, 1963; Buchmann, 1983; King, 1993) and defence (Schneider, 1972; Sen Sarma et al., 2002). During these vibrations, the wings are usually folded over the abdomen, which results in them decoupling from the indirect flight mechanism (Snodgrass, 1956). Generally, such non-flight vibrations are characterized by a main frequency component higher than that of the flight vibrations (King, 1993; King et al., 1996; Nachtigall, 2003), which was explained by an increase in resonant frequency of the oscillating system as a consequence of wing folding. The folding and decoupling of the wings reduces the inertial load on the oscillating system (Esch and Wilson, 1967; Nachtigall, 2003) and the thoracic capsule might be stiffened through an increased muscle tension associated with wing adduction (Seeley and Tautz, 2001).

In sonograms of the sounds and vibrations produced by bees, however, both an increase in the main frequency component during the build-up of ‘sound’ pulses and a decrease during their decay phase can be detected (Esch and Wilson, 1967; Seeley and Tautz, 2001; Sen Sarma et al., 2002; Hrnčir et al., 2006a; Hrnčir et al., 2006b). This evident, but never described, change in frequency points to driven oscillations: an oscillating system driven by a periodic force with a frequency higher than its natural frequency will vibrate at this excitation frequency as long as the force is applied. As soon as the force stops, however, the vibration will decay and drop to the resonant frequency of the system (Nocke, 1971).

In the present study, we analysed details of the thoracic oscillations associated with wing movements during stationary flight and those associated with 'sound' production during two different types of non-flight vibrations (annoyance buzzing, recruitment sounds). We asked the following two questions. (1) To which extent are resonances involved in both flight and non-flight vibrations? (2) How similar to or different from flight vibrations are vibrations generated in different behavioural contexts?

MATERIALS AND METHODS

Study site and bee species

The experiments were conducted on the campus of the University of São Paulo in Ribeirão Preto, Brazil, between January and April 2005, and in April and May 2007. Bees from four colonies (two colonies in 2005, two different colonies in 2007) of *Melipona seminigra* Friese 1903 were used for the measurements. The colonies were kept in wooden boxes inside a laboratory building (for details, see Hrncir et al., 2004), and were connected to the outside *via* plastic tubes through the laboratory wall. A glass-covered acrylic observation box ($10 \times 5 \times 4$ cm³) was inserted between the nest and the entrance/exit tubes for recording the thorax vibrations of returning foragers.

Sling-tethering for stationary flight and annoyance buzzing

Stingless bees reliably produce annoyance buzzing when tethered around the neck (Fig. 1). A lasso-sling was formed using a nylon thread (diameter: 0.35 mm) through an injection needle (1 mm i.d.), and loosely tightened around the neck of a bee. The thread was fixed by attaching the injection needle to the syringe. As soon as the sling-tethered bees touched any kind of substrate with their tarsi, they usually started to generate thorax vibrations. Pulsed annoyance buzzing was emitted for about 10 min before the bees stopped – probably due to exhaustion. Stationary flight was initiated by removing the substrate from underneath the tarsi.

Laser vibrometry

Vibrations were recorded as velocities using a portable Laser Doppler Vibrometer (PDV-100, Polytec, Waldbronn, Germany). The foragers collected unscented sugar solution (50% cane sugar w/w; refractometer: HR 25/800, Krüss Optronic, Hamburg, Germany) at an artificial food source 10 m away from the nest entrance. Their vibrations were measured during trophallactic contacts with hive bees inside the observation box (for details, see Hrncir et al., 2006b). The individuals used for stationary flight and annoyance buzzing were taken from the observation box and instantly tethered. These bees were foragers, potential recruits or food storing bees. Their vibrations were measured on the scutum, with the laser beam oriented perpendicular to the surface of the thorax. In some of the experiments, the vibrations of the wings and legs were recorded simultaneously with those of the thorax, using an identical, second laser vibrometer. The output of the vibrometers was fed into a notebook (Pentium IV, 2.4 GHz) using a 24-bit stereo soundcard (PSC 805, Philips, Amsterdam, The Netherlands) and the software Soundforge 7.0 (Sony Pictures Digital Inc., Madison, WI, USA), at a sampling rate of 44.1 kHz. For analysis of the recorded vibrations we used the software SpectraPro 3.32 (Sound Technology Inc., Campbell, CA, USA), SigmaPlot 2001 (SPSS Inc., Chicago, IL, USA) and SigmaStat 3.10 (Systat Software Inc., San Jose, CA, USA).

Thoracic vibrations

To evaluate similarities or differences between the different types of thoracic vibrations (stationary flight, FL; annoyance buzzing, AB;

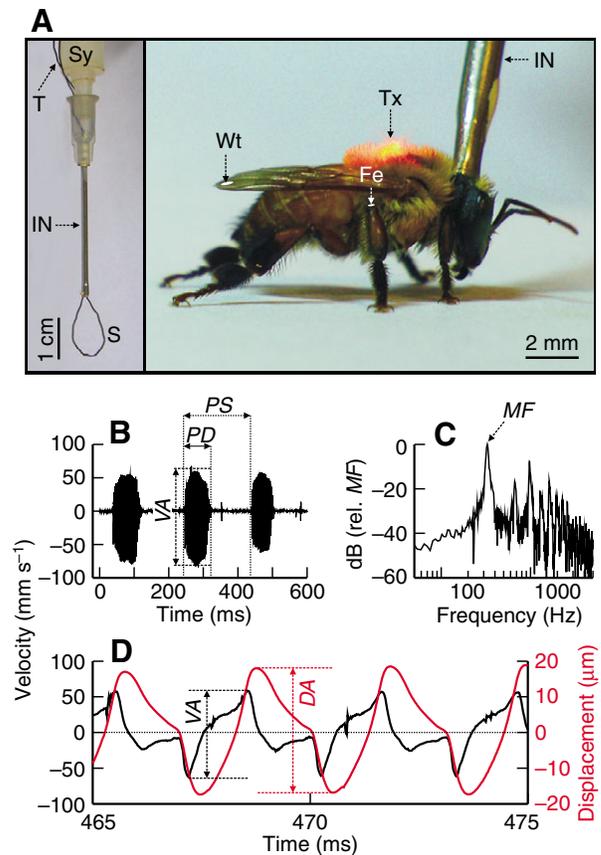


Fig. 1. Stingless bees of the genus *Melipona* generate pulsed annoyance buzzing when tethered by a sling around their neck. (A) Sling-tethering method, showing the sling (S) formed by a nylon thread (T) guided through an injection needle (IN). Sy, syringe for fixing the thread. Vibrations were measured on the thorax (Tx), the distal mesothoracic femur (Fe), and the wingtips (Wt) using a laser vibrometer. Photo showing a sling-tethered worker of *M. rufirentis*. (B) The following parameters of the pulsed vibrations were analysed: velocity amplitude (VA), duration of single pulses (PD), and pulse sequence (PS). In addition the frequency spectra (C) provided the main frequency component (MF). (D) The displacement component (red line; DA: displacement amplitude) of the vibrations was derived by integrating the vibration velocity recorded by the laser vibrometer (black line).

forager vibrations, FO), the following parameters of the oscillations were analysed (Fig. 1): the main frequency component (MF, defined as the frequency with highest energy content in the power spectrum), the velocity amplitude (VA), and the displacement amplitude (DA). The displacement of the oscillation was derived through an integration of the recorded vibration velocity using the software Microsoft® Office Excel 2003 (Microsoft Corporation, Bellevue, WA, USA). The resulting waveforms showing the displacement oscillations (Fig. 1D) were printed out on paper, and the displacement amplitudes (peak to peak, p-p) were measured to the nearest μm .

In all three experimental groups (flight, annoyance buzzing and forager vibrations), changes in amplitude as well as main frequency during the build-up and the decay phase of the vibratory pulses were studied as indicators of resonance. In the non-flight groups (annoyance buzzing and forager vibrations) we measured the velocity values peak to peak (p-p) of the first 15 cycles (build-up) and of the last 15 cycles (decay phase) of a vibrational pulse (Fig. 2), considering only pulses with at least 30 cycles. In case of the thorax

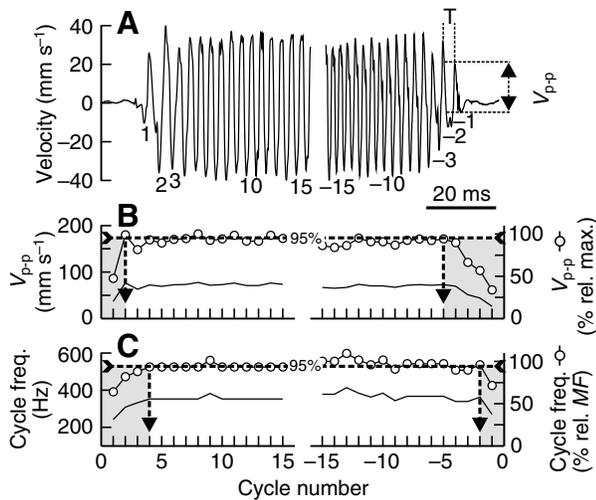


Fig. 2. Build-up and decay phase of vibratory pulses. The first 15 and the last 15 cycles of a vibratory pulse (A) were analysed regarding cycle amplitude (B) and cycle frequency (C). Line-plots, left y-axis: absolute values for velocity (V) and cycle frequency (Cycle freq.). Open circles, right y-axis: relative values (percentage of maximum of velocity V_{p-p} , and main frequency MF , respectively). Arrows indicate the cycle numbers where the 95% threshold of build-up and decay is attained. Size of gap: 0–600 cycles.

vibrations during stationary flight, we measured the first 30 cycles and the last 30 cycles of the thoracic oscillations. Cycle frequency was calculated in a simple way from the time between two subsequent peaks (Fig. 2A). Because of inter-individual differences, we normalized the measurements taking the maximum velocity amplitude and the main frequency component as 100%. Both the magnitude and cycle frequency of the vibrations fluctuated slightly after having reached the maximum values (Fig. 2). We therefore defined the build-up as the phase of continuous increase in magnitude and frequency from the onset of the pulse up to 95% of its maximum value. Correspondingly, the decay was defined as the continuous decrease from 95% to the cessation of the vibration (Fig. 2).

Wing removal and mass loading experiments

In sling-tethered individuals, the natural frequency of the oscillating system was modified either by first cutting one and then the second pair of wings close to the thorax (decreasing the inertial load on the system), or by attaching additional mass to the thorax. To increase the thoracic mass, a tiny piece of lead (average mass: 31.4 mg) was glued onto the thorax of a bee. Taking the thoracic mass of *M. seminigra* into account (33.6 mg, mean of 15 thoraces, all additional body parts removed), the oscillating thorax was almost doubled in mass by this experimental treatment. We measured MF , VA and DA (see above) of the sling-tethered individuals during stationary flight and during annoyance buzzing, both before the respective experimental treatment and after wing removal ($N=12$ bees) or after additional loading ($N=10$ bees). For each of the tested individuals, we recorded the thoracic vibrations during both stationary flight and annoyance buzzing.

Leg- and wing vibrations

Using two identical laser vibrometers, we simultaneously recorded the vibrations of the thorax and the distal part of the mesothoracic femur, or of the thorax and the wingtip. The femoral measurement point was chosen because it is easily accessible for the laser beam

during trophallaxis (Hrnčir et al., 2006b). We analysed the gain in vibration velocity between thorax and femur/wingtips, and the transmission of the temporal signal pattern (PD , pulse duration; PS , pulse sequence; MF , main frequency component; Fig. 1B) from the thorax to the femur/wingtips. The transmission was quantified as:

$$\text{Gain} = 20 \times \log(VA_{Fe/Wt}/VA_{Tx}) \text{ dB}, \quad (1)$$

where Gain is amplification; $VA_{Fe/Wt}$ is velocity amplitude (p-p) at femora, or at wingtips; VA_{Tx} is velocity amplitude (p-p) of thorax.

Statistics

For each tested individual the mean value of the respective vibration parameter was calculated from 15–30 vibratory pulses. The statistical tests were performed using these representative mean values. When the data showed normal distribution (Kolmogorov–Smirnov test, $P>0.05$) and equal variance (Levene median test, $P>0.05$), we applied parametric tests (Student t -test; paired t -test; one-way repeated-measures ANOVA followed by a Tukey test for pairwise comparison). If one of these requirements was not met, we applied non-parametric tests (Mann–Whitney U -test; Wilcoxon Signed Ranks test; Friedman repeated measures ANOVA on Ranks; Kruskal–Wallis test followed by a Dunn's test for pairwise comparison). The degree of correlation between signal parameters (PD , PS , MF) registered simultaneously on the thorax or the wings and legs, was tested using the Spearman rank correlation. The correlation coefficient (r_s) indicates the degree of correlation between the simultaneously measured vibrations ($r_s=1$, highly correlated; $r_s=0$, not correlated). Throughout the text, values are presented as median [1st quartile/3rd quartile]. N refers to the number of different individuals tested, and n to the number of pulses included in the calculations of the mean values. The level of significance of differences was taken as $P \leq 0.05$.

RESULTS

Thorax oscillations

The main frequency component (MF), velocity amplitude (VA) and displacement amplitude (DA) of the thoracic vibrations differed significantly between stationary flight, annoyance buzzing and forager vibration. MF was highest in forager vibrations, whereas VA and DA reached their highest values in annoyance buzzing (Table 1). In all three types of vibrations, the time course of the velocity varied considerably among individuals, between almost clean sinusoidal oscillations and complex oscillations with up to four high frequency components superimposed (Fig. 3).

The role of resonances in thoracic vibrations

To find out about the potential role of resonances involved in the generation of the thoracic vibrations, we analysed (1) the changes in both velocity amplitude and main frequency during the build-up and the decay of the vibrations, and (2) the changes in main frequency, velocity amplitude and displacement amplitude due to a decrease in inertial loading by wing removal or due to an increase in the mass of the oscillating system.

Build-up and decay of vibratory pulses

Flight

The velocity of the thoracic vibrations associated with wing movements had an average build-up of about 14 cycles and a decay phase of about 16 cycles (Fig. 4; Table 1). The main frequency, on the other hand, showed almost no changes at the beginning and at the end of the vibration. After the first cycle the average cycle frequency increased from 177 Hz [164/191] to its maximum value

Table 1. Thoracic vibrations of *Melipona seminigra* during stationary flight, annoyance buzzing and forager signalling

	Thorax vibrations			Kruskal–Wallis test		
	Stationary flight	Annoyance buzzing	Forager vibrations	<i>H</i> (d.f.=2)	<i>P</i>	<i>N</i> (FL/AB/FO)
Vibration parameters						
<i>MF</i> (Hz)	182 [175/186] ^a	305 [283/330] ^b	487 [454/499] ^c	76.4	<0.001	27/42/21
<i>VA</i> _{p-p} (mm s ⁻¹)	39 [35/47] ^a	209 [134/261] ^b	115 [69/167] ^c	62.5	<0.001	27/42/21
<i>DA</i> _{p-p} (μm)	20 [13/27] ^a	50 [32/75] ^b	18 [10/28] ^a	40.0	<0.001	27/42/21
Build-up and decay						
<i>VA</i> build-up	14 [14/17] ^a	5 [4/7] ^b	7 [4/9] ^b	26.5	<0.001	15/15/15
<i>VA</i> decay	16 [13/21] ^a	6 [4/7] ^b	8 [7/10] ^b	29.5	<0.001	15/15/15
<i>MF</i> build-up	1 [0/2]	2 [1/2]	2 [0/5]	3.8	0.15	15/15/15
<i>MF</i> decay	2 [0/5]	4 [3/4]	6 [3/6]	5.7	0.06	15/15/15

FL, stationary flight; AB, annoyance buzzing; FO, forager signalling; *MF*, main frequency; *VA*, velocity amplitude; *DA*, displacement amplitude. Values are medians [1st quartiles/3rd quartiles]; *N*, number of individuals. The build-up and decay phase of the oscillations (*VA*, *MF*) were determined through counting the number of cycles between the onset of the vibrations and 95% of their maximum values, and from 95% until their cessation. The same superscript letters (a–c) in each row indicate those groups that were statistically not separated (Dunn’s test for pairwise comparison: *P*>0.05).

of 181 Hz [175/186]. During the last two cycles, the cycle frequency dropped from 181 Hz [163/185] to 160 Hz [153/175].

Annoyance buzzing and forager vibrations

In the non-flight vibrations, the vibratory pulses showed short build-up and decay phases of both their velocity and main frequency (Fig. 4). In both annoyance buzzing and forager vibrations, the build-up phase took an average of six cycles for velocity and an average of two cycles for the main frequency (Fig. 4; Table 1). The decay in velocity took six oscillation cycles in the case of annoyance buzzing and eight cycles in the case of the forager vibrations (Fig. 4; Table 1). In annoyance buzzing, the cycle frequency dropped during the last four cycles from the main frequency of 298 Hz [240/324] to a final frequency of 215 Hz [202/223], and in the forager vibrations from the main frequency of 462 Hz [392/490] to a final value of 225 Hz [213/254] within the last six cycles (Fig. 4). There was no difference in final cycle frequency between the two types of non-flight vibrations (Dunn’s test: AB vs FO: *Q*=1.3; *P*>0.05).

Comparison flight with non-flight vibrations

The build-up and the decay in velocity were significantly longer in the vibrations during stationary flight than during the non-flight vibrations (Table 1). However, there was no significant difference in cycle number among the three different types of vibrations during the build-up and decay in main frequency (Table 1). The final cycle frequencies of annoyance buzzing (AB) and forager vibrations (FO) were significantly higher than that of the flight vibrations (FL) (Kruskal–Wallis test: *H*=23.4; *P*<0.001; d.f.=2; *N*_{AB}, *N*_{FO}, *N*_{FL}=15; Dunn’s test: AB vs FL: *Q*=3.4; *P*<0.05; FO vs FL: *Q*=4.7; *P*<0.05).

Wing removal and loading experiments

After wing removal, the main frequency component (*MF*), the velocity amplitude (*VA*) and the displacement amplitude (*DA*) of the thoracic vibrations during stationary flight changed significantly (*MF*, one-way repeated-measures ANOVA: *F*_{2,11}=135.1; *P*<0.001; Fig. 5A; *VA*, one-way repeated-measures ANOVA: *F*_{2,11}=3.75; *P*=0.04; Fig. 5B; *DA*, Friedman repeated-measures ANOVA on ranks: $\chi^2=8.17$; *P*=0.017; Fig. 5C). In the case of annoyance

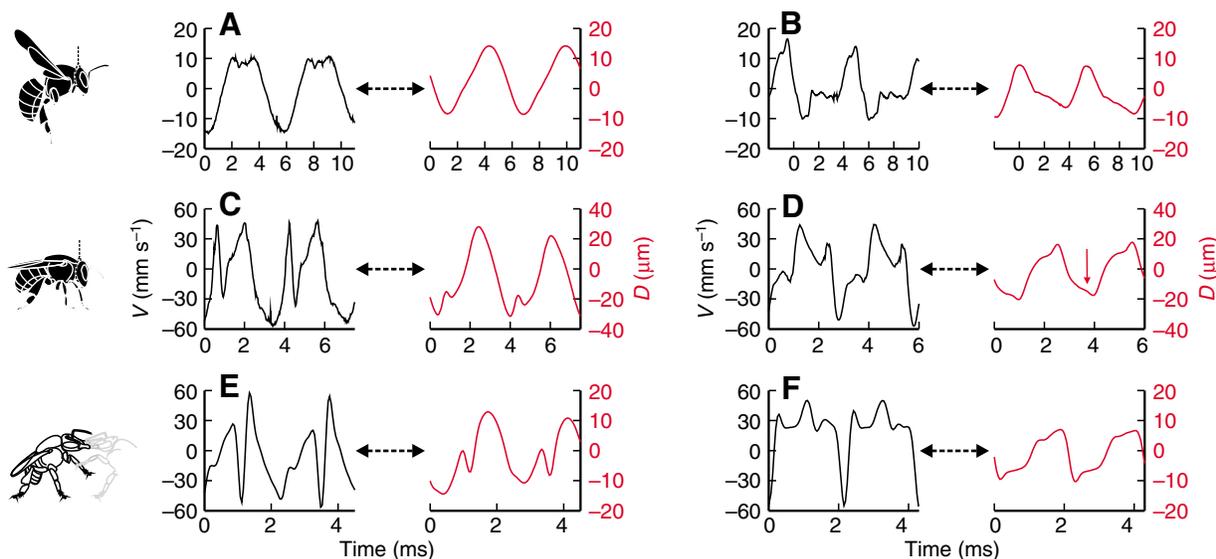


Fig. 3. Examples for thoracic vibrations during flight (A,B), annoyance buzzing (C,D) and forager vibrations (E,F). (A–F) Two cycles of the different types of thoracic vibrations showing the velocity (*V*, black lines) and the displacement (*D*, red lines). Arrows indicate high frequency components superimposed on the fundamental oscillation.

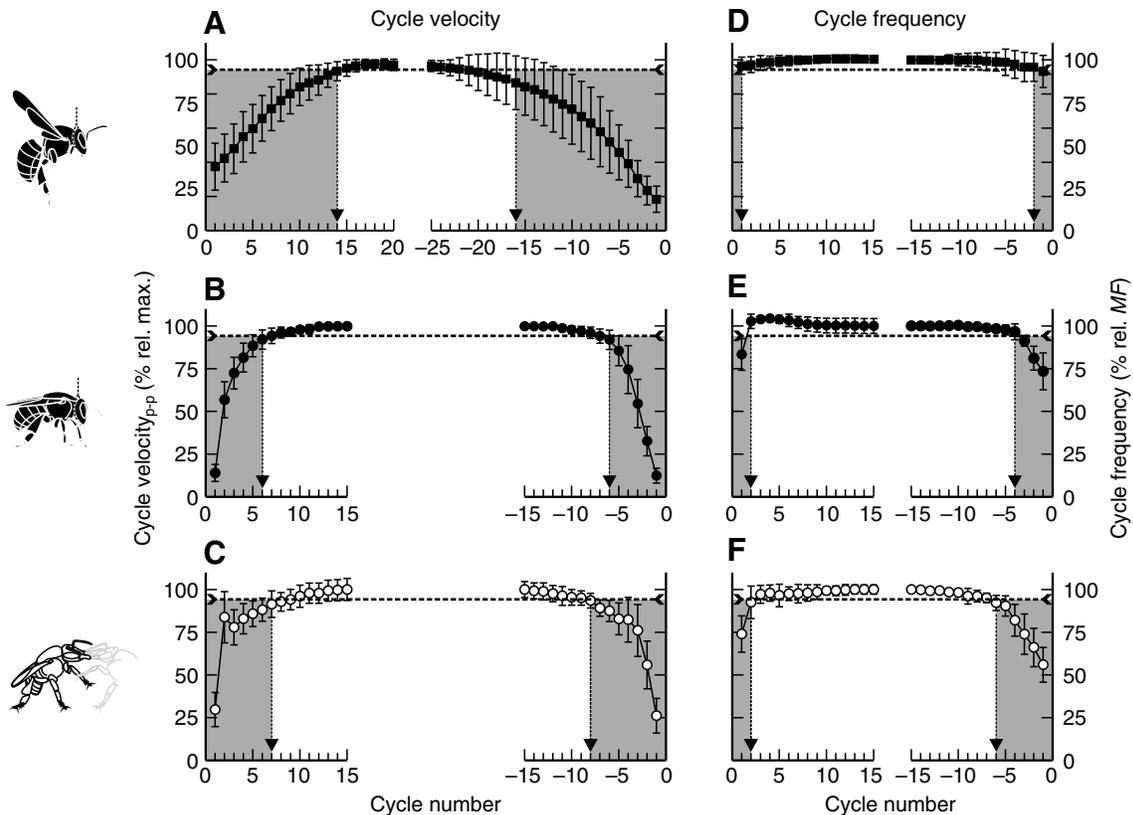


Fig. 4. Build-up and decay (shaded area) of cycle velocity (A–C) and cycle frequency (D–F) of thoracic oscillations during stationary flight (A,D; filled squares, $N=15$), during annoyance buzzing (B,E; filled circles, $N=15$), and during forager vibrations (C,F; open circles, $N=15$). Graphs show the means \pm s.d. of relative values (percent of the maximum velocity or of the main frequency, MF). Broken lines indicate 95% of maximum.

buzzing, however, neither of the investigated vibration parameters changed due to the removal of the wings (MF , one-way repeated-measures ANOVA: $F_{2,11}=1.75$; $P=0.20$; Fig. 5D; VA , one-way repeated-measures ANOVA: $F_{2,11}=2.18$; $P=0.14$; Fig. 5E; DA , Friedman repeated-measures ANOVA on ranks: $\chi^2=2.00$; $P=0.368$; Fig. 5F).

Doubling the thoracic mass resulted in a significant decrease in the main frequency component and of the velocity amplitude of the vibrations during stationary flight. The displacement amplitude of the oscillations in this type of thoracic vibrations, however, did not change after the experimental treatment (MF , paired t -test: $t_9=4.02$; $P=0.003$; Fig. 6A; VA , paired t -test: $t_9=2.87$; $P=0.019$; Fig. 6B; DA , paired t -test: $t_9=0.51$; $P=0.62$; Fig. 6C). In the case of annoyance buzzing, we observed a significant decrease in velocity amplitude and displacement amplitude after attaching a piece of lead to the thorax. The main frequency component of the vibrations, however, remained unaffected by this experimental treatment (MF , Wilcoxon signed ranks test: $W=-15$; $P=0.49$; d.f.=9; Fig. 6D; VA , Wilcoxon signed ranks test: $W=-55$; $P=0.002$; d.f.=9; Fig. 6E; DA , paired t -test: $t_9=3.45$; $P=0.007$; Fig. 6F).

Leg and wing oscillations

The distal part of the mesothoracic femur (Fe) oscillated in synchrony with the thorax (Tx) during both annoyance buzzing and forager vibrations. The velocity amplitudes of vibrations picked up at these two locations did not differ from each other in annoyance buzzing (AB) and forager vibration (FO) (AB , $VA_{Tx}=103 \text{ mm s}^{-1}$ [84/146], $VA_{Fe}=111 \text{ mm s}^{-1}$ [85/298]; paired t -test: $t_6=-1.838$,

$P=0.116$; FO , $VA_{Tx}=112 \text{ mm s}^{-1}$ [65/126], $VA_{Fe}=112 \text{ mm s}^{-1}$ [109/141]; paired t -test: $t_8=-2.02$, $P=0.074$; $\text{Gain}_{AB}=1.17 \text{ dB}$ [-0.50/4.83]; $N=7$; $\text{Gain}_{FO}=0.01 \text{ dB}$ [-0.34/4.70]; $N=9$; Mann–Whitney U -test: $U=17$, $P=0.832$; Fig. 7A). The main frequency component (MF), as well as the temporal pattern (PD , PS) of the vibrational pulses at the two measurement points, were highly correlated (Spearman rank correlation: $r_s=0.89\text{--}0.99$; Fig. 7A).

Similar to the femora, the wingtips (Wt) of both foragers and sling-tethered bees oscillated synchronously with the bees' thorax. In both vibration types, the velocity amplitude of the wingtip oscillations was significantly higher than that of the thorax vibrations (AB , $VA_{Tx}=96 \text{ mm s}^{-1}$ [74/182], $VA_{Wt}=688 \text{ mm s}^{-1}$ [496/865]; paired t -test: $t_{15}=-11.9$, $P<0.001$; FO , $VA_{Tx}=98 \text{ mm s}^{-1}$ [70/115], $VA_{Wt}=660 \text{ mm s}^{-1}$ [580/741]; paired t -test: $t_{15}=-24.0$, $P<0.001$; Fig. 7B). The velocity gain of the vibrations between the thorax (Tx) and the wingtips (Wt) was the same for annoyance buzzing and forager vibrations (AB , $\text{Gain}=16.2 \text{ dB}$ [14.4/17.9]; $N=16$; FO : $\text{Gain}=17.9 \text{ dB}$ [15.6/18.5]; $N=16$; t -test: $t_{30}=-1.128$, $P=0.181$; Fig. 7B). The main frequency component (MF), as well as the temporal pattern (PD , PS) of the thorax vibrations, was highly correlated with those of the wingtips in both types of vibration (Spearman rank correlation: $r_s=0.94\text{--}1.0$; Fig. 7B).

DISCUSSION

The thoracic oscillations that drive the indirect wing mechanism in bees have been the subject of detailed investigations (Snodgrass, 1956; Nachtigall et al., 1998; Nachtigall, 2003). Conclusions about

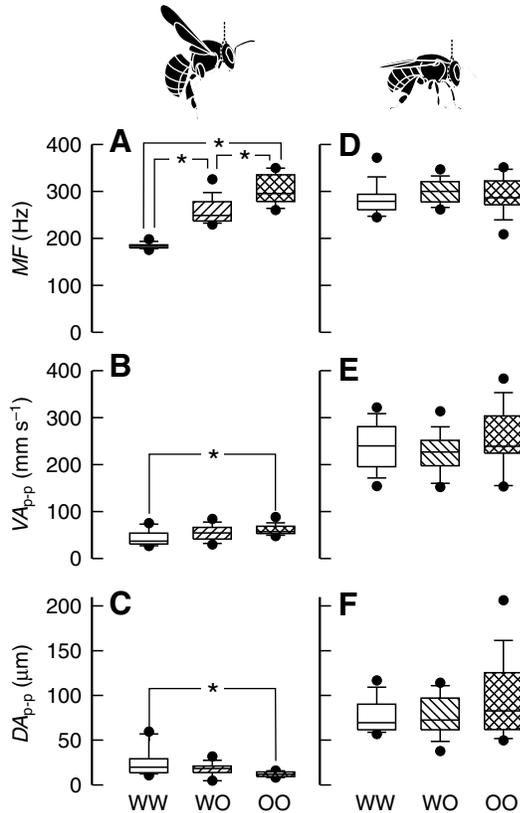


Fig. 5. Effect of wing removal on the thoracic vibrations during flight (A–C), and during annoyance buzzing (D–F) of 12 sling-tethered bees. (A,D) Main frequency; (B,E) velocity amplitude; (C,F) displacement amplitude; WW, intact bees; WO, OO, bees after removal of one or both wing-pairs. Asterisks indicate significant differences between the indicated treatments (Dunn’s test for pairwise comparison: $P < 0.05$). See text for statistics. Box plots indicate inter-quartile range (box), the median value (horizontal line), 95% range (whiskers) and outliers of all data.

non-flight thoracic vibrations, which eusocial bees generate in a variety of behavioural contexts, are primarily based on findings regarding the thoracic oscillations associated with the wing movements (Esch and Wilson, 1967; Seeley and Tautz, 2001; Nachtigall, 2003). Now a detailed comparison of the thoracic oscillations during flight with those during ‘sound’ production (non-flight vibrations) in the stingless bee *Melipona seminigra* provides evidence of mechanical differences between these two types of thoracic oscillations.

Different role of resonances in flight and non-flight vibrations

Different from the thoracic oscillations associated with wing movements during flight, non-flight vibrations in *M. seminigra* were not generated at the natural frequency of the oscillating system. Thus, resonances do not play an important role in the latter type of vibrations. Whereas all assumptions in favour of resonant vibrations tested in the present study were met in the thoracic oscillations associated with flight, none of the criteria applied to the non-flight vibrations.

When a simple oscillating system is excited by a periodic force at its natural frequency, the cycle frequency does not change during the build-up and the decay phase of the pulses. Driven by a periodic force at a frequency higher than its natural frequency, the system

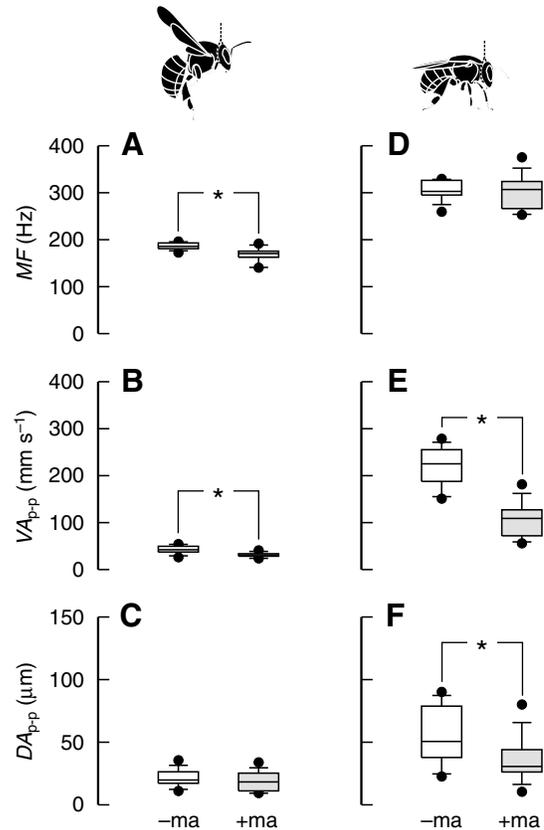


Fig. 6. Effect of increasing the mass of the oscillating system on the thoracic vibrations during flight (A–C), and during annoyance buzzing (D–F) of 15 sling-tethered bees. (A,D) Main frequency; (B,E) velocity amplitude; (C,F) displacement amplitude; –ma, bees before adding mass; +ma, bees after gluing a tiny piece of lead onto the thorax. The additional mass almost doubled that of the thorax. Asterisks indicate significant differences between the treatments (paired *t*-test: $P < 0.05$). See text for statistics. Box plots indicate inter-quartile range (box), the median value (horizontal line), 95% range (whiskers) and outliers of all data.

will vibrate at the excitation frequency as long as the force is applied. As soon as the force stops, the vibration will decay and drop to the resonant frequency of the system (Nocke, 1971; Bennet-Clark, 1999). Already the first oscillation cycle of the flight movements in *M. seminigra* was executed at the average flight frequency (Fig. 4D; Table 1). Admittedly, the ‘lift-off’ in our experiments, which occurred through the sudden deprivation of the contact between the bees’ tarsi and the substrate, is not the natural situation. During a natural take-off, as shown by detailed studies in flies (reviewed in Nachtigall, 2003), the stiffness and consequently the resonant properties of the thorax change within the last 50 ms prior to the onset of the indirect wing mechanism due to the increasing tension of the thoracic pleurosternal and tergotrochanter muscles. Yet, the indirect wing mechanism only starts to operate after a ‘jump-start’ of the animal, or in other words, only after the tarsi have lost the contact with the substrate. This jump to initiate flight is typical not only for flies but also for various other insects (Nachtigall, 2003), and probably also for bees. Therefore, the ‘forced take-off’ applied in our experiments can be considered a proper replicate of the natural situation.

Although in *M. seminigra* the oscillation frequency remained approximately constant during the entire stationary flight, it decreased from 181 Hz to 160 Hz (by about 12%) during the last

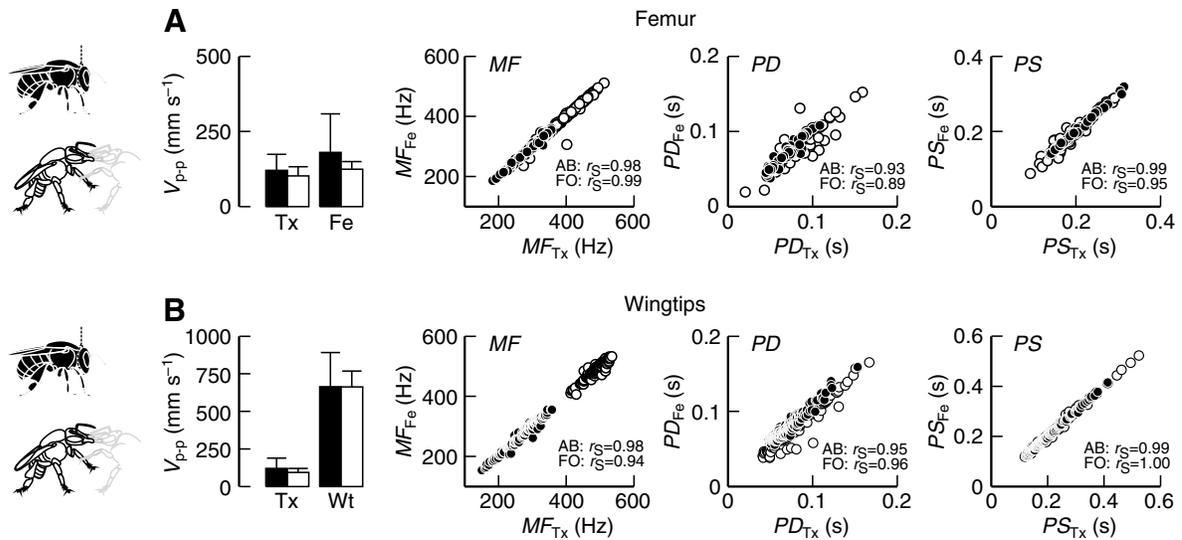


Fig. 7. Oscillations of legs and wings going along with thorax vibrations. Comparison of various parameters of vibrations registered simultaneously on the thorax Tx, and on the mesothoracic femur Fe (A), or on the wingtips Wt (B). Filled symbols: annoyance buzzing, AB (A: $N=7$, $n=140$; B: $N=16$, $n=320$); open symbols: forager vibrations, FO (A: $N=9$, $n=200$; B: $N=16$, $n=320$). r_s , correlation coefficient. See text for statistics. V , velocity; MF , main frequency; PD , pulse duration; PS , pulse sequence.

two cycles of the vibrations (Fig. 3B). Similar to our findings, the cycle frequency of the thoracic vibrations during flight of *Calliphora* and *Eristalis* decreased by about 13% during the last 2–4 cycles [measured from fig. 3 in Roeder (Roeder, 1951)]. Therefore, either flight vibrations are driven at a frequency slightly above the natural frequency of the oscillating system, or changes in the stiffness of the system occur during these last few cycles due to changes in muscle tension (Nachtigall and Wilson, 1967).

Both cutting the wings and increasing the mass of the oscillating system resulted in changes of the main frequency component of flight vibrations (Figs 5, 6). From this we conclude that resonances are indeed important for the generation of this type of thoracic oscillation. In contrast to the flight vibrations, however, thorax oscillations underlying annoyance buzzing remained largely uninfluenced by our experimental treatments. Doubling the mass of the thorax experimentally did not reduce the main frequency component of annoyance buzzing (Fig. 6D), only its displacement amplitude (Fig. 6F). An explanation for this could be that the driving force remained more or less constant during annoyance buzzing, and did not compensate for the additional mass on the oscillating system (simplified as: $F=ma \approx \text{constant}$, where F =force, m =mass and a =acceleration, which is proportional to displacement amplitude \times main frequency).

As with bumble bee queens (Schneider, 1975), ablation of the wings did not influence the main frequency component of annoyance buzzing in *M. seminigra*. Although the wings are folded above the abdomen during this type of thoracic vibration and thus decoupled from the indirect wing mechanism [(Snodgrass, 1956); for a comparative study of the thoracic muscles and their function in bees see Wille (Wille, 1956)], they still are attached to the thoracic capsule. This inevitably leads to oscillations of the wings along with the thorax (Fig. 7) which, consequently, influences the natural frequency of the oscillating system through the damping caused by the mass of air moved by the wings. Hence, a reduction of the wing area not only decreases the inertial load on the thorax during flight but also, to a minor extent, during annoyance buzzing. Even so,

wing removal had no influence on the main frequency component of the annoyance buzzing (Fig. 5D).

Our results suggest that in *M. seminigra* the natural frequency of the oscillating system during non-flight vibrations is higher than that during flight vibrations, and that non-flight vibrations are driven at a frequency higher than the natural frequency of the oscillating system. The final cycle frequency was very similar in annoyance buzzing (215 Hz) and in the forager vibrations (225 Hz). This indicates that the natural frequency of the oscillating thorax is similar during these two types of non-flight vibrations despite differences observed between their main frequency components (Table 1). The elevated natural frequency of the thorax during non-flight vibrations, in comparison to that during flight vibrations, supposedly is a result of an increased tension of the thoracic muscles (Nachtigall and Wilson, 1967; Seeley and Tautz, 2001).

In insects with an indirect flight mechanism, the wingbeat frequency increases with the frequency of motor action potentials (Roeder, 1951; Esch and Bastian, 1968; Bastian and Esch, 1970). The damping of the vibrating system can be deduced from the decay in amplitude at the end of the thoracic vibrations. In flight vibrations of *M. seminigra*, this decay took an average of 16 cycles (Fig. 3A; Table 1) or about 0.09 s, which corresponds to a motor input every 10–20 wingbeats (action potential frequency: 8–16 Hz), as found in honey bees and flies (Roeder, 1951; Esch, 1964). In the non-flight vibrations, the decay took an average of six cycles (~ 0.02 s) in the case of annoyance buzzing and eight cycles (~ 0.02 s) in that of forager vibrations. To offset this damping in the non-flight vibrations, the neural excitation would have to occur at a rate of about 50 Hz. This complies with electromyogram recordings during non-flight buzzing ('sonication') in bees of the genera *Bombus* and *Xylocopa* (King et al., 1996). Here, the authors measured a maximum excitation frequency of the indirect flight muscles of approximately 60 Hz. Therefore, the higher main frequency component found in non-flight vibrations compared to the flight vibrations can be explained by the higher action potential rate rather than by changes in the natural frequency of the oscillating thorax.

Analysis of signal generation and signal transmission under controlled conditions

In stingless bees of the genus *Melipona*, thoracic vibrations related to recruitment behaviour are supposedly transmitted to hive bees through substrate vibrations (Lindauer and Kerr, 1958), airborne signals (Esch, 1967), or direct contact during trophallaxis (Hrnrcir et al., 2006b). With the exception of the third pathway, neither the transformation of the thoracic vibrations into relevant signals (substrate-borne or airborne) nor their actual transmission to potential recruits has been studied in detail within their natural context. This is largely due to the fact that the trophallactic food transfer involves movements by the foragers themselves, and also by the hive bees in their proximity. Therefore, the accurate positioning of measuring devices close to the vibrating forager is an almost impossible task. The limitations for exact measurements around foragers within the nest call for alternative methods that allow a detailed study of the signal generation and signal transmission in a controlled laboratory situation (Michelsen, 2003).

Annoyance buzzing and forager vibrations are very similar in terms of the mechanism of their generation, and they result in similar vibrations of the legs and wingtips (Fig. 7), which transform thoracic vibrations into substrate vibrations and airborne sounds (Michelsen et al., 1987; Tautz et al., 2001; Michelsen, 2003; Hrnrcir et al., 2008). Our data suggest that thoracic vibrations are transformed into mechanical signals in both annoyance buzzing and forager vibrations with similar efficiency. Annoyance buzzing of sling-tethered bees, therefore, permits study mechanisms of signal generation and transmission under controlled conditions in stingless bees, as recently demonstrated for the transformation of thoracic vibrations into air particle movements (Hrnrcir et al., 2008).

LIST OF ABBREVIATIONS

AB	annoyance buzzing
DA	displacement amplitude
Fe	femur
FL	stationary flight
FO	forager vibrations
MF	main frequency component
p-p	peak to peak
PD	pulse duration
PS	pulse sequence
Tx	thorax
V	velocity
VA	velocity amplitude
Wt	wingtip

We would like to thank Sidnei Mateus for his patience and omnipresent help. This study complies with the current laws of Brazil, where the experiments were carried out. It was supported by FAPESP grants 06/50809-7 and 06/53839-4 to M.H. and by FWF grant P17530 to F.G.B.

REFERENCES

- Bastian, J. and Esch, H.** (1970). The nervous control of the indirect flight muscles of the honey bee. *Z. Vergl. Physiol.* **67**, 307-324.
- Bennet-Clark, H. C.** (1999). Resonators in insect sound production: how insects produce loud pure-tone songs. *J. Exp. Biol.* **202**, 3347-3357.
- Buchmann, S. L.** (1983). Buzz pollination in angiosperms. In *Handbook of Experimental Pollination Biology* (ed. C. E. Jones and R. J. Little), pp. 73-113. New York, Cincinnati, Toronto, London, Melbourne: Van Nostrand Reinhold.
- Chadwick, L. E. and Williams, C. M.** (1949). The effects of atmospheric pressure and composition on the flight of *Drosophila*. *Biol. Bull.* **97**, 115-137.
- Darveau, C.-A., Peter, W., Hochachka, P. W., Welch, Jr, K. C., Roubik, D. W. and Suarez, R. K.** (2005). Allometric scaling of flight energetics in Panamanian orchid bees: a comparative phylogenetic approach. *J. Exp. Biol.* **208**, 3581-3891.
- Esch, H.** (1961). Über die Schallerzeugung beim Werbetanz der Honigbiene. *Z. Vergl. Physiol.* **45**, 1-11.
- Esch, H.** (1964). Über den Zusammenhang zwischen Temperatur, Aktionspotentialen und Thoraxbewegungen bei der Honigbiene (*Apis mellifica* L.). *Z. Vergl. Physiol.* **48**, 547-551.
- Esch, H.** (1967). Die Bedeutung der Lauterzeugung für die Verständigung der stachellosen Bienen. *Z. Vergl. Physiol.* **56**, 199-220.
- Esch, H. and Bastian, J.** (1968). Mechanical and electrical activity in the indirect flight muscles of the honey bee. *Z. Vergl. Physiol.* **58**, 429-440.
- Esch, H. and Wilson, D.** (1967). The sounds produced by flies and bees. *Z. Vergl. Physiol.* **54**, 256-267.
- Hrnrcir, M., Jarau, S., Zucchi, R. and Barth, F. G.** (2004). Thorax vibrations in stingless bees (*Melipona seminigra*). II. Dependence on sugar concentration. *J. Comp. Physiol. A* **190**, 549-560.
- Hrnrcir, M., Barth, F. G. and Tautz, J.** (2006a). Vibratory and airborne sound-signals in bee communication. In *Insect Sounds and Communication: Physiology, Behaviour, Ecology, and Evolution* (ed. S. Drosopoulos and M. Claridge), pp. 421-436. Boca Raton, London, New York: CRC Press, Taylor & Francis Group.
- Hrnrcir, M., Schmidt, V. M., Schorkopf, D. L. P., Jarau, S., Zucchi, R. and Barth, F. G.** (2006b). Vibrating the food receivers: a direct way of signal transmission in stingless bees (*Melipona seminigra*). *J. Comp. Physiol. A* **192**, 879-887.
- Hrnrcir, M., Schorkopf, D. L. P., Schmidt, V. M., Zucchi, R. and Barth, F. G.** (2008). The sound field generated by tethered stingless bees (*Melipona scutellaris*): inferences on its potential as a recruitment mechanism inside the hive. *J. Exp. Biol.* **211**, 686-698.
- King, M. J.** (1993). Buzz foraging mechanism of bumble bees. *J. Apic. Res.* **32**, 41-49.
- King, M. J., Buchmann, S. L. and Spangler, H.** (1996). Activity of asynchronous flight muscle from two bee families during sonication (buzzing). *J. Exp. Biol.* **199**, 2317-2321.
- Lindauer, M. and Kerr, W. E.** (1958). Die gegenseitige Verständigung bei den stachellosen Bienen. *Z. Vergl. Physiol.* **41**, 405-434.
- Michelsen, A.** (2003). Signals and flexibility in the dance communication of honeybees. *J. Comp. Physiol. A* **189**, 165-174.
- Michelsen, A., Kirchner, W. H. and Lindauer, M.** (1986). Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **18**, 207-212.
- Michelsen, A., Towne, W. F., Kirchner, W. H. and Kryger, P.** (1987). The acoustic near field of a dancing honeybee. *J. Comp. Physiol. A* **161**, 633-643.
- Michener, C. D.** (1962). An interesting method of pollen collecting by bees from flowers with tubular anthers. *Rev. Biol. Trop.* **10**, 167-175.
- Nachtigall, W.** (2003). *Insektenflug*. Berlin, Heidelberg, New York: Springer Verlag.
- Nachtigall, W. and Wilson, D. M.** (1967). Neuro-muscular control of dipteran flight. *J. Exp. Biol.* **47**, 77-97.
- Nachtigall, W., Wisser, A. and Eisinger, D.** (1998). Flight of the honey bee. VIII. Functional elements and mechanics of the 'flight motor' and the wing joint-one of the most complicated gear-mechanisms in the animal kingdom. *J. Comp. Physiol. B* **168**, 323-344.
- Nocke, H.** (1971). Biophysik der Schallerzeugung durch die Vorderflügel der Grillen. *Z. Vergl. Physiol.* **74**, 272-314.
- Roeder, K. D.** (1951). Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull.* **100**, 95-106.
- Schneider, P.** (1972). Akustische Signale bei Hummeln. *Naturwissenschaften* **59**, 168-169.
- Schneider, P.** (1975). Versuche zur Erzeugung des Verteidigungstones bei Hummeln. *Zool. Jb. Physiol.* **79**, 111-127.
- Seeley, T. D. and Tautz, J.** (2001). Worker piping in honey bee swarms and its role in preparing for liftoff. *J. Comp. Physiol. A* **187**, 667-676.
- Sen Sarma, M., Fuchs, S., Werber, C. and Tautz, J.** (2002). Worker piping triggers hissing for coordinated colony defence in the dwarf honeybee *Apis florea*. *Zoology* **105**, 215-223.
- Snodgrass, R. E.** (1956). *Anatomy of the Honey Bee*. Ithaca, NY: Comstock Publishing Associates.
- Soltavala, O.** (1952). The essential factor regulating the wing-stroke frequency of insects in wing mutilation and loading experiments and in experiments at subatmospheric pressure. *Ann. Zool. Soc. Vanamo* **15**, 1-67.
- Tautz, J., Casas, J. and Sandeman, D. C.** (2001). Phase reversal of vibratory signals in honeycomb may assist dancing honeybees to attract their audience. *J. Exp. Biol.* **204**, 3737-3746.
- Wenner, A. M.** (1962). Sound production during the waggle dance of the honeybee. *Anim. Behav.* **10**, 79-95.
- Wille, A.** (1956). Comparative studies of the thoracic musculature of bees. *Univ. Kansas Sci. Bull.* **38**, 439-499.
- Wille, A.** (1963). Behavioral adaptations of bees for pollen collecting from *Cassia* flowers. *Rev. Biol. Trop.* **11**, 205-210.