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ORIGINAL PAPER

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Thorax vibrations of a stingless bee (*Melipona seminigra*). II. Dependence on sugar concentration

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Abstract Using a laser vibrometer we studied the influence of the food's sugar concentration on different parameters of the thorax vibrations produced by foragers of *Melipona seminigra* during trophallaxis in the nest. The concentrations tested (20–70% sugar w/w) were within the biologically relevant range. They substantially influenced different parameters of the thorax vibrations. An increase of energy gains at the food source due to an increased sugar concentration was followed by an increase of both the pulse duration and the duty cycle and by a decrease of the pause duration between two subsequent pulses. These findings further support the hypothesis that the temporal pattern of the thorax vibrations reflects the energy budget of a foraging trip rather than food source distance. Likewise, the steep increase of pulse duration variability with sugar concentration is hard to reconcile with the assumption that pulse duration conveys reliable information about food source distance when bees collect at high-quality food sources.

Keywords Communication · *Melipona* · Stingless bee · Sugar concentration · Vibration

Introduction

Thorax vibrations in *Melipona*

When returning from a successful foraging trip stingless bees of the genus *Melipona* emit pulsed thorax vibrations inside the nest (Lindauer and Kerr 1958; Esch et al. 1965; Esch 1967; Nieh and Roubik 1998; Hrncir et al. 2000; Aguilar and Briceño 2002; Nieh et al. 2003). Esch et al. (1965) and Esch (1967) were the first to quantify these vibrations in *M. quadrifasciata* and *M. seminigra*. According to these authors the duration of the vibration pulses correlates positively with the distance of a food source from the nest. Later these results could not be confirmed for either *M. quadrifasciata* (Hrncir et al. 2000; H. Velthuis, personal communication) or *M. seminigra* (Samwald 2000; Strauß 2002). For *M. panamica* Nieh and Roubik (1998) proposed the communication of food source height by variation of the longest pulse's duration during trophallactic contacts whereas after trophallaxis the same parameter correlated with the food source's distance. However, pulse duration, the most obvious aspect of the mechanical signals of *Melipona*, varied considerably in all cases studied (Table 1) which renders its capacity to carry reliable information doubtful. Evidence that bees of the genus *Melipona* inform recruits about the exact location of a food source is weak (Dyer 2002). Experiments have only demonstrated that the foragers' recruitment behavior leads to an increasing number of bees leaving the nest (Lindauer and Kerr 1958; Esch 1967; Pereboom and Sommeijer 1993) and/or arriving at a food source (Lindauer and Kerr 1958; Nieh and Roubik 1995; Jarau et al. 2000, 2003; Nieh et al. 2003). Recruited bees preferred a feeder previously visited by foragers over an unvisited control feeder at a different position (Nieh and Roubik 1995; Jarau et al. 2000). However, odors later shown to be left at the food source as foot prints by the foragers (Hrncir et al. 2004a; Jarau et al. 2004) and likely to bias the searching recruits towards the food source

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Table 1 Coefficient of variation (CV = 1SD×100/mean) of pulse duration of thorax vibrations in *Melipona* (*n* = number of pulses)

Species	CV (%)	*n*	Reference
M. seminigra	4–40	154–1,714	Esch 1967a
M. quadrifasciata	23–42	257–2,223	Esch 1967a
M. panamica	117–238	1,146–2,379	Nieh and Roubik 1998
M. scutellaris	80–94	39–209	Hrncir et al. 2000
M. quadrifasciata	104–227	118–537	Hrncir et al. 2000
M. costaricensis	55–181	134–1,484	Aguilar and Briceño 2002

were not excluded in these studies (Dyer 2002). It remains unclear whether and how spatial information is transmitted by *Melipona* to nestmates, and what role the vibrations actually play in the communication and recruitment process.

Thorax vibrations in honeybees

Similar to stingless bees, successful honeybee foragers (*Apis mellifera*) produce pulsed thorax vibrations upon their return to the hive. These signals are limited to the wagging phase of the dances (Esch 1961; Wenner 1962; von Frisch 1965). The duration of the wagging as well as the duration of the vibration signal and the number of vibration pulses are all correlated with the distance of a food source (Wenner 1962; von Frisch 1965). However, although the total duration of signal production is clearly correlated to the distance of a food source, the temporal pattern of the vibrations (duration of single pulses and interpulses and pulse rate) is not (Esch 1961). Instead, it depends on the sugar concentration of the collected food (Esch 1963; Waddington and Kirchner 1992). The duration of the pulses, pulse rate and the pulses' main frequency increase significantly with sugar concentration up to 60% w/w (Waddington and Kirchner 1992). The duration of the dance and the number of sound pulses, both parameters correlating with food source distance (Wenner 1962), remain uninfluenced by sugar concentration (Waddington and Kirchner 1992).

The information carried

Both these findings in honeybees and our previous failure to demonstrate spatial information within the vibrations of *Melipona* and thus symbolic "language" (Hrncir et al. 2000, 2004b; Samwald 2000; Strauß 2002) prompted a new approach of studying the relevance of the vibrations in stingless bees (Hrncir et al. 2002). Recently published studies (Aguilar and Briceño 2002; Nieh et al. 2003) point to an influence of food quality on the signal structure (mainly on the duration of pulses and interpulses) in various *Melipona* species. However, these studies lacked control groups and did not take into account factors significantly influencing recruitment signals in honeybees (such as time of the day, extranidal climatic conditions or intranidal food supply and climatic conditions; Lindauer 1948). It therefore remains uncertain whether food quality really was the principal factor accounting for the differences in the signal patterns found. In a recent study (Hrncir et al. 2004b) we proposed that in *M. seminigra* thorax vibrations reflect the energy budget (gains and costs) of a foraging trip. Bees that had collected inside flight tunnels substantially reduced their flight velocity. The resulting higher energy consumption (Nachtigall et al. 1995) was suggested to account for the decrease of both pulse duration and duty cycle and the increase of the pauses between two subsequent pulses (Hrncir et al. 2004b). Raising the energetic gains at a food source while keeping the energy consumption constant should have the opposite effect on the temporal pattern of the thorax vibrations if it really reflects the energy budget of a foraging trip.

The present study was undertaken to answer three questions. 1. Is the temporal pattern of the thorax vibrations of *M. seminigra* indeed related to the energetic gains (sugar concentration) at the food source? 2. Does the biologically relevant range of sugar concentration (*Melipona*: 20–70% sugar w/w; Roubik and Buchmann 1984; Roubik et al. 1995; Biesmeijer et al. 1999) have a comparable effect on the signals in different individuals? 3. Could pulse duration be an adequate parameter to carry reliable information even when taking signal variability into account?

Materials and methods

Animals

All experiments were carried out on the Ribeirão Preto campus of the University of São Paulo, Brazil. We used two colonies of a hitherto undescribed subspecies of *Melipona seminigra* Friese 1903 (Camargo, unpublished work). Both colonies had about 500 to 700 individuals and were kept in wooden boxes inside the laboratory building. A glass covered plexiglass box (observation box: 10×5×4 cm) was placed between the nest box and the entrance/exit tube through the laboratory wall. In most cases the returning foragers distributed the collected food among nestmates inside this box. When recording thorax vibrations the glass cover was removed, which did not affect the bees' behavior.

Laser vibrometry and signal analysis

Stingless bees produce thorax vibrations which simultaneously result in both airborne sound and substrate vibrations (Lindauer and Kerr 1958; Esch and Wilson 1967). The temporal patterns of these three forms of the same signal are very similar (Hrncir et al. 2000, 2004b; Hrncir 2003). In the following we refer to thorax vibrations only, although in different previous studies these were not measured as such but as airborne sound (Esch 1967; Nieh and Roubik 1998; Aguilar and Briceño 2002; Nieh et al. 2003), substrate vibrations and airborne sound (Hrncir et al. 2000) or thorax vibrations (see Hrncir et al. 2004b).

The bees' thorax vibrations during trophallaxis were recorded as velocities using a portable laser vibrometer (Polytec; PDV 100) (for details see Hrncir et al. 2004b). The vibrometer's output signal was fed into a notebook (Pentium III, 800 MHz) using a 32-bit sound card and Soundforge 4.0 software (Sonic Foundry). The following parameters of the pulsed thorax vibrations were analyzed (software: Spectra Pro 3.32; Sound Technology): pulse duration (PD); dominant frequency (MF) contained in pulses (peak frequency in the spectrogram); velocity amplitude of pulses, peak-to-peak (VA); interpulse duration, pause between two subsequent pulses (IPD). From these values additional parameters were calculated pulse sequence, PS (time from the beginning of one pulse to the beginning of the following pulse, PD + following IPD); and DC, duty cycle (PD/PS) (Fig. 1).

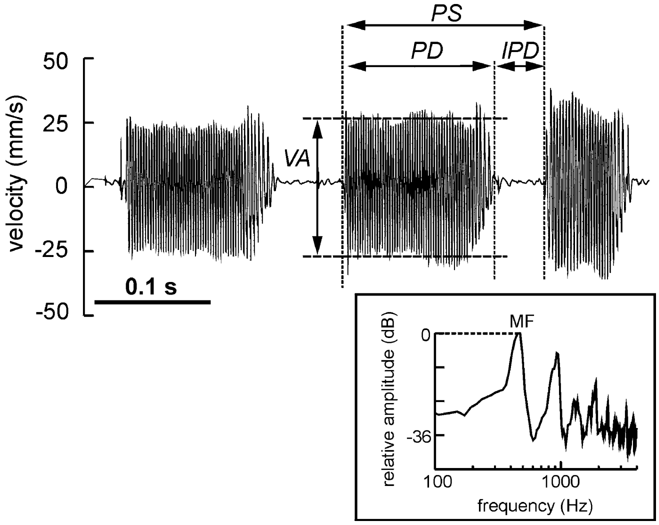


Fig. 1 Signal analysis. Typical recording showing the signal parameters examined: pulse duration (*PD*), velocity amplitude (*VA*), interpulse duration (*IPD*). From the measurements pulse sequence (*PS*) and duty cycle ($DC = PD/PS^{-1}$) were calculated. *Inset*: frequency spectrum of a pulse (FFT, 1024 pts) with main frequency (MF, 0 dB) and its harmonics

Food intake at different sugar concentrations

When analyzing the food intake behavior of the bees at different sugar concentrations a feeder was placed at a distance of 20 m from the nest entrance. On a plexiglass disc the bees were offered 70 µl of a solution containing 20, 40, 50, 60, or 70% (w/w) unrefined sugar (São João, Açucar cristal superior: 99.8% sucrose; 0.1% glucose/fructose; 0.1% mineral salts) in water. To reduce evaporation the solutions were offered only when a bee actually arrived at the feeding place. The time taken by a bee to imbibe the solution was measured to the nearest 0.1 s (digital stop watch). The left over sugar water was taken up with microcapillaries (Sigma Microcaps, 50 µl), and instantly measured to the nearest microliter. From the values for *imbibing time* and *crop load* (crop load = 70 µl$-x$ $\mu l_{\text{remaining sugar water}}$) we calculated the *solution intake rate* ($\mu l_{\text{solution}}\ s^{-1}$), the *total sugar intake* (mg_{sugar}), and the *sugar intake rate* ($mg_{\text{sugar}}\ s^{-1}$) (Weast et al. 1989). We observed ten different bees on different days in May and June 2002 (one bee per day), offering each of them all of the above mentioned sugar concentrations up to three times in different sequences.

Thorax vibrations at different sugar concentrations

The pulse duration of thorax vibrations in *M. seminigra*, the previously postulated symbolic indicator of food source distances, had been demonstrated to change substantially only at distances close to the nest (increase of pulse duration by an average of 370% between 0 and 20 m and by 9% between 20 and 100 m; Esch 1967). Therefore, bees were trained to different feeding sites at a distance of 20 m from the nest entrance in six independent experiments (Table 2), using a 20% w/w sugar solution. During the experiments the concentration of the sugar solution was changed every 30 min in different

Table 2 Details on the experiments

Experiment	Colony	Date	Feeder (*N*)	Control feeder (*N*)	Sequence of concentrations offered
A1	X	01.04.02	SE (4)	E (4)	40%–60%–40%–20%
A2	Y	13.05.02	SE (4)	E (4)	40%–60%–40%–20%
A3	X	15.05.02	NW (4)	E (4)	40%–20%–60%–40%
B1	X	03.05.02	E (3)	-	40%–20%–60%–70%–60%
B2	Y	18.05.02	E (3)	-	20%–40%–60%–70%–60%
B3	X	20.06.02	SE (4)	-	40%–50%–60%–50%

Colony used, date of experiment, position of feeder, and control feeder at a distance of 20 m from the nest entrance, number of bees (*N*), and sequence of the sugar solution concentrations offered At the control feeder the sugar concentration was constant at 40% w/w during the entire experiment. The concentrations of the sugar solutions are given in percentage weight on weight sequences (Table 2). The concentrations used were 20, 40, 50, 60, and 70% w/w (measured to the nearest 0.5%; refractometer: HR 25/800; Krüss Optronic). The experiments were always performed in the afternoon (training started at 1:00 p.m.) to exclude the influence of differences in the time of day on the bees' behavior (Lindauer 1948). The temperature at the food source(s) was recorded every 30 min (digital thermometer: Minipa MT-242). Foraging bees were used for one experiment only in order to avoid pseudoreplications.

Are changes in signal pattern causally related to changes in sugar concentration?

In experiments A1–A3 (Table 2) bees collected at the experimental feeder. Simultaneously, an equally sized control group collected at a different food source offering 40% w/w sugar water during the entire experiment. We compared the thorax vibrations produced by the experimental group foragers with those of the control group foragers. Changes in signal structure due to the change of the sugar concentration were expected to occur in the experimental group only. Changes in signal structure due to climatic conditions, however, should occur in both the experimental and the control group.

Dependence of signal pattern on sugar concentration

In order to study in more detail the effect of sugar concentration within the biologically relevant range (20–70% w/w) on the signal structure of different individuals (experiments B1–B3, Table 2) we compared the thorax vibrations of individually marked bees collecting at an experimental feeder. If the signal pattern depends on sugar concentration, the different individuals should modify their signal pattern in a similar way.

Does sugar concentration influence signal variability?

From the thorax vibrations recorded during experiments B1–B3 we calculated the variation of the signal parameters at each sugar concentration (coefficient of variation, CV = 1SD×100/mean).

Statistical analysis and considerations

The following statistical tests were used (software: SigmaStat for Windows 3.0, SPSS; STATGRAPHICS Plus 5.1, Statistical Graphics). Differences among sample means or medians were tested using one-way ANOVA (normally distributed data, food intake behavior) or Kruskal-Wallis test (not normally distributed data, signal parameters). In the case of significant differences among the samples (one-way ANOVA, Kruskal-Wallis test; $P < 0.05$) post-hoc tests (Tukey-, or Dunn's pairwise comparisons) specified which samples differ. Regression analysis was used to calculate the dependence of the signal parameters (dependent variable) from the sugar concentration or the temperature at the food source (independent variable). The degree of dependence is indicated by the coefficient R^2_{adj} (R^2 adjusted to the sample size) and is given in percent. Spearman rank order correlation (nonparametric correlation analysis for not normally distributed data) was used to measure the strength of association between sample means and the respective standard deviation. The coefficient of correlation (rS) indicates the degree of association ($rS = 0$, no association; $rS = 1$, high association). Levene's test was performed to test whether the variability of the signal parameters is equal at different sugar concentrations (homogeneity of variance). The level taken for the significance of difference between data was $P < 0.05$. N refers to the number of bees, n to the number of analyzed pulses.

Vibratory signals are highly variable in *Melipona*. The pulse duration measured in bees collecting at the same food source varied around the mean value by up to more than 200% in different studies (Table 1). Data were pooled and large sample sizes were used (Table 1) in a way which renders the unambiguous interpretation of these results difficult. First, mean values obtained from pooled data, or even more so theories based on the variation of the data and on outlier values, might be strongly influenced by the atypical behavior of one or few individuals: in *M. panamica* foragers collecting at ground level produced longer pulses than foragers collecting at the canopy level in 6 out of 102 performances (Nieh and Roubik 1998, data from 1995). However, it is not clarified whether these 6 performances, that form the basis of a height communication theory in *Melipona*, are due to a single forager only. Our approach therefore was to investigate whether there is a general trend typical of all (or most) individual foragers. Second, statistical tests detect differences between sets of data more easily when operating with great sample sizes (Sokal and Rohlf 1995). However, the biological relevance may be questionable in such cases (Fig. 2a, b). When comparing the thorax vibrations on the individual level (experiments B1–B3) there are at least three possible sources of variation: (1) variable signal production by the same individual under identical experimental conditions; (2) differences between different individuals under the same conditions; and (3) differences between individuals due to different conditions. We consider (1) and (2) as sources of "noise" in the communication system. If in communication systems the signal-to-noise ratio is 0 then the probability of a correct detection of the signal is 50% (i.e., by chance). An increasing separation of the signal from the noise increases the probability of correct "understanding" of the information encoded in the signal (Wiley and Richards 1982; Gerhardt and Huber 2002; Greenfield 2002; Heldmaier and Neuweiler 2003).

The values for the various signal parameters were compared using a Kruskal-Wallis test. This test analyses whether the variation *within* samples (signals of one individual under the same condition) is larger or smaller

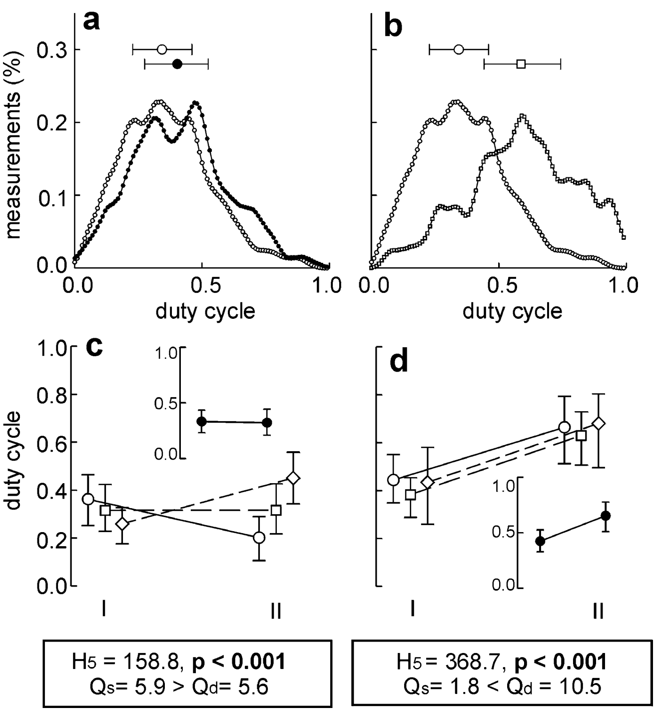


Fig. 2a–d Statistical considerations. Measurements of the duty cycle for **a** two individuals collecting at the same sugar concentration (40% w/w), and **b** for one individual collecting at two different sugar concentrations (*open circles*: 40% w/w, redrawn from **a**, *open squares*: 60% w/w). In both cases differences between the two sets of data are highly significant (Mann-Whitney *U*-test; **a**: $U_{304,588}=48127$, $P<0.001$; **b**: $U_{304,652}=159330$, $P<0.001$). Note almost overlapping distribution of data in **a**. *Symbols* above curve: median value, 1st and 3rd quartile of respective duty cycles. **c**, **d** Medians (1st/3rd quartile) of the duty cycle of three different bees under two different experimental conditions (*I*, *II*) (*inset*: values pooled for the three bees). In both cases samples differ significantly (Kruskal-Wallis test). Differences (mean of *Q* values from Dunn's pairwise comparison, SigmaStat 3.0) between different individuals under the same experimental condition (Q_s) or between experimental conditions (Q_d) are compared. $Q_s>Q_d$: differences are due to differences between the individuals. $Q_d>Q_s$: differences are due to differences between the treatments. *H* values from Kruskal-Wallis test, and Q_s and Q_d values given below figure

than the variation *between* different samples (signals of different individuals under the same conditions, or of one individual under different conditions). In the present case the Kruskal-Wallis test therefore analyses whether (1) is larger or smaller than (2) or (3): (1) > (2) or (3) accounts for no significant differences between samples (Kruskal-Wallis test; $P>0.05$). The variation in the data is due to the variable signal production by the same individual under identical conditions. If $P<0.05$ (significant difference between the data) then (1) < (2) or (3): the variation is due to differences between individuals (2), or to differences between experimental conditions (3). However, the Kruskal-Wallis test does not discriminate between these two possibilities. A post-hoc test (Dunn's pairwise comparison) calculates the differences between the different samples (*Q*, numeric value corresponding to the difference between two samples; SigmaStat for Windows 3.0). As an estimate for the difference between individuals (2) we calculated the mean differences between individuals under the same experimental conditions, Q_s (Q_{same}, mean of *Q* values from Dunn's pairwise comparison). As an estimate for the difference between different experimental conditions (3) we calculated the mean difference for individual bees between different experimental conditions, Q_d ($Q_{\text{different}}$). If $Q_s>Q_d$ then (2) > (3): differences between individuals account for significant differences in the data (Kruskal-Wallis test: $P<0.05$, Fig. 2c). If $Q_d>Q_s$ then (3) > (2): differences between different experimental conditions account for significant differences in the data (Kruskal-Wallis test: $P<0.05$).

Results

Food intake at different sugar concentrations

At ambient temperatures between 30°C and 32°C the *crop load* did not change with the concentration of the sugar solution (one-way ANOVA: $F_{4,142}=1.24$, $P=0.30$; Fig. 3a). The *imbibing time* (time needed to fill the crop), however, increased significantly with increasing sugar concentrations (one-way ANOVA: $F_{4,142}=94.1$, $P<0.001$; Fig. 3a). The solution *intake rate* (solution/time) remained constant up to a sugar concentration of 50% w/w and decreased significantly at still higher concentrations (one-way ANOVA: $F_{4,142}=154.4$, $P<0.001$; Fig. 3b). The *total amount of sugar* collected increased significantly with increasing sugar concentration (one-way ANOVA: $F_{4,142}=322.4$, $P<0.001$; Fig. 3c). The sugar intake rate (sugar/time) reached a maximum at 50% w/w sugar concentration (Fig. 3c). The differences between the sugar intake rates were significant at all sugar concentrations (one-way ANOVA: $F_{4,142}=112.3$, $P<0.001$; Tukey comparison, $P<0.05$) except those between 40% and 60% w/w (Tukey comparison, $P>0.05$).

Relation between signal pattern and sugar concentration

Is there a relation at all?

In experiments A1–A3 a group of bees collected at a food source where the sugar concentration was changed every 30 min (Table 2). Simultaneously, at a different feeding site at the same distance to the nest, an equally sized control group collected sugar water of constant concentration (40% w/w). The thorax vibrations of foragers of the experimental group were compared to those emitted by the control group during the same 30 min (Fig. 4). For all measured signal parameters the difference between both groups was highly significant (Kruskal-Wallis test: $P<0.001$; Fig. 4). Except for the velocity amplitude the signal parameters of both the bees from the experimental- and the control group, were barely influenced by the ambient temperature at the food

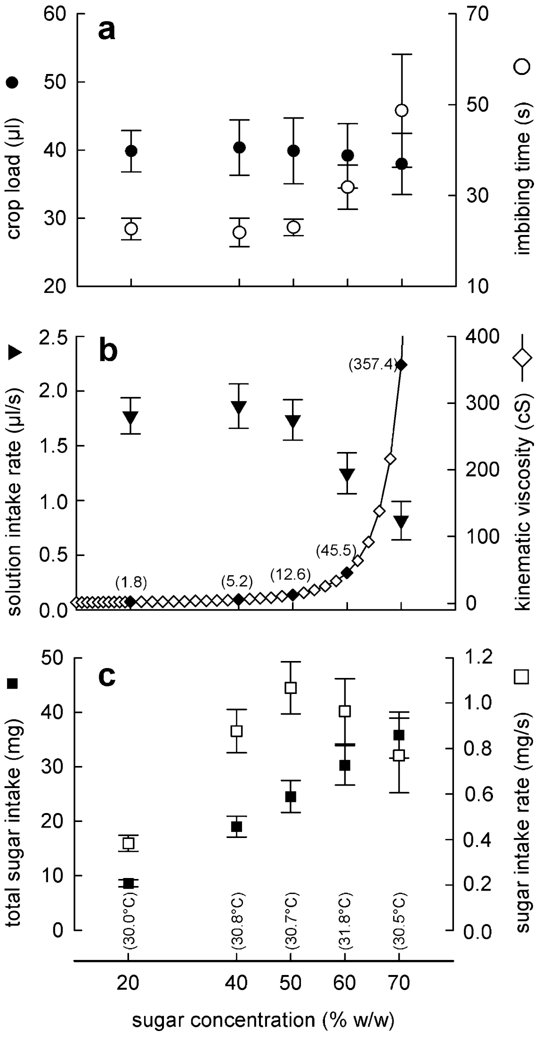


Fig. 3a–c Feeding behavior of *M. seminigra* at different sugar concentrations. Mean ± 1SD of **a** imbibed volume (crop load, *filled circles*), imbibing time (*open circles*), **b** solution intake rate (volume/time, *filled triangles*), **c** total sugar intake (*filled squares*), and the sugar intake rate (mass sugar/time, *open squares*). In **b**, kinematic viscosity (*open diamonds*) is given for different sugar concentrations (0.5–70% w/w at 20°C solution temperature; Weast et al. 1989). *Filled diamonds*, values for concentrations used in the study. Mean temperatures prevailing during experiment given in brackets in **c**

source (regression analysis for experimental groups: $R^2_{\mathrm{adj}} = 0.35\%_{[\mathrm{PD}]}$, $0.04\%_{[\mathrm{MF}]}$, $22.3\%_{[\mathrm{VA}]}$, $0.35\%_{[\mathrm{IPD}]}$, $1.28\%_{[\mathrm{PS}]}$, $0.03\%_{[\mathrm{DC}]}$; for control groups: $R^2_{\mathrm{adj}} = 0.24\%_{[\mathrm{PD}]}$, $0.78\%_{[\mathrm{MF}]}$, $12.2\%_{[\mathrm{VA}]}$, $1.81\%_{[\mathrm{IPD}]}$, $2.24\%_{[\mathrm{PS}]}$, $0.25\%_{[\mathrm{DC}]}$).

How do different parameters change with sugar concentration?

We compared the influence of the sugar concentration on the thorax vibrations of different individuals. This way we tried to avoid a possible misinterpretation of pooled data and were able to determine whether significant differences among the samples were due to differences between individuals or to different sugar concentrations (see Materials and methods). In experiments B1–B3 individually marked bees were fed with sugar water of different concentrations and given in different sequences (Table 2). For most values of the vibration parameters (PD, MF, IPD, PS, DC) the differences between experimental conditions (different sugar concentrations) were larger than the differences between individuals during the same treatment (Kruskal-Wallis test: $P < 0.001$; Dunn's comparison: $Q_d > Q_s$, Table 3). All ten individuals tended to produce longer pulses (PD), shorter interpulses (IPD) and a larger duty cycle (DC) with increasing sugar concentration (Figs. 5, 6; regression analysis: same sign of slopes, Table 3). These parameters (PD, IPD, DC) also showed the strongest dependence on sugar concentration (Table 3). The signal's main frequency only decreased when changing the concentration of the sugar solution from 60% to 70%, whereas it remained constant between 20% and 60% (Fig. 6).

Does the sugar concentration influence the signal variability?

Thorax vibrations showed a high degree of variation in the present study (Fig. 5). The variability of the signal parameters differed significantly among different sugar concentrations (Levene's test: $F_{4,348} = 117.4_{[\mathrm{PD}]}$, $177.6_{[\mathrm{MF}]}$, $91.3_{[\mathrm{VA}]}$, $168.5_{[\mathrm{IPD}]}$, $98.0_{[\mathrm{PS}]}$, $65.3_{[\mathrm{DC}]}$, $P > 0.001$). The standard deviations of pulse duration, interpulse duration, pulse sequence and main frequency were found to significantly correlate with their respective mean values (353 signals, Spearman Rank correlation: $rS = 0.94_{[\mathrm{PD}]}$, $0.38_{[\mathrm{VA}]}$, $0.92_{[\mathrm{IPD}]}$, $0.88_{[\mathrm{PS}]}$, $P < 0.001$; $rS = -0.52_{[\mathrm{MF}]}$, $0.31_{[\mathrm{DC}]}$, $P > 0.05$). In order to obtain comparable values of the variability for the different signal parameters we calculated the coefficient of variation (CV), which is a measure of the variation independent from the mean value ($\mathrm{CV} = 1\mathrm{SD} \times 100/\mathrm{mean}$). The variation of the pulse duration increased with sugar concentration (Fig. 7a). The variations of the main frequency, velocity amplitude, interpulse duration and pulse sequence (Fig. 7b–e) were lowest when the bees were offered a 50% w/w sugar solution and largest at the lowest and highest sugar concentrations (20% and 70% w/w). The variation of the duty cycle decreased with the sugar concentration up to 60% and increased again slightly at 70% w/w sugar solutions (Fig. 7f).

Discussion

Melipona foragers produce thorax vibrations inside the nest when returning from a rich food source. Here we show that the vibrations strongly depend on the sugar concentration of the collected food. All studied signal parameters, velocity amplitude excepted, changed with sugar concentrations between 20% and 70% w/w, which corresponds to the range found in the nectar naturally

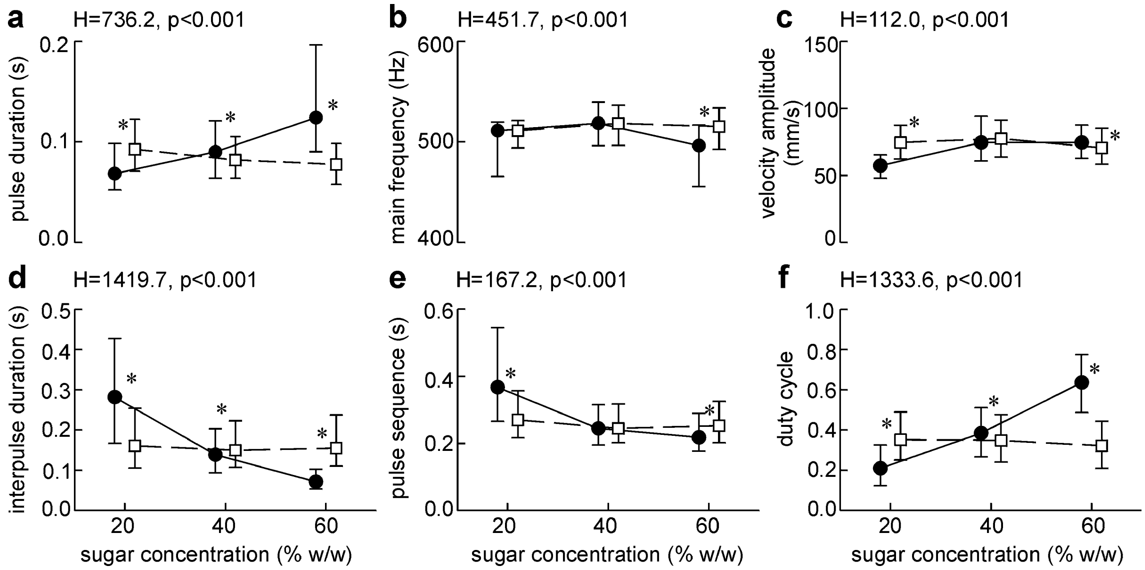


Fig. 4a–f Experiments A1–A3. Comparison of thoracic vibrations of bees collecting at the experimental feeder (*filled circles*, $N = 12$, $n_{20,40,60\%} = 474$; 1,521; 1,389) with those of the bees simultaneously collecting at the control feeder (*open squares*, $N = 12$, $n = 1{,}226$; 1,638; 1,170). Median values (1st/3rd quartile) are plotted against the sugar concentrations at the experimental feeder. Asterisks indicate significance of differences between experimental group and control group at the respective sugar concentration in the experimental group (Kruskal-Wallis test, $P < 0.01$; Dunn's comparison, $P < 0.05$). H values of the Kruskal-Wallis test are given in the figure ($df = 5$). Mean temperatures at the food sources were: 28.5°C (20%), 29.1°C (40%), and 29.3°C (60%) at the experimental feeder, and 28.9°C, 29.1°C, and 29.0°C at the control feeder

collected by bees of the genus *Melipona* (Roubik and Buchmann 1984; Roubik et al. 1995, Biesmeijer et al. 1999). These findings support the hypothesis advanced after having demonstrated the non-influence of visual flow on the thorax vibrations (Hrncir et al. 2004b) and suggesting that the temporal pattern of thorax vibrations reflects the energy budget of a bee's foraging trip.

Sugar concentration and temporal pattern of vibrations

Bees collecting at a food source with variable sugar concentration modified the temporal structure of their thorax vibrations while bees simultaneously collecting at a sugar solution of constant concentration did not (Fig. 4). This, for the first time, demonstrates the effect of sugar concentration independent of both extranidal climatic conditions and intranidal food supply and climatic conditions (Lindauer 1948; von Frisch 1965; Esch 1967). The signal parameters most affected by sugar concentration were pulse duration, interpulse duration and, accordingly, the duty cycle (Figs. 5, 6). Both pulse duration and duty cycle increased, whereas interpulse duration decreased with increasing sugar concentration. These findings agree with other studies on the vibrations produced by various *Melipona* species (*M. quadrifasciata*: W.H. Kirchner, personal communication; *M. subnitida*: J.C. Nieh, personal communication). In *M. costaricensis* longer pulses and shorter interpulses with increasing sugar concentration were described as well but are hard to interpret because the authors (Aguilar and Briceño 2002) publish negative values for pulse duration and the separation of long pulses (>0.1 s) from short pulses (<0.1 s) seems quite arbitrary. Nieh et al. (2003) studied the effect of both sugar concentration and food source distance on the vibrations produced by foragers of *M. mandacaia* and *M. bicolor*. Although these authors found that pulse duration increases with both sugar concentration and food source distance in both species they conclude that pulse duration reliably encodes food source distance once a recruit

Table 3 Comparison of the signal pattern of individuals collecting at different sugar concentrations using Kruskal-Wallis test (*H* values) and Dunn's pairwise comparison (*Q* values)

	Individual bees ($N = 10$, $n = 8857$, $df = 35$)					
	H	Q_s	Q_d	Slope mean	Slopes (+/-)	R^2_{adj} (%); mean
PD	$2{,}187.0^{***}$	5.51	6.39	0.0045	(10/0)	16.44
MF	$2{,}824.3^{***}$	4.32	7.45	−2.12	(2/8)	4.97
VA	$1{,}772.3^{***}$	5.06	4.27	−0.024	(5/5)	1.41
IPD	$2{,}840.1^{***}$	4.72	6.69	−0.0058	(0/10)	14.42
PS	$1{,}280.1^{***}$	3.86	4.35	−0.0017	(4/6)	2.89
DC	$2{,}807.3^{***}$	4.47	7.69	0.010	(10/0)	24.47

$^{***}P < 0.001$

PD pulse duration; *MF* main frequency; *VA* velocity amplitude; *IPD* interpulse duration; *PS* pulse sequence; *DC* duty cycle

Means of *Q* values (see text for details) indicate differences between individuals when feeding at the same sugar concentration (Q_s) or differences of individuals when feeding at different sugar concentrations (Q_d)

Regression analysis was performed to estimate the dependence (R^2_{adj}) of the signal parameters of each individual bee on the sugar concentration. Given are mean slopes and coefficients (R^2_{adj}) of the regressions, averaged for all bees

A comparison of the number of positive and negative slopes (+/-) demonstrates for how many bees the tendency of a signal parameter was to increase (positive slope) or to decrease (negative slope) with increasing sugar concentration

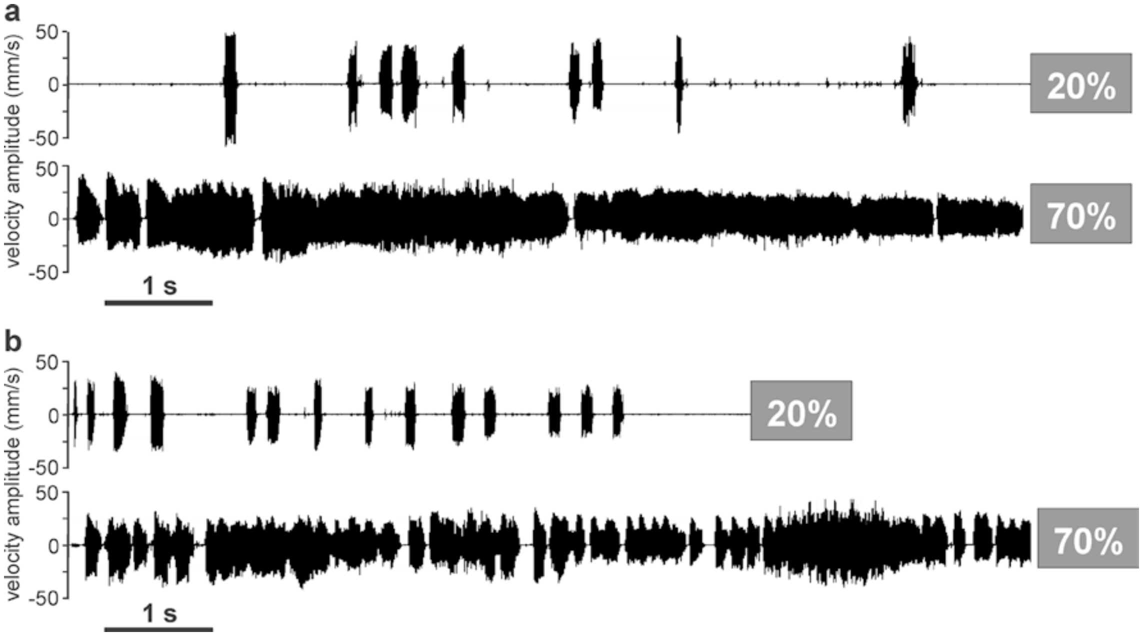


Fig. 5a, b Differences in the temporal signal structure as a function of sugar concentration. Typical examples of signals produced by two different individuals **a** and **b** when collecting at a 20%, and a 70% w/w sugar solution, respectively. Note higher variability of pulse duration when bees collected the 70% sugar solution

has distinguished between a high- and poor-quality food source. According to our findings pulse duration steadily increases with sugar concentration over the whole range of naturally collected sugar concentrations (Figs. 5, 6a). Accordingly, reliable distance information contained in pulse duration and depending on the distinction between a good and a bad food source seems rather doubtful.

Sugar concentration and energy budget

Inside a flight tunnel foragers substantially reduce their flight velocity as compared to their flight in the open (Hrncir et al. 2004b). The resulting higher energy consumption (Nachtigall et al. 1995) at similar energy intake was suggested to account for the decrease of pulse duration and duty cycle of the thoracic vibrations and for the increase of interpulse duration. Following this theory, raising the energetic gain at a food source while keeping the energy consumption constant should have an effect opposite to that of increased energy expenditure at similar energy intake. Our present data demonstrate this effect, that is an increase of pulse duration and duty cycle and a decrease of interpulse duration with increasing sugar concentration of a food source at constant distance. The total amount of sugar loaded at the food source (sugar gain) increases linearly with sugar concentration (Fig. 3c). Individual variability and interindividual differences in the pattern of thorax vibrations (Fig. 6) are suggested to reflect differences in energy intake when the bees collect sugar solution ad libitum either at artificial food sources (Fig. 3) or at natural food sources which they often leave with an only partly filled crop (Roubik and Buchmann 1984; Schmid-Hempel et al. 1985; Varjú and Núñez 1991)

Sugar concentration and signal variability

Behavioral patterns with signal function tend to be stereotyped (Lewis and Gowner 1980). In order to transmit unambiguous information to a receiver and to reduce the probability of misunderstanding and false alarm low signal variability is necessary. When assuming that thorax vibrations in *Melipona* contain information about the location of a food source (Esch 1967; Nieh and Roubik 1998; Nieh et al. 2003) a low degree of variation should speak for its “communicative value”. However, the temporal pattern (pulse duration, interpulse duration, pulse sequence and duty cycle) of thorax vibrations of *Melipona* is highly variable (Fig. 7, Table 1).

The variation of pulse duration, the signal parameter so far considered the most relevant one in all studies (Esch 1967; Nieh and Roubik 1998; Hrncir et al. 2000; Aguilar and Briceño 2002; Nieh et al. 2003), considerably increased with increasing sugar concentration (Fig. 7). Therefore, pulse duration is unlikely to reliably encode the distance of a food source, even more so at high-quality food sources (high sugar concentrations), despite a recent claim to the contrary (Nieh et al. 2003). Nieh and Roubik (1998) found that in *M. panamica* the variation of pulse duration during trophallaxis depends on the height of the food source above ground, and the variation of pulse duration after unloading the food on its distance. In *M. seminigra* the different variations of the signal parameters, including pulse duration, were certainly not an effect of food source location (height/distance). In all experiments the food was offered at the

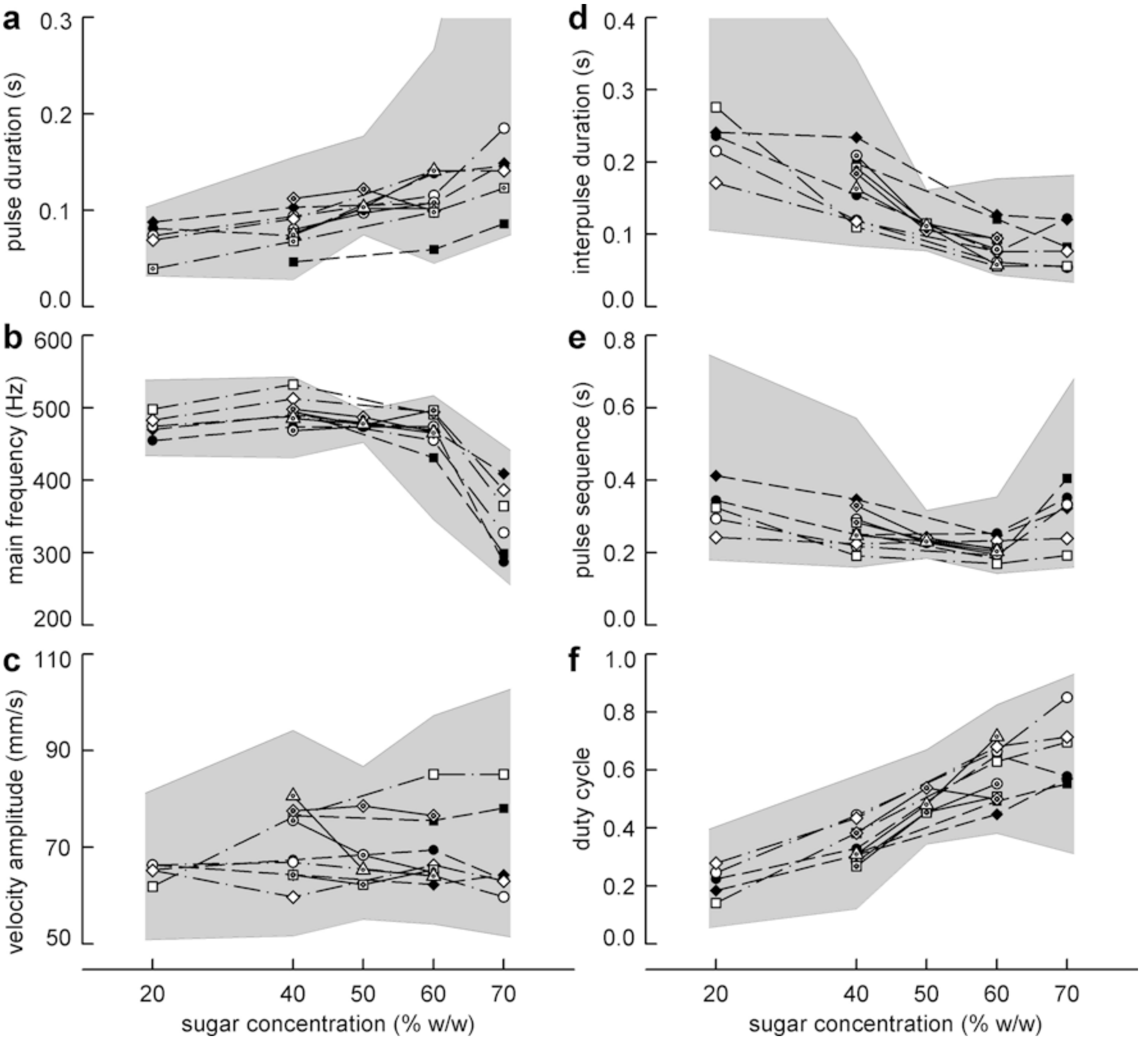


Fig. 6a–f Dependence of different signal parameters on sugar concentration in individual bees. Different symbols indicate median values for individuals; *shaded area*, variability of a signal parameter (interquartile range) at the respective sugar concentration; *filled symbols*, experiment B1 ($N=3$, $n=723$, 508, 697); *open symbols*, B2 ($N=3$, $n=883$, 968, 664); *dotted symbols*, B3 ($N=4$, n = 1,191; 996; 1,130; 797)

same distance and height. When interpreting the correlation of signal variation with food source location the fact that in *Melipona* the standard deviation of the pulse duration correlates with its mean value (Fig. 8) independent of food source location has to be considered. This seems not to be the case in the study on *M. panamica* (Nieh and Roubik 1998).

In different arthropods (crickets and spiders) the duty cycle of acoustical or vibratory signals has been demonstrated to be a parameter important for signal recognition (crickets, e.g., *Tettigonia caudata*: Schul 1998; spiders, e.g., *Cupiennius salei*: Schüch and Barth 1990; Shimizu and Barth 1996). In the present study, the signals' duty cycle was the temporal parameter that showed the smallest degree of variation (Fig. 7) and depended to the highest degree on sugar concentration (compare R^2_{adj} values in Table 3). However, the actual communicative value of this signal parameter as an indicator of food quality remains to be investigated.

Thorax vibrations in *Melipona* and *Apis*

Both stingless bee and honeybee foragers produce pulsed thorax vibrations upon their return from a valuable food source. The temporal patterns of these vibrations of both stingless bees and honeybees are strongly influenced by sugar concentration (Esch 1963; Waddington and Kirchner 1992; Aguilar and Briceño 2002; Hrncir et al. 2002; Nieh et al. 2003; present study). In honeybees the total time of vibration as well as the number of pulses are independent of sugar concentration but correlate with the distance of a food source (Wenner 1962). They are therefore assumed to transmit the information about the distance of a food source to the bees following the dance in the darkness of the hive (Esch 1961; Wenner 1962). In *Melipona*, however, neither the entire duration of the vibration performance nor the number of pulses per performance correlate with food source distance (*M. quadrifasciata*, *M. scutellaris*: Hrncir et al. 2000; *M. costaricensis*: Aguilar and Briceño 2002). They rather

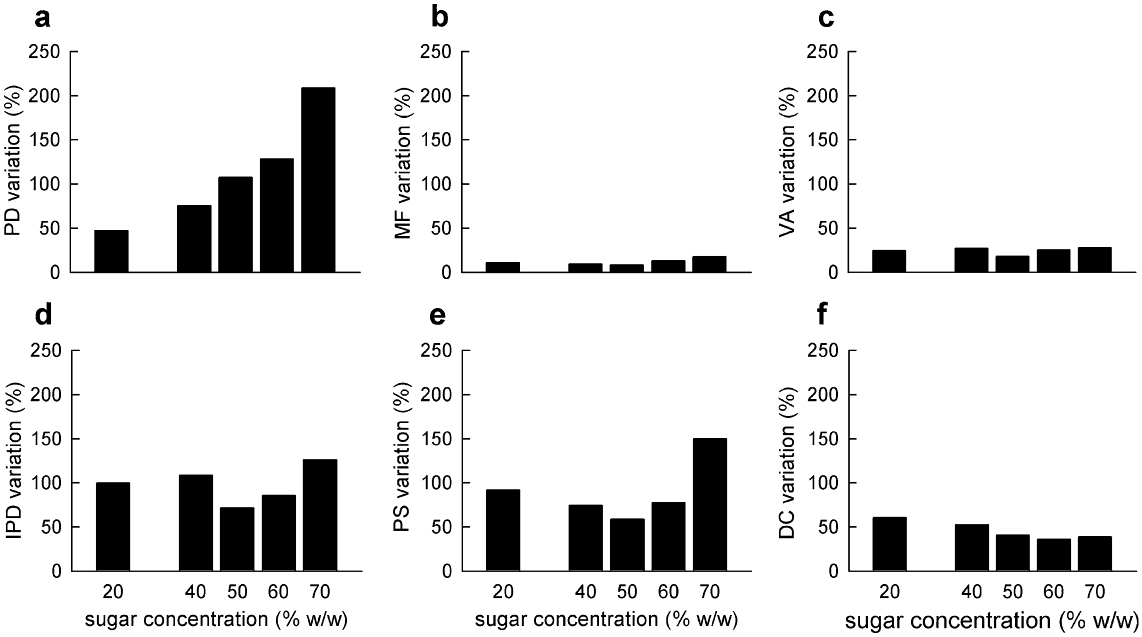


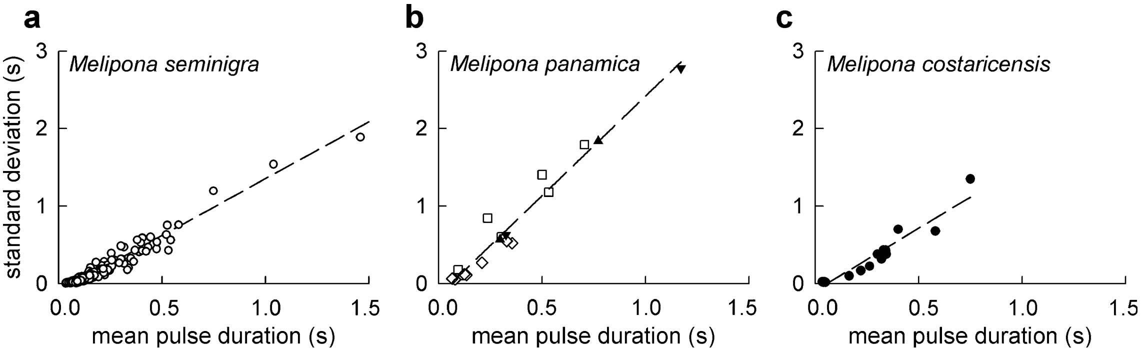
Fig. 7a–f Variation of signal parameters with sugar concentration. The variation of the signal parameters is plotted against sugar concentration. For the comparison of the variations of different signal parameters the coefficient of variation (CV) was calculated which is a measure of variation independent from the mean value (CV = 1SD×100/mean). *Bars* indicate the variation calculated from the data of experiments B1–B3 ($N = 10$, $n = 8857$)

depend on sugar concentration (*M. costaricensis*: Aguilar and Briceño 2002). A possible explanation for the influence of sugar concentration on the temporal pattern of the thorax vibrations could be the correlation between the foragers' thorax temperature and sugar concentration (Stabentheiner and Hagmüller 1991; Stabentheiner et al. 1995). In poikilothermic animals, like spiders, the ambient temperature effect the temporal pattern of vibratory signals (Shimizu and Barth 1996). So far, however, there is no experimental evidence for such an influence in bees, although studies in honeybees demonstrated an effect of body temperature on the amplitude of flight muscle potentials (Esch 1988) and on the wingbeat frequency (Soltavalta 1954).

Impact of thorax vibrations on foraging decisions

So far studies on the vibrations in *Melipona* concentrated on their dependence from various properties of the food source. Their actual role in the recruitment process per se has hardly been investigated. The results of the present study and of Hrncir et al. (2004b) demonstrate that the temporal pattern of the thorax vibrations in *M. seminigra* is strongly affected by the energy intake and consumption during a foraging trip. Information on the profitability of the food source is used in the individual bee's decision whether to forage or not (Biesmeijer et al. 1998). Thorax vibrations that reflect the energy budget of a foraging trip and thus the profitability of a food source therefore convey a message important for this decision process.

Fig. 8a–c Highly significant correlations between the mean pulse duration and the respective standard deviation were found in different *Melipona* species. **a** *M. seminigra* (present study); Spearman Rank correlation: $rS = 0.94$, $n = 353$, $P < 0.001$. **b** *M. panamica* (data from Nieh and Roubik 1998); Spearman Rank correlation: $rS = 0.92$, $n = 18$, $P < 0.001$; means and standard deviations from all pulses in the distance experiments (*open squares*), from the pulses after the unloading of food (*open diamonds*), from all pulses in the height experiments (top-foragers: *filled triangles*; bottom foragers: *inverted filled triangles*). **c** *M. costaricensis* (Aguilar and Briceño 2002); Spearman Rank correlation: $rS = 0.91$, $n = 19$, $P < 0.001$



Indeed, colonies of *Melipona* react to the foragers' vibrations with increased flight activity (*M. scutellaris*: Lindauer and Kerr 1958; *M. quadrifasciata*: Esch 1967). Also, the recruitment success of different *Melipona* species is influenced by the sugar concentration (*M. fasciata*: Biesmeijer and Ermers 1999; *M. scutellaris*, *M. quadrifasciata*: Jarau et al. 2000; *M. mandacaia*, *M. bicolor*: Nieh et al. 2003). Unfortunately, there is no direct proof yet that it is the differences in thorax vibrations of foragers collecting at different sugar concentrations which account for the differences in recruitment success when offering different sugar concentrations. It should also be considered that the role of the thorax vibrations in stingless bees may not be restricted to the recruitment of new foragers. The honeybee dance not only provides information about the food source (its location and quality), but also serves to increase the attention of food receivers, and thus to initiate an important information exchange between the foragers and their nestmates (Farina 2000). During trophallactic contacts nestmates receive information about properties of the food source (its odor, sugar concentration, and nectar flow rate: von Frisch 1965; Farina and Núñez 1991; Farina 1996; Wainselboim and Farina 2000), whereas foragers receive information about the nutritional status of the colony (via their nestmates' readiness to receive nectar; Farina 2000). Information about the colony's food supply is considered essential for the motivation of the foragers and their decision to continue or to cease foraging (Núñez 1970).

Recruitment processes in social insects are highly complex and a multitude of signals might be involved. In order to fully understand them it is essential to know exactly which signals or signal parameters are used to alert the nestmates and to stimulate them to leave the nest, and which (if any) signals or signal parameters are used to actually guide recruits to a food source. Describing properties and dependencies of potential signals, such as the thorax vibrations, contributes to our understanding. However, in order to better understand the particular role played by the vibrations in the recruitment communication of *Melipona* it remains to be investigated whether and how different signal patterns influence the behavior of recruited bees.

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