BEHAVIOUR-LOCKED SIGNAL ANALYSIS REVEALS WEAK 200–300 Hz COMB VIBRATIONS DURING THE HONEYBEE WAGGLE DANCE

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Summary

Waggle-dancing honeybees produce vibratory movements that may facilitate communication by indicating the location of the waggle dancer. However, an important component of these vibrations has never been previously detected in the comb. We developed a method of fine-scale behavioural analysis that allowed us to analyze separately comb vibrations near a honeybee waggle dancer during the waggle and return phases of her dance. We simultaneously recorded honeybee waggle dances using digital video and laser-Doppler vibrometry, and performed a behaviour-locked Fast Fourier Transform analysis on the substratum vibrations. This analysis revealed significantly higher-amplitude 200–300 Hz vibrations during the waggle phase than during the return phase ($P=0.012$). We found no significant differences in the flanking frequency regions between 100–200 Hz ($P=0.227$) and 300–400 Hz ($P=0.065$). We recorded peak waggle phase vibrations from 206 to 292 Hz (244 ± 28 Hz; mean ± S.D., $N=11$). The maximum measured signal − noise level was $+12.4$ dB during the waggle phase (mean $+5.8\pm2.7$ dB). The maximum vibrational velocity, calculated from a filtered signal, was $128\,\mu\mathrm{m\,s^{-1}}$ peak-to-peak, corresponding to a displacement of $0.09\,\mu\mathrm{m}$ peak-to-peak at 223 Hz. On average, we measured a vibrational velocity of $79\pm28\,\mu\mathrm{m\,s^{-1}}$ peak-to-peak from filtered signals. These signal amplitudes overlap with the detection threshold of the honeybee subgenual organ.

Key words: honeybee, *Apis mellifera*, communication, signal, vibration, waggle dance, behaviour.

Introduction

Social bees build nests that store food, house brood and serve as a substratum for the transmission of mechanical signals. Such mechanical signals are predicted to vary in strength according to the nature of the audience (Markl, 1985). For example, honeybee signals targeted at a broad audience, such as the tooting and quacking of queens, are broadcast over large areas of the comb and therefore employ a high-amplitude signal (Michelsen et al., 1986a). However, if signals are targeted locally, they should have a relatively low amplitude. We expected to find such a locally targeted signal in the honeybee waggle dance because a waggle dance communicates a specific resource location to a nearby set of dance followers (von Frisch, 1967).

The honeybee waggle dance communicates the distance and direction of good resources (typically pollen and nectar) to nestmates. The waggle dance consists of a looping figure-of-eight movement with a central, straight 'waggle run' (the waggle phase; Tautz et al., 1996) during which distance and direction information is communicated (von Frisch, 1967). During the waggle phase, the dancer moves her body in 15 Hz waggling motions while vibrating her wings in short pulses (20 ms duration) at frequencies ranging from 200 to 300 Hz

(Michelsen et al., 1987; Spangler, 1991). These wing vibrations generate weak near-field sounds that dance followers may be able to detect when they are close to the waggle dancer (Michelsen, 1993). The wing vibrations also superimpose a 200–300 Hz vibrational signal on the 15 Hz movements of the dancer (Esch, 1961; Wenner, 1962).

Because many hymenopteran communication systems exploit multiple sensory channels (Hölldobler, 1999; Nieh, 1998), we were intrigued by the possibility that waggle dancers might also transmit waggle vibrations *via* the comb to signal their presence. A series of experiments led us to focus on the role of comb-transmitted waggle vibrations. (i) Biomechanical studies on empty combs showed that freed combs facilitated vibrational transmission whereas attached combs damped vibrations, and honeybees freed the comb from vibration-dampening attachments precisely in those areas of the nest used for recruiting other foragers (Sandeman et al., 1996). (ii) Empty cells transmit vibrations better than capped brood cells (Sandeman et al., 1996), and dancers on open, empty cells recruit three times as many nestmates as dancers on capped brood cells (Tautz, 1996). (iii) The distance from which a dancer attracts follower bees is greater if the dance takes place

on empty cells as opposed to filled and sealed cells (Tautz and Rohrseitz, 1998).

These experiments led us to consider the relative importance of the 15 Hz and 200–300 Hz waggle-dance vibrations. Rohrseitz (1998) showed that the 15 Hz waggle-dance vibration is transmitted through the honey comb (with a maximum peak-to-peak displacement amplitude of 1.4 μm within a few millimetres of the dancer), but was not able to detect 200–300 Hz comb vibrations associated with the waggle phase. However, the honeybee vibration-sensitive subgenual organ is very insensitive to vibrations below 100 Hz, although it is quite sensitive to vibrations in the range 200–1000 Hz (Kilpinen and Storm, 1997). Moreover, 'silent' waggle dances that produce the 15 Hz waggle component but no acoustic 200–300 Hz component do not recruit bees (Esch, 1961). Finally, biomechanical studies on empty combs revealed an impedance minimum to horizontal comb vibrations between 230 and 270 Hz (Sandeman et al., 1996). We therefore focused on detecting 200–300 Hz waggle vibrations transmitted through the comb.

Previous analyses of the waggle dance have not detected a comb-transmitted 200–300 Hz waggle vibration signal (Michelsen et al., 1986b; Kirchner, 1993; Rohrseitz, 1998), and we suspect that three factors have contributed to the elusiveness of the signal. (i) The 200–300 Hz waggle vibration signal should be very weak since it is targeted at an extremely local audience: dances typically occur very close to each other without confusing follower bees. (ii) The enormous amount of background vibrational noise in an intact honeybee colony (Michelsen et al., 1986b; Kirchner, 1987; Rohrseitz, 1998) should mask weak signals. (iii) The 200–300 Hz waggle vibration signal is difficult to detect because it occurs as brief pulses superimposed upon the 15 Hz waggle motion. These 200–300 Hz pulses typically have a duration of only 20 ms and occur at a rate of 30 pulses s^{-1} during the 1–2 s of a typical waggle phase (Michelsen et al., 1987). Thus, it is essential to choose a precise behaviour-locked time window that will exclude non-waggle phase vibrations.

To overcome these problems, we developed a method of searching for short and very weak vibrational signals locked to a specific phase of behaviour in a very noisy environment. Using this method, we were able to detect and measure the 200–300 Hz waggle vibration signal produced by honeybees foraging for natural food sources.

Materials and methods
Colony and laser vibrometry apparatus

We placed a 44.6 cm×44.6 cm observation hive with an *Apis mellifera carnica* Pollm. colony of approximately 1000 bees on a Spindler & Hoyer vibration-dampening table. We maintained a low-density colony to prevent colony members from obscuring dancing bees. The colony entrance/exit was a semi-flexible tube leading through an opening in the laboratory wall. We provided no frames or comb foundations, and the colony therefore built a single large comb within the

observation hive (Fig. 1). We collected all data from waggle dancers recruiting for natural food sources around the Bee Research Station at the Universität Würzburg during the summer of 1998.

The face of the colony was covered with a clear plastic window containing four rotating circular panes. Each pane consisted of a freely moving circle set within a larger circle (Fig. 1). The small circle was pierced by a 3 cm long tube that excluded other bees from a 10 mm diameter circle. The beam from the laser vibrometer was deflected 90° by a small mirror glued to the tube. Thus, we aimed the beam perpendicular to the wall of an individual comb cell and measured vibrations in the horizontal comb plane. We reflected the beam off a small piece of reflective tape (3 mm×3 mm) attached to the outer rim of a cell. This arrangement allowed us to direct the laser at any point within the larger circle and prevented bees from walking into the beam. The multiple panes allowed us to direct the laser at almost any region of the comb.

To minimize vibrational noise, we also mounted the Polytec OFV2100 laser vibrometer on the vibration-dampening table. The laser vibrometer output was routed through a Hewlett Packard attenuator (model 350D) and then through a Rockland dual high/low filter (model 452) to remove all frequencies above 5 kHz. The filtered and attenuated laser output was
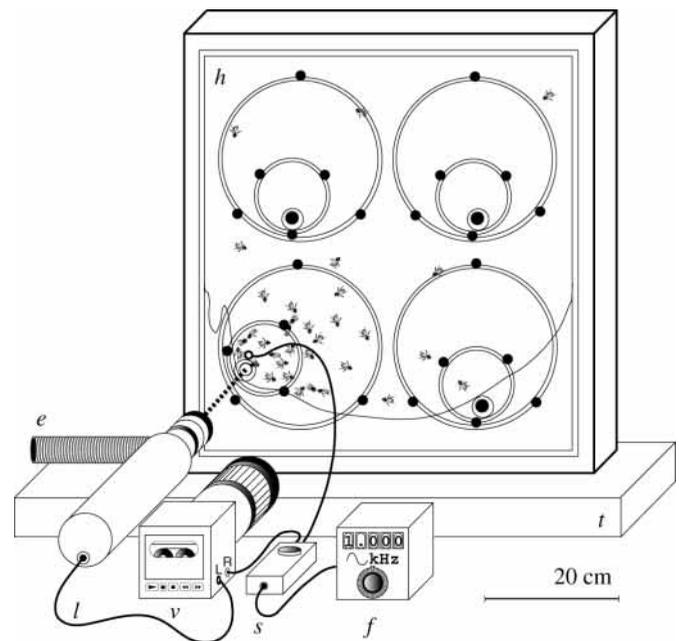

Fig. 1. Illustration of the apparatus used for measuring waggle vibrations. The letters indicate the observation hive (*h*), hive entrance/exit (*e*), laser vibrometer (*l*), digital video camera (*v*), switch (*s*) for passing a 1 kHz sound pulse to the video camera and simultaneously illuminating a light-emitting diode, function generator (*f*) and the vibration-dampening table (*t*). The laser vibrometer head was mounted on the same vibration-dampening table. All other items were mounted on the floor. Only the observation hive is shown to scale, all other items are at approximate scale.

routed through a Sony digital video camera (model DCR VX1000E; PAL video format, manual gain adjust set to 0.5). To provide an acoustic marker, a 1 kHz signal generated by a Hewlett Packard dynamic signal analyzer (model 35670A) was passed through a switch box and then filtered to remove all frequencies below 800 Hz before the input was passed to the video camera. Depressing the button on the switch box simultaneously allowed the 1 kHz signal to pass through and turned on a light-emitting diode (LED) taped to the plastic window directly next to the laser tube. Thus, we simultaneously recorded an acoustic and visual signal onto the video tape for the temporal calibration of acoustic and visual events before each waggle sequence. We also monitored the direct output of the laser vibrometer and the signal being recorded on the video tape using a Gould oscilloscope (model DSO 1604). This allowed us to watch for signal 'clipping' and to adjust the input attenuation accordingly.

Calibration measurements

To determine the frequency response of the digital video camera, we generated a 40.16 mV (peak-to-peak) reference sine wave (1 Hz to 20 kHz) with the Hewlett Packard dynamic signal analyzer, attenuated the signal by 15 dB, and filtered it to remove all frequencies below 5 Hz before passing it into the stereo input of the video camera. We recorded the vibrations onto the video tape and then played them back for comparison with the reference signal on the Gould oscilloscope. The video camera had a flat frequency response from 35 Hz to 16 kHz, with no measurable phase shift up to 14.5 kHz.

We used the Hewlett Packard dynamic signal analyzer to generate a sine-wave signal (1 Hz to 20 kHz) driving a Brüel & Kjaer mini shaker (type 4810) to calibrate and determine the frequency response of the entire system. The laser vibrometer targeted a reflective foil on the moving shaker head, and we compared the direct laser output with the output of the video camera/laser system. We generated vibrations with peak-to-peak displacements ranging from 0.032 to 0.350 μm since known honeybee substratum vibrations have magnitudes within this range (0.08–1.40 μm peak-to-peak; Rohrseitz, 1998). At peak-to-peak displacements of 0.032–0.350 μm, the entire video/laser system had a flat frequency response from 35 Hz to 16 kHz, with no measurable phase shift up to 14.5 kHz.

Video analysis

To analyze vibrations in synchrony with behaviour, we used the time code recorded on the digital video tape. Our PAL video system generated a 25 frames s^{-1} time code and thus yielded a temporal resolution of ±20 ms. Before each sequence of interest, we searched for an LED/sound calibration signal and noted the frame number at which the LED first turned on and the frame numbers at which relevant behaviours occurred. We then rewound the video camera several frames and digitized the entire acoustic sequence. The frame at which the LED first turned on corresponds to the beginning of the acoustic trigger signal, and visual events could therefore be

linked to acoustic events with a temporal accuracy of ±20 ms. From the video tape, we measured the distance of the waggle dancer from the laser (taking the distance from the centre of the dancer's thorax to the centre of the laser mirror) and the angular orientation of the dancer's longitudinal axis with respect to the laser mirror (0–90 °). All the data used in our analyses came from dancers on open cells on the lower end of the comb, where the vibrations were least restricted by comb attachment to the wooden hive walls (Fig. 1).

Vibration analysis

We digitized the vibrations recorded on digital video tapes using a Macintosh Powerbook G3 computer sampling at 22.1 kHz (with a calibrated flat frequency recording response between 1 Hz and 10 kHz), generating separate vibration files for the waggle phase and the return phase. Our overall recording system had a flat frequency response between 35 Hz and 5 kHz. All vibrations were analyzed using the program Canary v1.2.4 (Cornell University, Laboratory of Ornithology) and were calibrated using test signals of known amplitude generated by the Hewlett Packard dynamic signal analyzer. The following settings were used to generate spectra from each vibration file: filter bandwidth 10.9 Hz (frame length 372.4 ms); frequency grid resolution 0.3357 Hz (Fast Fourier Transform, FFT, size 16 384 points); and Hamming window function, clipping level −10 dB, logarithmic amplitude. We then selected waggle phase recordings in which we could hear the waggle phase signal and in which we could also detect a spectral peak. Each waggle phase was paired with the subsequent return phase. For better comparisons between FFT analyses, the return phase files were trimmed so that the total duration of the return phase matched the total duration of the preceding waggle phase. In three out of 11 cases, the return phase was shorter than the preceding waggle phase, and we therefore substituted the preceding return phase. Because different waggle phases naturally have different durations, even within the same dance performance, the FFT spectra for different waggle phases have slightly different numbers of peaks. However, this is due to different signal durations, not to different filter windows.

For each spectrum, we measured consistent equipment-generated spectral peaks at 100, 200 and 400 Hz and used these three peaks to calibrate the signal levels between the waggle phase signal and the subsequent return phase signal. On average, these calibration signals had a waggle phase to return phase ratio of 1.01±0.03. For each waggle and return phase pair, we calculated a calibration correction factor on the basis of the average amplitude difference between these three signals. We then measured spectrum peaks in three frequency regions in the waggle and return phases: 100–200 Hz, 200–300 Hz and 300–400 Hz. In each case, we excluded the limit frequencies. For example, we measured all frequencies greater than 200 Hz and less than 300 Hz. We then applied the calibration correction factor to all return phase spectrum peaks.

To compare the general strength of signals within each frequency range (100–200 Hz, 200–300 Hz and 300–400 Hz)

between the waggle phase and the return phase, we averaged all spectral peaks within each frequency range for the waggle phase and return phase separately. In the waggle phase recordings, we took the highest spectral peak between 200 Hz and 300 Hz to be the waggle signal. To obtain the noise level within the waggle phase, we averaged the amplitudes of all spectral peaks (excluding the maximum peak) greater than 200 Hz and less than 300 Hz.

Because of high vibrational noise levels, we were not able to measure the waggle signal amplitude directly from an unfiltered signal (see Fig. 2). We therefore bandpass-filtered each waggle phase recording to remove all signals not within ±20 Hz of the peak waggle signal and measured the signal amplitude from the resulting waveform. This narrow filtering tends to attenuate the signal and therefore yields only an estimate of true signal strength. Throughout this paper, all amplitudes are given as peak-to-peak values, all dB measurements are made relative to a 0 dB reference velocity of $0.1 \, \mu m \, s^{-1}$, and values are reported as means ±1 standard deviation (S.D.).

Results

In the 200–300 Hz frequency range, the mean amplitude of all waggle phase spectrum peaks was significantly greater than the mean amplitude of all return phase spectrum peaks (two-tailed sign test, $P=0.012$, mean difference 2.1±1.7 dB, $N=11$). This significant amplitude elevation is exclusive to frequencies from 200 to 300 Hz. We averaged the amplitudes of all spectral peaks in the flanking frequency regions and found no significant difference between the waggle phase and return phase levels at 100–200 Hz (two-tailed sign test, $P=0.227$, mean difference 1.2±1.8 dB, $N=11$) or at 300–400 Hz (two-tailed sign test, $P=0.065$, mean difference 1.3±1.8 dB, $N=11$).
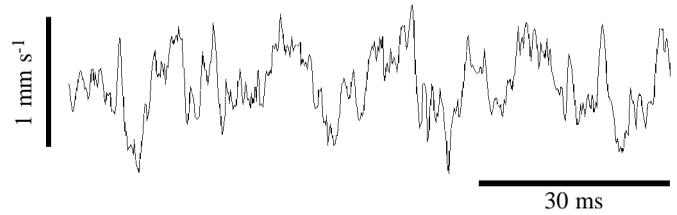

Fig. 2. An unfiltered sample vibrational waveform from recording T3W19-5. The amplitude is given as vibrational velocity.

Thus, a small but significant difference exists between vibrations measured during the waggle phase and return phase only within the 200–300 Hz range.

Table 1 summarizes the data for our strongest 11 waggle signals. The frequency of these signals ranged from 206 to 292 Hz (mean 244±28 Hz). The velocities of the filtered signals ranged from 48 to 128 $\mu m \, s^{-1}$ (mean 79±28 $\mu m \, s^{-1}$). Vibrational displacements ranged from 0.03 to 0.09 μm. The mean waggle signal − noise level was 5.8±2.7 dB.

Fig. 2 shows an unfiltered waveform of the vibrations generated by waggle dancer 19 during waggle run 5. During this recording, the dancer was 18 mm away from the laser at an angle of 30° to the direction of maximum vibrational sensitivity (at 0°, the waggle dancer's waggle movements would be parallel to the laser beam and, therefore, in the direction of maximum measurement sensitivity). This waveform illustrates the extreme noisiness of vibrational signals measured from the comb. Even with a signal − noise level of 12.4 dB, the peak frequency of 223 Hz (corresponding to a period of 4.5 ms) is not visually evident.

However, Fig. 3 shows that a spectral peak can be detected in the FFT spectrum of the waggle phase, especially in comparison with the FFT spectrum of the return phase. This

Table 1. *The frequencies and strengths of waggle vibration signals*

Recording	Waggle signal frequency (Hz)	Waggle signal filtered velocity ($\mu m \, s^{-1}$)	Waggle signal filtered displacement (μm)	Waggle signal − noise level, $dB_{signal} - dB_{noise}$	Distance from the laser (mm)	Angle from the laser (degrees)
T3W18-4	206	97	0.07	+4.3	12	10
T3W19-5	223	119	0.09	+12.4	18	30
T3W18-5	223	128	0.09	+6.6	18	30
T2W23-1	227	91	0.06	+3.3	30	30
T4W1-5	229	48	0.03	+4.0	23	30
T4W1-14	231	48	0.03	+7.4	17	20
T3W19-9	236	60	0.04	+5.6	15	60
T3W19-3	265	57	0.03	+7.4	20	30
T3W19-6	272	91	0.05	+3.3	18	60
T3W19-4	280	81	0.05	+6.2	22	0
T1W4-5	292	52	0.03	+3.2	17	70
Mean ± S.D.	244±28	79±28	0.05±0.02	5.8±2.7	19±5	34±12

The waggle signal is the highest peak within the 200–300 Hz range of the waggle phase.
The 0 dB reference velocity is 0.1 $\mu m \, s^{-1}$.
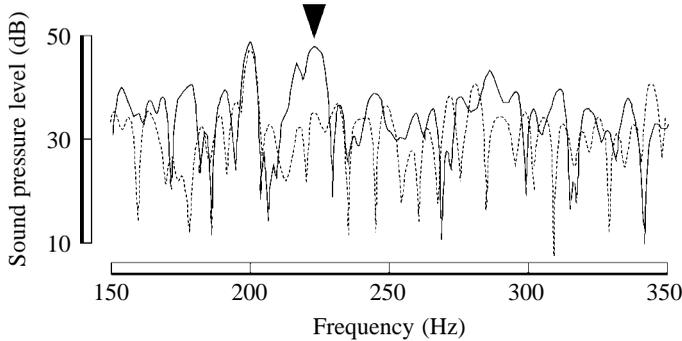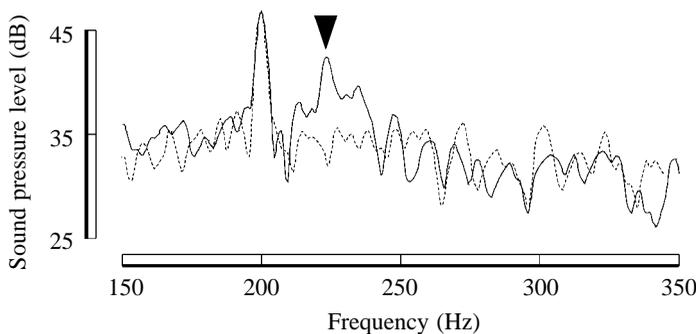All amplitudes are peak-to-peak measurements.

Fig. 3. Fast Fourier Transform (FFT) spectra of comb vibrations produced by waggle dancer 19 during her fifth waggle run 1 cm away from the free edge of the comb (recording T3W19-5). The waggle phase spectrum is shown as a solid line and the return phase spectrum as a broken line. The laser was positioned at an angle of 30°, 18 mm away from the waggle dancer. The arrowhead indicates the peak waggle dance frequency between 200 and 300 Hz. The 200 Hz peak is a consistent, equipment-generated frequency peak used to calibrate signal levels.

recording of waggle dancer 19 during waggle run 5 reveals a maximum vibrational peak at 223 Hz with a signal − noise level of +12.4 dB within the 200–300 Hz range during the waggle phase. The vibrational velocity of the filtered 223 Hz vibration signal is 119 µm s^{-1}, corresponding to a displacement of 0.09 µm. The conspicuous 200 Hz spectral peak is a consistent, equipment-generated frequency peak used to calibrate signal levels.

Fig. 4 similarly shows a spectral peak in the waggle phase that is absent in the return phase. This recording of waggle dancer 18 during waggle run 5 (recording T3W18-5) reveals a maximum vibrational peak at 223 Hz with a signal − noise level of +6.6 dB. The vibrational velocity of the filtered 200–300 Hz waggle vibration signal is 128 µm s^{-1},

Table 2. *Results of shifting the analysis time window by 50 %*

Recording	Signal duration (s)	Waggle signal − noise level, dB$_{signal}$ − dB$_{noise}$		B−A (dB)
		No time shift, A	50 % time shift, B	
T3W18-4	2.77	4.3	1.3	−3.0
T3W19-5	0.53	12.4	3.8	−8.6
T3W18-5	2.67	6.6	5.6	−1.0
T2W23-1	1.23	3.3	−3.7	−7.1
T4W1-5	1.73	4.0	−2.2	−6.2
T4W1-14	1.40	7.4	8.3	1.0
T3W19-9	0.67	5.6	4.0	−1.6
T3W19-3	0.73	7.4	2.8	−4.6
T3W19-6	0.77	3.3	2.6	−0.8
T3W19-4	1.07	6.2	3.0	−3.2
T1W4-5	1.74	3.2	−4.3	−7.5
Mean ± S.D.	1.39±0.77	5.8±2.7	1.9±3.9	−3.9±3.2

For each example, the analysis time window was shifted down by 50 % so that half of the 'signal' comes from the waggle phase and half comes from the return phase. The durations of the shifted and unshifted time windows are the same.

corresponding to a displacement of 0.09 µm at 223 Hz. As before, the 200 Hz spectral peak is a consistent, equipment-generated frequency peak used to calibrate signal levels. Spectrograms show that these vibration signals are only present during the waggle phase, not during the preceding or subsequent return phases (Figs 5, 6).

The importance of using only the waggle phase for analysis is shown in Table 2. Here, we shifted the analysis time window by 50 % so that half the 'signal' comes from the waggle phase and half from the return phase. For each comparison pair, we maintained the same total time duration, although times varied between pairs because of natural variation in waggle phase

Fig. 4. Fast Fourier Transform (FFT) spectra of comb vibrations produced by waggle dancer 18 during her fifth waggle run 1 cm away from the free edge of the comb (recording T3W18-5). The waggle phase spectrum is shown as a solid line and the return phase spectrum as a broken line. The laser was positioned at an angle of 30°, 18 mm away from the waggle dancer. The arrowhead indicates the peak waggle dance frequency between 200 and 300 Hz. The 200 Hz peak is a consistent, equipment-generated frequency peak used to calibrate signal levels.
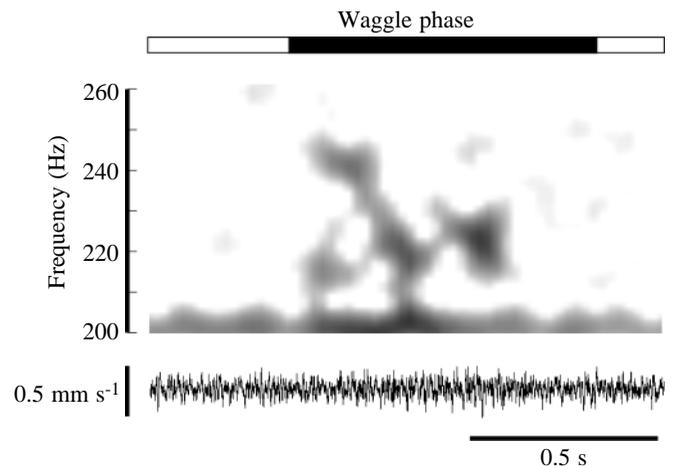

Fig. 5. Fast Fourier Transform (FFT) spectrogram and oscillogram of comb vibrations produced by waggle dancer 19 before, during and after her fifth waggle run (recording T3W19-5). The filled bar indicates the waggle phase, and the open bars indicate the return phases.
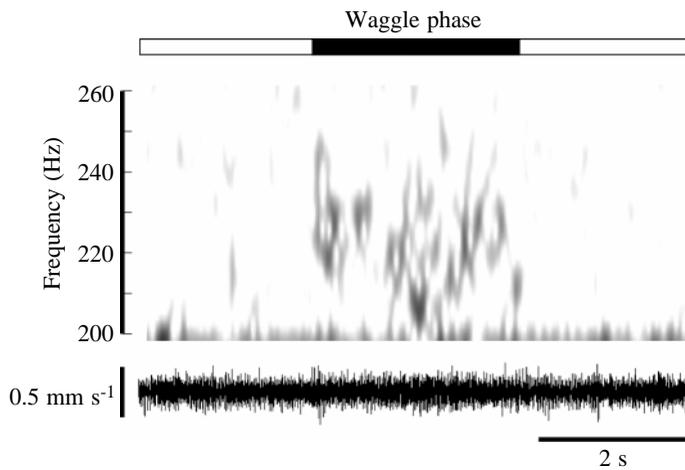
Waggle phase

Fig. 6. Fast Fourier Transform (FFT) spectrogram and oscillogram of comb vibrations produced by waggle dancer 18 before, during and after her fifth waggle run (recording T3W18-5). The filled bar indicates the waggle phase, and the open bars indicate the return phases.

durations. By shifting signals from 0.27 s to 1.39 s (mean shift 0.70±0.39 s, N=11), we significantly decreased the waggle signal − noise level (two-tailed sign test, P=0.012), The mean waggle signal − noise level decreased by 67 % (down to 1.9±3.9 dB). The 50 % time shift also causes the significant difference between the 200–300 Hz waggle phase and 200–300 Hz return phase vibrations to disappear (the mean difference is reduced to 0.72±1.1 dB, two-tailed sign test, P=0.227). Thus, a mean error of only 0.70 s will cause the 200–300 Hz waggle signal to vanish within the noise.

Discussion

Honeybee waggle dancers produce a wide variety of cues and signals that may assist followers in finding, orienting towards and following the waggle dancer. These cues and signals include temperature (Stabentheiner and Hagmüller, 1991), odour (von Frisch, 1967), tactile contact (Rohrseitz and Tautz, 1999), near-field sound (Michelsen et al., 1987) and vibration (Figs 3–6; Table 1). Thus, waggle dancers and dance followers use multiple sources of information to find and maintain contact with each other within the noisy, crowded bustle of the dance floor. Some of these cues and signals (near-field sound and tactile antennal contact) may inform dance followers about the waggle dance angle and the duration of the waggle run (Michelsen et al., 1987; Rohrseitz and Tautz, 1999). However, other cues and signals, such as temperature, odour and dance vibrations, may simply assist followers in detecting and localizing the dancer. As such, waggle dance vibrations should be detectable only locally. A strong, long-range waggle vibration signal could cause confusion and needlessly contribute to noise on the densely packed dance floor where different waggle dancers are often only 5–6 cell diameters away from each other. As predicted, behavioural evidence suggests that waggle dance vibrations provide a local

signal. Tautz and Rohrseitz (1998) reported that waggle dances on open cells attracted 90 % of all followers from within 27 mm, whereas dancers on sealed cells attracted 90 % of all followers from within 18 mm. The difference in attraction distance may derive from the poorer transmission of horizontal vibrations through sealed cells than through open cells (Tautz and Rohrseitz, 1998).

The waggle dancer produces vibrations in two frequency ranges: 15 Hz and 200–300 Hz. Rohrseitz (1998) compared horizontal comb vibrations measured during waggle dancing with vibrations measured when no waggle dances occurred. She found significantly higher-amplitude horizontal comb vibrations in the 13–18 Hz frequency range during the waggle dance than during a period with no waggle dances. She found no differences between dance and no-dance recordings in the 250–270 Hz frequency range, perhaps because the waggle phases and return phases were not sharply separated in her analyses and because a different type of measurement technique was employed. Since the 15 Hz movement is produced continuously during the waggle phase, it is not surprising that it is detectable even when the waggle phase and return phase are combined. However, the brief and intermittent 200–300 Hz waggle signal will be difficult to discriminate from noise if the analysis time window conflates the waggle phase and return phase (Table 2).

Behavioural data, physical measurements of the comb and measurements of the vibration-sensitive subgenual organ suggest that the 200–300 Hz waggle vibration component may be more important for dance followers than the 15 Hz waggle vibration component. (i) Esch (1961) showed that 'silent' waggle dances that produce the 15 Hz waggle component, but no 200–300 Hz acoustic component, do not recruit bees. (ii) The comb best transmits vibrations in the range 230–270 Hz and significantly attenuates vibrations below 150 Hz (Sandeman et al., 1996). (iii) The honeybee subgenual organ is a vibrational detector (Autrum and Schneider, 1948) and can detect vibrations transmitted horizontally across the comb (Rohrseitz and Kilpinen, 1997). Thus, it is well-suited for detecting the horizontally propagating waggle vibration signal. Yet this organ is quite insensitive to frequencies below 100 Hz (Kilpinen and Storm, 1997). Although other mechanoreceptors may be able to detect low-frequency vibrations, the insensitivity of the subgenual organ, coupled with the other two observations, suggests that the 15 Hz waggle vibrations are not as important as the 200–300 Hz vibrations.

We were able to detect a weak, previously unreported, 200–300 Hz comb-transmitted vibration signal produced during the waggle phase. This 200–300 Hz waggle signal is variable (ranging from 206 to 292 Hz) and is not necessarily confined to a single frequency within a given waggle phase. This signal is also brief and intermittent (Figs 5, 6), is produced within an extremely noisy environment (Fig. 2) and has a maximum filtered velocity of 128 μm s^{-1}, corresponding to a displacement of 0.09 μm at 223 Hz. As a result, this waggle signal is not detectable above comb noise (Michelsen et al., 1986b) unless special care is taken to analyze only vibrations

produced during the waggle phase and to measure vibrations close to the dancer along the horizontal plane in a region of comb that maximizes vibrational propagation. Under these conditions, it is possible to record a 200–300 Hz waggle vibration signal that is, on average, 5.8 dB greater than the level of background noise (Table 1).

Interestingly, the amplitudes of the 200–300 Hz waggle vibration signals overlap with the detection threshold of the subgenual organ. Kilpinen and Storm (1997) report that the electrophysiological sensitivity threshold of the subgenual organ ranges from 100 to 81 $\mu m\,s^{-1}$ peak-to-peak for stimuli between 200 and 300 Hz respectively (displacement of 0.08–0.04 μm peak-to-peak). Our 200–300 Hz waggle signal velocities of 48–128 $\mu m\,s^{-1}$ peak-to-peak overlap with this sensitivity range. However, because many of the 200–300 Hz waggle signals fall just at the detection threshold of the subgenual organ, other mechanoreceptors on the honeybee leg may be involved in transducing these faint vibrations (Sandeman et al., 1996).

The ability of honeybees to discriminate weak vibration signals from the noisy background remains a fascinating, though unresolved, issue. To date, studies have focused on the ability of an individual leg to transduce vibrations, yet honeybees have six legs that are all capable of sensing vibrations. This suggests an intriguing possibility: perhaps bees can integrate vibration information received from multiple legs. By comparing these multiple inputs, it may be possible for a bee to cancel out some of the comb noise and detect more easily the weak 200–300 Hz waggle vibration signal. Work on this question is currently being undertaken.

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