The Journal of Experimental Biology 204, 3737–3746 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3573

Phase reversal of vibratory signals in honeycomb may assist dancing honeybees to attract their audience

J. Tautz[1,*], J. Casas[2] and D. Sandeman[3]

[1]*Biozentrum, Zoologie II, Am Hubland, 97074 Würzburg, Germany,* [2]*Université de Tours, Institut de Recherches sur la Biology* and [3]*School of Biological Science, University of New South Wales, Sydney, NSW 2052, Australia*

*e-mail: tautz@biozentrum.uni-wuerzburg.de

Accepted 9 August 2001

Summary

Forager honeybees dancing on the comb are able to attract dance-followers from distances across the comb that are too remote for tactile or visual signals to play a role. An alternative signal could be the vibrations of the comb at 200–300 Hz generated by dancing bees but which, without amplification, may not be large enough to alert remote dance-followers. We describe here, however, an unexpected property of honeycomb when it is subjected to vibration at around 200 Hz that would represent an effective amplification of the vibratory signals for remote dance-followers. We find that, at a specific distance from the origin of an imposed vibration, the walls across a single comb cell abruptly reverse the phase of their displacement and move in opposite directions to one another. Behavioural measurements show that the distance from which the majority of remote dance-followers are recruited coincides with the location of this phase-reversal phenomenon relative to the signal source. We propose that effective signal amplification by the phase-reversal phenomenon occurs when bees straddle a cell across which the phase reversal is expressed. Such a bee would be subjected to a situation in which the legs were moving towards and away from one another instead of in the same direction. In this manner, remote dance-followers could be alerted to a dancer performing in their vicinity.

Key words: waggle dance, honeycomb vibration, recruitment, communication, honeybee, *Apis mellifera carnica*.

Introduction

Honeybee foragers returning to their hives can communicate the nature, direction and distance of the food source to their nest mates. This process is complex, has many facets and has captured the imagination of generations of biologists. One important element in the link between the forager and her nest mates is the curious and characteristic ‘dance’ carried out by the forager on the surface of the comb during which she is able to communicate to bees that ‘follow’ the dance much of the essential information they require to go out on their own and find the food source (von Frisch, 1967).

An obvious requirement, if the dancer is to be successful in communicating with dance-followers, is that they are in attendance. This is complicated by the fact that only a small proportion of the worker bees in the hive are potential dance-followers (Thom et al., 2000) and these may not be close when the dancer commences her performance.

There is evidence that several strategies are employed to bring dancers and dance-followers together. Certain regions of the hive, for example, are selected each day by the first foragers that return to the hive and are ‘marked’, probably by pollen clinging to the dancing foragers and the extrusion from glands on the dancer’s tarsi of chemicals that are taken up in the surface layers of the wax comb (Tautz and Lindauer, 1997). Such markers could provide cues about the location of future dances for waiting dance-followers. Also, not just any area of the comb is chosen to be a ‘dance floor’. In natural hives, the areas of the combs on which the bees most frequently dance are near the entrance to the hive; these cells are empty and uncapped and are close to the lower and freely suspended margin of the comb (Seeley and Morse, 1976). In commercial hives, comb templates are supplied to bees in wooden frames. In these frames, the combs are attached around all four sides so that the lower edges of the combs are fixed. It has been found that bees will modify such combs by removing the wax along the lower edge of the part of the comb on which they perform their dances, perhaps in an attempt to achieve a situation closer to that prevailing in natural hives (Sandeman et al., 1996).

Many of the observations that have been made on bee dancing have been carried out using an observation hive in which two frames containing combs, mounted one above the other, can be observed from either side through transparent walls. The bees do not seem to be unduly disturbed, provided that lighting conditions are subdued, and continue to perform their dances and various activities associated with the maintenance of brood and the defence of the hive. In a natural hive, however, there is far less light than in observation hives, raising the question of how a forager can pass on information in the dark that is so important for the nourishment of the colony. Even in well-lit observation hives, it is doubtful whether potential recruits that are more than a few body lengths from the dancing bee are able to see her, given the crowded conditions that often prevail on the combs.

Behavioural measurements on bees in both illuminated and non-illuminated observation hives have shown that potential dance-followers signal that they have detected a dancer in their vicinity by turning their heads towards the dancer and then moving close and following her through the turns of the dance (Tautz and Rohrseitz, 1998; Rohrseitz and Tautz, 1999). Many of these dance-followers head towards the dancer from some distance away, providing strong evidence for the existence of some signal or signals other than vision or direct tactile stimulation that advertises the presence of a dancer.

One of the possibilities is that the low-frequency (approximately 200–300 Hz) signals that bees emit from their thoraces during the ‘waggle’ stage of the dance (Esch, 1961; Wenner, 1962; Michelsen et al., 1986; Spangler, 1991) are transmitted laterally across the comb to nest mates that can perceive these through proprioceptors in their legs (Sandeman et al., 1996). This hypothesis is attractive because of the resonant properties of open cell combs (particularly along an unsupported edge), the sensitivity of some leg proprioceptors to low-frequency displacement and the greater efficiency with which forager bees recruit nest mates on empty, in comparison with filled and sealed, combs (Tautz, 1996).

The amplitude of the comb displacements produced by dancing bees, however, is small and barely exceeds the threshold of the receptors that could be responsible for their detection (Sandeman et al., 1996; Nieh and Tautz, 2001). However, the receptors from which recordings have been made may not be those that the bees use to detect the displacements, or the receptors may be much more sensitive in intact animals. Alternatively, the bees may have some sophisticated way of extracting the low-frequency displacement from the general noise.

A further possibility is that, as a result of an inherent elasticity in the wax of the comb, the cell walls do not act as a rigid structure in which the displacement moves at infinite velocity across the surface, but that some delay, or phase lag, is introduced between the displacement of one cell wall and that of the next. A bee straddling a cell would, therefore, be subjected to a signal in which, for a very short period of the cycle, pairs of its legs would be moving in opposite directions with respect to one another, effectively doubling the size of the input signal. Such an event would not have been detected in earlier measurements that employed a single laser to measure the amplitude first at the displaced cell and then at various distances along the cell row.

This study was initiated to explore the above possibility by employing two identical laser systems that could simultaneously monitor the displacement of the cell walls along a row of open comb cells. The result was the discovery of a remarkable and unexpected phenomenon: the lateral displacement of the comb walls proceeds across the comb at high velocity but, at a particular distance from the signal source and at a frequency within the range of that emitted from the bee’s thorax during the waggle dance, the phase of the lateral displacements of the cell walls is abruptly reversed across a single cell.

This discovery led us to re-examine a large amount of the collected behavioural data on the distances and directions from which dance-followers will come to follow dancers. These show that the region from which most dance-followers are attracted coincides with the predicted location of the phase-reversal phenomenon.

Materials and methods

Displacement and laser measurements

A framed honeycomb (40 cm×20 cm) that had been constructed by bees and used by them for one season was used for all the measurements presented in this report. The cells of the combs were empty and uncapped, and part of the lower edge of the comb had been freed from the frame by the bees themselves. Measurements repeated on a second comb yielded the same results.

The experimental arrangement was the same as that used in the previous study described in detail by Sandeman et al. (Sandeman et al., 1996). Briefly, the frame was supported horizontally, and the cell wall of one cell was displaced in the plane of the comb face using a bilayered piezo-electric transducer (BM/ML 60/40/300; Piezomechanik, München). Two identical laser-Doppler vibrometers (Ometron VS 100) were used, and their beams were directed separately through mirrors onto small reflective strips placed on the rims of the cells. One laser beam was focused on the wall of the stimulated cell (the reference laser), while the other (the difference laser) was focused on the walls of cells at different distances from the stimulated cell. Many regions of the comb were tested, but the data reported here come from three horizontal rows of cells in the centre of the comb (Fig. 1).

The forces exerted on the walls of comb cells by a honeybee during a waggle dance are small and have been calculated from careful measurements of the motion and mass of living honeybees (Storm, 1998). At the lowest fundamental frequency of the waggle (approximately 15 Hz), the maximum lateral force that is exerted by the legs on the walls ranges from 0.5 mN through the prothoracic leg to 1.5 mN for the mesothoracic leg and 2 mN for the metathoracic leg. These forces are achieved at the peak of the lateral excursion of the bee during the repeated side-to-side oscillation of the waggle dance. The amplitude of the lateral displacement of the walls can be estimated from the lateral impedance of the comb [80–100 N s m^{-1} at 15 Hz (Rohrseitz, 1998)], and is in the region of 0.5 μm for vibration by a single leg.

The above values were taken into account in designing a

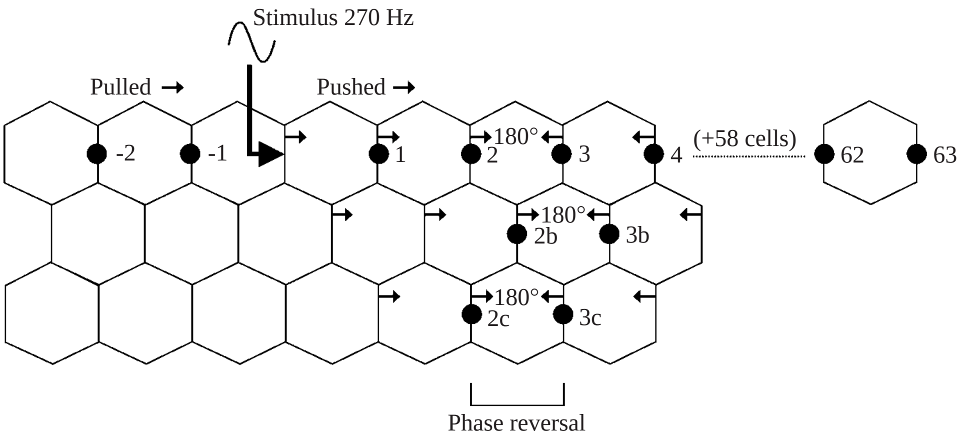

Fig. 1. Diagram of the comb showing the location of the stimulus probe and the three rows of cells from the walls of which measurements were taken. The cell rows are oriented along the same horizontal axis as they would be in the hive. The large arrow shows the point of application of the lateral sinusoidal displacement to the top rim of a cell wall. The power stroke of the stimulus is in the direction of the arrow, thus ‘pulling’ the cell walls on the left and ‘pushing’ the cell walls on the right. The return stroke of the probe allows the comb to move back to its starting position. The phase reversal occurs across walls 2 and 3, walls 2b and 3b and walls and 2c and 3c. No phase reversal was found to occur across cells on the ‘pull’ side of the stimulus.

method of imposing vibrations on the comb that would approximate the natural situation. There are, nevertheless, several unknowns and constraints that make a precise replication of the natural situation difficult. First, the bee exerts a force on the comb through more than one leg for each lateral excursion, and we limited ourselves to a single probe. Second, the amplitude of the cyclical excursion of the cell wall in the natural situation is calculated to be approximately 0.5 μm. The accurate production and measurement of cyclical displacements in the region of 0.5 μm were close to the limits of the apparatus we used.

Our compromise was to apply the probe of the piezo-electric transducer laterally at the centre of the rim of a cell wall in the direction of the horizontal long axis of the comb and in the plane of the comb face. The probe was advanced laterally against the rim with a force of 1 mN, which lies between the extremes (0.5–2 mN) calculated for the low-frequency oscillation of the bee (Storm, 1998). The force of the probe against the comb was constant, and the vibratory stimulus was superimposed on it. The ‘stimulated’ cell was chosen to lie half-way along the long axis of the comb, and measurements of the displacement of individual cell walls were taken on both sides of the stimulated cell. Hence, we could observe cells that were ‘pulled’ behind when the probe moved against the cell wall and also those that were ‘pushed’ ahead when the probe moved against the cell wall.

The frequency of the vibratory stimulus was held at 270 Hz because our previous study showed this frequency to be optimal for transmission along the face of the comb and in the centre of the range of frequencies that the bees produce during dancing. The amplitude of the stimulus was maintained at 2 μm, peak to peak, and was measured directly with one of the laser vibrometers. While larger than the cyclical amplitude estimated for vibration by a single leg, it is still close to the range that could be produced by a bee with more than one leg gripping the wall. Also, the impedance of honeycomb for vibration at 200–300 Hz is between 20 and 30 N s m^{-1} (Rohrseitz, 1998), which is approximately 3–4 times lower than for the waggle frequency. Hence, the cyclical amplitude for the 200–300 Hz signals would be expected to be proportionately larger than 0.5 μm. Large-amplitude displacements (20 μm) produce the same result as the small-amplitude displacements, suggesting that, over this range, the amplitude of the imposed vibrations is not a critical factor in the production of the phase reversal.

Behavioural observations

Behavioural observations on the dancers and the dance-followers were made in the same way as reported by Tautz and Rohrseitz (Tautz and Rohrseitz, 1998) but are described here in some detail where the results have relevance to the laser measurements of the comb displacement. The colony on which the observations were made contained approximately 4000 bees (*Apis mellifera carnica* Pollm.) and occupied a two-frame observation hive located in a laboratory from which the bees were unable to view the sky but from which they could gain access to the outside along a short tunnel. A high-speed video camera (HSV 400) was focused on the ‘dance floor’, an area of the comb nearest the entrance at the lower edge of the hive where the bees normally danced. An area of 75 mm×60 mm, or approximately 180 cells, was included in the field of view. Video recordings were made at 200 frames s^{-1} under daylight conditions or with additional visible stroboscopic light, which improved the quality of the images. A CCD camera was used with infrared illumination provided by infrared light-emitting diodes (λ=875 nm) to obtain pictures under ‘dark’ conditions.

The cells of the comb that constituted the dance floor varied from being open, empty cells (referred to as open cells) located near the lower freed edge of the comb to the capped brood cells (referred to as sealed cells) located closer to the centre of the comb. Videotaping was undertaken over 10 days during warm and sunny weather. During this period, the lower comb (with the dance floor) needed to be exchanged three times because the bees filled the cells with honey. Videotaping was resumed on the day following comb exchange.

Twenty marked forager bees were trained to a feeder (on non-scented 2 mol l^{-1} sugar solution) 1 km away from the hive. The dances of these foragers were videotaped.

We needed to determine the moment a dance-follower became interested in a dancer and her location relative to the dancer at that moment. We achieved this by inspecting the video recording until we found a dance-follower that followed the dancer for at least one waggle phase and one return phase of the dance. We then reversed the video recording so that we could track the image of the dance-follower, frame by frame, backwards in time, until we detected her first overt reaction, namely a turn of her head towards the dancer. Because the head turn always preceded movement of the dance-follower towards the dancer, we took this as the criterion of her becoming aware of a dancer in the vicinity.

The distance between the dance-follower and the dancer was measured as the shortest line between any part of the bodies (head, thorax or abdomen) of the dance-follower and dancer. We also determined the position of the head of the dance-follower within a system of polar coordinates centred on the thorax of the dancer. In this system, which was divided into 30 ° sectors, 0 ° was directly ahead of, and 180 ° directly behind, the dancer.

In all, 471 dance-followers in 132 dances were analysed. Of these, 40 dance-followers were videotaped under infrared conditions. The density of the bees (0.8 ± 0.1 bees cm^{-2}) within the field of view of the camera was the same in all cases for the different lighting conditions and dance floors.

Details of the statistics used in the analysis of the behaviour are given by Tautz and Rohrseitz (Tautz and Rohrseitz, 1998).

Results

The phase-reversal phenomenon

Applying a pure sine-wave displacement to the wall of one of the cells along a horizontal row of cells results in a movement of the walls of all the cells that are parallel to the displaced wall, right across the comb. The signals spread rapidly across the face of the comb at a velocity of approximately 230 m s^{-1}, which we calculate from the 0.5 ms delay between the displacement of the stimulated cell wall and that of the wall of a cell 115 mm away (Fig. 2).

The two lasers used to measure the simultaneous displacements of the wall of the stimulated cell and that of a cell some distance from it revealed that a sudden phase change occurred as the signals were propagated across the face of the comb. Fig. 1 shows the point at which the displacement was applied and recorded with the laser and also the points to the left and right of the stimulated cell from which measurements were taken with the two lasers. These simultaneous recordings for seven full cycles are depicted in the series in Fig. 3 that shows the displacement velocities (in mm s^{-1}).

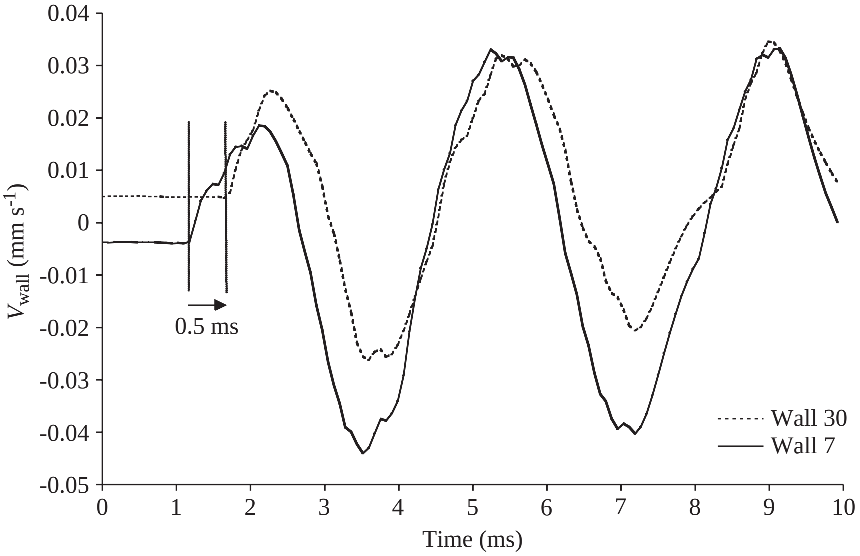

Fig. 2. Displacement velocity V_{wall} of wall 7 compared with that of wall 30 measured simultaneously using two identical lasers. The 0.5 ms time delay between the forward displacement of wall 7 and that of wall 30 at the onset of the series provides a measure of the conduction velocity of the signal across the comb. In this case, 230 m s^{-1}.

The cell wall immediately to the right of the stimulated wall (i.e. wall 1) moves in phase with the stimulus and also preserves the form of the stimulus fairly accurately (Fig. 3A). Shifting the focus of the lasers to rest on the rims of walls 1 and 2 shows that the sinusoidal form of the stimulus is well preserved in the displacement velocity of walls but that some distortions of the waveform are introduced in the displacement velocity. There is also a small phase advance of the major peaks of wall 2 with respect to wall 1. Measurement of the excursions of the cell walls to the left of the probe (i.e. the 'pull' direction) shows the same phenomenon (Fig. 1). A detailed analysis of the many distortions measured in the applied and recorded signals is beyond the scope of this investigation, but they probably have their origin in the way in which the stimulus probe was applied to the side of the cell wall and the overall mechanical complexity of the honeycomb system. The forward, or power, stroke forced the wall to the right, and we measured this as a positive displacement. The return stroke, however, represents a removal of the force, during which the comb is allowed to return under its own elasticity to the starting position. Some driven movement to the left occurs because the wall adheres to the probe and is pulled after it on the return stroke.

Focusing the lasers on walls 2 and 3 reveals the abrupt phase shift in the displacement velocity that is introduced at this distance from the stimulated cell. The distortions of the original signal introduced at cell 2 are carried over into the displacement velocities of wall 3, and additional peaks appear in some excursions. A comparison between the displacement velocities of walls 3 and 4, however, shows that these two are again nearly in phase with one another. Thus, the 180 ° phase shift introduced across walls 2 and 3 is now preserved across walls 3 and 4. No further phase change was detected, although

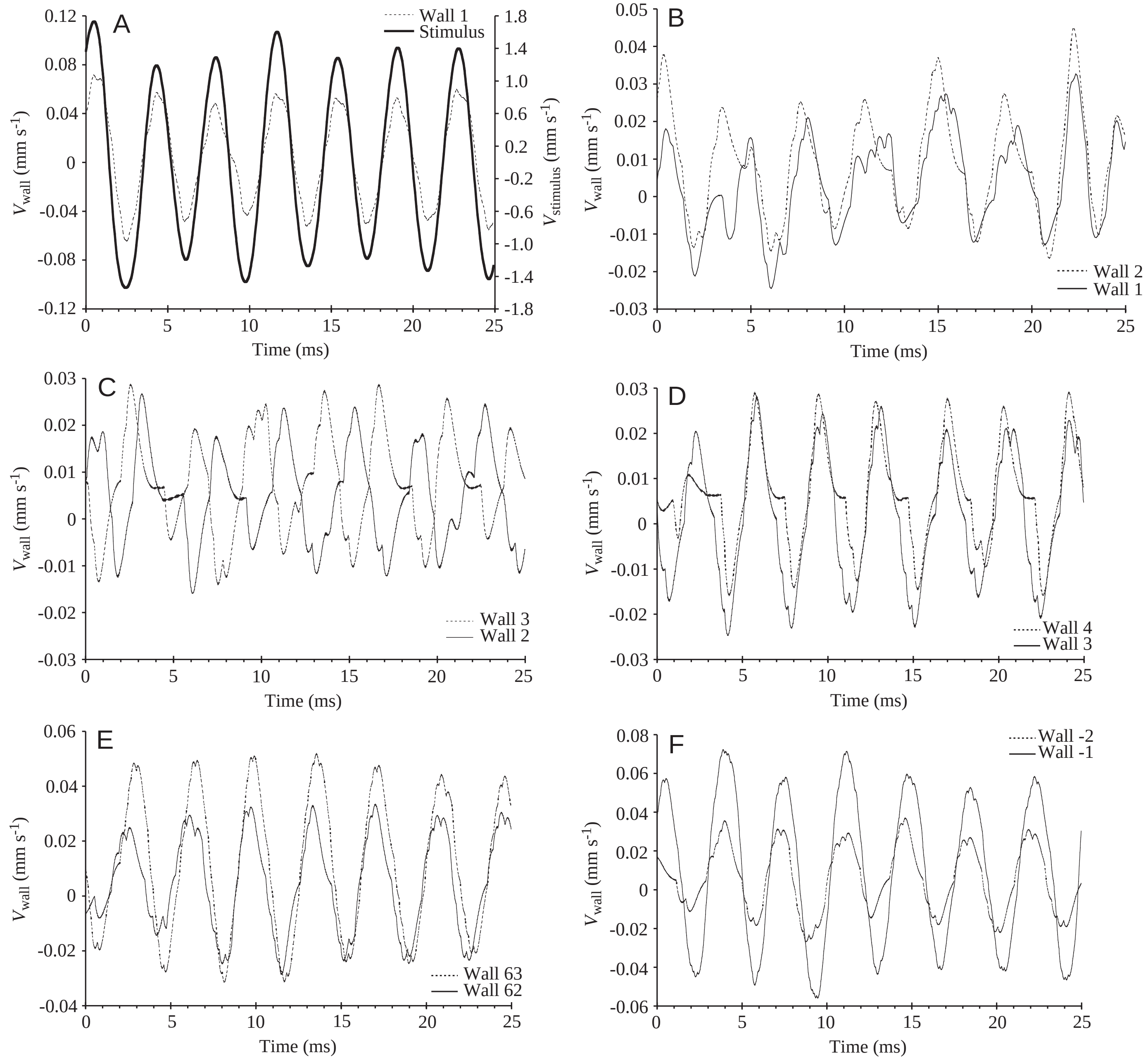

Fig. 3. (A–F) Comparison of the simultaneously measured displacement velocities V_{wall} of cell walls at different distances from the stimulus. (A) Time course of the stimulus and movement of the wall opposite to it (wall 1). There is no phase lag, but the wave form is already distorted. V_{stimulus}, velocity of the stimulus. (B) A sequence of wall 1 excursions compared with those occurring at wall 2. The displacement of wall 2 (broken line) exhibits a small phase lead over that of wall 1 (solid line). (C). The small phase lead of wall 2 over wall 1 advances suddenly so that the displacements of walls 2 and 3 are about 180 ° out of phase. (D). One cell further along the line from the stimulus (walls 3 and 4), the cell wall displacements are again in phase with one another. (E). Sixty-two cells away from the stimulus, the cell walls are still in phase with one another. The small phase lag between walls 62 and 63 is introduced by the finite conduction velocity of the signal across the comb. (F) Displacement velocities in the ‘pull’ direction (to the left of the stimulated cell in Fig. 1) between wall −1 and wall −2. No phase reversal was found in this direction at any distance from the stimulus.

measurements were taken up to 63 cells from where the displacement was applied.

The phase reversal that occurred across walls 2 and 3 along the primary row, horizontal to the stimulus, was also found to be represented between cell walls of secondary and tertiary horizontal cell rows, which lie lateral to the primary row (cells 2b and 3b and cells 2c and 3c, Fig. 1). The same type of distortions that appeared in the primary horizontal row were also found in the displacement velocities of the cell walls in both secondary and tertiary rows (Fig. 4A,B). When screening an entire comb, seven cell widths was the greatest distance between the stimulus and a phase-reversal cell.

The phase-reversal phenomenon is frequency-sensitive. Measurement of the phase relationship between wall 2 and wall 3 showed that these were 180 ° out of phase over the frequencies 175–275 Hz. Phase shifts of over 150 ° are found over the frequency range 100–300 Hz. Stimulus frequencies lower than 100 Hz result in a rapid decrease in the phase difference. For stimulus frequencies above 300 Hz, the phase change decreases more gradually, approaching 90 ° at 370 Hz. With increased frequency, the phase difference between the two cell walls approaches zero and finally changes sign to lag by 50 ° at 500 Hz (Fig. 5). A change in the amplitude of the driving signal (and thus the force applied to the wall), however, did not alter the phase difference.

The behaviour of dance-followers

Dance-followers in video recordings all exhibited the same response. At a particular moment, they abruptly turned their head towards the dancer and then ran rapidly in her direction until they contacted her with their antennae. All the bees we observed in the video recordings that turned their head towards the dancer behaved in this way and became followers in our definition, i.e. they followed the dancer for at least a full dance round including the waggle and return runs. Not included here are bees that were already in antennal contact with the dancer or that made antennal contact with her as she danced past them.

We have re-examined the video recordings taken by Tautz and Rohrseitz (Tautz and Rohrseitz, 1998) in relation to the phase-reversal phenomenon. The question we pose here is whether the responses of the dance-follower bees observed in video recordings would in any way suggest the possible presence of a phase-reversal signal in the comb vibrations that may occur under the natural conditions of a bee dance. We explore an answer to the question in terms of the effectiveness of the dancers in attracting followers from a particular distance and direction across open cells. We chose these variables because they are both tied to a location, as is the phase-reversal phenomenon itself, relative to the signal source (i.e. the dancing bee).

To estimate the direction and distance from which dance-followers were attracted to the dancer, we counted the number of individuals attracted in each sector over a range of distances from the dancer. Each set of columns in Fig. 6A–F therefore represents the numbers of dance-followers in a single sector that came in from a particular distance. The distance, in cell widths, across which the dance-followers ran to attend the dance is given on the abscissa. The region over which the phase reversal would be expected to occur is shown by the line below the abscissa stretching from one to seven cell widths away from the dancer. Statistical differences between the columns were tested using a χ^2-test in which $P<0.05$ is taken to be significant. Testing each sector against every other shows that all combinations are significantly different except sector 2 against sector 3 and sector 4 against sector 5.

The proportion of dance-followers that are attracted from close to the dancer (1 cell away) is low in all sectors except for sector 6 (Fig. 6F), and this may represent a special case (see below). The highest number of dance-followers that were

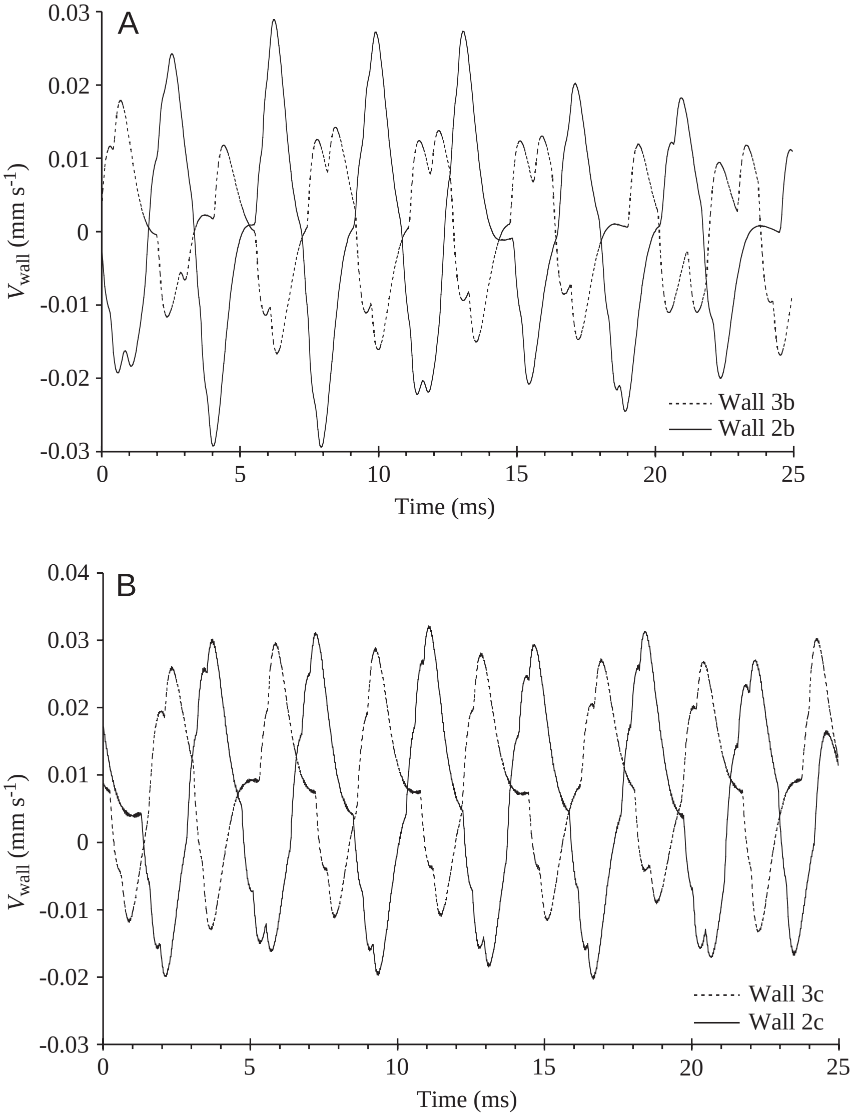

Fig. 4. (A,B) Laser traces of signals from wall 2b to wall 3b (A) and from wall 2c to wall 3c (B) show that the cell walls in rows lying below the point of application of the displacement also exhibit the phase-reversal phenomenon. V_{wall}, displacement velocity.

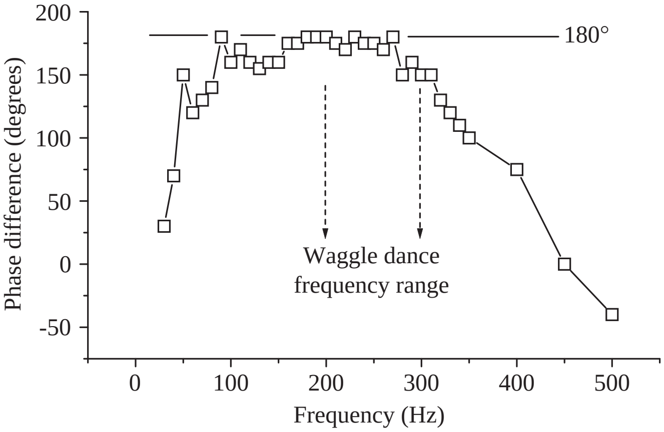

Fig. 5. The dependence of the phase-reversal phenomenon on the frequency of the displacement velocity measured across those cell walls at which the phase reversal occurs. The small phase lead of 30 ° at 30 Hz increases rapidly with an increase in displacement frequency, with a phase lead of 180 ° being maintained from 170 to 270 Hz. The arrows indicate the range of frequencies known to occur during the waggle dance. This coincides with the greater part of the range within which the phase reversal is exhibited.

attracted to the dancer in sector 1 (Fig. 6A) come from 2–3 cell widths from the dancer. Half this number come from four cell widths away and between 0 and 20 % from 6–9 cell widths away. Attraction in sectors 2 and 3 (Fig. 6B,C) are the broadest; here, a relatively large number of dance-followers come from distances of up to five cell widths away from the dancer. Two new aspects emerge from these graphs. The first is that more dance-followers in all sectors (except sector 6) run in to the dancer from a distance of between two and three cell widths from the dancer than from areas closer to or further from the dancer. Second, dance-followers that come in from sectors 2 and 3, which are lateral and slightly ahead of the dancer, also come in from over a greater range of distances than from sectors 1 (almost straight ahead) and 4 (Fig. 6D). Dance-followers in sector 5 (Fig. 6E) are fairly narrowly grouped around the three cell width distance.

Dance-followers in sector 6 (almost directly behind the dancer) do not fit the pattern. Most come from very close (one cell away), and the number attracted falls away with distance. Our suggestion is that these dance-followers could be responding to a different signal (see Discussion).

Discussion

Phase-reversal and recruiting distance

The greater efficiency with which foragers recruit nest mates on empty in comparison with filled and sealed combs (Tautz, 1996) and the overt response of remote dance-followers to dancers (Tautz and Rohrseitz, 1998; Rohrseitz and Tautz, 1999) indicate that some signals caused by the dancing bee are transmitted *via* the comb. The suggestion that these signals could be substratum vibration is supported by the demonstration that low-frequency (200–300 Hz) vibrations are conducted for some distance across the comb (Sandeman et al., 1996; Rohrseitz, 1998) and that vibrational signals do emanate from dancing bees (Nieh and Tautz, 2001).

Our demonstration of the phase-reversal phenomenon leads to the question of whether this phenomenon could play a role in alerting dance-followers to the presence of a dancer with whom they are in neither bodily nor visual contact. In the context of this question, it is important to take into account that the phase-reversal phenomenon was observed under conditions that only approximated those prevailing in the hive. The comb in which it was demonstrated consisted entirely of open cells, the oscillatory input displacements of the walls were continuous, and somewhat larger than, those calculated for a dancing bee and were applied precisely at a right angle to a single point on the rim of the cell wall, and the comb was not loaded with bees.

Nevertheless, if the phase-reversal phenomenon occurs under natural conditions and is being detected by dance-followers, we could expect to measure improved recruitment from those distances and directions where we would expect the phase reversal to occur. This is precisely what we find. Superimposing the histograms of Fig. 6 into a single graph (Fig. 7) shows that the region where we would expect phase reversal to occur corresponds with the distance from which most dance-followers (except from sector 6) run in to attend the dance. Sectors 2 and 3 have a second optimum around 4–7 cell widths from the dancer, suggesting a particularly effective spread of the vibratory signal along these radii in open cell combs.

The recruitment pattern from sector 6 is clearly different and, given that sector 6 lies almost behind the dancer, dance-followers here could be responding predominantly to other signals such as the air movements that have been measured to flow in this direction from the dancer's wings (Storm, 1998; Michelsen, 1999).

Why waggle?

To an observer, the waggle dance is one of the most striking behaviours that take place on the surface of the comb. A large literature has accumulated on the function of the waggle dance in terms of transmitting the distance and direction of the food source to the followers. Why should bees spend the effort in waggling during the straight run of their dance? Two obvious reasons come to mind why this could be useful: (i) that the waggle run distinguishes that part of the dance from the return run and (ii) that the waggle identifies foragers. In the context of the present paper, we would like to propose two additional possibilities.

The first of these is that the waggle may enhance the spread of the 270 Hz vibratory signal by improving the transmission of the signal from the vibrating thorax through the legs and to the walls of the comb (Tautz et al., 1996). The 270 Hz signal is emitted by the bee during that part of the waggle in which the bee is fully laterally displaced (Esch, 1961). The mass of the bee gains momentum through its lateral motion, and the bee must brace itself by gripping the walls of the cells;

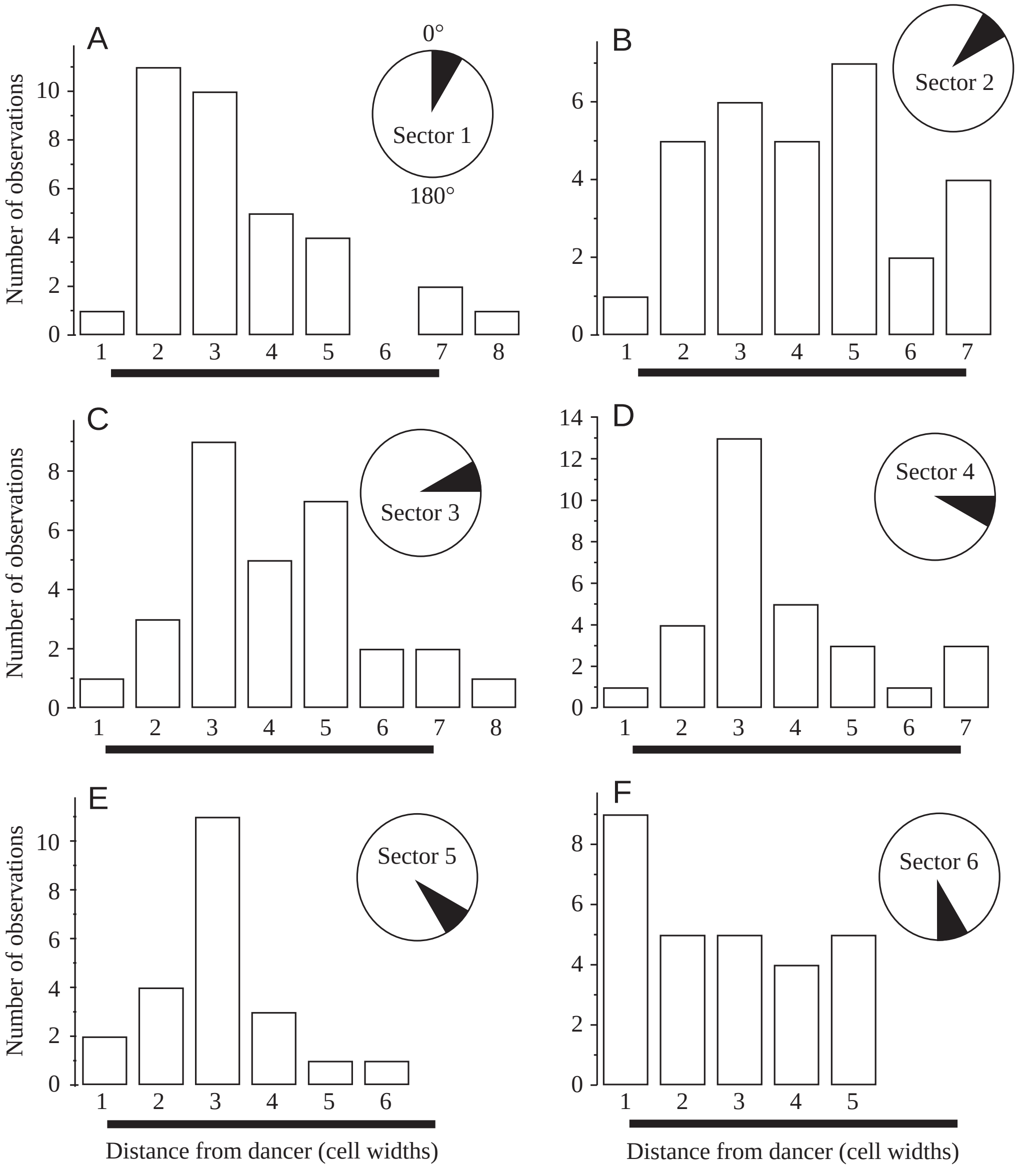

Fig. 6. (A–F) Histograms showing the recruitment of dance-followers. Each part of the figure represents the results from a single sector, indicated in the inset. Each column in each figure shows the number of dance-followers that turned their heads and ran in to follow the dancer. The distance from which they came is indicated along the abscissa. The data were analysed into 5 mm ‘bin widths’ because this represents the approximate width of a single cell. The distance is, nevertheless, given here as ‘cell widths’ to simplify comparison with the previous figures. (A). Sector 1. Most of the dance-followers in this sector are attracted from a distance of 2–3 cells away. *N*=34. (B). Sector 2. The attraction in the 30–60 ° sector is less narrow than that in the 0–30 ° sector, and dance-followers come from distances of 2–5 cells away. *N*=30. (C). Sector 3. Recruitment of dance-followers in this sector is also broadly distributed over distances ranging from three to five cells. *N*=30. (D,E). Sectors 4 and 5. Dance-followers in these two sectors are recruited predominantly from the 2–4 cell distance. *N*=30 (D), *N*=22 (E). (F). Sector 6. Most of the dance-followers in this sector are recruited from close behind the dancer, suggesting the presence of an attractant in addition to, or in place of, substratum vibratory signals. *N*=28. The line parallel to the *x* axis indicates the range over which phase reversal would be expected to occur.

otherwise, it would fall over. Storm’s (Storm, 1998) elegant calculations of the forces exerted by a bee on the rims of the cell walls show that it is both pulling and pushing with its mesothoracic legs, but mainly pulling with its prothoracic and metathoracic legs. The maximum application of force occurs at the peak of the lateral excursion of the waggle. As a result, the bee has optimised the conditions for ‘injecting’ the vibratory signal in its thorax into the comb through an increase in its effective mass and a stiffened mechanical link (the actively extending or flexing legs) with the walls of the comb. A similar strategy is seen in bees that transmit the 370 Hz ‘piping’ signals across the comb by pressing their vibrating thoraces down against the rims of the cells (Esch, 1964; Pratt, 1996). If the above is true, then recruitment of followers to the dancing bee would be expected to be better in those areas of the comb that lie to the side and slightly in front of the waggling bee because it is these areas that Storm (Storm, 1998) has shown receive the greatest force applied to them during the dance. The behavioural observations confirm that this is the case.

The second possibility concerns the difficulty of using the comb as a transmitting system given that it is loaded with a large number of active bees and probably constitutes a very noisy environment. A frequently used strategy in such systems is to pulse-modulate a tone. Instead of a continuous signal to which receptors quickly adapt, or do not recognise against the noise in the first place, the tone is represented as a ‘flashing’ signal that is readily perceived because of the added temporal properties (Ronacher et al., 2000). The 270 Hz signal is indeed pulsed and in phase with the waggle frequency. As such, it would be more

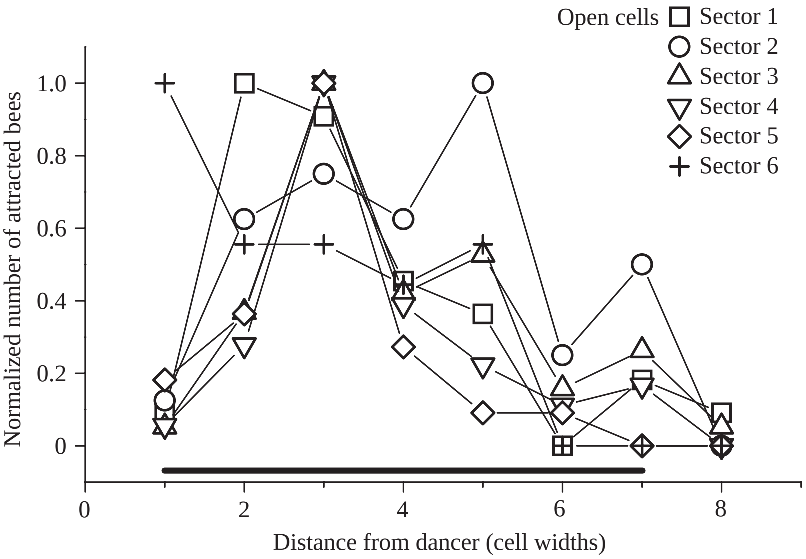

Fig. 7. Results of Fig. 6 superimposed in a single graph in which the data from the different sectors have been normalised by expressing the columns in each sector (which record the numbers of dance-followers) as a percentage of the tallest column in that sector. This figure shows that a high percentage of dance-followers from all sectors are recruited from a distance of three cell widths from the dancer. The distribution of percentages over all sectors varies, with sectors 1 and 2 being the broadest. The recruitment of dance-followers from sector 6 suggests that other factors are at work. The line parallel to the *x* axis indicates the range over which phase reversal would be expected to occur.

easily distinguished against the general background noise in the comb, which is relatively continuous (Nieh and Tautz, 2001).

Alternative hypotheses

The mechanical properties of honeycomb are complex, and the response of such a system to imposed vibration is certainly affected by many variables. We have shown that, under conditions that approximate the natural situation, the vibratory signal that spreads along a row of cells undergoes an abrupt phase shift after it has been transmitted across a number of cells. We also show that, in the natural situation, the majority of the ‘remote’ dance-followers (i.e. those not in immediate contact with the dancer) come from the region where we would predict a phase reversal to occur. A conclusion that can be drawn from this is that the two are causally related, a hypothesis that could be tested in the natural situation by, for example, jamming the natural signals with imposed comb vibrations. If there is no causal relationship between the phase reversal and the recruitment of the remote bees, then one is faced with explaining why the majority of the remote bees come from this particular distance and direction.

Vibratory comb signals emanating from a dancing bee are certainly not the only clue used by dancers to advertise their presence. A dancing bee raises her temperature (Stabentheiner and Hagmüller, 1991), which would make her immediately identifiable to her nest mates that are close to her but not to those that are remote. The increased temperature of the dancer would also increase the rate at which volatile substances accumulated at the food site would diffuse from her body into her neighbourhood. Such odours would be spread relatively broadly by diffusion and by air currents generated by the vibrating wings. However, neither a temperature increase nor the diffusion of odours would explain the distribution of the recruited bees that has been recorded. Indeed, odour diffusion would be expected to be concentrated behind the animals because air currents from the vibrating wings are directed there and not to the side and in front of the dancers. Air currents themselves are a possibly potent source of information but, again, particularly for followers that are situated behind the dancer (Michelsen, 1999).

Dancers will use every means they have at their disposal to attract dance-followers and may employ different strategies in different combinations to suit different situations. Our demonstration of the phase-reversal phenomenon in the vibratory signals in the comb suggests that substratum vibrations cannot be ruled out as a communication pathway simply because their amplitude appears to be too small.

This paper is dedicated to the memory of Jesper Storm, friend, colleague and scientist, and we thank him for permission to use the material from his thesis. We thank Thomas Fischer and Imen Djemai for help with the experiments. The DFG (Ta 82/7-2) and the Procope-program of the EU provided financial support.

References

Esch, H. (1961). Über die Schallerzeugung beim Werbetanz der Honigbiene. *Z. Vergl. Physiol*. **45**, 1–11.

Esch, H. (1964). Beiträge zum Problem der Entfernungsweisung im Schwänzeltanz der Honigbiene. *Z. Vergl. Physiol*. **48**, 534–546.

Michelsen, A. (1999). The dance language of honeybees: recent findings and problems. In *The Design of Animal Communication* (ed. M. D. Hauser and M. Konishi), pp. 111–113. Cambridge, MA, London: MIT Press.

Michelsen, A., Kirchner, W. H. and Lindauer, M. (1986). Sound and vibrational signals in the dance language of the honey bee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **18**, 207–212.

Nieh, J. and Tautz, J. (2001). Behaviour-locked signal analysis reveals weak 200–300 Hz comb vibrations during the honeybee waggle dance. *J. Exp. Biol.* **203**, 1573–1579.

Pratt, S. (1996). Worker piping associated with foraging in queenright colonies. *Apidologie* **27**, 13–20.

Rohrseitz, K. (1998). Biophysikalische und ethologische Aspekte der Tanzkommunikation der Honigbienen (*Apis mellifera carnica* Pollm.). PhD thesis, Universität Würzburg.

Rohrseitz, K. and Tautz, J. (1999). Honey bee dance communication: waggle run direction coded in antennal contacts? *J. Comp. Physiol.* **184**, 463–470.

Ronacher, B., Krahe, R. and Hennig, R. M. (2000). Effects of signal duration on the recognition of masked communication signals by the grasshopper *Chorthippus biguttulus*. *J. Comp. Physiol.* **186**, 1065–1072.

Sandeman, D. C., Tautz, J. and Lindauer, M. (1996). Transmission of vibration across honeycombs and its detection by bee leg receptors. *J. Exp. Biol.* **199**, 2585–2594.

Seeley, T. and Morse, R. (1976). The nest of the honey bee (*Apis mellifera* L.). *Insectes Sociaux* **23**, 495–512.

Spangler, H. G. (1991). Do honey bees encode distance information into the wing vibrations of the waggle dance? *J. Insect Behav*. **4**, 15–20.

Stabentheiner, A. and Hagmüller, K. (1991). Sweet food means 'hot dancing' in honeybees. *Naturwissenschaften* **78**, 471–473.

Storm, J. (1998). The dynamics and flow field of the wagging dancing honeybee. PhD thesis, Odense University, Denmark.

Tautz, J. (1996). Honeybee waggle dance: recruitment success depends on the dance floor. *J. Exp. Biol.* **199**, 1375–1381.

Tautz, J. and Lindauer, M. (1997). Honeybees establish and maintain specific sites on the comb for their waggle dances. *J. Comp. Physiol*. **180**, 537–539.

Tautz, J. and Rohrseitz, K. (1998). What attracts honeybees to a waggle dancer? *J. Comp. Physiol.* **183**, 661–667.

Tautz, J., Rohrseitz, K. and Sandeman, D. C. (1996). One-strided waggle dance in bees. *Nature* **382**, 32.

Thom, C., Seeley, T. and Tautz, J. (2000). A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers *versus* individual foraging activity. *Apidologie* **31**, 737–738.

von Frisch, K. (1967). *The Dance Language and Orientation of Bees* (translated by L. E. Chadwick). Cambridge, MA: Belknap Press of Harvard University Press.

Wenner, A. M. (1962). Sound production during the waggle dance of the honey bee. *Anim. Behav.* **10**, 79–95.