Decoding information in the honeybee dance: revisiting the tactile hypothesis

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The waggle dance of honeybees, *Apis mellifera*, is one of the most remarkable communication systems of the animal kingdom. In this study, we focused on a major gap in the understanding of this striking behaviour: the question of the sensory modality by which meaningful information is transferred from dancers to followers. We revisited the hypothesis that tactile stimuli convey information about the direction relative to gravity and the length of the waggle phase. It has long been suggested that followers use tactile stimuli arising from the wagging movements of a dancing bee to decode information in the dance. Yet, the questions of whether and to what extent such movements are mapped to the tactile experience of the followers have never been resolved. Using high-speed video techniques, we found that the higher the number of the dancer's wagging movements, the higher the number of the followers' antennal deflections. We also documented that most followers faced the dancers laterally and experienced a fairly regular pattern of tactile stimuli; a much smaller proportion of followers faced the dancers from behind and became the subject of a different, although still regular, pattern of tactile stimuli. From these observations, we conclude that tactile mechanosensory input from the antennae, presumably processed by neurons of the antennal joint hair sensilla and the neck hair plates, enables bees to estimate both the direction relative to gravity and the length of the waggle phase.

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Karl von Frisch (1946) discovered that a highly stereotyped motion pattern that honeybees, *Apis mellifera*, perform on the comb surface conveys to human observers the circular coordinates of relatively well-defined locations where food or a potential new nest site is found. The term 'waggle dance' denotes a form of this pattern that conveys information about goals located fairly far from the hive (von Frisch 1967). It enables a colony to coordinate the activity of its members during foraging and nest site selection (Seeley 1995). This is possible because those colony members that keep close contact with a dancing bee, called dance followers, detect a variety of signals emitted by the dancer and process them in such a way that their ensuing behaviours depend greatly upon the content of these signals (von Frisch 1967).

Several components of the waggle dance are thought to convey spatial information (e.g. von Frisch 1967; Seeley et al. 2000; Michelsen 2003). For a human observer, such information is encoded in the waggle phase (von Frisch 1967; Tautz et al. 1996). In this phase, a dancing bee moves forward on the comb surface and waggles its body from side to side at about 13 times/s. Each of these lateral displacements is called a 'wagging movement'. The average orientation of the dancer's successive waggle phases relative to the direction of gravity approximates the angle between the direction towards the goal and to that of the azimuth of the sun. Also, the average number of wagging movements of such waggle phases approximates, and increases with, the distance to the goal (von Frisch 1967).

Evidence indicates that followers can estimate the direction of and distance to the dancer's goal using the direction relative to gravity and the number of wagging movements of the waggle phase, respectively (von Frisch 1967). More than six decades after von Frisch's (1946) original report, however, the process of decoding spatial information in the dance remains obscure. We still do not know how followers estimate the orientation and length of the waggle phase, or how the behaviour of a dancer is actually mapped to that of its followers (see De Marco et al. 2008).

We know that followers detect three-dimensional fields of air particle oscillations and narrow jet air flows produced by a dancer's vibrating wings (Michelsen et al. 1987; Kirchner & Towne 1994; Michelsen 2003), and that they probably use these stimuli to gather meaningful information from dancers. Most likely, they use tactile mechanosensory input from their physical contacts with the dancer too (von Frisch 1967; Božič & Valentinčič 1991; Rohrseitz & Tautz 1999). Here, we refer to the latter hypothesis as the tactile hypothesis. Initially suggested by von Frisch (1967), it is consistent with the fact that the waggle phase involves vigorous body movements by the dancer, and that the followers stand so close to the dancer's body that they frequently touch it. Therefore, they may be exposed to meaningful mechanosensory input.

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Although evidence has been reported supporting the view that followers use tactile stimuli arising from the wagging movements of the dancer (e.g. von Frisch 1967; Božič & Valentinčič 1991; Rohrseitz & Tautz 1999), interpreting such evidence has not been straightforward. This is because the questions of whether and to what extent the wagging movements of a dancing bee are tactilely transmitted to the followers have never been resolved. In this study, we asked whether there is a correlation between the dancer's wagging movements and the pattern of antennal deflections of the followers. Also, we present information about how followers orient themselves towards dancers, which would prove useful for characterizing the sensory experience of the followers in further studies.

METHODS

The experiments were performed during the summer (July−August) and comply with the *Principles of Animal Care* (publication No. 86-23, revised 1985) of the National Institutes of Health and the corresponding current national laws. We used a colony of *A. m. carnica* bees housed in a two-frame observation hive. We trained bees to forage on an outdoor feeder placed at 135, 248 or 360 m from the hive. The feeder offered unscented 1.8 mol/litre sucrose solution. All bees foraging on the feeder were marked with number tags. Observers at the feeder and the hive communicated through walky-talkies and confirmed the identity of each bee both at the feeder and at the hive. At the hive, the waggle dances of the marked bees foraging on the feeder were filmed at 125 and 250 frames/s under red illumination (ca. 630−690 nm). Most of the recordings were done at 125 frames/s, a frame rate that was appropriate for our analyses and helped to optimize the rate of information transfer from the camera to the computer in which the digital videos were stored. Data were obtained by analysing the video recordings in slow motion. We analysed the behaviour of 33 dancers and 398 followers involved in 40 dances (see Table 1). We defined a follower as a bee that moves along with a dancer and faces it at a maximum distance of one cell width. This definition excludes 'attendees', bees that are close to the dancer but do not follow the dance manoeuvres (von Frisch 1967; Božič & Valentinčič 1991; Božič & Abramson 2003; our definition neither implies that followers are

successfully decoding the information in the dance, nor that they will become recruited to the food source being exploited by the dancer).

We focused on four behavioural variables: (1) the waggle phase duration (in ms), defined as the time elapsed between the beginning and the end of all the wagging movements of a waggle phase; (2) the return phase duration (in ms), defined as the time elapsed between two consecutives waggle phases; (3) the number of wagging movements occurring in the waggle phase; and (4) the oscillation period (in ms), defined as the time interval between when the body of a wagging bee moves from one side to the other, and vice versa.

In quantifying the behaviour of the followers, we defined three 'zones' of analysis relative to the body of a dancing bee: 1, 2 and 3 (Fig. 1). According to this criterion, 'Zone 1' (henceforth, Z1) denotes the area surrounding the dancer's head and thorax, 'Zone 2' (henceforth, Z2) denotes the area corresponding to the sides of the dancer's abdomen, and 'Zone 3' (henceforth, Z3) denotes the area behind the tip of the dancer's abdomen. An active follower is thus said to be in 'Zone 1' (henceforth, Z1-bee) when it faces the dancer's head or thorax, in 'Zone 2' (henceforth, Z2-bee) when it faces either one side or the other of the dancer's abdomen, and in 'Zone 3' (henceforth, Z3-bee) when it faces the tip of the dancer's abdomen.

To quantify the tactile stimuli arising from the wagging movements of a dancing bee, we recorded the physical contacts between the followers' antennae and the dancer's body at the end of each wagging movement. For each follower, we measured the angle between the scapes of the antennae, in degrees, as the angle formed by the directions of the left and right scapes (henceforth, S−S angle). Next, we calculated: (1) the deflections of the antennae occurring in the waggle phase, in degrees, as the difference between two consecutive S−S angles; (2) the magnitude of such deflections, in degrees, as the average of all the antennal deflections recorded in the waggle phase; and (3) the variability of the deflections, in degrees, as the mean standard error from all of the antennal deflections recorded in the waggle phase.

We also examined the pattern of antennation of each follower. To this end, we first recorded whether the follower touched, or did not touch, the body of the dancer at the end of each of its consecutive wagging movements. If the follower did touch the dancer, then we recorded whether it did so with one or both antennae. In the present context, a 'simultaneous' pattern of antennation refers to a situation in which the follower first touches the dancer with both antennae at the end of any given wagging movement, and then loses contact with it at the end of the next wagging movement. By contrast, an 'alternate' pattern of antennation refers to a situation in which the follower first touches the dancer with only one antenna at the end of any given wagging movement, and then touches it only with the contralateral antenna at the end of the next wagging movement.

To examine further the behaviour of the followers in the waggle and return phases, we also measured the body orientation of each follower in both phases (in degrees), as the angle formed by the body axes of the follower and the dancer (the body axis being an imaginary line crossing a bee's head and thorax). We depict these angles clockwise relative to the body axis of the dancer, where $0°$ corresponds to the situation in which the follower faces the dancer's head, so that both body axes are aligned with each other in the same direction. We also measured the number of followers involved in both phases, as the mean number of bees surrounding the dancer both in the waggle and in the return phases. These measurements took place every 120 and 240 ms in the waggle and the return phases, respectively. We used a time interval of 120 ms for the waggle phase recordings because we aimed to measure the above two variables at least twice during the waggle phase, on the

Table 1
Number of recorded bees and dances, together with mean values ± SEM of various dance parameters and the number of followers

	Distance (m)		
	135	248	360
Dancers	8	17	8
Dances	9	20	11
Waggle phases	30	35	20
Return phases	21	15	9
Followers	116	162	120
Waggle phase duration (ms)*	338.5±30.7	426.6±23.9	658.7±42.2
Wagging movements †	10.1±0.7	12.8±0.7	19.7±1.0
Period (ms)‡	69.3±1.4	68.1±1.1	67.9±2.0
Return phase duration (ms)§	1370.1±51.5	1319.1±100.1	1821.3±126.7
Followers during waggle phases**	5.4±0.2ᵃ	5.1±0.3ᵃ	6.5±0.4ᵇ
Followers during return phases††	5.3±0.3	5.5±0.3	6.4±0.4

* Linear regression: $F_{1,31} = 36.3$, $P < 0.001$, $r^2 = 0.54$; slope: $160.1 ± 23.6$.
† Linear regression: $F_{1,31} = 42.5$, $P < 0.001$, $r^2 = 0.58$; slope: $4.8 ± 0.7$.
‡ Linear regression: $F_{1,31} = 0.34$, $P = 0.6$.
§ Linear regression: $F_{1,21} = 7.2$, $P = 0.01$, $r^2 = 0.26$; slope: $210.0 ± 78.0$.
** One-way ANOVA (different lowercase letters indicate Tukey's multiple comparisons: $P < 0.05$): $F_{2,82} = 5.04$, $P = 0.008$.
†† One-way ANOVA: $F_{2,42} = 2.74$, $P = 0.07$.

(a) Zones   (b) Waggle phase   (c) Return phase
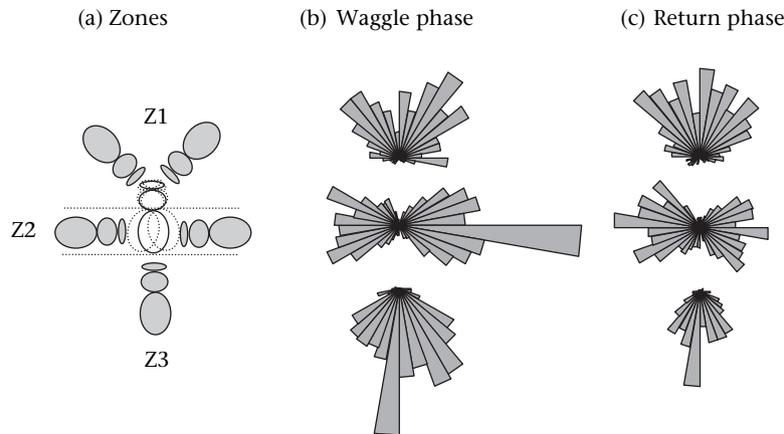
Figure 1. (a) The three 'zones', Z1, Z2 and Z3, used to quantify a follower's position relative to a dancer's body. 'Z1' denotes the area surrounding the dancer's head and thorax, 'Z2' the area corresponding to the sides of the dancer's abdomen, and 'Z3' the area behind the tip of the dancer's abdomen. (b, c) The orientation with respect to the dancer's body of followers found within each zone during (b) the waggle phase, measured every 120 ms, and (c) the return phase, measured every 240 ms (see Methods). In the waggle phase (b), the followers' mean orientation $\pm$ SEM was $7.1 \pm 2.7°$ in Z1, $89.9 \pm 1.4°$ in the right side of Z2, $268.8 \pm 2.0°$ in the left side of Z2 and $175.4 \pm 1.6°$ in Z3, whereas in the return phase (c), it was $359.5 \pm 2.6°$ in Z1, $94.8 \pm 2.2°$ in the right side of Z2, $266.3 \pm 2.0°$ in the left side of Z2 and $175.6 \pm 2.2°$ in Z3.

one hand, and the shortest waggle phase recorded lasted 240 ms. The time interval of 240 ms for return phase recordings allowed us to measure the first and second variables at least five times during any given return phase.

Finally, we computed the percentage of Z1-, Z2- and Z3-bees at the start and the end of each waggle phase, as calculated from the total number of followers. We defined the start and the end of a waggle phase as the time of the first and the last wagging movements of the dancer, respectively. Based on the number of bees present within each of the three zones of analysis (Z1, Z2 and Z3), we calculated the percentage of followers that either moved across zones or remained within the same zone in the waggle phase.

We analysed the data using either one- or two-way ANOVAs followed by Tukey's multiple comparisons, linear regressions followed by ANCOVAs, and paired t tests and Kruskal–Wallis tests (Zar 1984). The number of recorded dancers, dances, waggle and return phases and followers are shown in Table 1.

RESULTS

Our analysis first focused on the physical contacts between the wagging body of a dancing bee and the antennae of the followers. We closely examined all of the single antennal contacts of 80 followers that kept their positions within each of the above described zones of analysis (i.e. Z1, Z2 or Z3) during an entire waggle phase (Fig. 1). We separately analysed the data from the followers positioned within each zone, as well as the data from the dances for 135 m, 248 m and 360 m. As expected (von Frisch 1967; De Marco et al. 2008), both the number of wagging movements and the duration of the waggle phase increased together with the distance to the feeder (Table 1). By contrast, the oscillation period did not change across distances (Table 1).

Figure 2 shows examples of the tactile experience of Z1-, Z2- and Z3-bees following waggle phases for 135 m, 248 m and 360 m. The S–S angles of the Z1-bees did not vary systematically throughout the waggle phase, and we found no consistent pattern of either simultaneous or alternate contacts with the dancer (Fig. 2a–c). By contrast, the S–S angles of the Z2-bees did vary systematically throughout the waggle phase. They were maximal when the body of the dancer moved close to the heads of the Z2-bees and minimal when it moved it away from them (Fig. 2d–f).

We thus found a consistent pattern of simultaneous contacts between the Z2-bees and the dancer (Fig. 2d–f). These followers touched the dancer with both antennae each time a wagging movement moved the dancer's abdomen close to their heads and lost contact with the dancer when the next wagging movement moved the dancer's abdomen away from their heads. This pattern of antennation was consistent throughout the entire waggle phase, meaning that it was invariant to the number of wagging movements (Fig. 2d–f). Finally, the S–S angles of the Z3-bees did not vary systematically throughout the waggle phase (Fig. 2g–i). However, we found a consistent pattern of alternate contacts between the dancer and the Z3-bees. These bees touched the tip of the dancer's abdomen alternately with their left and right antennae during most of the waggle phase (Fig. 2g–i). The time elapsed between these alternate contacts did not differ across waggle phases for different distances. The corresponding mean values $\pm$ SEM were 15.6 ± 1.8 ms ($N = 6$), 13.7 ± 0.7 ms ($N = 11$) and 14.2 ± 0.4 ms ($N = 10$) in waggle phases for 135, 248 and 360 m, respectively (Kruskal–Wallis test: $H2 = 0.3$, $P = 0.8$).

We then asked how the number of antennal deflections of a follower correlates with the number of wagging movements of a dancing bee. To answer this question, we first quantified both the number of antennal deflections of each follower and the number of wagging movements occurring in the waggle phase. In quantifying the number of antennal deflections, we took into account their magnitudes, simply because the relationship between the magnitude of any given antennal deflection and the ensuing mechanosensory input remains unknown. Thus, we quantified the number of deflections larger than $10°$, $20°$, $30°$ and $44°$. We used the smallest and the largest mean deflections recorded ($20°$ and $88°$, respectively) as a reference to define such angles. We divided the two reference angles by 2, and used the resultant angles of $10°$ and $44°$ as threshold angles to quantify whether a deflection occurred, or not. For the sake of comparison, we also used intermediate threshold angles of $20°$ and $30°$.

Figure 3 shows the relationship between the number of antennal deflections of the followers and the number of wagging movements of the dancer (Fig. 3a–c). Clearly, there is a positive linear relationship between the numbers of deflections and wagging movements (Fig. 3a–c). This relationship is arguably invariant to the position of the followers relative to the dancer. In Z1-bees, we found significant linear regressions for the four
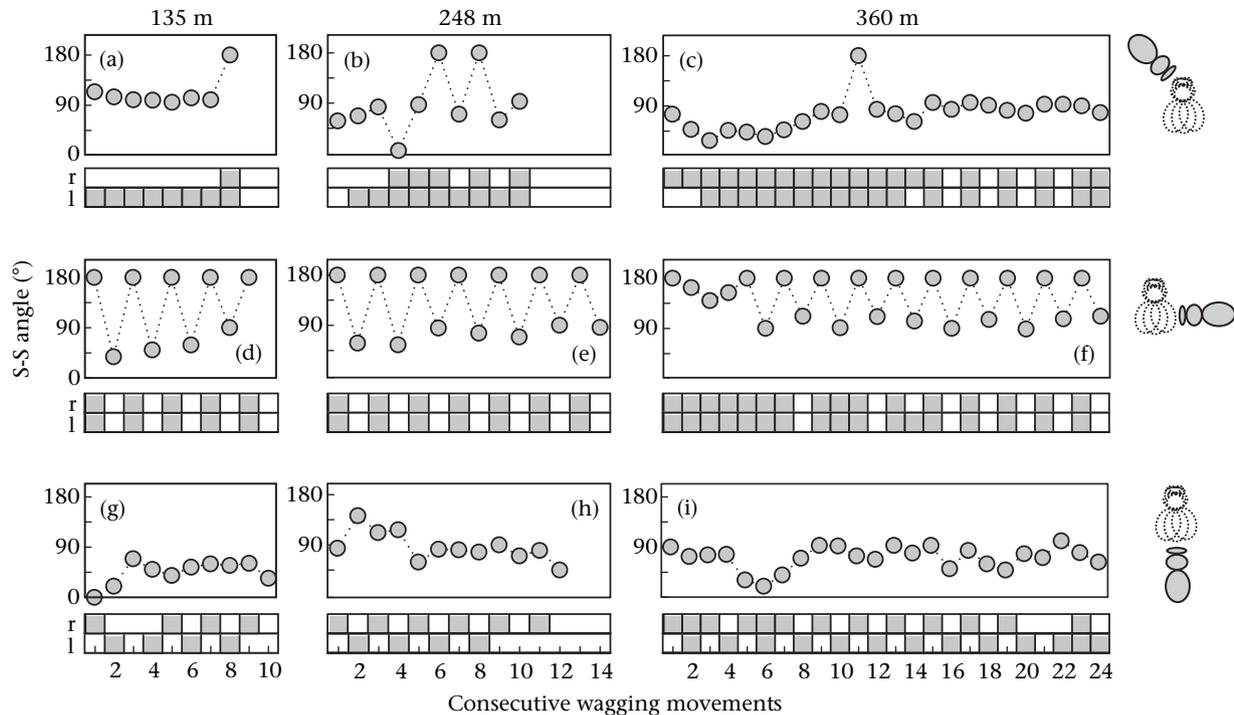
Figure 2. Examples of the observed variation in the angles between the scapes of a follower's antennae (S–S angles, in degrees, circles) throughout the consecutive wagging movements of a dancing bee. The series of grey squares at the bottom of each graph denote the pattern of contacts between the follower's right (r) and left (l) antennae and the dancer's body throughout the entire waggle phase. Each square corresponds to data from a single wagging movement. (a–c) Z1-bees, (d–f) Z2-bees and (g–i) Z3-bees following dances for 135, 248 and 360 m, respectively.

deflection magnitudes, although the slope of the regression line from the deflections larger than 10° was significantly higher than the slopes of the lines from the deflections larger than 20°, 30° and 44° (ANCOVA: $F_{2,76} = 2.8$, $P = 0.04$; Fig. 3a, black versus white, grey and dark-grey circles, respectively). In Z2-bees, we also found significant linear regressions for the four deflection magnitudes. The corresponding slopes varied between 1 and 0.89, and did not differ statistically from each other (ANCOVA: $F_{3,116} = 0.77$, $P = 0.5$; Fig. 3b, black, white, grey and dark-grey circles). Thus, the relationship between the numbers of antennal deflections and wagging movements was fully independent of the deflection magnitude when the followers faced the dancers laterally (Z2-bees). In Z3-bees, we found significant linear regressions for deflections larger than 10°, 20° and 30°, but not for deflections larger than 44°, and the slope of the deflections larger than 10° was significantly higher than those of the other three groups (ANCOVA: $F_{2,75} = 6.8$, $P = 0.001$; Fig. 3c, black versus white, grey and dark-grey circles). Moreover, for deflections larger than 10°, the Z2-bees showed higher slopes than the Z1- and Z3-bees (ANCOVA: Z1 versus Z2: $F_{1,48} = 8.2$, $P = 0.006$; Z2 versus Z3: $F_{1,54} = 17.2$, $P = 0.0001$; Z1 versus Z3: $F_{1,44} = 2.7$, $P = 0.1$; Fig. 3).

Next, we asked how the pattern of either alternate or simultaneous contacts correlates with the number of wagging movements. To answer this question, we quantified the numbers of simultaneous contacts both in Z1- and Z2-bees, and of alternate contacts in Z3-bees. We found a linear relationship between each of these two patterns and the number of wagging movements of the dancer (Fig. 3d–f). This relationship did not change drastically with the position of the followers relative to the dancer, although it seemed less robust in Z1-bees, as seen from the fact that the regression slope of Z1-bees was smaller than those of Z2- and Z3-bees (ANCOVA: Z2 versus Z3: $F_{1,55} = 0.0003$, $P = 0.98$; Z2 versus Z1: $F_{1,49} = 8.4$, $P = 0.006$; Z3 versus Z1: $F_{1,44} = 6.1$, $P = 0.002$; Fig. 3d–f).

We then asked how robust these patterns of antennation actually are. To answer this question, we calculated a 'reliability' index applicable to the numbers of antennal deflections and simultaneous or alternate contacts. We defined this index as '$I = (a/(n - 1))/(b + 1)$', where a corresponds to either the number of antennal deflections ($>10°$) or that of simultaneous or alternate contacts, n corresponds to the number of wagging movements, and b to the number of times in which either the deflections ($>10°$) or the simultaneous or alternate contacts do not occur in the waggle phase. This index has minimum and maximum values of 0 and 1, respectively. The higher its value the higher the fraction of the waggle phase during which the follower experiences the tactile stimuli arising from the wagging movements. First, we calculated the two indices for each follower: one based on its antennal deflections and another on its simultaneous or alternate contacts with the dancer. Next, we averaged the values from the bees positioned within each of the three zones that followed the waggle phases for the three distances (Table 2). We found that the means of the two indices changed across the zones of analysis (Z1, Z2 and Z3), but not across the waggle phases for different distances (Table 2). Both indices gave higher values in Z2-bees than in Z3- or Z1-bees (Tukey's multiple comparisons: antennal deflection: Z1 versus Z2: $P = 0.0001$; Z1 versus Z3: $P = 0.5$; Z2 versus Z3: $P = 0.0001$; simultaneous or alternate: Z1 versus Z2: $P = 0.0001$; Z1 versus Z3: $P = 0.008$; Z2 versus Z3: $P = 0.0008$).

To characterize further the tactile experience of the followers, we also calculated the average magnitude and variability of their antennal deflections (see Methods). We did so in relation both to the position of the followers relative to the dancer and to the distance of the feeder indicated by the waggle phase (Table 3). The average deflection magnitude changed across the zones of analysis (Z1, Z2 and Z3), but not across the waggle phases for different distances (Table 3). The mean magnitude was maximal in Z2-bees
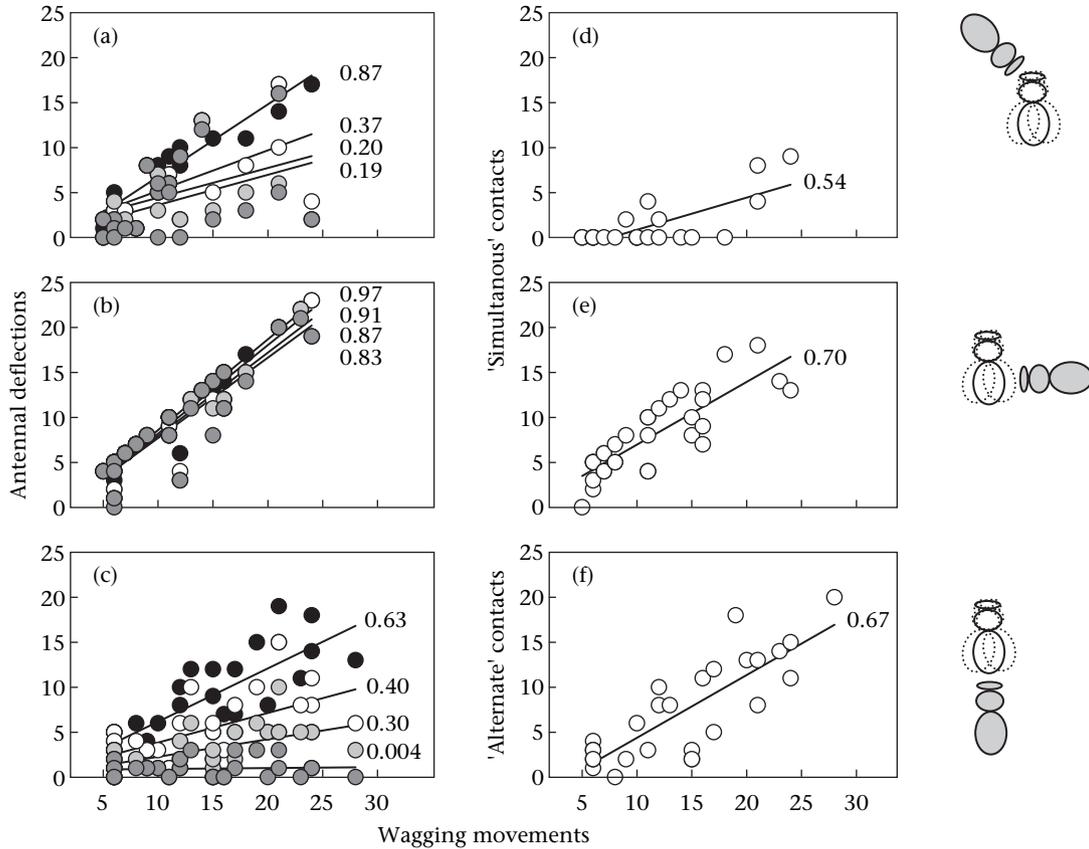
Figure 3. Relationship between the number of wagging movements of a dancing bee and (a–c) the number of antennal deflections of a follower and (d–f) the number of either 'simultaneous' (Z1- and Z2-bees) or 'alternate' (Z3-bees) contacts between the follower's antennae and the dancer's body. (a, d) Z1-bees, (b, e) Z2-bees, (c, f) Z3-bees. In (a–c), the numbers of antennal deflections larger than 10°, 20°, 30° and 44° are represented by black, white, grey and dark-grey circles, respectively. The numbers on the right of the lines correspond to the r^2 values of the linear regressions: (a) 10°: slope = 0.80 ± 0.07, F_{19} = 132.5, P < 0.0001; 20°: slope = 0.45 ± 0.13, F_{19} = 11.36, P = 0.003; 30°: slope = 0.33 ± 0.15, F_{19} = 4.96, P = 0.04; 44°: slope = 0.34 ± 0.16, F_{19} = 4.46, P = 0.04; (b) 10°: slope = 0.88 ± 0.06, F_{27} = 168.9, P < 0.0001; 20°: slope = 0.82 ± 0.07, F_{27} = 131.0, P < 0.0001; 30°: slope = 0.81 ± 0.08, F_{27} = 106.5, P < 0.0001; 44°: slope = 0.73 ± 0.07, F_{27} = 98.45, P < 0.0001; (c) 10°: slope = 0.60 ± 0.09, F_{25} = 43.5, P < 0.0001; 20°: slope = 0.33 ± 0.08, F_{25} = 16.67, P = 0.0004; 30°: slope = 0.20 ± 0.06, F_{25} = 10.8, P = 0.003; 44°: slope = 0.01 ± 0.03, F_{25} = 0.1, P = 0.8; (d) slope = 0.36 ± 0.07; F_{19} = 22.8, P = 0.0001; (e) slope = 0.69 ± 0.008, F_{30} = 70.9, P < 0.0001; (f) slope = 0.69 ± 0.1, F_{25} = 50.9, P < 0.0001.

(88.9 ± 4.6°), intermediate in Z1- (40.7 ± 5.7°) and minimal in Z3-bees (20.8 ± 7.8°; Tukey's multiple comparisons: Z1 versus Z2: P = 0.0001; Z1 versus Z3: P = 0.002; Z2 versus Z3: P = 0.0001). The variability of the deflections changed both across zones and waggle phases for different distances (Table 3). Z3-bees experienced less

variable antennal deflections (4.7 ± 0.4°) than Z1- (9.2 ± 1.2°) and Z2-bees (8.7 ± 0.9°; Tukey's multiple comparisons: Z1 versus Z2: P = 0.8: Z1 versus Z3: P = 0.01; Z2 versus Z3: P = 0.04). Also, such variability was higher in the waggle phases for 135 m and 248 m (8.6 ± 0.9° and 8.2 ± 0.9°, respectively) than in the waggle phases

Table 2
Mean values ± SEM of an index that weighs the reliability of the tactile stimulation arising from a dancer's wagging movements

Distance (m)	Zone	Index	
		Antennal deflections*	Simultaneous or alternate contacts†
135	Z1-bees	0.37±0.1	0.06±0.06
	Z2-bees	0.86±0.06	0.58±0.09
	Z3-bees	0.46±0.1	0.32±0.1
248	Z1-bees	0.58±0.1	0.05±0.03
	Z2-bees	0.83±0.09	0.85±0.09
	Z3-bees	0.49±0.1	0.32±0.06
360	Z1-bees	0.49±0.2	0.16±0.07
	Z2-bees	1±0	0.47±0.1
	Z3-bees	0.19±0.04	0.40±0.09

We applied this reliability index to both the number of antennal deflections and the number of simultaneous or alternate contacts (see Results).
 * Two-way ANOVA: Zone effect: $F_{70,2}$ = 23.4, P < 0.001; distance effect: $F_{70,2}$ = 0.59, P = 0.5; interaction: $F_{70,4}$ = 1.9, P = 0.1.
 † Two-way ANOVA: Zone effect: $F_{71,2}$ = 23.8, P < 0.001; distance effect: $F_{71,2}$ = 0.8, P = 0.4; interaction: $F_{71,4}$ = 2.1, P = 0.08.

Table 3
Mean values ± SEM of the magnitude and variability of the followers' antennal deflections

Distance (m)	Zone	Antennal deflections (°)	
		Magnitude*	Variability†
135	Z1-bees	32.3±7.1	11.2±3.1
	Z2-bees	95.2±6.6	9.3±1.0
	Z3-bees	20.3±3.0	4.9±0.8
248	Z1-bees	46.3±10.7	9.4±1.4
	Z2-bees	84.5±9.2	9.4±2.2
	Z3-bees	23.7±2.8	6.0±0.6
360	Z1-bees	42.2±11.7	6.1±0.6
	Z2-bees	80.1±3.2	5.4±1.0
	Z3-bees	18.2±1.8	3.1±0.2

 * Two-way ANOVA (data log transformed): Distance effect: $F_{70,2}$ = 0.5, P = 0.6; zone effect: $F_{70,2}$ = 60.0, P < 0.001; interaction: $F_{70,4}$ = 0.9, P = 0.4.
 † Two-way ANOVA (data log transformed): Distance effect: $F_{70,2}$ = 4.86, P = 0.01; zone effect: $F_{70,2}$ = 8.44, P = 0.0005; interaction: $F_{70,4}$ = 0.43, P = 0.8.

for 360 m (4.5 ± 0.4°; Tukey's multiple comparisons: 135 m versus 248 m: $P = 0.9$; 135 m versus 360 m: $P = 0.04$; 248 m versus 360 m: $P = 0.02$).

Finally, we asked how the followers distribute themselves around the dancers. Of the 398 bees that we followed (Table 1), 79% followed at least one entire waggle phase, and 40% followed at least one entire return phase. We found that the number of followers was higher in the waggle phases for 360 m than in the waggle phases for 135 or 248 m (Table 1). The number of bees following return phases did not change across the dances for different distances (Table 1). Also, the number of followers did not differ between waggle and return phases, irrespective of the distance to the indicated feeder (paired t test: 135 m: $t_{20} = 0.9$, $P = 0.4$; 248 m: $t_{14} = 0.9$, $P = 0.3$; 360 m: $t_8 = 0.8$, $P = 0.4$).

We also examined how the positions of the followers relative to the dancer changed during the waggle phases (Fig. 4). We observed that most followers (ca. 80%) faced the head (Z1), thorax (Z1) and abdominal sides (Z2) of the dancer at the beginning of the waggle

phase, as seen from the fact that the percentage of Z1- and Z2-bees at the beginning of the waggle phase was significantly higher than that of Z3-bees, irrespective of the waggle phase length, that is, the distance of the indicated feeder (Fig. 4, 'start'). The percentage of Z1-bees and Z3-bees decreased and increased during the waggle phase, respectively, whereas the percentage of Z2-bees (ca. 40%) remained constant. Moreover, the rise in the percentage of Z3-bees that occurred during the waggle phase increased together with the waggle phase length (Fig. 4, 'end'; Tukey's multiple comparisons: 135 m versus 248 m: $P = 0.7$; 135 m versus 360 m: $P = 0.01$; 248 m versus 360 m: $P = 0.02$). The overall distribution of followers between the start and the end of the waggle phase did not seem to differ across waggle phases for different distances (Fig. 4, 'middle'). Of the followers identified as Z1-bees at the beginning of the waggle phase, 25–45% remained within Z1 and 49–65% moved from Z1 to Z2. Of the Z2-bees, 33–49% remained within Z2 and 48–67% moved from Z2 to Z3. Of the Z3-bees, 80–100% remained within Z3 and 0–20% moved from Z3 to Z2.
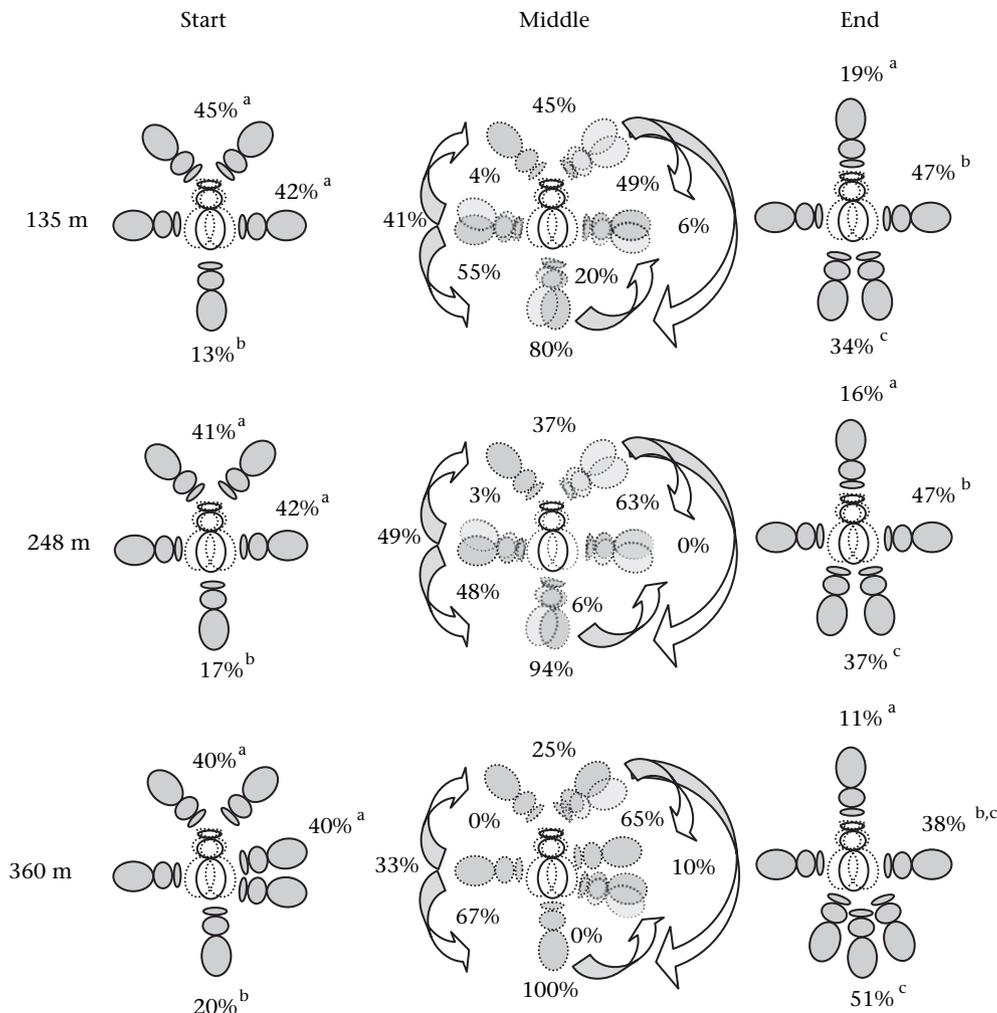
Figure 4. Schematic representation of the followers' positions relative to the body of a dancing bee at the start, the middle and the end of a waggle phase for a goal placed 135 m, 248 m and 360 m from the hive. The start and the end of a waggle phase are the times before the first and the last wagging movement of the dancer, respectively. The percentages of Z1-, Z2- and Z3-bees at the start and the end of each waggle phase were calculated from the total number of followers in dances for the different distances. Lowercase letters denote Tukey's multiple comparisons: $P < 0.05$ after two-way ANOVA: 'start': distance effect: $F_{2,210} = 0.01$, $P = 0.1$; zone effect: $F_{2,210} = 40.0$, $P < 0.0001$; interaction: $F_{4,210} = 0.7$, $P = 0.6$; 'end': distance effect: $F_{2,210} = 0.005$, $P = 0.1$; zone effect: $F_{2,210} = 41.5$, $P < 0.0001$; interaction: $F_{4,210} = 2.8$, $P = 0.03$. Likewise, based on the number of bees present within each of these three zones at the start and at the end of the waggle phase, we calculated the percentage of bees that moved across zones, as well as that of the bees that remained within the same zone during the entire waggle phase ('middle'). For example, 45% of the followers identified as Z1-bees at the beginning of waggle phases from 135 m remained in Z1, while 49% and 6% moved to Z2 and Z3, respectively; 41% of Z2-bees remained in Z2, while 4% and 55% moved to Z1 and Z3, respectively; finally, 80% of Z3-bees remained in Z3, while the remaining 20% moved to Z2.

DISCUSSION

Our work focused on a major gap in the study of dance behaviour and concerned the question of the sensory modality by which meaningful information may be transferred from dancers to followers. We re-examined the hypothesis that it is mechanosensory, or tactile, stimuli that convey information about the orientation and length of the waggle phase. Using high-speed video techniques, we documented how the wagging movements of a dancing bee correlated with the tactile experience of the followers. We found that the higher the number of wagging movements, the higher the number of antennal deflections of the followers (Figs 2, 3), and that the higher the number of wagging movements, the higher the number of either simultaneous or alternate contacts between the followers and the dancer (Figs 2, 3). We also found that the reliability of such a tactile mechanosensory input depended upon the positions of the followers relative to the dancer (Table 2). Most followers faced the sides of the dancer and experienced a fairly regular pattern of tactile stimuli in the waggle phase. The followers that faced the dancer from behind also experienced a regular, although still different, pattern of tactile stimuli (Figs 2–4, Table 2). These findings are in close agreement with previous observations by Rohrseitz & Tautz (1999), who reported that followers touch the body of the dancer with either one or both antennae during most of the waggle phase, and that the ensuing pattern of antennation depends upon their positions relative to the dancer. Followers facing the dancer from the side touch it simultaneously with both antennae more frequently than other followers do. From these observations, Rohrseitz & Tautz (1999) concluded that followers may estimate the waggle phase orientation using tactile stimuli from the dancer's wagging movements. Our results clearly support this view, but because we found a robust correlation between the number of wagging movements and graded components of the tactile experience of the followers, one can conclude that followers may use tactile mechanosensory input to estimate both the direction relative to gravity and the length of the waggle phase.

Božič & Valentinčič (1991) observed that followers positioned perpendicularly to the dancer remain in contact during longer periods than followers positioned behind the dancer, and proposed that followers have to face the dancer laterally to decode information in the waggle phase. Judd (1995), by contrast, suggested that only bees closely following the dancer from behind can successfully decode the spatial information in the dance. Judd (1995), however, observed the behaviour of bees following dances for a feeder located 150 m away from the hive; his measurements, made every 1 s, did not allow him to register the complete pattern of movements of the followers, simply because a waggle phase for such a short distance lasts only fractions of a second (e.g. von Frisch 1967; De Marco & Menzel 2005). Recently, Tanner & Visscher (2009) repeated Judd's experiment with a feeder placed 260 m away from the hive. They found that the recruited bees had previously followed dancers both from the side and from behind (Tanner & Visscher 2009). Moreover, 36% of the recruited bees never followed dancers from behind, while all of them followed dancers from the side, at least for a short time (Tanner & Visscher 2009).

As a rule, followers maintain a close distance between their heads and the body of a dancing bee, so that they touch it both during the waggle and return phases (Figs 2, 4; see also Božič & Valentinčič 1991; Rohrseitz & Tautz 1999; Božič & Abramson 2003). For a follower facing the tip of a dancer's abdomen, to keep such a close distance means to run behind it, but this would never be the case if the follower faces the dancer's head and thorax, or the sides of its abdomen. The absolute position of the followers that face dancers laterally changes only slightly during the waggle phase. It is the dancer that moves past them. As a result, the positions of these followers relative to the body of the wagging bee are passively shifted from the sides towards the tip of the dancer's abdomen (Fig. 4; see also Michelsen et al. 1987; Božič & Valentinčič 1991; Rohrseitz & Tautz 1999). This is why followers appear to move towards the rear of the dancer during the waggle phase (Fig. 4; see also Michelsen et al. 1987; Božič & Valentinčič 1991; Rohrseitz & Tautz 1999; Tanner & Visscher 2009).

Our results document how the pattern of antennation, on the one hand, and the magnitude and variability of antennal deflections, on the other, changed together with the positions of the followers relative to the dancer (Fig. 2, Table 3). The magnitude of the followers' antennal deflections increased when they faced the dancers laterally (Table 3), and the variability of such deflections diminished when they faced the dancers from behind, especially if the waggle phase was long (Table 3). These results indicate that followers can be exposed to different tactile mechanosensory inputs sequentially during the waggle phase, if they face dancers first from the side and then from behind. They also indicate that the complete tactile experience of both successful and unsuccessful followers (i.e. recruited and nonrecruited followers, respectively) has to be resolved in order to speculate further on how followers decode information about the direction and length of the waggle phase.

The tactile hypothesis also concerns the question of whether followers actively touch the dancers, or, instead, whether their antennae are simply hit by the dancers' wagging bodies. We observed that it was the wagging bodies of the dancers that bent the followers' antennae and occasionally even struck the heads of those followers facing them from the sides. These observations are in close agreement with previous reports (Božič & Valentinčič 1991; Rohrseitz & Tautz 1999) showing that the followers' antennal deflections occur passively, owing to the short distance that separates the followers' heads and the dancers' wagging bodies. Yet, we did not observe signs of collision avoidance responses by the followers. On the contrary, they kept a fairly regular distance from the wagging body of the dancer (data not shown). Therefore, the followers' mechanosensory input results from active and passive phenomena occurring simultaneously. It seems that the followers actively regulate the distance between their heads and the dancer's wagging body, and it is this that determines the limits of variation of the available input. Next, their antennae are passively hit by the dancers' wagging bodies.

We still do not know how information about the direction relative to gravity and the length of the waggle dance is transferred from dancers to followers. There is a striking variability of signals associated with the waggle dance: (1) three-dimensional fields of air particle oscillations are produced by the dancers' vibrating wings (Michelsen et al. 1987; Kirchner & Towne 1994; Michelsen 2003); (2) narrow jet air flows are directed behind the dancers and oscillate from side to side with the frequency of their wagging bodies (Michelsen 2003); (3) tactile stimuli originate from the physical contacts between dancers and followers (von Frisch 1967; Božič & Valentinčič 1991; Rohrseitz & Tautz 1999); and (4) substrate-borne vibrations of ca. 12–15 and 200–300 Hz arise from an interaction between the comb structure and the movements of wagging dancers (Tautz 1996; these vibrations do not encode information about the speed and direction of the dancers' movements (Nieh & Tautz 2000), but followers are attracted by them when they face dancers laterally (Tautz & Rohrseitz 1998; Rohrseitz 1998)). The inputs from the three-dimensional fields of air particle oscillations and the narrow jet air flows are presumably processed by neurons of the Johnston's organ (Johnston 1855; Dreller & Kirchner 1993), whereas that of the substrate-borne vibrations is

processed by neurons of the chordotonal organ (Schön 1911; Sandeman et al. 1996). The input from tactile stimuli is probably processed by neurons of the antennal joint hair sensilla and the neck hair plates. Whereas the sensilla gather input from the bees' antennal deflections (Kloppenburg 1995), the neck hair plates are part of the gravity–proprioception system (Lindauer & Nedel 1959; Sandeman et al. 1997). We found that the number of wagging movements of a dancing bee correlates well with the pattern of iterative antennal deflections of the followers (Fig. 3). An integration of such input over time may allow followers to estimate the waggle phase length. If they combine this estimate with information about their own orientation in space, then they may also estimate the waggle phase direction relative to gravity (Rohrseitz & Tautz 1999). The question of whether the processing of information about the direction and length of the waggle phase relies on only one or, instead, on several, albeit parallel pathways in the honeybee brain has not yet been resolved. Together with analyses of the sensory systems and the brain circuitry underlying the honeybee dance (Brockmann & Robinson 2007), further analyses on the behaviour of the followers, both inside and outside the hive, will prove useful to shed light on these issues.

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