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# Displacement activities during the honeybee transition from waggle dance to foraging

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Displacement activities, which are typically locomotory, grooming and object-manipulation behaviours, have been shown to reduce stress. However, their function within the motivational system remains unclear. I tested the hypothesis that displacement activities have a functional role during transitions between motivational states, using the honeybee model system. I observed a number of locomotory and grooming behaviours performed when foraging honeybees returned to the hive to dance. These focal behaviours occurred significantly more frequently during the period of transition from waggle dancing to exiting the hive to forage than during the periods before the waggle dance or during the waggle dance. By contrast, the control behaviour, trophallaxis, was distributed across time periods significantly differently, occurring with equal frequency in all periods. These results are consistent with the hypothesis that displacement activities have a functional role during motivational transitions. Evidence from other species suggests that the most likely function is facilitation, rather than inhibition, of the transition. The wide range of species in which displacement activities have been identified suggests that they are a universal feature of motivational control.

Behaviour plays an important role in the control of motivation (e.g. Timberlake 1993; Dayan & Balleine 2002; Anselme 2008). Wilz (1970a, b, c) hypothesized that displacement activities facilitate motivational transitions. Displacement activities are identified as locomotory behaviours, cleaning behaviours (e.g. grooming) and manipulation of objects (e.g. chewing or carrying), which sometimes are performed rapidly or incompletely, and which occur in the presence of external and/or internal stressors (Tinbergen 1951; Wilz 1970b; Berridge et al. 1999; Troisi 2002; Judge & Mullen 2005; Anselme 2008). Displacement activities reduce stress (Troisi 2002). They also decrease and increase in a dose-dependent manner on application of anxiolytic (stress-reducing) and anxiogenic drugs (Schino et al. 1996). Modern evidence for the anxiolytic function of displacement activities suggests that Wilz's hypothesis that displacement activities facilitate motivational transitions is plausible and should be revisited.

Displacement activities were originally described as 'nonfunctional,' or 'out of context' (Tinbergen 1951). Rowell (1961 and citations therein; see also van Iersel & Bol 1958) modified this view, concluding that underlying low-level stimulation and the duration of motivational equilibrium determine what type and intensity of displacement is displayed. Thus at the level of the discrete behaviour, displacement activities are functional in the sense that the animal is responding to a low-level stimulus. Tinbergen (1951), in calling displacement activities 'out of context', took the point of view of an isolated behavioural sequence within which a displacement activity is unlikely and unnecessary. However, at the level of the interaction (i.e. coincidence in time) between two different motivational states or behavioural sequences (see von Holst & von St Paul 1963), displacement activities are probable and predictable. This suggests they could have a functional role in at least some proportion of motivational transitions, for example, high-stress motivational transitions. Wilz (1970a) found that the number of displacement activities in sticklebacks (*Gasterosteus aculeatus* L.) increased with the number of courtship dances performed, suggesting that displacement activities increase to provide more facilitation as the difficulty to complete the transition from aggression to copulation increases. A weakness of this study was that it lacked a control condition in which individuals were prevented from being able to perform any displacement activities. In general such control conditions are not feasible, since preventing locomotion, object manipulation and grooming, even if possible, would not allow the subjects to perform the other behaviours being studied. Here I used a 'smoking gun' approach (Cleland 2001) to test the hypothesis that displacement activities have a functional role during behavioural transitions in honeybees, *Apis mellifera* L.

I compared the behaviours performed during the transition from dancing to foraging (hereafter 'focal behaviours') to trophallaxis, or nectar-sharing events, which serve as a control (de Marco & Farina

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2001; Wainselboim & Farina 2003). Specifically, I expected to observe behaviours that would (1) predominantly take the form of locomotory, manipulative and cleaning behaviours, (2) have a different distribution than that of trophallaxis and (3) occur more frequently during the transition period than during other periods of activity.

## METHODS

The honeybee dance is easy to observe, is repetitive, occurs between bouts of a different behaviour (foraging) and is well studied, making it a model system for researching displacement activities. After foraging bouts, honeybees communicate to nestmates both the distance and the angle of resources from the sun's azimuth by performing the waggle dance and its variants in the hive, as described by several authors (von Frisch 1967; Seeley 1995; Tautz et al. 1996; Srinivasan et al. 2000; Dyer 2002). Bees known as attendees obtain information from the dance. When each dance is finished, the forager, and possibly one or more attendees, exits the hive to return to or begin foraging, respectively. Trophallaxis, or nectar sharing, occurs throughout a returned forager's time in the hive (de Marco & Farina 2001; Wainselboim & Farina 2003). Trophallaxis was chosen as the control behaviour as it is the only frequent behaviour other than waggle phases performed during returned foragers' visits to the hive, and because it is not a locomotory, object manipulation or grooming behaviour.

Data were obtained from honeybees taking part in training for an unrelated experiment in which the bees flew down a 3 m optic flow channel papered with random checkers to elicit waggle dances in the hive (see Srinivasan et al. 2000), and then were transported to a second 3 m optic flow channel, which they flew down back to the hive (M. Collett, unpublished data). Dances were scattered, but all other aspects of the bees' behaviours were typical of dances performed under standard research conditions. The bees were housed in an outdoor two-frame hive with glass on one side for observational purposes, located in a field near East Lansing, Michigan, U.S.A. The majority of the bees had been marked with individual numbers for the purposes of the unrelated experiment, which allowed them to be identified individually. The bees fed from a single sucrose feeder accessed via a single optic flow channel on 2 September 2001, under sunny conditions at 24 °C. Ambient noise was minimal. Video of dancing in the hive was taken between 1306 and 1350 hours in dim, diffuse light conditions provided by a white cloth tent erected over the hive and camera, using a Sony Digital 8 Handicam on Fujifilm Hiwa8 P6 cassettes, and transferred to high-quality videotape. I observed individual bees continuously on video playback, using the frame-by-frame function, and transcribed their behaviours. All behavioural sequences that were not observed in totality (from the bee's entrance into the hive to its exit from the hive) or that contained no dances were omitted from the analysis, yielding a total of 73 behavioural sequences.

The transcribed behavioural sequences were divided into three temporal categories: Entrance, Dance and Transition. Entrance was defined as behaviours occurring before the first observed waggle phase. Dance was defined as the behaviours between (and including) the first waggle phase and the last observed waggle phase. Transition included those behaviours that were observed after the last observed waggle phase. The number of behaviours (see Table 1) in each temporal category was recorded. As a control, the number of trophallaxis events in each category was recorded. No behaviours other than dance phases, trophallaxis and the focal behaviours described in Table 1 were observed. To reduce pseudoreplication, I calculated a single average distribution of behaviours for each marked bee and a single average for all unmarked bees over the temporal categories. This resulted in a sample size of 21 individuals (20 marked bees and an unknown number of unmarked bees averaged together). A two-sample, one-tailed Kolmogorov–Smirnov test for unequal sample sizes was used to compare the focal and control behaviours' distributions across temporal categories (Siegel 1956). Siegel (1956) notes that the Kolmogorov–Smirnov test for unequal sample sizes is conservative. The test was repeated excluding the composite datum from unmarked bees, with no resulting difference. This datum was therefore retained for all calculations. The entry for each category was rounded to the nearest integer during the calculation of the Kolmogorov–Smirnov test. A chi-square value was calculated and the significance was assessed using $df = 2$, following Siegel (1956). To test in which temporal categories focal behaviours and trophallaxis occurred more, I used the signed-ranks test with a correction for multiple comparisons (Siegel 1956).

## RESULTS

The distribution of focal behaviours between temporal categories differed significantly from that of the control behaviour, trophallaxis (Kolmogorov–Smirnov two-sample test, one-tailed, unequal sample sizes: $\chi^2_2 = 8.567$, $P < 0.01$; Fig. 1). Focal behaviours occurred disproportionately in the Transition category (Entrance versus Transition, Wilcoxon signed-ranks test: $T = 0$, $N = 21$, $P < 0.0001$; Dance versus Transition: $T = 0$, $N = 21$, $P < 0.0001$; Bonferroni correction: accept $P < 0.025$ as significant). Trophallaxis was distributed evenly between the Dance and Transition categories (Entrance versus Transition: $T = 3$, $N = 10$, $P = 0.005$; Dance versus Transition: $T = 36$, $N = 9$, $P > 0.025$; Bonferroni correction: accept $P < 0.025$ as significant). Of the 73 sampled behavioural sequences, only four (5.5%) had no observed focal behaviour in the Transition category. Of the seven observed types of focal behaviours (Table 1), two involved cleaning (foreleg and hindleg grooming), four involved locomotion (exaggerated walk, climb over, direct walk and wander) and one fit in neither category (lone waggle), showing a majority of cleaning and locomotory behaviours as predicted. No behaviours of the remaining common type of displacement activity, object manipulation, were observed. Bees performed a mean ± SE of 12.6 ± 1.52 waggle phases per visit to the hive.

## DISCUSSION

Consistent with the hypothesis that displacement activities have a functional role during motivational transitions, honeybees

**Table 1**
Ethogram of focal behaviours

| Behaviour | Appearance |
|---|---|
| Foreleg grooming | Bee moves forelegs while stationary; forelegs and possibly antennae are groomed |
| Hindleg grooming | Bee moves abdomen in a jerky circular motion while stationary; hindlegs are cleaned against one another |
| Exaggerated walk | Bee moves forward in an agitated, swaggering manner, but does not perform waggling |
| Climb over | Bee raises its forelegs over the bodies of surrounding bees and usually proceeds to climb onto surrounding bees and walk over them |
| Direct walk | Bee walks rapidly in a relatively straight line |
| Wander | Bee walks for an extended period, neither towards the dance floor nor towards the hive entrance |
| Lone waggle | Bee performs an isolated waggling not at the angle of the waggles in the dance |