



Displacement activities during the honeybee transition from waggle dance to foraging

Meredith Root-Bernstein*

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, U.S.A.

ARTICLE INFO

Article history:

Received 17 June 2009

Initial acceptance 3 August 2009

Final acceptance 12 January 2010

Available online 19 February 2010

MS. number: A09-00396R

Keywords:

Apis mellifera
displacement activity
honeybee
trophallaxis
waggle dance

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.

© 2010 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

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

* Correspondence and present address: M. Root-Bernstein, Department of Ecology, Pontificia Universidad Católica de Chile, Avenida Libertador Bernardo O'Higgins 340, Santiago, Chile.

E-mail address: mrootbernstein@bio.puc.cl

2001; Wainseboim & 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 nest-mates 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; Wainseboim & 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 \pm 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

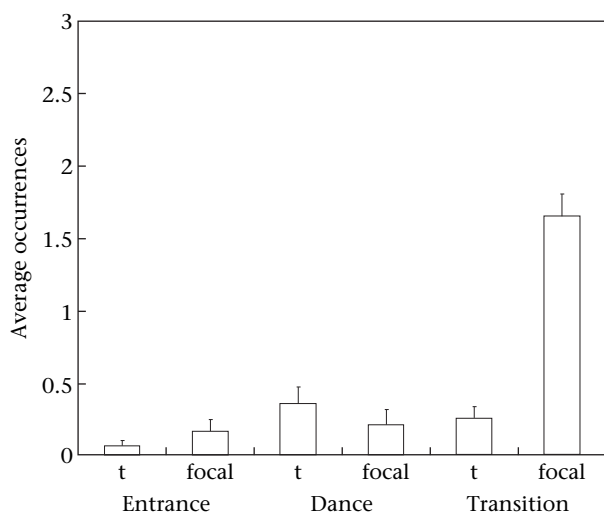


Figure 1. Mean \pm SE occurrences of trophallaxis (t) and focal behaviours for each temporal category.

transitioning between dancing and returning to forage performed grooming and locomotory behaviours, which occurred significantly more during the transition period than during other periods, unlike the control behaviour, trophallaxis.

The focal behaviours observed in this study may also be functional at the level of discrete behaviours. For example, foreleg and hindleg grooming behaviours may be a result of becoming dirty during or prior to the dance, and being clean may aid flight during foraging. A common misunderstanding is that if a displacement activity is functional at the level of a discrete behaviour (i.e. the displacement behaviour is a response to an external stimulus), it cannot also have a function at the level of the interaction between motivational states or behavioural sequences. However, this assumption does not stand up to the evidence. Displacement activities reduce stress (Troisi 2002), which shows functionality at the motivational level. Displacement activities also respond to background stimulation (van Iersel & Bol 1958; Rowell 1961 and citations therein). The results of the present study provide positive evidence for a role specifically during a motivational transition. Why or how responses to low-level stimulation that elicit locomotion, grooming and object manipulation also reduce stress associated with changing motivations is a separate, and interesting, question.

An interesting issue is the rarity of displacement activities in the Entrance period, which corresponds to the transition between foraging and dancing. There are two possible explanations. One explanation is that the foraging–dancing transition may be a low-stress transition. This could be the case because this transition coincides with many signals from the environment, such as signals associated with arriving at the hive, and honeybees might associate such signals with the transition to dancing, facilitating waggle dancing (Timberlake 1993). The dancing–foraging transition, by contrast, does not coincide with the appearance of external cues, and thus, may be more stressful. The other possibility is that displacement activities do occur, but they occur before the bee has arrived on the comb, in which case I would not have observed them.

There were not enough examples of each type of observed focal behaviour to test whether there were patterns in the occurrence of each type. Some evidence suggests that different types of displacement activities may have different proximate effects, but redundant functions. Berridge et al. (1999), for example, prevented the expression of chewing, a displacement activity, in stressed mice by removing chewable objects from the test arena, but the subjects showed a significant rise in grooming compared to stressed mice

that were allowed to chew objects. Mice that were allowed to chew showed less stress-related dopaminergic activation in the prefrontal cortex than mice that were prevented from chewing. Grooming does not appear to attenuate this prefrontal cortex dopaminergic response, but the authors concluded that the replacement of one displacement activity by another suggests that grooming has some other anxiolytic effect not measured in the study (Berridge et al. 1999).

In this study I did not specifically test whether the functional role proposed for displacement activities is facilitatory, as hypothesized by Wilz (1970a). Is there another plausible function of anxiolytic behaviours? There is evidence that stress can be an inhibitor or a facilitator of behaviour. Animals that are very neophobic (stressed by novelty) tend to perform less exploratory behaviour, learn less quickly and are slower to approach stimuli and consume foods in novel environments than less-neophobic individuals (Mettke-Hofmann et al. 2005; Boogert et al. 2006; M.R.-B., personal observation), while stressed rats may show either enhancement or reduction in sexual behaviours, depending on the stressor (Freidin & Mustaca 2004). Neophobia results from conflicting motivations as a response to novelty, and thus provides more relevant evidence for the case of motivational transitions than reactions to external stimuli such as pain, which are used as stressors in studies of rat sexual behaviour. These findings suggest that a reduction in stress during motivational transitions is likely to have a facilitating, rather than an inhibiting, function. A key question is whether subjects induced to perform more displacement activities than they would normally perform for a given stress level would also show a decrease in time to transition between motivational states. Affiliative grooming in aroused baboons (*Papio hamadryas hamadryas* L.), which could be interpreted as 'extra' displacement-like grooming, reduced the incidence of displacement activities by the subject in the following time period, compared to individuals that did not affiliate (Judge & Mullen 2005), suggesting that increased displacement-like feedback leads to a more rapid decrease in arousal. This result is consistent with the interpretation that increases in displacement activities facilitate changes in motivational state.

There are few other examples of displacement activities in invertebrates. Among arthropods, Gordon (1955) described displacement feeding in four species of crab, and Bastock & Manning (1955) noted grooming behaviours in male *Drosophila melanogaster* that they suggested could be a form of displacement, but they presented only anecdotal evidence. Displacement activities have been observed in vertebrates such as fish (e.g. Wilz 1970a), birds (e.g. van Iersel & Bol 1958; Rowell 1961) and mammals (e.g. Berridge et al. 1999; Judge & Mullen 2005). This wide taxonomic range suggests that displacement activities are a universal feature of motivational control.

To my knowledge, displacement activities have not previously been reported in the dance–foraging transition of honeybees. The ability to alternate between behavioural modes and tasks, such as foraging and dancing, is an important aspect of honeybee behaviour (Seeley 1995). There is wide variation in dance durations (measured as number of waggle phases) to a single source, 26% of which is due to variation within bees (Seeley 1995). A possible application of the behaviours observed here is to use them as indicators of individual foragers' stress under different foraging, nectar unloading or dancing conditions, which could be used to further understand the relationship between individual behaviours and coordination at the hive level.

Acknowledgments

I thank Matthew Collett for the use of his video and Jim Gould for comments on versions of this research, statistical advice and

equipment. Helpful and stimulating comments by several anonymous referees improved the manuscript. The work reported here was conducted in accordance with the laws of the country where it was performed, and in accordance with ASAB/ABS *Guidelines for the Use of Animals in Research*.

References

- Anselme, P.** 2008. Abnormal patterns of displacement activities: a review and reinterpretation. *Behavioural Processes*, **79**, 48–58.
- Bastock, M. & Manning, A.** 1955. The courtship of *Drosophila melanogaster*. *Behaviour*, **8**, 85–111.
- Berridge, C. W., Mitton, E., Clark, W. & Roth, R. H.** 1999. Engagement in a non-escape (displacement) behavior elicits a selective and lateralized suppression of frontal dopaminergic utilization in stress. *Synapse*, **32**, 187–197.
- Boogert, N. J., Reader, S. M. & Laland, K. N.** 2006. The relation between social rank, neophobia and individual learning in starlings. *Animal Behaviour*, **72**, 1229–1239.
- Cleland, C. E.** 2001. Historical science, experimental science, and the scientific method. *Geology*, **29**, 987–990.
- Dayan, P. & Balleine, B. W.** 2002. Reward, motivation and reinforcement learning. *Neuron*, **36**, 285–298.
- Dyer, F. C.** 2002. The biology of the dance language. *Annual Review of Entomology*, **47**, 917–949.
- Freidin, E. & Mustaca, A. E.** 2004. Frustration and sexual behavior in male rats. *Learning & Behavior*, **32**, 311–320.
- von Frisch, K.** 1967. *The Dance Language and Orientation of Bees*. Cambridge, Massachusetts: Belknap Press.
- Gordon, H. R. S.** 1955. Displacement activities in fiddler crabs. *Nature*, **176**, 356–357.
- von Holst, E. & von St Paul, U.** 1963. On the functional organization of drives. *Animal Behaviour*, **11**, 1–20.
- van Iersel, J. J. A. & Bol, A. C. A.** 1958. Preening of two tern species. A study on displacement activities. *Behaviour*, **13**, 1–88.
- Judge, P. G. & Mullen, S. H.** 2005. Quadratic postconflict affiliation among bystanders in a hamadryas baboon group. *Animal Behaviour*, **69**, 1345–1355.
- de Marco, R. J. & Farina, W. M.** 2001. Changes in food source profitability affect the trophallactic and dance behaviour of forager honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology*, **50**, 441–449.
- Mettke-Hofmann, C., Ebert, C., Schmidt, T., Steiger, S. & Stieb, S.** 2005. Personality traits in resident and migratory warbler species. *Behaviour*, **142**, 1357–1375.
- Rowell, C. H. F.** 1961. Displacement grooming in the chaffinch. *Animal Behaviour*, **9**, 38–63.
- Schino, G., Perretta, G., Taglioni, A. M., Monaco, V. & Troisi, A.** 1996. Primate displacement activities as an ethopharmacological model of anxiety. *Anxiety*, **2**, 186–191.
- Seeley, T. D.** 1995. *The Wisdom of the Hive: the Social Physiology of Honey Bee Colonies*. London: Harvard University Press.
- Siegel, S.** 1956. *Nonparametric Statistics for the Behavioural Sciences*. London: McGraw-Hill.
- Srinivasan, M. V., Zhang, S., Altwein, M. & Tautz, J.** 2000. Honeybee navigation: nature and calibration of the “odometer”. *Science*, **287**, 851–853.
- Tautz, J., Rohrseitz, K. & Sandeman, D. C.** 1996. One-strided waggle dance in bees. *Nature*, **382**, 32.
- Timberlake, W.** 1993. Behaviour systems and reinforcement: an integrative approach. *Journal of the Experimental Analysis of Behavior*, **60**, 105–128.
- Tinbergen, N.** 1951. *The Study of Instinct*. Oxford: Clarendon.
- Troisi, A.** 2002. Displacement activities as a behavioural measure of stress in nonhuman primates and human subjects. *Stress*, **5**, 47–54.
- Wainelboim, A. J. & Farina, W. M.** 2003. Trophallaxis in honeybees, *Apis mellifera* (L.), as related to their past experience at the food source. *Animal Behaviour*, **66**, 791–795.
- Wilz, K. J.** 1970a. Causal and functional analysis of dorsal pricking and nest activity in the courtship of the three-spined stickleback *Gasterosteus aculeatus*. *Animal Behaviour*, **18**, 115–124.
- Wilz, K. J.** 1970b. The disinhibition interpretation of the ‘displacement’ activities during courtship in the three-spined stickleback, *Gasterosteus aculeatus*. *Animal Behaviour*, **18**, 682–687.
- Wilz, K. J.** 1970c. Self-regulation of motivation in the three-spined stickleback (*Gasterosteus aculeatus* L.). *Nature*, **226**, 456–466.