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Nest-mate recognition template of guard honeybees (*Apis mellifera*) is modified by wax comb transfer

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In recognition, discriminators use sensory information to make decisions. For example, honeybee (*Apis mellifera*) entrance guards discriminate between nest-mates and intruders by comparing their odours with a template of the colony odour. Comb wax plays a major role in honeybee recognition. We measured the rejection rates of nest-mate and non-nest-mate worker bees by entrance guards before and after a unidirectional transfer of wax comb from a ‘comb donor’ hive to a ‘comb receiver’ hive. Our results showed a significant effect that occurred in one direction. Guards in the comb receiver hive became more accepting of non-nest-mates from the comb donor hive (rejection decreased from 70 to 47%); however, guards in the comb donor hive did not become more accepting of bees from the comb receiver hive. These data strongly support the hypothesis that the transfer of wax comb increases the acceptance of non-nest-mates not by changing the odour of the bees, but by changing the template used by guards.

Keywords: nest-mate recognition; *Apis mellifera*; recognition template

1. INTRODUCTION

Throughout the natural world, organisms can enhance their fitness by making appropriate recognition decisions, such as rejecting allospecific mating partners, parasites (Sherman *et al.* 1997), and, at group-level recognition, discriminating ‘friend from foe’ (Lubbock 1882). Honeybee (*Apis mellifera*) colonies are a model system for studying group recognition, as they possess entrance guards to exclude intruders while allowing nest-mates to enter (Butler & Free 1952; Moore *et al.* 1987). Guarding is vital as colonies face threats from allospecific intruders, such as wasps (Ono *et al.* 1995; Ken *et al.* 2005), and conspecific robber bees from other hives (Free 1977; Seeley 1985). Both frequently kill victim colonies.

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Recognition requires the evaluator to obtain sensory information from the cue bearer. Guard bees discriminate nest-mates from intruders by comparing their odours with a template representing the colony odour (Getz 1982; Lacy & Sherman 1983). Greater similarity of the odour to that of this template increases the probability of acceptance (Crozier & Pamilo 1996; Gamboa 2004).

Wax, secreted by workers and made into combs, plays a major role in honeybee recognition (Breed *et al.* 1988, 1995, 1998). When combs are experimentally swapped between hives, guards become more accepting of non-nest-mate workers from the swap partner hive, but not from the control hive (D’Ettorre *et al.* 2006). This increased acceptance could potentially be caused by two mechanisms. Exchanging combs may make the odours of the workers in swap partner hives more similar or it may change the template of the guard bees, making them more accepting even if the odours are unchanged.

Our study tests these two competing but non-exclusive hypotheses. We measured the rejection rates of nest-mate and non-nest-mate worker bees by entrance guards before and after a unidirectional comb transfer from a ‘comb donor’ hive to a ‘comb receiver’ hive. Our results showed a significant effect that was consistent across eight independent trials. The effect occurred in one direction. Guards in the comb receiver hive became more accepting of non-nest-mates from the comb donor hive; however, guards in the comb donor hive did not become more accepting of bees from the comb receiver hive. These data strongly support the hypothesis that the transfer of wax comb increases the acceptance of non-nest-mates by changing the recognition template.

2. MATERIAL AND METHODS

Methods (see electronic supplementary material) were similar to D’Ettorre *et al.* (2006) and used a standard behavioural assay of discrimination by natural entrance guards (Downs & Ratnieks 2000; D’Ettorre *et al.* 2006), where an observer, blind to the source of the bees (Gamboa *et al.* 1991), classifies the introduced workers as accepted or rejected by guards.

Each trial used a trio of hives, with two involved in the comb transfer and the other as a control. We ran two trials simultaneously. In October 2004, we tested acceptance levels in hives A, B and C and hives D, E and F before and after a unidirectional comb transfer. The entire process was replicated in November with the same trios but with transfer in the other direction (A to B and D to E in October and B to A and E to D in November). Based on the life cycle of honeybees and evidence that guards only work for less than a day (Breed *et al.* 1992), the data from these trials are independent. We repeated the entire procedure during October/November 2005 with six different hives, resulting in eight trials over the 2 years. We introduced a total of 3462 bees (1662 in 2004 and 1800 in 2005).

Each experimental hive received one nest-mate, one non-nest-mate from the hive that was involved in the comb transfer and one non-nest-mate from the control. The control hive received one nest-mate and two non-nest-mates from the experimental hives. This was repeated with the other trio of hives to give a series. Depending on the duration of suitable conditions, 4–11 series were completed per day.

After several days of introductions to establish baseline acceptance levels, we moved four frames of wax combs without brood from the donor hive to the receiver hive, from which we removed seven frames. The combs were alternated with the colony’s combs to ensure that the bees had contact with both the sets. We removed four frames from the control hives within each trio so each hive ended up with six to seven frames. Data collection resumed the next morning. It was important to gather data as soon as possible as the effect is strongest immediately after the swap (D’Ettorre *et al.* 2006). Once the rejection rates had returned to pre-transfer levels, we returned the combs to the original hives and waited 10 days

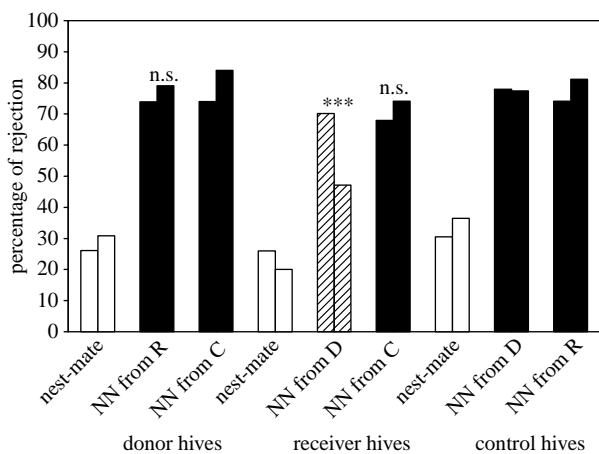


Figure 1. Rejection of introduced bees by guards before (first bar) and after (second bar) wax comb transfer. Data have been pooled across all trials. White bars represent nest-mates and black/hatched bars represent non-nest-mates. Hatched bars highlight the comb transfer effect where guards from the comb receiver hive became more accepting of non-nest-mates from the comb donor hive. This effect was observed in all eight trials and was highly significant ($p=0.001$). NN, non-nest-mate; R, receiver hive; D, donor hive; C, control hive; n.s., not significant.

before performing the experiment again with donor and receiver hives reversed.

We carried out a one-way ANOVA (MINITAB v. 14) with a single response variable for each of the three treatment groups (nest-mate and two types of non-nest-mate) for all three hive types (donor, receiver and control) for each trial by subtracting percentage of rejection after comb transfer from before comb transfer to obtain an index of eight values per treatment per hive.

3. RESULTS

Figure 1 presents the pooled data for all eight trials. Each pair of bars represents the introduction of three types of bees (nest-mate and two types of non-nest-mate) in the three types of hives (donor, receiver and control). The two bars are the proportion of the introduced bees rejected before and after the comb transfer. Across the eight trials, there was a decrease in the rejection, from 70 to 47% on average, of non-nest-mate bees from the comb donor hive by guards in the comb receiver hive. Overall, this effect is highly significant (one-way ANOVA, d.f. = 2, $p=0.001$). In contrast, the comb transfer did not cause a significant change in the rejection in the donor (74–79%; one-way ANOVA, d.f. = 2, $p=0.666$) or the control (75%; one-way ANOVA, d.f. = 2, $p=0.258$) hives. Figure 2 shows the same data but pooled per day across hives in reference to comb transfer (day 0) and shows the immediate effect and decay of the manipulation. The rejection rates had returned to pre-transfer levels within a week (figure 2), similar to the previously reported results (D'Ettoire *et al.* 2006). Figure 3 in the electronic supplementary material presents data per hive.

4. DISCUSSION

Our results show a significant comb transfer effect, in agreement with that of D'Ettoire *et al.* (2006). However, the effect was only observed in one direction. Guards in the comb receiver hive became

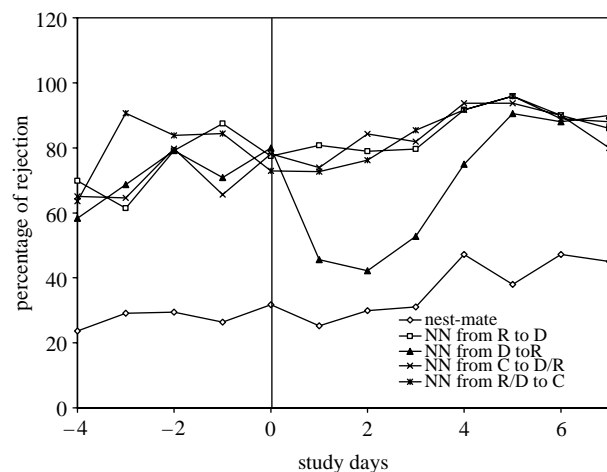


Figure 2. Rejection of types of bees per study day before and after comb transfer pooled for all eight trials. Abbreviations are the same as given in figure legend 1. Open diamonds represent percentage of rejection of nest-mates. The vertical line represents the evening on which the comb transfer occurred. The comb transfer effect was immediate for the non-nest-mates from the comb donor hive (black filled triangles) by guards from the comb receiver hive and decayed after several days.

significantly more accepting of bees from the comb donor hive, which carried no new odours, after the comb transfer. In contrast, guards in the comb donor hive did not become more accepting of bees from the comb receiver hive. This pattern was consistent in all the eight trials.

These results strongly support the template change hypothesis. The results do not support the odour change hypothesis because there was no increase in the acceptance of bees from the comb receiver hive. These results could not be due to environmental factors because no effects were observed within the control hives. The results cannot be explained by a general increase in guard acceptance because there was no change in the acceptance of non-nest-mate bees from the control hives.

Our results are surprising. We had expected both a guard template effect and a bee odour effect because previous research had shown not only that the guard template can change when a guard is exposed to new compounds (Breed *et al.* 2004a), but also that bee odour can change. Newly emerged workers are accepted into any hive, but exposure to wax for 5 min is sufficient to give the bee a chemical profile as evidenced by behavioural and chemical data (Breed *et al.* 1998, 2004b; Dani *et al.* 2005).

Perhaps both guard template and bee odour can change. For acceptance to increase, the changes must lessen the chemical distance between the two (D'Ettoire *et al.* 2006). In a unidirectional comb transfer, the changed template of the guard is sufficient to increase the acceptability of the non-nest-mate. Possibly, adult templates are easier to update by experience than odours. It would be interesting to see whether this is a general phenomenon in other species with group-level recognition.

Our results suggest that guards update their colony odour template directly from the wax comb rather

than indirectly from novel nest-mate odours. One way to test this hypothesis would be to prevent guard access to transferred comb so that their only contact is via odours carried on the bodies of nest-mates. Another important question is the location of the template. Previous studies have assumed the involvement of the central nervous system (Vandermeer *et al.* 1989; Breed *et al.* 2004a). However, in the carpenter ant *Camponotus japonicus*, antennal sensillae respond and eventually habituate to recognition chemicals, indicating that nest-mate discrimination may be a peripheral sensory process (Ozaki *et al.* 2005). Such evidence not only demonstrates the importance of the template, but it also questions the traditional idea that a guard is 'learning'.

Our study confirms the role of wax combs in honeybee nest-mate recognition (D'Ettoire *et al.* 2006) and demonstrates the importance of the colony odour template of guard bees as the main factor in changing acceptance. A changeable template would be adaptively valuable by increasing the discriminator's accuracy. Our study provides a methodology for investigating the acquisition of templates to address these and other questions.

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