

Research article

## Old comb for nesting site recognition by *Apis dorsata*? Field experiments in China

F. Liu<sup>1</sup>, D.W. Roubik<sup>2,\*</sup>, D. He<sup>1</sup> and J. Li<sup>1</sup>

<sup>1</sup> Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Xuefu Rd. 88, Kunming 650223, P. R. China; flliu@xtbg.ac.cn

<sup>2</sup> Smithsonian Tropical Research Institute, Ancon, Balboa, Republic of Panama; roubikd@si.edu

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**Abstract.** The Asian giant honey bee, *Apis dorsata*, often conducts seasonal, long-distance migrations in southern China, between a preferred tree (having more than one nest) and alternate sites. Although worker bees cannot make a round-trip journey, colonies re-utilize preferred trees after an absence of several months. We performed comb experiments in which bases and all abandoned combs were entirely scraped off trees and their sites covered with plastic, or comb was moved to trees of the same species. Swarms of giant honey bees investigated trees where combs were removed and continued to nest on the same trees. In contrast, placing combs in nets on previously used trees, or on nearby trees of the same species, did not attract more swarms. The same number of colonies that left them returned to previously occupied trees. Our findings suggest that direct olfactory or sensory contact with old comb bases might regulate nest establishment, but individual trees, lacking normal visual or chemical cues of old nests, are relocated using behavioral devices that remain to be elucidated.

**Keywords:** *Apis dorsata*, China, comb experiments, navigation, migratory tropical honey bees.

### Introduction

Insect navigation is one of the better understood subjects in animal navigation (Collett and Collett, 2006). Comparatively little is known about the orientation of migratory insects (Holland, 2006a). A dissimilarity between insect and most vertebrate migrations is that few

individual insects can make a round-trip journey, thus in the return migration, insects have no first-hand knowledge of former sites (Bingman and Cheng, 2005). Furthermore, well-known navigation mechanisms, including path integration, landmark recognition, or compass orientation, provide no cues for displacement into completely unfamiliar territory, when no outgoing path can be memorized to use in the return trip (Wehner et al., 1996; Walker et al., 1997). Migratory insects must develop navigational mechanisms that are not based on the past experience of sites.

Studies have shown that migratory animals are capable of compensating for an artificial displacement into unfamiliar territory (e.g. Phillips et al., 1995; Boles and Lohmann, 2003; Lohmann, 2004; Holland et al., 2006b). Such compensation requires that animals have both a sense of direction (“compass”) for maintaining their travel direction and a sense of geographic position (“map”) that permits them to know their location relative to their destination (Walker et al., 1997). That remarkable navigational map can be obtained from environmental gradient(s) of olfactory or magnetic cues, and can be extrapolated by the animals for navigation in the area beyond an individual’s experience (Phillips et al., 2006).

The Asian giant honey bee *Apis dorsata* often migrates between ‘preferred’ or aggregated-nest trees or cliffs, and alternate sites, sometimes separated by hundreds of kilometers (Koeniger and Koeniger, 1980). Although few individuals live long enough to make a round-trip journey to their original site, some colonies nonetheless return exactly to their former trees (Paar et al., 2000; Neumann et al., 2000). The honey bee, *Apis mellifera*, relies on a sun compass to guide travel and a unique dance language to provide orientation information (von Frisch, 1993). In contrast, migratory giant honey bees do not signal accurate spatial information in their

\* Author for correspondence.

migratory dance (Dyer and Seeley, 1994). Those migratory bees must therefore create a navigational map to locate their previously occupied trees. Published studies suggest that odor information of old combs, and the comb base materials that remain after the elements or natural enemies have removed the comb itself, likely provide the cue for bees to select the tree again (Paar et al., 2000; Neumann et al., 2000). Further, for returning colonies, the visual, chemical and tactile cues presented by the former comb base, which may persist for years, constitute a navigational map coordinate.

## Methods

In this study, we conducted comb transfer experiments to test whether old combs provide a cue for immigrant colonies at Xishuangbanna Tropical Botanical Garden (XTBG) in southwestern China. In fragmented forest of XTBG consisting of ~9 km<sup>2</sup>, more than ten large trees are selected by the bees to build combs in the dry season. The combs fall from trees after emigration of colonies, but some comb may persist on trees for several months, and darkened marks on the trunk are visible years afterward (personal observations). One large *Bombax ceiba* (Bombacaceae —recently placed in Malvaceae, Fig. 1a) holds a noteworthy aggregation and has hosted over 20 colonies for each of the past 17 years, a second nearby tree of this species has hosted 3 colonies for the past four years. Our study utilized the nesting trees of this species by making the following manipulations: 1) all 23 combs were removed on the most preferred *Bombax* tree, 2) all three combs were removed and their bases scraped completely clean with a knife, on a second preferred *Bombax*, 3) combs removed from the most preferred tree were reattached, with fine nets and wire, to a) the second preferred tree (13 combs), b) the original, most-preferred tree (2 combs), and c) each of two nearby *Bombax* on which no combs were previously built (2 combs, each).



**Figure 1.** Four trees of *Bombax ceiba* in Xishuangbanna Tropical Botanical Garden selected for comb transfer experiments (A). Tree 3 was the most preferred tree, followed by tree 1. Trees 2 and 4 were not explored by the bees to build their nests. The removal of old combs from tree 3 did not prevent the return of bees to the tree (B). The returning colonies could exactly find their former nesting location upon nesting branches if old comb area was not covered with plastic wrap (C).

In September of 2005 after the emigrating colonies left, we removed all combs. Our re-attachment sites of those combs were not previously occupied, and combs were not protected from decay or attack, but were maintained in the netting. Colonies returning to the

area had access to all four experimental trees, with a maximum separation of 310 meters. From December 2005 to February 2006, we monitored immigrant colonies. We recorded colonies on the four experimental bee trees, and also noted behavior as bees arrived or investigated these sites.

## Results

Immigrant colonies first arrived to the sites near the most favored *Bombax* then resided in the forest understory, for approximately two months, before nesting. Swarms first came to the XTBG on December 8 of 2005, noted when forager bees appeared at flowers of *Calliandra surinamensis* (Mimosaceae). The preferred trees, however, remained unoccupied.

The artificially attached combs did not alter nest site attractiveness, and the preferred *Bombax* trees received a number of nesting colonies equivalent to that of the previous season, prior to migration. Five swarms came first to the most preferred tree on 7 February (Fig. 1c). After swarms arrived near the previous nest trees, worker bees investigated the original comb branches. Many visited a re-attached comb at the most preferred tree (figure not shown). By 20 February, colonies completed occupancy of that tree, with 23 active nests. Immediately afterwards, additional colonies moved to other trees. Although three old combs on the less preferred tree were removed, and 13 were re-attached, originally from the preferred tree, only three colonies arrived. Each of two large *Bombax* with two attached combs from the preferred tree received no swarms.

## Discussion

A stopover at a site near preferred nest trees may allow bees to search for the trees before nesting. Any patch of forest with large trees may constitute such a habitat. Previous work has suggested that visual information is used by immigrant colonies to find a place where nesting has proven successful, although odor and tactile or chemical cues associated with the material of old combs (resin, pollen, wax, pheromones, microbes) seemed more likely to guide the final choice (see Paar et al., 2000; Neumann et al., 2000). If so, we expected that comb removal and wrapping the comb base scars to remove all potential stimuli left by previous colonies would disturb the decision making process of immigrant colonies. However, colonies still found the same nesting trees and settled in equivalent number. This is not remarkable, since the local Dai people remove the old combs of the bees from large trees for the production of temple candles year after year, but colonies still return to the same trees. Old combs were not there to provide cues, but their darkened ‘scars’ on the bark were present on the most preferred *Bombax*. In addition, the wrap with black plastic might have resembled such a natural visual cue on

the less preferred *Bombax*. Swarms at least accepted that tree, with no exposed scars and the addition of a plastic wrap, plus 13 old comb remnants in nets. Two of three colonies that arrived eventually abandoned the tree. Only one established a nest.

Chemical orientation, involving direct sensory contact with comb scars and their contents, was evidently not a decisive factor for a colony to establish a nest on a tree in one case of the three observed at our plastic-wrapped nest tree. In contrast, 23 colonies arrived and nested near scars, which signify the bases of former combs, on the most preferred *Bombax*. Our data suggest the cues left by the comb itself, the comb base, or any conceivable visual, microbial or other chemical/tactile cue were secondary, and not decisive for tree relocation. Further work is required to test how nest establishment is controlled by one or more of those variables. As for visual information, the altered visual and chemical characteristics of the less preferred experimental tree suggest that colonies do not have a precise template of traits that feature prominently in a reliable and preferred nesting site. At the very least, swarms were not repulsed visually by a lack of normal features on a *Bombax* previously used for nesting.

What determines whether a tree is used for giant honey bee nesting? We can conclude the tree itself held a significant component which was not the function of a particular odor or substrate. After the colonies found the trees, worker bees flew around the tree branches. Some worker bees investigated the comb area or re-attached combs. If the comb base area was covered by plastic, most colonies moved away in one or two days, but if comb bases were present, a remarkable consistency in site occupancy was carried on. Thus olfactory and/or direct sensory gradients of old comb bases, but not the wax combs themselves, seemed to provide an important map coordinate to a returning colony, or a powerful stimulus for a new colony to take up residence. Our study may help to provide new insights into potential navigation mechanisms of migratory insects, including *Apis mellifera*, which is often migratory in its tropical range.

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