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RESEARCH ARTICLE

Both olfactory and visual cues promote the hornet *Vespa velutina* to locate its honeybee prey *Apis cerana*

Z. W. Wang · G. Chen · K. Tan

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Abstract Predators use olfactory, visual and sometimes acoustic cues from the preys to assess food information. However, it is not known if the aggressive hornets (*Vespa* spp.) use olfactory, visual, or both types of information to find and recognize prey. In the present study, we trained hornet workers (*Vespa velutina*) to a feeding area. Once the hornets began consistently foraging at this feeding area, we determined whether they located prey (bees, *Apis cerana*) via olfactory or visual cues. We did this by testing whether hornets were attracted to a dummy bait (bee dummy bait or non-bee dummy bait) treated with extracts of honeybee cuticular hydrocarbons. We then tested whether hornets could distinguish between bee dummy bait and cotton ball dummy bait, both treated with bee odors. Hornets preferred the dummy treated with bee odors, and bee dummies (with bee images) were more attractive to the hornet than the cotton ball dummies with only bee odors. These results clearly indicate that a combination of olfactory and visual cues helps the hornet to locate its prey.

Keywords *Vespa velutina* · Predator · Food location · *Apis cerana* · Bee extractions

Z. W. Wang · K. Tan (✉)
Plant Protection College, Yunnan Agricultural University, Kunming 650201, People's Republic of China
e-mail: kentan@xtbg.ac.cn

K. Tan
Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming 650223, Yunnan, People's Republic of China

G. Chen
Kunming Botanical Garden, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, Yunnan, People's Republic of China

Introduction

Social insects rely on olfactory and visual cues when seeking and assessing information on potential nest sites, nestmates, or resources (Leonhardt et al., 2010). Bees use volatile compounds as well as visual cues, to find flowers suitable for pollen and nectar collection. Visual and olfactory cues are also used to identify enemies and situations where predators are present [reviewed by (Breed et al., 2004b)]. Stingless bees use olfactory cues to locate or recognize specific tree resin profiles (Leonhardt et al., 2010).

Predator species may use olfactory cues to locate their prey and deposit odors to mark prey location. For example the giant hornet's (*Vespa mandarinia japonica*) foraging sequence, includes a hunting phase in which a lone forager finds a honeybee colony, kills individual bees and takes them to the hornet's nest. How the hornets locate their target in the first place is yet to be described. Hornets forage alone or in a group of only a small number of individuals (Toh and Okamura, 2003). After several individual return visits, the hornet recruitment phase begins. The foraging hornet marks the site by rubbing its van der Vecht gland on or near the bee colony, helping other foraging hornets to find it (Ono et al., 1995). Previous research also reported that hornets (*Vespa* spp.) hunt for live honeybees and forage for fruit to feed their larvae (Perrard et al., 2009). Honeybee colonies are attractive targets for both hornets as they contain nutritious brood and stores of honey and pollen. Thus, hornets are frequent predators of honeybee colonies. Hornets often hunt adult honeybees at the hive entrance, or enter honeybee colonies to carry off developing larvae and pupae (Li et al., 2008). The majority of previous studies on the interaction between hornet and honeybee have focused on the defensive behavior of the honeybees (Ono et al., 1995; Tan et al., 2012; Li et al., 2008) and not on how hornets find these colonies.

How do these predators find their prey? Food-borne volatiles are attractants for species such as *Vespula germanica*, *V. maculifrons*, *Dolichovespula maculata* and *Polistes fuscatus* (Day and Jeanne, 2001). The social wasp *Vespula germanica* uses a combination of visual cues and conspecific odors to locate food sources (D'adamo et al., 2003). These wasps responded at much higher rates when presented with both visual and olfactory cues than when they were presented with either visual or olfactory cues, suggesting some synergy between these two types of cue (D'adamo et al., 2003). The European beewolf, *Philanthus triangulum*, may use the odor of honeybee cuticle to find their prey (Herzner et al., 2005). Olfactory cues allow the social paper wasp, *Mischocyttarus flavitarsis*, to locate its caterpillar prey over short and long ranges (McPheron and Mills, 2007). Richter and Jeanne (1985) reported that the tropical vespid wasp, *Polybia sericea*, used visual cues to direct intensive aerial search, and olfactory cues to elicit landing.

Olfaction plays a significant role in how hornets identify prey, how hornets communicate and how prey species can recognize and deal with predatory hornet threats. Hornets mark an attractive foraging site with odors to return to it and to recruit nestmates (Ono, 2006; Ono et al., 1995). Thus, it is likely that *V. velutina* uses olfactory cues to maximize their foraging and hunting efficiency. In addition, hornet site-marking pheromones can be recognized by other hornet species such as the yellow hornet *V. simillima* as well as by honeybees (Ono, 2006; Ono et al., 1995). These prey species can use this ability to help protect their colonies. After a giant hornet places an odor mark near a yellow hornet colony, yellow hornet workers from other colonies will join to protect their colonies from the predatory hornets (Ono, 2006).

However, no studies to date have determined how hornets detect honeybees. Knowing how they do this is important because *V. velutina* is now an invasive predator in France where it is beginning to affect honeybees (Monceau et al., 2013a; Monceau et al., 2013b). Here, we investigated whether *V. velutina* uses olfactory cues as well as visual cues to locate food sources.

Materials and methods

Vespa velutina colony preparation and experimental site

A *V. velutina* colony containing about 600 workers was trapped in the forest of a northern suburb of Kunming (Yunnan, China) in July and August of 2012, and then moved to the Kunming Botanical garden. The colony was housed in a 10 × 6.4 × 2.4 m flying room with walls and ceiling of nylon netting so that the hornets were exposed to outdoor conditions, but could not fly out. Four plates with scentless sucrose solution (w/w, 30 %) and a plate with flesh water were placed close to the colony to feed the hornets. Experiments commenced a week later to allow the hornets to calm down and resume foraging.

We trained hornet workers to a feeding area (40 × 40 cm) about 2 m away from the colony. Feeding plates were removed when trials started and replaced after trials finished at the end of each day. Once hornets began to consistently forage from the feeding area, three sets of experiments were conducted to determine whether they used olfactory or visual cues to locate prey (honeybee workers, *Apis cerana*).

Experiment 1

Cuticular hydrocarbons were extracted from honeybee workers by soaking them in 10 ml dichloromethane for about 90 s (Herzner et al., 2005). The extracts, as well as the bees from which they were extracted, were retained for subsequent experiments. Two extracted bees were attached together with a thin string 15 cm in length and hung on the top end of a straight metal wire 50 cm long. Two wires with hanging bees were inserted into a foam cube such that the two baits were about 40 cm apart, like two fishing rods. One bait held two dummy bees that had been treated with 100 μl dichloromethane as the solvent control (Breed et al., 2004a). The other bait held two bees treated with 100 μl bee extracts in dichloromethane (two bee equivalents of cuticular hydrocarbons).

Experiment 2

To determine whether the hornets need visual cues at all to find prey, we paired cotton ball dummy baits treated with 100 μl dichloromethane and the other cotton ball dummy baits treated with 100 μl bee extractions.

Experiment 3

This experiment utilized the same procedure above, except that we employed dummy baits to determine if *V. velutina* is attracted to a combination of bee image and scent or to the scent alone. For these trials, baits containing a cotton ball (cotton ball dummy), about the size of two bees, treated with 100 μl of bee extracts were paired with baits containing two bees treated with bee extracts.

At least 10 trials were performed for each experiment (experiment 1: 11 trials, experiment 2: 10 trials and experiment 3: 11 trials). Trials lasted for 15–20 min, during which the positions of the baits were swapped every 5 min to eliminate position bias. If a hornet flew to within 5 cm of the bait and then flew away, this was termed as an 'approach'. Hornets that approached, left the bait and then returned to it were only counted once, unless they returned to the nest before returning to the bait. If a hornet grasped or landed on the bait to bite it, this was termed as a 'locate'. Trials were conducted from 13:00 to 16:00 on sunny days when the ambient temperature was around 25°, optimal conditions for foraging hornets.

Statistics

For each trial, we calculated the proportion of approaches or locates per bait. For example, we calculated locate focal bait/(locate bait 1 + locate bait 2). For experiments 1 and 3 the focal bait was bee with bee odor, and in experiment 2 it was a cotton ball with bee odor. For example, in experiment 1, the proportion was calculated as: locates on bee with bee odor/(locates on bee with bee odor + locates on bee without bee odor). For experiment 1 and 2, we used a single sample *t* test with an expected mean of 0.5 to determine if bees preferentially approached or located one of our two baits. Our data on locates for experiment 2 were significantly non-normal, so we used a non-parametric Wilcoxon signed rank test with an expected mean of 0.5.

To determine if the effect driven by cues (olfactory or visual cues) only, or by model differences (bee model or cotton ball), we combined the data of experiment 1 and 2, to compare the model effects via unvaried analysis of variance (Univariate ANOVA), models were treated as random factors, and treatment (with and without bee odors) was treated as fixed factors. We used post hoc test (least significant differences, LSD) to determine if there were differences between the two different models.

Results

In experiment 1, hornets were significantly more attracted to bee dummies treated with bee odor extract than those treated with pure dichloromethane for both approach (61.61 % to approach focal bait, $t = 3.78$, $df = 10$, $P = 0.004$) and locate (66.43 % to locate focal bait, $t = 2.18$, $df = 10$, $P = 0.05$) (Fig. 1).

In the second experiment, when all baits consisted of cotton balls, hornets again had significantly higher preference for the cotton balls treated with extract for both approach (66.82 % to approach focal bait, $t = 8.97$, $df = 10$, $P < 0.001$) and locate (74.24 % to locate focal bait, $W = 10.5$, $df = 10$, $P = 0.013$) (Fig. 1).

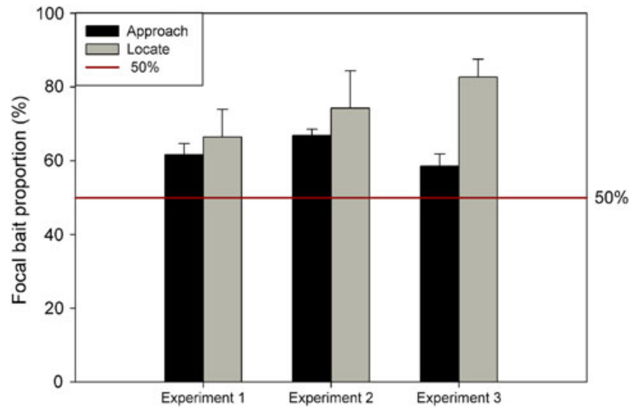

Fig. 1 Hornet preferences for locating prey. Mean proportions of focal bait (±SE) of approach and locate are shown. The *line* indicates the null hypothesis expectation of 50 %. In experiment 1, the hornet approaches and locates the bee with bee odor more frequently than the bee without any bee odor. In experiment 2, the hornet showed significantly different preference to cotton ball dummy with olfactory cues than the dummy without cues in both approach and locate way. In experiment 3, the hornet preferred the prey with bee image to cotton ball dummy, even both baits retreated with bee extracts

In the third experiment, the hornets showed a significantly greater preference for the baits containing bees over those with cotton balls for both approach (58.52 % to approach focal bait, $t = 2.58$, $df = 9$, $P = 0.03$) and locate (82.67 % to locate focal bait, $t = 6.76$, $df = 9$, $P < 0.001$) (Fig. 1).

Experiment 1 and 2 combined data analysis showed no model differences when hornets chose to approach ($F_{1, 41} = 0.53$, $P = 0.47$), while the hornets tend to approach baits with odor significantly higher than baits without odor ($F_{1,41} = 25.72$, $P < 0.001$). However, baits locating showed both model effect ($F_{1,41} = 10.46$, $P = 0.002$) and treatment effect ($F_{1,41} = 17.02$, $P < 0.001$).

Discussion

Our experiments show that hornets use a combination of olfactory and visual cues to locate their prey. Our results (experiments 1 and 2) are consistent with other studies showing that hornets can efficiently use olfactory cues to locate prey: the European beewolf, *P. triangulum* (Herzner et al., 2005), social paper wasp, *M. flavitarsis* (McPheron and Mills, 2007), spider-hunting wasp, *Sceliphron caementarium* (Uma and Weiss, 2010) and the tropical vespid wasps, *P. sericea* (Richter and Jeanne, 1985). However, adding the visual appearance of bees facilitated hornet prey recognition. Hornets were most attracted to baits containing dummy bees treated with extract (Fig. 1) and which, therefore, provided visual and olfactory cues. This has also been reported in the social wasp *Vespula germanica*, which uses a combination of visual cues and conspecific odors to locate food (D'adamo et al., 2003).

When presented with baits that consisted of the same image, but different odors (Experiments 1 and 2), hornets were more likely to approach and locate baits with the bee odor (Fig. 1). However, when given the choice of baits with the same odors but different images (Experiment 3), hornets preferentially located those with the bee image (Fig. 1). We, therefore, show that bee odor can significantly enhance hornet prey preferences. When given a choice, hornets prefer the prey item that looks and smells like a bee to one that only smells like a bee. Similarly, the digger wasp, *Liris niger*, first uses visual cues to orient toward its prey, then uses chemical cues for more accurate prey localizations (Anton and Gnatzy, 1998). The hornet showed no model effect, but only olfactory preference when approach prey in a short distance. However, hornet locates prey influenced by both model effect and treatment.

The relative importance of olfactory and visual cues in allowing wasps to locate food is difficult to determine from our data. When we used baits containing only cotton balls, the hornets took longer time to reach similar frequencies of approaches and touches than in the other two experiments. This suggests that the image of the bee as a prey item is important. Whether this search image is learned or innate is unclear, but honeybee workers can innately recognize hornets as predators (Tan et al., 2013). Hornets may possess innate search images of prey, particularly given that *V. velutina* and *A. cerana* co-evolved in the same habitat (Tan et al., 2012; Tan et al., 2013). Hornets can use this search image, whether acquired or innate, even on a new prey species. As an invasive species in Europe, *Vespa velutina* is able to hunt the European honey bee *A. mellifera* (Monceau et al., 2013a; Tan et al., 2012). The ability of *V. velutina* to learn new prey odors or use an innate set of odors to find prey is worthy of future study, particularly as this hornet species invades new ecosystems.

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