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Social but not solitary bees reject dangerous flowers where a conspecific has recently been attacked

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Social bees are known to avoid inflorescences marked with dead conspecifics or their smell. The avoidance response could be triggered by alarm signals actively given by attacked bees or by substances passively released through injuries as a by-product of the attack. To discriminate between these two options we note that both social and solitary bees are expected to react to nonsignalling cues associated with predation risk, while only social bees are expected to give alarm signals. We simulated risky inflorescences by pinching a landing bee with forceps, and compared the rate at which bees visited these experimental inflorescences and unmanipulated control inflorescences. We conducted the experiment with four species of social bees, *Apis mellifera*, *Apis dorsata*, *Apis florea* and *Bombus terrestris* and with three species of solitary bees, *Eucera* sp., *Panurgus* sp. and *Nomia strigata*. We found that while the three species of solitary bees responded similarly to control and experimental inflorescences, all four species of social bees strongly rejected inflorescences where we simulated a predation attempt. The finding that only social species avoided landing on dangerous inflorescences strongly suggests that the release of the alarm cue has been selected for its signalling value in social bees.

Animals rely on cues to detect their predators and avoid fatal encounters (Lima & Dill 1990). Such cues can belong to different sensory domains, such as the visual, auditory or chemical domains (Chivers & Smith 1998; Barbosa & Castellanos 2005). The cues to which prey respond can be produced by the predator itself or by other prey (Kats & Dill 1998; Wyatt 2003; Barbosa & Castellanos 2005). Prey-produced alarm cues can, at least in principle, be divided into two groups, according to whether their release is merely a by-product of the predation attempt (i.e. body fluids that have escaped through skin injuries) or has been selected for its signalling value (Chivers & Smith 1998; Kats & Dill 1998; Wyatt 2003). In this study, we investigated the use of alarm signals by foraging bees.

Aggressive alarm pheromones, which trigger attacks to intruders during colony defence, have been well described in species with a high level of social development, such as bees from the tribes Apini and Meliponini. (Koeniger et al. 1979; Roubik et al. 1987; Schmidt 1998; Schorkopf et al. 2009). In contrast, the role of evasive alarm pheromones, triggering an escape response, remains less clear in social bees. It is known that certain substances, such as 2-heptanone, a pheromone released through the mandibular glands of *Apis mellifera* bees, repel bees at the foraging site (Butler 1966; Simpson 1966; Rieth et al. 1986; Vallet et al. 1991). Although it has been suggested that foragers may deposit this compound on visited flowers to signal nectar depletion, this hypothesis has not been conclusively demonstrated (Balderrama et al. 1996; Stout & Goulson 2001; Gawleta et al. 2005). In particular, it seems likely that evasive alarm pheromones play a role in the predator avoidance response of foraging bees. Thus, evasive alarm pheromones are used by some Asian *Apis* species (Suwannapong et al. 2011a) which do not appear to mark visited flowers (Suwannapong et al. 2011b) and, in *A. mellifera* and certain species of Meliponini bees, these evasive substances are released in response to an experimental disturbance (Lindauer & Kerr 1960, page 31; Balderrama et al. 1996). Furthermore, honeybees and bumblebees reject flowers with a crushed conspecific or its smell (Stout et al. 1998; Dukas 2001; Abbott 2006), and honeybees, *A. mellifera*, reject flowers where a crab spider has previously struggled with another honeybee (Llandres & Rodriguez-Girones 2011).

Available evidence therefore suggests three nonexclusive interpretations: bees mark visited flowers to increase colony foraging efficiency, bees use alarm pheromones to mark dangerous flowers, or bees can detect, and avoid, the smell of a crushed conspecific. To discriminate between these hypotheses, we note that they make contrasting predictions for social and solitary bees.

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According to Hamilton's (1964a, b) rule, upon detection of a potential predator an individual will benefit from alerting group members if the cost to the sender is smaller than the cumulative benefit to group members, discounted by the appropriate coefficients of relatedness. We should therefore expect the evolution of alarm signals in social, but not in solitary insects (Wyatt 2003). Indeed several studies have shown that the active release of alarm signals has evolved in eusocial and group-living animals (reviewed in Blum 1969, 1974a, b; Verheggen et al. 2010), while we are not aware of any study that has specifically looked for the presence of alarm signals in solitary insects. As mentioned above, eusocial bees are known to use aggressive alarm pheromones (Koeniger et al. 1979; Roubik et al. 1987; Schmidt 1998; Schorkopf et al. 2009), which have not been found in social species with small colonies such as bumblebees (Maschwitz 1966). In contrast, alarm signals or predation cues are involved in the predator avoidance response of honeybees and bumblebees (Stout et al. 1998; Dukas 2001; Abbott 2006).

While the release of alarm signals is more likely to evolve in social than in solitary insects, both social and solitary species are expected to respond to any cue that signals the presence of a predator. Indeed, in the particular case of bees, it has been shown that solitary bees should accept lower levels of predation risks while foraging than social species (Clark & Dukas 1994; Rodríguez-Gironés & Bosch 2012). We should therefore expect solitary bees to respond more strongly than social bees to the presence of cues signalling the proximity of predators, but only social bees to give alarm signals to warn their conspecifics. A similar argument would suggest that social, but not solitary bees should mark visited flowers to increase colony foraging efficiency. To discriminate between marks left to inform about danger or resource depletion, we note that in the former case bees should only avoid flowers where a conspecific has been attacked, while in the latter flower rejection should depend on the number and duration of previous visits.

The main aim of this study was to determine whether social bees release deterrent substances as alarm signals to communicate the presence of a dangerous flower. Of particular interest is the possible use of alarm signals by bumblebees, which do not appear to use aggressive alarm pheromones for nest defence (Maschwitz 1966). A subsidiary aim was to confirm that solitary bees make no use of such alarm signals. To do this, we compared the number of bees, from different social and solitary species, visiting and rejecting control inflorescences that had been visited by a bee and inflorescences where we had simulated a predator attack by pinching a bee with forceps. If bees marked all flowers they exploited, control and experimental inflorescences should be treated alike. If bees release some volatile cues as a side-effect of the attack, there should be no association between the release of such compounds and the sociality status of the bees: we would expect no differences in the response of social and solitary bees to the manipulation. If, on the other hand, the substance is actively released as a warning signal, only social bees should respond to the manipulation.

METHODS

Study Sites and Species

We conducted the experiments in six different geographical areas: Baza (Granada, Spain), Almeria (Spain), Villuercas-Ibores (Extremadura, Spain), Cannonvale (Queensland, Australia), MacRitchie Reservoir Park (Singapore) and Xishuangbanna (Yunnan province, China) between May 2009 and May 2011. In each locality we conducted trials in patches as distant as possible in order to avoid pseudoreplication. The distance used between patches for each species was approximately 20, 50, 100, 100, 1000, 5000 and 5000 m for *Nomia strigata*, *Panurgus* sp., *Apis dorsata*, *Apis florea*, *Bombus terrestris*, *Eucera* sp. and *A. mellifera* bees, respectively. Our data set comprised a total of 233 experimental and 236 control trials from seven species of bees foraging at different flower species (see Table 1). We selected *A. mellifera*, *A. dorsata*, *A. florea* and *B. terrestris* as species representative of social bees and *Eucera* sp., *N. strigata* and *Panurgus* sp. as species representative of solitary bees. We selected our species from three different families: Apidae, Halictidae and Andrenidae (see Table 1).

Experimental Procedure

For each trial we selected and marked one inflorescence (hereafter referred to as the focal inflorescence) and assigned it to the experimental or control treatment in pseudorandom order: treatment was allocated randomly to odd inflorescences, and even inflorescences were assigned to whatever treatment had not been used for the previous observation. For the control treatment, we waited until a bee landed on the inflorescence and left it. For the experimental treatment, we waited until a bee landed on the selected inflorescence and carefully held it on the inflorescence for 2–10 s, grasping the bee with forceps over the thorax. We did not visibly harm bees: they left the area flying as soon as we released them. Social bees emitted a strong distinctive smell when pinched (a similar observation was reported by Abbott 2006), although one of the authors of this study was unable to detect it.

Once the bee left, we recorded the number of bees approaching and visiting the focal inflorescence during the trial. After a bee approached the inflorescence, we distinguished two bee responses: visits and rejections. We considered that a bee visited an inflorescence when it approached and landed on it, and that the bee rejected the inflorescence when it approached it, hovered for a few seconds in front of it (sometimes touching it with its forelegs) and then left without landing. Trials lasted 10–30 min depending on the visit rate of each bee species (see Table 1). All trials were conducted during sunny weather, at the peak time of bee activity. In most trials, several bees approached the focal inflorescence. The total number of bees approaching control and experimental inflorescences for each bee species is given in Table 1.

Table 1
Bee species, bee family, flower species, duration of trials (min) and sample size of experimental and control trials performed for each bee species

Bee species	Family	Flower species	Experimental treatment (*N*)	Control treatment (*N*)	Duration of trials (min)	Site of collection
Apis mellifera	Apidae	*Bidens alba*	35 (105)	35 (96)	30	Australia
Apis dorsata	Apidae	*Calliandra emarginata*	35 (116)	35 (102)	15	China
Apis florea	Apidae	*Alchornea tiliifolia*	30 (84)	30 (80)	15	China
Bombus terrestris	Apidae	*Teucrium fruticans*	40 (104)	40 (112)	20	Almeria, Spain
Eucera sp.	Apidae	*Lavandula stoechas*	5 (10)	7 (17)	20	Extremadura, Spain
Panurgus sp.	Andrenidae	*Launaea pumila*	40 (48)	40 (45)	20	Granada, Spain
Nomia strigata	Halictidae	*Melastoma malabatrichum*	48 (152)	49 (140)	10	Singapore

The total number of bees that approached experimental and control inflorescences in each experiment is given in parentheses after the sample size.