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Response to competing conspecific cues depends on social context in the honey bee *Apis mellifera*

Rebecca R. Westwick*, Gavin P. Brackett, Cameron E. Brown, Bethany J. Ison, Clare C. Rittschof

Department of Entomology, University of Kentucky, Lexington, KY, U.S.A.

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Animals exist in a world that is replete with sensory information. Not all of this sensory information is relevant to the organism at a given time, though. Understanding how animals are able to pick out 'the signal from the noise' has been of interest to behaviour and neuroscience researchers for decades. This problem may be especially challenging when the conflicting sensory 'noise' is also a conspecific signal, given that organisms often show heightened sensitivity to conspecific cues. We challenged nurse honey bees who were performing larval caretaking behaviours with honey bee alarm pheromone, a conspecific cue that they are able to detect but show low behavioural sensitivity to compared with other honey bee workers like guards and soldiers. We found that nurse bees that originated from high-aggression colonies decreased their larval caretaking behaviours in the presence of alarm pheromone, while nurses from low-aggression colonies did not show this change. Our work highlights the importance of considering social context when examining how organisms respond in the face of a sensory-rich world.

Communication, the process of sending and receiving informative signals, is critical for social species. This is particularly true for species that live in large, complex societies (Marler & Vandenbergh, 1979; O'Donnell & Bulova, 2007). Successful communication requires a receiver that is able to both detect and correctly process the relevant signal (Kaplan, 2014; Seyfarth & Cheney, 2003). For a receiver, this process can be difficult when competing information is present, a concept known in human systems as 'the cocktail party problem' (Cherry, 1953). Competing stimuli can cause interference at multiple points along neurosensory pathways, from peripheral sensory systems (as with energetic masking, information overload and olfactory receptor antagonism) to central processing (as with informational masking, distraction and cross-modal interference) (Milinski, 1990; Oka et al., 2004; Rosa & Koper, 2018). Competing information is often a pervasive, relatively persistent feature of the environment the organism is in. For example, anthropogenic noise is detrimental to an enormous variety of organisms, impairing everything from intraspecies communication to predator avoidance (Butler & Maruska, 2020; Chan et al., 2010; Kunc & Schmidt, 2019). Similarly, background odours (such as plant volatiles) create an 'odourscape' that can alter behaviours that rely on olfaction such as foraging and mate finding (Conchou et al., 2019; Deisig et al., 2014; Schröder & Hilker, 2008). But competing information can also come from a more transient source, such as a sudden noise that causes a startle response or a cue from another animal (Elwood et al., 1998; Moorhouse et al., 1987). With so many places along the neurosensory trajectory for interference to occur, any stimulus that causes particularly strong activation of the nervous system has the potential to alter behaviour or impair communication. One such example can be found with conspecific cues.

Many animals show special sensitivity to conspecific information across multiple sensory modalities (Braaten & Reynolds, 1999; Hattori et al., 2010; Kano & Call, 2014). This special sensitivity can involve greater precision in discriminating between similar stimuli. This phenomenon has been especially well documented in olfaction, a modality with high potential for specificity where receivers are commonly (but not always) tuned narrowly to precise blends of pheromones (Buchinger & Li, 2020; Endler et al., 1993; Li et al., 1995). Special sensitivity can also manifest as lower response thresholds, where organisms are sometimes able to detect

* Corresponding author.
E-mail address: r.r.westwick@gmail.com (R. R. Westwick).

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conspecific information at remarkably low signal strength (Kaissling & Priesner, 1970; Stengl, 2010). This special sensitivity comes about via multiple levels of sensory organization, from the tuning of olfactory receptors to neuronal organization that selectively amplifies conspecific pheromones (Sakurai et al., 2014; Tabuchi et al., 2013). Given this heightened sensitivity, conspecific signals could represent an especially potent source of conflicting information that could affect behavioural responses, but this idea remains untested.

Honey bees (*Apis mellifera*) provide a unique opportunity to study how different conspecific signals can interfere with each other, particularly in the context of olfactory signalling. Advanced eusociality in bees (e.g. honey bees, stingless bees) is associated with elaborated pheromone signalling (Fischman et al., 2011; Wittwer et al., 2017). Honey bees live in large, dense, enclosed nests where at least a dozen different pheromones can be in play at once (Bortolotti & Costa, 2014). Some of these pheromones are primer pheromones that are constantly present in the background and play out their effects over longer timescales, such as larval esters that suppress worker ovary development and have slow-acting effects on forager effort to collect different resources; other pheromones have acute releaser effects, and these are often more specific to particular in-hive tasks (reviewed in Slessor et al., 2005). Individual worker bees temporarily specialize on these different tasks at distinct times in their adult lives, but all tasks occur in the colony simultaneously (Seeley, 1995). This creates a system where individuals are exposed to a large suite of social signals but need to attend to only a subset of them at a given time. The prevailing model suggests that individuals show different sensitivity thresholds to cues that induce each task (Beshers & Fewell, 2001). Given the same level of a stimulus, some individuals will be more likely to respond than others despite similar perceptual abilities, allowing colonies to distribute labour demands among many thousands of individuals. Hormonal variation associated with behavioural specialization alters the probability that an individual worker will respond to a task-specific stimulus, allowing that individual to focus on task-specific social cues; as workers age and transition to new specializations, their hormonal milieu and stimulus thresholds shift in parallel (Robinson, 1987b). There is also evidence that perceptual abilities differ among specialists. For example, workers performing different jobs have different proteomic signatures in their antennae (Iovinella et al., 2018). This finding suggests that olfactory receptor protein abundance varies with task, potentially facilitating specialization. Overall, the conventional view is that behavioural specialists parse diverse cues in the nest and pay attention and respond primarily to task-relevant information.

Despite this conventional view, however, there is evidence that certain types of social information cross the lines of behavioural specialization and that workers pay attention to a greater range of information than previously appreciated. One example of such complexity is in the context of honey bee defensive aggression. Guards and soldiers are two types of defensive specialists that preferentially respond aggressively to threats to the colony (Breed et al., 2004). They stand near the entrance of the nest and perform characteristic attack behaviours in response to threats (Moore et al., 1987). Both specialist types also emit and respond to a social cue, the honey bee 'alarm pheromone', a blend of compounds in which isopentyl acetate (IPA) is a primary component (Boch et al., 1962; Boch & Shearer, 1971). The primary function of alarm pheromone is to recruit additional defensive specialists, especially soldiers, in response to an escalating or persistent threat (Breed et al., 2004). However, ample evidence shows that nondefensive specialists, including honey storers, individuals in the brood nest, returning foragers and laboratory-reared young and middle-aged workers, also sting and/or respond to alarm pheromone in certain contexts (Allan et al., 1987; Breed et al., 1990; Burrell & Smith, 1994). This generalized response to alarm cues could suggest that these are particularly salient social cues for honey bee workers. Recent studies show that even pre-adult (larval and pupal) worker bees are sensitive to the level of defensiveness (and thus potentially the degree of alarm pheromone emission) displayed by their natal nestmates: individuals that develop in a relatively high-aggression colony show behavioural and physiological consequences in adulthood (Rittschof et al., 2015, 2019). As larvae largely lack sensory structures (Betts, 1923; Eichmütler & Schäfer, 1995), one explanation for this effect is that nurse bees (brood care specialists) alter their interactions with larvae in high-aggression colonies, possibly through differential response to alarm pheromones released by nestmates. This result would be surprising, however, as nurses are classically considered to be nondefensive brood care specialists (Johnson, 2008; Pearce et al., 2001). Our goal in the current study was to assess whether nurses do indeed pay attention to alarm cues, a response that may ultimately shape the phenotype of the developing larvae under their care.

Nurses check on and feed the larvae within the brood nest, responding in part to a putative 'begging pheromone', e-β-ocimene, that is released by starving larvae and provokes nurse visits (He et al., 2016). Nurses show relatively low behavioural responsiveness to alarm pheromone and are much less likely to behave aggressively in general compared to guards and soldiers (Collins, 1980; Pearce et al., 2001; Robinson, 1987a). However, this lowered responsiveness is not a matter of detection abilities, as assessed using electroantennogram assays (Robinson, 1987a). In the current study, we assessed the possibility that alarm pheromone competes with larval olfactory cues to alter nursing behaviour. We tested and compared individuals from relatively high- and low-aggression colonies to evaluate whether colony level variation in alarm cue sensitivity is reflected in nurse behaviour. Such a result would suggest a more complex system of cue integration than previously appreciated in the honey bee.

METHODS

Overview

We used observation hives and video recordings to measure variation in nursing behaviour directed towards individual honeycomb cells in three treatment groups that differed in the quantity of begging cue: (1) larvae, (2) larvae supplemented with begging pheromone and (3) empty cells (a control). We further observed these behaviours with and without whole-colony exposure to an interfering social signal, alarm pheromone. We evaluated nurses from high- and low-aggression colonies (colonies that are more or less responsive to defence-inducing cues as described below) to determine whether colony response thresholds predict nursing behaviour generally and/or the nurse behavioural response to alarm pheromone. A diagram of our experimental treatments can be found in the Appendix (Fig. A1).

Honey Bee Sources

We performed experiments in Lexington, Kentucky, U.S.A. during July–October 2019 and 2020. The colonies from which we sourced the nurses and brood had mostly been installed as packages at the beginning of the season (strains advertised as 'Italian' and 'Russian Hybrid'). Remaining colonies were of mixed local genetic stock. All colonies were maintained according to standard management practices and parasite control measures as suggested by the Honey Bee Health Coalition. Only colonies that were at full mature size and healthy at the last check were used in the

experiment (i.e. queenright, not showing any overt signs of disease, not undergoing active mite treatment).

Identifying High- and Low-aggression Colonies for Nurse Bee Collection

Following Rittschof et al. (2015), we surveyed ~30 colonies for response to alarm pheromone, which is a measure of defensive aggression (Collins & Kubasek, 1982). Briefly, we photographed the landing board of each hive to measure the baseline activity level of a colony, which is the number of bees that could be seen on the landing board of the hive in the photograph. We then introduced a small piece of filter paper with 3 µl of 1:10 isopentyl acetate (hereafter IPA, a primary component of the honey bee alarm pheromone; Boch et al., 1962) in mineral oil and gave the bees 1 min to respond. We then took a second picture of the number of bees on the landing board. This amount of IPA is within the standard range for field aggression tests (Boch & Rothenbuhler, 1974; Collins & Kubasek, 1982; Collins et al., 1987). It is the estimated amount of IPA released by guard and soldier bees during a strong colony level defensive response (Allan et al., 1987; Collins & Rothenbuhler, 1978). When IPA is placed at the entrance, bees emerge from the entrance in response, typically congregating at the site of the filter paper or crawling up the front of the hive. Because the bees rarely take flight, the second photo of the entrance captures the IPA response (Collins & Kubasek, 1982; Guzmán-Novoa et al., 2003). We calculated the colony's response score as the difference between the number of bees after the IPA was placed and the number of bees at baseline on the front of the hive or landing board. We define an experimental 'round' as being the nurses from a single colony tested over 2 days (see Nurse Behaviour Assay and Recordings). For each pair of experimental rounds, we selected one colony with the highest and one colony with the lowest IPA response score to be the source colonies for nurse bees. No colony was used more than once as a source colony during the study. Overall, we included six colonies per aggression level ($N = 12$ colonies total, three high- and three low-aggression colonies in 2019 and three high- and three low-aggression colonies in 2020). Trials were conducted within 2 weeks of an aggression assay, as colony aggression level can vary over the season (Napier et al., 2021; Pearce et al., 2001; Schneider & McNally, 1992).

Larval Treatments and Manipulation of Begging Pheromone

We generated three larval treatments that differed in the quantity of begging pheromone to assess variation in nurse bee behaviour with and without interference from alarm pheromone. Honey bee larvae develop in individual honeycomb cells (one larva/cell). Our treatments included (1) a larva alone (unmanipulated), (2) a larva to which we added 10 µl 1:10 e-β-ocimene (hereafter EBO) in mineral oil, gently pipetted on the sides of the honeycomb cell or (3) a naturally empty honeycomb cell (control). The amount of EBO was based on previous work (He et al., 2016; Maisonnasse et al., 2009; Traynor et al., 2014) and a small pilot study where we supplemented larval cells with EBO across a concentration gradient and compared nurse visits (see Appendix). We selected the EBO dose that increased visits relative to untreated larval cells in this pilot test. In early trials of our main experiment (4 total rounds out of 12), we included larvae treated with two forms of e-β-ocimene, a pure form (Toronto Research Chemicals, O150025) and a racemic mixture used in previous studies (Sigma-Aldrich, Inc., St Louis, MO, U.S.A., W353901; as used in He et al., 2016). Early results did not suggest a difference between the two types, so we treated results from both EBO sources the same in analysis and continued using only the racemic mixture in later trials (see Appendix).

Because larval age impacts nurse bee visiting behaviour, we standardized larval age across the entire experiment. To do this, we chose a honey bee colony that was not otherwise used in the experiment (a different queen was used for each experimental round). We located the queen and placed her in a cage with an empty honeycomb frame (standard deep frame, ca. 48.3 × 23 cm) for 24 h to allow her to lay eggs. The cage has holes that are too small for the queen to pass through but large enough to allow workers access to the frame and larvae. Following the 24 h period, the queen was released back into the hive and the frame was placed back in the cage to prevent further laying (Rittschof et al., 2015). When the eggs had hatched and the larvae on the frame were approximately 2 days old (96–120 h postlaying), we removed the frame from its natal colony and performed the larval treatments. We assigned up to 30 cells on the frame to one of three treatments (see above, $N =$ approximately nine cells of each treatment).

The location of the cells for treatment on the honeycomb frame was necessarily constrained by the laying pattern of the queen. We selected cells covering the entire width of the brood area since proximity predicts similar offspring age. We avoided selecting focal cells that were immediately adjacent when possible to minimize potential interference of the EBO between cells, since the EBO was pipetted on the wall of the cell. Cells that contained larvae were randomly assigned to the unaltered or EBO treatment. Control cells were selected as any naturally empty cells that were not adjacent to other treatment cells and were distributed across the brood area as evenly as possible based on the queen's laying pattern.

Nurse Collection and Observation Hive Set-up

We inserted the honeycomb frame containing our treated larvae into the selected nurse source colony (either high-aggression or low-aggression, see above) for 10 min to draw nurse bees onto the frame (as in He et al., 2016). The frame was then removed and placed in the top portion of an Ulster observation hive (Fig. 1). This type of hive has an enclosed five-frame, queenright colony (known as a 'nuc') in a wooden box below a single glass-panelled viewable frame mounted on top. The queenright colony provides the blend of typical hive and queen pheromones that are required for the nurse bees to behave normally, as bees quickly begin to change their behaviour if they detect that their colony is queenless (Butler, 1954; Cejrowski et al., 2018). The top and bottom portions of the Ulster hive are separated by a mesh screen that allows air to pass freely and some physical contact between the bees (which is required for queen pheromone transfer; Ferguson & Free, 1980) but does not allow the bees to mix. We did not observe any overt aggression between bees at the nexus of the top and bottom portions. The same small colony was maintained in the bottom portion of the hive throughout the season, one for each year. Throughout the experiment, the colony was kept inside of a small shed but was allowed to forage freely through a tube that connected to the outside (including during assays). Nurses in the top portion of the hive were provided with supplemental honey via a drip feeder and bee-collected pollen rolled into balls (Betterbee) ad libitum. The honey feeder was removed during the acclimation period and the ~35 min behavioural assay and video recording (see below), although the nurses would still have access to any food that had previously been stored on the frame and were able to exchange food with the lower hive bees via trophallaxis.

Once the larval frame with nurses was placed in the top of the observation hive, the hive was kept under red light and allowed to acclimate for at least 30 min and until we observed normal nursing behaviour. We placed a transparent sheet of plastic with guide marks over the side of the observation window to allow video scorers (see below) to identify the treated cells.

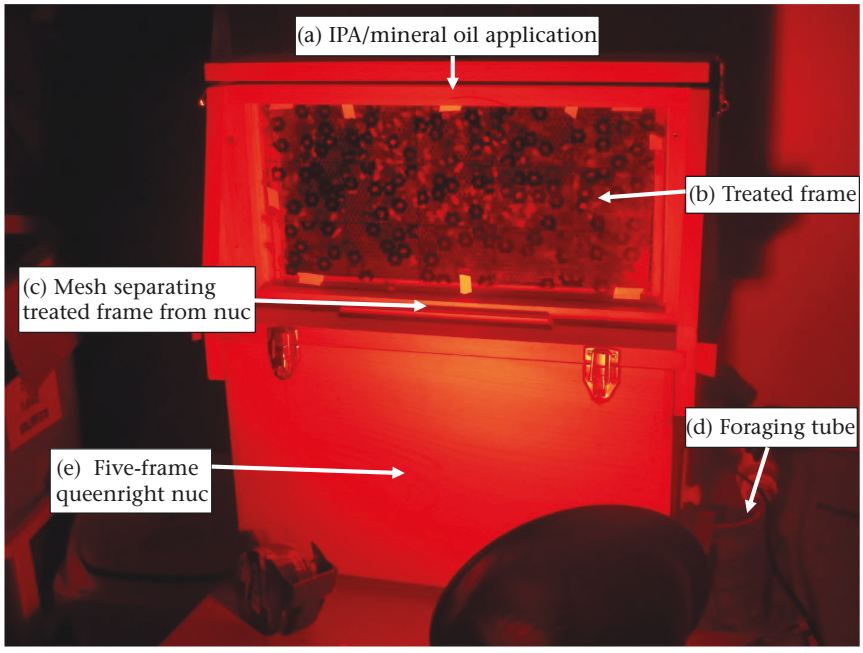

Figure 1. Picture of observation hive set-up under red light. (a) Alarm pheromone (isopentyl acetate, IPA) or mineral oil as a control was applied to a small piece of filter paper that was placed on a mesh ventilation hole at the position indicated by the arrow. (b) The treated frame with the nurses from high- and low-aggression colonies can be seen. A transparent sheet with guide marks covers the outside of the observation hive to highlight the selected empty, larval and begging signal-augmented larval cells. (c) The position of the mesh section that separates the treated frame from the nuc (a small, five-frame queenright colony). The mesh allows odour signals and limited physical contact between the target nurses and the bees from the nuc but does not allow them to mix. (d) Plastic and PVC tube that terminates outside the shed to allow the bees in the nuc to forage freely. This tube was always open, even during assays, but the nurses on the top frame could not leave due to the mesh that separated them from the nuc. (e) The nuc that provided the normal suite of background social signals found in queenright colonies that is necessary for typical nurse behaviour to occur.

Nurse Behaviour Assay and Recordings

We recorded nurse bee behaviour using a Panasonic HC-V770 video camera. In total, we videorecorded the larval frame and nurse bee behaviour continuously for about 35 min following the acclimation period. This included 2–5 min before an application of IPA or mineral oil control and 30 min following application. The initial 2–5 min period was included to allow ample time to set up the camera properly and prepare and apply the IPA/mineral oil application. We analysed the first 10 min following IPA or mineral oil application to assess how nurse behaviour changes as a function of source colony aggression, cell EBO treatment and IPA exposure (see below).

The alarm pheromone exposure treatment involved pipetting 10 µl of mineral oil (control, Sigma, M8410) or a 1:100 dilution of isopentyl acetate (IPA, Sigma, 112674) in mineral oil onto a small piece of filter paper, which was then immediately placed over a screened ventilation hole in the centre of the top of the larval frame with nurses (see Fig. 1) (Collins & Rothenbuhler, 1978). This amount of IPA falls within the range of a realistic dose of alarm pheromone that would be released by bees responding to an aggressive threat based on the sting-equivalent dose per bee, although we used a lower dose than in the colony level aggression assays due to the proximity of the exposure point to the nurses (Allan et al., 1987). The location of nurse bees and the brood nest inside a honey bee colony can vary from a few centimetres from the entrance to more than half a metre away, but, on average, nurses are more distant from alarm cues than guards or soldiers. During each experimental round, we treated nurses once with IPA and once with mineral oil. The IPA and mineral oil applications were applied to the same group of nurses in a random order spaced 24 h apart to allow recovery from the stimulus (Alaux et al., 2009; Collins & Rothenbuhler, 1978).

Ethical Note

All honey bee colonies used to source bees for this study were maintained according to recommendations set forth by the Honey Bee Health Coalition. These recommendations are designed to minimize ecological impacts of beekeeping as well as colony mortality and stress resulting from inadequate nutrition and/or high parasite and disease pressure. No permits, licenses or pre-approval at the level of the institution or government were required to carry out this study. Nevertheless, we minimized our stress and mortality impacts by using no more colonies than necessary to obtain a reasonable number of observations with a robust experimental design; the processes used to move and house the bees in this experiment are all standard practices. Worker bees were gathered with minimal disturbance based on which ones chose to enter the experimental frame. Frames with adult bees were moved from the source hive to the observation hive inside a dark, insulated box to minimize distress. The observation hive environment is very similar to a normal hive in terms of conditions such as temperature, humidity, density of individuals and sensory enrichment, thus it is a low-stress experimental context. Furthermore, all manipulations surrounding the observation hive (introduction to the hive, maintenance, experimental procedures and removal at the end of the study) were carried out under red light conditions to further prevent any additional stress (also a common practice). Throughout the experiment, bees had access to natural food sources ad libitum to minimize the possibility of nutritional stress. Exposure to alarm pheromone induces aggression, which could be considered a stressed state, but exposure to alarm pheromone is common in natural conditions at the level used in our study. All bees were returned to their natal hives upon completion of the observations. Few individuals died during the study, and the removal and return

of experimental bees had no discernible impacts on their home colonies.

Behaviour Scoring from Videos

The videos were scored by observers who were blinded to the nurse source colony, the identity of the treatment cells and the IPA versus mineral oil application. To score the data on nursing behaviour, the observers began watching from the moment the IPA or mineral oil was applied (excluding the first 2–5 min of preparation in the video) and observed for 10 min. The observers scanned each treatment cell ($N = \sim30$, see above) for nursing behaviour, where a worker bee placed her whole head (and in some cases, thorax or abdomen) inside one of the treatment cells. Observers did not track individual nurses but rather tallied the total amount of nursing attention a cell received. Observers recorded the number of times any nurse bee placed her head in each cell type, as well as the time stamp and duration of each visit to the nearest second. Inspection of the video scoring revealed that false positives were far more common than false negatives due to a grooming behaviour that can look similar to a nursing visit. In the grooming behaviour, the nurse necessarily angles her head downward (appearing to go towards a cell) as she lifts her abdomen high, rubbing her back legs together and against the bottom and sides of her abdomen. In a nursing visit, the bee only moves her head downward while keeping her abdomen mostly parallel to the comb's surface and without rubbing her legs (unless she climbs fully into the cell, which is unmistakably nursing behaviour). A separate observer who was trained to distinguish the grooming behaviour checked each recorded nurse visit to determine whether it was a true observation.

We additionally analysed the activity level of the bees surrounding the presentation of alarm pheromone (or its mineral oil control counterpart). For this analysis, observers would count the number of times any bee crossed a horizontal line on the frame and would note the direction of the cross (up or down). These observations were completed for 15 s at six time points for each video: 2 min before stimulus presentation, 1 min before stimulus presentation, directly at the moment of stimulus presentation, 1 min after stimulus presentation, 2 min after stimulus presentation and 5 min after stimulus presentation.

Statistical Analysis

We performed statistical analyses with R version 4.1.2 (R Core Team, 2021). To evaluate how pheromone treatments and source hive aggression impacted the frequency of nurse visits and the latency to the first visit, we used the 'glmmTMB' package to create generalized linear mixed models (GLMMs) with a negative binomial distribution with quadratic parameterization (Brooks et al., 2017; Hardin & Hilbe, 2007). Our response variable for this analysis included nursing observations following the IPA or mineral oil application. We included nurse source colony aggression level (high versus low), alarm pheromone application (IPA versus mineral oil), cell treatment (larva, larva with EBO, empty) and their interactions as fixed effects. All interactions (including the three-way interaction) were included in the global model. We additionally included source colony identity (ID) (a unique identifier of the colony the nurses came from), year and IPA versus mineral oil application order as random effects.

In our experiment, source colonies were derived from a variety of genetic strains (see Honey Bee Sources above). Because genetic strain is correlated with aggression in some studies (Alaux et al., 2009; Harpur et al., 2020; Locke, 2016), we considered including it as a factor in our models. However, preliminary examination of the data showed that the high- and low-aggression source colonies used in our experiment were distributed evenly across strains, suggesting no clear association between aggression and strain. Therefore, we omitted genetic strain from our models.

We took the global model and created alternative candidate models by progressively removing interaction terms. We then used Akaike information criterion corrected for small samples (AICc)-based model selection criteria to select the final model with the 'AICcmodavg' package (Mazerolle, 2020). Model diagnostics were assessed using the 'DHARMa' package, which includes a Q–Q plot, Kolmogorov–Smirnov test, dispersion test, outlier test, within-group uniformity test and Levene test (Hartig, 2022). We used the 'car' package to run a type III ANOVA on the final model to estimate significance values (Fox & Weisberg, 2019). We used the 'performance' package to examine whether our data set showed zero inflation (Lüdecke et al., 2021). Post hoc comparisons were carried out using the 'multcomp' and 'emmeans' packages for Tukey tests and estimated marginal means (EMM) comparisons, respectively (Hothorn et al., 2008; Lenth, 2022).

To evaluate the duration of nurse visits, we categorized visits into groups based on how long the visit lasted. When a nurse enters a honeycomb cell with a larva (called a 'visit'), she may be quickly checking the feeding status or health of the larva, feeding the larva or sleeping or performing thermoregulation activities (Gilliam et al., 1983; Lindauer & Watkin, 1953; Siefert et al., 2021). The nature of the nurse's visit can be assessed using the duration of the visit: anything shorter than 20 s is likely a brief check to assess the health of the larva and/or its feeding status (hereafter 'inspection'), anything between 20 s to 3 min is characteristic of larval feeding (hereafter 'feeding visit'), and anything longer than 3 min is an indication of sleeping or thermoregulation (hereafter 'sleeping/thermoregulation') (Brouwers et al., 1987; Gilliam et al., 1983; Lindauer & Watkin, 1953; Siefert et al., 2021). Similar to previous work (Brouwers et al., 1987; Huang & Otis, 1991; Lindauer & Watkin, 1953; Riessberger & Crailsheim, 1997; Schmickl et al., 2003), we further subdivided the 'inspection' category into two parts: 'short inspections' (1 s or less, where a nurse is likely very briefly using olfactory cues to rule out whether a larva is hungry or diseased before moving on) and 'long inspections' (2–20 s, where a nurse is likely taking more time to assess how much food a larva has to determine whether it requires more). Within each group, we used individual chi-square tests to compare the levels for each of our major factors (nurse source hive aggression, IPA application, cell type, as described above). None of these initial comparisons were significant, so we chose not to further examine the duration data for interaction effects among factors. We performed our tests using chi-square tests of independence with the 'chisq.test()' function (R Core Team, 2021).

To assess the activity level, we built linear mixed models using the 'lme4' package (Bates et al., 2015). The data were log-transformed to improve the data distribution. The number of crosses was our response variable. We used a similar AIC-based process to achieve our final model from a global model that contained aggression, alarm pheromone application, time point, direction and all possible interactions as fixed effects, plus colony ID and treatment order as random effects. Model diagnostics and significance values were determined as described above. We again used the 'emmeans()' package to carry out post hoc comparisons.

Figures were generated using the 'ggplot2' package (Wickam, 2016).

RESULTS

Impacts of Competing Pheromone Information on the Number of Nurse Visits

We built a GLMM to examine how competing pheromone information impacts the number of nurse visits to larval cells. The fixed effects that were included in our final model are shown in Table 1. The full global model and the random effects for the final model can be found in the Appendix. As expected, cell type (variation in begging pheromone emission: empty cell, larva, larva + EBO) significantly impacted the number of nurse visits (ANOVA: Wald $\chi^2_2 = 16.6$, $P = 0.0002$; range 0–28 visits; Fig. 2a). We expected that EBO-treated larval cells would show the greatest number of visits, followed by untreated larval cells and empty cells (He et al., 2016). However, a Tukey post hoc analysis indicated that, while untreated larval cells showed significantly more visits than empty cells (Tukey test: $P < 0.001$), EBO-treated larval cells received significantly fewer visits than untreated larval cells and were not significantly different from empty cells (Tukey test: larva-EBO: $P = 0.01$; EBO-empty: $P = 0.53$). We suspected that this phenomenon might have been caused by EBO-treated cells being visited first due to the heightened strength of the signal. Most cells that were visited at least once received only one visit (one visit: 54%; all other numbers of visits: 46%). If these early visits occurred during the acclimation period when we were not observing visits, the result would be reduced visits during the actual observation window. We therefore analysed the proportion of cells that received zero visits during the observation window (versus cells that received any number of visits). Although the data set overall was not zero-inflated (ratio of observed to predicted zeroes = 1.01), we found that there were nearly 50% more zeroes in the EBO-treated larval cells than in the untreated larval cells (chi-square test: $\chi^2_2 = 19.37$, $P = 0.00006$; post hoc comparisons: EBO versus larva: $P_{adj} = 0.00005$; larva versus empty: $P_{adj} = 0.01$; EBO versus empty: $P_{adj} = 0.15$; Fig. 3). Figure 2b shows the rate of visits only to cells that received at least one visit.

In addition to their response to brood signals, we found evidence that nurses also react to alarm pheromone and that their colony of origin influences this behaviour: we found a significant interaction between nurse bee source colony aggression and IPA application on the number of nurse visits (ANOVA: Wald $\chi^2_1 = 8.2$, $P = 0.004$). Nurse bees from high-aggression colonies decreased the number of visits to larvae in the presence of IPA relative to the mineral oil control, while nurses from low-aggression colonies did not show this change (Fig. 2a). Nurses from high-aggression colonies made 35% fewer visits in the presence of IPA compared to the mineral oil control (high aggression: EMM log contrast = −0.44, $P = 0.045$). In contrast, nurses from low-aggression colonies showed a nonsignificant tendency towards increasing their visits in the presence of IPA, making 62% more visits during trials with IPA than during trials with mineral oil (Fig. 2a) (low aggression: EMM log contrast = 0.48, $P = 0.055$). We found no evidence that the response to IPA was influenced by begging cues. Rather, IPA decreased nurse activity with no additional influence of EBO treatment. This finding, that nurse bees from high-aggression colonies decreased visits in the presence of IPA while nurses from low-aggression colonies did not, remained true when considering only cells that received one or more visits (Fig. 2b).

Table 1
Fixed effects from the final GLMM used to evaluate how competing pheromone information and nurse source colony aggression impact the number of nurse visits with Wald χ^2 values and ANOVA-determined P values

Factor	Wald χ^2	*df*	*P*
Nurse source colony aggression	0.98	1	0.32
IPA application	0.07	1	0.79
Cell type	16.6	2	**0.0002**
Nurse source colony aggression*IPA application	8.2	1	**0.004**

IPA: isopentyl acetate. Significant outcomes are shown in bold.

Impacts of Competing Pheromone Information on the Duration and Timing of Nurse Visits

Almost all of the visits we observed (505/519, 97%) were inspections. Of these, 341 (67.5%) were short inspections (<1 s long) and 164 (32.5%) were long inspections (2–20 s long). The ratio of short to long inspections was not affected by any of our explanatory variables (chi-square test: nurse source colony aggression: $\chi^2_1 = 0.06$, $P = 0.81$; IPA application: $\chi^2_1 = 0.03$, $P = 0.87$; cell type: $\chi^2_2 = 2.33$, $P = 0.31$). Because we found an interaction effect between nurse source colony aggression and IPA application in the analysis of the number of nurse visits (above), we additionally performed a chi-square test on nurse source colony aggression separated out by IPA application (one for only IPA trials, one for only trials with the mineral oil control). The number of visits was similar within the mineral oil application comparing between high- and low-aggression nurse source colonies (Fig. 2). Meanwhile, there was a strong difference between the number of visits by nurses from high- and low-aggression colonies within the IPA application (Fig. 2). Dividing the visit duration chi-square tests in this way would allow us to see whether there was a similar pattern in the duration data. However, these tests were additionally insignificant (chi-square test: nurse source colony aggression, IPA only: $\chi^2_1 = 0.41$, $P = 0.52$; nurse source colony aggression, mineral oil only: $\chi^2_1 = 0.03$, $P = 0.85$).

We identified 12 feeding visits (2.3% of total visits), a frequency that is consistent with previous observations of nursing behaviour (Brouwers et al., 1987; Huang & Otis, 1991; Lindauer & Watkin, 1953). Nurses from low-aggression source colonies performed feeding visits at over three times the frequency of nurses from high-aggression source colonies, but likely due to the small total number of feeding visits, this pattern was not statistically significant (chi-square test: $\chi^2_1 = 2.88$, $P = 0.09$). Neither IPA application nor cell type showed significant differences in the number of feeding visits (chi-square test: IPA application: $\chi^2_1 = 0.43$, $P = 0.51$, cell type: $\chi^2_2 = 0.06$, $P = 0.97$). Only 2 out of 519 total visits (0.4%) fell into the sleeping/thermoregulation category, precluding further statistical analysis.

We also examined the timing of visits. We first tested whether nurse source colony aggression, IPA application and cell type impacted the latency to the first nursing visit for each cell that received at least one visit. The latency to the first visit was not significantly affected by any of these explanatory variables nor their interactions (see Appendix). Additionally, we visually examined the distribution of all visits within the 10 min window. We saw no clear directional trend, suggesting that the depression of visits seen in high-aggression colonies on IPA days lasted at least 10 min (Fig. 4). Finally, we assessed whether visits differed within each combination of aggression level and IPA application based on treatment order (i.e. if IPA was applied on the first or second day). We found no effect of treatment order within any combination of IPA application and source colony aggression (high aggression, IPA: EMM log contrast = 0.02, $P = 0.96$; high aggression, mineral oil: EMM log contrast = −0.54, $P = 0.22$; low aggression, IPA: EMM log contrast = 0.29, $P = 0.62$; low aggression, mineral oil: EMM log contrast = −1.14, $P = 0.08$). Because we saw no difference in the rate of visits on mineral oil control days when they fell after the alarm pheromone treatment (as opposed to before), we can infer that the rate of nursing behaviour had returned to baseline within 24 h of a perceived threat.

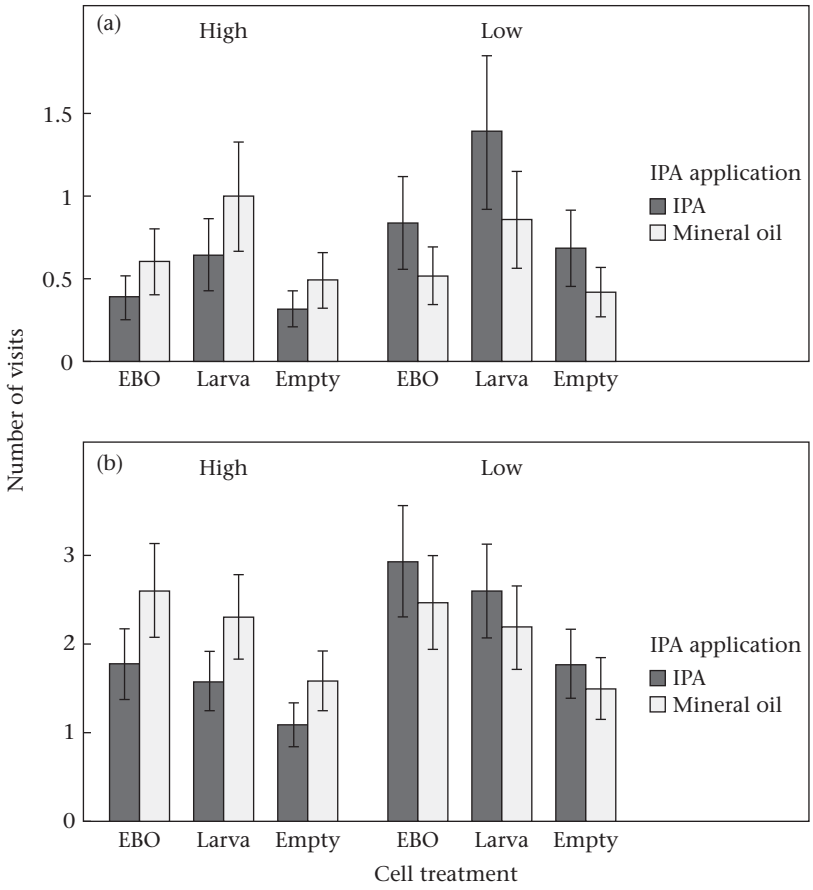

Figure 2. Least-squares mean (± SE) visits by nurses from high- and low-aggression colonies to cells that contained a larva with added e-β-ocimene (EBO), an untreated larva or an empty cell on days where alarm pheromone (isopentyl acetate, IPA) or mineral oil was applied (a) for all observations and (b) with cells that received zero visits being removed.

Impacts of Alarm Pheromone on the Activity Level of Honey Bees

To gain a clearer understanding of the behaviour of the bees in the immediate aftermath of the alarm pheromone presentation, we measured the activity level and gross movement patterns (towards versus away from the site of the alarm pheromone) before and after stimulus presentation. We found that the application of alarm pheromone affected the total activity level of bees in low-aggression colonies but not in high-aggression colonies. Our final linear mixed model included aggression, alarm pheromone application, direction and the interaction between aggression and alarm pheromone application as fixed effects, with colony ID and treatment order as random effects. We found a significant interaction effect between aggression and alarm pheromone application, suggesting that the addition of alarm pheromone affects the overall activity level of bees from different colony aggression levels in different ways (ANOVA: nurse source colony aggression: Wald $\chi^2_1 = 4.2$, $P = 0.04$; alarm pheromone application: Wald $\chi^2_1 = 0.10$, $P = 0.76$; direction: Wald $\chi^2_1 = 9.0$, $P = 0.002$; nurse source colony aggression∗alarm pheromone application interaction effect: Wald $\chi^2_1 = 60.7$, $P < 0.0001$). An estimated marginal means post hoc comparison revealed that overall activity level in low-aggression colonies was more than halved on alarm pheromone days compared to mineral oil days (8.0 crosses per 15 s compared to 16.4 crosses per 15 s). The activity level in high-aggression colonies was unaffected overall (12.4 crosses per 15 s versus 12.2 crosses per 15 s; high aggression: EMM log contrast = 0.02, $P = 0.76$; low aggression: EMM log contrast = −0.72, $P < 0.0001$). We additionally tested the pairwise differences between directions at each time point (i.e. up versus down at each time point for each combination of aggression and alarm pheromone application). We found only one time point with a difference: in high-aggression colonies on days where alarm pheromone was applied, during the 15 s

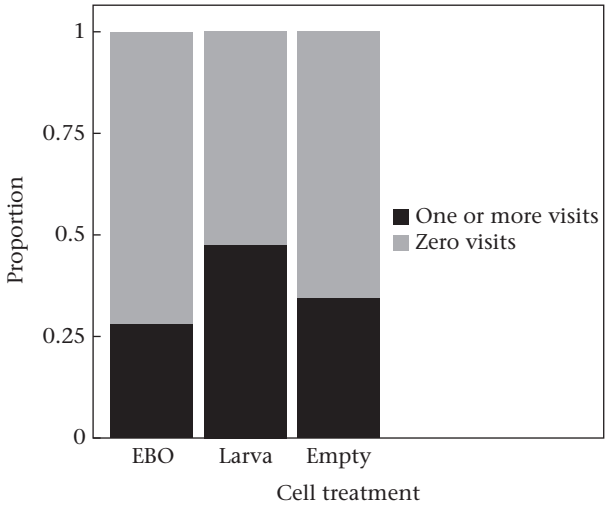

Figure 3. Proportion of each cell treatment (a larva with added e-β-ocimene, EBO, an untreated larva or an empty cell) that received zero visits versus cells that received any number of visits.

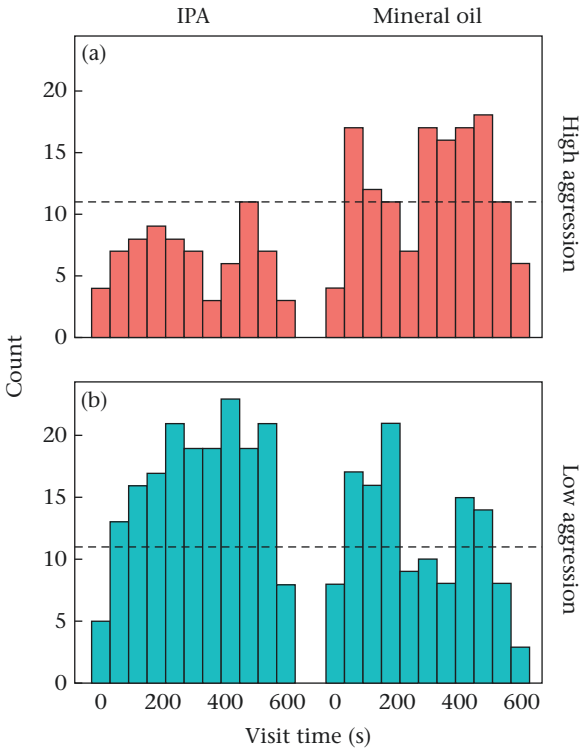

Figure 4. Average number of nursing visits to all cell types over the 10 min observation window (in seconds) in (a) high-aggression colonies and (b) low-aggression colonies on alarm pheromone (isopentyl acetate, IPA) days and on mineral oil days. Dashed line represents the mean value per bin on mineral oil (control) days.

immediately following the application of the pheromone, bees were significantly more likely to cross up (towards the pheromone source) rather than down (away from the pheromone source). They crossed up twice as often as down during this time point (EMM log contrast = 0.68, $P = 0.0096$, corrected with the Bonferroni method for the large number of comparisons; Fig. 5, see Appendix, Table A2 for a table of all P values). The bees had returned to equal numbers of crosses in each direction by the next measured time point 1 min later.

DISCUSSION

Here we show in a naturalistic colony context that nurse bees respond to social cues related to a separate specialization, colony defence. Even though nurses have a higher threshold for alarm response compared to defensive specialists (Robinson, 1987a), alarm information caused significant variation in the frequency of visits to larvae regardless of the intensity of begging cue emission. Importantly, we found that another source of variation in response thresholds to alarm signals, colony of origin, influenced this outcome: nurses collected from high-aggression colonies changed their larval care behaviour in the presence of alarm pheromone, while nurses from low-aggression colonies did not show this pattern. Overall, these results suggest behavioural specialists attend to a wider range of social cues than previously appreciated and that social and ecological information (e.g. predator threat levels) may have far-reaching and multigenerational colony level impacts.

There are at least two mechanistic explanations for why the nurses from high-aggression colonies change their larval care behaviour in the presence of alarm pheromone. First, it is possible that alarm pheromone interferes with their ability to detect cues emitted by larvae, decreasing their nursing response (i.e. masking, olfactory receptor antagonism) (Oka et al., 2004; Rosa & Koper, 2018). Future studies employing electroantennography could explicitly test whether there is any role of antennal sensitivity in the simultaneous detection of larval cues (such as e-β-ocimene) and alarm pheromone as well as the role that colony level aggression plays in modulating this sensitivity. A second explanation is that nurses are able to detect larval cues irrespective of alarm pheromone presence but are preferentially responding to the alarm pheromone cue (i.e. distraction) (Rosa & Koper, 2018). This hypothesis is partially supported by our finding that bees from high-aggression colonies more frequently moved towards the source of the alarm pheromone in the time period immediately after it was applied. Such an outcome may be particularly relevant in the context of nest defence, which relies on the successful recruitment of a critical mass of workers to fend off an attack (Breed et al., 2004). For example, several studies show that 1- to 2-day-old workers, which are relatively insensitive to alarm pheromone (Robinson, 1987a), respond behaviourally to alarm pheromone and intruder attack (Collins, 1980; Rittschof, 2017). Future studies could investigate the neural basis of prioritization of the alarm response in nurses and other workers. It would also be interesting to assess whether the response to nonspecialist cues demonstrated here is limited to alarm pheromone (due to its critical role in nest defence) or occurs more broadly.

Our finding that the nurse response to alarm cues depends on the colony of origin's aggression level suggests behavioural expression is the result of complex interactions between individual sensitivity thresholds and proximal social cues. Sensitivity thresholds can be shaped by genetic variation as well as social and ecological information (Calderone & Page, 1988; Page & Robinson, 1991; Scheiner & Erber, 2009; Wilson, 1985). Animals from fish to birds to mammals tailor both their signal production and cue response to health and body condition (Bachman, 1993; Brown et al., 2004; Burkhard et al., 2018; Seltmann et al., 2012). Both genetic and environmental mechanisms appear to influence alarm cue response in honey bees (Alaux & Robinson, 2007; Alaux et al., 2009; Guzman-Novoa & Page, 1994; Hunt et al., 2003). For example, Rittschof et al. (2015, 2019) showed that the developmental colony environment has lasting impacts on the threshold of individual responsiveness to alarm pheromone as well as immune system activity and pesticide tolerance. Alternatively, Giray et al. (2000) showed that genetic variation in worker developmental rate can cause differences in colony level aggression and foraging behaviour. Thus, the nurses in our study may have differed in their aggression response thresholds due to genetic differences or environmental factors such as infection, stress exposure or ecological conditions (Carr et al., 2020; Couvillon et al., 2008; Downs & Ratnieks, 2000; Garbuzov et al., 2020). High-aggression colonies may overall prioritize nest defence, manifesting as both strong guard/soldier response to threats and nurse prioritization of alarm cues over nursing cues. Interestingly, these results combined with the results of Rittschof et al. (2015) raise the possibility that threshold differences in alarm response among nurses could in turn influence the behaviour and health of the subsequent worker generation.

Although it was not statistically significant, we note that the nurses from low-aggression colonies showed an increase in visits in the presence of alarm pheromone. This effect is particularly remarkable given that total activity was suppressed in low-aggression colonies on days where alarm pheromone was applied relative to the control; that is, there were more nurse visits to cells at the same time as less total movement. If the trend of increased visits is a true phenomenon rather than noise in the data, one possibility is that it is due to olfactory priming. Olfactory cues can in

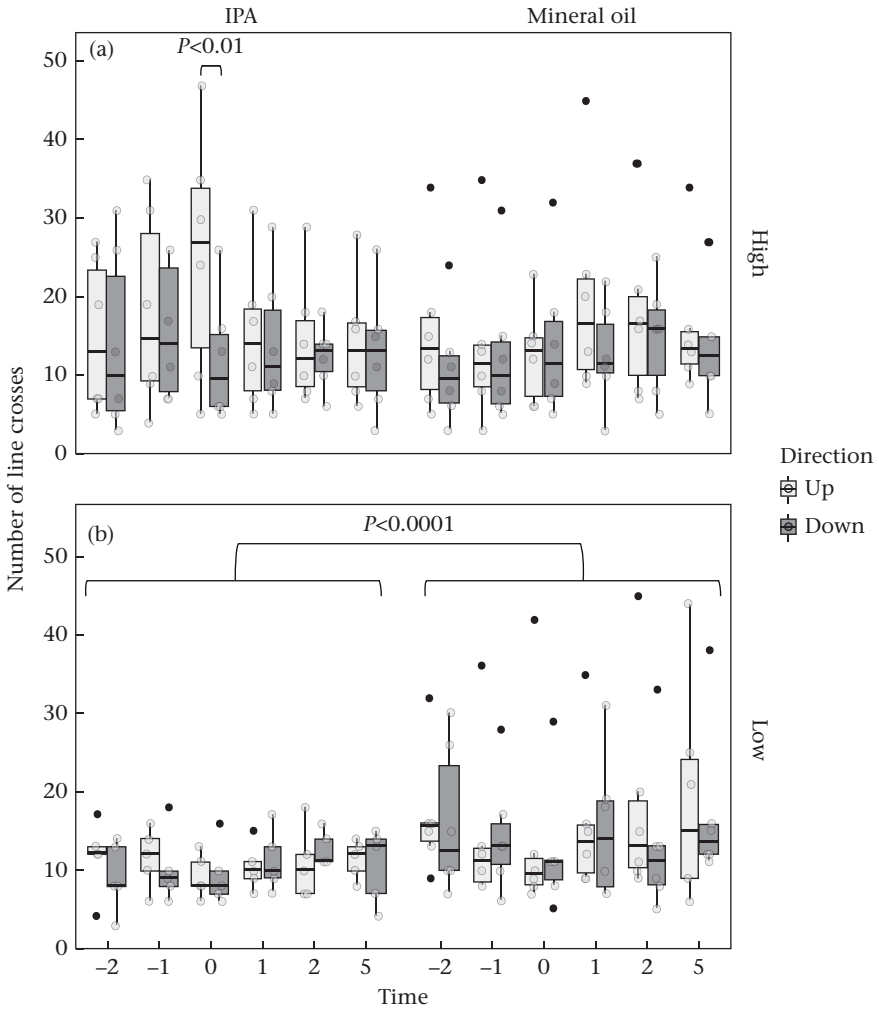

Figure 5. Total activity (number of line crosses) by bees in (a) high-aggression colonies and (b) low-aggression colonies within a 15 s interval on days when alarm pheromone (isopentyl acetate, IPA) or mineral oil was applied. Direction denotes movement towards ('Up') or away from ('Down') the treatment stimulus. Box plots show the median (interior line), 25% and 75% quartiles (outer box), 1.5 times the interquartile range (whiskers) and outliers (black circles) of the number of times a bee crossed a horizontal line in the viewable area over 15 s. This activity measurement was taken six times (noted as 'Time' on the *X* axis), with '0' being the moment of stimulus presentation. Other measurements were taken 1 min and 2 min before the stimulus as well as 1, 2 and 5 min after the stimulus presentation.

rare cases be enhanced by particular background odorants (Deisig et al., 2014; Schröder & Hilker, 2008). For example, male *Helicoverpa zea* moths show increased activity of a neuron specifically tuned to the female sex pheromone in the presence of linalool or hexanol, despite these two chemicals not activating that neuron in the absence of the pheromone (Ochieng et al., 2002). If these dynamics exist in this system, the presence of alarm pheromone could have enhanced the response to other larval cues. Additionally, a recent study identified that young larvae (instars 1–4) emit small amounts of isopentyl acetate (IPA, an important constituent of the honey bee alarm pheromone and the chemical used in our study) (Noël et al., 2023). It is therefore possible that this chemical serves a second function as a larval signal in addition to an aggressive signal, although this idea has not been explicitly tested. The addition of extra IPA in our study could have caused nurses in low-aggression colonies to overestimate the number of larvae present, generally increasing their nursing effort and leading to a higher number of visits. What is not clear from this scenario is how the directional dynamics of the pheromone application would have affected this process (as the alarm pheromone was applied at the top of the frame rather than directly over the larval area) as well as why this effect would have been limited to nurses from low-aggression colonies.

In the current study, we showed variation in nursing behaviour in response to a single, uniform application of alarm pheromone. However, the consequences of this variation may depend on the patterns of alarm cue signalling and response over the course of larval development, which lasts 5–6 days. For example, high- and low-aggression colonies could differ in the amount and timing of alarm pheromone release in a natural hive setting. High-aggression colonies may be exposed to more threats or, being more sensitive to threats, may release alarm pheromone more frequently (Alaux et al., 2009; Guzmán-Novoa et al., 2004). Additionally, additive effects of alarm pheromone release, where each responder releases additional alarm pheromone, can explain colony level variation in defensive aggression (Guzman-Novoa & Page, 1994). Such effects could increase the total amount of nurse bee alarm pheromone exposure in high-aggression colonies, even during a single antagonistic encounter.

Given the pheromone signalling dynamics in high-aggression colonies, the total amount of disrupted nursing time could add up substantially over the 6-day course of larval development.

However, nurse bee response to alarm pheromone may also be dynamic over time. Animals that are repeatedly exposed to the same stimulus often show habituation, a decrease in the magnitude of the behavioural response across repeated exposures (Thompson & Spencer, 1966). Alternatively, sensitization can occur, where the magnitude of the behavioural response increases across repeated exposures (Minoli et al., 2012; Russo & Ison, 1979; Walters et al., 2001). Both habituation and sensitization can be affected by the frequency and the intensity of the stimulus given (Groves et al., 1969; Pilz & Schnitzler, 1996; Thompson & Spencer, 1966). Nurses, which specialize on brood care for several days before switching to other tasks, may be subject to either process, but these possibilities remain to be tested (Seeley, 1982). Understanding alarm cue release and response dynamics over extended time frames will be required to interpret the extent to which nurse alarm pheromone response contributes to variation in individual larval development and colony level phenotypes.

We did not examine whether the short-term variation in nurse visits in our study affected larval outcomes. An individual honey bee larva is fed a little more than once per hour on average (Brouwers et al., 1987; Huang & Otis, 1991). We measured the disruption by alarm pheromone for 10 min. Although the larval signalling response to starvation occurs rapidly (the begging signal is released within 30 min of food deprivation), mortality impacts require longer periods of deprivation, on the time course of hours (He et al., 2016). It is possible that a brief disruption in the rate of nursing could be made up for by a temporary increase in the nursing workforce or increased effort by individual nurses once the perceived threat has passed, resulting in similar amounts of total food provisioned across high- and low-aggression colonies (Charbonneau & Dornhaus, 2015; Charbonneau et al., 2017; Harbo, 1986). Our study design cannot fully address this possibility. The disruption in high-aggression colonies caused by the addition of alarm pheromone lasted the full 10 min of observation, as the rate of nursing was depressed below the values seen on mineral oil days for the duration of our observation window. We also did not see an effect of treatment order: the rate of visits on mineral oil control days was similar whether they fell before or after the alarm pheromone treatment day. We therefore can say that the rate of nursing had recovered to baseline within 24 h after an alarm pheromone exposure. Thus, any increase in effort by the nurse bees would have had to have occurred between 10 min and 24 h post-threat, if at all. Periods of food deprivation could also accumulate over time in high-aggression colonies. For example, colonies face limitations to their provisioning abilities in other contexts such as a shortage of workers dedicated to brood care (Eischen et al., 1982, 1983) or brief disruptions in pollen availability; these both specifically impact nurse visits to the young larvae we examined here (Schmickl & Crailsheim, 2002). These limitations can result in physiological effects like decreased progeny life span and protein content (reviewed in Brodschneider & Crailsheim, 2010). Similar outcomes could occur in high-aggression colonies where nursing is disrupted by alarm pheromone.

The current study was premised on the idea that variation in nurse behaviour and accompanying impacts on larval physiology may underlie an adaptive larval response to colony social or ecological conditions. In honey bees, there is evidence of subtle responses to larval food deprivation that may have adaptive value. For instance, adult workers deprived of food as older larvae show increased starvation resilience, juvenile hormone titres and glycogen stores as adults (Wang et al., 2016). While this could reflect an adaptive physiological response to food scarcity, these characteristics also are associated with increased aggression in other honey bee studies (Pearce et al., 2001; Robinson, 1987a, 1987b). The adaptive value of such a shift in aggression is unknown, but it could give a competitive advantage to colonies under conditions of floral resource scarcity, as these circumstances increase the frequency of aggressive interactions among honey bee colonies (Garbuzov et al., 2020). Thus, nurses from high-aggression colonies may periodically and temporarily deprive larvae of food, causing increased aggression in response to environmental conditions (Rittschof et al., 2015). Interestingly, a variety of animals show a relationship between early-life nutritional deficits and adult aggression, suggesting a more general mechanistic and adaptive tie between these two characteristics (D'Eath & Lawrence, 2004; Randt et al., 1975; Shen et al., 2021).

Rather than the absolute degree of food deprivation, larval honey bees may also use the consistency of feedings in early-life as a source of information about the social or ecological environment they will experience in the future. Feeding disruptions may introduce uncertainty into a larva's assessment about the status of the environment, shaping their adaptive developmental trajectory (Trimmer et al., 2011). For example, spatiotemporal heterogeneity in environmental resource conditions, perceived through unpredictable nursing, could lead to a pessimistic cognitive bias, i.e. an expectation of poor resource conditions. Because such conditions are typically correlated with increased competition among colonies (Downs & Ratnieks, 2000; Willingham et al., 2000), an adaptive larval response would include increased aggression (Fawcett et al., 2014). Modelling studies indeed suggest that temporal variation in the environment across generations can select for pessimism (McNamara et al., 2011). Similarly, empirical studies demonstrate a positive correlation between uncertainty and aggression along with other covarying traits (Lewis, 2022; Mathot et al., 2012; Sih et al., 2015; Silk et al., 2019; Stamps & Frankenhuis, 2016; but see also Benus et al., 1991). Future studies could investigate the mechanistic and adaptive consequences of variation in nurse visits at both the individual and colony levels. For example, social insect colonies make decisions collectively, and it is possible that information acquired by other colony members may counteract or amplify the effects of nursing uncertainty experienced by cohorts of developing larvae (Marshall et al., 2009).

In addition to their response to alarm cues, our study suggests other sources of unexplored complexity in the cues nurses use to guide their interactions with larvae. For example, we observed that larval cells augmented with e-β-ocimene (EBO) were visited less frequently than unaltered larval cells. This effect could suggest that signals other than EBO play a role in modulating nurse visits. These signals could include other olfactory signals from the brood or the brood food as well as cues such as vibrations created as the larva moves and feeds (Heimken et al., 2009; Huang & Otis, 1991). Alternatively, there may be technical explanations for this effect. We found that a large proportion of cells in the EBO group received zero visits, possibly because visits occurred outside our observation time window. Most cells that were visited during our observations received only one visit. EBO cells, which have the strongest begging signal, may have been visited by nurses preferentially when they were first introduced to the frame during the acclimation period and outside our observation time frame. Although our EBO treatment mimicked previous studies, it is also possible that the presence of EBO repelled nurses from visiting cells. Follow-up studies should carefully examine how naturally emitted and supplemented EBO, in addition to other cues, alter nurse activity. For example, a two-choice experiment could directly compare nurse visiting preference for larvae with and without extra EBO.

The 'cocktail party problem' describes the increased difficulty in attending to a particular stimulus in the face of competing information, particularly when that competing information is also socially relevant (such as how it is more difficult to filter out other human conversations than ambient noise; Cherry, 1953).

Embedded within this framework, though, is recognition that highly relevant stimuli are able to break through the attention barrier, such as when a person's attention is diverted from a conversation by hearing their own name (Moray, 1959). Our research highlights that this phenomenon is true even in social insects. We did not find an interaction effect between adding additional begging pheromone and alarm pheromone treatment, but the larvae were still presumably releasing their own larval signals that the nurses were cueing into. Some nurse bees that were attending to this social information given off by the larvae were distracted by a different social cue, alarm pheromone, while others continued to focus on larval cues in the face of this alternative social information. Furthermore, the bees from low-aggression colonies were evidently affected by the alarm pheromone (as shown by the drastic decrease in total activity), and yet they maintained the level of nursing effort while under this effect. Individual variation has been found in human studies of the cocktail party phenomenon and is related to factors such as working memory (Conway et al., 2001). The variation seen in our study was associated with the larger-scale social factor of colony level aggression. It would be interesting to see whether broader social context affects this phenomenon in humans as well, or whether the colony level differences seen in honey bees are instead an emergent property of individual level cognitive differences similar to what has been found in humans.

Diverse, co-occurring signals can have a variety of effects on an organism. In some cases, a relevant signal must be 'extracted' from a milieu of distracting and possibly irrelevant cues (Conchou et al., 2019; Deisig et al., 2014; Gomes & Goerlitz, 2020; McDermott, 2009; Ord et al., 2007). In other cases where multiple cues are involved in mediating a complex process, a second cue can enhance the response to a primary signal, such as when host plant volatiles increase the response to sex pheromones (Schröder & Hilker, 2008). The current study highlights a unique case where both the target cue and the conflicting information are relevant to the organism but have historically been considered the domains of distinct task specialists. Our results suggest that these 'specialists' may be paying attention to a broader array of cues than previously appreciated, albeit with colony level variation related to cue sensitivity. Future experiments should consider how pheromone sensitivity tracks worker bee progression through various temporary behavioural specializations associated with adult temporal polyethism. Certain cues may be prioritized, not just because of behavioural specialization but because of collective colony level priorities. The regulation of diverse behaviour in the honey bee nest may be much more nuanced than previously appreciated.

Author Contributions

R. R. Westwick and C. C. Rittschof conceptualized and designed the methodology of the experiment. R. R. Westwick carried out the experimental staging and video data collection. G. P. Brackett, C. E. Brown and B. J. Ison completed the behavioural scoring from video data. R. R. Westwick and C. C. Rittschof completed the statistical analysis and manuscript writing. All authors agreed to the content within this paper.

Data Availability

Data for this study are available as Supplementary material.

Declarations of Interest

None.

Acknowledgments

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2023.09.015.

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Appendix

e-β-ocimene Pilot Study

We performed a small pilot study to test what concentration of e-β-ocimene (EBO) would elicit a response from our nurse bees. The methods were the same as used in the paper, except that we did not apply an alarm pheromone treatment. Briefly, we obtained a frame of brood and applied one of three EBO treatments to larval cells on the frame: 1) an untreated cell (control), 2) a cell to which we added 10 µl of 1:1000 EBO in mineral oil (amount calculated modified from the findings of He et al. (2016) to fit our methods), and 3) a stronger stimulus of 10 µl of 1:10 EBO ($N = 7$ per treatment). We then drew nurse bees onto the frame, sourced from a colony that was not otherwise used in the experiment. We placed the frame into our observation hive under red light conditions and video recorded the frame for 30 min. We counted every time a nurse bee visited each marked cell (as described in Methods).

In this pilot study, we found that only the 1:10 concentration increased visits (Appendix, Fig. A2) (ANOVA: $F_{2,18} = 4.79$, $P = 0.02$; Tukey post hoc comparisons: control versus 1:1000, $Q = 2.19$, $P = 0.29$; control versus 1:10, $Q = 4.38$, $P = 0.02$).

Pure versus Racemic e-β-ocimene Analysis

For the first four rounds of our experiment, we used both a pure form of EBO and a racemic mixture that had been used in previous experiments (as described in Methods). When these four rounds had been completed, we compared the number of nurse visits to cells with Pure EBO and racemic EBO to see if there was a difference in the response of the nurse bees to these two compounds ($N = 107$ cells). We found that there was no difference between the groups that received pure EBO and those that had received the racemic mixture (Mann–Whitney U test: $z = 0.19$, $N_1 = 56$, $N_2 = 51$, $P = 0.85$; Appendix, Fig. A3).

Models for Number of Nurse Visits

Global model: number of nurse visits = nurse source colony aggression + IPA application + cell type + nurse source colony aggression × IPA application + nurse source colony aggression × cell type + IPA application × cell type + nurse source colony aggression × IPA application × cell type + (1|year) + (1|colony ID) + (1|IPA treatment order).

Final model based on AICc: number of nurse visits = nurse source colony aggression + IPA application + cell type + nurse source colony aggression × IPA application + (1|year) + (1|colony ID) + (1|IPA treatment order).

Models for Latency to the First Visit

Global model: latency = nurse source colony aggression + IPA application + cell type + nurse source colony aggression × IPA application + nurse source colony aggression × cell type + IPA application × cell type + nurse source colony aggression × IPA application × cell type + (1|year) + (1|colony ID) + (1|IPA treatment order).

Final model based on AICc: latency = nurse source colony aggression + IPA application + nurse source colony aggression × IPA application + (1|year) + (1|colony ID) + (1|IPA treatment order). The results of the model can be seen in Table A1 and the data are displayed in Fig. A4.

Table A1
Fixed effects from the final GLMM used to evaluate how competing pheromone information and nurse source colony aggression affect the latency to the first nurse visit to honey bee larvae

Factor	Wald χ^2	*df*	*P*
Nurse source colony aggression	0.0003	1	0.98
IPA application	0.06	1	0.80
Nurse source colony aggression ∗IPA application	2.4	1	0.12

IPA: isopentyl acetate. Wald χ^2 values, degrees of freedom and ANOVA-determined *P* values are included.

Table A2
Pairwise analysis of directionality of crosses per time point

Time point	High aggression		Low aggression	
	IPA	Mineral oil	IPA	Mineral oil
−2 min	$P = 1$	$P = 0.67$	$P = 1$	$P = 1$
−1 min	$P = 1$	$P = 1$	$P = 1$	$P = 1$
0 min (exposure to stimulus)	$P = 0.0096$	$P = 1$	$P = 1$	$P = 1$
1 min	$P = 1$	$P = 0.21$	$P = 1$	$P = 1$
2 min	$P = 1$	$P = 1$	$P = 1$	$P = 0.86$
5 min	$P = 1$	$P = 1$	$P = 1$	$P = 1$

Estimated marginal means comparison of the number of crosses up versus down at each time point for each combination of aggression and alarm pheromone (isopentyl acetate, IPA) treatment. Values displayed were treated with a Bonferroni correction for multiple comparisons.

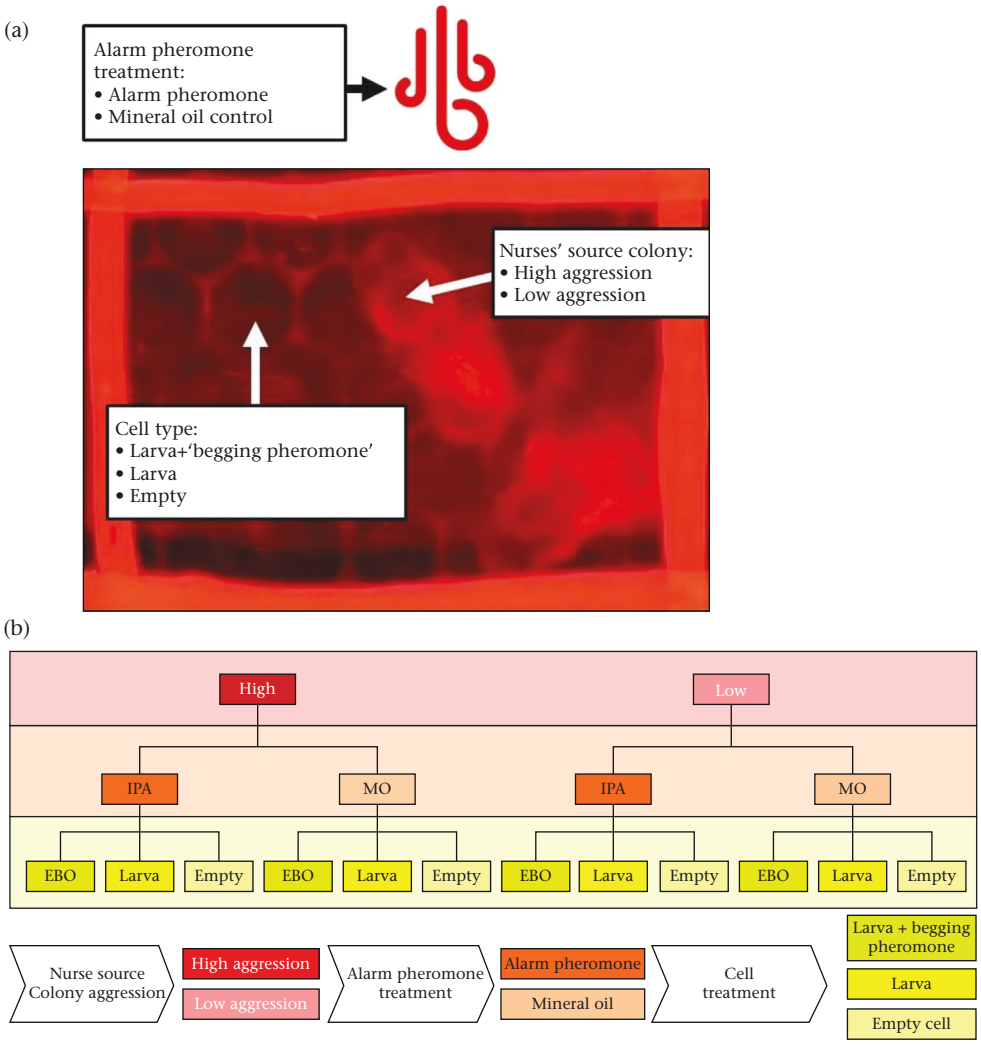

Figure A1. Diagram showing the experimental set-up for our experiment. (a) Three different variables were considered in this study. We examined effects of nurse source colony aggression (high/low), alarm pheromone treatment (isopentyl acetate, IPA 'alarm pheromone'/mineral oil (MO) control) and cell type (larva with extra e-β-ocimene (EBO) 'begging pheromone'/larva alone/empty cell control). (b) All combinations of all treatments were considered in this study, in addition to replication at the level of the colony.

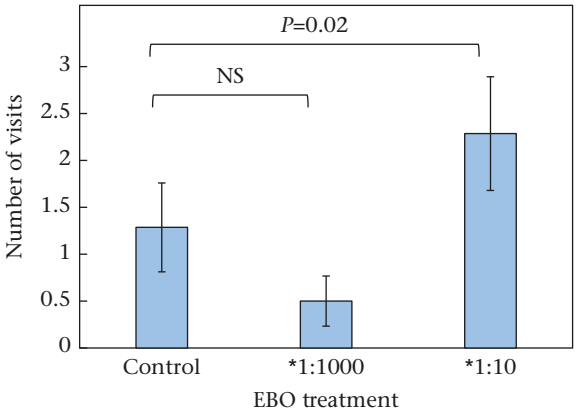

Figure A2. Bar chart showing the mean (± SE) number of visits by nurse bees to larvae that were augmented with e-β-ocimene 'begging pheromone' (EBO) diluted in mineral oil at a 1:1000 or 1:10 dilution versus control larvae that were unmanipulated.

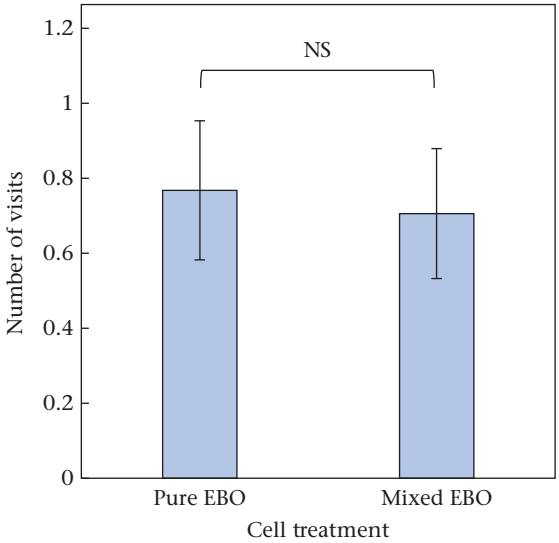

Figure A3. Bar chart showing the mean (± SE) number of visits by nurse bees to larvae that were augmented with either a pure form of e-β-ocimene 'begging pheromone' (EBO) or a racemic mixture of ocimene forms.

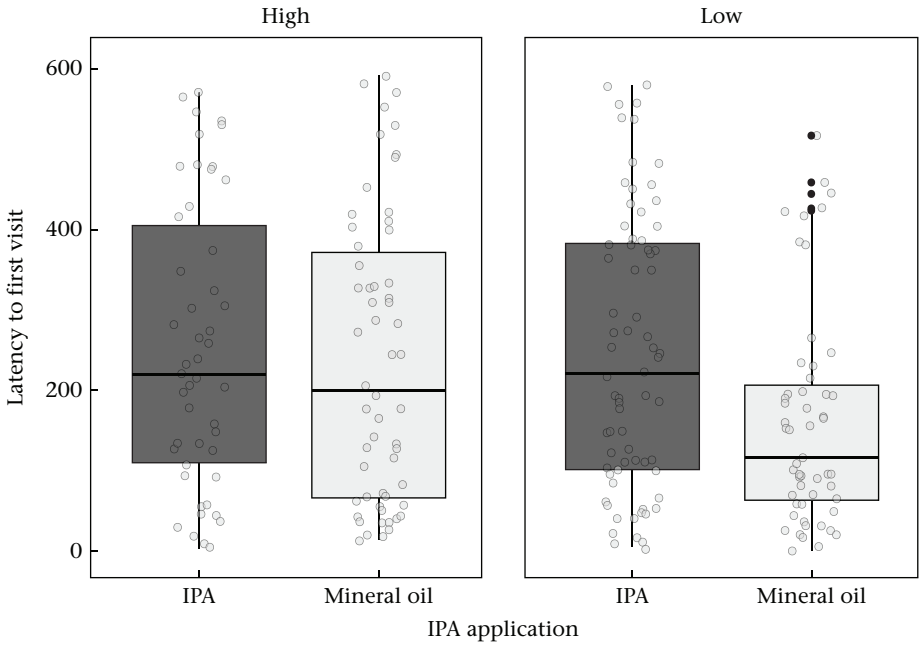

Figure A4. Box plots of median (interior line), 25% and 75% quartiles (outer box), 1.5 times the interquartile range (whiskers) and outliers (black circles) of the latency (in seconds) to first visit for each cell that received at least one visit. Nurses were sourced from colonies that were either high or low aggression and measurements were taken following application of alarm pheromone (isopentyl acetate, IPA) or mineral oil. The final model included nurse source colony aggression (high versus low), alarm pheromone (IPA) application and their interaction.