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Research

Decoding alarm signal propagation of seed-harvester ants using automated movement tracking and supervised machine learning

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Alarm signal propagation through ant colonies provides an empirically tractable context for analysing information flow through a natural system, with useful insights for network dynamics in other social animals. Here, we develop a methodological approach to track alarm spread within a group of harvester ants, *Pogonomyrmex californicus*. We initially alarmed three ants and tracked subsequent signal transmission through the colony. Because there was no actual standing threat, the false alarm allowed us to assess amplification and adaptive damping of the collective alarm response. We trained a random forest regression model to quantify alarm behaviour of individual workers from multiple movement features. Our approach translates subjective categorical alarm scores into a reliable, continuous variable. We combined these assessments with automatically tracked proximity data to construct an alarm propagation network. This method enables analyses of spatio-temporal patterns in alarm signal propagation in a group of ants and provides an opportunity to integrate individual and collective alarm response. Using this system, alarm propagation can be manipulated and assessed to ask and answer a wide range of questions related to information and misinformation flow in social networks.

Cite this article: Guo X, Lin MR, Azizi A, Saldyt LP, Kang Y, Pavlic TP, Fewell JH. 2022 Decoding alarm signal propagation of seed-harvester ants using automated movement tracking and supervised machine learning. *Proc. R. Soc. B* **289**: 20212176.
https://doi.org/10.1098/rspb.2021.2176

Received: 30 September 2021
Accepted: 23 December 2021

Subject Category:
Behaviour

Subject Areas:
behaviour

Keywords:
behaviour tracking, alarm behaviour, supervised machine learning, social insects, information flow networks

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5772172.

1. Introduction

Coordination in biological systems often depends on complex, decentralized processes for distributing information among system components [1]. The decentralized mechanisms of information distribution are critical to adaptive social function, but they also can be subject to manipulation, and under some conditions, they can fail. Deleterious self-perpetuating ‘cytokine storms’ can lead to multisystem organ failure and death [2], and misinformation in social networks can continue to spread even long after it has been retracted by its original source [3]. Being able to manipulate and assess biological communication networks in fine detail may unveil strategies converged upon by natural selection with connections to the spread of information and misinformation. Furthermore, understanding catastrophic failures of sharing information in one system can lead to new insights into analogous failures in others (e.g. cytokine storms and misinformation spread).

Social-insect colonies are ideal model systems for studying natural communication networks. Adaptive responses at the colony level emerge from

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the collective actions of individuals, each responding to local stimuli from their environment or from other individuals within the colony. As a result, large numbers of individuals can self-organize to regulate diverse aspects of task organization [4–6] including food distribution [7], social defence [8–10], collaborative house hunting [11] and foraging [12,13]. Two elements common to these collective phenomena are an underlying individual-to-individual contact network and a distributed system of information flow over the network. These attributes enable colonies to flexibly and adaptively respond to changing environments and social contexts [14].

By tracking individual movement and social interactions, object-tracking techniques have provided useful methodologies to study the dynamic structure of social networks in diverse social groups. In social insect colonies, the technique has been applied to European honeybees (*Apis mellifera*) [15], bumblebees (*Bombus ignitus*) [16], carpenter ants (*Camponotus fellah*) [17] and others. These tracking methodologies do not, by themselves, provide a reliable assessment of individual behavioural state during social interactions, nor do they assess the reliability of information exchange during encounters between individuals. Researchers have also developed more sophisticated statistical and mathematical modelling tools to develop theory about how social insects spread information across their communication networks [18,19]. Nevertheless, a gap between empirical and theoretical studies on information movement across the network constrains understanding of how a social-insect colony is organized as a complex adaptive system.

In many studies of vertebrate social networks, research has focused on several aspects of information flow, such as the transmissibility of information, individual-characteristic effects on information flow and the path of information flow [20–22]. In these cases, the focus has been primarily on dyadic relationships or association networks and how they shape behaviour. This is more difficult for the social insects, where individual relationships are usually ephemeral and task based [5] because a colony has numerous informational pathways operating simultaneously around different tasks. These are difficult to disentangle, and the observable behaviours used to make inferences about the impact of information flow are cryptic and difficult to discriminate from baseline behaviour. Consequently, tracking and analysing information dynamics using passive observations of social-insect colonies is often prohibitively complex.

Individual-level alarm status in ants can be characterized by observable changes in movement patterns and velocity [23–26], and so information flow within ants' alarm networks can be readily observed as they radiate out from an artificially initiated alarm event. The challenge comes, however, in how to capture the complexities of movement, contact and response within a social group, especially at the second-to-minute time scales relevant to the amplification and decay of a group-level alarm response.

One way to do this is to employ machine learning (ML) algorithms with complementary modelling techniques to classify behavioural states at a fine-grained level [27–29]. An ML approach has the advantage of automatic characterization of behavioural responses that normally require labour-intensive observation that introduce both intra- and inter-rater reliability issues [28,30]. Furthermore, the ML approach can combine assessments of behavioural (alarm) state with information on individual contacts, to simultaneously capture the movement of information throughout the social group and assess individual and group-level response to that information.

In this study, we match individual alarm strengths with contact networks in groups of workers from harvester ant colonies (*Pogonomyrmex californicus*) to (i) characterize the spatio-temporal dynamics of alarm spread, (ii) identify the relative contribution of different mechanistic pathways (e.g. chemical or physical) to alarm spread and (iii) evaluate varied individual sensitivity to alarm stimuli. Our methodology offers a way to more directly assess the influence of information spread on individual behaviour and to capture the speed of information transmission and response across a biological social network.

2. Materials and methods

(a) Animals and housing

Experiments were performed on three subsets, each containing 61 harvester ant workers, from three laboratory-reared colonies. All three subsets were assessed for colony-level response to alarm stimuli; one was additionally used to develop the ML model for alarm state and to build the associated alarm propagation network. The colonies from which the workers were selected were housed in a circular Plexiglas nest, containing water tubes. Colonies were fed with ad libitum Kentucky bluegrass seeds and provided mealworms weekly. Colonies and experimental subsets were maintained at a consistent temperature of 30°C. For test arenas, we used previously unoccupied Plexiglas nest chambers with no other contents (15 cm diameter). The arena was floored with plaster (thickness of 8 mm) and placed on top of a foam pad within an enclosed glass tank (24″ × 13″ × 17″) to prevent ants from being disturbed by the internal vibration due to ants' activities and the external vibration of the platform during experiments. A video camera (Panasonic HC-WXF990) was securely mounted above the glass tank to record all alarm events.

(b) Video capture of alarm events and general methods for machine learning development

To maintain a similar density as in the ants' original nests (0.23–0.42 ants cm^{-2}), we randomly selected 61 workers from each of the three colonies. Each selected ant was painted with unique colour combinations on the head, thorax and gaster using Sharpie oil-based paint markers. The paint-marked ants were transferred into the test arena and left to acclimate to the new environment overnight prior to testing; no food was provided during acclimation or testing. We video recorded 2 min of activity in the arena as a baseline assessment of individual- and group-level movement patterns prior to any manipulation. Preliminary experimental results indicated that this time period is sufficiently long to capture the entirety of an alarm event from initiation through decay to baseline activity levels.

After video recording, three ants were randomly selected to serve as initial alarm stimuli. These were carefully removed from the testing arena, using an aspirator to minimize disturbance, and placed into a separate Petri dish. We provided 25 min for ants in the testing arena to acclimate after the removal event. The three removed ants were then pinched gently with soft forceps until they displayed visible agitated movement and dropped into the centre of the test arena to initiate the alarm event. The group was video recorded for 2.5 min immediately after the disturbed ants were added to capture the alarm response.

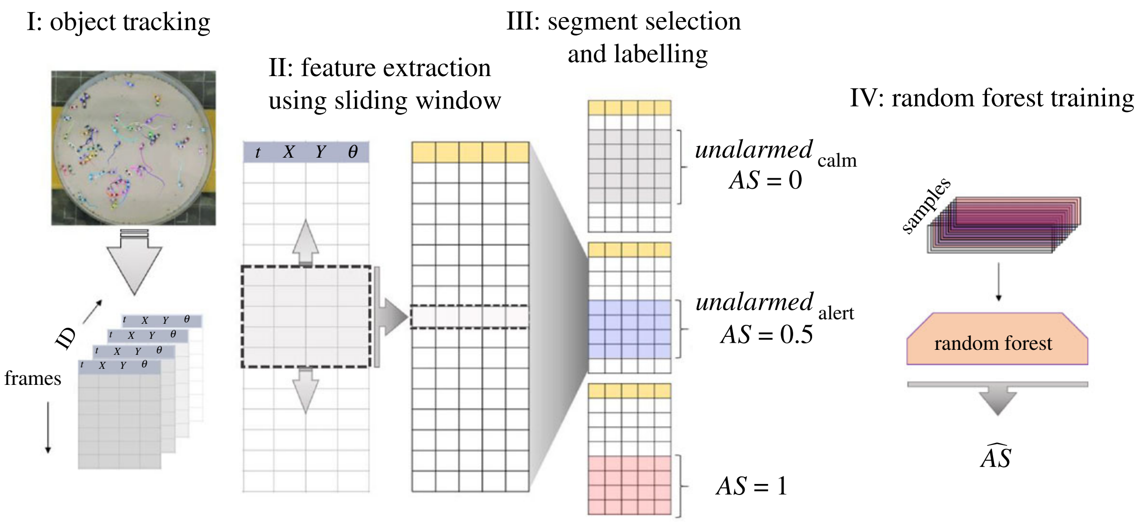

Figure 1. Stages of the algorithm development from data collection and encoding to estimation of alarm strength ($\widehat{AS}$). (*a*) Object tracking is applied to raw ant videos, producing a set of track matrices, each with size (no of frames × 4), representing the (*x*, *y*) position, speed and orientation data for each frame. (*b*) A sliding window of length 30 frames is moved across each track. At each frame, we extract a multi-dimensional feature vector containing five metrics computed over the window. (*c*) We select track segments that are visual exemplars of '$Unalarmed_{calm}$' ($AS = 0$), '$Unalarmed_{alert}$' ($AS = 0.5$) or '*Alarmed*' ($AS = 1$) behaviour and add them to the training and testing datasets with their respective *AS* numerical labels. (*d*) We train a random forest model to estimate these regression values from the presented feature vectors. (Online version is in colour.)

This protocol was replicated for subsets of ants from each of three colonies to validate its effectiveness of inducing a group-level alarm response and to assess whether we could consistently elicit a distinctive pattern of collective alarm response across replications. The group-level behavioural responses of all three colony subsets were assessed by measuring differences in the mean instantaneous speeds of ants during the baseline and alarm events.

The random forest ML (RFML) algorithm, a non-parametric method, has been applied widely and demonstrated to have equivalent or even better performance than parametric methods (e.g. a logistic model) [27,31]. We applied the RFML algorithm to the group of ants chosen from colony B, which showed the strongest alarm response. The methodological protocol, as outlined in figure 1, consisted of video recording the movement patterns of all individuals during baseline and alarm events and then choosing frame sequences in which individuals were visually identified as being alarmed or unalarmed and with low (calm) or intermediate (alert) movement speeds. We used a frame-by-frame assessment of movement features (a sliding window) to analyse movement features within the selected frame sequences and to train and test the RFML model. Our all subsequent analyses were based on the alarm behaviour of ants chosen from colony B and their temporal dynamics of alarm strength ($\widehat{AS}$) estimated by the RFML model.

(c) Behavioural characterization of alarm states

Because we cannot observe the internal state of any ant, we associate the alarm state of an individual ant with observable and consistent behavioural changes linked to the alarm response occurring in the groups. In our harvester ant groups, we defined that two ants have contact, and therefore are each others' neighbours, if their physical distance is less than or equal to 45 pixels (electronic supplementary material, §1). Based on this definition, an ant can have more than one contact at a time. Ants identified as alarmed move at more rapid and erratic speeds, with more frequent contacts with nest-mates. This aligns with the functional expectation of alarm behaviour as reacting to a potential threat and communicating that threat to others. To extract this behavioural pattern, we manually applied labels (*Alarmed*, $Unalarmed_{alert}$ and $Unalarmed_{calm}$) based on our visual assessments of behaviour to a set of video track segments in which the behaviours could be easily differentiated (electronic supplementary material, §2). These were used for ML training and testing.

Our raw data included tracked coordinates, instantaneous speed and body axis orientation for each ant. The behaviour as *Alarmed* presents visually as a distinct increase in movement speed, with a generally circular trajectory and increased contacts with other individuals. This characterization is consistent with ethological descriptions of alarm behaviour in other ant species, including *P. badius*, *C. obscuripes* and *C. japonicus* [23–25].

Outside of the context of alarm, ants vary considerably in their speeds and associated movement patterns. Therefore, we also subdivided ants not categorized as *Alarmed* into two sub-categories: $Unalarmed_{alert}$ and $Unalarmed_{calm}$. Those categorized as $Unalarmed_{alert}$ were observed moving at moderate speeds and potentially covering significant area in the nest but visually presented lower speeds, less frequent speed changes and lower levels of contact with other workers. Ants labelled as $Unalarmed_{calm}$ were stationary or moved at a low speed; they may have been engaged in social contacts disassociated with alarm, such as allogrooming, such that contact rate with neighbours depended more on task performance than movement.

(d) Object tracking and feature extraction

We used the multi-object tracking program ABCTracker [32] to obtain frame-by-frame movement data for each of workers chosen from three colonies. ABCTracker provides a sequence of time-stamped planar coordinates and body axis orientations for each ant tracked, which allows determination of instantaneous speeds, turning rates and the number of neighbours.

To detect behavioural transitions at a fine temporal resolution, we developed a sliding time-window method that creates a five-dimensional representation of each individual's movement characteristics and social context at each video frame. For a given ant at frame *t*, we take a local track window within frames [t, $t + 29$] (1 s) and construct a feature vector consisting of five metrics computed over this window. These include the frame-wise mean speed (MS), s.d. of frame-wise speeds (SS), s.d. of body axis orientations (SO), convex hull area (AR) and mean frame-wise number of contacts with

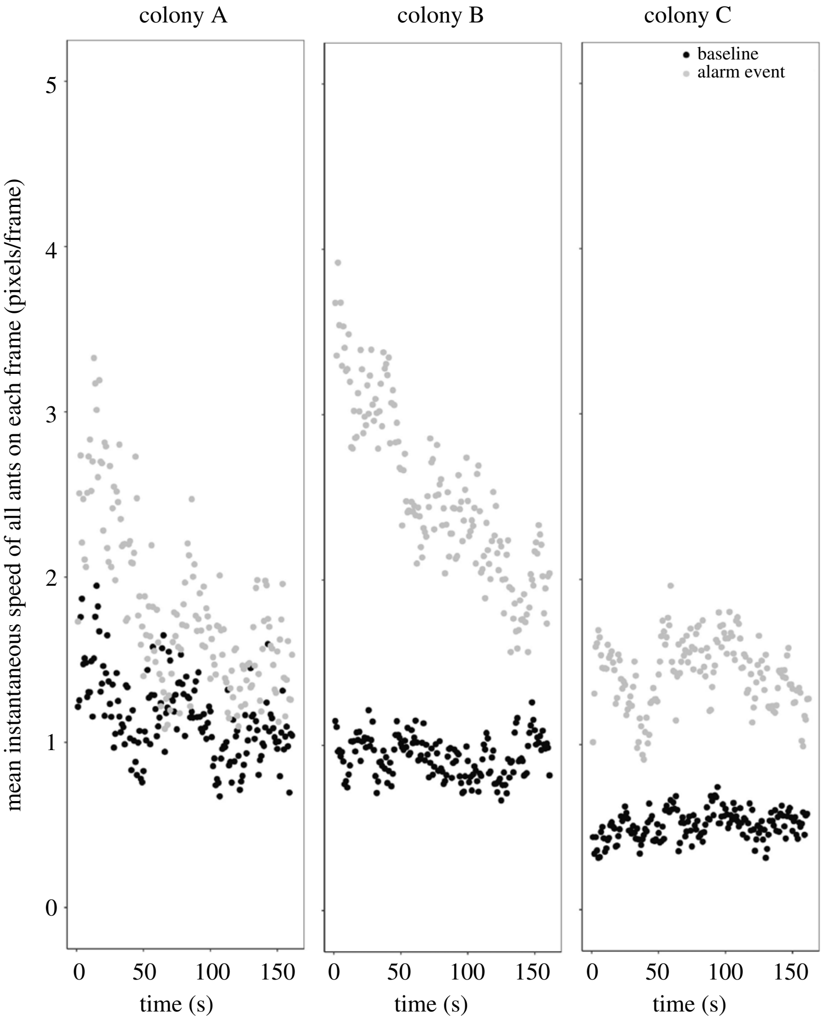

Figure 2. Comparing the mean speed of ants in subsets from three colonies at the absence (black dots) and the presence (grey dots) of the alarm event. Each frame of the plot refers to a sample of 61 ants from a distinct colony. There is a significant difference between the baseline and alarm event for all three subsets (randomization test, $p \leq 0.00001$) (1 pixel/frame = 4.1 mm s^{-1}).

neighbours (MC) over the window. Sliding the window over a track of length n produces $(n-30)$ feature vectors.

(e) Supervised machine learning

To create training and testing datasets, we selectively identified 16 track segments, each visually assessed to contain only one of the three visually identified behavioural states. All feature vectors extracted from a given sub-track were also assigned the same human-rated value as a measure of alarm strength for regression: $AS_{\text{calm}} = 0$, $AS_{\text{alert}} = 0.5$ or $AS_{\text{alarmed}} = 1.0$. Here, the ordinal value (alarm strength (AS)) represents a unitless measure of the probability that ants are labelled as alarmed [31].

Using our data labelled with categorical and ordinal values, we trained a RFML regression model to estimate continuous 'alarm strength' $(\widehat{AS})$ because of the versatility and simplicity of this model in categorical/ordinal data (RandomForest package in Rv. 3.5.0). We then applied the trained model to estimate $\widehat{AS}$ for unlabelled ant tracks over the entire video.

3. Results

(a) Validation of experimental procedure

The movement feature that is most often associated with alarm state in ants is velocity [23,24]. Therefore, we first employed the mean instantaneous speed of ants on each frame to estimate collective alarm responses (figure 2). Then, we employed the randomization test to examine the difference of average ant speed between the alarm treatment condition and the baseline condition ($ms_{t-c} = ms_t - ms_c$) in the groups of ants from colony A, B and C. The frequency distribution of 5000 randomly calculated ms_{t-c} gave the 95% CI of (0.044–0.037) for colony A, (−0.073 to 0.069) for colony B and (−0.0329 to 0.0361) for colony C. Furthermore, the ms_{t-c} assessed in the raw data was 0.657 for colony A, 1.58 for colony B and 0.752 for colony C, all of which were larger than the ms_{t-c} intervals from the randomly generated data, yielding $p = 0$. Those results demonstrate that ants' speed significantly increased, and their alarm responses were successfully induced via re-introducing three alarmed ants into the nest.

(b) Features extraction of alarm behaviour

By applying the sliding window technique on the raw data of time-stamped planar coordinates and body axis orientations for ants during the focal alarm event, we extracted 6462 vectors from track segments of the 16 focal ants. Each vector pairing with manual annotations of categorical alarm status and ordinal alarm strength value includes five feature variables: MS, SS, SO, AR and MC. Pairwise comparison within each feature variable after logarithmic

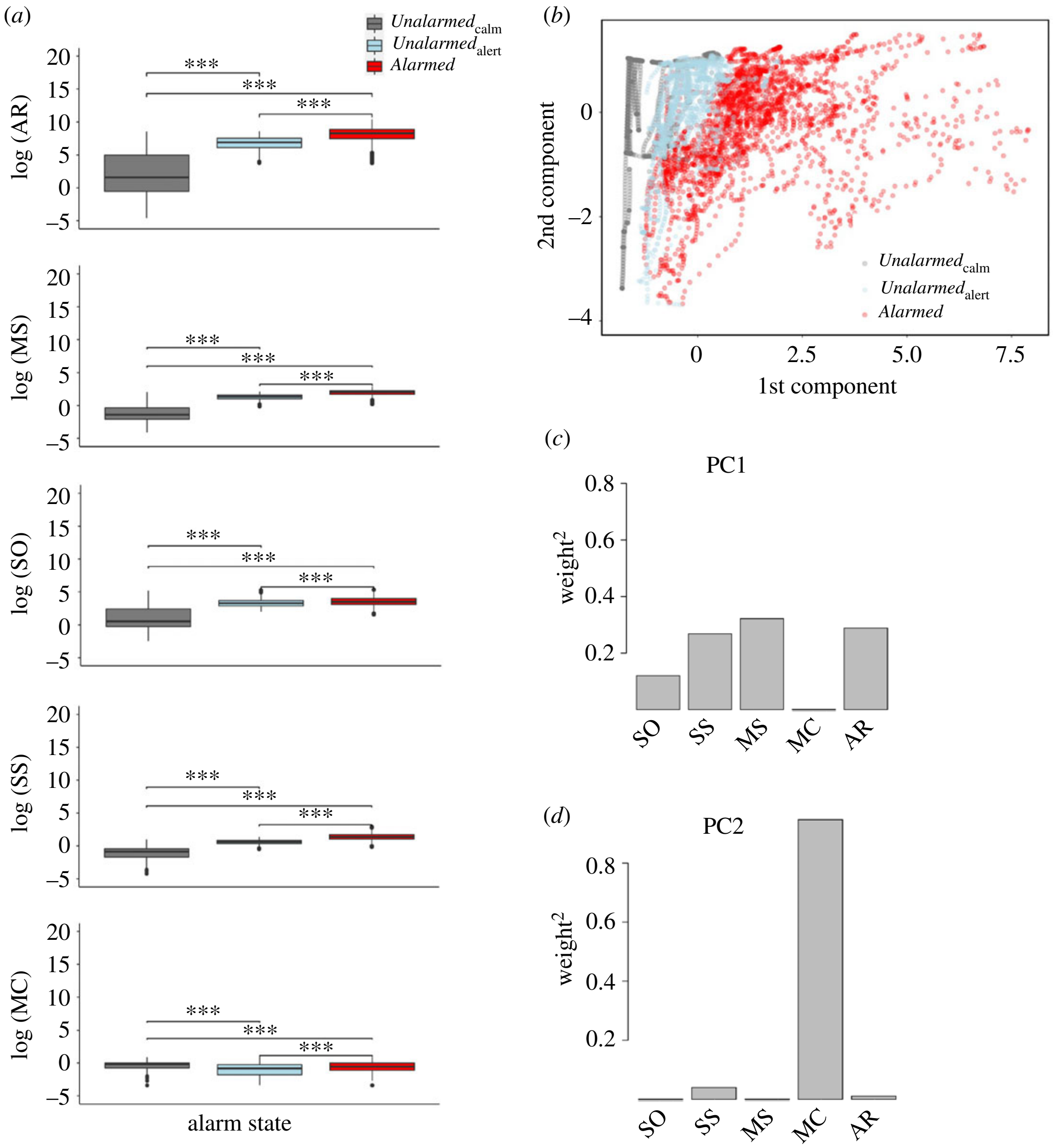

Figure 3. Pairwise comparison with a Wilcoxon test and principal component analysis on five feature variables after logarithmic conversion. (*a*) Pairwise comparison of each feature variable with a Wilcoxon test over three different alarm status, which were visually assessed as $Unalarmed_{calm}$ (grey), $Unalarmed_{alert}$ (light blue) and *Alarmed* (red). Three alarm states of ants differ from each other significantly within each feature variable (***$p < 0.0005$). (*b*) The five feature variables in the training data were plotted in the 2D subspace of first two principal components. Grey represents frames when ants were identified as $Unalarmed_{calm}$, light blue as $Unalarmed_{alert}$ and red as *Alarmed*. (*c*,*d*) PCA weights for the training data. PC1 is a measure of locomotion pattern, and PC2 is a measure of social context. SO, s.d. of body axis orientations; SS, s.d. of frame-wise speeds; MS, mean frame-wise speed; MC, mean frame-wise number of contacts with neighbours; AR, convex hull area over the sliding window. (Online version in colour.)

conversion over the three alarm states supports the validity of using these features to differentiate the ants' alarm status (Wilcoxon test with large-sample approximation [33]: $p <$ 0.005) (figure 3*a*). Principal component analysis of features indicates that those five features variables are effective predictors for classifying alarm status (figure 3*b*). Among the five principle components, the first two components explained over 80% of variance; PC1 represented variance in properties of locomotion patterns, with PC2 representing variance in the number of contacts with nest-mates (figure 3*c*,*d*). Therefore, we applied those feature vectors to train the random forest regression model (electronic supplementary material, §2).

(c) Alarm strength regression and classification

A random forest regression model was trained to estimate continuous alarm strengths of individual ants ($\widehat{AS}$), and its accuracy was estimated using the root mean square error (r.m.s.e. = 0.0276). The MS was ranked as the most significant feature variable in predictions followed by SS, AR, SO and finally MC. To identify alarmed ants and estimate their transition from an unalarmed to alarmed state, we applied the multiclass receiver operator characteristic (ROC) pairwise analysis to find the best threshold value for differentiating alarmed ants from unalarmed ants (ants in states of $Unalarmed_{calm}$ and $Unalarmed_{alert}$) [34]. On an ROC curve for the comparison between alarmed and unalarmed states, a threshold of classification (0.749) was estimated corresponding to the Youden index, *J*, a metric identifying the maximum potential effectiveness of the classification [35]. The area under the ROC curve for this comparison was 0.8906 (electronic supplementary material, §3). From this, we categorized ants with $\widehat{AS} \geq 0.749$ as alarmed, and those with lower $\widehat{AS}$ as unalarmed.

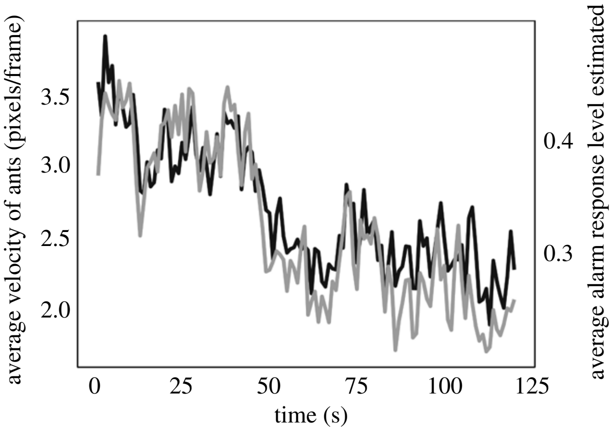

Figure 4. The average $\widehat{AS}$ per second estimated by RFML model (grey line) and the average velocity per second obtained by ABCTracker from experiments (black line) (1 pixel/frame = 4.1 mm s^{-1}).

We also used the Granger causality test to examine the relation between the group average speed $v_{\text{group}}(t)$ and the group average alarm strength $\widehat{AS}_{\text{group}}(t)$ [36] (figure 4). The highly significant causal connection between them ($F_{1,117} = 46.596$, $p < 0.00001$) validates the significance of velocity in random forest regression model. Meanwhile, the average alarm response, as expected, Granger-causes average velocity, but average velocity does not Granger-cause average alarm response because alarm strength of ants estimated in the RFML model carries the information about movement and social context of ants beyond that of the information contained in velocity alone.

(d) Spatio-temporal pattern of individual alarm response

Alarm behaviour in a social group functions in part to transmit information to others about potential danger. Here, we examined the impact of the presence of alarmed ants that entered the neighbouring space of unalarmed ants (electronic supplementary material, §§S1 and S4). We identified 20 approaching events between unalarmed ants and their alarmed neighbours. We then measured the s.d. of $\widehat{AS}$ for the unalarmed ant $\sigma_{AS}(A_j^u)$ during the time of approach and used the nonlinear least-squares fit of model to data. In the model, unalarmed ants have an increased tendency to advance in alarm strength as they approach an alarmed ant, with this effect falling off roughly exponentially with distance from the alarmed ant (d_{min}) (figure 5; equation (3.1); $\beta = 0.94878$, $F_{1,19} = 13.8137$, $p = 0.00146$). This result demonstrates the importance of proximity in the transmission of alarm between ants.

$$\sigma_{\text{AS}}(A_j^u) = -\ln(\beta) \times \beta^{(d_{\text{min}})}. \tag{3.1}$$

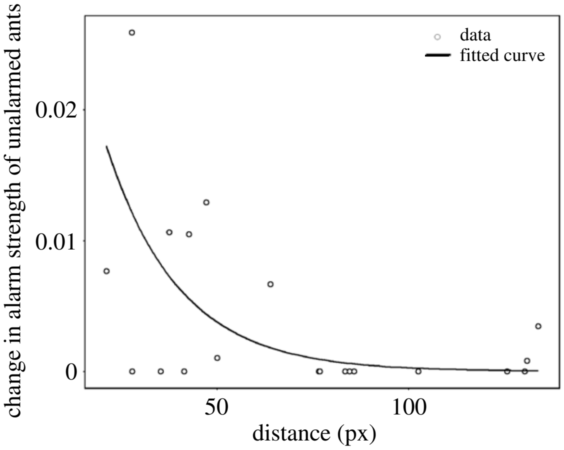

Figure 5. Spatial characteristics of alarm recruitment. Variation in unalarmed ants $\widehat{AS}$ during the time of approaching their alarmed neighbours. Each point indicates one unalarmed ant. The *y*-axis shows the variation in $\widehat{AS}$ during the time the two ants were near each other, and the *x*-axis shows the minimum distance between the two ants during that time. Unalarmed ants which came closer to alarmed neighbours varied more in their $\widehat{AS}$ (7.3 pixel = 1 mm). Circles represent observations; curve represents expectations in exponential decay model equation (3.1).

It has been found that alarm-sensitive neurons exhibit spike activity of 0–4 s in response to alarm stimulation in the ant brain [37]. Therefore, to study the tendency of ants to relay alarm signals received from others, we focus on ants that become alarmed within 4 s of a prior ant's own transition into the alarmed state. We use the resulting temporal associations between alarmed ants to estimate alarm response latency. Our results showed 46 ants eventually became alarmed after the introduction of three alarmed seed ants. Among those 46 ants, 39 unalarmed ants transitioned to the alarmed state within 1.51 ± 0.1 s after contact-mediated alarm stimulations, and seven unalarmed ants were induced to be alarmed with no contact-mediated alarm stimulations or exhibited alarm behaviour at timescales beyond the 4 s response window.

(e) Network of alarm signal propagation

To assess how alarm propagates through the group, we constructed a time-ordered propagation network with weighted and directed pathways (electronic supplementary material, §§S5 and S6). Ants were considered to have transmitted an alarm signal if the behavioural state of contacted unalarmed ants changed within 4 s, considered from our rule above. In the time-ordered propagation network, successful alarm state transitions occurred primarily via contact-mediated interactions (83%), with approximately 17% occurring as independent excitement events. These independent excitations may have been caused by alarm pheromone diffusion from the alarmed ant. Tracing the path of alarm excitation from three initially alarmed ants in the time-ordered propagation network indicated that a burst of multiple alarm transitions and rapid spreading dynamics happened initially, and the intensity of propagation declines precipitously after the initial events (figure 6*a*; electronic supplementary material, figure S7 and movie).

The 61 ants were highly interconnected with each other in the social contact network (average degree = 48.98, cluster coefficient = 0.862) (figure 6*b*). The alarm propagation network was built as a subset of the larger social contact network by assessing the primary pathway of alarm propagation via contact-mediated interactions (figure 6*b*,*c*) (electronic supplementary material, §§S5 and S6). During the progression of alarm signal spread, the longest path length from initial signal senders to receivers was four edges (figure 6*c*). The number of signal receivers on each

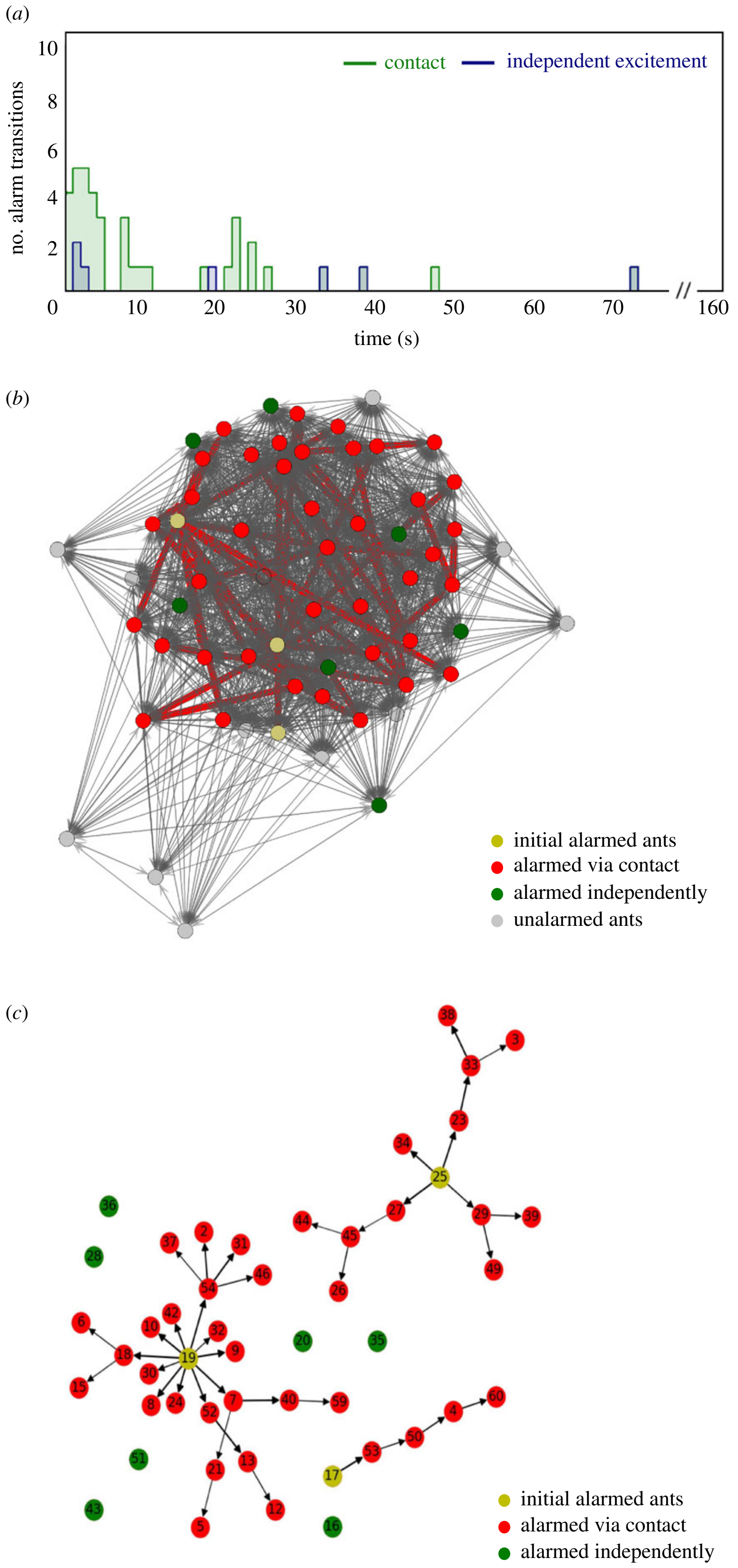

Figure 6. Dynamics of alarm signal propagation. (*a*) The temporal dynamics of alarm propagation via physical contact or independent excitement. Green bars indicate the alarm transition via contact-mediated interactions. Blue bars indicate the independent excitations. (*b*) The background social contact network of 61 ants (nodes) with 4140 social contacts (grey edges) within 2.5 min, and alarm propagation network residing above the social contact networks after edge pruning. (*c*) The alarm propagation network extracted from (*b*). Yellow nodes represent initially alarmed ants placed into the test arena. Green nodes represent ants which become alarmed independently of alarm contact. Red nodes represent ants transited to alarmed via contacting with an alarmed neighbour. Grey nodes indicate unalarmed ants. Edges highlight the propagation pathways via contact-mediated interactions. (Online version in colour.)

network distance showed a significant pattern of linear decline (slope = 5.1, intercept = 22.5, $p < 0.025$, $R^2 = 0.95$).

The time-ordered propagation network allows for quantification of varied individual alarm responsiveness, potentially indicating variation in alarm sensitivity, which can be measured by the quantity of key sensory cues before a response is elicited [38]. Before each alarm state transition, the number of contacts an unalarmed ant made with alarmed neighbours was used as a measure of alarm responsiveness. In other words, an unalarmed ant that underwent fewer alarm stimulations prior to becoming alarmed would have a higher responsiveness. We estimated 46 ants transitioned to the alarmed state after a mean of 5.17 ± 1.04 contact-mediated alarm stimulations. In figure 7, a geometric distribution was regressed on the data by using the Lilliefors-corrected K-S test, and the expected and observed frequencies were not significantly different ($D = 0.33$, d.f. = 46, $p = 0.12$). This right-skewed geometric distribution shows many ants demand less than five alarm contacts, while a few ants require more, to transition to the alarm status.

4. Discussion

Alarm behaviour in social-insect colonies functions to rapidly communicate information about emergent potential threats among individuals both proximal and distal to the threat. It triggers further explorative and scattering behaviours, such as ‘panic alarm’ [39], or defensive behaviours, such as mobilizing a collective aggression [40], or leads to a qualitative reconfiguration of the defence system [41]. In laboratory-reared *P. californicus* colonies, alarm behaviour can be experimentally induced and observed by introducing alarmed individuals into a quiescent group or colony. The three colony subsets tested within this study consistently showed an immediate increase in alarm behaviour after the introduction of alarmed ants, which gradually decayed back to a resting state within 2.5 min. This behavioural profile is consistent with other descriptions of alarm behaviour [23,24] and is illustrative of the ability of colonies to respond immediately to potential threats and also to quickly damp alarm response to spurious threat stimuli.

As with many complex social phenomena, it is not trivial to study the rapid sequence of interactions during alarm events using visual assessments or even with motion-tracking analyses alone. Using the RFML model, we were able to consistently quantify changes in individual-level alarm behaviour across timeframes of seconds or less from tracking data and categorical/ordinal annotations [31], which allowed us to identify the significance of individual motion and interaction most critical to alarm spread and decay. We trained our RFML model using large sets of high-resolution motion data from ants that were each subjectively scored by human raters into different levels of alarm. A PCA uncovered relationships between machine-identifiable features of motion and the human ratings of alarm, which we then leveraged to train our RFML model so as to be a reliable, highly repeatable automatic labeller for alarm state in ants (as an example that could possibly be replicated for other social systems).

Our findings demonstrate that, unlike the chemical diffusion of volatile alarm pheromone, as the classic mechanism for broadcasting alarm signals in social insects [23,42,43], the transmission of signals in the context of potential within-colony threats for *P. californicus* ants also requires contact-mediated interactions. By contrast to large-scale defensive signalling via alarm pheromones, these individual-to-individual contacts may function to locally scrutinize cryptic potential intruders without generating an immediate and costly full-scale response. Contact-based propagation may thus allow a moderate collective alarm response and damp quickly when no threat is confirmed. Thus, our results confirm the utility of using proximity-based social networks as proxies for potential information transfer in in-nest contexts where other communication modalities might also be used (e.g. acoustic or chemical).

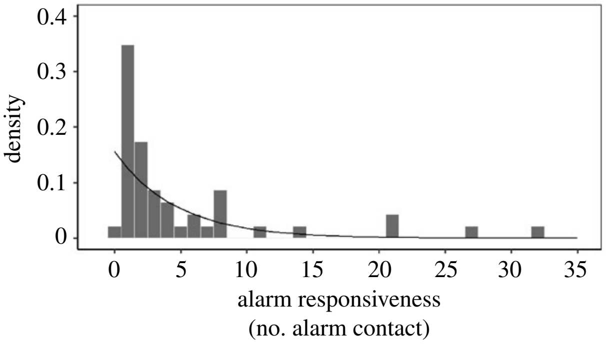

Figure 7. A right-skewed distribution of individual alarm responsiveness. The Lilliefors-corrected K-S test on the alarm responsiveness, indicates observed frequencies are not significantly different from expectations in a geometric distribution ($D = 0.33$, d.f. = 46, $p = 0.12$). Bars represent the proportion of observations; curve represents expectations in a geometric distribution.

As in the physical interactions used in foraging recruitment by the desert ant *Cataglyphis niger* [44] and alarm recruitment by *Atta insularis* ants [26], the alarm signal transmission in our seed-harvester ants primarily involved contact-mediated interactions. Over 80% of alarm transition events occurred maximally 4 s after physical-mediated contact. However, harvester ants also use a high-volatile contact pheromone (4-methyl-3-heptanone) as part of their signalling process [45], and our results showed that a small subset of individuals became alarmed independently of physical contact likely via contact with alarm pheromone. Thus, it may be that these ants mix some degree of local and non-local spread, or it may be that the local signalling mechanism is modulated by interactions with non-local signalling.

Generating synchronous traces of liquid food and trophallaxis events helps to characterize the process of food dissemination in laboratory-reared carpenter ant colonies (*C. sanctus*) [46]. Similarly, the co-occurrence of alarm transition and contact-mediated interactions allows us to characterize information spread dynamics. In our alarm signal propagation network, the number of nodes was observed to linearly decay with network distance from the source of alarm signal, which suggests that sustained alarm spread requires updating, which could theoretically serve to differentiate initial perception of threat from sustained information that a threat is real.

The temporal network analysis also offered opportunities to evaluate the sensitivity of the alarm response. The geometric distribution of alarm responses in the 46 ants we characterized is right-skewed, which indicates that most individuals will transition to an alarmed state after only a few contacts with other alarmed individuals, while a few individuals are far less sensitive and require many more contacts to become alarmed. A higher individual sensitivity may increase the speed of alarm propagation, as more ants will be triggered into alarm with fewer contacts during any alarm event. However, such increased sensitivity may also lead to longer propagation of false alarms. The significant variation in alarm sensitivity opens the question of how inter-individual heterogeneity may contribute to adaptive modulation of alarm propagation [47,48]. Methodologies developed here allow us to bridge from varied individual responses and adaptive collective responses to unveil potential modulatory effects of individual variation on group-level propagation of important signals (and damping of false signals).

Data accessibility. Data and electronic supplementary material are available at ASU Library Research Data Repository: https://doi.org/10.48349/ASU/OYZWEK.

Authors' contributions. X.G.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft; M.R.L.: formal analysis, investigation, methodology, visualization, writing—review and editing; A.A.: formal analysis, visualization, writing—review and editing; L.P.S.: software, visualization, writing—review and editing; Y.K.: writing—review and editing; T.P.P.: writing—review and editing; J.H.F.: funding acquisition, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. Funding for this research was provided by contract number FA8651-17-F-1013 from the United States Air Force/Eglin AFB/FL and in part by NSF (1716802 and 1558127) and The James S. McDonnell Foundation (UHC Scholar Award no. 220020472).

Acknowledgements. We thank Min Shin, Lance Rice and David Farynyk for technical supports on tracking; Kaitlin Baudier, Ioulia Bespalova, Nathan Smith, Madeleine Ostwald and Colin Lynch for their comments on the manuscript.

References

1. Camazine S, Deneubourg J-L, Franks N, Sneyd J, Theraula G, Bonabeau E. 2001 *Self-organization in biological systems*. Princeton, NJ: Princeton University Press.
2. Nazinitsky A, Rosenthal KS. 2010 Cytokine storms: systemic disasters of infectious diseases. *Infect. Dis. Clin. Pract.* **18**, 188–192. (doi:10.1097/IPC.0b013e3181d2ee41)
3. Lewandowsky S, Ecker UK, Seifert CM, Schwarz N, Cook J. 2012 Misinformation and its correction: continued influence and successful debiasing. *Psychol. Sci. Public Interest* **13**, 106–131. (doi:10.1177/1529100612451018)
4. Gordon DM. 1996 The organization of work in social insect colonies. *Nature* **380**, 121–124. (doi:10.1038/380121a0)
5. Fewell JH. 2003 Social insect networks. *Science* **301**, 1867–1870. (doi:10.1126/science.1088945)
6. Crall JD, Gravish N, Mountcastle AM, Kocher SD, Oppenheimer RL, Pierce NE, Combes SA. 2018 Spatial fidelity of workers predicts collective response to disturbance in a social insect. *Nat. Commun.* **9**, 1–13. (doi:10.1038/s41467-017-02088-w)
7. Cassill DL, Tschinkel WR. 1999 Information flow during social feeding in ant societies. In *Information processing in social insects* (eds C Detrain, JL Deneubourg, JM Pasteels), pp. 69–81. Berlin, Germany: Springer.
8. Hermann HR. 1984 *Defensive mechanisms in social insects*. New York, NY: Praeger Publishers.
9. Sasaki T, Hölldobler B, Millar JG, Pratt SC. 2014 A context-dependent alarm signal in the ant *Temnothorax rugatulus*. *J. Exp. Biol.* **217**, 3229–3236.
10. Hager FA, Krausa K. 2019 Acacia ants respond to plant-borne vibrations caused by mammalian browsers. *Curr. Biol.* **29**, 717–725. (doi:10.1016/j.cub.2019.01.007)
11. Pratt SC. 2005 Quorum sensing by encounter rates in the ant *Temnothorax albipennis*. *Behav. Ecol.* **16**, 488–496. (doi:10.1093/beheco/ari020)
12. Pinter-Wollman N, Bala A, Merrell A, Queirolo J, Stumpe MC, Holmes S, Gordon DM. 2013 Harvester ants use interactions to regulate forager activation and availability. *Anim. Behav.* **86**, 197–207. (doi:10.1016/j.anbehav.2013.05.012)
13. Kolay S, Boulay R, d'Ettorre P. 2020 Regulation of ant foraging: a review of the role of information use and personality. *Front. Psych.* **11**, 734. (doi:10.3389/fpsyg.2020.00734)
14. Pinter-Wollman N *et al.* 2014 The dynamics of animal social networks: analytical, conceptual, and theoretical advances. *Behav. Ecol.* **25**, 242–255. (doi:10.1093/beheco/art047)
15. Gernat T, Rao VD, Middendorf M, Dankowicz H, Goldenfeld N, Robinson GE. 2018 Automated monitoring of behavior reveals bursty interaction patterns and rapid spreading dynamics in honeybee social networks. *Proc. Natl Acad. Sci. USA* **115**, 1433–1438. (doi:10.1073/pnas.1713568115)
16. Azarcoya-Cabiedes W, Vera-Alfaro P, Torres-Ruiz A, SalasRodrıguez J. 2014 Automatic detection of bumblebees using video analysis. *Dyna* **81**, 81–84. (doi:10.15446/dyna.v81n187.40475)
17. Mersch DP, Crespi A, Keller L. 2013 Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* **340**, 1090–1093. (doi:10.1126/science.1234316)
18. Richardson TO, Gorochowski TE. 2015 Beyond contact-based transmission networks: the role of spatial coincidence. *J. R. Soc. Interface* **12**, 20150705. (doi:10.1098/rsif.2015.0705)
19. Guo X, Chen J, Azizi A, Fewell J, Kang Y. 2020 Dynamics of social interactions, in the flow of information and disease spreading in social insect colonies: effects of environmental events and spatial heterogeneity. *J. Theor. Biol.* **492**, 110191. (doi:10.1016/j.jtbi.2020.110191)
20. Ward P, Zahavi A. 1973 The importance of certain assemblages of birds as 'information-centres' for food-finding. *Ibis* **115**, 517–534. (doi:10.1111/j.1474-919X.1973.tb01990.x)
21. Deutsch JC, Nefdt RJ. 1992 Olfactory cues influence female choice in two lek-breeding antelopes. *Nature* **356**, 596–598. (doi:10.1038/356596a0)
22. Dindo M, Thierry B, Whiten A. 2008 Social diffusion of novel foraging methods in brown capuchin monkeys (*Cebus apella*). *Proc. R. Soc. B* **275**, 187–193. (doi:10.1098/rspb.2007.1318)
23. Wilson EO. 1958 A chemical releaser of alarm and digging behavior in the ant *Pogonomyrmex badius* (latreille). *Psyche* **65**, 41–51. (doi:10.1155/1958/57483)
24. Mizunami M, Yamagata N, Nishino H. 2010 Alarm pheromone processing in the ant brain: an evolutionary perspective. *Front. Behav. Neurosci.* **4**, 28.
25. Mizutani H, Tagai K, Habe S, Takaku Y, Uebi T, Kimura T, Ozaki M. 2021 Antenna cleaning is essential for precise behavioral response to alarm pheromone and nestmate–non-nestmate discrimination in Japanese carpenter ants (*Camponotus japonicus*). *Insects* **12**, 773. (doi:10.3390/insects12090773)
26. Reyes A, Curbelo M, Tejera F, Rivera A, Simon G, Ramos O, Altshuler E. 2019 Transmission of danger information past physical barriers by ants. *arXiv* preprint arXiv:1904.03236
27. Hong W, Kennedy A, Burgos-Artizzu XP, Zelikowsky M, Navonne SG, Perona P, Anderson DJ. 2015 Automated measurement of mouse social behaviors using depth sensing, video tracking, and machine learning. *Proc. Natl Acad. Sci. USA* **112**, E5351–E5360. (doi:10.1073/pnas.1515982112)
28. Kabra M, Robie AA, Rivera-Alba M, Branson S, Branson K. 2013 Jaaba: interactive machine learning for automatic annotation of animal behavior. *Nat. Methods* **10**, 64. (doi:10.1038/nmeth.2281)
29. Blut C, Crespi A, Mersch D, Keller L, Zhao L, Kollmann M, Beye M. 2017 Automated computer-based detection of encounter behaviours in groups of honeybees. *Sci. Rep.* **7**, 1–9. (doi:10.1038/s41598-017-17863-4)

30. Burghardt GM, Bartmess-LeVasseur JN, Browning SA, Morrison KE, Stec CL, Zachau CE, Freeberg TM. 2012 Perspectives – minimizing observer bias in behavioral studies: a review and recommendations. *Ethology* **118**, 511–517. (doi:10.1111/j.1439-0310.2012.02040.x)
31. Malley JD, Kruppa J, Dasgupta A, Malley KG, Ziegler A. 2012 Probability machines: consistent probability estimation using nonparametric learning machines. *Methods Inf. Med.* **51**, 74. (doi:10.3414/ME00-01-0052)
32. Rice LA. 2016 A beginning-to-end system for efficiently gathering tracking data on multiple targets. Doctoral dissertation, The University of North Carolina at Charlotte.
33. Fahoome G. 2002 Twenty nonparametric statistics and their large-sample approximations. *J. Modern Appl. Statist. Methods* **1**, 248–268.
34. Landgrebe TC, Duin RP. 2007 Approximating the multiclass roc by pairwise analysis. *Pattern Recognit. Lett.* **28**, 1747–1758. (doi:10.1016/j.patrec.2007.05.001)
35. Youden WJ. 1950 Index for rating diagnostic tests. *Cancer* **3**, 32–35. (doi:10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3)
36. Granger CW. 1969 Investigating causal relations by econometric models and cross-spectral methods. *Econom.: J. Econom. Soc.* **37**, 424–438. (doi:10.2307/1912791)
37. Yamagata N, Nishino H, Mizunami M. 2007 Neural pathways for the processing of alarm pheromone in the ant brain. *J. Comp. Neurol.* **505**, 424–442. (doi:10.1002/cne.21500)
38. Scheiner R, Page RE, Erber J. 2004 Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* **35**, 133–142. (doi:10.1051/apido:2004001)
39. Wilson EO, Regnier Jr FE. 1971 The evolution of the alarm-defense system in the formicine ants. *Am. Nat.* **105**, 279–289. (doi:10.1086/282724)
40. Millor J, Pham-Delegue M, Deneubourg J-L, Camazine S. 1999 Selforganized defensive behavior in honeybees. *Proc. Natl Acad. Sci. USA* **96**, 12 611–12 615. (doi:10.1073/pnas.96.22.12611)
41. Sakata H, Katayama N. 2001 Ant defence system: a mechanism organizing individual responses into efficient collective behavior. *Ecol. Res.* **16**, 395–403. (doi:10.1046/j.1440-1703.2001.00404.x)
42. Sledge MF *et al.* 1999 Venom induces alarm behaviour in the social wasp *Polybioides raphigastra* (hymenoptera: Vespidae): an investigation of alarm behaviour, venom volatiles and sting autotomy. *Physiol. Entomol.* **24**, 234–239. (doi:10.1046/j.1365-3032.1999.00137.x)
43. Vander Meer RK, Breed MD, Winston M, Espelie KE. 2019 *Pheromone communication in social insects: ants, wasps, bees, and termites*. Boca Raton, FL: CRC Press.
44. Razin N, Eckmann J-P, Feinerman O. 2013 Desert ants achieve reliable recruitment across noisy interactions. *J. R. Soc. Interface* **10**, 20130079. (doi:10.1098/rsif.2013.0079)
45. McGurk D, Frost J, Eisenbraun E, Vick K, Drew W, Young J. 1966 Volatile compounds in ants: identification of 4-methyl-3-heptanone from *Pogonomyrmex* ants. *J. Insect. Physiol.* **12**, 1435–1441. (doi:10.1016/0022-1910(66)90157-0)
46. Greenwald E, Segre E, Feinerman O. 2015 Ant trophallactic networks: simultaneous measurement of interaction patterns and food dissemination. *Sci. Rep.* **5**, 12496. (doi:10.1038/srep12496)
47. Pinter-Wollman N. 2015 Persistent variation in spatial behavior affects the structure and function of interaction networks. *Cur. Zool.* **61**, 98–106. (doi:10.1093/czoolo/61.1.98)
48. Sendova-Franks AB, Hayward RK, Wulf B, Klimek T, James R, Planqué R, Britton NF, Franks NR. 2010 Emergency networking: famine relief in ant colonies. *Anim. Behav.* **79**, 473–485. (doi:10.1016/j.anbehav.2009.11.035)