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Longevity and detection of persistent foraging trails in Pharaoh's ants, *Monomorium pharaonis* (L.)

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Pheromone trails provide a positive feedback mechanism for many animal species, and facilitate the sharing of information about food, nest or mate location. How long pheromone trails persist determines how long these environmental memories are accessible to conspecifics. We determined the time frame over which Pharaoh's ant colonies can re-establish a long-lived trail and how the behaviour of individual workers contributes to trail re-establishment. We used artificially constrained pheromone trails on paper to investigate trail longevity and individual responses. Trails formed by traffic of 1000–2000 ant passages could be re-established after 24 h, and after 48 h for 4000–8000 ant passages. Only 27.5% of individual foragers were highly successful in a bioassay testing the ability to detect trails established 24 h earlier. Trail-finding ability was significantly correlated with a low antennal position. Long-lived trail detection scores increased significantly in 57% of foragers after 5 h of food deprivation and isolation, but declined again after feeding. In a control study, only 9% of foragers changed their scores, when isolated with food present. A high degree of conservatism was found such that, regardless of treatment, 21% always failed the bioassay and 17% always succeeded. Our demonstration of long-lived components in Pharaoh's ant trails and of a behavioural specialization in 'pathfinding' shows that pheromone trails are more complex at the individual level than is generally recognized.

Pheromones are ubiquitous signalling molecules used for communication between individual animals within a species, which modulate the 'pattern of behaviour in another organism in an adaptive fashion' (Wilson 1970, pp. 233–234). Study of pheromones has increasingly highlighted their pivotal roles in social organization, recognition, mate choice, aggregation and territoriality (Wyatt 2003). Animals make sophisticated use of pheromones to communicate information of adaptive value to conspecifics (Hölldobler & Wilson 1990; Johnston et al. 1999). For example, the pheromonal control of group activity in insect societies is often used to illustrate the concept of self-organization, where the colony displays an emergent global behaviour not present at the lower level (Camazine et al. 2001).

Pheromone trails spectacularly illustrate how animal behaviour can be modulated using pheromones. Trails deposited on a substrate are used for both orientation and recruitment by many central place foragers including ants, termites, social caterpillars, slugs and snails. Trails are also used in aggregation, courting and mate finding by many snakes, lizards and salamanders (Wyatt 2003).

The pheromone trails of caterpillars, slugs, ants and termites have been extensively studied and modelled as single pheromone systems (for example: Pasteels et al. 1987; Nicolis & Deneubourg 1999). However, as found in the pheromone mixtures used for social recognition and mate choice (Silverstein 1977), animal species usually produce trails containing multiple pheromones, frequently derived from diverse glandular sources (Vander Meer 1986; Hölldobler & Wilson 1990; Wyatt 2003). These pheromones vary in volatility (and stability) and elicit different behavioural responses that are dependent upon concentration, context and their proportion in synergistic mixtures (Van Vorhis Key et al. 1981; Hölldobler & Wilson 1990; Vander Meer et al. 1990). In the fire ant, *Solenopsis invicta*, for example, putative roles have been assigned to six different pheromones used in trail marking (Vander Meer et al. 1981, 1990). Improved understanding of communication via trail pheromones requires accurate quantification of both the physical parameters of the chemical

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components used and the behavioural responses they elicit. It is timely that study of individual pheromones is extended to investigate how trail pheromone blends work, particularly now that the molecular basis of odour coding in insects is understood (Hallem et al. 2004).

A parameter critical to the function of a trail is its persistence, and trail longevity must be matched to the foraging ecology of a particular species. Indeed, in ants trail longevity varies from minutes in *Aphaenogaster albisetosus* (Hölldobler et al. 1995) to several weeks in some *Eciton* species (Torgerson & Akre 1970). Short-lived trails can rapidly modulate recruitment to ephemeral food sources, whereas long-lived trails will be more suited to persistent, or recurrent, food sources. In social caterpillars, these trail types have been demonstrated in the closely related lasiocampid species *Gloveria* sp. and *Malacasoma americanum* (Fitzgerald & Underwood 1998a). *Gloveria* sp. has very long-lived trails that take many hours to abandon once exhausted, whereas *M. americanum* trails are short lived and rapidly abandoned. The trail pheromones used are ideally suited to the foraging ecology of each species. *Gloveria* sp. consumes a generalist diet of abundant plants, whereas *M. americanum* feeds on a small range of plants with a patchy spatial distribution. In theory, a combination of both long-lasting and short-lived pheromones could allow animals to remember routes to sites that were previously rewarding but also to shift focus rapidly to sites of immediate value. This combined system would be of most benefit to opportunist animals that forage on a wide range of resources.

Pheromone trails are analogous to learning, but the memory of a successful route to food is externalized into the environment. By leaving trails, individual animals no longer need to store visual memories of foraging routes and in doing so unselfishly share an externalized memory with conspecifics. However, marking trails with multiple pheromones raises the problem of which signal takes priority and in which context. Furthermore, in a heterogeneous biological community individual variation in the trail-following behaviour of foragers might result from variable response thresholds, which may be influenced by nutritional status, experience and other factors. Differences in pheromone detection thresholds could affect the temporal dynamics of trail usage, because the likelihood (or fidelity) of individual trail following would then be determined by trail strength.

The Pharaoh's ant, *Monomorium pharaonis* L. (Formicidae, Myrmicinae), is a widely studied model system for investigating pheromone trails (Fourcassié & Deneubourg 1994; Jeanson et al. 2003; Jackson et al. 2004). At least seven chemicals elicit trail following in *M. pharaonis*, although the specific role of each has never been satisfactorily clarified (Ritter et al. 1977a; Jones & Blum 1982; Hölldobler & Wilson 1990). Faranal, the most active pheromone in eliciting trail following, was identified in Dufour's gland extracts at trace levels, and is now widely regarded as the 'true' trail pheromone of *M. pharaonis* (Hölldobler 1973; Ritter et al. 1977b). However, six alkaloid monomorines (and their homologues) abundant in the poison gland also elicit trail-following activity (Ritter et al. 1977a).

The diverse blend of chemicals eliciting trail following in *M. pharaonis* suggests that their trails should have complex characteristics and there is evidence of the retrieval of information over long and short time frames. Jeanson et al. (2003) found that *M. pharaonis* trails made on newsprint decayed within 8 min and those on polycarbonate plastic decayed within 25 min. In contrast, several researchers have noted that *M. pharaonis* trails are long lived (Sudd 1960; Ritter et al. 1973; Talman et al. 1974; Fourcassié & Deneubourg 1994). Blum (1966), in particular, noted that natural trails could be reused more than 24 h later, although trails derived from individual poison gland extracts had only weak activity after 2.5 h. Different methodologies adopted in these contrasting studies may have highlighted different characteristics of the trail pheromone blend.

We tested two main hypotheses. Essentially we investigated how a multiple pheromone system might operate from the individual to the population level. First, at the colony level, we determined the time frame over which *M. pharaonis* colonies can re-establish a long-lived trail, as a function of the ant traffic that initially formed it. We hypothesized that *M. pharaonis* colonies use long-lived and short-lived pheromone trails to optimize their foraging efforts. Second, at the individual level, we determined how the behaviour of individual workers contributed to trail reestablishment. We hypothesized that variations in individual trail-following behaviour will affect trail use over time and could efficiently allocate worker resources.

METHODS

Study Species

We studied colonies of *M. pharaonis* from a culture maintained for 7 years at the Laboratory of Apiculture and Social Insects, University of Sheffield, U.K., and which originated from the culture maintained by the Central Science Laboratories, U.K. *Monomorium pharaonis* has small monomorphic workers (ca. 2 mm body length). Colonies are easily maintained in the laboratory, and readily form trails to food. Manipulation of colony size is simple, owing to the absence of nest mate recognition in this unicolonial ant (Hölldobler & Wilson 1990). Frequent splitting and combining of the colonies meant that the six colonies studied were very similar in genetic composition. Study colonies contained 1200–2500 workers, with brood of all stages and multiple queens (12–50), and were housed in wooden nestboxes (11 $\times$ 8 cm and 2 cm high) held within a large plastic foraging box (45 $\times$ 30 cm and 15 cm high), with Fluon-coated walls to prevent escape. The experimental room was maintained at 24 $\pm$ 2 °C, relative humidity approximately 30%, and a 12:12 h light:dark regime. Colonies were given fresh water ad libitum in glass tubes sealed with cotton wool and fed daily with mealworm larvae, *Tenebrio molitor*, weekly with apple sauce, and monthly with dried egg yolk. During experimental trials, colonies also received sugar syrup (1 M sucrose, molecular weight 342) from perforated Eppendorf tubes as part of the experimental procedure.

Colonies

Trail production

The foraging trails of *M. pharaonis* are always sinuous, except where they follow an edge (Sudd 1960; Talman et al. 1974). We aimed to determine whether trails are re-established and for this purpose it was desirable to have straight trails in precisely known locations. To do this, we used the apparatus shown in Fig. 1. The colony's foraging box was temporarily linked to a foraging arena (140 × 70 cm) by a bridge (18 × 3 cm). Part of the arena was covered with two A4 (29.5 × 21.0 cm) sheets of ECF (eucalyptus chlorine-free) paper photocopied with a 2-mm grid. This is a standard paper without surface finish or chlorine treatment, which might affect pheromone degradation. Two parallel polycarbonate strips (60 × 4 cm and 0.5 cm thick), internally coated with Fluon to prevent ants from climbing them, were placed to give a 4-mm-wide corridor leading to a syrup feeder within a section of Fluon-coated plastic pipe (8 cm in diameter). Ants crossing the bridge were guided into the corridor by two Fluon-coated plastic barriers. We counted total ant traffic passing the midpoint of the corridor in both directions. *Monomorium pharaonis* lays trails in both directions (outward and returning) when exploring (Fourcassié & Deneubourg 1994) and could also do so when foraging. Traffic was counted in both directions because total trail laid will be a function of this parameter, whether trail is laid on both legs of a foraging trip or only on the return leg. When the required number of ant passages (500, 1000, 2000, 4000, 8000) had been counted all ants were shaken from the paper; 4000 ant passages typically took approximately 2 h to complete. The paper was then stored, away from ants but exposed to the air and light, in the ant room.

Trail longevity

Trail longevity was bioassayed according to the procedure shown in Fig. 2a. We cut 8-cm sections from the stored, straight trails at intervals of 1, 2, 4, 6, 24, 48 and 96 h as required. Individual trail sections were placed on the empty arena floor with a syrup feeder at the rightmost extremity of the trail and the bridge was lowered on to the leftmost extremity. All colonies presented with trails were in the same motivational state; they had been deprived of syrup for the previous 2 days and shared the same feeding regime. We could clearly distinguish between the successful re-establishment of a long-lived trail and the formation of a new foraging trail. Foraging trail re-establishment occurred within 2 min of the first ant stepping from the bridge on to the paper. A dense trail rapidly formed to the syrup feeder in exactly the same place as the original trail (marked line ± 2 mm), whereas very few foraging ants (<10%) on the paper explored away from the re-established trail. In contrast, a new trail was sinuous and discernible only after ca. 20 min of the first ant stepping on to the paper. In addition, approximately 90% of all foragers on the paper were exploring away from the trail at this time. The build-up of trail traffic on the bridge during re-establishment also far exceeded that seen in new trail formation. During re-establishment bidirectional traffic on the bridge was 50–100 ants/min at 2–3 min whereas this measure never exceeded 20 ants/min during new trail formation. Furthermore, new trails only ever reached flow rates greater than 50 ants/min after 15 min. In this case, foragers searched the large arena for food without the aid of a trail, so only a small proportion succeeded in locating food and recruitment of further foragers was slow. In summary, the re-establishment of a trail was much faster than new trail formation and a re-established trail was always formed in exactly the same location as the original trail. We performed 10 replicates for each traffic frequency (500, 1000, 2000, 4000 and 8000) at the seven time intervals.

Individuals

Individual behavioural analysis

The methodology is illustrated in Fig. 2b. As before, we established straight trails (4000 ant passages) on ECF copier paper and stored them for 24 h before use. The next day, before 1000 hours, we took 20 ants from the foraging box floor (these ants were thus foragers, not ants performing within-nest tasks) and placed them in empty plastic boxes prior to their five trials. Individual ants were then carefully transferred, on a 4 × 2-cm section of thin plastic,

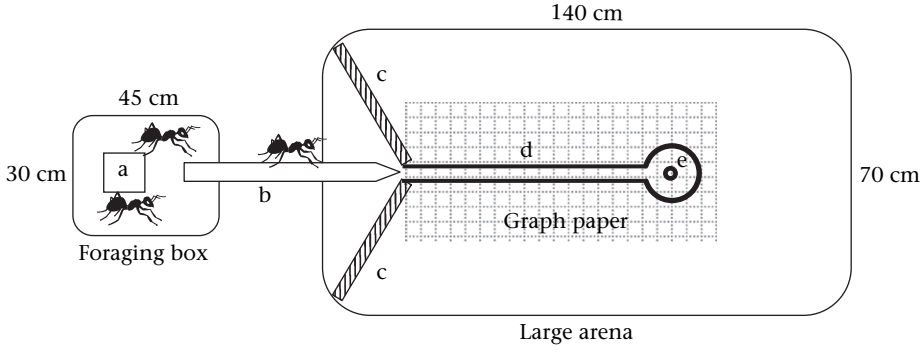

Figure 1. Experimental apparatus used in the production of straight foraging trails on paper. Foraging ants (not to scale) leaving the nestbox (a) crossed an 18 × 3-cm bridge (b) into a 4-mm-wide corridor (with Fluon-coated walls) in the foraging arena (d), which guided them to a syrup feeder (e). The barriers (c) prevented the majority of ants from walking outside the corridor. Acetate sheets prevented the few that did from laying pheromone on the paper covering the arena.

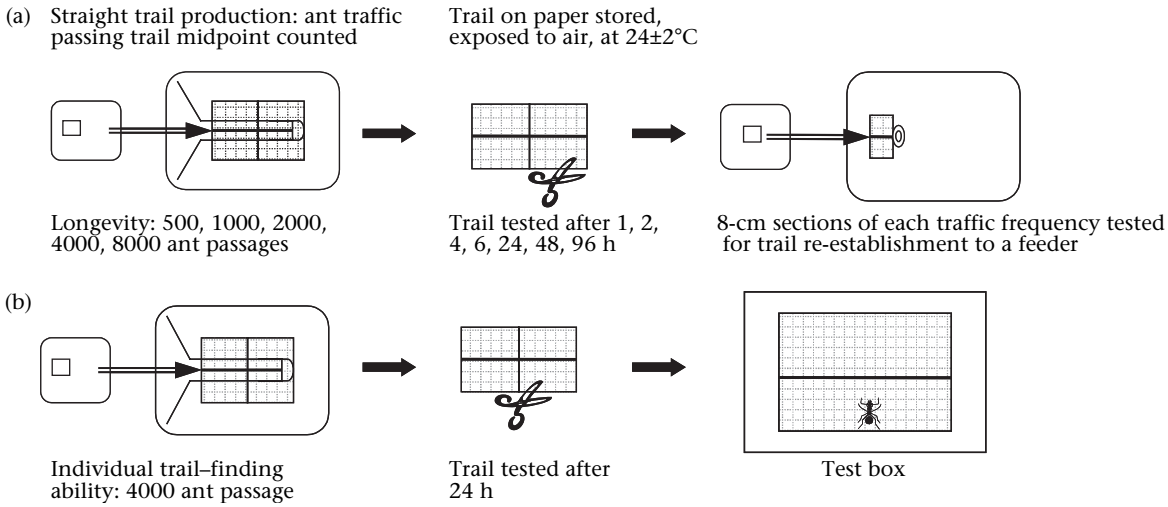

Figure 2. Experimental procedures used to determine (a) trail longevity and (b) individual trail-finding ability. In (b) an individual ant was presented 4 cm from the midpoint of a 20-cm trail. Successful trail finding was defined as following the trail without deviation for ≥8 cm. Each ant tested made five separate trials. For detailed set up of (a) see Fig. 1.

to a separate test box, with fresh grid-marked ECF paper on the floor, and allowed to settle before we measured their walking speed (time taken to cover 10 cm in 2-mm grid markings). We also directly observed the position of their antennae for the next 20 s, which we quantified as following: down = always carried low or even dragged on the surface; up = always held high and never touching the surface; up/down = both positions observed. A 20 × 10-cm section of paper with a 4-mm-wide trail running down the centre for its entire length was then placed in the box. We guided the ant, with sections of plastic placed on the paper as barriers, to within 4 cm of the trail and allowed it to cross the trail (at ca. 90°) on five occasions. If, upon crossing the trail, the ant followed the trail without deviating more than 2 mm from the trail centre for a distance of 8 cm we considered it to have successfully located the 24 ± 2-h-old trail. After each trial we removed the ant from the trail on a piece of plastic, and immediately retested it by placing it 4 cm from the trail, until five trials had been made per ant. (Test trail sections were replaced after every five ants received their five trials to reduce the possibility that ants might lay trails that subsequent ants could follow.) In total, 200 ants were each tested five times.

Manipulated individuals

We then studied individual ants over time to determine whether their trail-finding abilities were affected by two circumstances, isolation and feeding, likely to influence their motivation and behaviour. We took 20 ants from each of five colonies from the foraging box floor, before 1000 hours, and tested them for their trail-finding ability with a 24 ± 2-h-old trail. After testing we placed them in individual compartments of an ice cube tray, with Fluon coating the walls to prevent escape. Ants remained isolated in these compartments without food or water for 5 h before retesting with a 24 ± 2-h-old trail. Subsequent to retesting, each ant was provided with a droplet of 0.1-M sucrose solution and allowed to feed for 30 min before being tested a third time with a 24 ± 2-h-old trail. A control group of equal size, which received sucrose solution throughout their isolation period of 5 h, was also tested for trail-finding ability at the beginning and end of the isolation period.

Manipulated colonies

While founding new colonies, we had the opportunity to study the trail-finding abilities of ants in colonies lacking brood. We took 20 ants from the foraging box of two such colonies (one containing 1200 workers and 20 queens and the other 750 workers and eight queens) and tested individuals for their trail-finding ability with a 24 ± 2-h-old trail. These colonies were again tested according to the same protocol 4 weeks later when the colonies contained brood. In this study, we could not compare individuals over time, but the workers tested were sampled from the same population as the original ants tested in broodless conditions.

RESULTS

Trail Longevity

The probability of trail re-establishment declined to zero over a period of 96 h (Fig. 3). In general, trails established by more traffic lasted longer. The traffic a trail has received is thus a predictor of the longevity of a trail (generalized linear model, binomial error structure: $W_1 = 11.77$, $P < 0.001$, $R = 0.82$). The data allowed us to determine the interval that gave a re-establishment probability of 50% for each traffic frequency. The longevity (t) of a trail (h), re-established with a 50% probability, can be predicted from the total ant traffic (n): $t = 13.954 \ln(n) - 81.437$. Trail longevity (as hours after formation, trail re-established with probability 0.5) is a linear function of the log-transformed ant traffic ($R^2 = 0.9387$, $P < 0.01$; Fig. 4).

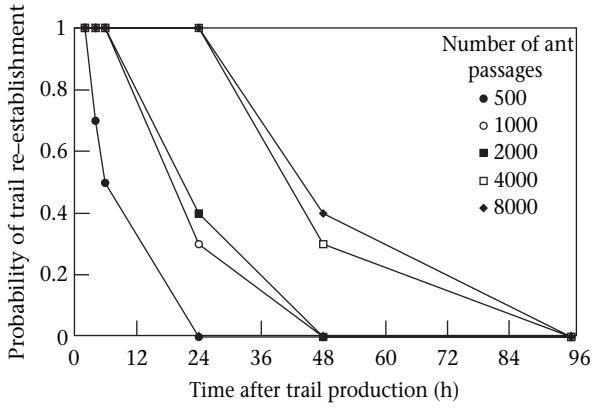

Figure 3. Probability of re-establishment of straight *M. pharaonis* foraging trails established on paper. Each traffic level (500, 1000, 2000, 4000 and 8000 ant passages) received 10 replicates at elapsed intervals of 1, 2, 4, 6, 24, 48 and 96 h.

Individual Trail-finding Ability

The trail-finding ability of individual foraging ants was scored from 0 to 5 according to the number of trials in which they detected the trail they crossed. There is a clear bimodal distribution in trail-finding score (Kolmogorov–Smirnov test for normality: $d = 0.452$, $N = 200$, $P < 0.01$; Fig. 5). The majority of foragers (72.5%) failed to detect the trail even once, while the remainder detected the trail three to five times (mean score $\pm$ SD $= 1.25 \pm 2.07$; no ants scored 1 or 2). Ant trail-finding scores were significantly affected by the position of their antennae (ANOVA: $F_{2,194} = 6.78$, $P = 0.001$). Those ants that held their antennae down ($N = 40$) were most successful at finding the trail, with 85% scoring 5. In contrast, those that held their antennae up ($N = 37$) always failed to detect trails. Of those switching between the two positions (up/down, $N = 123$), 17.1% scored 3–5. There was a significant difference between the walking speeds of the ants in the two classes (mean speed $\pm$ SD for 0 successes: 9.60 ± 2.65 mm/s; 3–5 successes: 7.10 ± 2.29 mm/s), with those

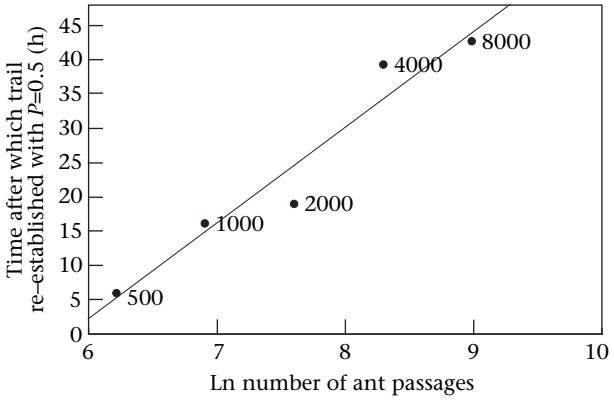

Figure 4. Estimated period after which trails could be re-established with 50% probability as a function of the traffic that formed the trail. Data points represent 50% probability re-establishment estimates derived from the decay functions for each traffic frequency shown in Fig. 3: 500, 1000, 2000, 4000 and 8000.

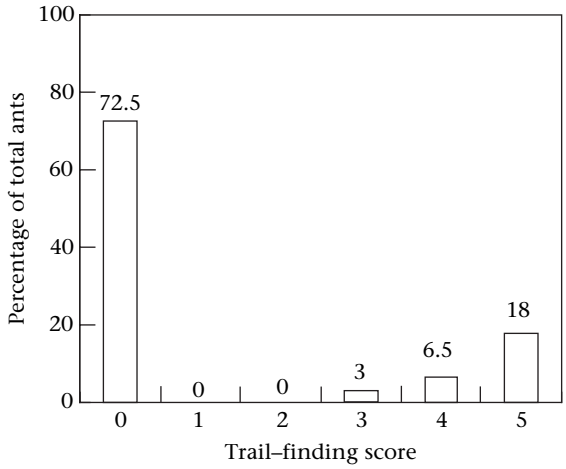

Figure 5. Trail-finding ability of 200 *M. pharaonis* foraging workers, sampled from the foraging box floor before 1000 hours. The score for each ant was the number of times it joined an established trail (unused for 24 h previously) on crossing, in five trials.

that succeeded in detecting trails moving significantly more slowly (ANOVA: $F_{1,194} = 3.91$, $P = 0.049$). However, there was no significant interaction effect between antennal position and walking speed, on the trail-finding score (ANOVA: $F_{2,194} = 2.35$, $P = 0.098$). These results show that the major factor determining high trail-finding score is low antennal position.

Manipulated Individuals

After isolation/food deprivation, there was a highly significant difference from initial scores (comparison of individual scores before and after treatment using Wilcoxon signed-ranks test: $Z = -4.522$, $N = 100$, $P < 0.001$; Fig. 6a, d). Of 100 individual ants, 61 changed score, most (93.4%) increasing. Subsequent to feeding there was again a highly significant change ($Z = -4.472$, $N = 100$, $P < 0.001$) with 59 ants changing score, most (58) decreasing. However, a comparison of initial and final (fed) scores found no significant difference ($Z = -1.723$, $N = 100$, $P = 0.083$; pairwise comparison by ANOVA with Bonferroni correction: mean difference $\pm$ SE $= -0.14 \pm 0.071$; $P = 0.155$). Only 19 of 100 differences from initial scores were found (sign test: $P = 0.169$). Regardless of treatment, 21% of the individual ants tested consistently scored 0/5 and 17% always scored 5/5.

There was no significant change in scores (Wilcoxon signed-ranks test: $Z = -0.378$, $N = 100$, $P = 0.705$; Fig. 6b, f) in the control group of manipulated individual ants, which were isolated with food. Only nine of 100 ants changed scores, all of them showing a reduction. These control data show that isolation alone has little effect on trail-finding score, but food deprivation with isolation has a large effect. Although the distribution of scores achieved after feeding by food-deprived ants (Fig. 6e) and by the fed control group (Fig. 6f) were statistically different (Mann–Whitney U test: $U = 4058$, $Z = -2.778$, $N = 100$, $P < 0.05$), the two distributions were similar. Most workers

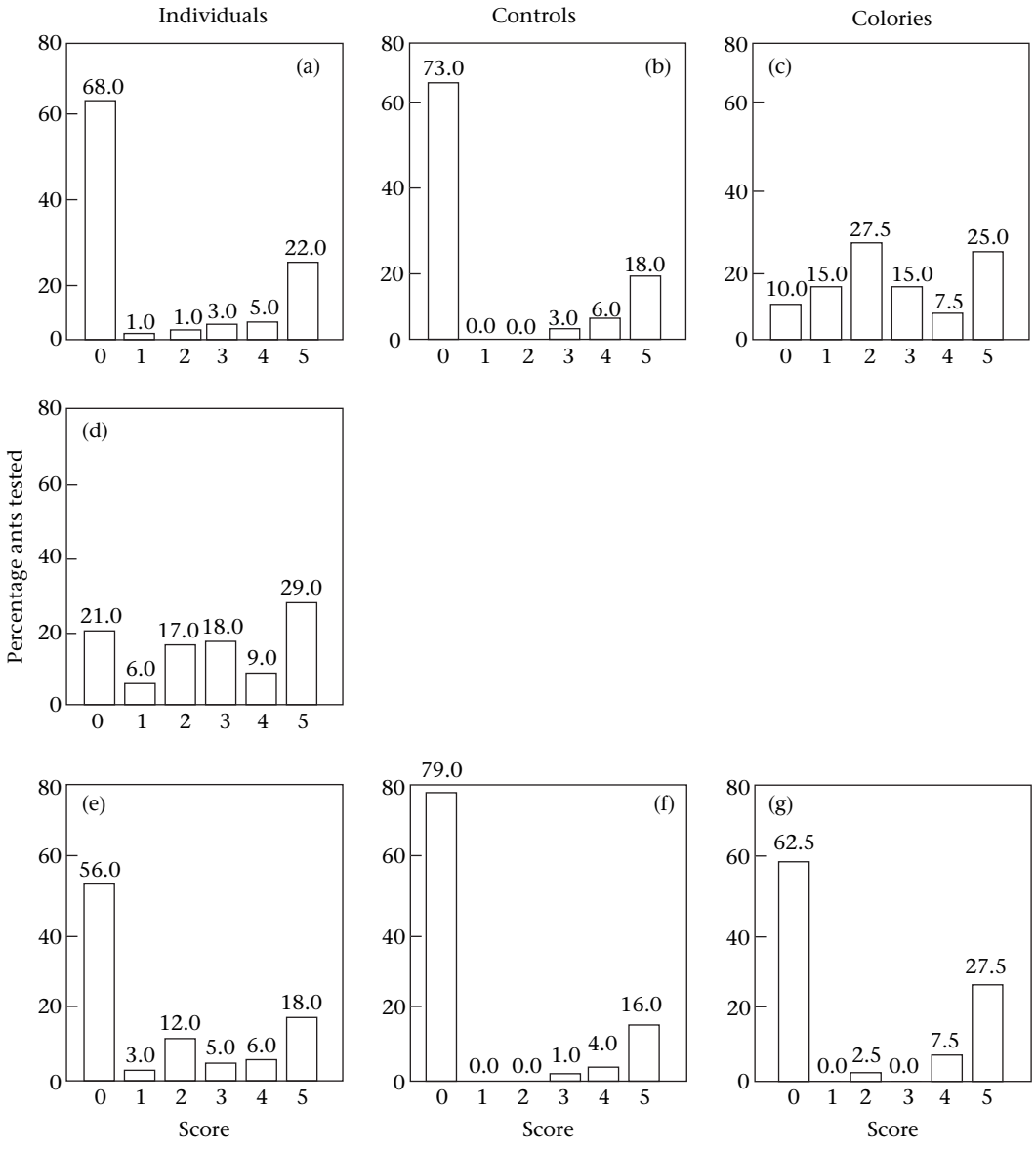

Figure 6. Trail-finding score distributions of manipulated individual foragers and colonies. (a) Initial trail-finding scores of individual *M. pharaonis* foragers ($N = 100$); (d) scores, after food deprivation/isolation and (e) scores after subsequent feeding. (b) Scores of control group ($N = 100$) and (f) after isolation with syrup. (c) Scores for foragers ($N = 40$) sampled from newly founded colonies when no brood was present and (g) 4 weeks later when brood was present.

in both groups were found in the zero success class (56% of manipulated individuals and 79% of the control group, respectively) and the proportion scoring 5/5 was also very similar (18% of manipulated individuals and 16% of the control group, respectively).

Manipulated Colonies

The initial distribution of grades (Fig. 6c) when brood was absent was significantly different to that generally observed in colonies with brood (Fig. 5; chi-square test: $\chi^2_5 = 104.7$, $P < 0.001$), and more closely resembled that found when individuals were deprived of food and held in isolation. The most striking finding is that only 10% of the ants tested wholly failed to detect the trail. However, the distribution of scores 4 weeks later (Fig. 6g), when brood was present, was not significantly different to the usual distribution (Fig. 5; $\chi^2_5 = 9.547$, $P = 0.891$), but was significantly different to that observed in the same colony when brood was absent ($\chi^2_5 = 328.6$, $P < 0.001$).

DISCUSSION

Our results clearly show that foraging trails of *M. pharaonis* can persist for long periods, up to 48 h, when established by more than 4000 ant passages. The longevity of a trail was a linear function of the logarithm of the ant traffic that formed it (Fig. 4). Our results also permit quantitative

predictions. For example, for a trail to have a 50% chance of being re-established after 24 h of nonuse would require 1900 ant passages, or approximately 1000 foraging trips, but for trails to persist for 96 h or longer would require more traffic, ca. 330 000 passages, than can realistically be made by a species with nests that generally contain less than 3000 workers (Peacock et al. 1955).

Our demonstration of long-lived trails in *M. pharaonis* makes a striking contrast to the findings of Jeanson et al. (2003) who found that trails decayed within 8 min and 25 min on newsprint and polycarbonate plastic, respectively. However, the two studies were very different in methodology. In particular, Jeanson et al. (2003) investigated the ability of individual ants to choose between the branches of a Y junction, in one of which a decaying pheromone trail led to a syrup feeder. The experimental set-up used by Jeanson et al. (2003) was constantly accessible to ants in the ant foraging box (as occurs in nature), so would have been marked, because *M. pharaonis* always lays trails when exploring new territory (Blum 1966; Fourcassié & Deneubourg 1994). This long-lived marking would have been a constant background, almost equally abundant on both branches of the Y (masking occurred only during experimental trials), allowing the longevity of the short-lived pheromone to be studied.

We have shown that the trail laid by *M. pharaonis* also contains a pheromone component with long-term persistence, serving a different role to the short-lived pheromone component. The trail substance of the Texan leaf-cutting ant, *Atta texana*, also comprises volatile and nonvolatile fractions, both of which are followed by worker ants (Moser & Blum 1963). Workers fail to respond to the volatile fraction on an artificial *A. texana* trail 60 min after its deposition, whereas the nonvolatile fraction remains fully active 6 days later and partially so after 12 days. Trails of the slug *Limax pseudoflavus* also contain two pheromone components with differing persistence that elicit different trail-following responses (Cook 1994). Our study is the first to quantify trail longevity as a function of trail traffic, clarifying the dose dependency of trail persistence. Here, we have shown a second level of response in *M. pharaonis* pheromone trails which makes the dynamics of this system highly complex.

The best candidates for the long-lived *M. pharaonis* trail pheromone are the alkaloid trail pheromones originating in the poison gland, which are very stable, low-volatility compounds (Blum 1966). The most active of these in eliciting a trail-following response is *trans*-2-pentyl-5 (5′-hexenyl)-pyrrolidine, known as monomorine III (Ritter et al. 1977a). Another alkaloid, 5-methyl-3-butyl-octahydroindolizine (monomorine I), is also highly active in eliciting trail following (Ritter et al. 1977a). However, the long-lived pheromone may be other chemicals or a blend of chemicals.

The second part of our study identified a simple but fundamental behavioural specialization of individual foragers, the position of their antennae, which mediated their ability to detect long-lived trails. We found a clear bimodal distribution in forager success at detecting a trail produced 24 h earlier. Only 27.5% of foragers were highly successful and the majority (72.5%) wholly failed to detect the trail. Similarly, in a study on the periwinkle *Nodilittorina unifasciata*, 75% of individuals followed fresh trails but only 8% followed trails 2–3 h old; those snails following the old trails also showed previously unobserved patterns of behaviour (Chapman 1998).

Successful trail detection by *M. pharaonis* occurred only in foragers with antennae held low and making frequent contact with the substrate. Of ants adopting this posture, 85% scored 5/5 in the trail relocation bioassay. This contrasts with the total lack of success (0% scoring 3–5) in trail relocation shown by those ants carrying their antennae above the substrate, and the poor success rate of those switching between the two antennal positions (17% scoring 3–5). These findings are in contrast to ants following well-defined trails, which generally 'walk at a continuous pace making infrequent antennal contacts with the substrate' (Torgerson & Akre 1970, page 5).

Because only ants with their antennae touching the substrate can detect it, our results strongly suggest that the active space for the long-lived pheromone is very small, as a consequence of low volatility. This is important because ants are widely considered to follow pheromone trails without their antennae touching the ground. For example, classic experiments using the fire ant, *Solenopsis saevissima*, showed that they follow a pheromone that persists in the air, diffusing from the volatile trail below creating a cloud with a maximum radius of 1 cm (Wilson 1962; Bossert & Wilson 1963). The ability to follow this pheromone cloud is restricted by the ant's threshold for detection, such that a vapour tunnel of pheromone exists (the active space).

In our study, ants capable of trail relocation were observed to deviate little from the centre of the trail. That this behaviour is characteristic of long-lived trail following is supported by Sudd (1960) who described how a small number of *M. pharaonis* foragers leave the nest early in the morning and explore the trunk trails used the previous day in order to locate any persistent food sources. These foragers leave the nest 'along definite routes which they follow more slowly and hesitantly than ants on a true scent trail' (Sudd 1967, page 132). A comparable situation was found in *Messor capitatus*, where only a minority of workers successfully detected trails made from sternal gland extracts, and followed them slowly, making frequent contacts with their antennae (Grasso et al. 1998).

We then investigated how motivational state (as affected by isolation and food deprivation) influenced an individual forager's trail-finding score. After 5 h of isolation, 57.0% of the individual ants showed an increase in score, but after feeding this was matched by a highly significant lowering of score. Importantly, we found a considerable degree of conservatism where, regardless of treatment, 21% always scored 0/5 and 17% always scored 5/5. We hypothesize that the 17% of foragers that could locate and follow the long-lived trails, even after being fed, are specialized 'pathfinder scouts'. We use the term pathfinder to emphasize that these scouts were following existing trails rather than locating new food sources independently of trails. We cannot be certain that the behaviour of pathfinders was permanent, but their frequency in the forager population of ca. 20% was repeatedly confirmed. The

exact role of pathfinders in foraging is suggested in Sudd (1957), where ants exploring early in the morning returned to the nest after finding food and rapidly recruited further foragers, by physical contact.

Hunger also increases responsiveness to trails in other trail-following species. For example, in the snail *N. unifasciata* trail following was more frequent (86%) in snails deprived of food for 4 days than in freshly collected snails (56%; Chapman 1998). Trail-following frequency in hungry slugs *L. pseudoflavus* was similarly high at 80–90% (Cook 1994). We suggest that a behavioural response to hunger in *M. pharaonis* is lowering of the antennae to make greater contact with the substrate. This could also be the case in other trail-following species using contact chemoreception in trail following, such as caterpillars (Roessingh et al. 1988).

In the final part of our study, we investigated the influence of brood on the path-finding ability of individual foragers. Scores were higher when brood was absent, with only 10% of the ants failing to detect the trail. When brood was present the distribution of scores returned to that usually observed. Brood affects a colony's foraging behaviour by its nutritional demands. Without brood a major source of social interaction is absent and workers may forage independently to satisfy their own nutritional requirements (Dreller 1998). Indeed if brood is absent the only remaining task is foraging and if more ants are available to forage then more may be likely to act as pathfinder scouts. However, the adaptive significance and mechanisms behind the behavioural differences we observed, in the presence or absence of brood, are unclear.

Our study shows novel aspects of a highly sophisticated system and suggests there must be important benefits from using multiple pheromones with widely differing decay rates. Clearly it is adaptive to lay persistent trails to rich feeding sites enabling their reuse the next day because *M. pharaonis* colonies are diurnal (Sudd 1960). It is probably also of adaptive significance that *M. pharaonis* trails do not persist beyond a few days, because if a trail is unrewarding for 2 or maybe 3 days, it may be better to lose this trail. A functionally similar system to that of *M. pharaonis* is found in the Madrone butterfly, *Eucheria socialis*, caterpillar, which produces a persistent network of silk threads that are selectively marked with trail pheromone during foraging (Fitzgerald & Underwood 1998b).

Long-lived trails are accessible only to ants showing the appropriate behaviour (low position of antennae when walking slowly), which may be a specialized subcaste of foragers. This is important, as it would be costly to have a large proportion of the worker population slowly exploring long-lived trails, exposed to predators. This may explain why the majority of ants are not pathfinders and can enter this role (and then poorly) only when deprived of food. However, if pathfinders are unsuccessful in finding food the probability of locating a new resource would be increased if further ants scout the trail network.

We have shown that the trail pheromone system of *M. pharaonis* is a more complex and sophisticated communication medium than previously understood. The competitive advantage that pheromone trails confer on foraging ants explains this sophistication. We speculate that further information could be subtly transmitted over time by variation in decay rates and also contextually. Our research informs the many areas of animal behaviour governed by pheromone signals. We also suggest that individual behavioural variations in responsiveness to trail pheromones influence decentralized control in the foraging-trail system. Our shift in focus to studying differences in individual behaviour shows that pheromone trails are more complex at the individual level than generally recognized.

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