

RESEARCH ARTICLE

What counts for ants? How return behaviour and food search of *Cataglyphis* ants are modified by variations in food quantity and experience

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SUMMARY

When finding more food than one is able to carry home, should one come back to the site to exploit it further? This question is crucial for central place foragers that provide for a home place with brood or nest mates. The benefit of returning has to be weighed against the chance of finding food elsewhere and the resources available. Desert ants *Cataglyphis fortis* are well-studied examples when it comes to navigating back and forth between their nest and a foraging area, due to their primary reliance on path integration in the open and featureless desert habitat. The ants use path integration not only for a safe return from their foraging trips but also for future returns to plentiful feeding sites. The direction from the nest that has previously yielded food items is preferred for future foraging trips, a phenomenon termed sector fidelity. What prompts the ants to return to a particular site, and how faithfully they search for that place, has not been well studied. We examine the evaluation of food sources in channel experiments by varying both the number of food items in a feeder and the number of visits to the feeder before testing search distances of foragers returning to the feeding site. Ants exhibited more focused searches for plentiful food sources than for sources with only few food items upon their first return visit. After several successful visits, the ants always searched thoroughly for the food source, independent of the amount of food offered. Thus, desert ants consider both food abundance and reliability of food encounter, with corroborative learning of reliability gradually overriding the initial preference for plentiful feeders. The density of food items appears to be used by the ants as a proxy for food abundance. On the level of our analysis, the searches performed in the experimental channels are indistinguishable from those performed in the open desert terrain. The present results not only demonstrate how otherwise well-studied desert ants assess yield and experience with reliability of food sources, but also establish a model system for future study of how itemised food sources are exploited.

Key words: desert ant, food evaluation, food search strategy, navigation.

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INTRODUCTION

Desert ants (*Cataglyphis fortis*) live in North African salt pans often devoid of landmarks, making path integration, or dead reckoning, their primary means of navigation. Path integration allows the ants to return to their nest after foraging runs that may cover hundreds of metres and last for more than an hour. The ants also use this navigation system to return to previously visited food sites if these are plentiful (Cheng and Wehner, 2002; Wolf and Wehner, 2000; Wolf, 2008). What may prompt the ants to return to a particular site, and how faithfully they search for that place, has not been well studied. Food quality may be one of the significant factors, although difficult to quantify (Schmid-Hempel, 1984). Here, we examine what elicits return visits and local search patterns for a feeding site by manipulating both the amount of food available in the feeder and the experience the ants have had with the feeder location. It turns out that desert ants consider the abundance of food available as well as their experience with the food location, namely the corroborative learning of the reliability of finding food. Searches for plentiful food sources are initially more focused than searches for sparse ones although, over time, the reliability of food encounter overrides food quantity in this regard.

MATERIALS AND METHODS

Cataglyphis fortis (Forel 1902) ants (Wehner, 1983) were studied near the Tunisian village Maharès (34°32'N; 10°32'E WGS84) in

the months from July to September in 2010 and 2011. Ants were marked individually with a colour code using car paint. Only novice foragers were used for all experiments.

To address possible changes in search behaviour (Wehner and Srinivasan, 1981) brought about by restricting the ants' foraging behaviour to linear channels used in most experiments (below), we initially recorded food searches performed in the open salt pan habitat. Otherwise, the conditions were identical to those outlined below for the channel experiments. That is, a feeder filled with many cookie crumbs was placed into a shallow ground depression of the desert floor at a distance of 10 m from an ant nest. The depression snugly accommodated the feeder without any edges protruding above the floor and providing orientation landmarks. A 20×20 m grid (spacing 2 m) was painted on the desert floor with wall paint to record the ants' walking trajectories on grid paper. Thirty-one ants that had encountered the feeder by chance just once were followed on their first return to the feeder position, and their searches were recorded. The feeder had been removed before the animals commenced the recorded foraging trip. Search density plots (e.g. Wolf and Wehner, 2000) were constructed from the recordings by digitisation with a graphics tablet (Wacom Intuos 3; Wacom Europe, Krefeld, Germany). For comparison with the searches in channels, the searches were projected onto the nest–feeder axis and evaluated as if they had been channel recordings.

In all subsequent experiments, the setup consisted of a training channel (~11.50 m length) and a parallel test channel (~35.5 m length), connected via a Y-shaped switch to the nest (channels made of U-shaped aluminium profile, 7 cm wide and with 7 cm high walls) (Fig. 1). The channels were assembled daily and randomly from uniform 2 m channel pieces, thus preventing ants being trained to any inconspicuous marks on the channel segments. A feeder was placed in the training channel at a 10 m distance from the nest. The test channel was always on the upwind site of the training channel to prevent any odour interfering with the tests. The channel arrangement assured travel in a straight direction and selective assessment of the distance component, or odometer, in the ants' navigation. It also meant that search behaviour might be altered with regard to the normal search of desert ants in the open field of a salt pan (Wehner and Srinivasan, 1981). Possible changes in search behaviour due to the channel arrangement were scrutinised by the first experiment (above; see also Results and Fig. 2).

The feeder consisted of a 3.2 cm Petri dish. In different experimental sessions, the feeder was supplied either with a single food crumb, or with five, 25 or many (>800) standardised food items. The food items were cookie (Tunisian brand Saida; Sotubi Biscuiteria, Megrino, Ben Arous, Tunisia) crumbs sieved to ~1.5 × 1.5 mm size and soaked with a little mango juice. In a subset of experiments, a much smaller feeder was used (8 mm in diameter) to increase the density of food items (actually 16-fold).

Ants that had found the feeder, taken up a food item and returned to the nest were followed further. The animals were tested either on their next foraging trip or they were allowed a defined number of further visits to the feeder. Preliminary experiments had shown that after five visits the food search did not change further (see also Wolf, 2008). Therefore, ants were allowed to forage either once, or at least five times, before being tested. For testing, the ants were guided individually into the test channel running in parallel close to the training channel. The animals were guided into the test or training channels through a Y-shaped channel segment with a switch door. The switch door allowed selection of the appropriate channel without disturbing the ants. In the test channel, the animal's search for food was recorded until it had completed its sixth turning point (defined as a U-turn with at least 40 cm walking distance into the new direction). We noted the turning point distances with regard to the nest to the next 10 cm by means of a measuring tape.

For each ant, the median of the initial six turning point measurements was calculated and taken as the search centre for statistical analyses. In addition, the variance of the initial six turning point measurements (intra-individual variance) was calculated and evaluated further. The variance of the individual ants' search was considered as a proxy for search focusing (cf. Sommer and Wehner, 2004).

For statistical comparisons between experimental groups Kruskal–Wallis ANOVA on ranks was used. Comparisons of variances were performed as described in Sachs (Sachs, 1999). To

test whether the variances of normally distributed data differed, the quotient of the variances was tested with the *F*-test (Sachs, 1999). To test for different variances in not normally distributed data, the difference of the search centre to the median of the whole group was first calculated. These differences were then tested with the Mann–Whitney rank sum test (Sachs, 1999). To compensate for multiple comparisons, the Holm–Bonferroni correction for multiple comparisons was used (Motulsky, 2010). Sigma Plot 9.01 with Sigma Stat 3.11 integration software (Systat Software, San Jose, CA, USA) was employed for calculation of significance levels and for visualisation as box plots. Box plots show medians and 25th and 75th percentiles, and whiskers indicate 10th and 90th percentiles.

RESULTS

Food searches in the open desert terrain and in channels

The main results presented in this study rely on experiments performed in 7 cm-wide channels that connected the ant nest to a feeder or to a test channel without food (Fig. 1). While such channel experiments have frequently been performed in the past (e.g. Cheng and Wehner, 2002; Sommer and Wehner, 2004; Steck et al., 2009; Wittlinger et al., 2006), we wanted to ascertain that the searches in the channels are similar to the searches in the open field, or, if there were quantitative differences, we wanted to be able to assess these. Fig. 2 illustrates that search behaviour in the field and in the channels is indeed similar. To compare searches performed in the two situations, *C. fortis* foragers were allowed to find a feeder established 10 m from the nest and retrieve a food morsel from a large number of cookie crumbs (>800). Ants reliably search for such a plentiful feeding site on their next foraging trip, which was recorded with the feeder being removed. The searches recorded in the open field (Fig. 2A) are clearly centred on the previous location of the feeder. And when the ants' search trajectories are projected onto the nest–feeder axis, the resulting search distribution (Fig. 2B) is very similar to the search distribution recorded in the channel (Fig. 2C). This concerns both medians and spreads (details in Fig. 2 legend).

Food abundance at the feeding site

Desert ants are apparently able to judge the yield of a feeding site upon their first visit. This is demonstrated by the data in Fig. 3A,D. The search median was centred on the previous nest–feeder distance only if the feeder had been supplied with many food items (>800) (Fig. 3A). If just one, five or 25 food items had been offered, the ants centred their searches farther away from the nest, between 13 and 14 m, and the search spread was larger (details in Fig. 3 legend). The groups with one, five or 25 food items in the feeder differ significantly from the group with a full standard feeder (ANOVA on ranks with Dunn's *post hoc* test, $P < 0.01$ for one and 25 food items, $P < 0.05$ for five food items against the group with a filled feeder).

The same tendency is discernible for the variances of the individuals' searches (Fig. 3D). Ants that had previously visited a

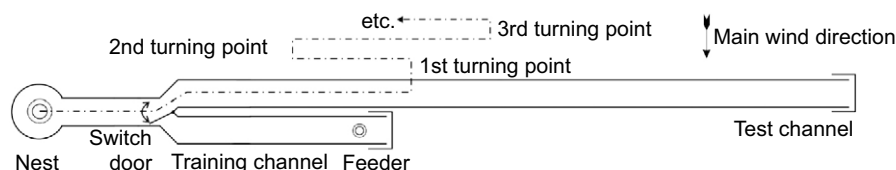


Fig. 1. Schematic diagram of the channel setup. Arrangement of training and test channels; length of channels is not to scale; nest–feeder orientation 180 deg compass direction. For further details, see Materials and methods.

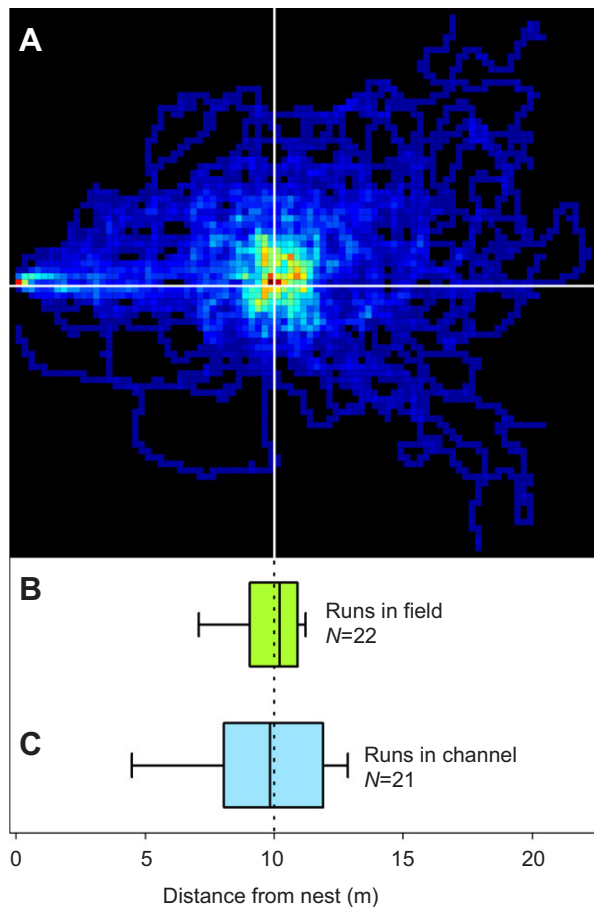


Fig. 2. Desert ants' search behaviour in the open field and in channels. For the two-dimensional search density plot (A), the numbers of ants' visits to each 25×25 cm pixel of the feeder surrounds was recorded, summed and normalised to the maximum number of visits per pixel observed in the plot. The darkest red represents the highest density (100%), the darkest blue just a single visit (note individual walking trajectories discernible near the margin), and black areas were not searched at all (0%). Recordings lasted for 2.5 min after the animal had left the nest (red pixel on the left-hand margin); nest–feeder distance was 10 m. The ants ($N=31$) had visited the full feeder (>800 cookie crumbs) once before the recordings were made. (B) To construct the box plot, the data in A were projected onto the nest–feeder axis; i.e. any movements along the axis perpendicular to the nest–feeder direction were disregarded. Turning points were only recognised if the respective animal walked more than 40 cm into the new direction along the nest–feeder axis, in correspondence to the channel recordings (see Materials and methods); only those animals that performed at least 6 turning points according to this criterion were evaluated; this reduced the number of ants from 31 in A to 22 in B. (C) The box plot presents searches recorded in the test channel used in all the other experiments described in this report. The ants had visited a full feeder once in the training channel before the recordings were made. Note the similarity of the plots in B and C, attesting to comparable search behaviour in the channel and in the open field on the level of the present analysis. Box plots show medians, spreads (+75th, –25th percentiles) and whiskers (+90th, –10th percentiles).

full feeder exhibited consistently small search variances (low variance and low spread of variances), while the searches for the feeders equipped with 1–25 food items were altogether more variable. No significant differences were observed here, however (ANOVA on ranks, $P=0.088$).

From these data, two tendencies are evident. With a large number of food items, (1) the search tended to be centred more accurately

on the position where the food had been presented during the first visit, rather than on positions farther away from the nest, and (2) spread and variance decreased, indicating a more focused search.

Experience with the feeding site

The reliability of food available at the feeder appears to focus the search in a similar way to a high abundance of food in the feeder, perhaps even more strongly (Fig. 3B,E). After five or more training visits to the feeder, the ants that had experienced a full feeder and the ants that had encountered just one or five food crumbs showed no significant differences in their search behaviours. The searches were focused on nest–feeder distances of ~ 12 m, i.e. noticeably further from the nest than the original feeder position. Compared to the group with just one training run, the search medians thus shifted, from 9.85 m to 11.85 m in the group with many food crumbs, and from 14.43 m to 12.30 m for the group with five food crumbs. The variances of the searches also decreased significantly [test for variances (after Sachs, 1999) with Holm–Bonferroni correction for multiple comparisons; five food crumbs, $P<0.01$; many food crumbs, $P<0.05$; for values for percentiles and variances, see Fig. 3 legend]. In the group with one food crumb, no notable shift occurred (12.75 m to 12.78 m) and the changes in variance did not yield significant differences either (even though intra-individual variances decreased, see below).

The most striking features in Fig. 3B and Fig. 3E are the reduced variances of the search distributions in the experienced ants. This is illustrated most clearly in Fig. 3E, with the search variances being similar to the situation with a full feeder visited just once (Fig. 3D, bottom box plot). These reductions were significant for the groups with five and with many food crumbs in terms of variance in search medians (Fig. 3A,B) and for the groups with one food crumb in terms of intra-individual variance (Fig. 3D,E).

Is food density a proxy for food abundance?

Ants do not literally count (e.g. Franks et al., 2006), raising the question of how *Cataglyphis* assesses food abundance. As an initial enquiry into this question, we changed the density of food items but kept their number constant (Fig. 3C,F). Twenty-five food crumbs were offered in a standard feeder of 32 mm diameter or in a small feeder of 8 mm diameter, thus increasing food density 16-fold. The ants that had paid a single visit to the small feeder exhibited a search pattern that significantly shifted towards the pattern elicited by a full feeder. The search median changed from 14.10 m (large feeder) to 11.60 m (small feeder) (the value for one visit to a full feeder was 9.85 m). This shift of the search medians was statistically significant (t -test, $P=0.013$) and the variances of the search medians also decreased significantly ($P<0.02$) (Fig. 3C). Changes in intra-individual variances were not significant, however (Fig. 3F).

DISCUSSION

Parameters that determine food searches: abundance and reliability

We scrutinised which parameters elicit searches for a feeder. The parameters food abundance and reliability of food encounter appeared as obvious possibilities and were tested in this study [while food quality had been examined to some extent previously (Schmid-Hempel, 1984)]. Food abundance was manipulated by varying the number of food items in the feeder from one to either five, 25 or >800. Reliability apparent to the ants was manipulated by allowing different numbers of training runs to the feeder before testing the ants, either after only one or after at least five training visits. An important initial test confirmed that *Cataglyphis* search behaviour

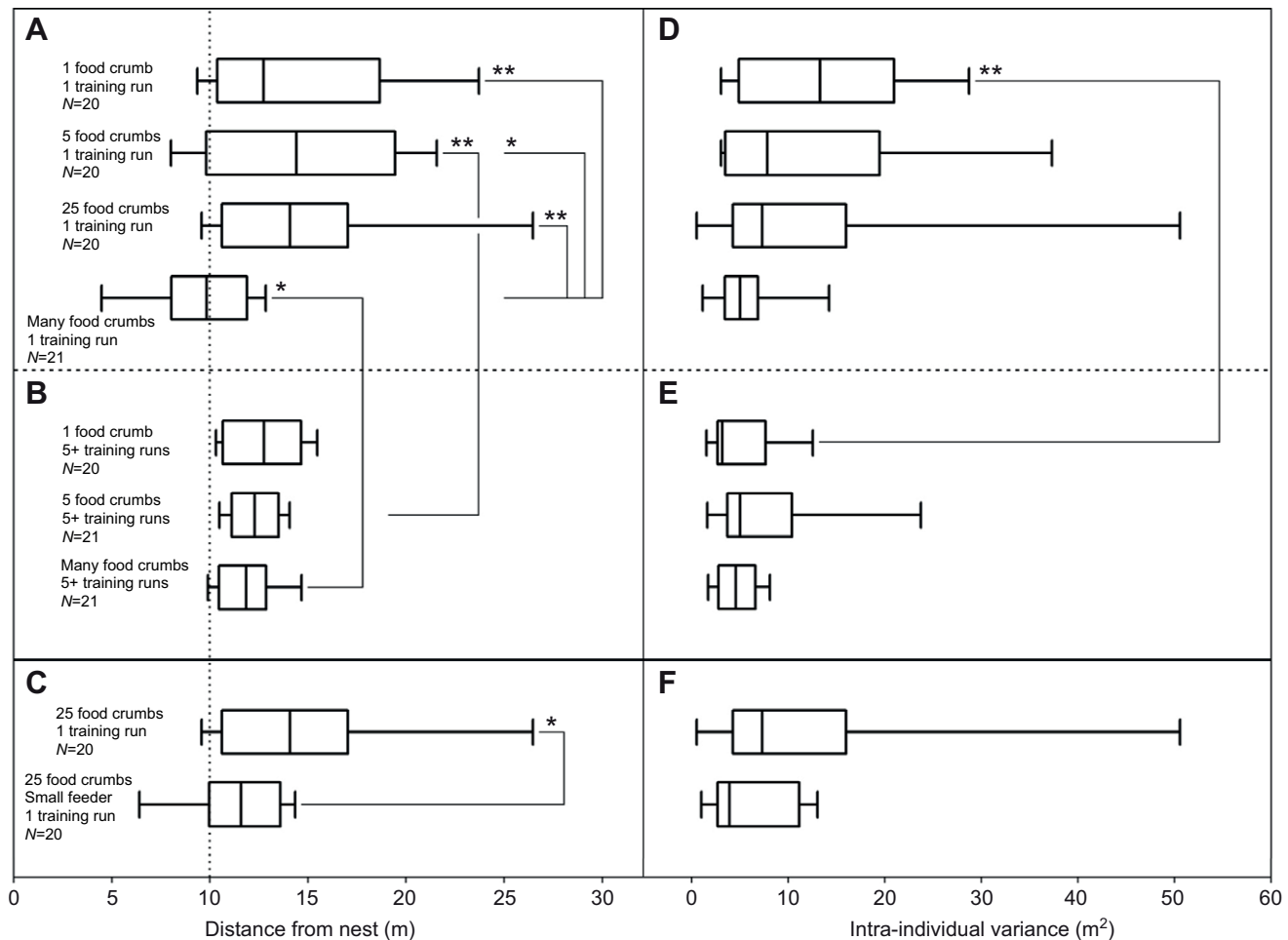


Fig. 3. Distributions of food searches in the different experimental groups: (A–C) search distances; (D–F) intra-individual variances of ants' searches in the corresponding experimental groups. (A,D) Data from ants that had performed a single (training) visit to a feeder located in a channel, 10 m from the nest. The experimental groups differed in the amount of food available in the feeder, as noted on the left; further noted are numbers of experimental animals. Medians, spreads (+75th, –25th percentiles) (A), and medians and spreads of intra-individual variances (D) for the different groups are as follows: many (>800) food crumbs, 9.85 m (+2.03 m, –1.80 m) and 5.02 m² (+1.84 m², –1.52 m²); 25 food crumbs, 14.10 m (+2.85 m, –3.42 m) and 7.32 m² (+7.70 m², –2.72 m²); five food crumbs 14.43 m (+4.22 m, –4.60 m) and 7.85 m² (+9.97 m², –4.31 m²); one food crumb 12.75 m (+5.0 m, –2.15 m) and 13.32 m² (+7.40 m², –7.86 m²). (B,E) Data from ants that had performed five or more (training) visits; the experimental groups differed in the amount of food available in the feeder. Medians and spreads (B) and medians and spreads of intra-individual variances (E) are: many (>800) food crumbs, 11.85 m (+0.99 m, –1.34 m) and 4.60 m² (+1.84 m², –1.80 m²); five food crumbs 12.30 m (+1.15 m, –1.16 m) and 5.03 m² (+5.12 m², –1.23 m²); one food crumb 12.78 m (+1.78 m, –2.03 m) and 3.17 m² (+4.44 m², –0.48 m²). (C,F) Data from ants that had visited a feeder equipped with 25 food items once before being tested. The feeder was either of standard size (32 mm diameter; same data as in A) or smaller (8 mm). Corresponding medians and spreads (C) and medians and spreads of intra-individual variances (F) were: 14.10 m (+2.85 m, –3.42 m) and 7.32 m² (+7.70 m², –2.72 m²), and 11.60 m (+1.88 m, –1.63 m) and 3.92 m² (+6.83 m², –1.22 m²), respectively. Significant differences are indicated by brackets and asterisks (**P*<0.05; ***P*<0.01). Absence of significant difference is not indicated; all experimental groups were tested against each other within A, B, C, D, E and F, respectively. We also tested the corresponding pairs between A and B and between D and E; i.e. groups of experimental animals with the same number of food items in the feeder. Box plots show medians, spreads (+75th, –25th percentiles) and whiskers (+90th, –10th percentiles).

is very similar in the channel arrangement used here and in many previous studies (e.g. Cheng and Wehner, 2002; Sommer and Wehner, 2004; Steck et al., 2009; Wittlinger et al., 2006) and in the open desert terrain (Fig. 2).

The amount of food appears to be of primary importance upon the first encounter with the food source, as shown by the data in Fig. 3A,D. Ants that had encountered a full feeder searched quite precisely at the position of the feeder in their subsequent test run. By contrast, ants that had encountered only one, five or 25 food crumbs searched over a much larger area. The two groups showed significant differences with regard to positions of the search centres and (intra-individual) variances of their searches. Evidently, the ants

are able to assess the abundance of food upon their first visit to the feeder.

Offering 25 food crumbs in a small feeder, 8 mm diameter compared with the standard 32 mm, reduced search variance (*P*<0.02). This indicates that the ants do not evaluate the number of food particles – they do not literally count items (Franks et al., 2006) – but instead judge something like food density.

If food was presented reliably over five or more visits, the above differences between feeders with different food abundances disappeared (Fig. 3B,E). This is in good agreement with previous results that demonstrated that the approach to a feeding site stabilises with regard to the approach trajectory after about five

foraging trips (Wolf, 2008). For desert ants, a reliable feeder with one food crumb available upon each visit thus appears as valuable as a reliable feeder with a much larger amount, such as more than 800 food items. It also means that ants consider both food abundance and corroborative learning of the reliability of a food source in future food searches.

Search centres and search spreads

An intriguing observation was the fact that increased experience with a feeding site shifted the search area further away from the true nest–feeder distance, in the present case towards 12 m from a 10 m nest–feeder distance. This finding was unexpected as one would assume that with increased experience the ants' accuracy in localising the food source should also increase, focusing the search on 10 m (cf. Wolf, 2008). And while the ants indeed narrowed their search, as expressed in the search variances (see Fig. 3), their search distances were well past 10 m.

These observations suggest independence of the two main aspects of a food search: position of the search centre and search spread, or variance. With regard to search variance, a narrow search would appear to indicate high motivation or high confidence regarding goal location. Higher motivation is assumed to be brought about by a larger number of successful previous visits, regardless of the reasons for this success, reliability or food abundance. This is borne out by the present data (Fig. 3).

With regard to search distance, the extension might be interpreted as reflecting the foragers' general tendency to expand their search territory over time as long as the visited segment of the nest surrounds provides food (Schmid-Hempel, 1984; Wehner et al., 1983; Wehner et al., 2004). Recent experiments suggest, however, that the extended search distance, compared with the true nest–feeder distance, may rather represent two competing pieces of navigational information. One is the animals' path integrator centring the search on a distance of 10 m from the nest. The other is guidance by the channel walls, prompting the ants to follow the channel until the feeder is reached. This interpretation

is suggested by experiments comparing food searches with and without guidance by extended landmark-like structures, such as the channels used here.

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