Current Biology

Report

Absence of visual cues motivates desert ants to build their own landmarks

Highlights

- *Cataglyphis* ants face high mortality during long foraging runs
- Nests in the flat salt pan have taller nest hills than nests in structured areas
- Ants use the nest hill as a visual cue to navigate home
- Adding landmarks to nests in the salt pan suppresses hill-building behavior

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In brief

Freire et al. show that *Cataglyphis* ants build taller nest hills in environments with fewer visual cues to use them as landmarks, allowing them to locate their nests more efficiently in environments void of natural cues.

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Report

Absence of visual cues motivates desert ants to build their own landmarks

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SUMMARY

The desert ants *Cataglyphis fortis* inhabit the harsh salt pans of Tunisia. The individually foraging ants rely on path integration to navigate back to their nest.[1–4] However, as path integration accumulates errors[5] at a rate that increases with distance traveled,[6,7] it is supplemented by visual and olfactory cues.[8–13] We show that despite their impressive homing accuracy, ants returning from long foraging journeys face a mortality rate of up to 20%. To facilitate homing, colonies inhabiting the featureless center of the salt pan build tall nest hills as visual cues. Removing these hills triggers rebuilding, but visual artificial landmarks placed near the nest entrance are sufficient to suppress the ants' rebuilding activity. Our data suggest that the desert ant builds its own landmark on purpose in a featureless environment to increase its chances of successful homing and survival.

RESULTS AND DISCUSSION

Accuracy of long homing runs and survey of nests in a salt pan

To explore the efficiency of long-distance foraging ants that rely primarily on path integration, we tracked the foraging behavior of *Cataglyphis* ants using a GPS app (Geo Tracker). We placed food crumbs in locations several hundred meters deep within the salt pan, waited until retrieval by an ant, and subsequently monitored the ant's homing run to its nest. We observed 80% of ants successfully returning to their nests from this remote area, while 20% became lost and died (Figure 1A, n = 20; Table S1). The longest homing run observed was 2.16 km. However, this ant did not successfully return to its nest and died, likely due to desiccation after 2 h of nest search. The longest successful homing run recorded was 1.1 km, which due to its straightness almost corresponded to the distance of the ant from its nest when it picked up the food crumb. *Cataglyphis* ants have been previously reported to penetrate the salt pan up to a distance of 350 m, a distance likely constrained by the ants' physical limits.[14,15] Our observations reveal that foraging distances are longer than previously reported but that extended distances involve higher mortality rates for navigating ants. Within the preceding 2 years, local precipitation has been noted as comparatively low, which may further contribute to food deprivation within the salt pan and increase pressure on ants to forage further and thus face associated mortality risks.

Several tracked ants returned to nests that were situated deep within the salt pan. We noticed that these nests had unusually high nest hills (Figure 1B). We, therefore, surveyed the salt pan habitat for additional nests and found 19 located deeper in the salt pan and 14 located near the shoreline, totaling 33 surveyed nests. Given that homing *Cataglyphis* ants in this specific habitat have been previously shown to not respond to shoreline cues when they are more than 40 m away,[15] nests situated further than 60 m from any shoreline were classified as "salt pan nests," and nests within this distance range to the shoreline were classified as "shore nests" (Figure 1C). Nests situated between 40 and 60 m away from any landmark were not considered for analysis to ensure some leeway between the two categories. We then measured nest hill heights for both groups and found that salt pan nests had significantly taller nest hills compared with shore nests (Figure 1D).

Absence of a nest hill impairs the navigation of salt pan ants

As ants are known to use visual cues to pinpoint their nest entrance[8] and to especially rely on such cues after extremely long foraging runs,[15] we asked whether the ants might use their nest hills for homing. To do this, we studied the role of the nest hill in the homing behavior of ants returning to one nest of each category: a salt pan nest or a shore nest in their native environment. We captured ants from both types of nests before nest entry and displaced these so-called zero-vector ants (foragers that ran off their path integrator completely) by 5, 7.5, or 10 m from the nest. The ants were allowed to search for their nest, either in the presence or absence (after artificial hill removal) of their nest hill (Figures 2A–2F). When we compared the straightness of homing runs of ants from the salt pan nest, tested either with or without their nest hill, we found that at all displacement distances the ants homed less directly when their nest hill was removed (Figures 2G–2I). When testing ants from a shore nest, we found such an effect only in ants displaced by 5 m (Figure 2G), while the absence or presence of the nest hill did not affect those ants displaced by longer distances (Figures 2H and 2I). In the experimental group with nest hills removed, we also observed that ants from the salt pan nest were less straight in navigating back home than shore nest ants. This may be explained by the

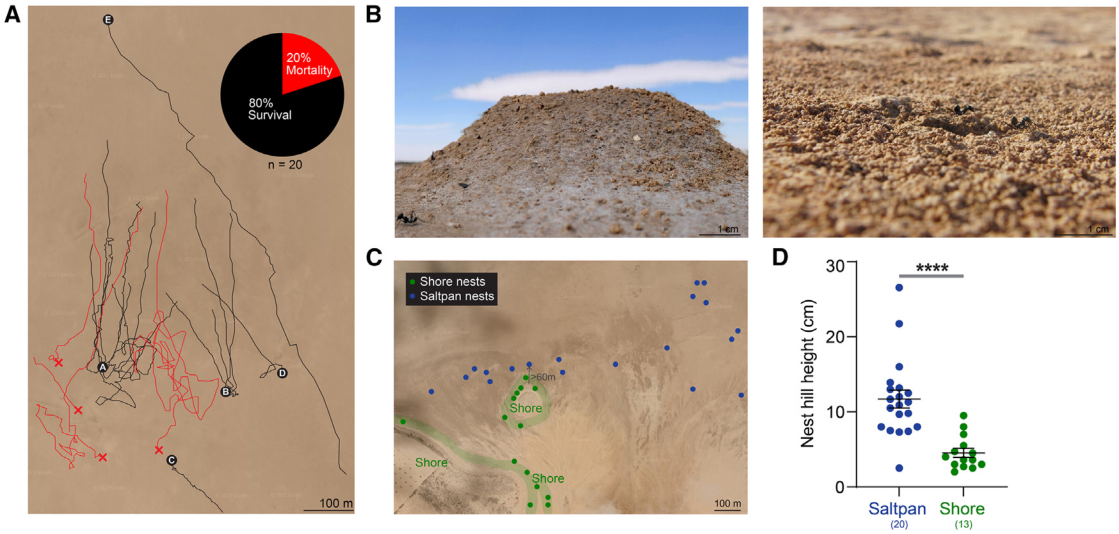

Figure 1. Accuracy of long homing runs and survey of nests in a salt pan

(A) GPS tracks of long foraging runs (Geo Tracker, Google Earth) (see also Figure S1). Each black dot represents a different nest. Black lines, successful homing runs; red lines, unsuccessful homing runs that resulted in the ant's death. The track that leads to colony E is displaced to fit closer to the other paths for visualization purposes.

(B) Photographs of a typical salt pan nest (left) and a typical shore nest (right, photo credit: Cornelia Buehlmann).

(C) Map of the salt pan with dominant environmental features (i.e., vegetation and terrain elevations) and classifications of two nest types. Nests located at least 60 m away from any shoreline are classified as salt pan nests (blue) and nests less than 40 m away from any shoreline are classified as shore nests (green). Nests situated between 40 and 60 m from any shoreline are not included in the analysis.

(D) Height of nest hills at salt pan nests and shore nests (unpaired t test, n = 33; ****$p < 0.0001$; bars indicate median and SD).

See also Table S1.

use of visual cues present at the shoreline, which are absent from the areas deep within the salt pan that are free of visual features (Figures 2G–2I). Lastly, when displaced by a 10-m distance, ants from the salt pan nest returned significantly less successfully when they could not rely on the nest hill (Figure 2L), while the success rate of shore nest ants was unaffected by nest hill removal (Figures 2J–2L). We conclude that ants use the nest hill to pinpoint their nest entrance and that the importance of the nest hill is higher for those ants that return to a salt pan nest within an otherwise featureless environment.

Ants build their own landmark by increasing their nest hill's height

Nest hills of ants often fulfill a function in thermoregulation.[16] Thus, a driving factor for higher nest hills in the salt pan might be a microhabitat that differs from that at the shoreline. Concurrently, higher groundwater levels within the salt pan might increase damage below ground to the nest chambers, resulting in more digging activity by the ants. However, having shown that ants use the nest hill as a cue for homing and especially rely on it in the featureless environment deep within the salt pan, we asked whether the visual surroundings of a nest might also govern the ants' hill-building activity, i.e., whether the ants build the nest hill on purpose when they lack other nest-defining cues. To test this hypothesis, we removed the hills of 16 salt pan nests and divided them into two equal groups. For one group, we installed two black cylinders (50 cm height, 20 cm diameter) in the nest vicinity as artificial landmarks (Figure 3B). For the other group, nests were not provided with any landmarks. Artificial visual landmarks have been widely used in research on *C. fortis*, and previous studies have shown that the ants are able to perceive and associate them with their nest entrance.[8,17] After 3 days, we measured the rebuilding effort at all nests. Where artificial landmarks were present, ants had started to rebuild the hill at only two of eight nests. In contrast, with artificial landmarks absent, ants from seven of eight nests had made a rebuilding effort (Fisher's exact test, $p < 0.05$). Next, for every nest, we measured the rebuilt nest-hill height 3 days after removal and compared it with its initial height (i.e., percent of previous height rebuilt). We found hills at nests without artificial landmarks were higher than those at nests with artificial landmarks (Figure 3C). We conclude that ants do not only use their nest hills as cues for homing but also purposefully increase their size when other visual cues for navigation are absent.

The phenomenon of functional landmark building in animals is seldom addressed in literature, although a few examples exist. For instance, male fiddler crabs build mud hoods at burrow entrances to attract females for mating. These mud hoods are also utilized later by the crabs for pinpointing their burrow after foraging excursions.[18–20] Likewise, thatched entrance structures of leaf-cutting ants (*Acromyrmex*) have been suggested to serve as visual homing cues.[21] Landmark building in animals is therefore not novel. However, it remains unclear whether animals build such structures for navigational purposes or whether improved navigation is merely a secondary consequence of structures built to fulfill unrelated functions, such as nest thermoregulation.

We were surprised that *Cataglyphis* ants not only build their own nest-associated landmarks but also do so readily when deprived of other visual cues necessary for navigation. A colony's investment into building a nest hill is justified when other

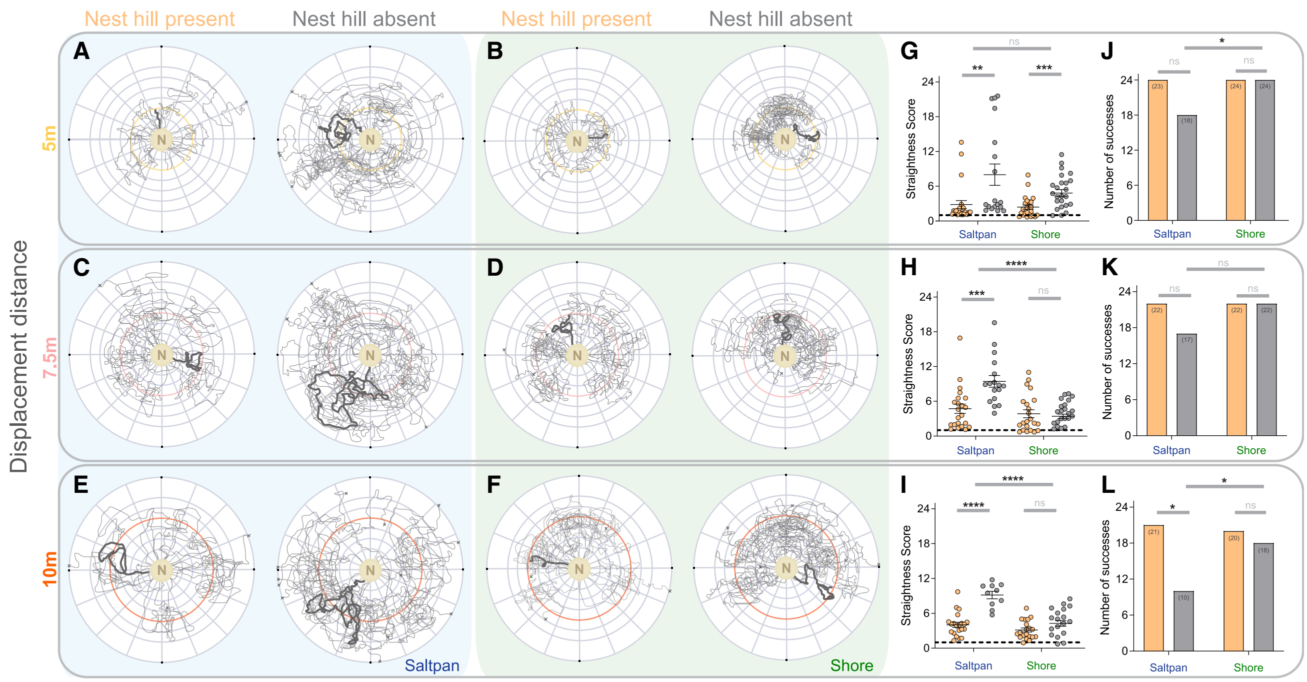

Figure 2. Absence of a nest hill impairs the navigation of salt pan ants
(A–F) Trajectories of zero-vector ants of a salt pan nest (A–C) or shore nest (D–F) with nest hill present (left) or absent (right) after 5, 7.5, and 10 m displacement (colored circumferences indicate each displacement distance). Highlighted in bold is a trajectory exemplar of an ant for each displacement distance.
(G–I) Straightness scores (i.e., run length divided by beeline) of homing ants of both nest types for the different displacement distances (Kruskal-Wallis test with Dunn's multiple comparisons test for selected pairs, $^{**}p < 0.01$; $^{***}p < 0.001$, bars indicate median ± SD).
(J–L) Success rates of homing ants of both nest types for the different displacement distances (Fisher's exact test, $^{*}p < 0.05$). Ants that during nest search left the tracking grid (radius, 20 m) around the nest entrance were classified as "unsuccessful" and are represented as crosses at the edges of the arenas.

guiding visual cues are absent, as fast and efficient homing is evidently paramount for survival in the harsh habitat of the salt pan. However, as soon as other visual cues are present, the investment does not seem justified anymore and no nest hill is rebuilt. Foraging is usually the last task in the life of a *Cataglyphis* worker, while the digging involved in building the nest hill is often performed by younger ants.[22] This calls for some kind of information flow between the older foraging ants that face the lack of visual cues surrounding the nest and their younger nestmates responsible for building the nest-defining landmark. One possibility is that information is passively shared by taking into account the number of foragers that do not return from foraging bouts. Alternatively, younger nest-building ants may perform short runs outside the nest to assess the surrounding landmarks. However, future research is needed to experimentally explore these mechanisms of information sharing. The purposeful building of landmarks adds another facet to the already complex navigational strategies of desert ants, highlighting the adaptive solutions that *Cataglyphis*[10] has developed to survive in one of the harshest environments.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - ○ Lead contact
  - ○ Materials availability
  - ○ Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.05.019.

A video abstract is available at https://doi.org/10.1016/j.cub.2023.05.019#mmc3.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.F. and M.K.; methodology, M.F. and M.K.; investigation, M.F. and A.B.; visualization, M.F. and M.K.; funding acquisition, M.K.; writing – original draft, M.F.; writing – review & editing, M.F. and M.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

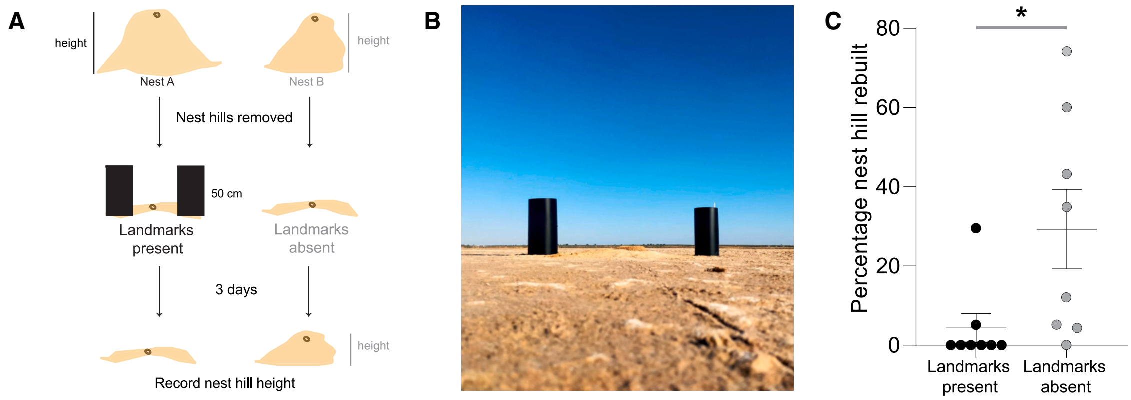

Figure 3. Ants build their own landmark by increasing their nest hill's height

(A) Experimental design. Nest hills were first measured (height) and removed from 16 salt pan nests of which only eight were afterward provided with two artificial landmarks each. 3 days later, we re-measured rebuilt nest hills.

(B) Photograph of the artificial landmarks added to a nest.

(C) Percentage of the original nest rebuilt at nests with landmarks present or absent (unpaired t test, *$p < 0.05$, bars indicate median ± SD).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Analyzed data	This paper	https://doi.org/10.17617/3.VHXFD3
Experimental models: Organisms/strains		
Cataglyphis fortis	Tunisian salt pan	N/A
Software and algorithms		
Geo Tracker, v. 5.1.5.2972	Geo tracker	https://www.geo-tracker.org
Google Earth, v. 9.180.0.0	Google Earth	https://earth.google.com
Statistical Software: GraphPad Prism 9.4.1	Graphpad	https://www.graphpad.com/scientific-software/prism/
Vector Graphics Editor: Adobe Illustrator CS5 15.0.0	Adobe Illustrator	https://www.adobe.com/products/illustrator/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to the Lead Contact, Markus Knaden (mknaden@ice.mpg.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any unique dataset or code.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The model organism of this study is the desert ant *Cataglyphis fortis*. The desert ants inhabit a saltpan in Tunisia close to the village Menzel Chaker (Sebkhet Bou Jemel, 34°96'N, 10°41'E) and experiments were performed in May, June, and August of 2022. Only female foragers were used as subjects in this study.

METHOD DETAILS

To track ants during long homing runs, we walked deep into the saltpan and searched for foraging ants. Once we located a foraging ant, we placed a cookie crumb in its proximity. As the ant picked up the crumb and began its homing run, we followed it using a GPS tracker (Geo Tracker, v 5.1.5.2972) while maintaining a safe distance to not disturb the ant's path (as shown in Figure 1A). We also surveyed the saltpan for ant colonies, logging each colony's location with the same GPS app. At each nest, we recorded the height of the nest hill by using two 30 cm rulers and logged the location of nearby landmarks and the distance of each nest to the nearest landmark in Google Earth (as shown in Figures 1B and 1C). All data are available at https://doi.org/10.17617/3.VHXFD3.

To investigate the role of the nest hill in the ant's homing accuracy, we selected two nests, one from the 'saltpan' category and the other from the 'shore' category. We built a round arena on the ground with a 20 m radius by measuring distances of 2.5, 5, 7.5, 10, 12.5, 15 and 20 m and then using ropes and a stick to lightly imprint the arena outlines on the saltpan floor. We then added 16 sectors to the arena for more precise path recording. Artificial feeders were created by digging shallow holes at the edge of each quadrant of the arena and adding cookie crumbs. The feeders ensured that ants maintained consistent back and forth foraging trips from the nest during the duration of the experiment. All feeders were active and visited throughout the experiment. For the first part of the experiment, the nest hill was present. We collected zero-vector ants (ants whose path integration information has run out) when they returned from the feeders just before they entered the nest, displaced them at 5, 7.5, 10 m and recorded their paths as they attempted to return to the nest entrance (as shown in Figures 2A–2F). All ants were displaced from the same direction they were travelling from. From these trajectories, we calculated a straightness score that corresponds to the fraction between the distance ran and the shorted distance to the nest entrance from the displacement point (Figures 2G–2I). We also recorded the number of successful returns to the nest entrance after displacement (as shown in Figures 2J–2L). If an ant was successful, we collected it and painted it to ensure that it was not tested again. If an ant was unsuccessful, it fell into one of these categories: exit the arena or exceed 20 minutes another ant. We also included an additional exclusion category when an ant got attacked or its food item stolen by another ant. These ants were also marked once the trial was over. We repeated this procedure for the four displacement distances and tested 24 ants for each distance. In the second part of the experiment, we removed the nest hill of both nests using a small shovel, ensuring that the nest entrance was not obstructed. The soil from the nest hill was disposed of far from the ants' nest. The nests were given 36 hours of rest after the nest hill removal before any experiment was conducted. After this resting period, the experimental design was identical to the first part of the experiment.

To investigate the ants' ability to rebuild their nest hills, we surveyed the saltpan in August and identified 16 active nests. Some nests found earlier in the season were not active at this point, so they were not regarded. We removed the nest hills from the active nests, and then added artificial landmarks to half of them. The landmarks were 50 cm tall black cylinders, placed one meter away from the nest entrance in opposite sides. The other half of the nests received no landmarks. After three days, we measured the rebuilding effort of each colony and re-measured the height of the nest hills. This information can be found in Figure 3.

QUANTIFICATION AND STATISTICAL ANALYSIS

To assess the difference between the heights of 'salt pan' and 'shore' nests we performed an unpaired t-test.

To compare the number of successful returns to the nest after displacement, we used Fisher's exact test. We also calculated the straightness score, which is the ratio of the actual distance traveled to the shortest possible distance. This score ranges from one to infinite, with one corresponding to the straightest path. We compared the straightness scores between "salt pan nest" and "shore nest" with a Kruskal-Wallis test with Dunn's multiple comparisons test for selected pairs.

The rebuilding efforts of the nest were also compared between the presence and absence of landmarks using Fisher's exact test. The percentage of the nest rebuilt was calculated by comparing the new height of the nest to the original height, i.e. if the original height was 10 cm and after three days we observed 2cm re-built, the percentage of height rebuilt is 20%, and then compared between the presence and absence of landmarks using an unpaired t-test.