



Desert ants possess distinct memories for food and nest odors

Roman Huber^a and Markus Knaden^{a,1}

^aEvolutionary Neuroethology, Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

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The desert ant *Cataglyphis fortis* inhabits the North African salt-pans where it individually forages for dead arthropods. Homing ants rely mainly on path integration, i.e., the processing of directional information from a skylight compass and distance information from an odometer. Due to the far-reaching foraging runs, path integration is error-prone and guides the ants only to the vicinity of the nest, where the ants then use learned visual and olfactory cues to locate the inconspicuous nest entrance. The learning of odors associated with the nest entrance is well established. We furthermore know that foraging *Cataglyphis* use the food-derived necromone linoleic acid to pinpoint dead insects. Here we show that *Cataglyphis* in addition can learn the association of a given odor with food. After experiencing food crumbs that were spiked with an innately neutral odor, ants were strongly attracted by the same odor during their next foraging journey. We therefore explored the characteristics of the ants' food-odor memory and identified pronounced differences from their memory for nest-associated odors. Nest odors are learned only after repeated learning trials and become ignored as soon as the ants do not experience them at the nest anymore. In contrast, ants learn food odors after a single experience, remember at least 14 consecutively learned food odors, and do so for the rest of their lives. As an ant experiences many food items during its lifetime, but only a single nest, differentially organized memories for both contexts might be adaptive.

navigation | olfaction | *Cataglyphis* | desert ants | learning

The desert ant *Cataglyphis fortis* inhabits the North African salt-pans where it individually forages for dead arthropods. During foraging ants take into account directional information from a skylight compass (1) and distance information from an odometer (2). By integrating both types of information the ants are always informed about their relative position to the nest entrance. However, as the foraging runs can cover more than 1.5 km (3), path integration due to accumulating errors guides the ants only to the vicinity of the nest. Close to the nest entrance homing ants then use the visual landmark panorama (4) as well as olfactory cues like carbon dioxide emanating from the nest entrance (5) or learned environmental odors (6, 7) to locate the inconspicuous nest entrance. Olfaction has also been shown to be involved in food location. Foraging *Cataglyphis* perform extensive cross-wind runs on a search for odor plumes derived from dead arthropods. Upon plume contact, the ants follow this plume to pinpoint the food item [a strategy that was also found in leaf cutter ants (8)]. It was found that the ants are highly sensitive to the food-derived necromone linoleic acid and use this compound to localize their dead prey in the saltpan (9). Although linoleic acid emanates from most insect carcasses, ants experience numerous different food sources and might learn additional olfactory characteristics that help them to find food efficiently.

Here we show that *Cataglyphis* indeed can learn the association of a given odor with food. After experiencing food crumbs that were spiked with an innately neutral odor, ants were strongly attracted by the same odor during their next foraging journey. We therefore explored the characteristics of the ants' food-odor memory and—when comparing it with the memory for nest

odors—identified pronounced differences regarding acquisition time, storage capacity, and memory duration.

Results and Discussion

Learning of Food Odors. *C. fortis* ants learn to associate different odors with the nest entrance and use this knowledge to pinpoint the nest during homing (6). Here, we asked whether ants also learn to associate odors with food. To address this question, we first trained ants to forage at a distant feeder [nest-to-feeder distance: 6 m; nest-to-feeder direction: perpendicular to wind direction (which was continuously tracked via a wind vane at the experimental area)] by placing a bucket containing a petri dish with biscuit crumbs in the ground (Fig. 1A₁). The ants could access these crumbs in the bucket via a ladder that they could climb up and down. Under this situation it takes about 1 h until most of the foraging ants of this nest continuously commute between nest and feeder along a rather straight line. We then approached ants on their way to the feeder with an open Eppendorf tube containing a single odor (2 μL diluted in 100 μL of hexane) and placed some 2 m upwind of the ant and observed whether this odor would elicit plume following (Fig. 1A₂). A short thin thread attached to the tube informed us about the current wind direction and ensured that we were approaching the ant from upwind. Turning into the plume was regarded as a positive response when it led the ants into a less than 5 cm distance to the tube. By doing this we tested the innate attraction of 32 common plant volatile odors (blue bars in Fig. 1B). We next spiked the food in the feeder with one of the odors by shaking the biscuit crumbs in a tube that contained 2 μL of the test odor (diluted in 100 μL of hexane). Ants were allowed to forage on the spiked crumbs for 15 min (i.e., about three to four training runs per ant) (Fig. 1A₃). For each trained odor, we set up a feeder at a new nest to guarantee that each nest was trained and tested with only one odor. Testing of the ants' responses to the

Significance

Desert ants have been shown to use olfaction to pinpoint food and find their nest after far-reaching foraging runs. Here we show that the ants can learn new food and nest odors and that they possess two distinct memories for both contexts. While ants need several training trials to learn a nest odor, and forget about this odor shortly after training has stopped, they learn food odors after one trial, remember them over a lifetime, and can remember at least 14 different food odors. These different memory characteristics make sense, as food is unpredictable and ants might experience many different food items in consecutive foraging runs, while the nest odor will not dramatically change during an ant's lifetime.

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¹To whom correspondence should be addressed. Email: mknaden@ice.mpg.de.

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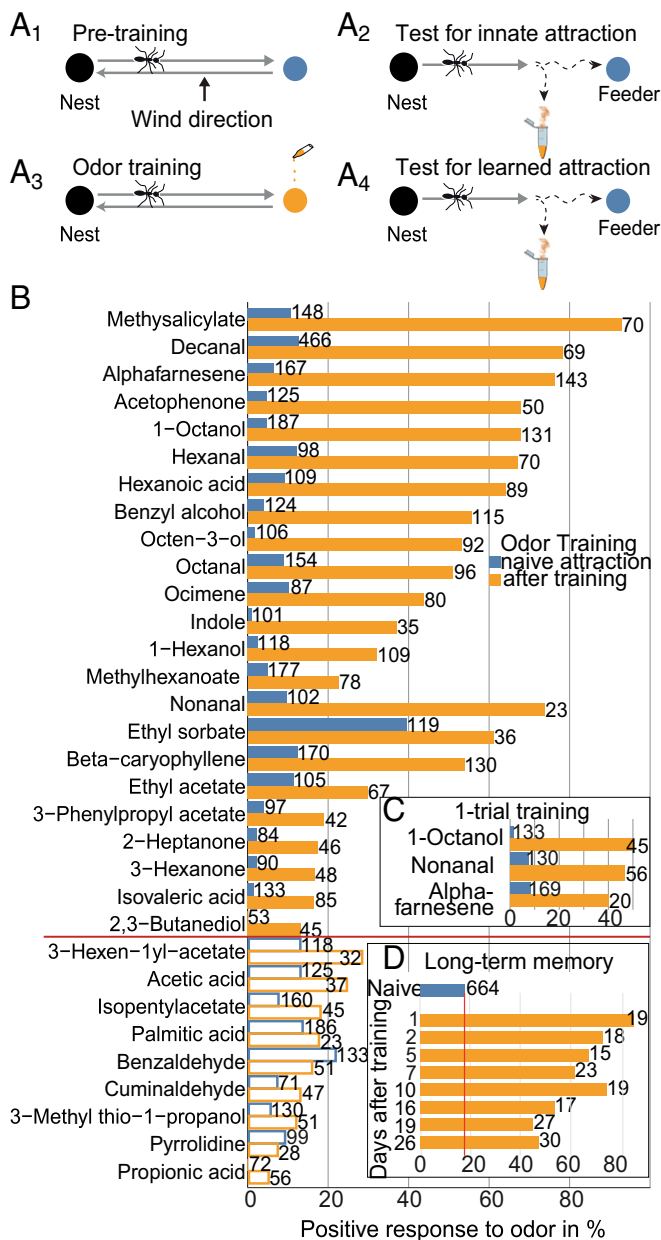


Fig. 1. Learning of food odors. (A₁–A₄) Training and test paradigms. (A₁) Pretraining to establish the feeder. Black circle: nest entrance; blue circle: dug-in bucket with cookie crumbs; nest-to-feeder distance: 6 m. (A₂) Test for innate attraction. Feeder-heading ants approach or do not approach a tube with odor placed 2 m upwind. (A₃) Odor training. Food crumbs in the feeder become spiked with training odor. Orange circle: dug-in bucket with odor-enriched cookie crumbs. (A₄) Test for learned attraction. Ants do approach or do not approach a tube with odor placed 2 m upwind of feeder-heading ants. (B) Innate and learned responses to odors. Blue bars: percentage of ants that approached an odor before training; orange bars: percentage of ants that approached an odor after they had been trained to recognize it; solid bars: learned odors with significant learning-induced differences ($P < 0.05$, test of equal or given proportions); open bars: odors that were not learned ($P > 0.05$); numbers next to bars: number of tested ants. (C) Responses of ants that had experienced the odor only during one foraging trip ($P < 0.05$, test of equal or given proportions); for color coding, see B. (D) Responses of ants that were tested with decanal 1–26 d after they had been trained to it ($P < 0.05$, test of equal or given proportions with Bonferroni correction for multiple comparisons with data of naive ants). For color coding, see B.

odors afterward (Fig. 1A₄) showed that training resulted in 23 of the 32 odors having a significantly elevated attraction (solid bars in Fig. 1B). In laboratory experiments using a Y-maze assay, *Camponotus* ants have been shown to associate odor with a sugar reward (10, 11). Our data reveal that such an association can be learned also in a natural context, where freely foraging ants are exposed to numerous cues from the environment. In proboscis extension experiments, harnessed bees were shown to learn some odors better than other odors (12). The authors (12) argued that either the bees had pre-experimental experience with the better-learned odors within the hive or that these odors were more reliably associated over evolutionary time with resources that are normally linked with a food reward. In our study we did not use any reported food or nest odors, to make an innate bias toward one of the training odors less likely. We, however, cannot exclude that the ants had experienced some of the experimental odors before.

Memory Acquisition Time. We next asked how fast ants can form this association. To do so, we again trained ants to forage at a feeder and waited until most of the foraging ants were commuting between nest and feeder. We then loaded the feeder with crumbs spiked with the training odor and immediately removed the ladder from the feeder. Ants facing this situation jumped into the feeder and took a spiked food crumb, but were unable to leave the feeder again. When all commuting ants were trapped in the feeder, the ladder was reinstalled, and all ants were allowed to return to the nest. We then tested the ants' responses to the training odor on their next foraging run, i.e., after they had experienced the food odor only once. Surprisingly, after a single training trial the ants already strongly pinpointed an odor that before training had been neutral (Fig. 1C).

Memory Duration. Having shown that the ants learn odors that quickly, we next asked, how long they would remember them. For that, we slightly modified the training procedure. Instead of spiking the food itself (which would result in foraging ants carrying the training odor into the nest), we did not shake the crumbs in the odor but applied the odor (0.8 μ L of the test odor diluted in 40 μ L of hexane) inside the bucket, but outside of the petri dish containing the food crumbs. Therefore, whenever picking up an unscented food crumb at the feeder, ants still experienced the training odor. We trained the nests for 2 h to assure that most ants of the nest's foraging force would have visited the feeder many times, marked all ants that visited the feeder with a color dot, and removed the odor from the feeder afterward. We next tested feeder-heading ants (that due to the color dot belonged to the former training force) 1–26 d after training. More than 80% of the ants followed the odor 1 d after training. However, even after 26 d, more than 45% of the ants were attracted by the odor that originally had been innately neutral (Fig. 1D). Obviously, the ants' olfactory memory remains for a very long time.

Memory Capacity. We then asked how many individual odors the ants' memory can store at the same time. We took advantage of our list of learned odors (Fig. 1B) and trained the ants with 14 well-learned odors consecutively, with the training odor on cookie crumbs being exchanged every 15 min. When testing the ants with one of the training odors afterward, the ants exhibited significantly increased attraction to each of the odors compared with before the training (Fig. 2A). To exclude that the ants after being trained with numerous odors just generalized between odors and, hence, started to follow any odor, we repeated the experiment by training the ants with only 7 of the 14 odors and testing all 14 odors individually afterward. Regardless of which seven odors that the ants were trained to recognize, the ants afterward mainly followed the training odors and either ignored

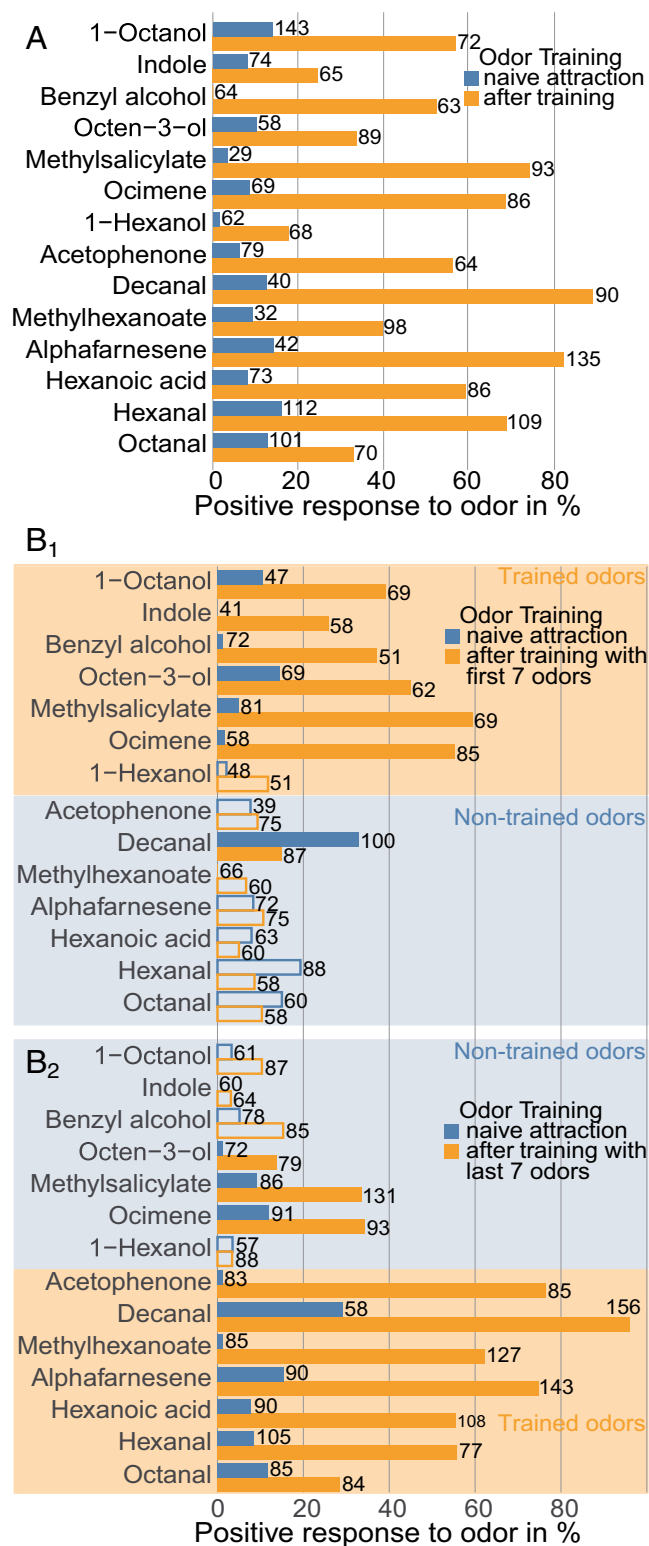


Fig. 2. Memory capacity for food odors. (A) Innate and learned responses after ants have been trained with 14 odors consecutively. Solid bars, learned odors with learning-induced significant differences ($P < 0.05$, test of equal or given proportions); numbers beside bars, number of tested ants. (B_1 and B_2) Tests for generalization. (B_1) Ants were trained with the first seven odors (box with orange background), but later were tested with trained (orange background) and nontrained (blue background) odors. (B_2) Ants were trained with the last seven odors (orange background), but were tested with trained and nontrained odors. For color coding, see Fig. 1B.

or weakly responded to those seven odors that were not included in the training procedure (Fig. 2 B_1 and B_2). We conclude that ants can store multiple food-odor associations simultaneously.

Nest-Odor Memory. As ants obviously can associate odors both with food items and with the nest (6), we next aimed to compare the memory characteristics of nest and food odors. Contrary to the single-trial learning of food odors (Fig. 1C), ants are known to associate an olfactory nest cue only after 10–15 foraging runs (13). Hence, both memories differ regarding memory acquisition time. Furthermore, the ants remembered a food odor almost 1 mo after they had learned it (Fig. 2). Would the ants remember nest odors that they had learned in the past, also? To test this, we performed experiments similar to those described by Steck et al. (6); i.e., we connected a nest entrance to the center of an open aluminum channel (length: 13 m; width: 7 cm; height: 7 cm) and trained the ants to forage at a petri dish with food crumbs (nest-to-feeder distance: 4 m).

We then pipetted 0.4 μL of the test odor (diluted in 20 μL of hexane) of either alpha-Farnesene (Fig. 3 B_1) or Methylsalicylate (Fig. 3 B_2) as an olfactory cue placed directly at the nest entrance. After at least 30 min of training (i.e., ~ 15 training runs), homing ants were caught when they started their return from the feeder and were released in a remote channel with the same odor but without a nest entrance (for a schematic of the training and test paradigm, see Fig. 3A). Under this situation the ants immediately unreleased their path integration vector and afterward started a highly stereotyped nest search. Ants relying on an odor cue exhibit a narrow search, while ants relying only on path integration exhibit a rather wide search (6). The distance of the first six turning points to the center of the search (i.e., the mean of the first six turning points) after an ant had passed the odor for the first time informed us about whether the ant associated the cue with the nest entrance. A second group of ants was trained under identical conditions. However, before testing these ants, the odor cue at the nest was removed, and the training was continued for another 30–120 min.

When testing these ants later with the formerly trained odor in the test channel, the distance between the turning points and the odor was significantly longer and did not differ anymore from ants, that had not been trained to the odor at all (Fig. 3 B_1 and B_2). We conclude that a nest-defining odor loses its behavioral significance for the ant when this odor is not present at the nest anymore. We also conclude that, while food odors are learned quickly and remembered for a long time and multiple food-odor associations can be stored, ants take longer to learn olfactory cues at the nest entrance and ignore former nest odors when these odors are removed from the nest entrance. Obviously, *Cataglyphis* exhibits two distinct olfactory memories for food and nest odors.

Different strategies regarding the memory of food and nest odors might be adaptive for this ant. During its lifetime as a forager [life expectancy for foragers of a closely related *Cataglyphis* ant in a similar habitat was calculated as only 6 d (14)] an ant might experience contact with different food items. While the type of food (dead arthropods such as spiders, cicadas, ant sexuals) is rather unpredictable, during some of our field excursions cicadas and ant sexuals suddenly appeared in the habitat of *C. fortis* in masses. It has been shown that predators [spiders (15), birds (16, 17)], by establishing visual search images of their prey, increase their foraging efficiency. It might therefore be beneficial for *Cataglyphis* foragers to establish an olfactory search image, i.e., learn and store the olfactory characteristics of a given food item, as this might help the ants to efficiently localize more items of the same type afterward. That the ants learned the food-odor association after a single foraging trip is in agreement with learning experiments with leafcutter ants (18, 19), bees (20), cockroaches (21), and mollusks (22, 23). However, the long

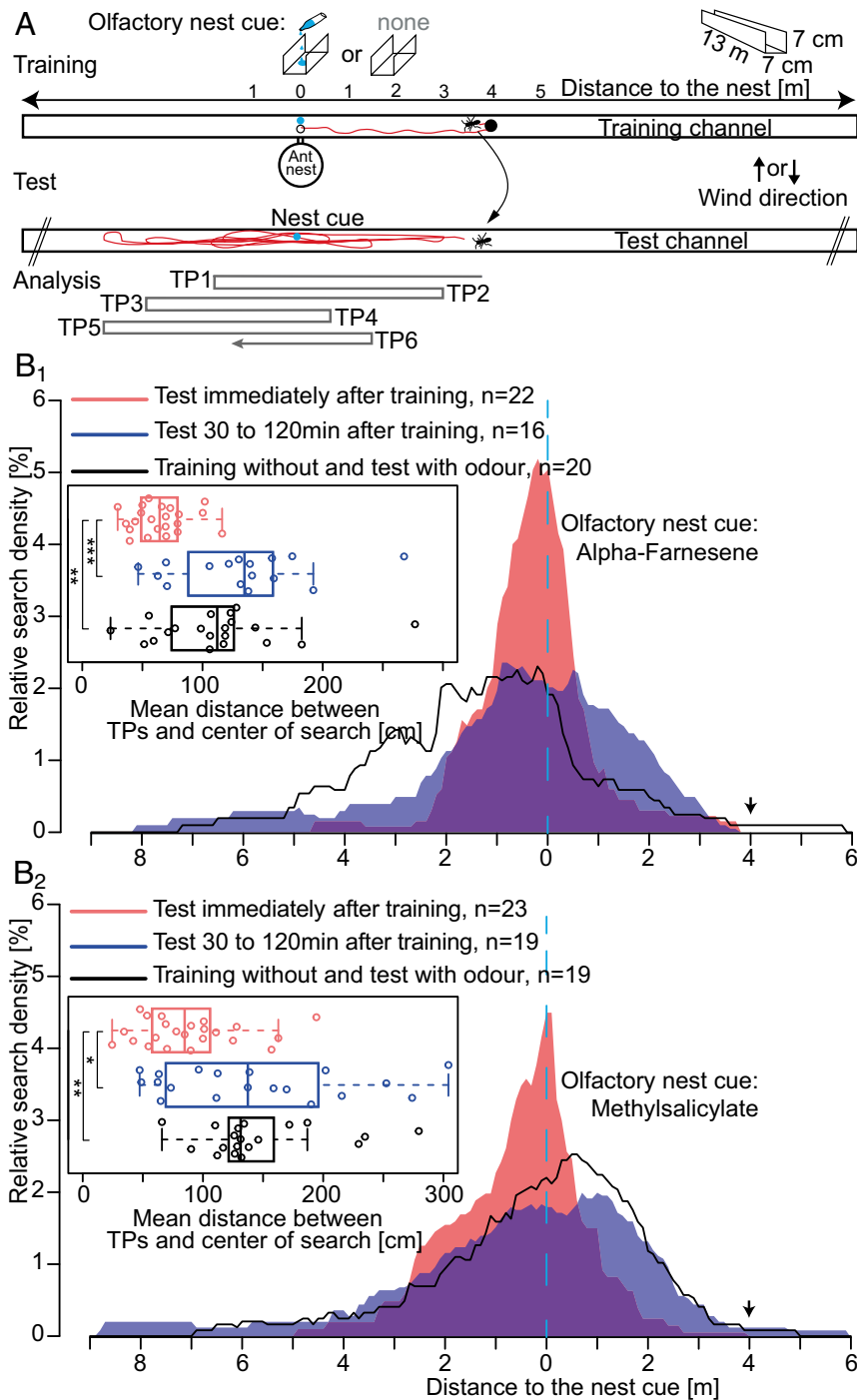


Fig. 3. Learning and memory of olfactory nest cues. (A) Training and test paradigm. (Top) Ants were trained to visit a feeder (solid black dot) 4 m from the nest entrance (open dot) in a linear channel. The exit from the training channel to the nest was marked by a nest-defining odor (blue dot). (Bottom) Example of a homebound run and nest search after displacement into a test channel (zigzag line: for simplicity projected outside the channel). Blue dot: position of fictive nest as defined by the path integrator and of nest-defining cue in test channel. (B₁ and B₂) Search density plots for ants that were tested immediately after training with a nest cue (red) were tested for 30–120 min after the nest cue had been removed from the training channel (blue) or were trained without a cue (black line). (B₁) Experiment with alpha-Farnesene as nest cue. (B₂) Experiment with methylsalicylate as nest cue. (Insets) Analysis of distances between turning points (TPs) and center of search as measurement of search fidelity (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; Kruskal–Wallis with Dunn’s post hoc test).

storage duration of at least 26 d that by far exceeds the foragers’ average life expectancy is surprising. To our knowledge the longest time for olfactory memory retrieval in insects has been reported in leafcutter ants (24). These ants collect leaves to culture a fungus and learn to avoid odors of plants that turned out to be harmful to the fungus. Saverschek et al. (24) observed

that this olfactory avoidance remained even after 18 wk. As the survival of the fungus is crucial for the ant colony, lifetime memory for the smell of plants that turned out to be poisonous seems to be highly adaptive. Lifetime olfactory memory, however, also has been reported for positive cues. Crickets can learn to associate an odor with a sugar reward and remember this

association even after 10 wk (25). Similar to our finding that *Cataglyphis* can remember the association of at least 14 odors, crickets have been further shown to remember a preference in a two-choice test for seven odor pairs (26). Obviously, a lifetime olfactory memory with storage capacity for numerous odors is conserved in insects.

Why, however, do the ants ignore nest odors already 30 min after the cues have been removed from the nest entrance? Contrary to the numerous food items that an ant experiences during its life as a forager, it will always return to the same nest entrance. Hence, there seems to be no need to remember numerous alternating cues or those cues that are not existing anymore. Contrary to our findings, Ziegler and Wehner (27) showed that ants that were first trained to nest-defining visual cues, and then kept in captivity for 20 d, still remembered the nest cues. However, as these ants were kept in captivity until they were tested, they did not experience the nest without cues between training and test. In another study, Bisch-Knaden and Wehner (28) showed that knowledge about nest-defining cues is not erased after a single foraging trip without these cues. As our ants were tested 30 min after cue removal, they most probably performed several foraging runs without the nest-defining odor before they were tested. Therefore, these datasets do not allow the comparison of the robustness of visual and olfactory memory of nest cues. Future studies will reveal whether olfactory nest

cues are remembered as long as visual ones, when the ants are kept in captivity directly after training and hence do not perform any foraging runs without cues before they are tested. This will tell us whether the ants indeed forget about nest odors after a short time or whether they rather always update their knowledge and hence learn after the removal of training odors that the nest is not smelly.

However, obviously it is adaptive for the ant to learn and remember multiple food odors, while the olfactory memory of nest odors is restricted to the last odor learned.

Materials and Methods

Experiments were performed in a saltpan close to the Tunisian village of Menzel Chaker (geographic coordinates, E010.411 N34.956). This area contains more than 900 nests and allowed us to use each ant nest for only one experiment to avoid individual ants becoming trained in different training paradigms. Ants were trained as described above. Each ant was tested only once and was excluded from further experiments. During the training ants were baited with crumbs (crumb size: ~8 mm³) of locally available biscuit cookies. All odorants used for the learning experiments were purchased from commercial sources (Sigma, www.sigma-aldrich.com and TCI America, www.tcichemicals.com/en/us/).

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