Odor Trail through Ant Country

The desert ant's use of its own built-in GPS – consisting of a sun-compass-based path integration system and visual landmarks – in locating its nest is a known phenomenon. Researchers recently ascertained, however, that this system also includes a sense of smell. Even more surprising is the discovery that these animals learn to distinguish between different odors in the nest environment, and use these like a map. **Markus Knaden** and his team at the **Max Planck Institute for Chemical Ecology** in Jena set out to search for clues in ant country.

A desert ant runs through the test channel to the feeder. The walls of the channel prevent the ant from seeing its environment. Under these test conditions, the ant can only navigate using its sense of smell.

TEXT **MARCUS ANHÄUSER**

Kathrin Steck looks a bit tired. That's not surprising – she has been up all night. The biologist has been traveling since 2:00 a.m. Her trip started with a two-hour taxi ride through the darkness from the Tunisian town of Maharès to Monastir. From there, she flew back to Germany, took a train from Leipzig airport to Jena and, instead of going home, went straight to the laboratory at the Max Planck Institute for Chemical Ecology.

The reason for the urgency is the small glass of clear liquid in her backpack. It contains invisible odors extracted from the nest entrance of a desert ant. "These must be cooled as much as possible and then quickly processed," says the 34-year-old post doc, who works with Bill Hansson, an olfaction expert.

Kathrin Steck and her colleagues Cornelia Bühlmann and Markus Knaden want to discover the substances that are contained in the nest odor of desert ants. Does every nest have its own odor? Or do all nests smell the same? What odor emanates from the entrance, a roughly two-centimeter hole in the encrusted ground of a desiccated salt lake? This is the typical habitat of the subject of their research – the feisty, shiny black ant *Cataglyphis fortis*.

A TRAIL OF ODORS FOR THEIR CONSPECIFICS

For two weeks, Steck stood all day, every day, in the blazing sunshine in temperatures of up to 50 degrees Celsius. Bühlmann extracted the odors at the nest entrance while Steck pursued the long-legged ants on their journeys across the salty desert floor.

The olfactory orientation of ants has long been established – even if it is not obvious to everyone. The ants pick up the scent of their food and identify friends and foes in ant country. "When they meet one another or arrive at the nest, they touch one another extensively with their antennae," says Knaden. Once a foraging ant finds a food source, it informs its companions in the nest by means of an attractant, a pheromone trail on the ground. The ants follow this odor trail to the food source and then carry the food back to the nest. The more animals use the trail, the more reinforced it becomes. The result is the familiar ant trails, teeming with insects.

However, the *Cataglyphis* genus does not deploy these types of ant trails at all. "They are lone warriors when it comes to food," says the behavioral ecologist. In the salt lake, only individual ants are seen flitting around, sometimes zigzagging around while foraging, sometimes hot-footing it toward the entrance hole of the nest. "A pheromone trail would quickly disappear in the salt pan, where temperatures reach almost 60 degrees," says Knaden. There are ant species that do leave odor trails in such territories,

Photo: MPI for Chemical Ecology

» *Cataglyphis* occupies a very special place in the world of ant research. It is known as a "navigational genius."

"but these are mostly species that are active in the evenings or at night." This means that they engage in foraging when temperatures cool down.

DESERT ANTS WITH BUILT-IN GPS

In addition, laying an odor trail is beneficial only if there are plentiful food sources to exploit, that is, for ants who are carrying in those seeds that are lying on the ground behind a bush. However, *Cataglyphis fortis* ants have a particular liking for dead insects. And they carry these back to the nest in one piece.

Markus Knaden has spent 14 years researching *Cataglyphis*, a genus with approximately 60 species, of which 6 can be found in the Sahara, its main habitat. *Cataglyphis* occupies a very special position in the world of ant research. It is known as a "navigational genius." This is probably a slight exaggeration; many other animal species have comparable orientation skills, but only a few of them are as well researched as the *Cataglyphis*.

Since the 1960s, scientists have been investigating how ants get their bearings in the desert. Back then, the father of *Cataglyphis* research, neurophysiologist Rüdiger Wehner from the University of Zurich, pitched his tents in the Tunisian town of Maharès and began to examine the navigational ability of these long-legged, elegant desert runners, which are roughly the same size as the common wood ants.

Over the decades, researchers discovered that the insect actually uses several techniques to cross the salt pans in search of rich pickings and return directly to its nest. "They use a path integration system – based on a sun compass for measuring directions and a step counter for measuring distances covered – and landmarks, to find the nest entrance," says Knaden, summarizing the ants' repertoire. Their facet eyes are thus bigger than those of many other ants. They also have very large, mobile antennae.

Yet, until now, hardly anybody had bargained on their sense of smell for guiding them across the hot, hostile salt flats. Not even Knaden. It was only when he arrived in Bill Hansson's department in 2006 that the use of the Formicidae nose as a homing device came to his attention. "I had hoped that the ants had learned not only visual landmarks, but also possibly odor cues," says the Max Planck scientist.

Knaden and Steck first began systematically researching the substances that ants actually smell – or don't smell. In a special laboratory such as the one operated by Bill Hansson they have all the equipment they need to systematically investigate odors. Steck tested a wide variety of substances from the odor libraries and, using 'electroantennograms,' demonstrated how the olfactory nerves in the antennae respond to odors.

The two researchers then concentrated on four odors on which they trained the ants for their experiments: methyl salicylate, decanal, nonanal and indole. "We took environmental odors that are not connected with food, but rather are produced, for example, by plants and are highly volatile at high temperatures," says Knaden. Food odors would have been too attractive. And the ants are not supposed to be attracted by the odor – they are supposed to be able only to identify it and remember it.

RESEARCH ON DRY LAND

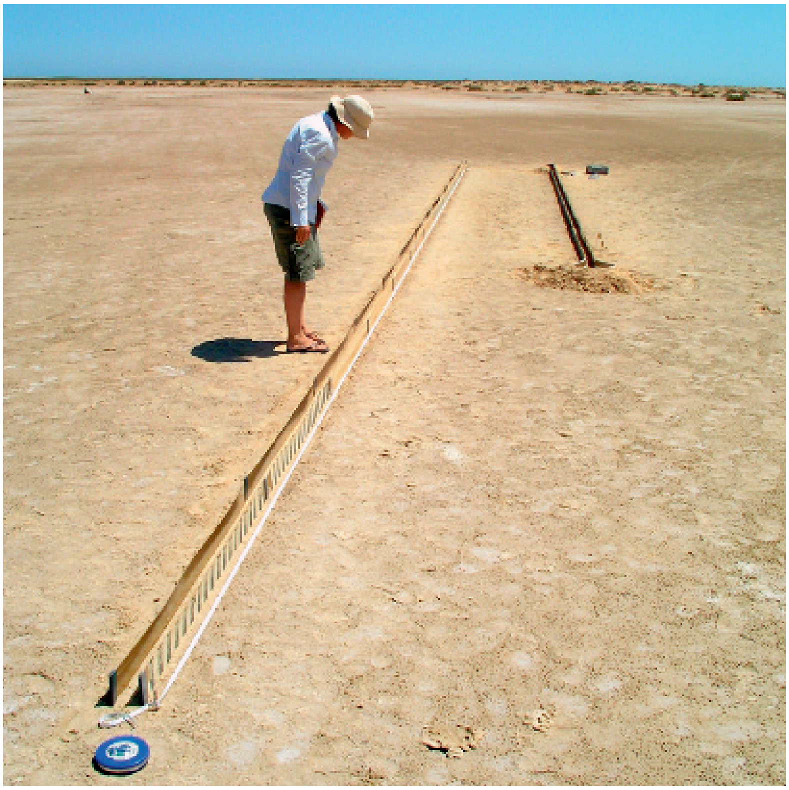
The odor researchers then wanted to find out whether the animals are capable of using these odors for orientation in the area around the nest entrance,

above: Different strokes: The entrances to many of these nests consist of only an inconspicuous, thumbnail-size hole in the ground; other ants of the same species create striking nest mounds. One possible reason could be that returning ants use the mound as a landmark and therefore find their way back faster to the safety of the nest.

right: Kathrin Steck marks individual ants. Using checklists, the researchers can see how often the individual animals have already performed the relevant training.

Photos: MPI for Chemical Ecology (2)

for example, in the same way that they use visual landmarks, such as a bush or a stone, to pinpoint the small entrance hole. The area studied is a desiccated salt lake in Tunisia, six kilometers long, three kilometers wide, about one hour



above: The test site shows the salt pan in the background. Several such salt pans are dotted throughout Tunisia. This one measures approximately three by six kilometers.

below: In temperatures of up to 50 degrees Celsius, Kathrin Steck stands in the blazing sunshine and tracks the ants' search runs in the channel

from the coast and the town of Maharès, where the scientists were staying. The mecca of *Cataglyphis* research. Rüdiger Wehner discovered the coastal area in 1968 and set up his base there. Markus Knaden, who completed his doctorate with Wehner and worked with him as a post doc, also began working with desert ants in this area.

When he moved to Bill Hansson's department in Jena in 2006, he initially had some bad luck: his ant population in Maharès was almost decimated. "We assume that it had something to do with the major use of pesticides in the nearby olive tree plantations," says

Photos: MPI for Chemical Ecology (2)

Large test fields painted on the ground allow the researchers to record the ants' extensive search runs. This method was established by Rüdiger Wehner, the father of *Cataglyphis* research in the late 1960s, and remains a proven technique to this day.

Photo: MPI for Chemical Ecology

Knaden. He and Steck were thus forced to search for a new population – and they found one at the said salt lake, 75 kilometers away. Knaden saw the advantage of their salt flat immediately on their first visit: "The nests there were literally exploding with ants." The nests were much bigger than those in Maharès, which is beneficial for carrying out spot checks and performing statistical evaluations.

SWITCHING TO LANDMARK ORIENTATION

Even though research conditions are better at the salt pan, the scientists from Jena don't want to move there lock, stock and barrel; their base camp continues to be in Maharès. "The coast is simply nicer," says Knaden. Better climate, a fishing port, a few restaurants. And the locals know the researchers, as they spend entire days standing in the scorching heat, watching ants. "We are the crazy people who come every year with new equipment and a jeep," says Knaden, smiling.

The scientists set up their experiment in the salt lake, which contains water only during the winter. For their first experiments, they needed only a round, five-millimeter-thick plastic and aluminum panel with a one-centimeter hole in the middle, which was placed over a nest entrance. Using this method, Bühlmann and Knaden were able to demonstrate that odor is actually a factor in helping the ants find the entrance when they return to the nest: once the sun compass and path integrator have brought the ant to within a meter of the nest, the animal then switches to landmark orientation. "The ant cannot actually see the hole itself, so it remembers a nearby stone or bush," says Knaden.

In the experiment, the researchers can exclude such information by covering the nest entrance with the panel. The ants then have no clues as to where the hole is. Nevertheless, they find it – because the nest has a particular smell. The researchers know this because each time the ants on the panel reach the wind vane, which indicates the wind direction in front of the entrance, they turn and scurry to the hole. On the leeward side, however, they wander around, searching, even if they are only a few centimeters from their own home. A first important finding.

FORAGING TRIP IN THE ALUMINUM CHANNEL

For the subsequent, more complex tests, the field researchers needed aluminum channels measuring two meters long and seven centimeters wide, U-shaped and open at the top, which are placed on the ground. These channels are connected via a tube with an upturned bucket positioned over the

top: The researchers paint several spots of color on the abdomen of each ant so that they can distinguish between the ants on the training runs.

bottom: Markus Knaden and Kathrin Steck look at the experimental data on the computer.

entrance hole to the *Cataglyphis* nest. The scientists use this test channel to hide all visual cues and the horizon from the ants' field of vision: "The animals scuttle out of their nest under the bucket. They go through the tube and arrive at the side wall of the aluminum channel through a tiny hole. There they look for the food that we have laid out and come back to this hole," says Markus Knaden, describing the experiment.

Would the ants also be guided by an odor that does not emanate from the hole itself, but originates from its environment? When Knaden and Steck examined this issue in their channel experiment, they made an exciting discovery that once again illustrates the complex sensory abilities of these small insects. But something first had to go wrong before they realized the ants' abilities.

The hole in the side wall of the aluminum channel is so small that it is very difficult for the insects to find unless it is marked. "It takes them forever to find it," says Markus Knaden. They keep running by the hole. However, they find it immediately if the researchers paint a black spot, for example, on the wall opposite the hole. They evidently use the spot as a landmark and link this to the location of the entrance. Would the same thing work with odors?

Because Knaden and Steck were not sure whether even one (and if so which) of the four odors identified in the laboratory – methyl salicylate, decanal, nonanal and indole – could ultimately be important, they drizzled the four substances separately to form an odor square in the training channel in front of the entrance hole. The ants learned very quickly that their entrance could be found in this odor field. When they returned after their foraging trip in the aluminum channel, they just searched inside the square for the entrance and found it immediately.

A MAP OF VARIOUS ODORS

Unfortunately, the control experiments yielded no conclusive results: the researchers offered an odor field again, but this time without an entrance. The ants did remember the odor and initially systematically frequented the odor field, "but each time they hurried out again over the lines of the square," says

Photos: MPI for Chemical Ecology (2)

The Jena-based odor specialists set up camp in the Tunisian coastal town of Maharès. It has been the mecca for *Cataglyphis* research since 1968, when Swiss zoologist Rüdiger Wehner discovered the town and decided to use it as a base for his research.

Photo: MPI for Chemical Ecology

Knaden, describing the ants' behavior. And then the researcher realized they had made a serious error: "We had mixed up the odors. In the control channel, they were in the wrong locations in relation to where the entrance hole was supposed to be."

The ants were visibly confused by this. When Kathrin Steck arranged the odors in the same order in the odor square as they were in the test channel in front of the entrance hole, everything was back to normal again: the ants looked for the nest entrance only within the odor field as they had learned to do previously. "We had not expected the odor array to be important for orientation purposes," says Knaden.

The animals clearly navigate not only on the basis of one or two odors in the mix and link these in their memory with the entrance hole, but they actually remember the individual locations of the odor drops. Or, in a nutshell: *Cataglyphis* smells in 3-D – a completely surprising and conclusive result that nobody had reckoned with after 40 years of research on *Cataglyphis'* navigational skills.

In order to be able to orientate themselves spatially in such an olfactory landscape, the animals need two separate sensory inputs – as they do for seeing – which means that they rely on both of their antennae. According to Knaden, "Ants that only had one antenna could no longer find their way around." This type of stereo smelling among animals has been known for a long time; researchers are sure that rats and even humans use such a method. What they did not realize is that ants also have this facility.

Yet a number of questions remain unanswered: Which landmarks are more important for the ants – visual or olfactory? And does the distribution of odors at the nest entrance in the natural environment really play a role? After all, the experiment with the aluminum channels is an artificial arrangement. It is possible that the ants may use such an odor map less at the nest entrance but more when foraging. A typical smell of home could also be enough for an ant to find its own nest.

Following his latest field trip, Markus Knaden is somewhat skeptical about this: "It looks like there's one general nest smell," he says. Steck, Bühlmann and he already suspect what the smell – or perhaps one should say stink – is: carbon dioxide, or stale air. "The animals breathe in the nest, food is consumed there – all of this causes the level of CO_2 in the air to rise," explains Knaden. That could account for the nest odor, but he isn't sure.

To find out exactly, Cornelia Bühlmann and Kathrin Steck collected the nest odors mentioned at the start of this article using an aspirator. Here in Jena, Steck puts the odors back into the refrigerator, and now there is just one more thing to do: go home and go to bed. ◂

GLOSSARY

Electroantennograms (EAG)
Electrophysiological discharge of so-called summating potential (superimposed action potential of several odor receptors) over the entire antenna when an odor is applied.

Path integrator
Desert ants use a path integration system. This consists of a compass, to determine where they are heading, and an odometer to measure the distances they have traveled. The compass uses a light phenomenon in the sky that is invisible to the human eye and stretches across the entire celestial hemisphere as a large-scale polarization pattern. *Cataglyphis* measures the distance it has traveled by integrating the steps it has taken on the journey. Rüdiger Wehner and his colleagues discovered this by artificially lengthening or shortening the length of the desert ants' legs before they returned to the nest from a feeder. The result was that animals manipulated in this way over- or underestimated the return distance by exactly the amount by which the animals had modified their step length due to the change in the length of their legs.