Starting foraging life: Early calibration and daily use of the navigational system in *Cataglyphis* ants

Start in den Außendienst: Zur anfänglichen Kalibrierung und alltäglichen Nutzung des Navigationssystems in *Cataglyphis*-Ameisen

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Summary

Cataglyphis ants are famous for their navigational abilities. They live in hostile habitats where they forage as solitary scavengers covering distances of more than hundred thousand times their body lengths. To return to their nest with a prey item – mainly other dead insects that did not survive the heat – *Cataglyphis* ants constantly keep track of their directions and distances travelled. The navigational strategy is called path integration, and it enables an ant to return to the nest in a straight line using its home vector. *Cataglyphis* ants mainly rely on celestial compass cues, like the position of the sun or the UV polarization pattern, to determine directions, and they use an idiothetic step counter and optic flow to measure distances. In addition, they acquire information about visual, olfactory and tactile landmarks, and the wind direction to increase their chances of returning to the nest safe and sound. *Cataglyphis*' navigational performance becomes even more impressive if one considers their life style. Most time of their lives, the ants stay underground and perform tasks within the colony. When they start their foraging careers outside the nest, they have to calibrate their compass systems and acquire all information necessary for navigation during subsequent foraging.

This navigational toolkit is not instantaneously available, but has to be filled with experience. For that reason, *Cataglyphis* ants perform a striking behavior for up to three days before actually foraging. These so-called learning walks are crucial for the success as foragers later on. In the present thesis, both the ontogeny and the fine-structure of learning walks has been investigated. Here I show with displacement experiments that *Cataglyphis* ants need enough space and enough time to perform learning walks. Spatially restricted novices, i. e. naïve ants, could not find back to the nest when tested as foragers later on. Furthermore, ants have to perform several learning walks over 1-3 days to gain landmark information for successful homing as foragers. An increasing number of feeder visits also increases the importance of landmark information, whereas in the beginning ants fully rely on their path-integration vector.

Learning walks are well-structured. High-speed video analysis revealed that *Cataglyphis* ants include species-specific rotational elements in their learning walks. Greek *Cataglyphis* ants (*C. noda* and *C. aenescens*) inhabiting a cluttered pine forest perform voltes, small walked circles, and pirouettes, tight turns about the body axis with frequent stopping phases. During the longest stopping phases, the ants gaze back to their nest entrance. The Tunisian *Cataglyphis fortis* ants inhabiting featureless saltpans only perform voltes without directed gazes. The function of voltes has not yet been revealed. In contrast, the fine structure of pirouettes suggests that the ants take snapshots of the panorama towards their homing direction to memorize the nest's surroundings.

The most likely hypothesis was that *Cataglyphis* ants align the gaze directions using their path integrator, which gets directional input from celestial cues during foraging. To test this hypothesis, a manipulation experiment was performed changing the celestial cues above the nest entrance (no sun, no natural polarization pattern, no UV light). The accurately directed gazes to the nest entrance offer an easily quantifiable readout suitable to ask the ants where

they expect their nest entrance. Unexpectedly, all novices performing learning walks under artificial sky conditions looked back to the nest entrance. This was especially surprising, because neuronal changes in the mushroom bodies and the central complex receiving visual input could only be induced with the natural sky when comparing test animals with interior workers. The behavioral findings indicated that *Cataglyphis* ants use another directional reference system to align their gaze directions during the longest stopping phases of learning-walk pirouettes. One possibility was the earth's magnetic field. Indeed, already disarraying the geomagnetic field at the nest entrance with an electromagnetic flat coil indicated that the ants use magnetic information to align their looks back to the nest entrance. To investigate this finding further, ants were confronted with a controlled magnetic field using a Helmholtz coil. Elimination of the horizontal field component led to undirected gaze directions like the disarray did. Rotating the magnetic field about 90°, 180° or -90° shifted the ants' gaze directions in a predictable manner. Therefore, the earth's magnetic field is a necessary and sufficient reference system for aligning nest-centered gazes during learning-walk pirouettes. Whether it is additionally used for other navigational purposes, e. g. for calibrating the solar ephemeris, remains to be tested. Maybe the voltes performed by all *Cataglyphis* ant species investigated so far can help to answer this question.

Zusammenfassung

Cataglyphis-Ameisen sind für ihre Navigationsfähigkeiten berühmt. Sie bewohnen lebensfeindliche Regionen in denen sie einzeln und über weite Strecken Futter suchen müssen. Um mit Beute (meist ein totes Insekt, das die große Hitze nicht überlebt hat) zu ihrem Nest zurückzukehren, bedienen sie sich einer Navigationsstrategie, die als Wegintegration bezeichnet wird. Dabei müssen die Ameisen die zurückgelegten Distanzen messen und jeden Richtungswechsel registrieren, um schließlich in gerader Linie nachhause zurückkehren zu können. Als Kompass nutzen sie Himmelsinformationen, wie den Stand der Sonne oder das UV-Polarisationsmuster, und für die Distanzmessung verwenden sie einen inneren Schrittzähler sowie optischen Fluss. Außerdem nutzen sie alle weiteren Informationen, die hilfreich sein könnten, um sicher zum Nest zurückzukehren. Dazu gehören visuelle, olfaktorische und taktile Landmarken sowie die Richtung des Windes. Die Navigationsleistungen von *Cataglyphis*-Ameisen sind insbesondere dann bemerkenswert, wenn man sich bewusst macht, dass sie die meiste Zeit ihres Lebens unter der Erde verbringen. Dort übernehmen sie Aufgaben im Nest bis sie dann schließlich alt genug sind, um draußen Futter zu suchen. Dann müssen sie ihre Kompasssysteme kalibrieren und alle Informationen lernen, die sie für eine erfolgreiche Futtersuche brauchen.

Dieses sogenannte Navigations-Toolkit steht den Ameisen nicht automatisch zur Verfügung, vielmehr müssen sie es mit eigener Erfahrung füllen. Dafür nutzen sie die ersten ein bis drei Tage außerhalb des Nestes. Während dieser Zeit suchen sie kein Futter, sondern vollführen sogenannte Lernläufe. Lernläufe sind unabdingbar, um später als Fourageur erfolgreich zu sein. In der vorliegenden Doktorarbeit wurde sowohl die zeitliche und räumliche Entwicklung der Lernläufe als auch deren Feinstruktur untersucht. Mit Versetzungsexperimenten konnte ich zeigen, dass Ameisen genügend Zeit und Raum brauchen, um Lernläufe durchzuführen. Wurden Neulinge während ihrer Lernläufe räumlich eingeschränkt, so konnten sie nicht zum Nest zurückfinden, wenn sie als erfahrene Fourageure getestet wurden. Außerdem brauchen die Ameisen ein bis drei Tage Zeit, um ein Landmarkenpanorama zu erlernen, das sie dann später erfolgreich zur Landmarkenorientierung nutzen können. Eine größere Anzahl an Besuchen am Futterplatz erhöht die Wichtigkeit von Landmarkeninformation für die Ameisen, die anfangs nur ihren Wegintegrator nutzen.

Lernläufe weisen eine beeindruckende Struktur auf. Mit High-Speed-Videoaufnahmen konnte gezeigt werden, dass *Cataglyphis*-Ameisen artspezifische Drehungen während der Lernläufe vollführen. Die griechischen *Cataglyphis*-Ameisen (*C. noda* und *C. aenescens*) leben in einem Pinienwald, der ihnen ein vielfältiges und landmarkenreiches Panorama bietet. Ihre Lernläufe beinhalten zwei Drehungsformen, nämlich sogenannte Volten (kleine gelaufene Kreise) und Pirouetten (enge Drehungen um die eigene Körperachse mit häufigen Stoppphasen). Während der längsten Stoppphase einer Pirouette schauen die Ameisen zurück in die Richtung ihres Nesteingangs, obwohl sie ihn nicht direkt sehen können. Die tunesischen *Cataglyphis*-Ameisen (*C. fortis*) leben auf einem landmarkenarmen Salzsee. Sie vollführen nur Volten und machen keine Pirouetten während ihrer Lernläufe. Die Funktion von Volten ist

noch unbekannt, wohingegen die Feinstruktur der Pirouetten die Vermutung nahelegt, dass die Ameisen sogenannte Schnappschüsse von der Umgebung ihres Nestes machen, um dorthin zurückkehren zu können.

Es schien wahrscheinlich, dass die Ameisen ihren Wegintegrator nutzen, um ihre Blickrichtungen zum Nest auszurichten. Während der Futtersuche bekommt der Wegintegrator seine Richtungsinformationen vom Himmelskompass. Daher wurde ein Experiment geplant und durchgeführt bei dem die Himmelsinformationen über dem Nesteingang manipuliert wurden (keine Sicht auf die Sonne, kein natürliches Polarisationsmuster oder kein UV-Licht). Die nestzentrierten Blickrichtungen der Ameisen ermöglichen es relativ einfach zu überprüfen, ob die Ameisen die Position des Nesteingangs kennen. Überraschenderweise schauten die Ameisen unter allen Bedingungen weiterhin zurück zum Nesteingang. Dies war insbesondere bemerkenswert, da die Himmelsmanipulation neuronale Veränderungen in den Pilzkörpern und dem Zentralkomplex (das sind Regionen im Gehirn der Ameisen, die visuelle Informationen verarbeiten) bewirkten bzw. diese verhinderten. Nur unter natürlichen Bedingungen, also bei freiem Blick auf die Sonne, gab es Unterschiede auf neuronaler Ebene zwischen den Testtieren und den Innendiensttieren, die als Kontrolle dienten. Die Ergebnisse des Verhaltensversuchs deuteten darauf hin, dass die Ameisen ein anderes direktionales Referenzsystem nutzen, um ihre Blickrichtungen zu kontrollieren. Eine Möglichkeit war das Erdmagnetfeld. Tatsächlich zeigte schon die experimentelle Streuung des Magnetfelds am Nesteingang mittels einer elektromagnetischen Flachspule, dass die Ameisen tatsächlich Magnetinformationen nutzen, um ihre Blicke auszurichten. Die Blickrichtungen während der längsten Stoppphasen waren nicht mehr zum Nesteingang gerichtet. Um dies genauer zu untersuchen wurden die Ameisen mit dem kontrollierten Magnetfeld einer Helmholtzspule konfrontiert. Die Eliminierung der Horizontalkomponente des Magnetfelds bewirkte wiederum, dass die Ameisen nicht zum Nesteingang zurückschauten. Wurde die Horizontalkomponente jedoch um 90°, 180° oder -90° gedreht, so folgten die Blickrichtungen der Ameisen dieser Drehung voraussagbar im selben Winkel. Dies zeigt, dass das Erdmagnetfeld tatsächlich das Referenzsystem für die Ausrichtungen der Blicke während der Lernlaufpirouetten darstellt. Ob es auch noch anderen Navigationszwecken, wie beispielsweise der Kalibrierung der solaren Ephemeris dient, muss zukünftig überprüft werden. Vielleicht können die Volten, die alle bisher untersuchten *Cataglyphis*-Ameisen ausführen, dabei helfen diese Frage zu beantworten.

1 General introduction

1.1 Preface

When introducing the research topic of my PhD thesis “How do desert ants learn to navigate?” to non-biologists, the first reaction I get usually is “you are an ant expert!? I have a problem with ants in my garden [house/kitchen/any other place conceivable]”. After explaining that I, unfortunately, do not know any secret tricks to convince six-legged intruders to live somewhere else, I usually highlight the fact that ants fascinating creature with astonishing abilities. They are social insects that, like humans, live in complex social societies (Hölldobler and Wilson, 1990). Furthermore, they accomplish fascinating tasks like e. g. farming. For example, leaf cutting ants feed on fungi that they culture by providing cut leaves and grasses (ibid. p. 596ff). Other ant species breed and defend aphids like humans rear cattle (ibid. 527ff).

My research objects – *Cataglyphis* ants – are not farmers, but they are to no lesser account impressive. *Cataglyphis* ants are famous navigators (for reviews: Ronacher, 2008; Wehner, 2003; Wehner, 2008; Wehner and Rössler, 2013). They are strictly diurnal and mainly guided visually. Like honey bees, and unlike many other ant species, these thermophilic ants use path integration for navigating during their extensive foraging excursions. This means that they have to continuously keep track of their directions and distances traveled in order to return safe and sound to their nest entrance that is a tiny hole in the ground invisible from the ants’ perspective. The facts that the ants use the sun and other celestial information not seen or used by humans for determining their homing direction, and that they can count their steps is acceptably impressive for laymen.

Moreover, the navigational performance of *Cataglyphis* ants becomes even more impressive if we consider the consequences of their age-related division of labor. Most of the time of their lives, the ants live within the nest underground and perform interior duties like serving as a food storages, so-called repletes, feeding the queen or the brood, cleaning and digging (Schmid-Hempel and Schmid-Hempel, 1984). Only after some weeks, the ants leave their nest for the first time to become foragers outside the nest. This task is very risky and only few foragers survive longer than a week. *Cataglyphis* ants still spent up to three days exploring the nest’s surroundings before they head out on first foraging trips. Why on earth, one might ask, do they waste so much time circling around the nest entrance?

This question marks the starting point of my research project, and as the present thesis will show, *Cataglyphis* ants do not wastefully meander around, but the naïve ants, so-called novices, carry out a distinct and important behavior – they perform learning walks. These walks are short trips around the nest entrance during which the novices never bring back food items to the nest; they acquire all information needed for foraging and calibrating their compass systems. Especially during transitions from one phase of life to another, animals face novel challenges and have to adapt and react to their environment in new manners. Changes in behavior, neuronal architecture and physiology are necessary in order to meet these challenges successfully. One prominent transition in the life of *Cataglyphis* ants is the time when they leave the nest to become foragers. Instead of continuing their indoor tasks underground in the darkness of the colony's nest, henceforth, individual foragers search for single food items in the nest's surroundings. During their extensive foraging excursions, they are mainly guided visually.

Cataglyphis ants are prime examples of solitary central place foragers, and they bring along an impressive toolkit for navigation, which will be introduced in more detail in the next section. Decades of research scrutinizing the navigational strategies and capacities of *Cataglyphis* ants has left the questions open how ants calibrate their compass systems at the beginning of their foraging career and how they acquire all information necessary for successful navigation as foragers later on. Here, the present PhD project has started by investigating spatiotemporal features of learning walks which *Cataglyphis* ants perform during their transition from interior to exterior workers and by revealing the importance of learning walks for the foragers' navigational abilities.

1.2 *Cataglyphis'* toolkit for navigation

Cataglyphis (Hymenoptera: Formicidae) ants (Foerster 1850) inhabiting the southern part of the Palearctic region are famous for their navigational performances (for reviews: Ronacher, 2008; Wehner, 2003; Wehner, 2008; Wehner and Rössler, 2013). As typical central place foragers, they use a variety of navigational strategies to navigate efficiently in their hostile habitats (figure 1.1A, B). Before investigating how the ants learn to navigate, it is crucial to understand how diverse the navigational strategies of foragers are, and how the cues used for navigation differ in respect of sensory modality, temporal and spatial availability, and reliability in general.

Cataglyphis' main navigational strategy is path integration (also called "dead reckoning" or "vector navigation") for which two pieces of information are needed, namely information about direction and about distance (Müller and Wehner, 1988). Already a century ago Felix Santschi found out that ants use visual information provided by the sun and other celestial cues – the latter unbeknownst to Santschi – to determine their direction (for a review on early ant research by Santschi: Wehner, 1990). Only after the crucial experiments in honeybees by von Frisch it became known that insects can use the polarization pattern of the sky for navigation (von Frisch, 1949). Today it is known that a specialized set of ommatidia in the so-called dorsal rim area detects the UV polarization pattern (for a review on polarization vision: Wehner, 2014). Sun compass and polarization compass interact with each other; informational direction can be transferred, and neither of both completely dominates over the other one (Lebhardt and Ronacher, 2013; Lebhardt and Ronacher, 2015; Wehner and Müller, 2006).

In contrast to the celestial compass systems discovered in the 20th century, only at the beginning of this millennium the ants' main mechanism for measuring distances was discovered. Elegant experiments with ants "on stilts and stumps" revealed that *Cataglyphis* ants use an idiothetic step counter for navigation (Wittlinger et al., 2006; Wittlinger et al., 2007). In addition, *Cataglyphis* ants can use the optic flow to measure the distances traveled (Pfeffer and Wittlinger, 2016; Ronacher and Wehner, 1995). The ant's path integrator is continuously updated during foraging excursions and reset to zero when the ant returns to the nest (Knaden and Wehner, 2006). However, if the ant does not find the nest entrance at the expected position – for example due to displacement by an experimenter – it is not completely lost, but starts

Figure 1.1: Habitats and *Cataglyphis* ants. (A) Saltpan in Tunisia. (B) Pine forest in Greece. (C) *C. fortis* forager at a piece of melon. (D) *C. noda* ants at the nest entrance.

its backup strategy, namely a systematic search (Müller and Wehner, 1994; Wehner and Srinivasan, 1981). The ants even adapt their search loops to the increasing uncertainty caused by long foraging distances (Merkle and Wehner, 2010; Merkle et al., 2006).

Path integration is especially important for ants inhabiting featureless habitats (for a review: Cheng et al., 2014), like *Cataglyphis* fortis living on North African saltpans (figure 1.1C). However, both *C. fortis* ants and their relatives inhabiting more cluttered environments, for example the Greek Cataglyphis noda (figure 1.1D), make heavy use of any other cue available. *Cataglyphis* ants use visual (e. g. Andel and Wehner, 2004; Bisch-Knaden and Wehner, 2003; Bregy et al., 2008; Collett, 2010; Collett et al., 1992; Knaden and Wehner, 2005; Wehner et al., 1996; Ziegler and Wehner, 1997), olfactory (e. g. Buehlmann et al., 2013; Buehlmann et al., 2014; Buehlmann et al., 2015; Steck et al., 2009), tactile (Seidl and Wehner, 2006), and even vibrational and magnetic landmarks (Buehlmann et al., 2012) as well as the wind direction (Müller and Wehner, 2007) to find their ways during foraging and homing.

All information gained for navigation has to be processed, stored, retrieved and re-adjusted in the ant's brain, which weighs only about 0.1 mg (Wehner et al., 2007). Despite its tiny size, the *Cataglyphis* brain is impressive from a structural as well as a functional point of view (for reviews: Wehner, 2003; Wehner and Rössler, 2013). As said before, *Cataglyphis* ants rely on different navigational systems during foraging which can operate independently from each other. This might indicate that the information used is also processed in different parts of the brain. The mushroom bodies – higher-order centers for sensory integration, learning and memory – and the central complex – a center in the middle of the insect brain mainly processing visual information relevant for orientation and navigation – have been identified as promising target regions for neurobiological investigations (for a review: Wehner and Rössler, 2013). Changes in *Cataglyphis'* mushroom bodies' synaptic complexes (microglomeruli) could be induced by light exposure (Stieb et al., 2010; Stieb et al., 2012), i. e. the sensory change ants' have to undergo when leaving the nest. Recently, the polarization pathway transferring the polarization information from the dorsal rim area in the eye via several stages to the central complex has been described in detail in *Cataglyphis* (Schmitt et al., 2016). This brief look into *Cataglyphis* brain research already reveals the complexity of the neuronal basis underlying the ants' astonishing navigational performance. *Cataglyphis* ants exhibit both behavioral flexibility and neuronal plasticity which together enable the ants' extreme lifestyle with rapid changes in sensory input and range of tasks.

1.3 Learning walks of novices: Transition from interior to exterior worker

The impressive toolkit for navigation is not completely available from day one outside the nest, but has to be filled by the ants with experience. Most of their life, *Cataglyphis* ants live underground and pass through different developmental stages (figure 1.2) in the darkness of their nest (described in detail for *Cataglyphis bicolor*: Schmid-Hempel and Schmid-Hempel, 1984). The first stage in the life of an ant is very short (about 24 hours). The so-called "callow" is easily recognizable, because it has a pale color. Afterwards, the ant becomes a "replete" (interior I worker) and its task is to store food. Repletes sit motionless with swollen gasters in the nest chambers. In contrast, interior II workers are very busy and perform a variety of tasks to take care of the brood and the queen and to maintain the nest. At some point interior II workers will start to dig or remove waste outside the nest so that they get exposed to the outside world. It is at that time, after about one month of underground life, that they start their foraging career which only lasts about a week (for forager survival, see fig. 29.1 in Wehner and Rössler, 2013).

The short life-time as foragers of *Cataglyphis* ants makes it especially important to adapt as soon as possible to the novel situation in order to accomplish the new tasks efficiently. However, *Cataglyphis* ants use up to three days to perform well-structured learning walks during which they do not bring home any food items (Stieb et al., 2012; Wehner et al., 2004). Instead the novices meander around the nest entrance and frequently include turns into their paths. With increasing experience, i. e. with an increasing number of explorative excursions around the nest entrance, the duration and the lengths of the walks increase as well. After exploring all directions around the nest sufficiently, ants eventually bring back a food item. As foragers they perform long outbound trips during which they stick to one foraging direction ("sector fidelity"). It is for this short time of successful foraging that they have to calibrate their compass systems and acquire a variety of information. This astonishing time investment in an intensive period of learning is the starting point of the present doctoral thesis.

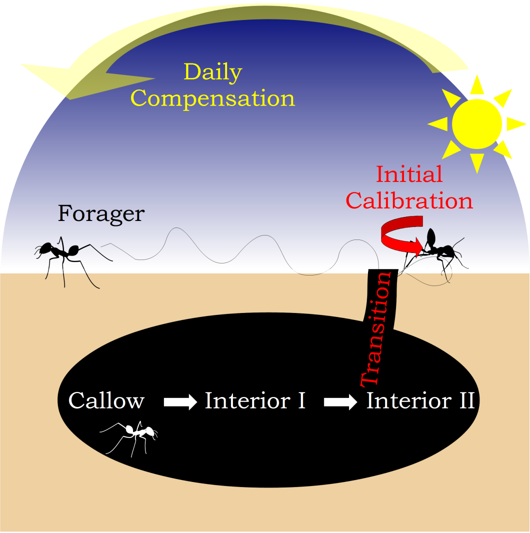

Figure 1.2: Ontogeny of *Cataglyphis* ants. The ants live underground for most time of their lives passing through different stages (callow, interior I, interior worker II). When leaving the nest, they perform learning walks to calibrate their compass systems and acquire all information needed for successful foraging. Foragers have to time-compensate the solar ephemeris to make use of their path integration system. For details see text.

1.4 Thesis outline

Within the past years, I performed several experiments investigating the learning phase of *Cataglyphis* ants after their first appearance outside the nest. I identified several challenges for the naïve ants, and I am deeply impressed by their navigational performance. In the following paragraphs, these challenges will be outlined, and in the following chapters the ants' solutions will be presented in detail.

When I started my PhD thesis, the first question I asked was which reference system the Tunisian desert ants (*C. fortis*) use for initial calibration of their celestial compasses (chapter 2). The problem with using the sun and the other sun-linked cues in the sky, i. e. the UV polarization pattern and the spectral gradient, is that they change their position over the day. Furthermore, this movement, which can be described as the solar ephemeris, is not linear, and it is dependent on the geographical position (latitude) as well as on the time of the year (season). For these reasons, information about the sun's daily course cannot be innately known by individual ants. However, relying on celestial information requires time-compensation (figure 1.2). This is a task which has been shown to be successfully performed by experienced foragers (Wehner and Lanfranconi, 1981). But how are they able to do so? Our hypothesis was that *Cataglyphis* ants use the horizon as an earthbound reference system to calibrate the solar ephemeris. To test this hypothesis, we setup a 10 m-channel on wheels which was supposed to be rotated with the sun's position in a way that the ants would never experience the movement of the sun. Unfortunately, the crucial experiment with the rotating channel could never be performed due to the political situation in Tunisia. However, the control experiments performed with the stationary channel indicate that the ants need space to perform learning walks – a crucial information very helpful for new experimental approaches in the following field seasons.

Parallel to the (not) rotating channel experiment, we investigated the learning walks performed by novices and recorded high-speed videos for detailed analysis. Before the experiment's start, I was told that the ants frequently turn to look back to the nest entrance – as do other desert ants like *C. bicolor* and *Ocymyrmex robustior* (Müller and Wehner, 2010; Wehner et al., 2004) – but we were not able to detect looks back to the nest entrance (Grob, 2014). It took us quite some time to understand that *C. fortis* ants do not look back to the nest entrance during learning walks. Actually, I only realized that it were not my limited observation skills, but a real difference in behavior, when first watching *C. noda* ants performing learning walks in Greece. In contrast to their North African relatives inhabiting saltpans, these ants clearly look back to the nest entrance. This discovery made me realize for the first time that being forced to switch from one field site to another might not only be troublesome, but that it can also be very rewarding. The comparison of learning walks performed by different *Cataglyphis* species living in Tunisia and Greece led to the exciting finding that environmental constraints may cause distinct differences in learning-walk behavior (chapter 3; Fleischmann et al., 2017). In this sense, evolution worked out for us as an experiment. It has been suggested that the looks back to the nest entrance are performed to take so-called snapshot of the nest's surroundings (Graham et al., 2010; Müller and Wehner, 2010). However, *C. fortis* ants can learn an artificial landmark panroama around the nest entrance without performing any looks back to the nest. In a displacement experiment we investigated the ontogeny of learning walks and the ability of ants to find the nest entrance position guided by landmarks at different stages of experience (Christian, 2015). This study revealed that the learning walks of *C. fortis* ants are spatially and temporally well-organized, and with that crucial for the acquisition of landmark information. Only after performing several learning walks, test ants were able to pinpoint the fictive nest entrance position after displacement to the test field (chapter 4, Fleischmann et al., 2016).

The Greek ants inhabit a much more cluttered environment, i. e. a pine forest, so that learning landmarks means to acquire detailed information about the panorama which changes drastically after moving only a few meters. Since I had to come up with a new experiment suitable to be performed in the forest, I decided to investigate the interaction of path integrator and landmark guidance at different stages of experience (chapter 5). When first starting the experiment, we wanted to test ants with a different number feeder visits, but it turned out that the pine forest ants were not as easily trained to a feeder as saltpan ants. To force them to come to the feeder, we built a glass channel which guided them to our feeder. When testing these ants, we found out that the ants that made only one feeder visit before the test followed the path integrator completely when being released. In contrast, ants that had visited the feeder at least ten times, stopped earlier to follow their path integrator and started to search systematically for the nest. They did not know where the nest was, but they recognized that they were not on their usual route homewards. To verify this observation, we went back to Greece one year later to restrict the ants' outdoor activities using a moat. The Biocenter's workshop had already contributed to my extraordinary experimental setups, but the moat was a new challenge, and it was worth doing it. After three months of training ants in differently sized moat setups, and recording their homing paths with pen and paper, we were able to confirm our impressions of the year before: ants need to perform learning walks in order to return to the nest guided by the landmark panorama from places where they have never been before. Furthermore, with an increasing number of feeder visits, ants are able to realize earlier during testing that they are walking into the wrong direction. This can be concluded from their behavior; they stopped earlier to follow their path-integration home-vector, and instead performed search loops or switched to landmark guidance, i. e. returned to the nest.

The last project I planned within the frame of my PhD project was to manipulate the learning walks of *C. noda* by changing the celestial cues above the nest entrance (chapter 6). The idea was to take away different aspects of the celestial cues, i. e. the sun, the polarization pattern, or UV-light, in order to take away the sky-compass cues to prevent ants from looking back to the nest entrance during learning walks. Furthermore, we planned to dissect the ants' brains in the field and immunolabel their brains with antibodies to synaptic proteins to subsequently quantify neuronal changes in visual integration centers induced by our experimental manipulations. Unexpectedly, it was not possible to alter the learning walk performance of the ants on a behavioral level, but we found remarkable changes on the neuronal level. Novices always looked back to the nest entrance, but neuronal plasticity in both the mushroom bodies and the central complex was only induced when the natural sky with the sun and the UV polarization pattern moving over the day was available (Grob, 2016; Grob et al., 2017). Eager to induce a behavioral change of the learning walks, we kept asking about the directional reference system and installed an electromagnetic flat coil around the nest entrance to alter the earth's magnetic field. When it was supplied with current, the ants continued to perform learning walks, but their gaze directions were no longer directed to the nest entrance any more.

The last project I performed in the frame of my PhD project was to manipulate the learning walks of *C. noda* by changing the earth's magnetic field at the nest entrance (chapter 7). We returned to Greece one more time to alter the earth's magnetic field systematically using a Helmholtz coil. Indeed, the manipulation of the horizontal field component led to a predictable change of gaze directions towards the position of the fictive nest entrance showing that the geomagnetic field, and not the sky compass, is used as a directional reference system during naïve learning walks.

With that the circle is complete. I started with the search for a reference system in Tunisia, and ended up finding a different one in Greece. Although the magnet experiment marks the end of my PhD thesis, it opens up many new research possibilities which I will sketch in the last part of the present thesis (chapter 8).

2 Manuscript I: What is the earthbound reference system for the daily time compensation of the celestial compass in *Cataglyphis*?

2 What is the earthbound reference system for the daily time compensation of the celestial compass in *Cataglyphis*?

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Using a celestial compass for navigation requires considering changes on the course of the sun and sun-linked celestial cues, like the polarization patter, during the day. Furthermore, these parameters vary from geographical location to another and may differ at different times of the year. *Cataglyphis* desert ants mainly rely on celestial cues as directional input to the path integrator during their extensive foraging excursions. To be able to rely on this compass system with the required precision, the ants must have precise information about the daily course of the sun. Since this information cannot be completely innate, they have to calibrate their compass system at the beginning of their foraging career. To be able to do so, they need an internal clock as a temporal reference system, and an external earthbound reference system. Here we ask whether the horizon as a visual skyline can provide this earthbound reference system by confronting the ants with a channel restricting the ants' view to the sky. The channel can be rotated with the sun in a way that the ants do not experience the actual movement of the sun and other celestial information. Consequently, the ants either have to rely on potentially innate information about the sun's course or cannot compensate for the sun's movement. When tested later, the homing directions should reveal the ants' knowledge about the sun course and whether the horizon provides the earthbound reference system needed. Whereas the crucial rotation experiment could not be conducted for infrastructural reasons, the control experiments demonstrate that free-field experienced ants are well-informed about the course of the sun and channel-restricted ants make systematic navigational errors. Furthermore, the time-compensation of channel-ants is less precise as compared to the free-field experienced ants. Based on preliminary data, we hypothesize that the possibility to perform learning walks might be crucial for correct compensation.

2.1 Introduction

The desert ant *Cataglyphis fortis* lives in an extremely hostile habitat, i. e. saltpans in Northern Africa, compared to their congeneric relatives (Dillier and Wehner 2004). Desert

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ants inhabiting such featureless environments must especially rely on path integration (PI) during navigation (for a review: Cheng et al. 2014). During this type of navigation, ants have to non-stop keep track of their directions and distances traveled (Müller and Wehner 1988). To meet the challenges of their hot habitat devoid of any obvious visual landmarks, forcing them to use PI, *C. fortis* ants are well adapted by having the longest legs relative to their body size (Sommer and Wehner 2012; Wehner 1987), and moving farther and faster during their extensive foraging trips compared to other ant species (Wehner 1987). Their excursions can lead the scavengers several hundred meters away from the nest until they find food (Buehlmann et al. 2014; Huber and Knaden 2015), traveling potential distances of up to 1200 m (Buehlmann et al. 2014). PI is a mechanism prone to errors (Müller and Wehner 1988). The longer the distances traveled, the larger are the errors (Merkle et al. 2006). Luckily for the ants, under natural conditions errors due to left and right turns of the freely moving forager cancel out each other (Müller and Wehner 1988). In addition, ants have back-up navigational strategies to find their nest entrance despite the uncertainties due to PI. On the one hand, they perform a systematic search when their home vector is zero and they have not yet reached their nest (Müller and Wehner 1994; Wehner and Srinivasan 1981). They even adapt their search strategy by widening their search pattern to the increasing uncertainties occurring due to longer foraging distances (Merkle and Wehner 2010; Merkle et al. 2006). On the other hand, ants use additionally available cues to pinpoint the nest entrance (e. g. visual cues: Bregy et al. 2008; Fleischmann et al. 2016; Knaden and Wehner 2005; Wehner et al. 1996, or olfactory cues: Steck et al. 2009). Nevertheless, one challenge remains unaffected by the ants' mechanisms to minimize their uncertainties due to the use of PI, and that is the fact that celestial cues, i. e. the position of the sun and the sun-linked polarization pattern, change with (i) the time of the day, (ii) the time of the year (date), and (iii) the geographical position (latitude). In order to use these cues as reliable directional input to the compass of the path integrator, foragers must time-compensate the daily course of the sun and the associated polarization pattern. To do so, three pieces of information are needed: an internal clock, an external earthbound reference system and exact information about the solar ephemeris (e. g. a table with values of the sun's position at any time). For the reasons mentioned above it seems impossible that individual ants are innately equipped with the solar ephemeris they need for their foraging trips at any time of the year and any place (Wehner and Lanfranconi 1981; Wehner and Müller 1993). *Cataglyphis*' foraging trips increase with experience in both spatial extent and duration (*C. fortis*: Fleischmann et al. 2016; *Cataglyphis bicolor*: Wehner et al. 2004). Since experienced foragers can stay outside the nest more than one hour (e. g. Wehner et al. 2004) and the sun's azimuth changes around solar noon for more than one degree per minute during midsummer at the test site of the present experiments, foragers must have precise information about the solar ephemeris in order to return to the nest successfully guided by celestial cues. Importantly, naïve ants may leave the nest for the first time at any day between spring and autumn, i. e. they are confronted with seasonally differences in the daily course of the sun depending of the starting point of their foraging career. *Cataglyphis* foragers compensate the movement of celestial cues correctly even if they have not seen the sky for several hours (Wehner and Lanfranconi 1981). Furthermore, ants that were restricted from their first appearance outside the nest to forage only in the early morning and were tested for the first time late in the afternoon, expected the sun's position to have moved about 180° (Wehner and Müller 1993). These results led to the hypothesis that desert ants (Wehner and Müller 1993) – as well as honeybees (Dyer and Dickinson 1994)

– have innate information about general spatiotemporal features of the sun's daily course and that they shape their internal representation of the actual solar ephemeris by individual experience. The general features of the sun's movement may be represented as a so-called "step-function" including the information that the sun's positions at sunrise and sunset lie about 180° apart, and that the rate of change of the sun is similar and slow in the morning and the afternoon, but rapid around solar noon (Dyer and Dickinson 1994; Wehner and Müller 1993; for a review: Dyer 1996). As mentioned before, animals using the position of the sun and the sun-linked polarization pattern, do not only need exact information about the sun's course, but also an internal clock and an earthbound reference system. Here, we wanted to ask which reference system is used by *C. fortis* for detection and compensation of the daily movement of the celestial cues. For honeybees it has been shown that they use the landmark panorama around the hive as a stable reference to detect and learn the daily movement of celestial cues (Dyer 1987; Dyer and Gould 1981). Our hypothesis is that the horizon, i. e. the visual panorama, provides the necessary reference system for *C. fortis* to initially calibrate and daily compensate the solar ephemeris. To test this hypothesis, we restricted the ants' foraging excursions to a 10 m-long channel on wheels which could be rotated with the sun's movement in a way that the ants would never experience the daily course of celestial cues. If they were captured at the feeder in the morning and kept for different durations until testing, their homing directions should reveal their innate expectation about the sun's course and proof whether the horizon indeed provides the reference system for calibration and compensation of the movement of the celestial cues.

2.2 Material and Methods

Test animals and study site Experiments were performed with *Cataglyphis fortis* (Forel 1902) desert ants in the summer of 2014 on a saltpan near Menzel Chaker, Tunisia (coordinates: 34°57'N 10°24'E). The colony used was located towards the middle of the saltpan where almost no natural landmarks occur in the nest's vicinity (fig. 2.1). Experienced foragers were marked with one color for at least three days before the experiment started. After this period, all unmarked ants were considered to be naïve, i. e. did not have experience above ground. These individuals, so-called novices, were marked with a unique three-dot color-code using car paint (Motip Lackstift Acryl, MOTIP DUPLI GmbH, Haßmersheim, Germany).

Experimental setup In order to prevent ants from uncontrollably experiencing the daily course of the sun and of the polarization pattern, the nest entrance was covered. Ants were allowed to leave the nest only through a tunnel connected to the channel (fig. 2.1B). The nest cover was permanently installed, but the experimental channel had to be set up and removed every day. The channel had a length of 10.00 m, a width of 0.12 m and a height of 0.06 m. It was installed on one axis of rotation near the nest entrance and five pairs of wheels. The edges of the channel were folded inwards to prevent ants from climbing out of the channel. Above the entrance to the channel a cover was installed to keep ants from seeing the sky from within the tunnel. The had to enter the channel in the shade without direct view of the sky. During the stationary control experiments (see details about the experimental procedure below), the channel was aligned with the solar azimuth at 09:00 local solar time (LST). During the last training before the test, ants foraged towards the sun (east) and returned to the nest in antisolar

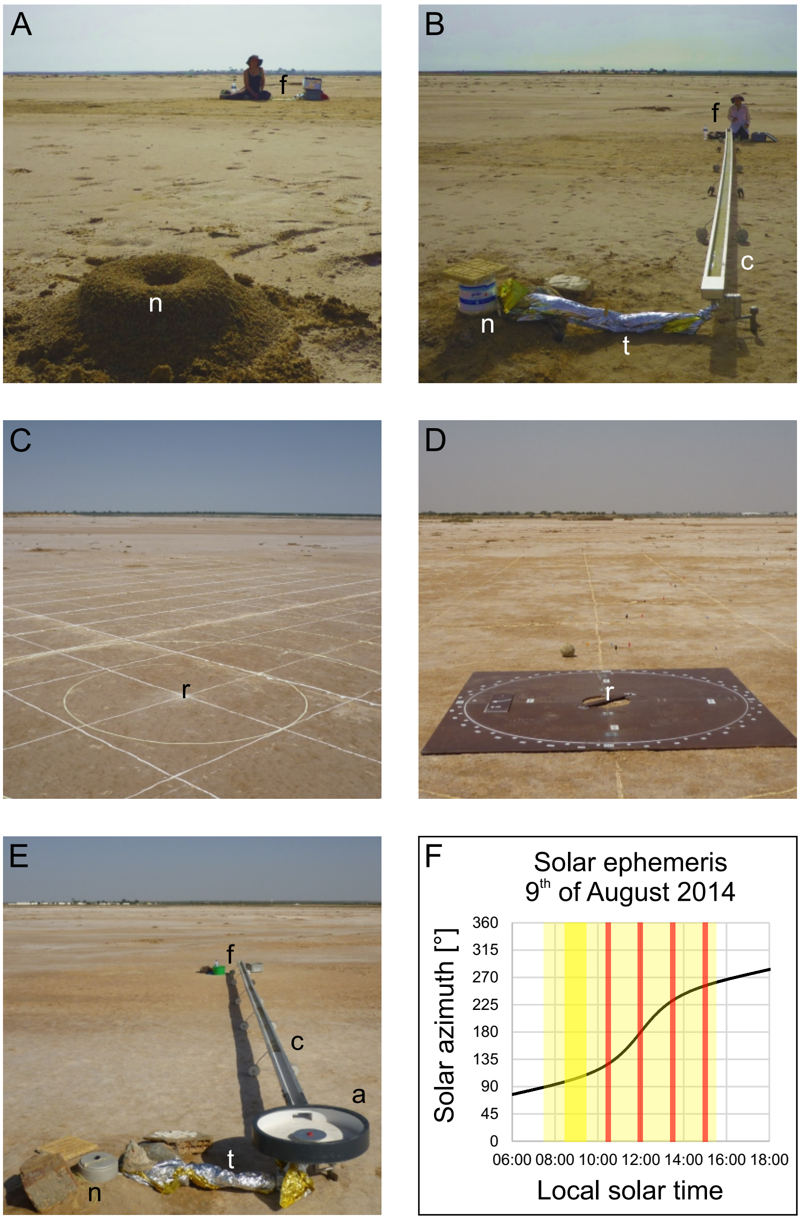

Figure 2.1: Experimental settings. (**A**) In the control experiment with free-field foragers, ants were trained to a feeder (f) 10 m east of their natural nest entrance (n). (**B**) In the two control experiments with the experimental channel (c), ants were trained to leave their nest entrance (n) via a tunnel (t) to reach the channel in which they had to forage 10 m until they reached the feeder (f). (**C**) For testing, ants were released at the center of the test field (r). The mesh width is 1 m. (**D**) After ants performed their home runs, their heading directions were measured directly at the field site with a goniometer. (**E**) As a preliminary experiment, an arena was installed instead of the linear first channel segment so that ants entered the channel (c) from their nest entrance (n) through the tunnel (t) in the middle of the arena (a). (**F**) Ants were trained to visit the feeder between 07:45 and 15:45 local solar time (LST) every day (yellow shaded area). Last training before capturing the ants was at 09:00 LST ± 30 minutes (yellow bar). The tests were performed at four times (red bars).

direction (west).

Experimental procedure Ants were trained every day from about 07:45 LST to about 15:45 LST towards a feeder 10 m to the east of the nest entrance (fig. 2.1F). At the feeder ants could collect cookie crumbles and were additionally offered a piece of fruit (e. g. water melon or cucumber) to soak some liquid. The feeder was observed non-stop during the experimental time by one person recording every visit of an individually marked ant. Before being tested, each ant had to visit the feeder at least on two days, and at different times of the day (i. e. in the morning meaning before 11:30 LST, around solar noon meaning between 11:30 and 12:30 LST and in the afternoon, i. e. after 12:30). The last training before testing was at 09:00 LST ± 30 minutes. Tests were performed at four different times: at 10:30 LST, 12:00 LST (solar noon), 13:30 LST and 15:00 LST, i. e. ants had to compensate for the change of the celestial cues over periods of about 1.5 h, 3.0 h, 4.5 h or 6.0 h. At every testing point, maximally five ants were tested subsequently to test them as punctually as possible. Each ant was only tested once. Ants were tested on a distant test field (20 m × 20 m) which had been painted with diluted wall paint on the desert ground (fig. 2.1C). Ants were released in the center of the test field in a plastic ring. They were offered a food item and released either when they took the food item or after one minute. Only ants carrying a food item were included in the statistical analysis to ensure that ants were motivated to return to the nest. The ant's path was recorded true to scale with pen and paper by one person. In addition, another person put little flags in the ground whenever the ant crossed a circle in 1 m to 10 m distance from the release point. Testing was stopped when the ant left the test field, i. e. followed the home vector for at least 10 m, or turned around and crossed a circle twice to search for the nest entrance systematically. After all ants at the time of one testing cohort had been tested, the positions of the flags were measured immediately by the two experimenters in the test field using a rope and a goniometer (fig. 2.1D).

Three control experiments To test the hypothesis that the horizon provides the reference system for the detection and compensation of the daily course of celestial cues, we planned to perform an experiment in which the experimental channel should be rotated with the solar azimuth. With this rotation, the ants would never experience the movement of the sun over the day. When being tested, their homing direction should reveal what they expected about the solar ephemeris innately and which role the horizon plays. This crucial rotational channel experiment (RCE) could not be performed, because we could not return to Tunisia again after 2014 due to the political situation.

In the field season of 2014 three control experiments and one preliminary experiment were performed and completed. All experimental ants were trained and tested as described above in the different experimental setups described below.

In the first control experiment, ants were trained to a feeder east from the nest entrance (100°) in the free field prior to testing at the test field (fig. 2.1A). All free-field foragers (FFF) were individually marked, but had an unknown age. This FFF experiment was conducted to test whether well-experienced ants of the colony used for the experiments can compensate the daily movement of the sun successfully as described before (Wehner and Lanfranconi, 1981), and without any bias. Ants trained in a channel are expected to make systematic, navigational errors when presented with only parts of the polarization pattern, e. g. an asymmetrical sector of the sky provided by the channel, due to the peculiarities of their polarization compass (Müller, 1989; Wehner and Müller, 2006). The consequences of this channel-induced error during navigation in the free field will be discussed in the discussion below. At this point it is just important to know that ants trained and captured in a channel offering an asymmetrical view of the sky will make systematical errors during homing at the test field.

In the second control experiment ants that had free-field experience were trained in the channel (106°) and tested at the test field. These free-field experienced, but channel-captured (FCE) ants had belonged to the FFF group, but had not yet been tested when the channel was installed to start the next experiment (fig. 2.1B). The orientation of the channel (106°) was chosen in a way that it was aligned with the solar azimuth during the last training. At 09:00 LST the sun's azimuth was 106°. It moved $\approx 12°$ during the one hour period of capturing test ants. Therefore, when ants were captured the polarization pattern was symmetrically visible from the channel.

Before the start of the third control experiment, ants in the channel were marked for three days with one color to ensure that all ants participating in the stationary channel experiment (SCE) did not have any experience in the free field. Ants were trained in the stationary channel (106°) and tested at the test field.

Recordings of learning walks in an arena In addition to the three control experiments described above, a preliminary experiment was performed during the last 1.5 days of the field season. Instead of the rather narrow linear channel segment, an arena (diameter: 0.60 m) was installed at the starting point of the experimental channel (fig. 2.1E). Since this experiment was performed at the end of the field season, ants could not be trained and tested. Instead, we recorded the learning walks performed by the ants in the arena using high-speed video (Lumix, DMC-FZ200, Panasonic Corp., Kadoma, Japan; temporal resolution: 100 fps, spatial resolution: 1080 × 720 pixels). The observer sitting next to the arena started the recording when a novice left the nest, and stopped it when the ant returned to the nest or left the recording area (about 20 cm × 30 cm) using a remote control (Wireless Remote Release DCCS System base, HAMA GmbH & Co KG, Monheim, Germany). To track the ant's path, a video was converted into an image stack using the Free Video to JPG converter (v. 5.0.99 build 823, DVDVideoSoft, DIGITAL WAVE LTD., London, UK). The position of the thorax was marked manually frame by frame using the Matlab (2015a, The MathWorks Inc., Natick, MA, USA) application Digilite (Jan Hemmi and Robert Parker, The Australian National University, Canberra, Australia). Additionally, the position of the artificial nest entrance was marked. The ant's path was visualized using Matlab 2015a.

Statistics Based on the recorded homing directions (headings of the ants at 4 m distance from the release point) of the test ants, the ants' individual expectations of the solar azimuth could be calculated. We tested whether the groups' results were randomly distributed using the Rayleigh test ($\alpha = 0.05$). If data was directed, we calculated the 95 % confidence interval to check whether the expected position of the sun lay between these limits.

2.3 Results

Control experiments 1-3 (FFF, FCE, SCE): Compensation of the sky's rotation Ants that had free-field experience before being tested, i. e. had the possibility to learn the daily movement of the sun and related celestial cues without any restrictions, compensated the changing position of the sun correctly – no matter whether they were caught in the free field or in the stationary channel (fig. 2.2a, b, respectively). The actual position of the sun lay always within the 95 % confidence interval (table 2.1) except for the FFF 10:30 group where the actual position just lay outside the interval (95 % CI -/+ 130.2°/140.9° versus actual position of the sun: 128.9°). Ants that had only foraged within the stationary channel were less well-informed about the position of the sun (fig. 2.2c). In three of four cases (SCE 10:30, SCE 13:30 and SCE 15:00) the actual position of the sun did not lie within the limits of the 95 % confidence intervals. In the last case (SCE 12:00) the sun's position lay between the limits, but the data covered a wide range (fig. 2.2c).

Preliminary experiment: Learning walks in the arena Many ants, both novices and foragers, performed (re-)learning walks when the arena had been installed. Novices meandered around the artificial nest entrance and included voltes in their learning walks (fig. 2.3).

2.4 Discussion

Compensation of the daily rotation of the celestial cues *C. fortis* foragers that had been trained in the free field (FFF and FCE) compensated the movement of the sun correctly as has also been shown before for both desert ants (Wehner and Lanfranconi 1981) and honeybees (Gould 1980; Lindauer 1957). In contrast, the ants that could only forage in the stationary channel (SCE) underestimated the sun's position in the morning and overestimated the sun's position in the afternoon. These errors may be explained with the systematic navigational errors occurring when ants had been trained in a channel providing them only with a partial view of the sky and the polarization pattern associated with it, and when they were then tested at the open test field (Müller 1989; Wehner and Müller 2006). When qualitatively

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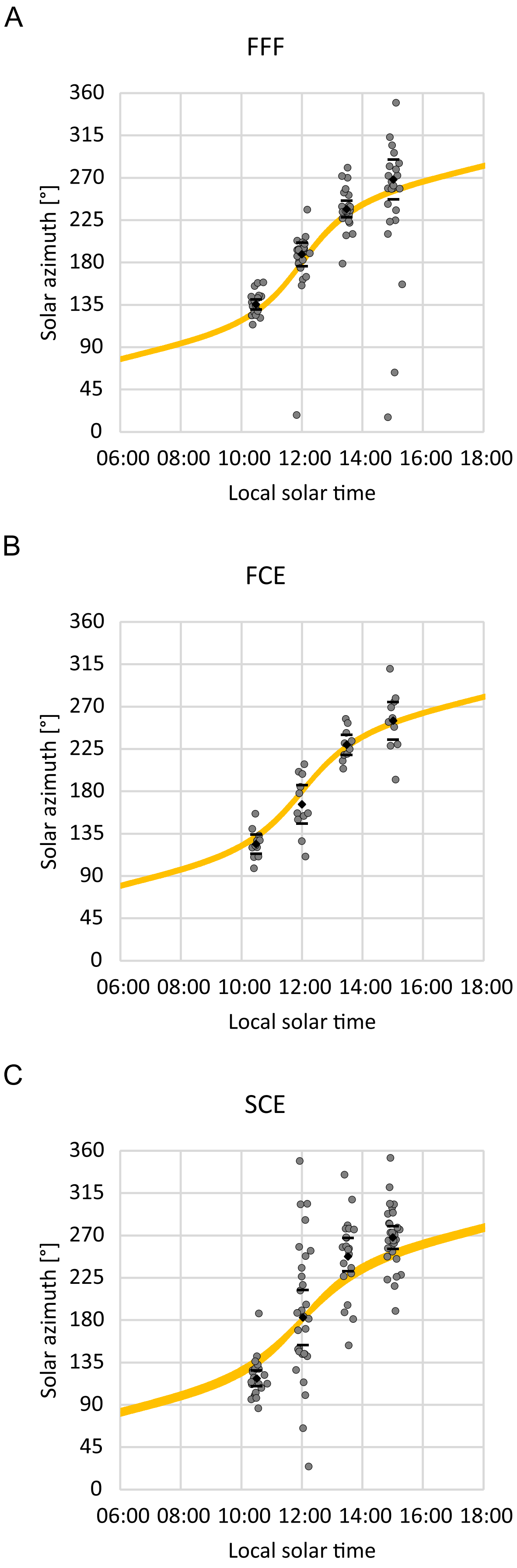
Figure 2.2 *(following page)*: Ants' compensation of the rotation of the celestial cues. Results of (**A**) the first control experiment with free field foragers (FFF), (**B**) the second experiment with free field foragers captured in the channel (FCE), and (**C**) the third experiment with ants that could only forage in the stationary channel (SCE). In each experiment ants were tested at four different times (10:30, 12:00, 13:30, and 15:00 local solar time). The expected solar azimuth by each ant is represented by one grey dot, means of the groups are shown as black diamond, and the 95 % confidence interval limits as black bars. The orange curves indicate the position of the sun in the experimental periods (A: 9th to 13th of August, B: 18th to 21st of August, C: 21st of August to 3rd of September 2014). For statistical details see table 2.1.


A
FFF
B
FCE
C
SCE
Solar azimuth [°]
0
45
90
135
180
225
270
315
360
06:00
08:00
10:00
12:00
14:00
16:00
18:00
Local solar time

Exp. group	n	μ	Z	p	95 % CI ll	95 % CI ul	Solar Azimuth		Corr.	Corr.
							1st exp. day	last exp. day	95 % CI ul	95 % CI ll
FFF 10:30	20	135.6	19.107	< 0.001	130.2	140.9	127.9	128.9	x	x
FFF 12:00	22	188.7	16.613	< 0.001	176.0	201.3	180.1	179.7	x	x
FFF 13:30	23	236.6	19.993	< 0.001	227.9	245.4	232.2	230.7	x	x
FFF 15:00	23	268.2	10.468	< 0.001	247.3	289.1	256.2	255.1	x	x
FCE 10:30	11	124.0	10.272	< 0.001	113.7	134.3	131.4	132.9	x	x
FCE 12:00	11	166.2	8.354	< 0.001	145.6	186.7	179.8	180.3	x	x
FCE 13:30	11	229.4	10.221	< 0.001	218.8	240.1	228.3	227.4	x	x
FCE 15:00	11	254.9	8.463	< 0.001	234.9	274.9	253.0	252.0	x	x
SCE 10:30	22	118.0	19.389	< 0.001	109.5	126.5	132.9	137.8	119.5	136.5
SCE 12:00	30	182.9	6.489	0.001	153.5	212.3	180.3	180.1	153.5	212.3
SCE 13:30	21	249.8	12.411	< 0.001	232.3	267.4	227.4	222.3	217.3	257.9
SCE 15:00	28	267.7	20.066	< 0.001	255.5	279.9	252.0	247.2	252.0	247.2

Table 2.1: Statistical details. Ants trained in the three different setups (FFF, FCE and SCE) were tested at four different times (10:30, 12:00, 13:30, and 15:00 LST). Each ant was only test once. For each group numbers of ants (n), mean vector (μ), the results of the Rayleigh test (Z and p), the lower and upper limit of the 95 % confidence interval (95 % CI ll / ul) and the solar azimuth at test time of the first experimental day (FFF: 9th of August, FCE: 18th of August, SCE: 21st of August), and the last experimental day (FFF: 13th of August, FCE: 21st of August, SCE: 3rd of September) are given. For the SCE groups the lower and upper limit of the corrected 95 % confidence interval (corr. 95 % CI ll / ul) are calculated to correct for the channel induced systematic errors (expected homing direction after correction: 10:30 LST: 296°, 12:00 LST (no correction necessary): 286°, 13:30 LST: 276°, and 15:00 LST: 284°).

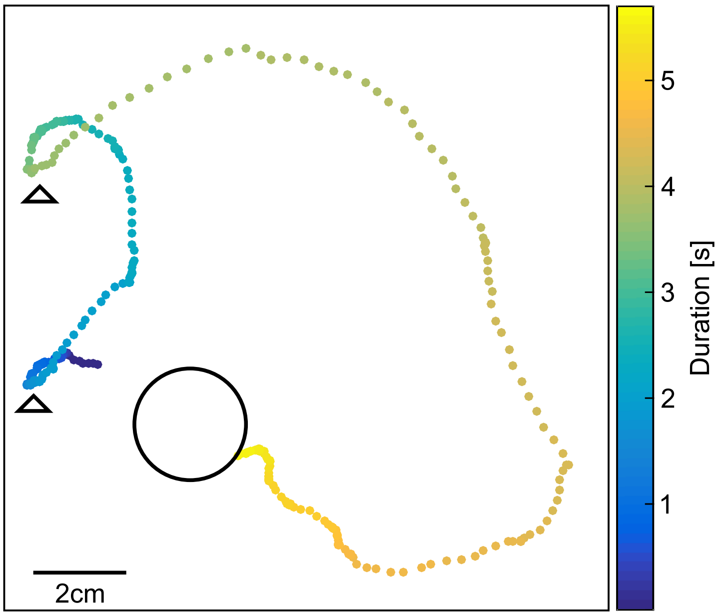


Figure 2.3: Learning walk of a novice performed in the arena. The unmarked ant left the artificial nest entrance (black circle) located in the center of the arena to perform a short learning walk including two voltes (indicated by the two triangles) until eventually returning to the nest after 5.6 s (time is color coded). Each dot shows the ant's position every 10 ms.

correcting for the navigational errors by using the polarization-induced error functions (fig. 2E in Wehner and Müller 2006), the actual position of the sun lies between the shifted limits of the 95 % confidence intervals in two of the three cases (SCE 10:30, expected homing direction by correction: 296° instead of the actual homing direction of 286°; SCE 13:30: 276° instead of the actual 286°). In the third case the actual position of the sun just lies outside the confidence interval (SCE 15:00: expected homing direction: 284° instead of the actual homing direction 286°; new 95 % confidence interval of the sun's azimuth +/-: 253.5°/277.9°, sun's position: 252.0°). Of course, the error functions should be calculated for the present setups (in the figure used the channel was oriented in a way that the homing direction was 270°, in the present experiment it was 286°), but as a first approximation and explanation this qualitative correction is applicable. The widely spread data in the SCE 12:00 group may indicate that the ants could not gain enough experience about the sun's course around solar noon and had to use their step function instead (Dyer and Dickinson 1994; Wehner and Müller 1993; for a review: Dyer 1996). It is possible that the ants need enough space to perform learning walks in order to learn the solar ephemeris and cannot learn it within the restricted channel. To test this hypothesis, we originally planned to perform an experiment with an arena around the artificial nest entrance. The preliminary results will be discussed below.

Learning walk performance in the arena We could show, for the first time, that *Cataglyphis* ants use an experimental arena with an artificial entrance to perform learning walks, as they do under natural conditions (Fleischmann et al. 2016; Fleischmann et al. 2017; Stieb et al. 2012; Wehner et al. 2004). The novices in the present study included voltes in their learning walks, which are typical elements of learning walks performed under natural conditions

(Fleischmann et al. 2017). The function of the voltes is not yet known, but it might be that they are needed to calibrate the celestial compass system at the beginning of the ant's foraging career (Fleischmann et al. 2017). The performance of learning walks in an experimental setup demonstrates two important things. First, it is proven that it is possible to guide ants into experimental settings and study their natural behavior. This will become especially important for experiments presented later in this thesis (chapters 7 and 8) or experiments that have been part of student courses and a bachelor thesis (Marschewski 2017), as well as for potential future projects that can be conducted in experimental arenas in the laboratory. Second, when the arena was installed many ants performed learning walks at the same time. This is remarkable for two reasons. The ants had hesitated to enter the channel setup with the nest covered and it had been quite difficult to train them in this setup. However, when the arena was installed ants kept coming, were very active and performed many learning walks (personal observation). There are two described changes in the environment to trigger the performance of learning (and re-learning) walks. If a landmark is newly installed, ants start to perform re-learning walks in order to learn the newly available landmarks (Fleischmann et al. 2016; Müller 1984; Müller and Wehner 2010). If ants are confronted with a linear polarization pattern that does not change over the day above their nest entrance for some days, and then the polarization pattern is turned experimentally both the number of learning walks performed by novices and the number of re-learning walks performed by foragers increase dramatically (Grob 2016; Grob et al. 2017). Maybe the ants trained in the channel in the present study were not only restricted spatially, but also prevented drastically from performing their learning walks. This assumption is further corroborated by the fact that *Cataglyphis noda* ants – that inhabit a cluttered environment – need to perform learning walks of a certain distance from the nest to acquire the landmark information necessary to return to the nest (cf. chapter 5). It would be very interesting to test, whether ants that had the chance to perform learning walks in the arena during training, would acquire the solar ephemeris comparable to the ants trained in the free field. If that was the case, they should compensate the sun's movement as did the FFF and FCE groups and without a channel-induced systematic error (like the SCE groups).

2.5 Conclusion and Outlook

The arena experiment was meant to be conducted together with the crucial rotating channel experiment in the summer of 2015. Possibly it might be conducted in the future. Until then, the question of what the earthbound reference system is for the daily time compensation of the celestial compass in *C. fortis* remains open. For honey bees it has been convincingly shown that the reference system is provided by the landmarks available around the hive (Dyer 1987; Dyer and Gould 1981; Towne and Moscrip 2008). Whether the horizon as a visual skyline actually provides the earthbound reference system necessary for the compensation of the sky's rotation remains elusive – and will be discussed later in this thesis (chapter 8).

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References

Bregy, P., Sommer, S., and Wehner, R. (2008). Nest-mark orientation versus vector navigation in desert ants. *The Journal of Experimental Biology*, 211:1868–1873.

Buehlmann, C., Graham, P., Hansson, B. S., and Knaden, M. (2014). Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. *Current Biology*, 24(9):960–964.

Cheng, K., Schultheiss, P., Schwarz, S., Wystrach, A., and Wehner, R. (2014). Beginnings of a synthetic approach to desert ant navigation. *Behavioural Processes*, 102:51–61.

Dillier, F.-X. and Wehner, R. (2004). Spatio-temporal patterns of colony distribution in monodomous and polydomous species of North African desert ants, genus *Cataglyphis*. *Insectes Sociaux*, 51(2):186–196.

Dyer, F. (1996). Spatial memory and navigation by honeybees on the scale of the foraging range. *The Journal of Experimental Biology*, 199:147–154.

Dyer, F. C. (1987). Memory and sun compensation by honey bees. *Journal of Comparative Physiology A*, 160(5):621–633.

Dyer, F. C. and Dickinson, J. a. (1994). Development of sun compensation by honeybees: how partially experienced bees estimate the sun's course. *Proceedings of the National Academy of Sciences*, 91(May):4471–4474.

Dyer, F. C. and Gould, J. L. (1981). Honey bee orientation: a backup system for cloudy days. *Science*, 214(4524):1041–1042.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *The Journal of Experimental Biology*, 219:3137–3145.

Fleischmann, P. N., Grob, R., Wehner, R., and Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *The Journal of Experimental Biology*, 220:2426–2435.

Gould, J. L. (1980). Sun compensation by bees. *Science*, 207(4430):545–547.

Grob, R. (2016). *Experience-dependent behavioural changes and neuronal plasticity in visual pathways of Cataglyphis desert ants*. Master thesis, Universität Würzburg.

Grob, R., Fleischmann, P. N., Grübel, K., Wehner, R., and Rössler, W. (2017). The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11:226.

Huber, R. and Knaden, M. (2015). Egocentric and geocentric navigation during extremely long foraging paths of desert ants. *Journal of Comparative Physiology A*, 201(6):609–616.

Knaden, M. and Wehner, R. (2005). Nest mark orientation in desert ants *Cataglyphis*: What does it do to the path integrator? *Animal Behaviour*, 70:1349–1354.

Lindauer, M. (1957). Sonnenorientierung der Bienen unter der Äquatorsonne und zur Nachtzeit. *Die Naturwissenschaften*, 44(1):1–6.

Marschewski, S. (2017). *Manipulation of Cataglyphis learning walks with a rotating disc*. Bachelor thesis, Universität Würzburg.

Merkle, T., Knaden, M., and Wehner, R. (2006). Uncertainty about nest position influences systematic search strategies in desert ants. *The Journal of Experimental Biology*, 209:3545–3549.

Merkle, T. and Wehner, R. (2010). Desert ants use foraging distance to adapt the nest search to the uncertainty of the path integrator. *Behavioral Ecology*, 21(2):349–355.

Müller, M. (1984). *Interokularer Transfer bei der Wüstenameise Cataglyphis fortis (Formicidae, Hymenoptera)*. Diplomarbeit, Universität Zürich.

Müller, M. (1989). *Mechanismus der Wegintegration bei Cataglyphis fortis (Hymenoptera, Insecta)*. Phd thesis, Universität Zürich.

Müller, M. and Wehner, R. (1988). Path integration in desert ants, Cataglyphis fortis. *Proceedings of the National Academy of Sciences*, 85(14):5287–5290.

Müller, M. and Wehner, R. (1994). The hidden spiral: Systematic search and path integration in desert ants, *Cataglyphis fortis*. *Journal of Comparative Physiology A*, 175:525–530.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Current Biology*, 20(15):1368–1371.

Sommer, S. and Wehner, R. (2012). Leg allometry in ants: extreme long-leggedness in thermophilic species. *Arthropod Structure & Development*, 41(1):71–77.

Steck, K., Hansson, B. S., and Knaden, M. (2009). Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Frontiers in Zoology*, 6:5.

Stieb, S. M., Hellwig, A., Wehner, R., and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 72(5):729–742.

Towne, W. F. and Moscrip, H. (2008). The connection between landscapes and the solar ephemeris in honeybees. *Journal of Experimental Biology*, 211(23):3729–3736.

Wehner, R. (1987). Spatial Organization of Foraging Behavior in Individually Searching Desert Ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In Pasteels, J. M. and Deneubourg, J.-L., editors, *From Individual to Collective Behavior in Social Insects*, pages 15–42. Birkhäuser Verlag, Basel Bostom.

Wehner, R. and Lanfranconi, B. (1981). What do the ants know about the rotation of the sky? *Nature*, 293(5835):731–733.

Wehner, R., Meier, C., and Zollikhofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecological Entomology*, 29:240–250.

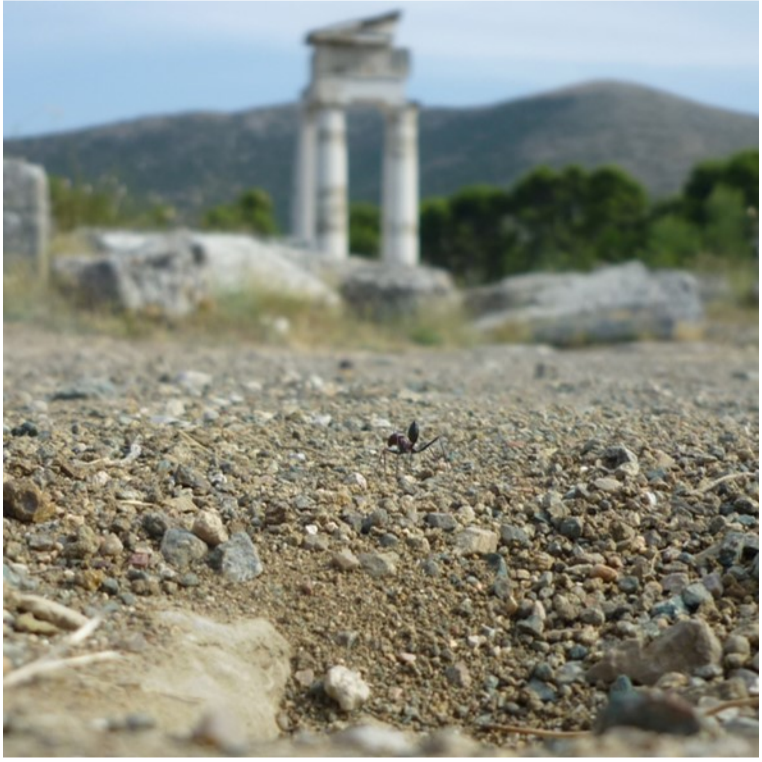
Wehner, R., Michel, B., and Antonsen, P. (1996). Visual navigation in insects: Coupling of egocentric and geocentric information. *The Journal of Experimental Biology*, 199:129–140.

Wehner, R. and Müller, M. (1993). How do ants acquire their celestial ephemeris function? *Naturwissenschaften*, 80:331–333.

Wehner, R. and Müller, M. (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proceedings of the National Academy of Sciences*, 103(33):12575–12579.

Wehner, R. and Srinivasan, M. V. (1981). Searching behaviour of desert ants, genus *Cataglyphis*. *Journal of Comparative Physiology A*, 142:315–338.

3 Manuscript II: Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants



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RESEARCH ARTICLE

Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants

Pauline N. Fleischmann[1,*], Robin Grob[1], Rüdiger Wehner[2] and Wolfgang Rössler[1]

ABSTRACT

Cataglyphis desert ants are famous navigators. Like all central place foragers, they are confronted with the challenge to return home, i.e. relocate an inconspicuous nest entrance in the ground, after their extensive foraging trips. When leaving the underground nest for the first time, desert ants perform a striking behavior, so-called learning walks that are well structured. However, it is still unclear how the ants initially acquire the information needed for sky- and landmark-based navigation, in particular how they calibrate their compass system at the beginning of their foraging careers. Using high-speed video analyses, we show that different *Cataglyphis* species include different types of characteristic turns in their learning walks. Pirouettes are full or partial rotations (tight turns about the vertical body axis) during which the ants frequently stop and gaze back in the direction of the nest entrance during the longest stopping phases. In contrast, voltes are small walked circles without directed stopping phases. Interestingly, only *Cataglyphis* ant species living in a cluttered, and therefore visually rich, environment (i.e. *C. noda* and *C. aenescens* in southern Greece) perform both voltes and pirouettes. They look back to the nest entrance during pirouettes, most probably to take snapshots of the surroundings. In contrast, *C. fortis* inhabiting featureless saltpans in Tunisia perform only voltes and do not stop during these turns to gaze back at the nest – even if a set of artificial landmarks surrounds the nest entrance.

KEY WORDS: Celestial compass, Central place forager, Panorama snapshot, Spatial orientation, Visual landmark, Vector navigation

INTRODUCTION

Hymenopteran central place foragers – such as bees, wasps and ants – perform so-called learning flights or walks whenever they have to memorize the landmark panorama surrounding a particular place, e.g. the location of their nest. Since the first descriptions over a century ago (Peckham and Peckham, 1898; Wagner, 1907), learning flights have been investigated in great detail in wasps (Tinbergen, 1932; Zeil, 1993a,b; Zeil et al., 1996), honeybees (Becker, 1958; Capaldi and Dyer, 1999; Lehrer, 1991, 1993; Opfinger, 1931; Vollbehr, 1975) and bumblebees (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Robert et al., 2017) using increasingly sophisticated techniques like harmonic radar (Capaldi et al., 2000; Degen et al., 2015, 2016; Osborne et al., 2013) or high-speed cameras (Riabinina et al., 2014; Stürzl et al., 2016). Much less is known about learning walks of ants (Fleischmann et al., 2016; Jayatilaka, 2014; Müller and Wehner, 2010; Muser et al., 2005; Nicholson et al., 1999; Stieb et al., 2012; Wehner et al., 2004). However, these few studies document that – like flying insects – some ant species exhibit a striking behavioral sequence at the beginning of their foraging life. Desert ants in particular, which are well known for their navigational performance (Ronacher, 2008; Wehner, 2008; Wehner and Rössler, 2013; Wehner et al., 1996), use a considerable amount (up to 3 days; Fleischmann et al., 2016; Stieb et al., 2012) of their short lives (often less than a week; Schmid-Hempel and Schmid-Hempel, 1984) outside the nest to perform learning walks. *Cataglyphis* ants are thermophilic scavengers searching for dead arthropods during their extensive foraging excursions. *Cataglyphis fortis* inhabiting North African chotts and sebkhas cover the largest distances compared with other desert ant species (Wehner, 1983, 1987a). Their paths can be longer than 1 km and their maximal foraging distances more than 300 m from the nest (Buehlmann et al., 2014). *Cataglyphis noda*, native to Greece, search for food closer to the nest, but still cover large distances (Wehner et al., 1983), as do *Cataglyphis bicolor* [Wehner, 1987a; note that in that publication (and others: Harkness, 1977; Wehner et al., 1983), *C. noda* belonging to the *bicolor* species group, i.e. to the same phylogenetical group as *C. bicolor*, is named *C. bicolor*]. Before the successful completion of these extensive foraging trips, *Cataglyphis* ants perform learning walks characterized by several typical features: naive ants (called 'novices' below) move slowly around the nest entrance and explore all directions around it in subsequent learning walks (Fleischmann et al., 2016; Wehner et al., 2004). These ants include repeated body turns in their small excursions and they do not bring back any food items (Fleischmann et al., 2016; Stieb et al., 2012; Wehner et al., 2004). However, in novices, the characteristics of the learning walks, including circular movements as well as saccadic rotations with distinct stopping phases, have not yet been analyzed in detail. Only a few studies have recorded these striking elements during learning walks in different desert ant species and report on their rotational motifs (*C. fortis*: Stieb et al., 2012; *Melophorus bagoti*: Wystrach et al., 2014; *Myrmecia croslandi*: Jayatilaka, 2014). Namibian desert ants, *Ocymyrmex robustior*, perform 're-learning walks' when presented with a new landmark situation around the nest (Müller and Wehner, 2010). These walks contain marked pirouettes with well-defined nest-fixating stopping phases.

Here, we used high-speed video recordings to reveal temporal and spatial characteristics of the learning walk of novices in different *Cataglyphis* species inhabiting different types of environments (cluttered and open). Our analyses show that the ant species performed different types of turn. Only ants inhabiting a cluttered environment (namely *C. noda* and *Cataglyphis aenescens*) performed both voltes (small walked circles) and pirouettes (tight rotations about the vertical body axis). They paused during the latter to gaze in the nest direction. In contrast, ants inhabiting a featureless

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habitat (*C. fortis*) only performed voltes and did not gaze back to the nest entrance even if artificial landmarks offered a prominent panorama.

MATERIALS AND METHODS

Test animals and study sites

To compare learning walks of different *Cataglyphis* species inhabiting different environments, we performed experiments in Tunisia and Greece. The experiments with *C. noda* (Brullé 1832) were performed in July and August 2015 in the Schinias National Park near Marathon, Greece (38°08′N, 24°02′E). We used two nests located on different clearings in the surrounding pine forest. The trees around the clearings offer prominent skylines with natural landmarks (Fig. 1A). In addition, during July 2016, we recorded learning walks of a smaller species, *C. aenescens* (Nylander 1849), inhabiting the same clearings as *C. noda*. The experiments with *C. fortis* (Forel 1902) were carried out in June and July 2014 at a saltpan near Menzel Chaker, Tunisia (34°57′N, 10°24′E). The saltpan offers an almost landmark-free habitat. We used two different colonies for the video recordings, both situated towards the middle of the saltpan where almost no natural landmarks occurred (Fig. 1B).

Data acquisition: video recordings

All foragers were marked with the same color (Motip Lackstift Acryl, Motip Dupli GmbH, Haßmersheim, Germany) over a period of 3–4 days before video recordings started. We considered unmarked ants appearing on subsequent experimental days at the nest entrance as novices and recorded their learning walks for 2–3 days. As these novices are easily disturbed and disappear immediately into the nest (Wehner et al., 2004), we usually did not mark them. Therefore, we usually could not decide whether a learning walk was the first or a subsequent one. However, as the walks were only included in the data if the ants stayed within the recording area, and the ants move further away with more experience (Fleischmann et al., 2016), these walks were most probably early learning walks. In one colony of *C. noda*, we marked some of the naive ants with an individual color code in order to record subsequent learning walks of individual ants. The experimental setup consisted of cameras mounted on one or two tripods (Manfrotto 190, Lino Manfrotto+co. Spa, Cassola, Italy) placed north of the nest entrance. A Full HD video camera (HDC-SD300, Panasonic Corp., Osaka, Japan) recorded the nest entrance and its surroundings (i.e. an area of about 100 cm×60 cm) at 25 frames s^{-1} and minimal zoom throughout the entire day. We used these videos to trace the learning walks in the case of one individually marked ant (see Fig. 2D–F), because its paths left the high-speed recording area. The high-speed camera (Lumix DMC-FZ200, Panasonic Corp.) recorded a smaller area (about 25 cm×43 cm) at 100 frames s^{-1} temporal and 1080×720 pixel spatial resolution. The observer sitting 5 m east of the nest watched the ants through binoculars (Condor 10×50 1821050, Bresser GmbH, Rhede, Germany), and started and stopped the high-speed recordings via a remote control (Wireless Remote Release DCCS System Base, HAMA GmbH & Co KG, Monheim, Germany) whenever learning walks occurred.

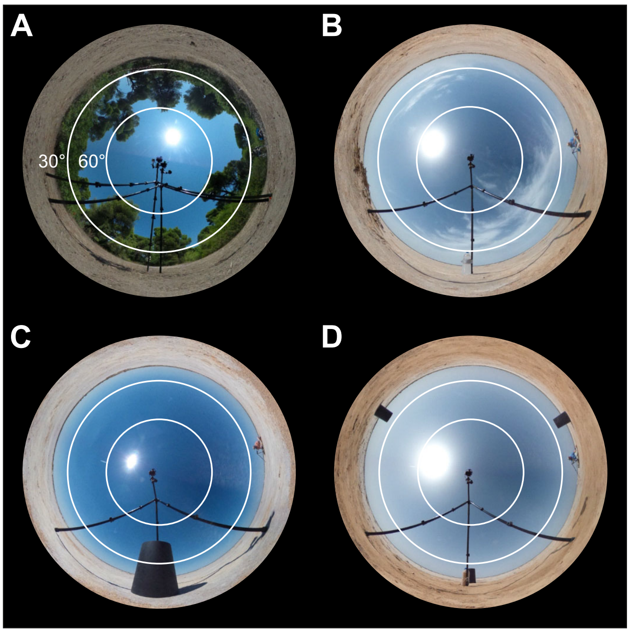

Fig. 1. Panoramic (360 deg) pictures of the different experimental setups. (A) In the pine forest in Greece, the trees and bushes offer a natural and prominent skyline. (B) In contrast, the saltpan in Tunisia lacks prominent natural landmarks. (C) One or (D) three black cylinders were offered as artificial landmarks close the nest entrance for the setup shown in B. The camera tripod stood north of the nest entrance in all setups. The experimenter sitting 5 m east of the nest watched the ants through binoculars to start high-speed recordings when naive ants performed learning walks at the nest entrance. The white lines indicate 30° and 60° latitudes above the horizon; the zenith is in the middle of the circles. The panoramic pictures in A and C are also shown in Movie 1.

In Tunisia, we performed three experiments with *C. fortis*: no artificial landmarks, one artificial landmark or three artificial landmarks were placed near the nest entrance. In the first case, the ants did not have any additional landmarks (Fig. 1B). In the second case, one black cylinder (height: 33 cm, diameter: 20 cm) was positioned 0.4 m north of the nest entrance (vertical angular size: 48 deg, horizontal angular size: 28 deg; Fig. 1C). In the third case, three black cylinders (height: 38 cm, diameter: 22 cm) were placed 2 m from the nest at 0 deg (north), 120 deg (southeast) and 240 deg (southwest) (for each cylinder: vertical angular size: 11 deg, horizontal angular size: 6 deg; Fig. 1D).

Data analysis

We cut the videos to the length of one learning walk or one turn and converted them into image sequences using QuickTime Player Pro 7.7.5 (Apple Inc., Cupertino, CA, USA). Both learning walks and individual turns were easily identifiable and were extracted by eye. A learning walk was defined as an explorative trip, i.e. no digging or foraging, of an unmarked ant from exiting the nest entrance until its return into the nest. A turn was defined as a circular movement during which an ant changed its gaze direction until eventually re-establishing its previous walking direction. We analyzed the image sequence frame by frame with the Matlab (MathWorks, Inc., Natick, MA, USA) application Digilite (Jan Hemmi and Robert Parker, The Australian National University). We tracked the ant's position by marking the mandibles as well as the thorax. Furthermore, the position of the nest and the north direction were marked. We used these four types of coordinates to determine the ant's position and its viewing direction relative to the nest (the nest direction was defined as 180 deg). We defined a stopping phase as a period of at least 100 ms during which the ant did not move forward and gazed in one direction. Of course, the duration of 100 ms is an arbitrary criterion. However, as *O. robustior* ants pause for about 150 ms (Müller and Wehner, 2010), and both *Cataglyphis* and *Ocymyrmex* desert ants are very fast (Wehner and Wehner, 2011), we are confident that we captured the vast majority of relevant stopping

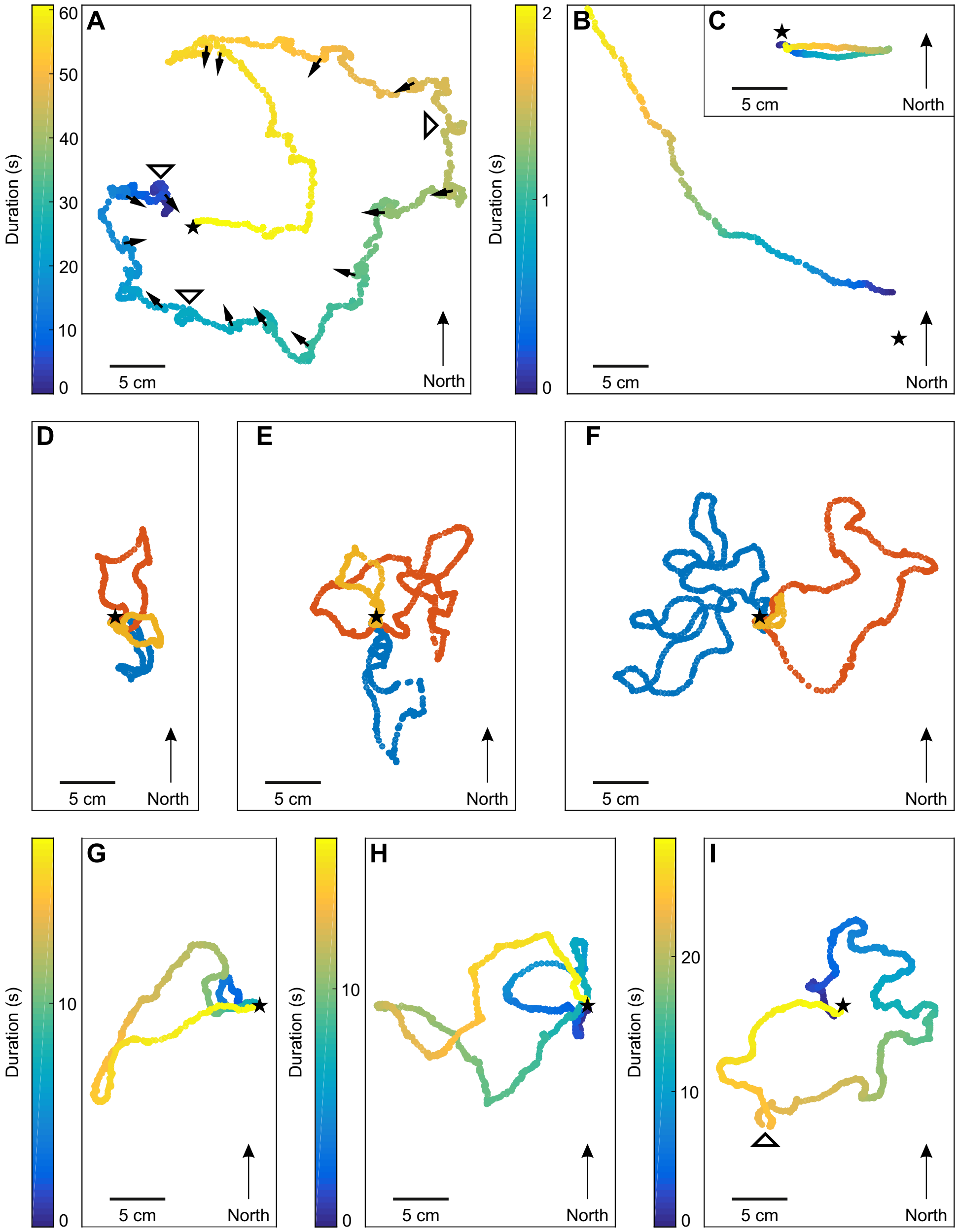

Fig. 2. Characteristics of learning walks in *Cataglyphis*. (A–C) Three paths walked by ants (*C. noda*) leaving the nest for different purposes recorded at 100 frames s^{-1}. (A) Typical learning walk circling around the nest entrance (black star), including several pirouettes, during which the ant looks back to the nest entrance (black arrows), and voltes, without stopping phases (white arrowheads). (B) An experienced forager leaves the nest in a straight line without any turns. (C) Typical path of a digger ant leaving the nest in a straight line, followed by a 180 deg turn and run back to the nest after depositing soil. Time is color coded in all traces. Note that the learning walk in A takes about 1 min, whereas the path of the forager in B and the digger in C disappears after 2 s outside the recording area or into the nest, respectively. (D–F) Successive learning walks of an individual ant (*C. noda*) during 1 day (18 July 2015) recorded at 25 frames s^{-1}. This individually marked ant was seen only once outside the nest in the morning before the recordings started. (D) The first three walks happened in short sequence at 11:05:05, 11:05:33 and 11:07:02 h local time. The durations were 14 s (yellow), 16 s (red) and 10 s (blue). After these explorative trips, the ant re-appeared outside the nest entrance at 11:07:21 h local time, became lost and searched for several minutes (4 min 55 s) to find its way back to the nest (not shown). (E) This ant immediately continued to perform learning walks at 11:12:26 h local time (7 s, yellow). The subsequent two learning walks were performed in short sequence, at 11:43:25 h (24 s, red) and 11:43:58 h (12 s, blue), respectively. (F) Three further learning walks directly followed the ones shown in E. The first of these three started at 11:45:06 h and was aborted (2 s, yellow) because the ant was frightened and returned to the nest immediately. The next learning walk occurred directly afterwards at 11:45:21 s (14 s, red). The last learning walk of this ant was recorded at 12:17:32 s (19 s, blue). During subsequent learning walks, the ant explored different sectors around the nest entrance. (G–I) Examples of three ants (*C. fortis*) performing a learning walk with no, one or three artificial landmarks recorded at 100 frames s^{-1}. (G) Without artificial landmarks. (H) One black cylinder, 0.4 m north of the nest entrance. (I) Three artificial landmarks positioned symmetrically at a 2 m distance around the nest entrance. Time is color coded in all traces. Note that the durations of learning walks depicted were slightly different (color bars). However, the learning walk durations of ants in different experimental groups were not significantly different between species and experimental setups (Fig. 3). A complete learning walk of *C. noda* recorded from the top as well as from the side is shown in Movie 1.

phases with this criterion. It is also noteworthy that the duration of the stopping phases in *Ocymyrmex* is temperature dependent (Müller and Wehner, 2010). However, the ground temperatures in Tunisia and Greece were similar [Tunisia: 41.7±9.3°C, 36.5–45.8°C; Greece: 47.2±21.6°C, 38.8–60.4°C; median±interquartile range (IQR: Q1–Q3), ranging from 31.3 to 51.4°C in Tunisia and from 26.6 to 66.3°C in Greece]. Concerning the gaze direction, we tolerated a deviation of 10 deg during a stopping phase. Multiple analyses of the same sequence showed that the accuracy for determining gaze direction is within this 10 deg limit. Based on these data, we also calculated the angular velocities of the turns.

Statistics

Because our data were not normally distributed, we report medians± IQR, calculated by subtracting the lower quartile (Q1) from the upper quartile (Q3). We compared the duration of learning walks and the number of turns per learning walk of our experimental groups using the Kruskal–Wallis test. The significance level was $\alpha=0.05$. If necessary, i.e. if there was a significant difference indicated by the Kruskal–Wallis test, we performed *post hoc* pairwise comparisons with the Mann–Whitney *U*-test with Bonferroni correction. The significance level was $\alpha=0.05$ (i.e. $\alpha=0.0084$ with Bonferroni correction, because we compared the four experimental groups: ‘*C. fortis* with no artificial landmark’, ‘*C. fortis* with one artificial landmark’, ‘*C. fortis* with three artificial landmarks’ and ‘*C. noda*’ pairwise with each other, resulting in six performed tests). We compared the number of stopping phases and the angular velocity of different types of turn pairwise within species using the Mann–Whitney *U*-test ($\alpha=0.05$). All these statistical tests were performed with Matlab R2014b (MathWorks, Inc.). To analyze gaze direction, we used the Rayleigh test to test for uniformity, i.e. whether the data were randomly distributed over the 360 deg. If the data were significantly directed ($\alpha=0.05$), we calculated the 95% confidence interval and checked whether the expected value, i.e. the direction of the nest (180 deg) was between the limits. All circular statistics were performed with Oriana (version 4.02, Kovach Computing Services, Pentraeth, UK).

RESULTS

Cataglyphis ants perform characteristic learning walks

Cataglyphis desert ants leave their nest for quite different reasons. When observing the happenings at the nest entrances of three *Cataglyphis* species (*C. noda*, *C. fortis* and *C. aenescens*) in two completely different environments (Fig. 1), three main reasons were identifiable, i.e. performing learning walks, digging/waste disposal and foraging (Fig. 2A–C). All these outdoor behaviors were clearly distinguishable. In this study, the focus was on the learning walks performed by the ants before starting their foraging careers (Fig. 2A). However, ants also left the nest to carry out nest-related tasks like waste disposal or digging behavior (Fig. 2C; see Harkness, 1977; Stieb et al., 2012), and of course to search for food (Fig. 2B). In addition, some ants carried out single nest mates or stood next to the nest entrance and guarded it from time to time as has been described in detail elsewhere (Harkness, 1977). Indoor workers that deposited soil or waste outside the nest moved very fast. They ran in a straight line outside the nest, dropped their item a few centimeters away [9.9±3.7 cm, 8.5–12.2 cm, median±IQR (Q1–Q3) dropping distance from the nest entrance, $n=20$], turned around by 180 deg immediately and ran back into the nest (Fig. 2C). Foragers also left the nest quickly and in a straight line. In contrast to diggers and learners, foragers moved far away from the nest entrance and left the recording area rapidly (Fig. 2B). Conversely, novices performed their learning walks at the beginning of their foraging career in close vicinity to the nest entrance, and therefore they remained inside our selected recording area, enabling us to analyze entire learning walks using high-speed video recordings. These explorative trips were very short [*C. noda* $n=25$: 10.4±9.3 s, 6.8–16.1 s; *C. fortis* without artificial landmarks $n=12$: 12.0±5.4 s, 11.2–16.6 s, median±IQR (Q1–Q3); Fig. 3A, Kruskal–Wallis test: duration of learning walks $\chi^2_3=3.98$, $n=66$, $P=0.27$]. No significant differences between the durations of the learning walks of the different species or setups were apparent (Fig. 3A). We marked some individual *C. noda* ants with a unique color code at their first appearance so that we could record subsequent learning walks of the same ants (Fig. 2D–F). Typically, *C. noda* moved further away from the nest entrance with increasing experience until they eventually

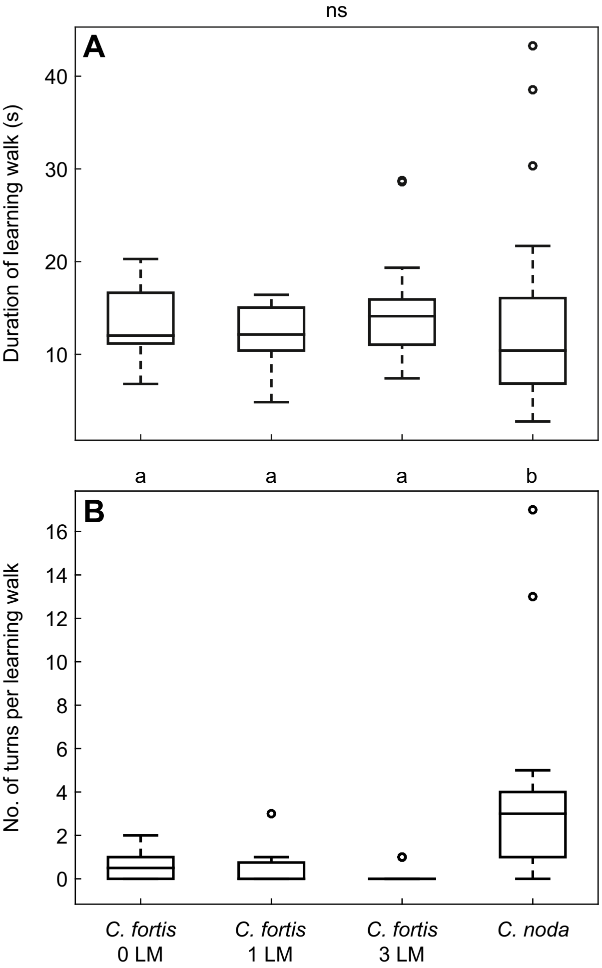

Fig. 3. Comparison of early learning walks performed by two different *Cataglyphis* species and under different conditions. The central mark of each boxplot represents the median, the edges of the boxes are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points (excluding outliers). Outliers are plotted individually as open circles. Different letters indicate significant differences between the groups when compared pairwise (Mann–Whitney *U*-test with Bonferroni correction, i.e. $\alpha=0.0084$). (A) The durations of early learning walks performed within our recording areas were not significantly different between the experimental conditions (*C. fortis* with no, one or three artificial landmarks) or between the two species. (B) The number of turns per learning walk was significantly different between species. However, there was no significant difference between the three experimental setups of *C. fortis*.

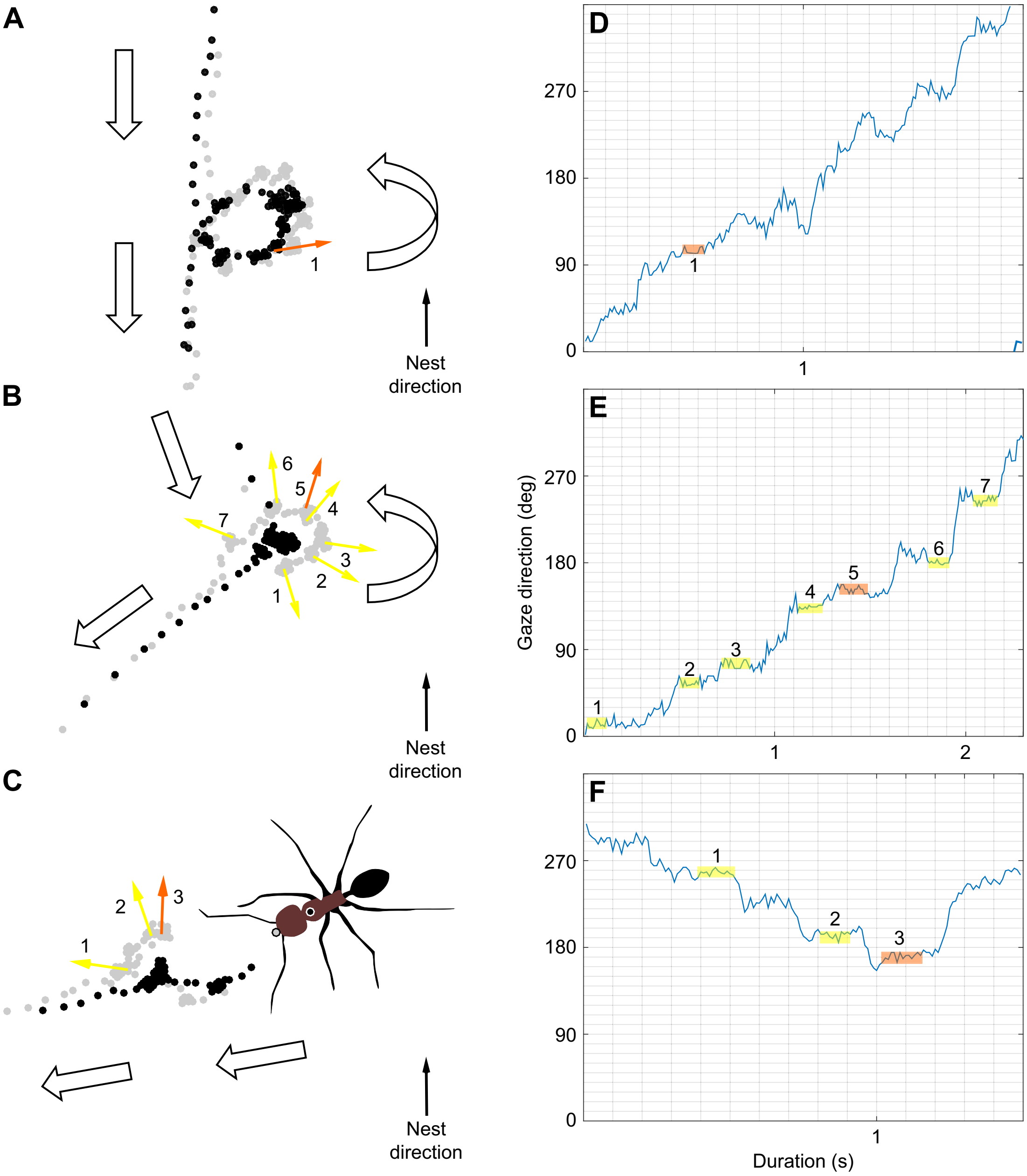

Fig. 4. Different types of turns. (A–C) The position of the mandibles and thorax was tracked to monitor the different types of turns. The dots show the position of the mandibles (gray) and the thorax (black) recorded every 10 ms (as illustrated in C). The walking and turning directions are indicated by white arrows. Black arrows point towards the nest. (A) A volte was defined as a walked circle. The tracks of the mandibles and thorax follow the same path. In contrast, (B) full or (C) partial pirouettes were defined as turns about the ant's own axis. The tracks of the mandibles circle around the position of the thorax. Stopping phases (>100 ms) are indicated by yellow and orange arrows (see D–F), and are numbered consecutively. (D–F) Gaze directions of the ants during these three turns with regard to the nest (180 deg) is plotted over time. A stopping phase is defined as an interval of at least 100 ms during which the ant does not move forward and does not change its gaze direction for more than 10 deg. The stopping phases of the turns are highlighted in yellow, and the longest stopping phase per turn is in orange. (D) The volte has only one stopping phase (100 ms), which is not directed towards the nest. (E) The full pirouette includes seven stopping phases of which no. 5 is the longest (180 ms). Stopping phase no. 6 (132 ms) is directed towards the nest. (F) The partial pirouette has three stopping phases of which the longest (no. 3, 185 ms) is directed towards the nest. In general, pirouettes include more stopping phases than voltes (Fig. 5). The underlying data are shown in Movie 1.

became foragers. The most obvious characteristic of *C. noda* learning walks were the frequent turns, during which the ants usually looked back to the nest. The ants performed up to 17 turns per learning walk in our recording area. The average number of turns was 3±3 [1–4, median±IQR (Q1–Q3), *n*=25; Fig. 3B]. In comparison to the Greek *C. noda* ants living in a pine forest, the Tunisian *C. fortis* ants inhabiting a saltpan performed fewer turns during their learning walks [0.5±1, 0–1, median±IQR (Q1–Q3), *n*=12; Fig. 3B; Kruskal–Wallis test: number of turns per walk χ^2_3=30.75, *n*=66, *P*<0.05, and *post hoc* pairwise comparison with Mann–Whitney *U*-test with Bonferroni correction: *C. noda* versus *C. fortis* without an artificial landmark (LM) z=3.41, n_{Cn}=25, $n_{Cf0\mathrm{LM}}$=12, *P*<0.0084; *C. noda* versus *C. fortis* with one artificial landmark z=4.03, n_{Cn}=25, $n_{Cf1\mathrm{LM}}$=15, *P*<0.0084; *C. noda* versus *C.*

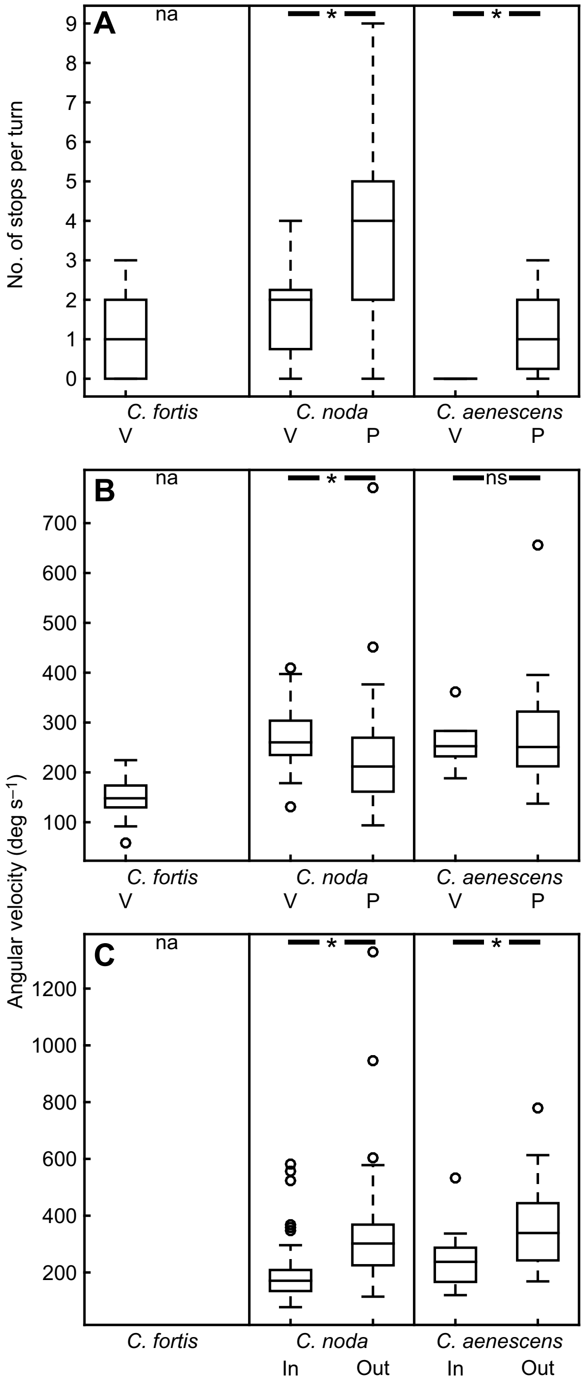

Fig. 5. Interspecies comparison of the different types of turn. The central mark of each boxplot represents the median, the edges of the boxes are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points (excluding outliers). Outliers are plotted individually as open circles. Data were compared pairwise within each species using the Mann–Whitney *U*-test when applicable. *Cataglyphis fortis* did not perform any pirouettes; therefore, no statistical comparison was possible (na, not applicable). Asterisks indicate significant differences ($P<0.05$), groups labeled 'ns' are not significantly different. (A) Number of stopping phases per turn. Pirouettes (P) of *C. noda* and *C. aenescens* include more stopping phases than do voltes (V). (B) Angular velocity during turns. The angular velocity of *C. noda* voltes was significantly higher than that of pirouettes, but there was no significant difference for *C. aenescens*. However, it is noteworthy that the angular velocities were quite different depending on the turning direction relative to the nest, i.e. whether the ant turned to the nest (turning-in) or back to continue the learning walk (turning-out). This becomes especially clear when examining the fine structure of the partial pirouettes performed by the Greek *Cataglyphis* ants. (C) Angular velocity of in- and out-turns of the partial pirouettes. Both species performing partial pirouettes, i.e. *C. noda* and *C. aenescens*, turn in with less speed than they turn out, as does the Namibian desert ant *O. robustior* (Müller and Wehner, 2010).

fortis with three artificial landmarks z=4.27, n_{Cn}=25, $n_{Cf3\mathrm{LM}}$=14, P<0.0084].

An artificial panorama in an otherwise featureless environment does not alter the learning walks in *C. fortis*

To check whether *C. fortis* did not perform frequent turns because of the absence of visual landmarks, we manipulated its natural habitat with two different settings of artificial landmarks. In one experiment, we installed a black cylinder 0.4 m north of the nest entrance (Fig. 1C; Movie 1); in another, three cylinders were placed at 2 m distance around the nest entrance (Fig. 1D). However, there was not any noticeable difference between the learning walks of *C. fortis* under the different conditions (Fig. 2G–I). The duration of the learning walks remained the same (Kruskal–Wallis test: duration of learning walks χ^2_3=3.98, n=66, P=0.27; Fig. 3A), and the number of turns per learning walk of *C. fortis* did not increase [one artificial landmark: 0±0.75, 0–0.75, median±IQR (Q1–Q3), n=15; three artificial landmarks: 0±0, 0–0, median±IQR (Q1–Q3), n=14; Fig. 3B]. There was no significant difference between the number of turns per walk in the three conditions under which *C. fortis* ants performed their learning walks (Fig. 3B; Kruskal–Wallis test: number of turns per walk χ^2_3=30.75, n=66, P<0.05, and *post hoc* pairwise comparison with Mann–Whitney *U*-test with Bonferroni correction: *C. fortis* without an artificial landmark versus *C. fortis* with one artificial landmark z=1.09, $n_{Cf0\mathrm{LM}}$=12, $n_{Cf1\mathrm{LM}}$=15, P=0.28; *C. fortis* without an artificial landmark versus *C. fortis* with three artificial landmarks z=1.55, $n_{Cf0\mathrm{LM}}$=12, $n_{Cf3\mathrm{LM}}$=14, P=0.13; *C. fortis* with one artificial landmark versus *C. fortis* with three artificial landmarks z=0.38, $n_{Cf1\mathrm{LM}}$=15, $n_{Cf3\mathrm{LM}}$=14, P=0.71). As stated above, *C. noda* made significantly more turns per learning walk than *C. fortis* even when artificial landmarks were available for the latter species (Fig. 3B).

Cataglyphis ants perform different types of turns with distinct features

Beside the difference in the number of turns per learning walk between *C. fortis* and *C. noda*, our high-speed recordings also revealed striking qualitative differences of turns across and within species. *Cataglyphis fortis* and *C. noda* included different types of turns in their learning walks (Fig. 4). *Cataglyphis fortis* ants performed only one type of turn during their learning walks: small walked circles, we termed voltes. During this type of turn, the ants moved forward on a circumference. Voltes were only rarely interrupted by stopping phases longer than 100 ms. The median number of stopping phases per volte of *C. fortis* was 1±2 [0–2, median±IQR (Q1–Q3), n=20; Fig. 5A]. *Cataglyphis noda* also performed this type of turn (Fig. 4A,D; Movie 1). During these voltes, *C. noda* stopped 2±1.5 times [0.75–2.25, median±IQR (Q1–Q3), n=17; Fig. 5A, Mann–Whitney *U*-test: *C. noda* voltes versus pirouettes z=−3.99, $n_{Cn\mathrm{V}}$=17, $n_{Cn\mathrm{P}}$=85, P<0.05]. However, *C. noda* much more frequently performed another, more saccadic, type of turn we termed pirouettes (as in Müller and Wehner, 2010). Over 80% of *C. noda* turns were pirouettes (85 out of 102 analyzed turns were pirouettes and only 17 were voltes). In contrast to a volte, a pirouette does not include any forward motion. Instead, to perform a pirouette, an ant stops walking and turns on its vertical axis without any translation (Fig. 4B,C; Movie 1). To re-establish its former walking direction, an ant has to turn back either continuing or reversing its direction of rotation. Therefore, pirouettes may be full 360 deg turns or partial turns (Fig. 4E,F; Movie 1). Usually, the ants took the shorter way back to re-establish their initial angular position – only in 12 partial pirouettes did ants cover an angle larger than 180 deg when turning back (n=74). Furthermore, the angular velocities of the back-turns were usually higher than those of the in-turns (Fig. 5C). Pirouettes were frequently interrupted by stopping phases defined as phases of at least 100 ms during which the ants did not move forward and gazed in one direction (±10 deg to compensate for tracking inaccuracies). *Cataglyphis noda* pirouettes included 4±3 [2–5, median±IQR (Q1–Q3), n=85] stopping phases, significantly more than their voltes (Fig. 5A). In addition, *C. noda* pirouettes had a lower angular velocity than their voltes (Fig. 5B, Mann–Whitney *U*-test: *C. noda* voltes versus pirouettes z=2.84, $n_{Cn\mathrm{V}}$=17, $n_{Cn\mathrm{P}}$=85, P<0.05). Interestingly, a smaller *Cataglyphis* species (*C. aenescens*) inhabiting the same clearings as *C. noda* in the Greek pine forest also performed both types of turns. Similar to *C. noda*, pirouettes in *C. aenescens* contained more stopping phases than voltes (Fig. 5A, Mann–Whitney *U*-test: *C. aenescens* voltes versus pirouettes z=−2.50, $n_{Ca\mathrm{V}}$=5, $n_{Ca\mathrm{P}}$=15, P<0.05); however, there was no significant difference in their angular velocity (Fig. 5B, z=0.00, $n_{Ca\mathrm{V}}$=5, $n_{Ca\mathrm{P}}$=15, P=1). In both *C. noda* and *C. aenescens*, the turning-in movements of the partial pirouettes were slower than the turning-out movement (Fig. 5C, Mann–Whitney *U*-test: *C. noda* turning-in versus turning-out z=−6.79, n_{Cni}=74, n_{Cno}=74, P<0.05; *C. aenescens* turning-in versus turning-out z=−2.04, n_{Cai}=14, n_{Cao}=14, P<0.05).

Only *Cataglyphis* species inhabiting cluttered environments perform pirouettes during which they turn back to the nest entrance

The striking feature of the saccadic pirouettes is that the ants turn back and look towards the nest entrance. As has been described for other desert ants (see *C. bicolor*: Wehner et al., 2004; *O. robustior*: Müller and Wehner, 2010), *C. noda* ants turned back to the nest (defined as the direction 180 deg), faced the nest entrance (which was invisible to them) and paused for a few hundred milliseconds [longest stopping phase per turn: 162±68 ms, 134–202 ms, median± IQR (Q1–Q3); minimum by definition 100 ms, maximum 469 ms, n=83]. However, *C. noda* ants only paused and fixated the position of the nest entrance during one type of turn, i.e. during pirouettes (Rayleigh test: z=30.48, n=83, P<0.05; 187.5 deg, mean vector, 174.7–200.2 deg, 95% confidence interval; Fig. 6A). In contrast, the gaze direction of *C. noda* ants during the voltes was randomly distributed (Rayleigh test: z=1.29, n=13, P=0.28; Fig. 6B). The same was true for *C. fortis* ants – they also did not fixate the nest direction during voltes (Rayleigh test: z=2.46, n=11, P=0.08; Fig. 6C). In contrast to *C. noda*, *C. fortis* never performed any pirouettes during which they stopped to look back to the nest entrance (Fig. 5). *Cataglyphis aenescens* ants inhabiting the same clearings in the Greek pine forest as *C. noda* also performed pirouettes and looked back to the nest entrance during the longest stopping phases (Rayleigh test: z=10.17, n=11, P<0.05; 183.5 deg, mean vector, 172.5–194.4 deg, 95% confidence interval; Fig. 6D).

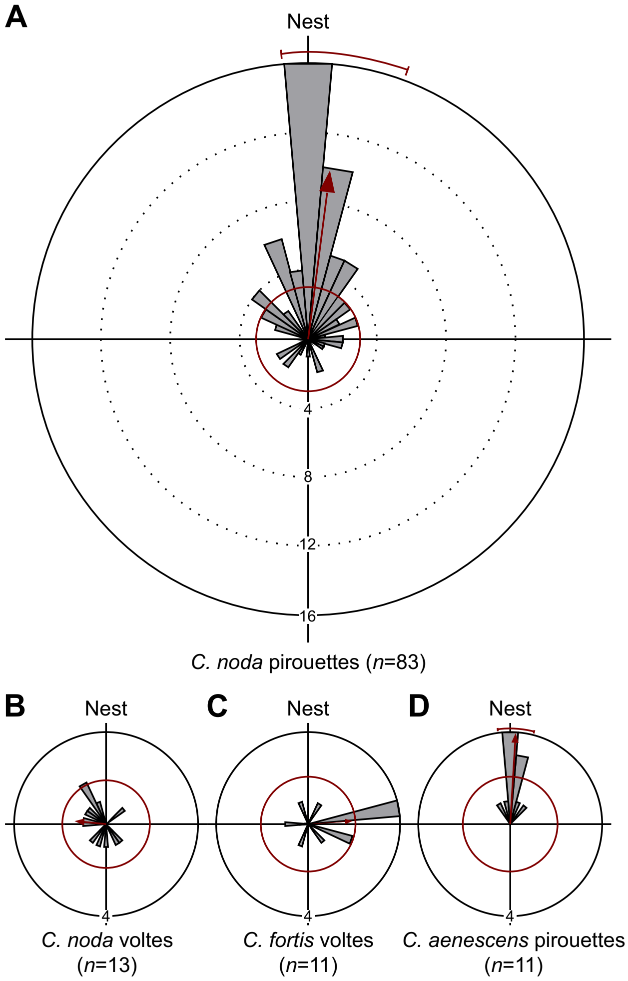

Fig. 6. Viewing directions during the longest stopping phases. Data are shown in gray and the corresponding statistics are shown in red. The width of the circular histogram bins was 10 deg. The red circle indicates Rayleigh's critical value α=0.05. The red arrow indicates the *r*-vector pointing in the mean direction. If the red arrow exceeds the red circle, the data are directed and not randomly distributed. In that case, the 95% confidence interval is also shown (red circular line). If the expected direction (nest) lies between the confidence interval limits, we assume that the ants look back at the nest entrance. (A) *Cataglyphis noda* mean gaze direction during the longest stopping phases of their pirouettes was not significantly different from the nest direction. (B) In contrast, *C. noda* volte gaze was non-directional. (C) The same was true for the gaze direction of *C. fortis* during the longest stopping phases of their voltes – there was no preferred viewing direction towards the nest. (D) *Cataglyphis aenescens* ants inhabit the same clearings as *C. noda* ants, and also looked back to the nest entrance during the longest stopping phases of their pirouettes. In these diagrams, only the turns that had at least one stopping phase (>100 ms) were included – all turns without stopping phases were disregarded here (see Fig. 5).

DISCUSSION

Conspicuous turns have been observed in several desert ant species (*C. fortis*: Fleischmann et al., 2016; Stieb et al., 2012; *C. bicolor*: Wehner et al., 2004; *C. aenescens*: Petrov, 1993; *Cataglyphis bombycina*: Wehner, 1994; Wehner and Wehner, 1990; *M. bagoti*: Wystrach et al., 2014; *O. robustior*: Müller and Wehner, 2010). However, these turns have never been directly compared with each other. In this study, we showed that both *C. noda* and *C. aenescens* inhabiting a pine forest in Greece (Fig. 1A) performed voltes as well as pirouettes during their learning walks. These different types of turns can be easily distinguished qualitatively – the former is a walked circle whereas the latter consists of a full or partial rotation about the body axis. However, there were also quantitative differences – the number of stops per turn was higher in pirouettes than in voltes in both species. Most importantly, *C.*

noda as well as *C. aenescens* only stopped to gaze back at the nest entrance during the pirouettes. Furthermore, in partial pirouettes, the turning-in rotations were slower than the turning-out rotations as is the case in *O. robustior* (Müller and Wehner, 2010). In contrast, *C. fortis* inhabiting a featureless saltpan in Tunisia (Fig. 1B) did not perform any pirouettes and did not look back at the nest. We manipulated the natural panorama around the nest entrance using artificial landmarks (Fig. 1C,D). However, even this enrichment of the visual environment did not induce the performance of pirouettes in *C. fortis* with pauses directed to the nest entrance, even though experiments showed that the ants learn these landmarks reliably during their learning walks (Fleischmann et al., 2016). It is noteworthy, however, that the artificial landmarks did not enrich the visual panorama comparable to the natural environment of *C. noda* (Fig. 1A versus B). Therefore, one might speculate that the panorama was not visually enhanced enough to induce the performance of pirouettes. We think this is unlikely, because other desert ants that live in cluttered but less structured environments than the pine forest of the Greek *Cataglyphis* ants in this study also perform turns and look back to the nest entrance repeatedly during their learning walks (Müller and Wehner, 2010; Wehner et al., 2004). Why is it that *C. fortis* does not invest any time in stopping and looking back at the nest entrance? As its natural habitat usually does not offer a prominent panorama and all directions look alike, it may not be worth making distinct snapshots of the panorama, as has been suggested for the Namibian desert ant *O. robustior* (Graham et al., 2010). These ants only stop once or twice during their back turns (Müller and Wehner, 2010). This suggests a possible correlation between the richness of the visual scene in the natural environment and the number of stopping phases during the frequent turns of the desert ants performing learning walks [i.e. *C. fortis*: no landmarks (saltpans and chotts) – exclusively voltes without stopping phases, *O. robustior*: few landmarks (a skyline of distant dunes and acacia trees) – only nest-centered stopping phases during pirouettes; Müller and Wehner, 2010; *C. noda* and *C. aenescens* (forest): many landmarks, i.e. a lot of visual information available – two types of turns with several stopping phases during pirouettes]. Further analyses should investigate whether fixation directions are associated with panorama features. Wood ants (*Formica rufa*), for example, look back to fixate a landmark when becoming familiar with a new feeder (Nicholson et al., 1999). Therefore, it is possible that desert ants might fixate prominent landmarks during the other stopping phases. However, systematic fixations of specific landmarks could not be observed by qualitative judgments, whereas the look back to the nest entrance behavior was as obvious in the Greek *Cataglyphis* species as in the Namibian *O. robustior*.

Besides the question of the purpose of the other stopping phases during the pirouettes, there are two other pressing questions: (1) what enables the ants to look back precisely towards the nest entrance?; and (2) why do *Cataglyphis* desert ants perform different types of turns? Concerning the first question, it has been proposed that the main navigational tool, i.e. path integration, offers the reference system needed to precisely gaze at the nest (Graham et al., 2010; Müller and Wehner, 2010). Further experiments need to be conducted to verify that path integration enables the Greek *Cataglyphis* ants in their landmark-rich habitat to turn back to the nest, as opposed to another mechanism, such as landmark guidance. Concerning the second question, it may be that there are anatomical reasons for performing different types of turn (Wehner, 1994). However, the behavioral trait to perform pirouettes is independent from morphological traits enabling different desert ant species to raise their gasters to improve their mobility (McMeeking et al., 2012). This indicates that the habitat, rather than anatomical constraints or phylogenetic relationships, exerts a selection pressure on the performance of the different types of turns.

Therefore, the second question has to be reformulated: what might be the function of the voltes performed exclusively (*C. fortis*) or in addition to other turns (*C. noda*, *C. aenescens*)? As mentioned above, the main navigational toolkit of *Cataglyphis* is path integration (Ronacher, 2008; Wehner, 2008). To determine the direction of their home vectors, the ants mainly rely on celestial cues, especially the polarization pattern and the azimuthal position of the sun (Wehner and Müller, 2006). In principle, compass information can be deduced from the polarization pattern by either a sequential or an instantaneous method (Wehner and Labhart, 2006). Voltes could provide the rotatory component necessary for the sequential method (Wehner, 1987b, 1994). Moreover, voltes may be used by desert ants to learn the configuration of landmarks close to the nest entrance even though they do not include stopping phases like pirouettes. Regardless, *C. fortis* ants learn the artificial landmarks surrounding the nest entrance even though they do not perform any pirouettes or other turns with distinct stopping phases (Fleischmann et al., 2016). It is not known how ants – or other animal species – store snapshots or other visual information in their brains. However, it was shown that short-term light exposure at the beginning of the ants' foraging careers has long-term effects on the synaptic architecture in visual subregions of the mushroom bodies, high order sensory integration and learning and memory centers (Stieb et al., 2010, 2012). Similarly, microglomerular synapses in the lateral complex, the last synaptic relay station in the neural pathway of the sky compass are influenced by light particularly in the UV region of the spectrum during first exposure (Schmitt et al., 2016). We hypothesize that visual information gained by different types of turns during learning walks may represent crucial elements triggering neuroplastic calibrations in visual pathways.

Interestingly, circular movements and rotatory motifs are important not only in the learning walks of desert ants but also in the orientation behavior of other arthropods. Learning flights of flying hymenoptera include repeated arcs, loops and turn-backs (honeybees: Becker, 1958; Capaldi and Dyer, 1999; Capaldi et al., 2000; Degen et al., 2015, 2016; Lehrer, 1991, 1993; Opfinger, 1931; Vollbehr, 1975; wasps: Peckham and Peckham, 1898; Stürzl et al., 2016; Tinbergen, 1932; Zeil, 1993a,b; Zeil et al., 1996; bumblebees: Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014; Robert et al., 2017; Wagner, 1907). Dung beetles perform rotations about their vertical axis before rolling a ball away from the dung pile (Baird et al., 2012), during which they take a snapshot of the celestial scenery (el Jundi et al., 2016). Desert spiders also perform learning walk-like behavior: they leave their burrows in sinusoidal paths when departing to unfamiliar terrains (Nørgaard et al., 2012).

In conclusion, different types of turns performed by desert ants (and other arthropods) during learning walks (or flights) are likely to serve different functions. Pirouettes during which the desert ants look back to the nest entrance are only performed by ants inhabiting cluttered environments (*C. noda* and *C. aenescens*). The stopping phases during pirouettes are most suited to taking snapshots (Graham et al., 2010; Müller and Wehner, 2010), suggesting that the ants take snapshots of the distant panorama around the nest entrance. Additionally, all *Cataglyphis* species investigated so far performed voltes, which may provide the rotational movement needed to successfully calibrate the celestial cues as compass tools or serve other navigational purposes like memorizing the configuration of nearby landmarks.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.N.F., R.G., R.W., W.R.; Methodology: P.N.F., R.G., R.W., W.R.; Software: P.N.F., R.G.; Validation: P.N.F., R.G.; Formal analysis: P.N.F., R.G.; Investigation: P.N.F., R.G.; Resources: W.R.; Data curation: P.N.F., R.G.; Writing - original draft: P.N.F.; Writing - review & editing: P.N.F., R.W., W.R.; Visualization: P.N.F.; Supervision: P.N.F., R.W., W.R.; Project administration: P.N.F., R.W., W.R.; Funding acquisition: R.W., W.R.

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Supplementary information

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References

Baird, E., Byrne, M. J., Smolka, J., Warrant, E. J. and Dacke, M. (2012). The dung beetle dance: an orientation behaviour? *PLoS ONE* **7**, e30211.

Becker, L. (1958). Untersuchungen über das Heimfindevermögen der Bienen. *Z. vergl. Physiol.* **41**, 1-25.

Buehlmann, C., Graham, P., Hansson, B. S. and Knaden, M. (2014). Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. *Curr. Biol.* **24**, 960-964.

Capaldi, E. A. and Dyer, F. C. (1999). The role of orientation flights on homing performance in honeybees. *J. Exp. Biol.* **202**, 1655-1666.

Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M. et al. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature* **403**, 537-540.

Collett, T. S., de Ibarra, N. H., Riabinina, O. Philippides, A. (2013). Coordinating compass-based and nest-based flight directions during bumblebee learning and return flights. *J. Exp. Biol.* **216**, 1105-1113.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H. et al. (2015). Exploratory behaviour of honeybees during orientation flights. *Anim. Behav.* **102**, 45-57.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H. et al. (2016). Honeybees learn landscape features during exploratory orientation flights. *Curr. Biol.* **26**, 2800-2804.

el Jundi, B., Foster, J. J., Khaldy, L., Byrne, M. J., Dacke, M. and Baird, E. (2016). A snapshot-based mechanism for celestial orientation. *Curr. Biol.* **26**, 1456-1462.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W. and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *J. Exp. Biol.* **219**, 3137-3145.

Graham, P., Philippides, A. and Baddeley, B. (2010). Animal cognition: multi-modal interactions in ant learning. *Curr. Biol.* **20**, R639-R640.

Harkness, R. D. (1977). The carrying of ants (*Cataglyphis bicolor* Fab.) by others of the same nest. *J. Zool. Proc. Zool. Soc. London* **183**, 419-430.

Hemple de Ibarra, N. H., Philippides, A., Riabinina, O. and Collett, T. S. (2009). Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. *J. Exp. Biol.* **212**, 3193-3204.

Jayatilaka, P. (2014). Individual foraging careers of the Jack Jumper ant, *Myrmecia croslandi*. *PhD thesis*, The Australian National University.

Lehrer, M. (1991). Bees which turn back and look. *Naturwissenschaften* **78**, 274-276.

Lehrer, M. (1993). Why do bees turn back and look? *J. Comp. Physiol. A* **172**, 549-563.

McMeeking, R. M., Arzt, E. and Wehner, R. (2012). *Cataglyphis* desert ants improve their mobility by raising the gaster. *J. Theor. Biol.* **297**, 17-25.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Curr. Biol.* **20**, 1368-1371.

Muser, B., Sommer, S., Wolf, H. and Wehner, R. (2005). Foraging ecology of the thermophilic Australian desert ant, *Melophorus bagoti*. *Aust. J. Zool.* **53**, 301-311.

Nicholson, D., Judd, S. P. D., Cartwright, B. A. and Collett, T. S. (1999). Learning walks and landmark guidance in wood ants (*Formica rufa*). *J. Exp. Biol.* **202**, 1831-1838.

Nørgaard, T., Gagnon, Y. L. and Warrant, E. J. (2012). Nocturnal homing: learning walks in a wandering spider? *PLoS ONE* **7**, e49263.

Opfinger, E. (1931). Über die Orientierung der Biene an der Futterquelle. *Z. Vgl. Physiol.* **15**, 431-487.

Osborne, J. L., Smith, A., Clark, S. J., Reynolds, D. R., Barron, M. C., Lim, K. S. and Reynolds, A. M. (2013). The ontogeny of bumblebee flight trajectories: from naïve explorers to experienced foragers. *PLoS ONE* **8**, e78681.

Peckham, G. W. and Peckham, E. G. (1898). *On the Instincts and Habits of the Solitary Wasps. Geological and Natural History Survey Bulletin, no. 2*, pp. 1-245. Madison, WI. http://dx.doi.org/10.5962/bhl.title.26182

Petrov, I. Z. (1993). Some remarks on the foraging strategy in *Cataglyphis aenescens* Nyl. (Hymenoptera, Formicidae). *Tiscia (Szeged)* **27**, 23-28.

Philippides, A., de Ibarra, N. H., Riabinina, O. and Collett, T. S. (2013). Bumblebee calligraphy: The design and control of flight motifs in the learning and return flights of *Bombus terrestris*. *J. Exp. Biol.* **216**, 1093-1104.

Riabinina, O., de Ibarra, N. H., Philippides, A. and Collett, T. S. (2014). Head movements and the optic flow generated during the learning flights of bumblebees. *J. Exp. Biol.* **217**, 2633-2642.

Robert, T., Frasnelli, E., Collett, T. S. and de Ibarra, N. H. (2017). Male bumblebees perform learning flights on leaving a flower but not when leaving their nest. *J. Exp. Biol.* **220**, 930-937.

Ronacher, B. (2008). Path integration as the basic navigation mechanism of the desert ant, *Cataglyphis fortis* (Forel, 1902) (Hymenoptera: Formicidae). *Myrmecological News* **11**, 55-62.

Schmid-Hempel, P. and Schmid-Hempel, R. (1984). Life duration and turnover of foragers in the ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Insectes Soc.* **31**, 345-360.

Schmitt, F., Stieb, S. M., Wehner, R. and Rössler, W. (2016). Experience-related reorganization of giant synapses in the lateral complex: Potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* **76**, 390-404.

Stieb, S. M., Muenz, T. S., Wehner, R. and Rössler, W. (2010). Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* **70**, 408-423.

Stieb, S. M., Hellwig, A., Wehner, R. and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* **72**, 729-742.

Stürzl, W., Zeil, J., Boeddeker, N. and Hemmi, J. M. (2016). How wasps acquire and use views for homing. *Curr. Biol.* **26**, 1-13.

Tinbergen, N. (1932). Über die Orientierung des Bienenwolfes. *Z. Vergl. Physiol.* **16**, 305-334.

Vollbehr, J. (1975). Zur Orientierung junger Honigbienen bei ihrem 1. Orientierungsflug. *Zool. Jb. Physiol.* **79**, 33-69.

Wagner, W. (1907). *Psycho-biologische Untersuchungen an Hummeln*. Stuttgart: E. Schweizerbartsche Verlagsbuchhandlung.

Wehner, R. (1983). Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise *Cataglyphis fortis* (Forel 1902) stat. nov. *Senckenbergiana biol.* **64**, 89-132.

Wehner, R. (1987a). Spatial organization of foraging behavior in individually searching desert ants, *Cataglyphis* (Sahara desert) and *Ocymyrmex* (Namib desert). In *From Individual to Collective Behavior in Social Insects* (ed. J. M. Pasteels and J.-L. Deneubourg), pp. 15-42. Basel, Bostom: Birkhäuser Verlag.

Wehner, R. (1987b). “Matched filters” - neural models of the external world. *J. Comp. Physiol. A* **161**, 511-531.

Wehner, R. (1994). The polarization-vision project: Championing organismic biology. In *Neural Basis of Behavioural Adaptations* (ed. K. Schildberger and N. Elsner), pp. 103-143. Stuttgart, Jena, New York: Gustav Fischer Verlag.

Wehner, R. (2008). The desert ant’ s navigational toolkit: Procedural rather than positional knowledge. *J. Inst. Navig.* **55**, 101-114.

Wehner, R. and Labhart, T. (2006). Polarisation vision. In *Invertebrate Vision* (ed. E. J. Warrant and D. E. Nilson), pp. 291-347. Cambridge: Cambridge University Press.

Wehner, R. and Müller, M. (2006). The significance of direct sunlight and polarized skylight in the ant’s celestial system of navigation. *Proc. Natl. Acad. Sci. USA* **103**, 12575-12579.

Wehner, R. and Rössler, W. (2013). Bounded plasticity in the desert ant's navigational tool kit. In *Invertebrate Learning and Memory* (ed. R. Menzel and P. R. Benjamin), pp. 514-529. New York: Academic Press.

Wehner, R. and Wehner, S. (1990). Insect navigation: use of maps or Ariadne's thread? *Ethol. Ecol. Evol.* **2**, 27-48.

Wehner, R. and Wehner, S. (2011). Parallel evolution of thermophilia: daily and seasonal foraging patterns of heat-adapted desert ants: *Cataglyphis* and *Ocymyrmex* species. *Physiol. Entomol.* **36**, 271-281.

Wehner, R., Harkness, R. D. and Schmid-Hempel, P. (1983). *Foraging Strategies in Individually Searching Ants Cataglyphis bicolor (Hymenoptera: Formicida)*. Stuttgart, New York: Gustav Fischer Verlag.

Wehner, R., Michel, B. and Antonsen, P. (1996). Visual navigation in insects: coupling of egocentric and geocentric information. *J. Exp. Biol.* **199**, 129-140.

Wehner, R., Meier, C. and Zollikofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor. Ecol. Entomol.* **29**, 240-250.

Wystrach, A., Philippides, A., Aurejac, A., Cheng, K. and Graham, P. (2014). Visual scanning behaviours and their role in the navigation of the Australian desert ant Melophorus bagoti. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* **200**, 615-626.

Zeil, J. (1993a). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) I. *Description of flight*. *J. Comp. Physiol. A* **172**, 189-205.

Zeil, J. (1993b). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) II. Similarities between orientation and return flights and the use of motion parallax. *J. Comp. Physiol. A* **172**, 207-222.

Zeil, J., Kelber, A. and Voss, R. (1996). Structure and function of learning flights in bees and wasps. *J. Exp. Biol.* **199**, 245-252.

4 Manuscript III: Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*

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Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*

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ABSTRACT

At the beginning of their foraging lives, desert ants (*Cataglyphis fortis*) are for the first time exposed to the visual world within which they henceforth must accomplish their navigational tasks. Their habitat, North African salt pans, is barren, and the nest entrance, a tiny hole in the ground, is almost invisible. Although natural landmarks are scarce and the ants mainly depend on path integration for returning to the starting point, they can also learn and use landmarks successfully to navigate through their largely featureless habitat. Here, we studied how the ants acquire this information at the beginning of their outdoor lives within a nest-surrounding array of three artificial black cylinders. Individually marked 'newcomers' exhibit a characteristic sequence of learning walks. The meandering learning walks covering all directions of the compass first occur only within a few centimeters of the nest entrance, but then increasingly widen, until after three to seven learning walks, foraging starts. When displaced to a distant test field in which an identical array of landmarks has been installed, the ants shift their search density peaks more closely to the fictive goal position, the more learning walks they have performed. These results suggest that learning of a visual landmark panorama around a goal is a gradual rather than an instantaneous process.

KEY WORDS: Landmark learning, Navigation, Experience-dependent behavior, Visual landmark, Central place forager, Path integration

INTRODUCTION

The crucial challenge for all central place foragers is to find their way back to their central place, e.g. the nest, successfully after foraging in the nest surroundings (for a review, see Wehner, 1992). In order to return safe and sound, a variety of information relevant for navigation must be detected, learned, memorized and retrieved by the animals in the right place at the right time.

After scavenging for dead insects in their hostile and largely featureless salt pan environment for distances of up to several hundred meters (Buehlmann et al., 2014), desert ants (*Cataglyphis fortis*) must successfully return to their inconspicuous nest entrance. It is this feat of navigation that made *C. fortis*, a prime example of a solitary central place forager, a model organism for navigation (for a review, see Wehner, 2008). The key to the ants' navigational success is path integration (Müller and Wehner, 1988; Wehner, 1982; for a review, see Collett and Collett, 2000).

The navigational performances of the desert ants become even more impressive if one considers the short time span within which the necessary information must be acquired. After undertaking tasks within the nest for about 4 weeks, the ants forage outside the nest only for less than a week (*Cataglyphis bicolor*: Schmid-Hempel and Schmid-Hempel, 1984). The life expectancy of *C. fortis* outside the nest has not been systematically determined yet, but Ziegler and Wehner (1997) mention 7.3 days (for a short survey comparing the forager survival frequencies of different desert ant species, see fig. 39.1 in Wehner and Rössler, 2013). During the rapid transition from indoor to outdoor life, the workers' behavior as well as its neuronal underpinnings change drastically (Stieb et al., 2010, 2012). In this transition phase, the ants exhibit a distinct behavioral trait, the so-called learning walks, similar to the learning flights described for bees and wasps (for two detailed recent studies, see Philippides et al., 2013; Stürzl et al., 2016). Such learning walks have previously been described for *C. bicolor* (Wehner et al., 2004). In the beginning, the ants perform several exploratory walks meandering around their nest entrance and including frequent body rotations. With increasing experience, the ants stay outside the nest for longer and longer times, and their foraging efficiency as well as their fidelity to a specific foraging sector increases. During the learning walks, the ants do not search for food, but most probably learn about the nest's surroundings (Wehner et al., 2004).

In contrast to their congeneric relatives, *C. fortis* ants occupy a featureless habitat devoid of shrubs or other prominent visual landmarks (Dillier and Wehner, 2004). Even though they are confronted with only a few obvious visual landmarks in their natural environment, and therefore rely predominantly on vector navigation (for a review, see Cheng et al., 2014), many studies have shown their ability to learn and use artificial landmarks successfully (for a review, see Wehner, 2008). It is important to note that landmarks may serve different navigational purposes. They may either help to pinpoint the goal, which may be the nest (Bregy et al., 2008; Knaden and Wehner, 2005; Wehner et al., 1996) or a feeding site (Bisch-Knaden and Wehner, 2003a; Wolf and Wehner, 2000), or guide the foragers *en route* (Andel and Wehner, 2004; Collett, 2010; Collett et al., 1992; Wehner et al., 1996). Furthermore, the ants may link so-called 'local vectors' to specific landmarks (Bisch-Knaden and Wehner, 2001; Collett et al., 1998, 2001). In general, the visual landmark memories of *C. fortis* are very stable and long lasting for up to the entire foraging life span (Ziegler and Wehner, 1997). A landmark memory is most robust when acquired close to the nest entrance (Bisch-Knaden and Wehner, 2003b). These studies consistently show that *C. fortis* uses visual landmarks as navigational aids. In the present study, we show how much experience *C. fortis* desert ants need in order to gather enough information about visual landmarks for finally pinpointing the position of the goal, i.e. the nest. We confronted the ants with an artificial landmark panorama around their nest entrance and designed a two-stage approach to investigate the following two

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aspects. (1) The movements of individually marked ants were analyzed through systematic observation and recording of paths for their entire life span outside the nest. (2) Displacement experiments were used in order to test how exactly the ants located the position of their nest entrance, after they had performed various numbers of learning walks. We found that the ants acquire the information about the landmark panorama around the nest entrance gradually rather than instantaneously.

MATERIALS AND METHODS

Test animals and study site

We performed this study with *Cataglyphis fortis* (Forel 1902) desert ants in the summer of 2014 at a saltpan near Menzel Chaker, Tunisia (34°57′N, 10°24′E). The two different colonies used for the experiment were located towards the middle of the saltpan where almost no natural landmarks in the nest's vicinity occur. The experienced foragers of the ant colonies were marked for 3 days before the experiment started. After this period, we considered all unmarked ants as newcomers, which did not have experience above ground. These individuals were caught and were either tested immediately or marked with a unique multi-color code using car paint (Motip Lackstift Acryl, MOTIP DUPLI GmbH, Haßmersheim, Germany).

Experimental procedure

In order to enable accurate recordings of the ants' paths, we painted a net (10 m×10 m) with diluted wall paint around the nest entrance of each colony (Fig. 1A). The grid had 1 m^2 squares and the inner 4 m^2 around the nest entrance were additionally subdivided with a grid size of 0.2 m. Three artificial landmarks which consisted of black cylinders (height: 38 cm, diameter: 22 cm) were placed at a 2 m distance north (0 deg), southeast (120 deg) and southwest (240 deg) of the nest entrance. The ants were able to leave the nest during the experiment in the daytime, but not in the evening, at night or in the early morning, as we covered the nest entrance. This enabled us to record all appearances of the ants, i.e. all learning walks and foraging trips, outside the nest. Furthermore, we recorded the paths of individual ants as often as possible using squared paper. The last walk of individually marked ants before the test was always recorded. We caught the test animals shortly before they entered their nest. Therefore, the ants' path integrator had been reset to zero ('zero-vector ants'), so that the animals had to rely solely on landmark information when being tested. The distant test field offered a similar array of three artificial landmarks (Fig. 1B). After capture, the ants were immediately transferred in the dark in plastic tubes to the test field. They were released at one of three possible positions to avoid a location-dependent bias. The release points were located 3 m away from the fictive nest position between the landmarks (60, 180 or 300 deg, respectively). After releasing the ant within a plastic ring, we offered a cookie crumb and noted whether the ant picked it up or not. Afterwards, we recorded the ant's path for 5 min. Each ant was tested only once and then removed from the experiment (i) to avoid recapture and (ii) to favor the occurrence of newcomers.

Data analysis

For digitizing the walks, the protocol sheets were scanned with a resolution of 300 dpi. The resulting images were then further processed in a customized application for Android devices written with MIT Appinventor (www.appinventor.mit.edu), which we designed and programmed for this task. Specifically, the scans were loaded into the application running on an Xperia Z2 tablet (Sony, resolution: 1920 pixels×1200 pixels) and the ants' walks were traced on the screen with a tablet pen (Jot Pro Fine Point Stylus, Adonit). With a physical size of 18 cm×18 cm, the representation of the 10 m×10 m grid on the screen was comparable to that on the datasheets, so the digitization procedure did not deteriorate the precision of the data. Before saving the data, the walks could be rotated so as to superimpose the release points. The dataset saved by the application contained both a list of coordinates along the path and a pixel array (200 pixels×200 pixels, 1 pixel=0.05 m) encoding whether the ant crossed a pixel or not. The former was used for a quantitative analysis of the results while the latter was used for creating false color maps.

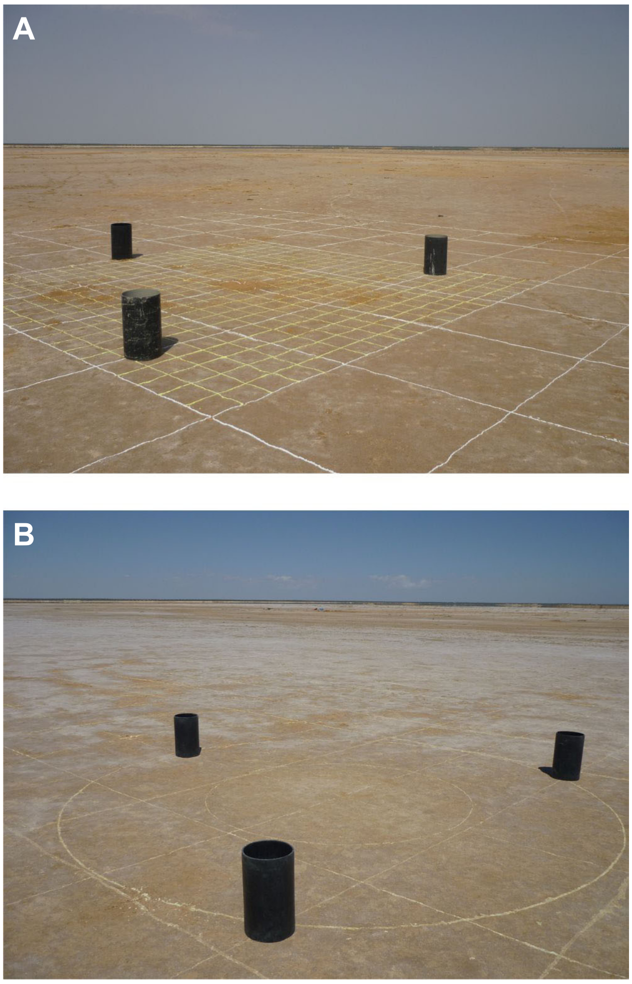

Fig. 1. Two identical landmark arrays. Three artificial landmarks offer a specific panorama at the nest (A) and the test field (B).

Categorization

We categorized the test animals based on the previous individual experience of the ants. (1) For the first category, unmarked ('black') ants were caught and tested after their first appearance, i.e. we considered them to be naive and without any experience in the field. They stayed within a 0.3 m radius around the nest entrance. (2) The second category contained individually marked ants that had performed only short learning walks less than 0.7 m away from the nest. They did not forage. (3) For the third category, we pooled all ants that performed long learning walks or their first foraging trip. These ants had moved more than 0.7 m from the nest before the test.

However, they did not leave the nest in a straight line, but followed a more circuitous route, including many loops. In this category, learning walks may blend into foraging walks (see e.g. Fig S1G–I). This shows that learning about nest-defining landmarks may continue during the first phase of foraging. (4) In contrast, the experienced foragers in the fourth category moved much faster and ran off in a straight line. They often stayed away for several minutes and usually returned with a food item before being tested. In Fig. S2A, the four categories of experience are correlated with the number of appearances outside the nest. Of course, any segmentation of a phenomenologically continuous process is subject to some degree of arbitrariness. However, as the categorization chosen here correlates very well with the learning success, we feel that the designation of categories 1–4 represents an appropriate approach. (5) The last category contained all ants that we marked before the start of the experiment. These ants were experienced foragers as well, but they first moved within their nest surroundings without the artificial landmark panorama and thus can be considered as 're-learners'. As we do not have any information about the individual history of this group of ants before the test, category 5 might be a rather inhomogeneous group of ants. As such, we include them only for a rough comparison.

Statistics

To compare the five categories of experience regarding the proportion of ants that (i) took a food item on the test field and (ii) directly crossed the fictive nest position during their search on the test field, we used Fisher's exact test (two-sided) with Bonferroni–Holm correction. The significance level was $\alpha=0.05$.

To compare the ants of different categories with regard to their search performance, we first plotted all searches of one category in a false-color map indicating how many ants crossed any given 0.2 m×0.2 m pixel on the test field. The origin of the false-color map is the fictive position of the nest. We then calculated the median position of the ants' search center from all x- and y-coordinates. Afterwards, we determined the search center of the groups, which is the median of the individuals' median search positions of one category (see Pfeffer et al., 2015). We compared the search accuracy as well as the search precision of the ants belonging to the different categories with the Kruskal–Wallis test and *post hoc* pairwise comparisons with the Mann–Whitney U-test with Bonferroni–Holm correction. The significance level was $\alpha=0.05$. The 'search accuracy' was defined as the median distance of the individual's search center to the fictive nest position, and the 'search precision' as the median distance of the individual's path to its corresponding search center (see Pfeffer et al., 2015). To evaluate search accuracy and precision, we used only the ants that stayed on the test field for the total time of 5 min. However, all test animals were included in the heat map visualization and the bar graphs. To compare the categories regarding other quantitative aspects (like number of appearances, number of turns and duration of trips outside the nest), we also used Kruskal–Wallis test and *post hoc* pairwise comparisons with the Mann–Whitney U-test with Bonferroni–Holm correction. All statistics were performed with Matlab R2014b (MathWorks, Inc., Natick, MA, USA).

RESULTS

At the nest site: ants exhibit a characteristic ontogeny of learning walks before they start foraging

Our long-term observation of what happened at the nest entrance revealed that the behavior of individually identifiable ants changed drastically over time when leaving the nest: with increasing experience (where 'experience' is defined as the number of appearances outside the nest; Fig. S2A), the ants moved further away from the nest entrance in a gradual manner and they did so with increasing straightness (Fig. 2; Figs S1 and S2). Based on these behavioral differences, we categorized the ants into five different categories (see Materials and methods). In the beginning, the ants (category 1; Fig. 2A; Fig. S1A–C) left the nest only for a short period of time (less than a minute; Fig. S2B) and moved a distance of only a few centimeters from the nest (less than 0.3 m). During these first learning walks, the ants were very timid and easily scared. When caught at their first appearance to be marked individually, only four of 42 ants reappeared on the same day. The vast majority, more than two-thirds (27 of 42 ants), returned the next day and started their foraging career then. The remaining test animals reappeared 2 or 3 days after being marked (eight and three ants, respectively). In category 2, the ants made up to four very short learning walks after being marked at their first appearance (Fig. 2B). These learning walks did not take them further than 0.7 m from the nest entrance (median maximal distance from the nest entrance: 0.41 m). With more experience, the ants covered longer distances (category 3; Fig. 2C–E; Fig. S1D–H). Nine of the 15 ants in category 3 left the nest field before being captured for the test (Fig. S2C). The remaining six ants moved a few meters from the nest (median maximal distance from the nest: 2.18 m). The characteristics of all these further learning walks were relatively slow movements and winding paths. The ants of category 3 had significantly less-straight paths than the experienced foragers (Fig. S2D). Furthermore, these paths included full-turns frequently and significantly more often than the paths of experienced foragers (in category 3 and 4, the median number of turns per run was eight and one, respectively; Mann–Whitney U-test: category 3 versus 4, $z=-3.571$, $N_3=15$, $N_4=20$, $P<0.05$). These turns occurred particularly in the beginning of the ants' outbound trip (see examples in Fig. 2 and Fig. S1). During the learning walks, ants seemed to explore the full range of azimuthal directions (though it was not possible to analyze dominant axes of the trajectories in detail; examples are given in Fig. 2E and Fig. S1D–F). If the ants came across a food item on the nest field, they returned to their nest in a straight line (Fig. 2D,F). The ants needed at least three appearances outside the nest before they abandoned their orientation behavior and started foraging successfully (Fig. S2A). However, some ants made up to seven extensive learning walks (category 3). Their paths straightened with increasing experience (an example is given in Fig. S1G–I). Usually, the ants started to forage around their second day outside the nest, then they disappeared from the nest field in the saltpan and left the nest for several minutes. The duration of the outbound trips increased significantly from category 1 to category 4 (Fig. S2B). Experienced foragers (category 4) generally left the nest field and returned to the nest in a straight line (Fig. 2F, green paths).

In the test field: increasing numbers of learning walks improve the accuracy of pinpointing the goal

The test animals were captured close to their nest entrance at different stages of experience and afterwards released in the distant test field. There, a landmark array identical to the one surrounding the nest entrance was set up. As the ants were devoid of any vector information (zero-vector ants), they had to rely solely on the landmarks when searching for the nest. A look at the search paths of individual ants reveals the main result: naive ants and ants with only a little experience (category 1 and 2, respectively) immediately searched at the release point (category 1, Fig. 3A, and category 2,

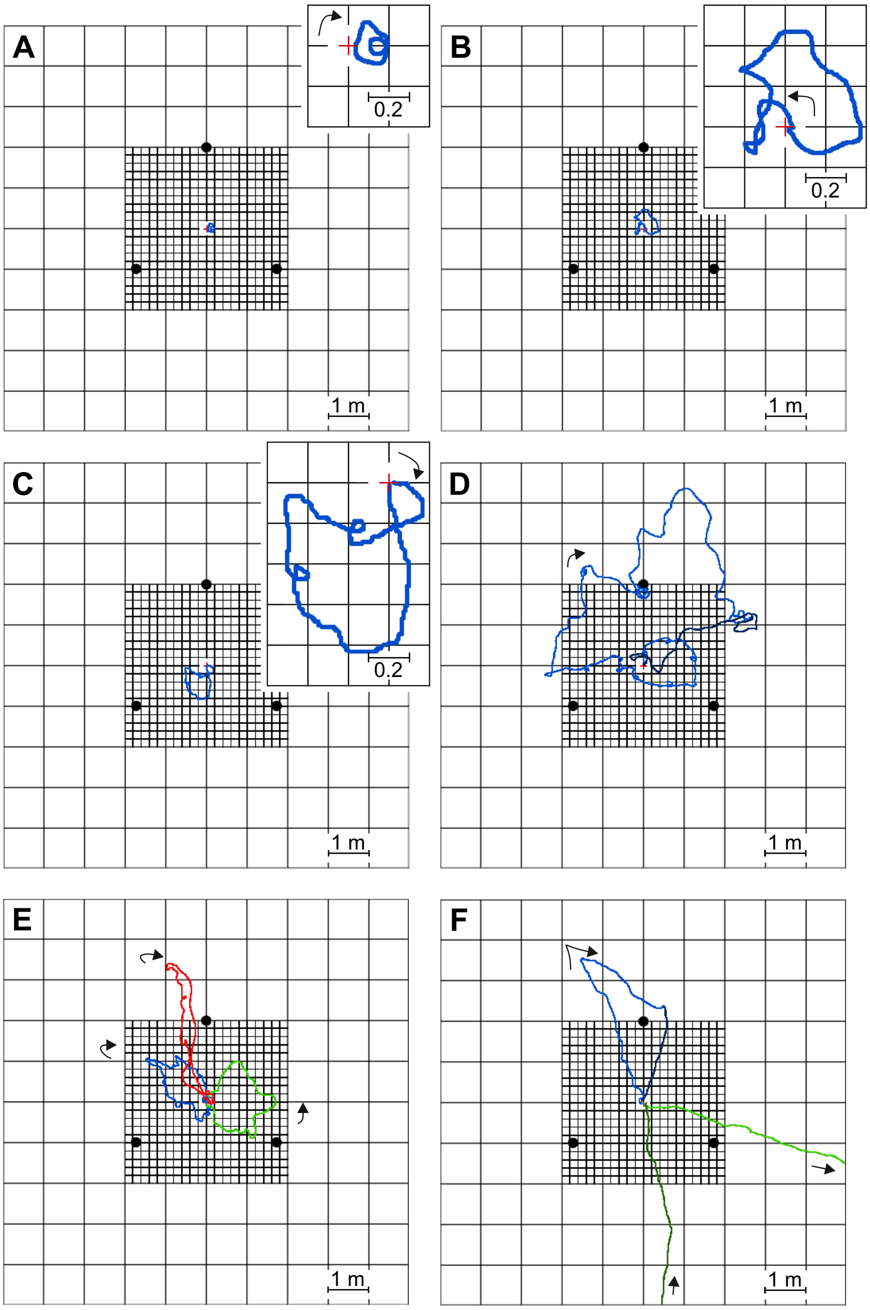

Fig. 2. Ontogeny of learning walks. (A) First learning walk of a naive ant (category 1). (B) Second learning walk of another ant (category 2). (C) Third and (D) fourth walk of another individual (category 3). (D) This ant found food for the first time before being tested (light blue: outbound walk, dark blue: homeward run after finding a food item). (E) Three successive learning walks of another ant (no. 4 – blue, no. 5 – green and no. 12 – red) and its (F) first (light blue: outbound walk, dark blue: homeward run after finding a food item) and last foraging trip (light green: outbound run, dark green: homeward run) (category 4). The nest entrance is located in the middle of the landmark array (+) and surrounded by three landmarks (black filled circles). The grid size corresponds to 1 m on the nest field (the inner 4 m×4 m are additionally subdivided into 0.2 m×0.2 m boxes). The insets in A–C show the short paths at higher magnification (grid size corresponds to 0.2 m). The black arrows show the direction of the ants' paths.

Fig. S3A–C), whereas experienced foragers directly proceeded to the fictive nest entrance position, where they started searching for their nest (category 4, Fig. 3C and Fig. S3G–I). Ants that had made long learning walks or had finished their first foraging trip before being tested (category 3) proceeded towards the fictive nest position in the middle of the landmark array, and their search was centered in between the release point and the fictive nest position (Fig. 3B; Fig. S3D–F).

Integrating the ants' searching paths of either category into false-color maps (Fig. 3D–F; Fig. S4) illustrates the results more impressively. With increasing experience in the nest area, the ants shifted their search centers more from the point of release toward the fictive position of the nest entrance (Fig. S5). Calculating the accuracy (Fig. 4A) and the precision (Fig. 4B) of the ants' searches allowed for statistical comparison of the groups, which revealed significant differences between them (Kruskal–Wallis test: search accuracy, χ_4^2=310,68, N=54, P<0.001; search precision, χ_4^2=14,42, N=54, P=0.007). Naive ants (category 1) and inexperienced ants (category 2) mainly focused their searches around the point of release (Fig. 3D; Fig. S4A; Mann–Whitney U-test with Bonferroni–Holm correction: category 1 versus 2, z=−0.888, N_1=12, N_2=7, P=0.375). Therefore, the distance between the fictive position of the nest entrance and their search centers was large and, consequently, the search accuracies differed from those of the more experienced foragers, which searched closer to the fictive nest entrance position (Mann–Whitney U-test with Bonferroni–Holm correction: category 1 versus 4, z=4.22, N_1=12, N_4=15, P<0.005; 1 versus 5, z=3.48, N_1=12, N_5=11, P<0.006; 2 versus 4, z=3.60, N_2=7, N_4=15,

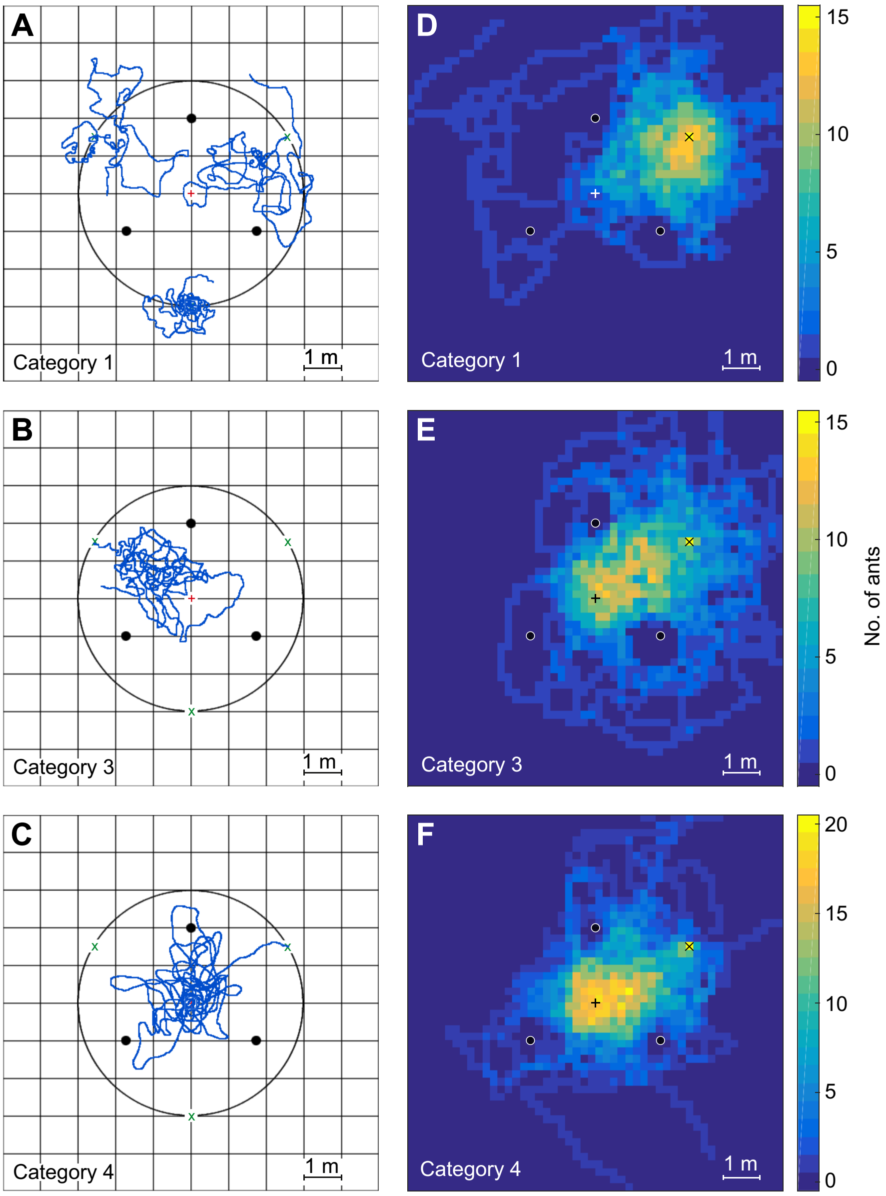

Fig. 3. Examples of searching paths on the test field and false-color maps of ants in three categories of experience. (A–C) Searching paths of three naive ants (category 1; A), an ant with more experience (category 3; B) and an experienced forager (category 4; C). (D–F) Superimposed searching paths of all ants in category 1 (naive ants, N=15; D), category 3 (ants that made long learning walks or their first foraging run, N=15; E) and category 4 (experienced foragers, N=20; F). The fictive position of the nest entrance is located in the middle of the test field (+) surrounded by an identical landmark array to that at the nest (black filled circles). The release points (×) lie 3 m from the fictive nest entrance position. The grid size in A–C corresponds to 1 m on the test field. Each pixel of the false-color map in D–F is equivalent to a 0.2 m×0.2 m square on the test field. Dark blue pixels have not been visited by any ant, whereas yellow indicates that all ants of a category crossed the pixel (the number of test ants for each corresponding color is given next to the color bar).

P<0.005; 2 versus 5, z=3.08, N_2=7, N_5=11, P<0.007). With increasing experience (category 3), the ants searched between the point of release and the fictive nest entrance position (Fig. 3E). Their search accuracy was not significantly different from that of the other groups (Mann–Whitney U-test with Bonferroni–Holm correction: category 1 versus 3, z=2.60, N_1=12, N_3=9, P=0.010; 2 versus 3, z=2.44, N_2=7, N_3=9, P=0.015; 3 versus 4, z=2.21, N_3=9, N_4=15, P=0.028; 3 versus 5, z=1, N_3=9, N_5=11, P=0.288). All experienced foragers (categories 4 and 5) concentrated their searches around the fictive nest entrance position (Fig. 3F; Fig. S4B). There was no significant difference between the search accuracies of ants that had learned only the artificial landmark array and those that had already foraged prior to the installation of the landmarks and hence had to re-learn the panorama around the nest (Mann–Whitney U-test with Bonferroni–Holm correction: category 4 versus 5, z=−0.83, N_4=15, N_5=11, P=0.407). All categories showed similar search precision, ranging from 0.5 to 1.4 m (Fig. 4B). The groups did not differ significantly when compared pairwise (Mann–Whitney U-test with Bonferroni–Holm correction: category 1 versus 2, z=0, N_1=12, N_2=7, P=1; category 1 versus 3, z=−2.45, N_1=12, N_3=9, P=0.015; category 1 versus 4, z=−2.22, N_1=12, N_4=15, P=0.027; category 1 versus 5, z=−2.49, N_1=12, N_5=11, P=0.013; category 2 versus 3, z=−2.11, N_2=7, N_3=9, P=0.035; category 2 versus 4, z=−2.32, N_2=7, N_4=15, P=0.020; category 2 versus 5, z=−2.08, N_2=7, N_5=11, P=0.038; category 3 versus 4, z=1.20, N_3=9, N_4=15, P=0.233; category 3 versus 5, z=0.23, N_3=9, N_5=11, P=0.820; category 4 versus 5, z=−1.34, N_4=15, N_5=11, P=0.178).

Experienced foragers (categories 4 and 5) were most successful in carrying food items homeward. Two measures illustrate their success in particular. First, with increasing experience, the ants were more likely to take a cookie crumb after being released on the test field than those with less experience (Fig. 5A). Naive ants (category 1) took a cookie crumb significantly less often than experienced foragers (Fisher's exact test with Bonferroni–Holm correction: category 1 versus 4, P<0.0055; 1 versus 5, P<0.005). The

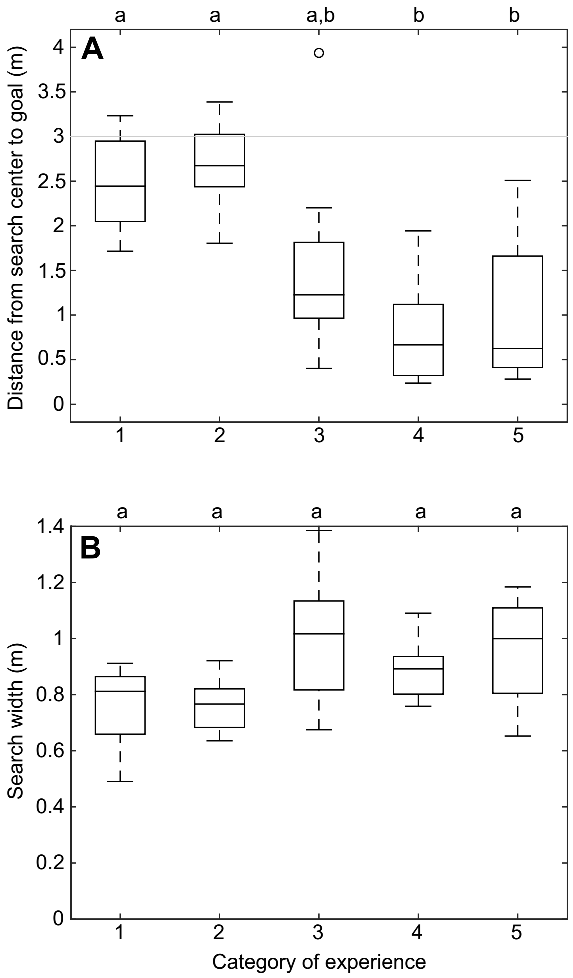

Fig. 4. Search accuracy and search precision of the ants in the five categories of experience. (A) Search accuracy was calculated as the distance between the search center and goal. (B) Search precision is the distance from the path to the search center (i.e. search width). The central mark is the median, the edges of the boxes are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points (excluding outliers). Outliers are plotted individually as circles. Number of ants: category 1, *N*=12; category 2, *N*=7; category 3, *N*=9; category 4, *N*=15; and category 5, *N*=11. Different letters indicate significant differences between the groups (*post hoc* Mann–Whitney *U*-test with Bonferroni–Holm correction; for details see Results).

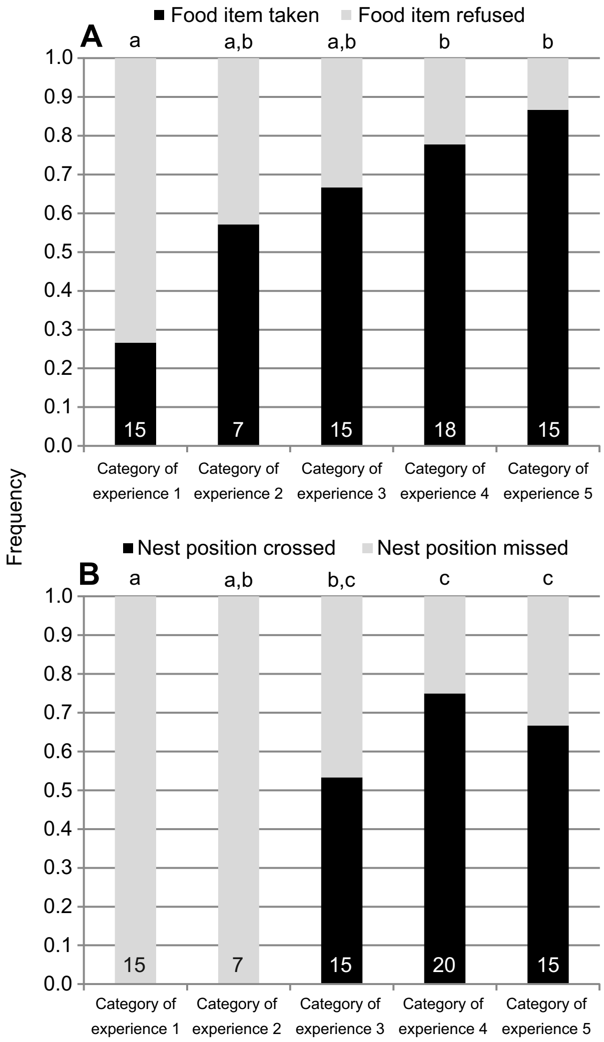

Fig. 5. Foraging success. Proportion of ants that (A) took a cookie crumb and (B) crossed the fictive nest position in the test field. Number of ants (*N*) is shown in the bars. Different letters indicate significant differences between the categories (Fisher's exact test with Bonferroni–Holm correction; for details see Results). The number of tested ants is given in parentheses (for two ants in category 4, it was not clear whether a crumb was taken or refused).

proportion of ants picking up a food item increased from category 1 to 5, but these differences were not significant between the other groups when compared pairwise (Fisher's exact test with Bonferroni–Holm correction: category 1 versus 2, *P*=0.343; category 1 versus 3, *P*=0.066; category 2 versus 3, *P*=1; category 2 versus 4, *P*=0.356; category 2 versus 5, *P*=0.275; category 3 versus 4, *P*=0.697; category 3 versus 5, *P*=0.390; category 4 versus 5, *P*=0.665). Second, with increasing experience, the ants were more likely to cross the fictive position of the nest on the test field (Fig. 5B). Only very few ants of categories 1 and 2 came close to the fictive position of the nest entrance (category 1, Fig. 3D and category 2, Fig. S4A; for an example of an individual path, see Fig. 3A ant starting at the northeast release point). However, even these ants never crossed the fictive nest entrance position and, hence, the results were significantly different from those of the more experienced foragers (Fisher's exact test with Bonferroni–Holm correction: category 1 versus 3, *P*<0.007; 1 versus 4, *P*<0.005; 1 versus 5, *P*<0.005; 2 versus 4, *P*<0.006; 2 versus 5, *P*<0.008). The increase in the number of ants crossing the fictive nest entrance from category 2 to 3 was visible, yet not statistically significant (category 2 versus 3, *P*=0.023). In addition, there were no significant differences between the proportion of ants reaching the fictive nest entrance in categories 3, 4 and 5 (category 3 versus 4, *P*=0.283; 3 versus 5, *P*=0.711; 4 versus 5, *P*=0.712).

DISCUSSION

The ants' rapid transition from intranidal workers to extranidal foragers offers the opportunity to comprehensively record the ants' first spatial activities outside the nest – the sequence of learning walks – and the subsequent foraging journeys, ideally throughout the ants' entire foraging lives. Here, we have taken this opportunity to investigate how the ants' accuracy in local visual homing increases with the number of learning walks performed by the ants around the nest entrance. We show that the number of learning walks, the distance by which the ants move away from the nest entrance during the learning walks and the accuracy with which they subsequently pinpoint the nest site are strongly correlated.

Cataglyphis fortis exhibits a characteristic ontogeny of learning walks

Several studies have investigated the learning flights of bees and wasps and the learning walks of ants at their nests or feedings sites (honeybees: Becker, 1958; Vollbehr, 1975; Lehrer, 1991, 1993; Capaldi and Dyer, 1999; bumblebees: Hempel de Ibarra et al., 2009; wasps: Zeil, 1993a, 1993b; Stürzl et al., 2016; wood ants: Judd and Collett, 1998; Nicholson et al., 1999; desert ants: Müller and Wehner, 2010). However, only very few studies have specifically described the ontogeny from the start of learning walks or flights at the beginning of the forager's career to the foraging trips of experienced foragers (desert ants: Wehner et al., 2004; honeybees: Capaldi et al., 2000; Degen et al., 2015; bumblebees: Osborne et al., 2013). The first learning walks and flights all share some common features: when leaving the nest for the first time, the animals meander around the nest entrance including circular movements or rotations in their trajectories. These motion sequences were described in great detail for the first time in solitary wasps (Zeil, 1993a). Since then, similar observations have been made for other species (desert ants: Müller and Wehner, 2010; bumblebees: Philippides et al., 2013). Usually, the newcomers explore the nest's surroundings without bringing back any food items. This distinction between the learning and foraging phase is not as clear in bumblebees as it is in honeybees and ants, because the former occasionally bring home pollen after their first trip outside the nest (Hempel de Ibarra et al., 2009; Osborne et al., 2013). With increasing experience, the bees' maximal range, distance traveled, area covered and ground speed increase significantly (honeybees: Capaldi et al., 2000; Degen et al., 2015; bumblebees: Hempel de Ibarra et al., 2009). Desert ants (*C. bicolor*) also stay longer outside the nest with more experience. They cover longer distances and they forage more efficiently. Furthermore, after exploring all directions during their learning walks, successful *Cataglyphis* ants preferentially stick for a considerable foraging time to one sector (Wehner, 1987; Wehner et al., 2004). Some basic features in the structure of *C. fortis*' learning walks have been described before (Stieb et al., 2012). Our constant and long-term observations close to the nest entrance confirm and extend these findings. We demonstrate that *C. fortis* ants undergo a distinct ontogeny of their learning walks until they become foragers when confronted with an artificial landmark panorama. The learning walks in the beginning of the desert ants' foraging life are slow, more twisted and much shorter than the foraging runs of experienced ants. With increasing experience, the ants move further away from the nest and eventually start foraging. The next step will be to examine the learning walks around the nest entrance by using high-speed video analyses, in order to analyze the fine structure of the walks in more detail. A recent computational study showed that successful orientation is best achieved with a large visual field and low resolution (Wystrach et al., 2015). Interestingly, *C. fortis*' visual field covers a huge area (about 93%) of the unit sphere and remains constant with varying body size of the ants, whereas the number of ommatidia, and hence the resolution of the compound eyes, varies with different body and thus eye sizes (Wehner, 1983; Wehner et al., 2014; Zollikofer et al., 1995). Therefore, *C. fortis* meets the conditions of a visual system that is evolutionarily optimized for navigation by panoramic landmark cues.

Landmark learning requires stable memory formation

In the beginning of their foraging lives, ants – and other insects – must adjust their orientation systems and acquire various kinds of visual information (for a review, see Zeil, 2012). Outside the nest, they have to learn possible landmark cues around their home, and they have to calibrate their celestial compass. Here, we used an artificial landmark panorama to show that the desert ants' learning walks are crucial prerequisites for successful homing. After only three to seven appearances and learning walks outside the nest, *C. fortis* starts to forage. The ants' rapid transition from a naive newcomer performing a few learning walks to an experienced and successful forager is thus another example of the impressive speed of navigational learning in insects with short (foraging) life spans (Collett, 1998). Intriguingly, the same amount of time that the ants need to perform their learning walks, i.e. usually 2–3 days, is necessary after first exposure to light pulses to induce neuronal changes in visual subregions of the mushroom bodies of *C. fortis* (Stieb et al., 2010, 2012). A recent computational study revealed that the desert ant mushroom body circuit has the capacity to store hundreds of independent images taken by an ant during its outbound trip, and to distinguish these from other deceptive, yet very similar images when looking off-route (Ardin et al., 2016). Formation of long-term memory (LTM) requires at least 1 day (early LTM), and 3 days (late LTM) to be consolidated as a stable LTM (for review, see Menzel, 2001). Studies in honeybees (Hourcade et al., 2010) and in leaf-cutting ants (Falibene et al., 2015) show that the formation of transcription-dependent, stable olfactory LTM is associated with structural synaptic changes in olfactory subregions of the mushroom bodies after 2–3 days. Hence, we hypothesize that the duration of the learning walks performed by the ants at the beginning of their foraging lives is correlated with the time needed to establish a robust LTM of stable nest surroundings, which in turn is necessary for successful homing.

Naive newcomers are not yet in foraging mood

Experienced foragers picked up a cookie crumb on the test field significantly more often than naive newcomers did. This difference in behavior certainly reflects the ants' motivational state of foraging. It has been shown before that navigational states can influence the motivation to perform other kinds of behavior. For example, if *C. fortis* ants are repeatedly forced to return to the nest guided exclusively by landmarks and hence to put their path integrator into an unusually large negative state, they drop their cookie crumb and express largely reduced escape behavior when approached by the experimenter (Andel and Wehner, 2004). Furthermore, the state of the path integrator can control the state of aggressiveness of *C. fortis*: zero-vector ants showed higher levels of aggressiveness than ants that had run off only a quarter of their home vector (Knaden and Wehner, 2004). Additionally, motivational state may determine whether an ant continues its trip inward or outward: Australian desert ants (*Melophorus bagoti*) link their route memories to inbound or outbound states of their foraging excursions, and these states may determine whether or not the ants retrieve their route memories (Wehner et al., 2006). Furthermore, wood ants (*Formica rufa*) choose different routes depending on their feeding state – if fed, they choose the homeward pattern, whereas unfed ants follow the route with the foodward pattern (Harris et al., 2005). Remarkably, landmark cues can also change the desert ants' motivation from homing to foraging. If *C. fortis* is disturbed on its way to a feeder, it usually returns to its nest. However, if landmarks are available to guide the ants to a familiar feeder, they continue their outbound trip (Merkle and

Wehner, 2008). In our experiment, all ants were motivated to return to the nest. However, experienced foragers were willing to accept food items when released on the test field, whereas less experienced ants refused food items and returned to their nest immediately. Hence, the ants' motivation to forage is dependent on their previous experience and may indicate their confidence in the ability to relocate the nest successfully.

With increasing experience, desert ants locate their goal more accurately and precisely

In most cases, experienced foragers pick up a food item, run straight into the middle of the landmark array and center their search on the test field around the fictive nest entrance (Cheng et al., 2014; Wehner and Räber, 1979; Wehner et al., 1996). In contrast, naive ants search at the release point. If desert ants are lost, they pursue a systematic search strategy. Their search pattern consists of several loops in all azimuthal directions starting and ending at the origin, i.e. the place where the ants presume the nest position to be (*Cataglyphis*: Müller and Wehner, 1994; Wehner and Srinivasan, 1981; *M. bagoti*: Schultheiss and Cheng, 2011). This symmetric search pattern was observed in our study as well (Fig. 3A–C; Fig. S3). All ants exhibited similar search widths, and there was not a significant difference between the categories of experience in regard to their search precision. In contrast, there are clear differences between the categories of experience with respect to search accuracy. The more experienced the ants are, the closer they search to the fictive position of the nest entrance (Figs 3, 4A; Fig. S3–S5). This shows that the ants have learned the landmarks and use the landmark information to search for the nest. Moreover, the increase of search accuracy from naive ants to experienced foragers does not occur instantaneously. If the learning process were instantaneous, ants in category 3 would split into two groups, namely (i) ants that have already learned the landmark panorama and (ii) ants that have not. However, with more experience, the ants shift their searches towards the fictive position of the nest entrance. Ants of category 3 searched at intermediate positions (see Fig. 3B,E; Fig. S3D–F), indicating a gradual (or stepwise) improvement of their learning success.

Novel learning and re-learning exhibit similar characteristics

The gradual acquisition of visual cues by desert ants orientating with artificial landmarks has been previously shown in re-learning paradigms. In these experiments, experienced foragers were confronted with an altered environment and consequently had to adapt their behavior to the new conditions. Faced with an artificial landmark array consisting of four cylinders, the Australian desert ant *M. bagoti* gradually increased its search performance for the nest on a test field when trained with a different number of trials over different numbers of days (Narendra et al., 2007). As *C. fortis* did in our replacement experiment, the proportion of ants crossing the fictive nest entrance between the landmarks increased with more experience (Narendra et al., 2007). Furthermore, the search time of the Namibian desert ant (*Ocymyrmex robustior*) until entering the nest decreased significantly from day to day when the entrance of a nest in a landmark-free environment had been furnished with two artificial cylinders (Wehner and Müller, 2010). As we found with our category 5 ants, and as a number of previous experiments (*C. bicolor*: Wehner and Räber, 1979; *C. fortis*: Wehner et al., 1996; *M. bagoti*: Narendra et al., 2007; for a review, see Cheng et al., 2014) have shown, desert ants can use artificial landmarks for orientation successfully, even if they had learned the natural surroundings of their nest before. Our observations suggest that the behavioral patterns exhibited during the acquisition as well as the recall of landmark orientation show striking similarities between new learners and re-learners. Future studies should investigate these similarities in more detail and examine whether specific sections of learning walks (e.g. the characteristic and frequent turns) are necessary prerequisites for the learning process, as has been shown recently in the learning flights of wasps (Stürzl et al., 2016). Furthermore, it will be interesting to investigate the interplay of the landmark learning and the initial calibration of the celestial compass that must happen at the same time. In addition, detailed neurobiological studies, preferably using the same animals that have participated in landmark learning paradigms, may help to us to understand the neuronal processes underlying the novel learning and re-learning behavioral traits.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

R.W. and W.R. led the study. R.W. and P.N.F. designed the experiment. P.N.F. and M.C. conducted the experiment. P.N.F. and V.L.M. designed and programmed the app for data analysis. P.N.F., M.C. and V.L.M. analyzed the data. P.N.F. wrote the manuscript, and R.W. and W.R. revised the manuscript. All authors participated in discussing the results and checking the paper.

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Supplementary information

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References

Andel, D. and Wehner, R. (2004). Path integration in desert ants, *Cataglyphis*: how to make a homing ant run away from home. *Proc. R. Soc. B Biol. Sci.* **271**, 1485-1489.

Ardin, P., Peng, F., Mangan, M., Lagogiannis, K. and Webb, B. (2016). Using an insect mushroom body circuit to encode route memory in complex natural environments. *PLOS Comput. Biol.* **12**, e1004683.

Becker, L. (1958). Untersuchungen über das Heimfindevermögen der Bienen. *Z. Vergl. Physiol.* **41**, 1-25.

Bisch-Knaden, S. and Wehner, R. (2001). Egocentric information helps desert ants to navigate around familiar obstacles. *J. Exp. Biol.* **204**, 4177-4184.

Bisch-Knaden, S. and Wehner, R. (2003a). Local vectors in desert ants: context-dependent landmark learning during outbound and homebound runs. *J. Comp. Physiol. A* **189**, 181-187.

Bisch-Knaden, S. and Wehner, R. (2003b). Landmark memories are more robust when acquired at the nest site than en route: experiments in desert ants. *Naturwissenschaften* **90**, 127-130.

Bregy, P., Sommer, S. and Wehner, R. (2008). Nest-mark orientation versus vector navigation in desert ants. *J. Exp. Biol.* **211**, 1868-1873.

Buehlmann, C., Graham, P., Hansson, B. S. and Knaden, M. (2014). Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. *Curr. Biol.* **24**, 960-964.

Capaldi, E. A. and Dyer, F. C. (1999). The role of orientation flights on homing performance in honeybees. *J. Exp. Biol.* **202**, 1655-1666.

Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M. et al. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature* **403**, 537-540.

Cheng, K., Schultheiss, P., Schwarz, S., Wystrach, A. and Wehner, R. (2014). Beginnings of a synthetic approach to desert ant navigation. *Behav. Processes* **102**, 51-61.

Collett, T. S. (1998). Rapid navigational learning in insects with a short lifespan. *Conn. Sci.* **10**, 255-270.

Collett, M. (2010). How desert ants use a visual landmark for guidance along a habitual route. *Proc. Natl. Acad. Sci. USA* **107**, 11638-11643.

Collett, M. and Collett, T. S. (2000). How do insects use path integration for their navigation? *Biol. Cybern.* **83**, 245-259.

Collett, T. S., Dillmann, E., Giger, A. and Wehner, R. (1992). Visual landmarks and route following in desert ants. *J. Comp. Physiol. A* **170**, 435-442.

Collett, M., Collett, T. S., Bisch, S. and Wehner, R. (1998). Local and global vectors in desert ant navigation. *Nature* **394**, 269-272.

Collett, T. S., Collett, M. and Wehner, R. (2001). The guidance of desert ants by extended landmarks. *J. Exp. Biol.* **204**, 1635-1639.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H. et al. (2015). Exploratory behaviour of honeybees during orientation flights. *Anim. Behav.* **102**, 45-57.

Dillier, F.-X. and Wehner, R. (2004). Spatio-temporal patterns of colony distribution in monodomous and polydomous species of North African desert ants, genus *Cataglyphis*. *Insectes Soc.* **51**, 186-196.

Falibene, A., Roces, F. and Rössler, W. (2015). Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. *Front. Behav. Neurosci.* **9**, 84.

Harris, R. A., Hempel de Ibarra, N., Graham, P. and Collett, T. S. (2005). Ant navigation: priming of visual route memories. *Nature* **438**, 302.

Hempel de Ibarra, N., Philippides, A., Riabinina, O. and Collett, T. S. (2009). Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. *J. Exp. Biol.* **212**, 3193-3204.

Hourcade, B., Münz, T. S., Sandoz, J.-C., Rössler, W. and Devaud, J.-M. (2010). Long-term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? *J. Neurosci.* **30**, 6461-6465.

Judd, S. P. D. and Collett, T. S. (1998). Multiple stored views and landmark guidance in ants. *Nature* **392**, 710-714.

Knaden, M. and Wehner, R. (2004). Path integration in desert ants controls aggressiveness. *Science* **305**, 60.

Knaden, M. and Wehner, R. (2005). Nest mark orientation in desert ants *Cataglyphis*: what does it do to the path integrator? *Anim. Behav.* **70**, 1349-1354.

Lehrer, M. (1991). Bees which turn back and look. *Naturwissenschaften* **78**, 274-276.

Lehrer, M. (1993). Why do bees turn back and look? *J. Comp. Physiol. A* **172**, 549-563.

Menzel, R. (2001). Searching for the memory trace in a mini-brain, the honeybee. *Learn. Mem.* **8**, 53-62.

Merkle, T. and Wehner, R. (2008). Landmark cues can change the motivational state of desert ant foragers. *J. Comp. Physiol. A* **194**, 395-403.

Müller, M. and Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proc. Natl. Acad. Sci. USA.* **85**, 5287-5290.

Müller, M. and Wehner, R. (1994). The hidden spiral: systematic search and path integration in desert ants, *Cataglyphis fortis*. *J. Comp. Physiol. A* **175**, 525-530.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Curr. Biol.* **20**, 1368-1371.

Narendra, A., Si, A., Sulikowski, D. and Cheng, K. (2007). Learning, retention and coding of nest-associated visual cues by the Australian desert ant, *Melophorus bagoti*. *Behav. Ecol. Sociobiol.* **61**, 1543-1553.

Nicholson, D., Judd, S. P. D., Cartwright, B. A. and Collett, T. S. (1999). Learning walks and landmark guidance in wood ants (*Formica rufa*). *J. Exp. Biol.* **202**, 1831-1838.

Osborne, J. L., Smith, A., Clark, S. J., Reynolds, D. R., Barron, M. C., Lim, K. S. and Reynolds, A. M. (2013). The ontogeny of bumblebee flight trajectories: from naïve explorers to experienced foragers. *PLoS ONE* **8**, e78681.

Pfeffer, S. E., Bolek, S., Wolf, H. and Wittlinger, M. (2015). Nest and food search behaviour in desert ants, *Cataglyphis*: a critical comparison. *Anim. Cogn.* **18**, 885-894.

Philippides, A., de Ibarra, N. H., Riabinina, O. and Collett, T. S. (2013). Bumblebee calligraphy: the design and control of flight motifs in the learning and return flights of *Bombus terrestris*. *J. Exp. Biol.* **216**, 1093-1104.

Schmid-Hempel, P. and Schmid-Hempel, R. (1984). Life duration and turnover of foragers in the ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Insectes Soc.* **31**, 345-360.

Schultheiss, P. and Cheng, K. (2011). Finding the nest: inbound searching behaviour in the Australian desert ant, *Melophorus bagoti*. *Anim. Behav.* **81**, 1031-1038.

Stieb, S. M., Münz, T. S., Wehner, R. and Rössler, W. (2010). Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* **70**, 408-423.

Stieb, S. M., Hellwig, A., Wehner, R. and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* **72**, 729-742.

Stürzl, W., Zeil, J., Boeddeker, N. and Hemmi, J. M. (2016). How wasps acquire and use views for homing. *Curr. Biol.* **26**, 1-13.

Vollbehr, J. (1975). Zur orientierung junger honigbienen bei ihrem 1. Orientierungsflug. *Zool. Jb. Physiol.* **79**, 33-69.

Wehner, R. (1982). Himmelsnavigation bei insekten. Neurophysiologie und verhalten. *Neujahrsbl. Naturf. Ges. Zürich.* **184**, 1-132.

Wehner, R. (1983). Taxonomie, funktionsmorphologie und zoogeographie der saharischen wüstenameise *cataglyphis fortis* (Forel 1902) stat. nov. *Senckenbergiana Biol.* **64**, 89-132.

Wehner, R. (1987). Spatial organization of foraging behaviour in individually searching desert ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). *Experientia Suppl.* **54**, 15-42.

Wehner, R. (1992). Arthropods. In *Animal Homing* (ed. F. Papi), pp. 45-144. London: Chapman and Hall.

Wehner, R. (2008). The desert ant's navigational toolkit: procedural rather than positional knowledge. *J. Inst. Navig.* **55**, 101-114.

Wehner, R. and Müller, M. (2010). Piloting in desert ants: pinpointing the goal by discrete landmarks. *J. Exp. Biol.* **213**, 4174-4179.

Wehner, R. and Räber, F. (1979). Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). *Experentia* **35**, 1569-1571.

Wehner, R. and Rössler, W. (2013). Bounded plasticity in the desert ant's navigational tool kit. In *Invertebrate Learning and Memory* (ed. R. Menzel and P. R. Benjamin), pp. 514-529. San Diego: Academic Press.

Wehner, R. and Srinivasan, M. V. (1981). Searching behaviour of desert ants, genus *Cataglyphis*. *J. Comp. Physiol. A* **142**, 315-338.

Wehner, R., Michel, B. and Antonsen, P. (1996). Visual navigation in insects: coupling of egocentric and geocentric information. *J. Exp. Biol.* **199**, 129-140.

Wehner, R., Meier, C. and Zollikhofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecol. Entomol.* **29**, 240-250.

Wehner, R., Boyer, M., Loertscher, F., Sommer, S. and Menzi, U. (2006). Ant navigation: one-way routes rather than maps. *Curr. Biol.* **16**, 75-79.

Wehner, R., Cheng, K. and Cruse, H. (2014). Visual navigation strategies in insects: Lessons from desert ants. In *The new visual neurosciences* (ed. J. S. Werner and L. M. Chalupa), pp. 1153-1163. Cambridge: MIT Press.

Wolf, H. and Wehner, R. (2000). Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. *J. Exp. Biol.* **203**, 857-868.

Wystrach, A., Dewar, A., Philippides, A. and Graham, P. (2015). How do field of view and resolution affect the information content of panoramic scenes for visual navigation? A computational investigation. *J. Comp. Physiol. A* **202**, 87-95.

Zeil, J. (1993a). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) I. Description of flight. *J. Comp. Physiol. A* **172**, 189-205.

Zeil, J. (1993b). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) II. Similarities between orientation and return flights and the use of motion parallax. *J. Comp. Physiol. A* **172**, 207-222.

Zeil, J. (2012). Visual homing: an insect perspective. *Curr. Opin. Neurobiol.* **22**, 285-293.

Ziegler, P. E. and Wehner, R. (1997). Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. *J. Comp. Physiol. A* **181**, 13-20.

Zollikofer, C., Wehner, R. and Fukushi, T. (1995). Optical scaling in conspecific *Cataglyphis ants*. *J. Exp. Biol.* **198**, 1637-1646.

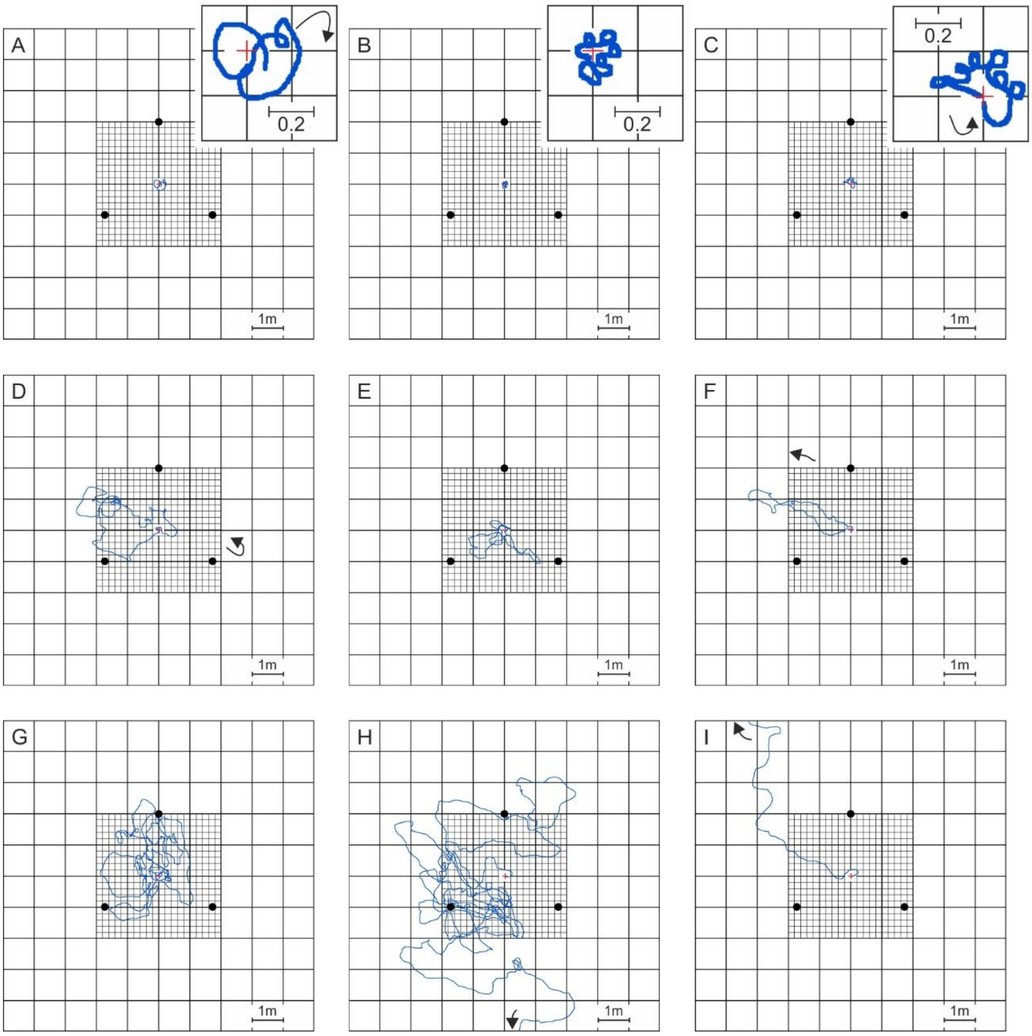

Fig. S1 Additional examples of learning walks and foraging trips on the nest field. (A-C) First learning walks of three ants (category 1). (D-F) Three subsequent walks of the same ant (category 3) exploring all directions. (G-I) Three trips no. 3 (G), no. 6 (H) and no. 7 (I) of the same ant. The nest entrance is located in the middle of the landmark array (+) and surrounded by three landmarks (black filled circles). The grid size corresponds to 1 m on the nest field (the inner 4 m x 4 m are additionally subdivided into 0.2 m x 0.2 m boxes). The insets in A, B and C show the short paths in higher magnification (grid size corresponds to 0.2 m). The black arrows show the direction of the ants' paths (where applicable).

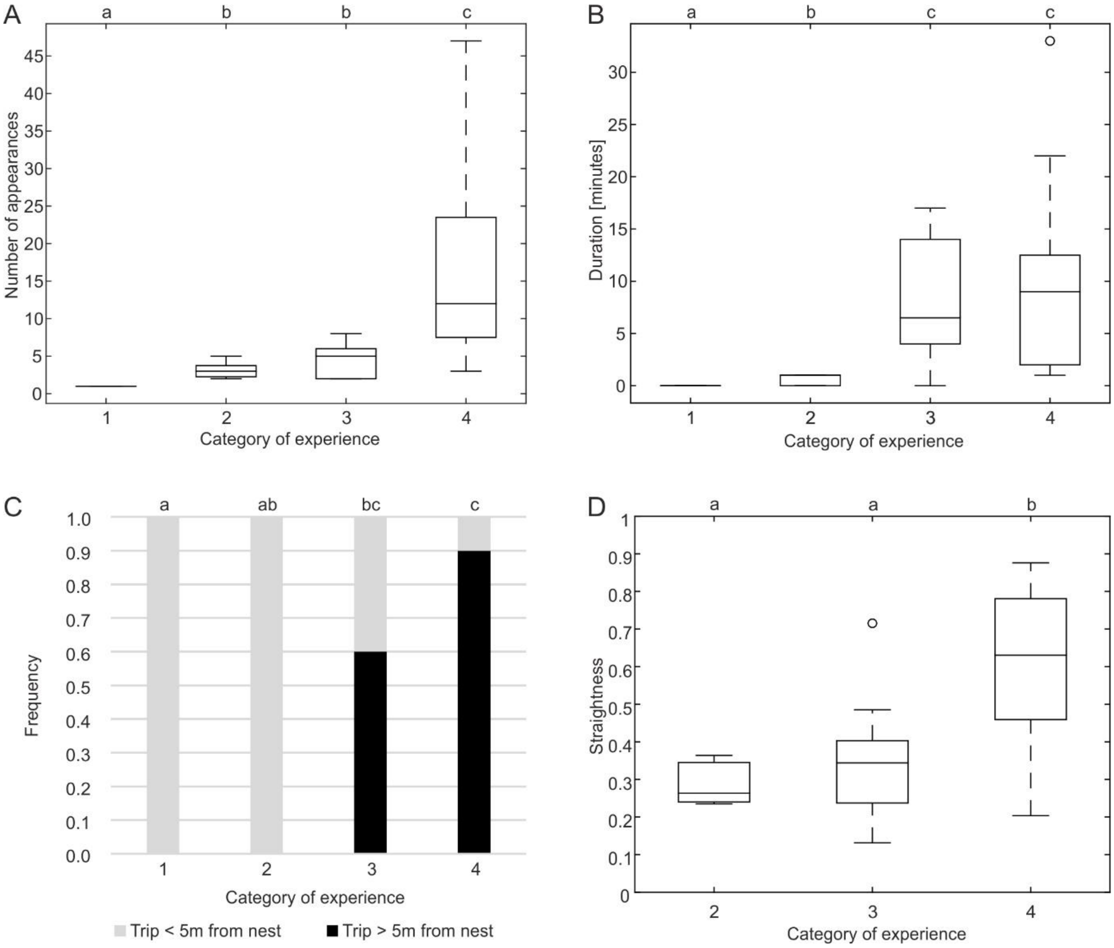

Fig. S2 Additional information concerning the learning walks of the test animals. (A) Number of appearances outside the nest of the ants in the different categories of experience. (B) Duration of the ants' trips outside the nest before being captured for the test. Since naïve (category 1) and unexperienced ants (category 2) leave the nest only for some seconds up to about a minute, the measurements of the duration outside the nest exact to the minute can only be a rough estimation for these categories. (C) Proportion of ants that left the nest field before the being captured for the test. (D) Straightness of the ants calculated as the proportion of maximal distance/length of path. If the ants left the nest field, straightness was calculated only for their outbound trip, whereas if the ants stayed on the nest field before being captured, straightness was calculated for the whole trip. The number of ants per category were n=15 for category 1, n=7 for category 2, n=15 for category 3, and n=20 for category 4. The central mark in the boxplots (A, B, and D) is the median, the edges of the boxes are the 25th and 75th percentiles, and the whiskers extend to the most extreme data points (excluding outliers). Outliers are plotted individually as „o“. The groups in the boxplots were statistically tested with the Kruskal-Wallis-Test (α=0.05) and compared post hoc with the Mann-Whitney-U-test with Bonferroni-Holm correction. The groups in the bar graph (C) were compared pairwise with Fisher's exact test with Bonferroni-Holm correction (α=0.05). Different letters indicate statistically significant differences between the groups.

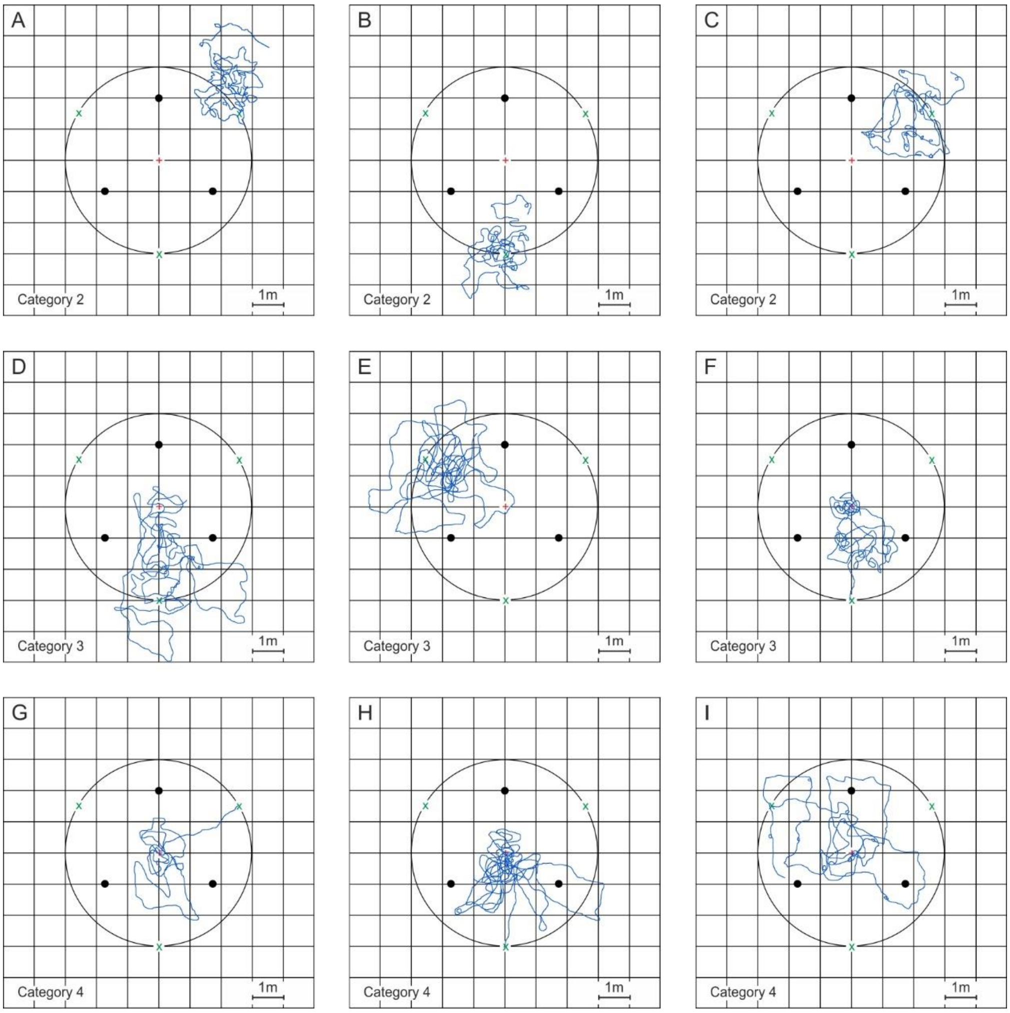

Fig. S3 Additional examples of searching paths on the test field. (A-C) Search paths of three ants in category 2. (D-F) Search paths of three ants in category 3. (G-H) Search paths of three ants in category 4. The fictive position of the nest entrance is located in the middle of the test field (+) surrounded by an identical landmark array as at the nest (black filled circles). The release points (x) lie 3 m away from the fictive nest entrance position. The grid size corresponds to 1 m on the test field.

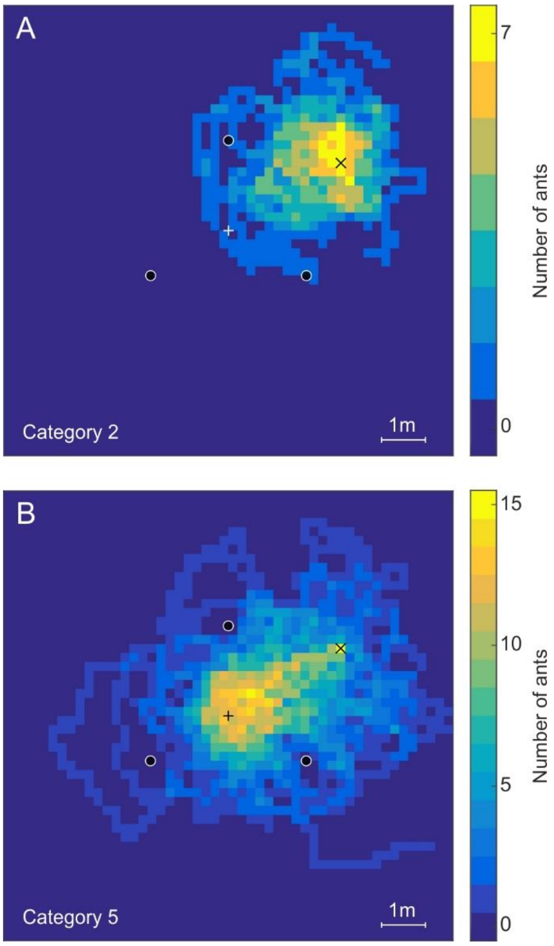

Fig. S4 Additional false-color maps of the superimposed searching paths of all ants in categories 2 (A, n=7) and 5 (B, n=15). The fictive position of the nest entrance is located in the middle of the test field (+) surrounded by an identical landmark array as at the nest (black filled circles). The release point (x) lies 3 m away from the fictive nest entrance position (all data is superimposed so that all ants start at the release point northeast of the fictive nest entrance). Each pixel of the false-color maps is equivalent to a 0.2 m x 0.2 m square on the test field. Dark blue pixels have not been visited by any ant, whereas yellow indicates that all ants of a category crossed the pixel (the number of test ants for each corresponding color is given next to the color bar).

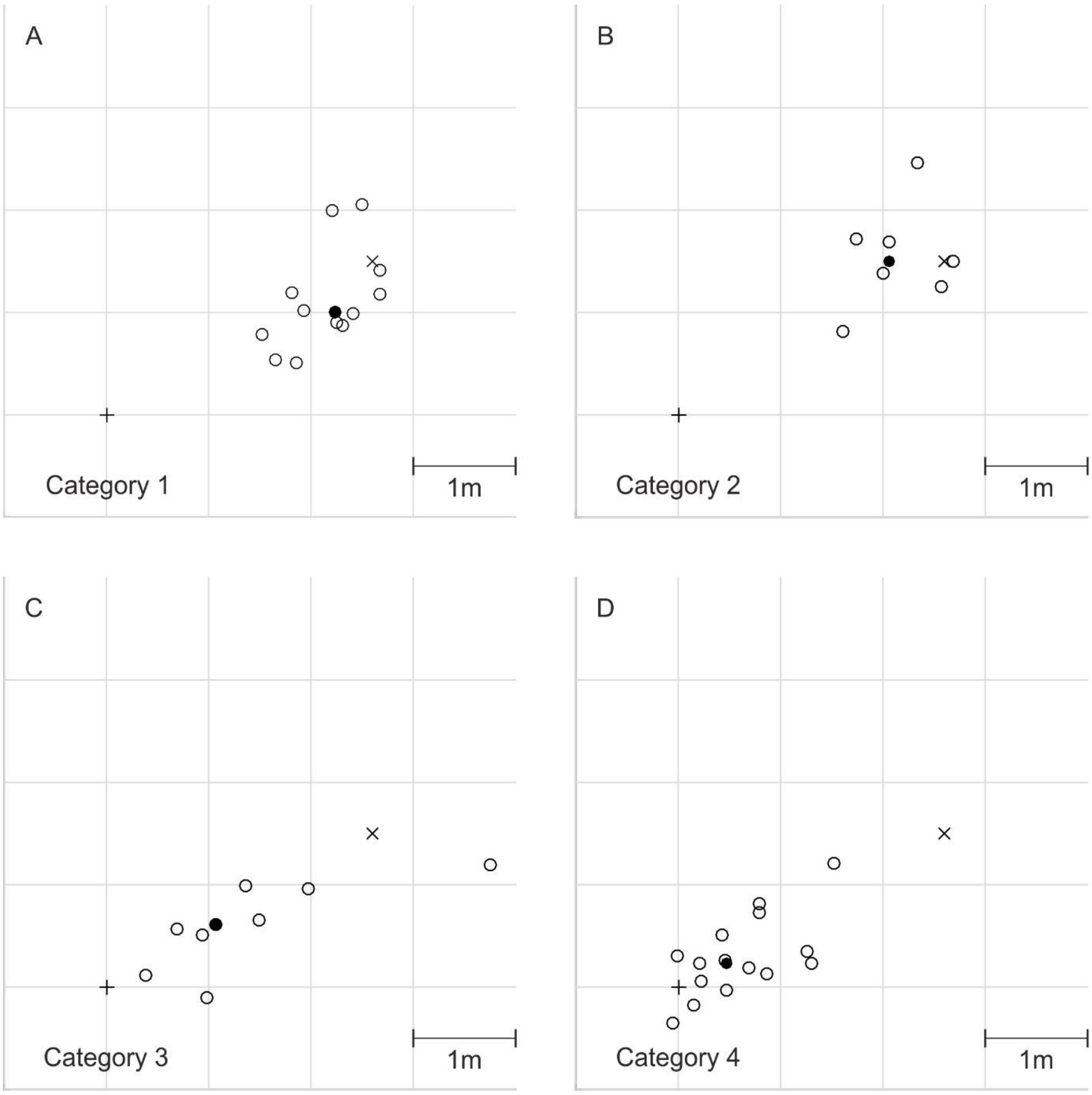

Fig. S5 Median search centers of the ants. The scatterplots (A) category 1 (n=12), (B) category 2 (n=7), (C) category 3 (n=9), and (D) category 4 (n=15) show the same data as the boxplots in fig. 4. The search centers of individual ants are shown as “o” and the search center of the corresponding categories is shown as a filled black circle. The release point (x) lies 3 m away from the fictive nest entrance position (+). All data is superimposed so that all ants start at the release point northeast of the fictive nest entrance. The grid size corresponds to 1 m on the test field.

5 Manuscript IV: Early in foraging life: spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*

5 Early in foraging life: spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*

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Within the powerful navigational toolkit unraveled in desert ants, path integration and landmark guidance are the key routines. Here we use cue-conflict experiments to investigate the interplay between these two routines in ants, *Cataglyphis noda*, which start their foraging careers (novices) and are then tested at different stages of experience. During their learning walks, the novices take nest-centered views from various directions around the nest. In the present experiments these learning walks are spatially restricted by arranging differently sized water moats around the nest entrance and the thus limiting the space available around the nest and the nest-feeder route. First, we show that the ants are able to return to the nest by landmark guidance only when the novices have had enough space around the nest entrance for properly performing their learning walks. Second, in 180° cue-conflict situations between path integration and landmark guidance, path integration dominates in the beginning of foraging life (after completion of the learning walks), but with increasing numbers of visits to a familiar feeder landmark guidance comes increasingly into play.

5.1 Introduction

Finding back to the nest after foraging is essential for all central place foragers. Ants, prime examples of central place foragers, cope with this task by pursuing several navigational strategies to return to their nest after searching for food (Wehner 2008; Graham 2010; Zeil 2012; Graham and Philippides 2017). *Cataglyphis* desert ants primarily rely on path integration (PI) involving a celestial compass for determining directions (Wehner and Müller 2006) and a stride integrator (Wittlinger et al. 2006) as well as an optic flow meter (Ronacher and Wehner 1995; Pfeffer and Wittlinger 2016) for gauging distances travelled. In addition, they make heavy use of landmark information. At the beginning of their forager career they learn landmark configurations around the nest entrance by performing well-structured learning walks when leaving the nest for the first time (Fleischmann et al. 2016). These learning walks include characteristic turns (Fleischmann et al. 2017). The most conspicuous ones in *Cataglyphis noda* are pirouettes (also described for *Cataglyphis bicolor*: Wehner

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et al. 2004, and *Ocymyrmex robustior*: Müller and Wehner 2010) during which the ants look back to the nest, presumably to take goal-centered snapshots from various locations around the nest (Graham et al. 2010; Müller and Wehner 2010; Fleischmann et al. 2017; Grob et al. 2017). The learning walks increase with experience, i. e. in subsequent trips the ants move further away from the nest entrance and cover larger areas (Wehner et al. 2004; Stieb et al. 2012; Fleischmann et al. 2016; Fleischmann et al. 2017) as do flying hymenopterans during learning flights (honeybees: Capaldi et al. 2000; Degen et al. 2015; bumblebees: Osborne et al. 2013). Here we investigate how the ants acquire spatial information at the beginning of their foraging career. We hypothesize that they start by relying on PI and that with increasing outdoor experience they gradually acquire landmark knowledge of their nest surroundings. In this line, we further hypothesize that when displaced to locations at which the ants have never been before, and at which the steering commands by PI and landmark guidance (LG) are set into conflict, the ants would gradually switch from relying on the former to using the latter. In particular, we investigate the spatial and temporal characteristics of this process, i. e. how the acquisition of landmark information depends on the space available to the ants for performing their learning walks and on the number of foraging journeys.

5.2 Material and Methods

Test animals, study site and general experimental procedure Experiments were performed with *C. noda* (Brullé 1832) in the summers of 2015 and 2016 in the Schinias National Park near Marathon, Greece, using three nests located in different clearings in the surrounding pine forest. The trees offered prominent skylines with natural landmarks. Experienced foragers were marked with one color for three days before experiments started. After this period, all unmarked ants were considered being naïve ("novices"). They were caught and marked with a unique multi-color code using car paint (Motip Lackstift Acryl, MOTIP DUPLI GmbH, Haßmersheim, Germany). All visits of these individually identifiable ants at the feeder (distance between nest entrance and feeder was always 5.0 m) were registered. After a specific number of visits (depending on the experimental paradigm), ants were caught for testing and released at different release points on the clearing (depending on the experimental paradigm). In order to facilitate the recording of the ants' search paths, a grid was constructed (about 20 m × 20 m, mesh width: 1 m). We recorded the ant's path with pen and paper true to scale until it returned into the nest or for a maximum of five minutes.

Free field experiment In the free field experiment (carried out at nest 1 in 2015), ants could freely explore the nest's surroundings and forage without any spatial restriction. An artificial feeder was set up at 5.0 m east of the nest entrance, and every visit of an ant was noted. After ten visits, the ant was captured at the feeder and released there. Its homebound run was recorded. The same ant was allowed to return to the feeder and bring home a food item from the feeder once before being captured again and released at one of the other release points (5.0 m south, west or north from the nest entrance). This procedure was repeated until the ant had been tested at every release point. Ten ants were tested at all four release points. In addition, 16 novices were tested when they occurred for the first time outside the nest and had not yet performed their learning walks, each at one release point. Since it was not possible to train the ants only in the direction of the feeder and catch them at their first feeder visit, a glass channel (height: 0.3 m, width: 0.3 m, length: 5.3 m, feeder 5.0 m north from the nest entrance) was installed at another nest (nest 2). The ants could explore the area within the channel and were captured when they visited the feeder for the first time. Ten ants were tested, each at one of three release points (5.0 m east, south or west from the nest entrance).

Moat experiment Since we were not sure, whether the walks in the glass channel might influence the ants' navigational performances by an altered panorama or reflecting light, we restricted the area around the nest entrance by using a moat filled with seawater. Three differently sized setups were used to test the influence of space available to perform learning walks on homing success during testing. At nest 2, we first installed a moat that offered the ants only a narrow runway (moat setup 1: width: 0.3 m, length: 5.3 m, feeder 5.0 m north of the nest entrance, fig. 5.1a). To offer ants more space for performing learning walks, two other setups were used. At nests 2 and 3, a water moat was installed that offered 1 m^2 free area around the nest entrance (moat setup 2, fig. 5.1b). At nest 3, a larger setup offering 4 m^2 free area around the nest entrance was set up using gutters (moat setup 3, fig. 5.1c). The feeder was placed 5.0 m west of the nest entrance at nest 3. These three setups offered different amounts of space available to the ants to perform their learning walks (fig. 5.1d). In moat setup 1, the ants could move in all directions 15 cm to 20 cm away from the nest entrance. In this way, novices could only perform their very first learning walks (Fleischmann et al. 2016; Fleischmann et al. 2017) without stumbling upon water moat restrictions. In moat setups 2 and 3, the ants could walk 50–70 cm (cf. learning walk category 2 in Fleischmann et al. 2016), and 100–140 cm in each direction, respectively, before they reached the water moat. If an ant stumbled into the water, it immediately returned to the nest.

To test the influence of experience gained over time, we applied three different testing regimes. Ants were assigned to one of three experimental test groups. The first experimental group of ants was tested multiple times sequentially after different numbers of visits at the feeder (six full-vector tests every second feeder visit until their fifth test, and additionally for the sixth time after the 16th feeder visit (FV I-VI), and subsequently one zero-vector test (ZV)). Ants of the second experimental group were tested once for the first time after their tenth feeder visit (FV 10+) and once as zero-vector ant (ZV 10+). Ants of the third group were only tested once after their first feeder visit (FV 1st). All experimental regimes were performed in all three setups (for details: table 5.2).

The general experimental procedure was the same in all three moat setups. After all foragers had been color-marked during three successive days, an ant leaving the nest for the first time (a "novice") was captured at the nest entrance and marked individually. When it reached the feeder in the following days and took a food item, this event was counted as the first feeder visit. Ants were tested at different stages of experience over time depending on their experimental group (for details: table 1). Test ants were transferred to the release point in a dark tube and released within a plastic ring (diameter: 16 cm). They were offered a food item and were released when they had picked up the food item or after five minutes. Their paths were recorded for five minutes or until they reached the moat.

Data analysis and statistics The homing success rate was measured in each experimental group by counting what proportion of the homing ants entered an area of 1.5 m around the nest entrance within five minutes. If the ants reached this area, they usually found back to the nest entrance or touched the water channel a few moments later. We compared the results

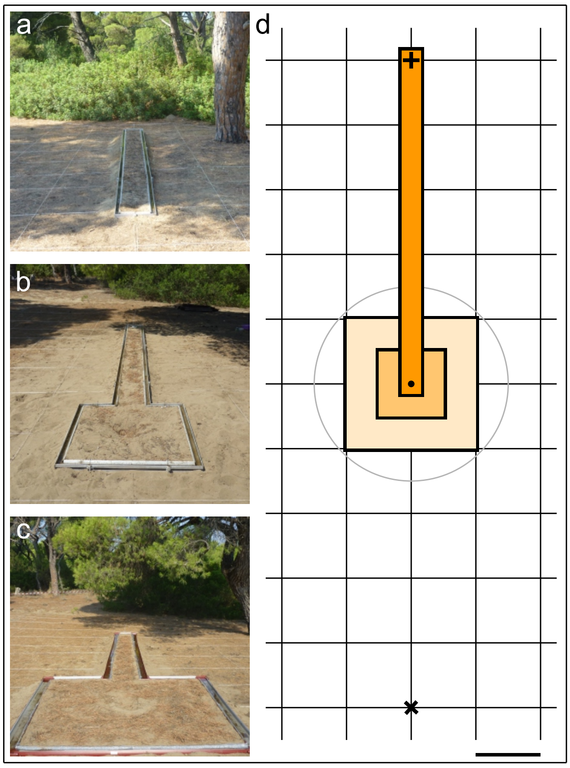


Figure 5.1: Moat setup. Three differently sized water moats (**a** moat setup 1, **b** moat setup 2, and **c** moat setup 3) offered ants space to perform learning walks around their nest entrance before being trained to a feeder and released in the test field later on. **d** The scheme illustrates the spatial relations: The distance between nest entrance (shown as black dot) and feeder (shown as black +) was always 5 m. The release point (shown as black ×) was 5 m of the nest entrance in the opposite direction of the feeder. Mesh size of the test grid was 1 m (shown by the scale bar). The runway had a width of 0.3 m and a length of 5.3 m. Ants trained in the moat setup 1 could only walk in the dark orange area. When moat setup 2 was installed, ants had additionally 1 m^2 around the nest entrance (light orange), and with moat setup 3 they even had 4 m^2 (completely colored area). The gray circle indicates the "nest area". Ants in all setups could reach this area without any restrictions and if they did, they were counted as returning successfully to the nest.

	Regime	Moat setup 1			Moat setup 2			Moat setup 3		
		Multiple tests	After 10^{th} visit	After 1^{st} visit	Multiple tests	After 10^{th} visit	After 1^{st} visit	Multiple tests	After 10^{th} visit	After 1^{st} visit
	n	10	21	33	7	16	32	21	18	33
Feeder visit no.	1	FV I	N	FV $^{1\text{st}}$	FV I	N	FV $^{1\text{st}}$	FV I	N	FV $^{1\text{st}}$
	2	N	N	-	N	N	-	N	N	-
	3	FV II	N	-	FV II	N	-	FV II	N	-
	4	N	N	-	N	N	-	N	N	-
	5	FV III	N	-	FV III	N	-	FV III	N	-
	6	N	N	-	N	N	-	N	N	-
	7	FV IV	N	-	FV IV	N	-	FV IV	N	-
	8	N	N	-	N	N	-	N	N	-
	9	FV V	N	-	FV V	N	-	FV V	N	-
	10	N	FV 10+	-	N	FV 10+	-	N	FV 10+	-
	11	N	N	-	N	N	-	N	N	-
	12	N	ZV 10+	-	N	ZV 10+	-	N	ZV 10+	-
	13	N	-	-	N	-	-	N	-	-
	14	N	-	-	N	-	-	N	-	-
	15	N	-	-	N	-	-	N	-	-
	16	FV VI	-	-	FV VI	-	-	FV VI	-	-
	17	N	-	-	N	-	-	N	-	-
	18	ZV	-	-	ZV	-	-	ZV	-	-

Table 5.1: Experimental regimes. Ants were either tested multiple times (six full-vector tests FV I-VI and one zero-vector test ZV) or once as full-vector (FV 1^{st} and FV 10+) ants. Ants tested only once were either tested after their first (FV 1^{st}) or their tenth (FV 10+) feeder visit. FV 10+ ants were additionally tested as zero-vector (ZV 10+) ants as were the multiple tested ants. Between tests, ants were allowed to return to their nest (N) from the feeder taking a food item home.

of the different experimental groups using Fisher's exact test (two-sided) with Bonferroni correction. The significance level was $\alpha = 0.05$. This test was also used for the comparison of the proportion of ants that took or refused food items when they were released.

The protocol sheets of the ants' search paths were scanned to process the data using GIMP 2.8.10. Examples of ants' paths were copied using the pencil tool (size 5.0).

The distance between release point and return point (the point where an ant stopped to follow one direction) was measured. The return point was determined by selecting a circle using the ellipse select tool and expanding it from the center at the release point until the ant's path followed the circle or touched it and reversed towards the inside of the circle. The distance between release point and return point was the radius of the circle. We compared the median distances of the first and sixth tests of the sequentially tested ants pairwise within the different experimental setups (i. e. moat setup 1, 2 and 3) using the Mann-Whitney-U test with a significance level of $\alpha = 0.05$. The same statistical test was performed for the first tests of ants tested once after their first feeder visit and the first tests of ants tested after their tenth visit at the feeder. All statistical tests were performed with Matlab R2014b (MathWorks, Inc., Natick, MA, USA).

Figures were edited with Corel Draw X6 (Corel Corporation, Ottawa, ON, Canada).

5.3 Results

5.3.1 Free field experiment

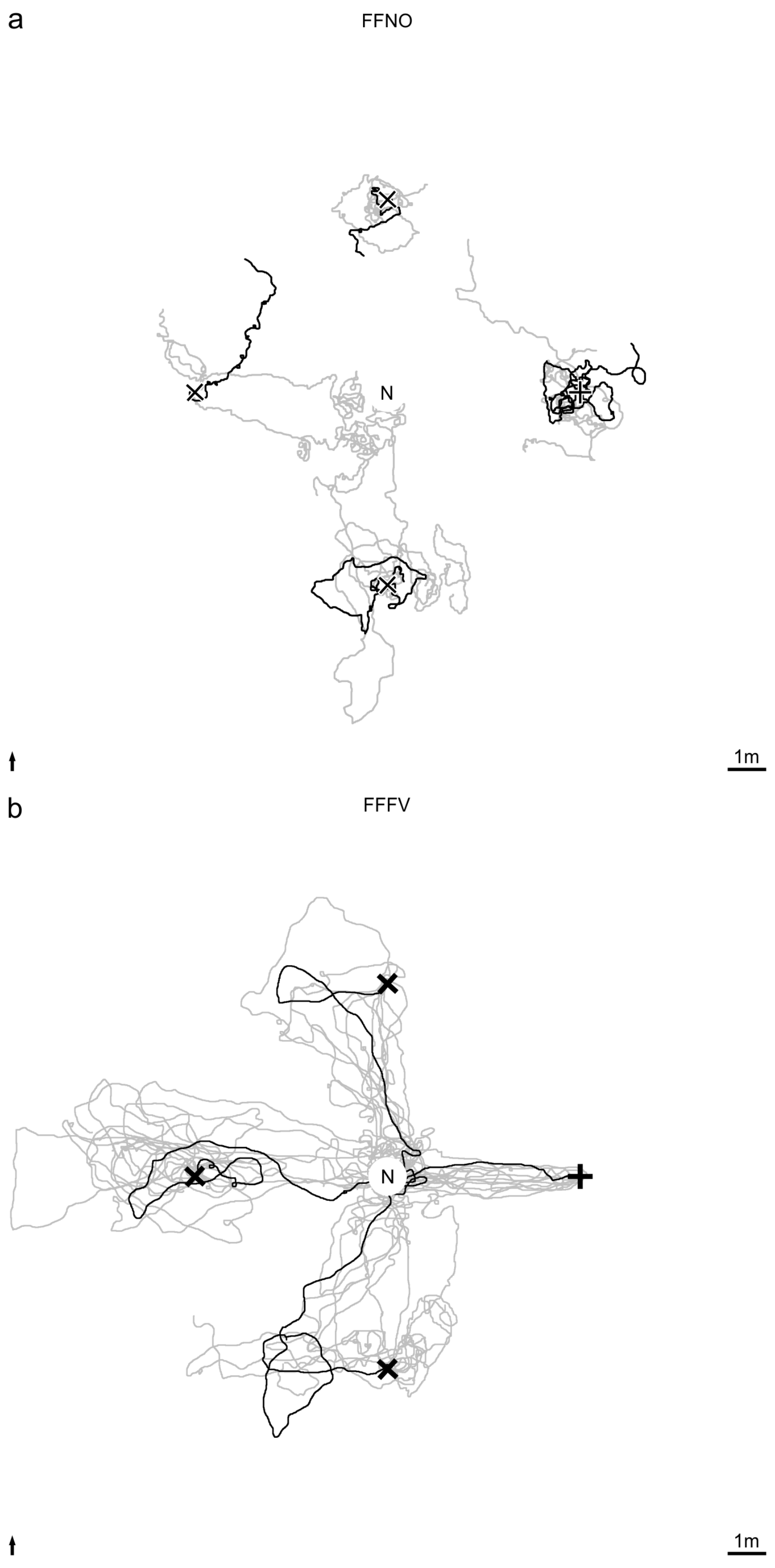
Free-field novices (FFNOs) captured at the nest when leaving it for the first time and transferred to release points in 5 m distance, usually did not find back to the nest (fig. 5.2a). Only 3 out of 16 ants reached the nest area (defined as a circle of 1.5m radius around the nest entrance) within 5 minutes (fig. 5.3a), and only one of them actually entered the nest entrance within 5 minutes. A quarter (4 out of 16) of FFNOs did not move to search for the nest, but hid under grass and pine needles. In contrast, free-field full-vector (FFFV) foragers captured at the feeder 5 m east of their nest entrance after more than ten feeder visits were usually able to return to the nest (fig. 5.2b). When released at the feeder all ants immediately returned to the nest carrying a food item (median duration: 61 s, ranging from 35 s to 137 s, n = 10, fig. 5.2b and fig. 5.3). FFFV ants were also successful in homing when released in the other cardinal directions of the nest in 5 m distance (fig. 5.2b). In 26 out of 30 tests (n = 10, each ant tested from north, south and west) the ants reached the nest area, and in 20 out of 30 tests they actually entered the nest entrance within 5 minutes. Usually, released ants followed their PI home vectors first and switched to landmark-guided navigation after few meters (measured as the distance between release and return point, i. e. the point where an ant stopped to follow vector direction: northern release point: 3.3 m ± 1.9 m, southern release point: 3.6 m ± 2.4 m, and western release point: 2.2 m ± 1.3 m, median ± IQR). The release of naïve ants and experienced foragers at four different release points showed that the homing success differed drastically. Significantly more FFFVs returned to the nest than FFNOs did (fig. 5.3a, for statistical details see below). Since it was not possible to train new ants to the feeder in the free field in a way that they only got to know the way to the feeder and no other direction around the nest, a glass channel was installed to guide the ants in one direction but allowing them to view the whole panorama. Glass-channel full-vector ants (GCFVs, n = 10) captured at the feeder 5 m north of the nest entrance after their first feeder visit, i. e. after they had picked up a food item at the feeder, and released at one of the three release points (east, south or west of the nest entrance) did not return to the nest. None of them entered the nest area (fig. 5.3a), but they followed their PI vectors in a straight line for 4.2 m ± 2.4 m (median ± IQR, ranging from 2.1 m to 6.0 m) and subsequently started a systematic search for their nest. Therefore, only FFFVs were able to return home from all directions, resulting in a significant difference in homing success between those foragers and both FFNO ants as well as first visitors at the feeder in the glass channel (GCFV) (fig. 5.3a, Fisher's exact test with Bonferroni correction: FFFV ($n = 10$) versus FFNO ($n = 16$): $p < 0.0167$; FFFV versus GCFV ($n = 10$): $p < 0.0167$; GCFV versus FFNO: $p = 0.2616$). However, to double-check whether GCFVs in the glass channel were actual foragers and not naïve ants lost in the channel, we compared their willingness to pick up a food item when being released with those of FFFVs and FFNOs. Both GCFVs and FFFVs took significantly more often food items than novices did before being tested (fig. 5.3b, Fisher's exact test with Bonferroni correction: FFFV ($n = 10$) versus FFNO ($n = 16$): $p < 0.0167$; FFFV versus GCFV ($n = 10$): $p = 0.4737$; GCFV versus FFNO: $p < 0.0167$).

In summary, these results first show that foragers rely on their path integrator from their first feeder visit onward. Second, experienced foragers can return from all directions to the nest even if their home vector initially leads them in another direction. However, they cannot return from release points at which they have never been before when they have been restricted by a glass channel while performing their learning walks. This raises two main questions to be answered next: (i) how much space do the ants need for performing their learning walks around the nest entrance, to be able to successfully return home by landmark guidance (LG), and (ii) does their landmark-based homing success improve with increasing numbers of feeder visits?

5.3.2 Moat paradigm

In order to answer these questions and to test under what spatial conditions (size of area available for learning walks) and what temporal conditions (number of feeder visits) LG is able to override the dictates of the path integrator (PI), we applied the following test paradigms. Ants were trained in three differently sized moat setups (moat setup 1, 2 and 3, fig. 5.1). They were captured at the feeder (F) – hence full vector (FV) ants – were released at a location (release point R) that was at the same distance (5 m) from the nest (N), but in the opposite direction, so that the $F \rightarrow N$ direction was 180° apart from the $R \rightarrow N$ direction. Hence, at R the PI home vector pointed in the direction opposite to the nest direction (maximal cue conflict). Ants were either tested multiple times (FV I – FV VI), once after their first feeder visit (FV 1^{st}), or after their tenth feeder visit (FV 10+). Ants that were captured and tested as FV ants after several feeder visits were additionally captured at the nest entrance and tested as zero-vector ants (ZV and ZV 10+, respectively).

In these test paradigms we assumed that the homing success rate would increase with the area that was available for performing learning walks. We also assumed that ants displaced for the first time (FV I and FV 1^{st}) would fully rely on their path integrator and run in the direction away from home, but with increasing numbers of visits to the feeder would stop following the home vector earlier and start to search for the nest (FV II – FV VI and FV 10+ ants). Furthermore, we expected more ants to return to the nest area when being tested as ZV ants (ZV and ZV 10+) than as FV ants. The results of our experiments confirmed all


a
FFNO
N
1m
b
FFFV
N
1m

three assumptions and the main results become already obvious when looking at the recorded search paths of the tested ants. In a nutshell, ants only returned to the nest area if they had space to perform learning walks (moat setup 2 and 3, versus moat setup 1, fig. 5.4, and fig. 5.5). Ants tested multiple times followed their home vector fully when tested for the first time (FV I in fig. 5.4 a moat setup 1, b moat setup 2 and c moat setup 3), but stopped following the home vector earlier with increasing experience, i. e. with an increasing number of feeder visits and tests (FV II – FV VI in fig. 5.4). The same effect could be observed when comparing ants tested once after their first feeder visit (FV 1^{st}) with ants tested once after their tenth feeder visit (FV 10+) (fig. 5.5, moat setup 1: a versus d, moat setup 2: b versus e, and moat setup 3: c versus f). More ants reached the nest area when tested as ZV ants (fig. 5.4 ZV and fig. 5.6) than when tested as FV ants.

In the next sections, we take a closer look at the effect of the spatial and temporal experiences that the ants could gain while performing their learning walks and their first foraging journeys.

Spatial aspects Ants trained in the moat setup 1 always followed their home vector and subsequently started a systematic search (fig. 5.4a, figs. 5.5a, d and fig. 5.7). Only one of in total 64 ants trained this way (FV 1^{st}: $n = 33$, FV 10+: $n = 21$, and FV ants tested multiple times, i. e. FV I and FV VI: $n = 10$) reached the nest area during testing. In contrast, some ants trained in moat setup 2 (fig. 5.4b, figs. 5.5b, e, and fig. 5.7) and always more than half of the ants trained in moat setup 3 (fig. 5.4c, figs. 5.5c, f, and fig. 5.7) reached the nest area within five minutes. Actually, the proportion of ants homing successfully was the higher, the larger the training setup had been (fig. 5.7). This increase in homing success was significant when ants had been trained in the large moat setup 3 compared to ants trained in the moat setup 1 (Fisher's exact test with Bonferroni correction: Ants tested multiple times, moat setup 1 ($n = 10$) versus moat setup 3 ($n = 21$), for each test (FV I, FV VI, ZV): $p < 0.0028$; ants tested once either after their first (FV 1^{st}) or tenth (FV 10+) visit, the latter additionally as zero-vector ants (ZV 10+): for each test (moat setup 1 FV 1^{st} ($n = 33$) versus moat setup 3 FV 1^{st} ($n = 33$), moat setup 1 FV 10+ ($n = 21$) versus moat setup 3 FV 10+ ($n = 18$), and moat setup 1 ZV 10+ ($n = 21$) versus moat setup 3 ZV 10+ ($n = 18$)) $p < 0.0042$). Furthermore, significantly more ants reached the nest area when comparing FV I of moat setup 2 with moat setup 3, and FV 10+ of moat setup 2 with moat setup 3 (Fisher's exact test with Bonferroni

Figure 5.2 *(preceding page)*: Recorded paths of novices (FFNO) (**a**) and experienced free field foragers (FFFV) (**b**). The nest entrance (N) was located in the middle of the test field (20 m × 20 m, scale bar: 1 m). The feeder (shown as a black cross) was 5 m east of the nest entrance (the black arrow point towards north). **a** Novices ($n = 16$) were released at four different release points (i. e. feeder in the east and 5 m north, south and west of the nest entrance shown as black ×s). Each novice was tested only once, i. e. each bold trajectory refers to a separate individual. **b** Experienced foragers ($n = 10$) were caught at the feeder and released at all four release points after visiting the feeder. The black paths show all four tests of one individual.

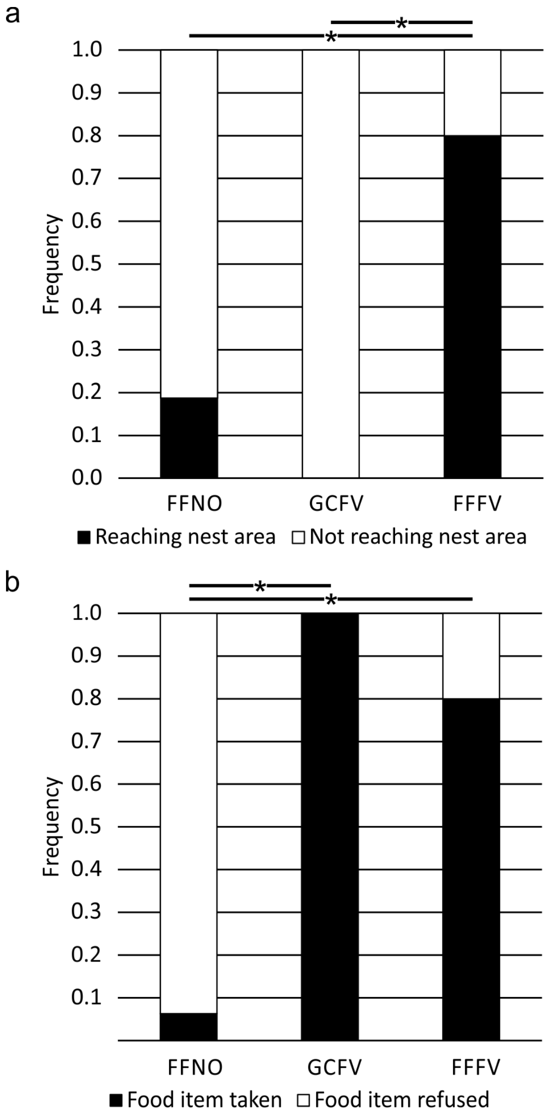


Figure 5.3: Foraging success during first or only test from a novel release point. **a** Frequency of ants reaching the nest area within five minutes. **b** Frequency of ants that took a food item when being released in the test field. Asterisks indicate significant differences between the groups when compared pairwise using Fisher's Exact test with Bonferroni correction ($\alpha = 0.05$, after correction 0.0167, free-field novices (FFNO): n = 16, glass-channel full-vector ants (GCFV): n = 10, free-field full-vector ants (FFFV): n = 10).

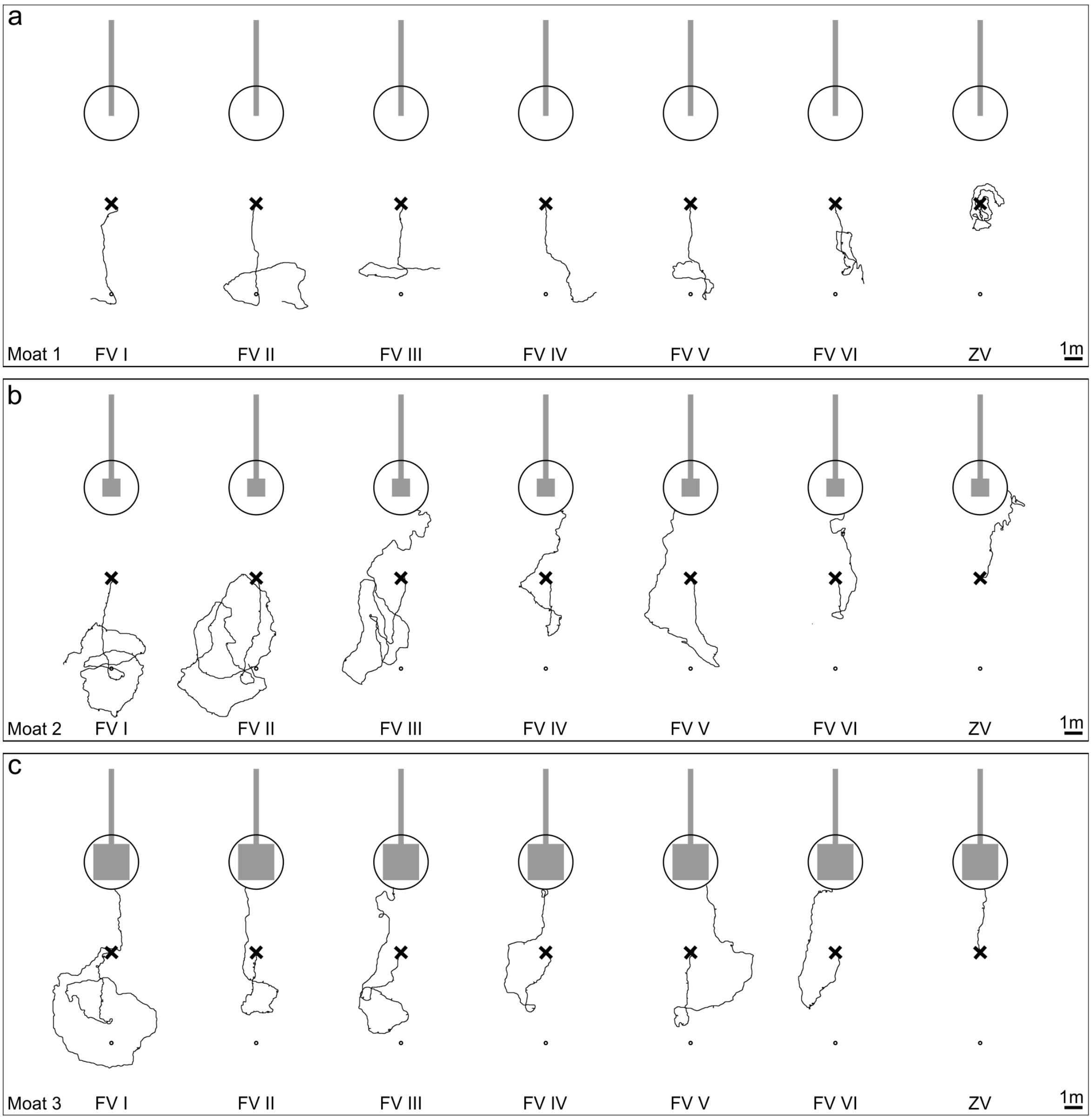


Figure 5.4: Examples of recorded paths of ants tested multiple times and trained in three different setups (**a** moat setup 1, **b** moat setup 2, and **c** moat setup 3). Setups are shown true to scale in grey. The large circle (radius: 1.5 m) includes the nest area in which the nest entrance is located in the middle. The release point is shown as a black × and the fictive nest position of the home vector as a small circle. The PI home vector points in the anti-nest direction. Paths of ants were recorded for 5 minutes or until ants reached the nest area. Each ant was tested six times as full-vector ant (FV I to VI) and afterwards once as zero-vector ant (ZV).

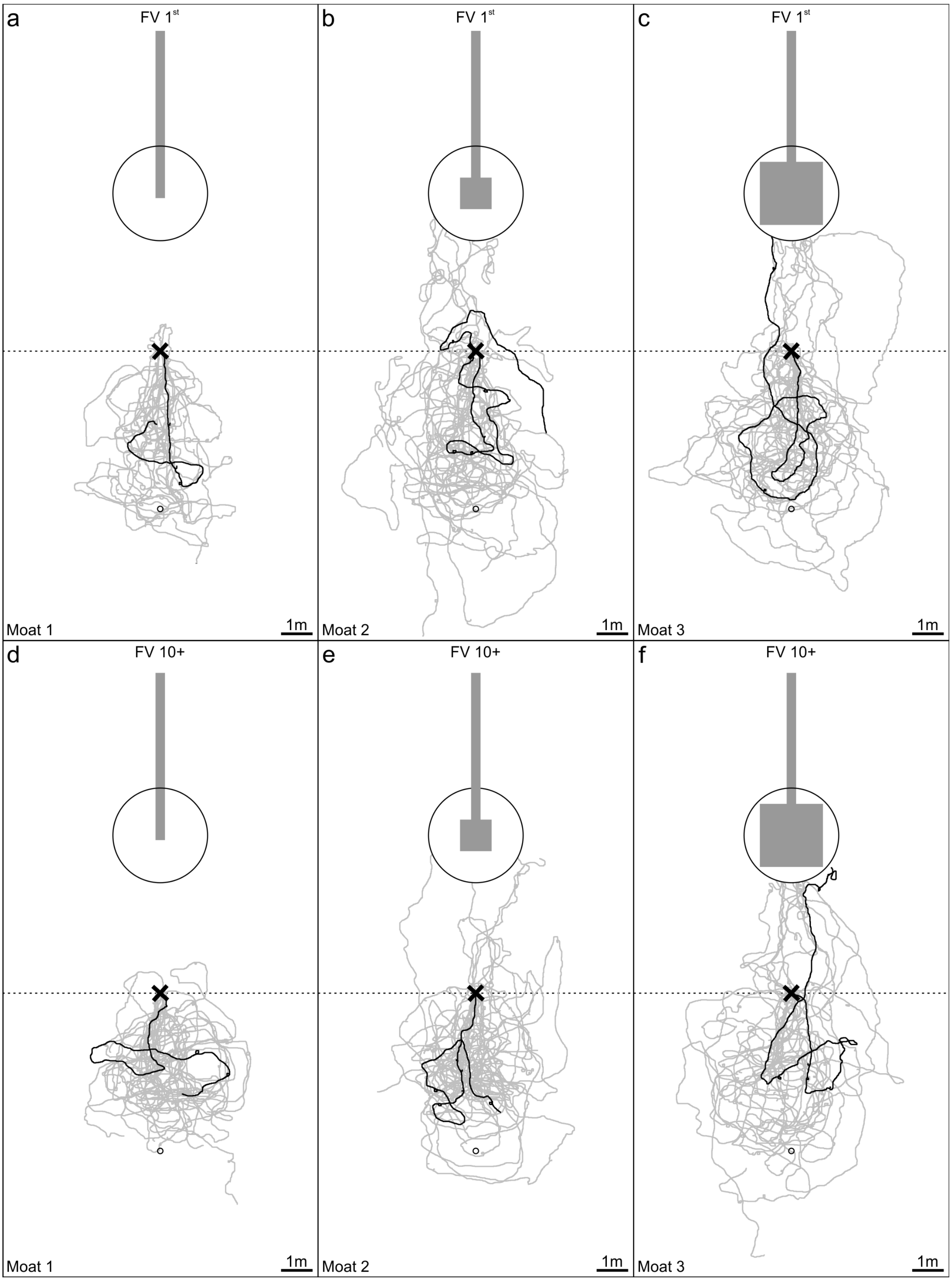


Figure 5.5: Recorded paths of FV ants tested once after their first (FV 1st: **a**, **b**, **c**) or tenth (FV 10+: **d**, **e**, **f**) feeder visit. Setups are shown true to scale in grey (**a** and **d**: moat setup 1, **b** and **e**: moat setup 2, **c** and **f**: moat setup 3). Each subfigure includes 15 examples, which were randomly chosen from all ants tested. One example is highlighted in black. For further conventions, see fig. 5.4.

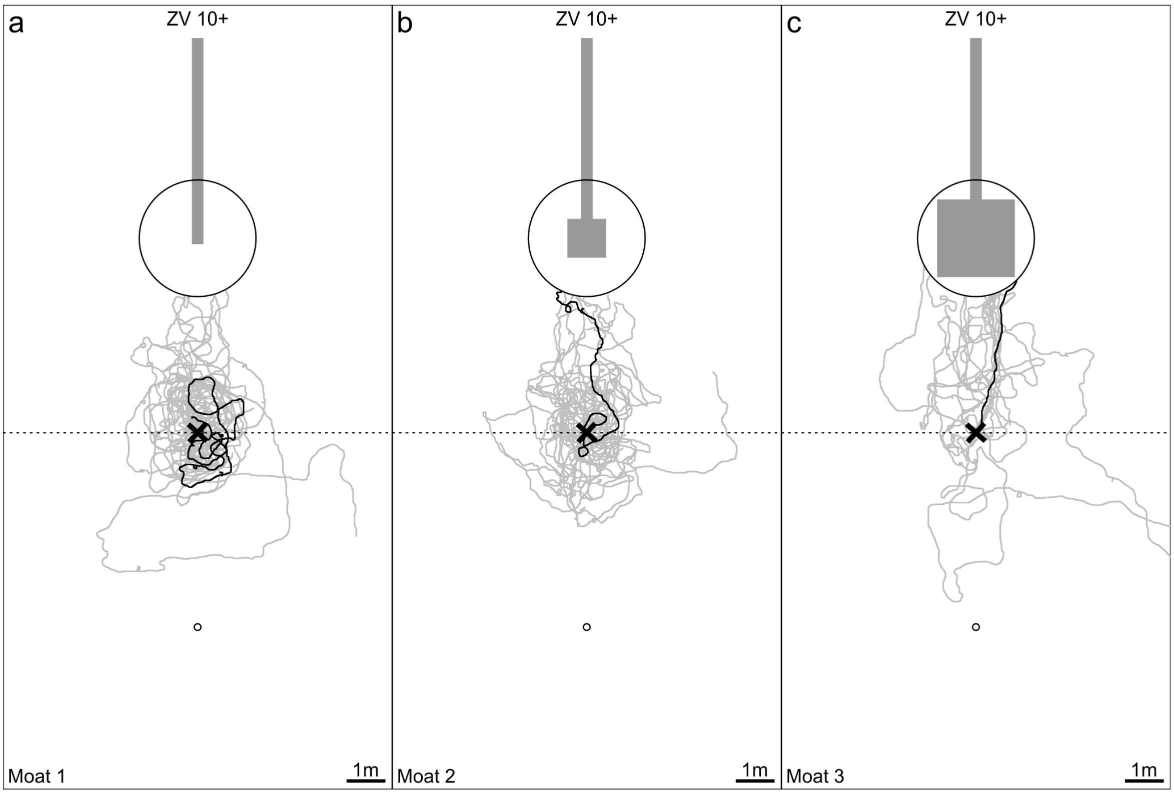


Figure 5.6: Recorded paths of ZV 10+ ants trained in three different setups (**a** moat setup 1, **b** moat setup 2, and **c** moat setup 3). For further conventions, see fig. 5.5. The examples highlighted in black show the paths of the same ants as in fig. 5.5d-f.

correction: FV I moat setup 2 ($n = 7$) versus moat setup 3 ($n = 21$): $p < 0.0028$; FV 10+ moat setup 2 ($n = 16$) versus moat setup 3 ($n = 18$): $p < 0.0042$). All other pairwise comparisons between the experimental groups in differently sized setups revealed no significant differences, although there is a tendency of more ants reaching the nest area when the training setup offered more room to perform learning walks during training. Ants that had visited the feeder multiple times before the tests were additionally tested as ZV ants (fig. 5.4: ZV, and fig. 5.6: ZV 10+). The homing success rate of ants without any PI vector information was always higher than that of ants tested as FV ants (fig. 5.7). This is due to the fact that after release the FV ants first follow their PI instructions for longer or shorter times (see section on "Temporal aspects"). However, the better homing performance of the ZV ants was only significant when comparing FV 10+ with ZV 10+ of moat setup 1 trained ants (Fisher's exact test with Bonferroni correction: moat setup 1 ($n = 21$) FV 10+ versus ZV 10+: $p < 0.0042$). The difference between the behavior of FV and ZV ants is not only borne out statistically by comparing the homing success rates, but also shown in the searching behavior. Moat setup 1 trained ants immediately started systematic searching around the release point (fig. 5.4a ZV and fig. 5.6a), whereas moat setup 3 ants usually approached the nest directly in a straight line (fig. 5.4c ZV and fig. 5.6c). Ants trained in moat setup 2 took an intermediate position. Sometimes they searched systematically and sometimes they approached the nest directly (fig. 5.4b ZV and fig. 5.6b).

Temporal aspects Ants tested for the first time after their first feeder visit followed their home vectors almost completely (fig. 5.8; moat setup 1: FV I ($n = 10$): 4.6 m ± 1.4 m; FV 1^{st} ($n = 33$): 4.4 m ± 2.0 m; moat setup 2: FV I ($n = 7$): 4.0 m ± 0.8 m; FV 1^{st} ($n = 32$): 4.6 m ± 1.7 m; moat setup 3: FV I ($n = 19$): 4.4 m ± 1.2 m; FV 1^{st} ($n = 32$): 3.8 m ± 1.7 m, median ± IQR). In contrast, ants that had gained more experience before being tested stopped to follow their vectors earlier (fig. 5.8; moat setup 1: FV VI ($n = 10$): 3.3 m ± 0.6 m; FV 10+ ($n = 21$): 3.3 m ± 1.2 m; moat setup 2: FV VI ($n = 7$): 2.6 m ± 0.6m; FV 10+ ($n = 16$): 3.2 m ± 1.1 m; moat setup 3: FV VI ($n = 21$): 3.3 m ± 1.6m; FV 10+ ($n = 17$): 2.9 m ± 1.8 m, median ± IQR). This shortening as shown in figs. 5.4 and 5.5 is statistically significant in five of six pairwise comparisons (fig. 5.8, Mann-Whitney-U test: moat setup 1: FV I versus FV VI: $z = 2.4226$, $n_{\mathrm{FV\,I}} = 10$, $n_{\mathrm{FV\,VI}} = 10$, $p < 0.05$; FV 1^{st} versus FV 10+, $z = 3.7411$, $n_{1^{st}} = 33$, $n_{10+} = 21$, $p < 0.05$ moat setup 2: FV I versus FV VI: $z = 2.6252$, $n_{\mathrm{FV\,I}} = 7$, $n_{\mathrm{FV\,VI}} = 7$, $p < 0.05$, FV 1^{st} versus FV 10+: $z = 3.1082$, $n_{1^{st}} = 32$, $n_{10+} = 16$, $p < 0.05$; moat setup 3: FV I versus FV VI: $z = 2.7529$, $n_{\mathrm{FV\,I}} = 19$, $n_{\mathrm{FV\,VI}} = 21$, $p < 0.05$, FV 1^{st} versus FV 10+: $z = 32.2085$, $n_{1^{st}} = 32$, $n_{10+} = 17$, $p = 0.2269$). Some ants were not only tested once after a specific number of feeder visits, but multiple times after every second feeder visit (FV I–VI). The most abrupt shortening of the PI-guided path segment occurred between the first test (FV I) after the first feeder visit and the second test (FV II) after the third feeder visit, i. e. after the ant had experienced the F→N route once. In subsequent tests, the ants followed their PI vectors less far, but never ignored the PI vector information completely and hence never relied exclusively on LG (examples of three individual ants are shown in fig. 5.4). As a result, with increasing number of feeder visits, FV ants follow their PI home vector for increasingly shorter distances, i. e. gradually increase their readiness to home by LG. Even though the ants stopped to follow their home vector earlier when they had performed more feeder visits before the tests, their homing success rates did not increase significantly in any experimental setup (fig. 5.7a, Fisher's exact test with Bonferroni correction: moat setup 1 ($n = 10$): FV I versus FV VI, $p = 1$;

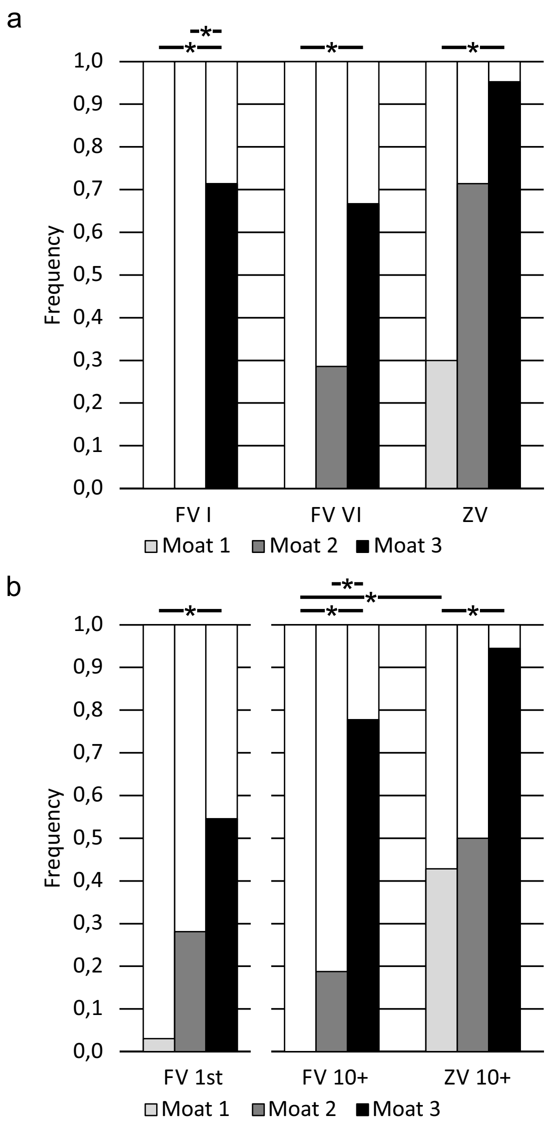


Figure 5.7: Homing success of ants trained in three differently sized moat setups. **a** Frequency of ants reaching the nest area tested multiple times (moat setup 1: n = 10, moat setup 2: n = 7, moat setup 3: n = 21). **b** Frequency of ants reaching the nest area tested once either after their first (FV 1st: moat setup 1: n = 33, moat setup 2: n = 32, moat setup 3: n = 33) or tenth (FV 10+: moat setup 1: n = 21, moat setup 2: n = 16, moat setup 3: n = 18) feeder visit. FV 10+ ants were additionally tested as zero-vector ants (ZV 10+) after two additional feeder visits. Asterisks indicate significant differences between the groups when compared pairwise using Fisher's Exact test with Bonferroni correction ($\alpha = 0.05$, after correction in a 0.0028 and in b 0.0042). Experimental groups were compared across setups at each test. Furthermore, the frequencies of the same ants tested more than once were compared.

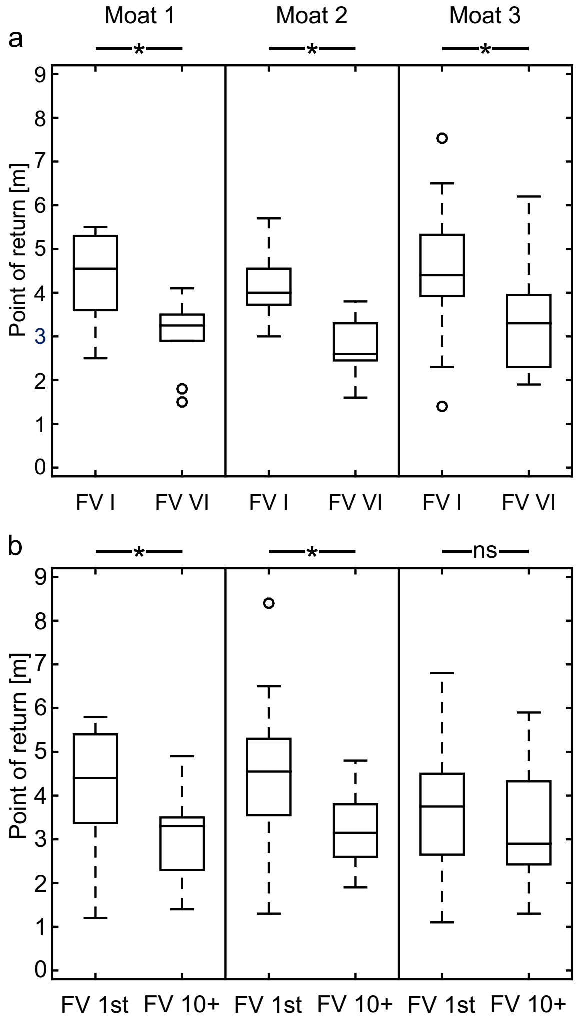


Figure 5.8: Points of returns of ants during testing in different testing regimes and different experimental setups. **a** Points of returns of ants that were tested after their first and subsequent visits at the feeder (FV I vs. FV VI) were compared in three different moat setups (left: moat setup 1 ($n_{\mathrm{FV\,I}} = 10$, $n_{\mathrm{FV\,VI}} = 10$), middle: moat setup 2 ($n_{\mathrm{FV\,I}} = 7$, $n_{\mathrm{FV\,VI}} = 7$), right: moat setup 3 ($n_{\mathrm{FV\,I}} = 19$, $n_{\mathrm{FV\,VI}} = 21$)). **b** Points of returns of ants that were tested once either after their first (FV 1^{st}: moat setup 1: n = 33, moat setup 2: n = 32, moat setup 3: n = 32) or tenth (FV 10+: moat setup 1: n = 21, moat setup 2: n = 16, moat setup 3: n = 17) feeder visit in three different moat setups (left: moat setup 1, middle: moat setup 2, right: moat setup 3). Groups were compared pairwise using Mann-Whitney-U test ($\alpha = 0.05$). Asterisks indicate significant differences between groups.

moat setup 2 (n = 7): FV I versus FV VI, p = 0.4616; moat setup 3 (n = 21): FV I versus FV VI, p = 1).

5.4 Discussion

The critical experimental parameters differing between test groups were on the one hand the space available around the nest entrance to perform learning walks before testing started, and on the other hand the number of visits at the feeder, and how often the ants were tested. Both dimensions of experience significantly influenced the homing abilities of the ants (see figs. 5.7 and 5.8 for spatial experience and experience gained over time, respectively).

5.4.1 Spatial requirements of learning walks

At the start of its foraging life a *Cataglyphis* ant performs a sequence of learning walks, with increasing length and duration from one learning walk to another (*C. bicolor*: Wehner et al. 2004; *C. fortis*: Stieb et al. 2012; Fleischmann et al. 2016; *C. noda*: Fleischmann et al. 2017). During these learning walks the ant stops at different places and distances and looks back to the nest entrance (Fleischmann et al. 2017). Most likely it is during these turn-back and-look events that the ants acquire and store goal-centered panoramic images (Graham et al. 2010; Müller and Wehner 2010; Fleischmann et al. 2017; Grob et al. 2017). The present study shows that enough space around the nest entrance is required for successfully taking the necessary panoramic views. By confining the ants' learning walks to differently sized areas around the nest entrance, we investigated how much space the novices need in their pine forest habitat to acquire the landmark memories necessary for successful homing. A glass channel and three different water moat configurations (fig. 5.1) restricted the learning walks to various degrees.

Only in moat setup 3 could the ants perform extensive learning walks up to 1 m distance in all directions from the nest entrance. As fig. 5.3a and fig. 5.7 show, the differences in space offered to the ants for performing their learning walks led to marked differences in homing success. Ants trained in a 0.3 m wide runway (moat setup 1 or glass channel) never returned to the nest by landmark guidance (LG) (fig. 5.4a, figs. 5.5a, d). Similarly, *C. fortis* novices cannot find back to the (fictive) nest entrance position guided by landmarks when they have been captured and tested after short learning walks (category 1: < 0.3 m, category 2: < 0.7 m, Fleischmann et al. 2016). In contrast, *C. noda* ants, which had free field experience (fig. 5.2b) or had performed their learning walks in the large setup (moat setup 3, fig. 5.4c and figs. 5.5c, f) reliably reached the nest area when displaced to a novel location. Hence, the first conclusion drawn on the basis of the results from the current experiments is that spatial restrictions of the learning walks decrease the ants' homing success significantly when the ants are released in the neighborhood (at a 5 m distance) of their nest located in the middle of a clearing in their pine forest habitat, but at places at which they have never been before. This is also the case when in full-vector (FV) ants path integration (PI) and LG systems are in conflict by indicating opposite directions.

When Australian jack jumper ants (*Myrmecia croslandi*) are displaced in their natural habitat within 10 m in various directions from the nest they return to their nest directly (Narendra et al. 2013). When released further away they pursue different strategies: Some ants return to the nest directly, some follow the PI vector first and subsequently correct toward the nest, and some only follow their PI vector until it is in zero state, and thereafter search systematically (Narendra et al. 2013). Since the experience of individual jack jumpers prior to testing was not known, the ants could have potentially visited the release sites before they had been experimentally transferred to them. In our study individually marked ants had been recorded from their first foraging trip onwards. It therefore can be excluded that they have ever been at a release site before (see test paradigms FV I, FV 1st and FV 10+, and fig. 5.4, fig. 5.5 and fig. 5.6). Depending on the space restrictions during the learning walks, in the PI vs. LG competition experiments the ants having initially followed their PI vector start either LG (under conditions of moat setup 2: fig. 5.4b and figs. 5.5b, e; moat setup 3: fig. 5.4c and 5.5c, f) or perform systematic searches (under condition of moat setup 1: fig. 5.4a and figs. 5.5a, d). In conclusion, the spatial restrictions of the moat influenced the homing success of the ants significantly (fig. 5.7). Under moat setup 1 conditions learning walks are virtually prevented. This enables us to ask what landmark knowledge the ants acquire with increasing number of foraging runs (feeder visits) alone. This is the kind of question to which we turn next.

5.4.2 Gradual transition from path integration to landmark guidance

Learning the landmark panorama around the nest or any other goal is not a one-shot event. As shown in *C. fortis*, the ants must perform a suite of nest-centered learning walks, in which they move in loops of ever increasing size around the nest, until they are finally able to locate the goal accurately and precisely on the basis of landmark information alone (Müller 1984; Fleischmann et al. 2016). Hence, landmark memories – memories of panoramic views later used in returning to the nest – are acquired gradually. In contrast, PI works from the very start of an ant's foraging career. As in fully fledged foragers LG can completely override the dictates of the path integrator (e. g. Andel and Wehner 2004), it is a likely hypothesis that early in an ant's foraging life LG gradually gains in importance relative to PI. We have tested this hypothesis by exposing ants, which had just started their foraging lives, to cue conflict situations, in which PI and LG information led the ant in opposite directions (different by 180°). As expected, all FV ants (trained in the free field or one of the experimental setups), which were displaced from the feeder to the release point, first followed the direction indicated by the PI home vector and then switched to LG or systematic search.

To test the influence of experiences gained over time during training on the homing abilities during testing, ants were captured after different numbers of feeder visits. FV ants tested for the first time, i. e. displaced after they had arrived at the feeder on their first foraging trip in life, selected the PI direction and reeled off their home vector to about 76 to 92 percent (FV I and FV 1st ants, fig. 5.8) before switching to another navigational strategy. When being tested after at least ten feeder visits, ants already stopped to follow the PI vector after 52 to 66 percent (FV VI and FV 10+ ants, fig. 5.8). With an increasing number of feeder visits ants stopped earlier to follow their PI home vectors as can be seen in the ants tested multiple times during their foraging career. Even though the homing behavior differed most obviously between ants being tested for the first and the second time (i. e. ants tested for the first time followed their PI home vectors almost completely whereas ants tested repeatedly stopped earlier to follow their PI vectors), the distance between release point and return point continued to decrease in subsequent tests (for individual examples: fig. 5.4). Importantly, FV ants always followed their PI home vectors to some degree of their home vector length (as did the free-field experienced foragers, fig. 5.2b). Therefore, landmark experience gained over time during multiple feeder visits gradually decreased the impact of PI guidance.

Homing success depends on the spatial experiences gained during learning walks (as discussed above). There was an obvious behavioral difference between ants trained in the moat setup 1 and ants trained in the large moat setup 3. Ants that had previously been able to perform their learning walks in the large moat setup 3 setup, followed their home vector, turned around, exhibited some search behavior, and finally returned to the nest by LG. In contrast, ants that had not had space to perform learning walks prior to testing, also followed their home vectors, but then exhibited search behavior centered on the fictive position of the nest entrance and never returned to the actual nest area – not even after extensive training and several tests (fig. 5.7). Therefore, extensive learning walks are necessary to enable ants to return to their nest by LG.

This conclusion is corroborated by ants that were tested as zero-vector (ZV) ants, which being devoid of PI information must rely exclusively on LG (see e. g. Wehner et al. 1996; Kohler and Wehner 2005; Wystrach et al. 2012). Due to the experimental schedule applied in the present study, all ZV ants had been at the release point before as FV ants. Furthermore, when starting their searches they were closer to the goal, i. e. the nest, than the FV ants. This is for the simple reason that the FV ants while initially following their PI vector had moved away from the nest for about 7 m to 10 m. Hence, it should have been easier for the ZV ants than for the FV ants to reach the nest, but this was not the case. In the moat setup 1 situation only 12 of 31 ZV ants returned to the nest (ZV (n = 10) and ZV10+ (n = 21)). The success rate was high only in ants that have had enough space to perform learning walks (i. e. were trained in moat setup 2 and 3) before foraging and testing (fig. 5.7). These ants did not return to the nest by systematic search behavior. Rather they approached the nest directly (fig. 5.4C ZV and fig. 5.6c). Hence, the ZV tests confirm the results obtained in the FV tests that learning walks are necessary for successful homing.

Several studies performed in different desert ant species have investigated the ants' navigational performance under PI-LG cue conflict conditions (e. g. *Cataglyphis*: Wehner et al. 1996, see fig. 11 therein, Wystrach et al. 2015; Melophorus: Kohler and Wehner 2005; Legge et al. 2014; Wystrach et al. 2012; *Myrmecia*: Freas et al. 2017; Narendra et al. 2013). But only two recent studies focus particularly on the influence that increased experience has on the ants' decisions (Freas and Cheng 2017; Schwarz et al. 2017). Both studies show that Australian red honey ants (*Melophorus bagoti*) rely on LG as compared to PI the more, the more frequently they have travelled a familiar feeder-nest route, as shown in the present study for *C. noda*.

In the first study (Freas and Cheng 2017) ants were trained to forage within an arena (diameter: 2 m) around the nest entrance, with a feeder included in the arena. When naïve FV ants were displaced to a release point located in the opposite direction to the feeder at an 8 m distance outside of the arena, these ants did not orient in the true nest direction as indicated by terrestrial cues, but followed their PI vector. Experiencing the route from release point to the nest once during testing did not change this result when thereafter the ants were tested for the second time. However, after a training procedure of several feeder visits and transfers to the release point as ZV ants, FV ants oriented towards the true nest direction. Hence, information acquired during training caused overriding a conflicting PI vector. The second study (Schwarz et al. 2017) shows that repeated travels along a familiar route let landmark scenes distant from the route appear more unfamiliar than before route learning has started. As deduced from LG-PI 180° cue-conflict experiments, with increasing route training a familiar scene becomes more readily distinguished from an unfamiliar scene. In particular, when naïve ants that were displaced to an unfamiliar distant test field after their first visit to a feeder (set up 8 m from the nest entrance), they followed their PI vector to about 80 percent before starting to search for the nest. In contrast, experienced ants that had visited the feeder for two days, ran off only about 40 percent of their PI vectors. These results are in principal accordance with the performance of the FV VI and FV 10+ ants in the present study.

In conclusion, differences in both spatial and temporal dimensions influence the navigational performance of *Cataglyphis* ants tested at different stages of experience. The ants need enough space to perform their learning walks around the nest entrance in order to later return to the nest reliably. More time for experiencing a foraging route reduces the impact of conflicting PI information. Therefore, both more space for performing learning walks and more time for repeatedly visiting a familiar site help ants to find back to the nest from places at which they have never been before. Comparison with similar results obtained in other desert ants indicate that using terrestrial cues for landmark guidance is a process that starts with the learning walks of novices and continues throughout the ants' entire foraging lives.

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Author Contributions

P. N. F. and R. W. designed the experiment. P. N. F. conducted the experiment. R. W. and W. R. led the study. P. N. F. analyzed the data. P. N. F. wrote the manuscript, and R. W. and W. R. revised the manuscript. All authors participated in discussing the results and checking the paper.

References

Andel, D. and Wehner, R. (2004). Path integration in desert ants, *Cataglyphis*: How to make a homing ant run away from home. *Proceedings of the Royal Society of London B: Biological Sciences*, 271(1547):1485–1489.

Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M., and Riley, J. R. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature*, 403:537–540.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H., Chamkhi, H., Greggers, U., and Menzel, R. (2015). Exploratory behaviour of honeybees during orientation flights. *Animal Behaviour*, 102:45–57.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *The Journal of Experimental Biology*, 219:3137–3145.

Fleischmann, P. N., Grob, R., Wehner, R., and Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *The Journal of Experimental Biology*, 220:2426–2435.

Freas, C. A. and Cheng, K. (2017). Learning and time-dependent cue choice in the desert ant, *Melophorus bagoti*. *Ethology*, pages 1–13.

Freas, C. A., Narendra, A., and Cheng, K. (2017). Compass cues used by a nocturnal bull ant, Myrmecia midas. *The Journal of Experimental Biology*, 220:1578–1585.

Graham, P. (2010). Insect Navigation. In Breed, M. and Moore, J., editors, *Encyclopedia of Animal Behaviour*, volume 2, pages 167–175. Oxford Academic Press.

Graham, P. and Philippides, A. (2017). Vision for navigation: What can we learn from ants? *Arthropod Structure and Development*, 46(5):718–722.

Graham, P., Philippides, A., and Baddeley, B. (2010). Animal cognition: multi-modal interactions in ant learning. *Current Biology*, 20(15):R639–R640.

Grob, R., Fleischmann, P. N., Grübel, K., Wehner, R., and Rössler, W. (2017). The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11:226.

Kohler, M. and Wehner, R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: How do they interact with path-integration vectors? *Neurobiology of Learning and Memory*, 83:1–12.

Legge, E. L. G., Wystrach, A., Spetch, M. L., and Cheng, K. (2014). Combining sky and earth: desert ants (*Melophorus bagoti*) show weighted integration of celestial and terrestrial cues. *The Journal of Experimental Biology*, 217:4159–4166.

Müller, M. (1984). *Interokularer Transfer bei der Wüstenameise Cataglyphis fortis (Formicidae, Hymenoptera).* Diplomarbeit, Universität Zürich.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Current Biology*, 20(15):1368–1371.

Narendra, A., Gourmaud, S., and Zeil, J. (2013). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. *Proceedings of the Royal Society B*, 280:20130683.

Osborne, J. L., Smith, A., Clark, S. J., Reynolds, D. R., Barron, M. C., Lim, K. S., and Reynolds, A. M. (2013). The ontogeny of bumblebee flight trajectories: From naïve explorers to experienced foragers. *PLoS ONE*, 8(11):e78681.

Pfeffer, S. E. and Wittlinger, M. (2016). Optic flowodometry operates independently of stride integration in carried ants. *Science*, 353(6304):1155–1157.

Ronacher, B. and Wehner, R. (1995). Desert ants *Cataglyphis fortis* use self-induced optic flow to measure distances travelled. *Journal of Comparative Physiology A*, 177:21–27.

Schwarz, S., Wystrach, A., and Cheng, K. (2017). Ants' navigation in an unfamiliar environment is influenced by their experience of a familiar route. *Scientific Reports*, 7(1):14161.

Stieb, S. M., Hellwig, A., Wehner, R., and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 72(5):729–742.

Wehner, R. (2008). The desert ant' s navigational toolkit: Procedural rather than positional knowledge. *Journal of The Institute of Navigation*, 55(2):101–114.

Wehner, R., Meier, C., and Zollikhofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecological Entomology*, 29:240–250.

Wehner, R., Michel, B., and Antonsen, P. (1996). Visual navigation in insects: Coupling of egocentric and geocentric information. *The Journal of Experimental Biology*, 199:129–140.

Wehner, R. and Müller, M. (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proceedings of the National Academy of Sciences*, 103(33):12575–12579.

Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. *Science*, 312(5782):1965–1967.

Wystrach, a., Beugnon, G., and Cheng, K. (2012). Ants might use different view-matching strategies on and off the route. *Journal of Experimental Biology*, 215(1):44–55.

Wystrach, A., Mangan, M., and Webb, B. (2015). Optimal cue integration in ants. *Proceedings of the Royal Society B: Biological Sciences*, 282(1816):20151484.

Zeil, J. (2012). Visual homing: an insect perspective. *Current Opinion in Neurobiology*, 22(2):285–293.

6 Manuscript V: The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies

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The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies

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Central place foragers are faced with the challenge to learn the position of their nest entrance in its surroundings, in order to find their way back home every time they go out to search for food. To acquire navigational information at the beginning of their foraging career, *Cataglyphis noda* performs learning walks during the transition from interior worker to forager. These small loops around the nest entrance are repeatedly interrupted by strikingly accurate back turns during which the ants stop and precisely gaze back to the nest entrance—presumably to learn the landmark panorama of the nest surroundings. However, as at this point the complete navigational toolkit is not yet available, the ants are in need of a reference system for the compass component of the path integrator to align their nest entrance-directed gazes. In order to find this directional reference system, we systematically manipulated the skylight information received by ants during learning walks in their natural habitat, as it has been previously suggested that the celestial compass, as part of the path integrator, might provide such a reference system. High-speed video analyses of distinct learning walk elements revealed that even exclusion from the skylight polarization pattern, UV-light spectrum and the position of the sun did not alter the accuracy of the look back to the nest behavior. We therefore conclude that *C. noda* uses a different reference system to initially align their gaze directions. However, a comparison of neuroanatomical changes in the central complex and the mushroom bodies before and after learning walks revealed that exposure to UV light together with a naturally changing polarization pattern was essential to induce neuroplasticity in these high-order sensory integration centers of the ant brain. This suggests a crucial role of celestial information, in particular a changing polarization pattern, in initially calibrating the celestial compass system.

Keywords: look-back behavior, desert ants, vector navigation, sky-compass pathway, memory, central complex, mushroom body, visual orientation

INTRODUCTION

Before starting their foraging career central place foragers, like bees, wasps and ants, have to acquire knowledge about the position of their nest in its surroundings and need to calibrate their navigational toolkit (Collett et al., 2013; Fleischmann et al., 2016). In order to do so, they perform learning flights or walks. Studies of this early learning behavior in bees (Opfinger, 1931; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Degen et al., 2015), wasps (Zeil et al., 1996; Stürzl et al., 2016) and ants (Wehner et al., 2004; Fleischmann et al., 2016, 2017) revealed striking parallels in the general sequence of this behavior (Zeil, 2012). When leaving the nest entrance for the first time honeybees (Lehrer, 1993), bumblebees (Hempel de Ibarra et al., 2009; Collett et al., 2013; Philippides et al., 2013) and wasps (Zeil et al., 1996; Stürzl et al., 2016) turn back immediately towards their nest entrance and look back before flying in multiple arcs parallel to the nest entrance. As walking insects do not walk sideways, ants perform repeated turns during their learning walk loops and make stops to look back towards their nest entrance (Wehner et al., 2004; Fleischmann et al., 2017). During these looks back the animals most probably learn the landmark panorama (honeybees: Opfinger, 1931; Lehrer, 1993; bumblebees: Collett et al., 2013; ants: Fleischmann et al., 2016, 2017). Over time the arcs or loops increase in size, and novices move farther away from the nest entrance, while still looking back towards it (Zeil et al., 1996; Wehner et al., 2004; Philippides et al., 2013; Fleischmann et al., 2016). Likewise, experienced foragers perform a learning behavior that includes looks back to the nest, e.g., when experienced animals had difficulties pinpointing their nest (Zeil, 1993; Zeil et al., 1996) or when the nest surrounding had changed drastically (Müller and Wehner, 2010; Narendra and Ramirez-Esquivel, 2017).

However, to determine the direction of the nest entrance from various positions in space, the animals need some kind of reference system. It has been previously proposed, that this system could be part of the path integrator (Graham et al., 2010; Müller and Wehner, 2010), which integrates information about the walked directions (compass) and the distance covered (odometer) into a vector pointing towards the starting point. In *Cataglyphis* ants the path integrator is the main navigational tool (Müller and Wehner, 1988). The ants use an odometer (Wittlinger et al., 2006) and optic flow (Pfeffer and Wittlinger, 2016) to determine the distance covered. By integrating the odometer information with information about the walked directions, for which the ants use the celestial compass (Müller and Wehner, 1988; Wehner et al., 1996; Wehner, 2003), they determine a vector pointing homewards. The celestial compass mainly relies on information about the position of the sun and the skylight polarization pattern in the UV-spectrum (Duelli and Wehner, 1973). This suggests that the skylight polarization pattern only in the UV-spectrum could provide a suitable reference system for the compass information of the path integrator to align gaze directions during learning walks.

The polarization direction of the UV-skylight is detected by specialized ommatidia in the dorsal rim area of the compound eye (Labhart and Meyer, 1999). The information is transferred by neurons forming the anterior optical tract (AOT) via several stages into the central complex (CX; Schmitt et al., 2016). In the CX polarization of the skylight is represented in a map-like pattern (Heinze and Homberg, 2007; Homberg et al., 2011; Heinze and Reppert, 2012). The CX was also shown to be involved in several tasks closely linked to orientation and navigation (Pfeiffer and Homberg, 2014; Fiore et al., 2017). In *Drosophila* the CX is additionally involved in landmark memory (Neuser et al., 2008), landmark orientation and angular path integration (Seelig and Jayaraman, 2015). Another prominent neuronal pathway in bees and ants, the anterior superior optical tract (asot), transfers visual information into the visual subregions of the mushroom bodies (MB; Gronenberg, 2001; Yilmaz et al., 2016). The MBs are centers for sensory integration, learning and memory. They undergo substantial neuronal changes when exposed first time to light (*Drosophila*: Barth and Heisenberg, 1997; *Apis*: Scholl et al., 2014; *Cataglyphis*: Seid and Wehner, 2009; Stieb et al., 2010, 2012) and during the formation of long-term memory (*Acromyrmex*: Falibene et al., 2015; *Apis*: Hourcade et al., 2010).

The duration of learning walk behaviors lasts for up to 3 days (Wehner et al., 2004; Stieb et al., 2012; Fleischmann et al., 2016). This correlates with the time needed for stable long-term memory formation (Menzel, 2001; Hourcade et al., 2010; Falibene et al., 2015; Scholl et al., 2015) and the time needed to induce neuronal changes in the visual subregions of the MBs after exposure to light pulses in *Cataglyphis fortis* (Stieb et al., 2010, 2012). Therefore, learning walks are perfectly suited to study brain-behavior-environment interactions. In this study, we restricted the input into the sky-compass of *Cataglyphis noda* during their early learning walks to ask, which reference system the ants use during this early learning phase to align their gaze directions. Ants that participated in the behavioral field experiments were subsequently used for neuroanatomical analyses. This allowed us to look at the interaction between the learning-walk behavior, the received information during these walks, as well as changes in the neuronal architecture in the terminal stages of two visual pathways, the CX and the MBs. The results suggest that natural skylight polarization information with the UV part of the light spectrum present induce structural changes in the CX and the MBs indicating their role in the initial calibration of visual pathways processing celestial information. However, exclusion of sky-compass information did not prevent *C. noda* from looking back towards their nest entrance suggesting, that celestial cues do not serve as the initial reference system for compass information during learning walks.

MATERIALS AND METHODS

Animals

Experiments were conducted with *C. noda* (Brullé 1832) (**Figure 1A**) in Schinias National Park, Marathonas, Greece from June–August 2016. A colony with a nest entrance in the middle

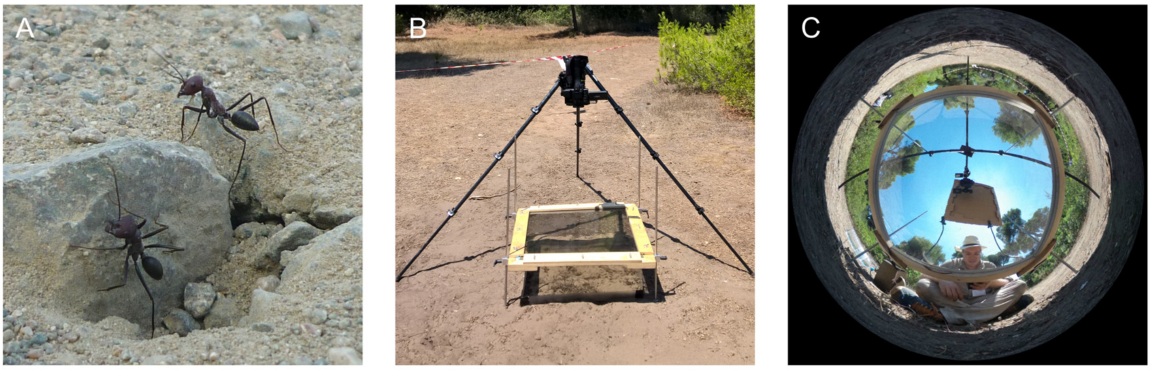

FIGURE 1 | Experimental setup for skylight manipulation experiments. **(A)** Unmarked *C. noda* ants at the nest entrance. **(B)** 30 cm above the nest entrance, a filter was placed in order to alter the skylight information. Learning walks were recorded with a high-speed 4K-camera. In addition, a HD-camcorder recorded the nest entrance for the whole day. **(C)** Panoramic image of the UV-block with sunshade setup (UVBS). The observer was located in the south to trigger the high-speed recording and to prevent unmarked ants from leaving the area covered by the filter through the opening in the fence, which was located in the south-west.

of a small clearing in the pine forest of the national park (38°08'N 24°01'E) was used for the experiments. In order to make sure that only novices (ants performing learning walks for the first time) were used, all ants leaving the nest were marked on at least three consecutive days before the experiment using car paint (Motip Lackstift Acryl, MOTIP DUPLI GmbH, Haßmersheim, Germany). Unmarked ants can then be considered to be naïve, as it was shown in previous studies (Fleischmann et al., 2016, 2017). The animals were allowed to perform learning walks for three consecutive days within an arena (60 cm × 60 cm) restricted by a transparent plastic fence. Only marked foragers were allowed to leave the restricted area through a small exit in the fence.

Manipulation of the Skylight

To manipulate the skylight the ants perceived during their learning walks, different filter systems (60 cm × 60 cm; **Table 1**) were placed 30 cm above the nest entrance from the third day of marking. Thereby, ants that did not leave the nest, but stayed inside of the nest entrance area would only perceive the altered skylight. The ants could still encounter the landmark panorama in the setup. As a control for the setup, a UV-permeable plexiglass was installed above the arena that did not alter the skylight perceived by the ants (UV100). To alter the skylight polarization pattern to an artificial, fixated one, a linear polarization filter was used. To test whether

TABLE 1 | Groups and filter systems used for skylight manipulation.

Group	Icon	Conditions	Analyses
DD	■	Interior workers that had not yet performed learning walks (excavated in the dark using red light);	Neuroanatomy
UV-Block with sunshade (UVBS)	UV	Three days of learning walks under a UV-light impermeable filter (Plexiglas (Gallery) 0A570 GT, Evonik Performance Materials GmbH, Essen, Germany) blocking 99.7% of the light below 420 nm with a sunshade, to additionally disguise the position of the sun;	Neuroanatomy Gaze direction
Diffusor (Dif)	◩	Three days of learning walks under a diffusor that lets UV-light pass (Plexiglas (GS) 2458 SC, Evonik Performance Materials GmbH, Essen, Germany), but diffuses any polarization pattern in the skylight;	Neuroanatomy
Polarization filter (P)	▥	Three days of learning walks under a polarization filter (OUV6060-C—HNP'B replacement, Knight Optical Ltd., Harrietsham, United Kingdom) that lets UV-light pass, but provides an artificial linear, fixed polarization pattern;	Neuroanatomy Gaze direction
UV100	□	Three days of learning walks under a UV-light permeable Plexiglas (Plexiglas (GS) 2458, Evonik Performance Materials GmbH, Essen, Germany), as a control for the setup;	Neuroanatomy Gaze direction
No filter	N	Three days of learning walks under natural conditions, as a control for the experiment;	Gaze direction

the full light spectrum without a polarization pattern had an influence on the ants' behavioral development, a UV-permeable plexiglass that diffused the skylight was installed. The skylight polarization pattern and the position of the sun was blocked using a UV-impermeable plexiglass with a sunshade (UVBS; **Figures 1B,C**). On the second day of marking a camera set-up was placed north to the nest entrance. Two cameras were installed: a 4K-camcorder (HC-X1000, Panasonic Corporation, Kadoma, Japan) that recorded learning walks of novices at 50 fps, and a Full-HD camcorder (HDR-CX330E, Sony Corporation, Minato, Japan) that recorded the nest area at 25 fps for the entire day. Every time an unmarked ant left the nest entrance, an observer positioned south of the experimental setup triggered recordings of the 4K-camcoder using the Panasonic Image App (Version 10.9.2, Panasonic Corporation, Kadoma, Japan) on a Sony Xperia Z1 smartphone (Sony Corporation, Minato, Japan). Since it was not possible to film through the diffusor (Dif), only observational data is available for this experimental trial.

Neuroanatomical Procedures

Anti-Synapsin Immunolabeling

On the third day of recording, novices that performed wide range learning walks reaching up the fence were captured under the filter setup and kept in the dark until the next day. This ensured, that the ants had performed several learning walks under the altered skylight conditions and that their brains had enough time to undergo structural changes (Stieb et al., 2012; Fleischmann et al., 2016, 2017; Schmitt et al., 2016). In addition, interior workers (DD) were collected from another nest in which, similar to the experimental nest, all ants leaving the nest were marked over three consecutive days. In order to get interior workers that had never seen daylight before, the nest was excavated in the night using red light. All ants were kept in a dark box until the next day.

To analyze neuroanatomical changes in the CX and MBs (all neuroanatomical nomenclature after Ito et al., 2014), the brains were stained using a primary antibody to synapsin (SYNORF1, kindly provided by E. Buchner, University of Würzburg, Germany) and a secondary antibody coupled to AlexaFluor 568 (A12380, Molecular Probes, Eugene, OR, USA) dye.

The ants were cooled down in a freezer and decapitated in the dark. Immediately afterwards the brains were carefully dissected and fixated in 4% formaldehyde in phosphate-buffered saline (PBS) for 1 day. The brains were then rinsed three times in PBS for 10 min, followed by one rinse in 2% Triton-X 100 solution in PBS and two rinses in 0.5% Triton-X solution, for 10 min each, to permeabilize cell membranes for antibody application on whole mount brains. To block unspecific binding sites, the brains were then incubated for 1 h at room temperature on a shaker in a 0.5% Triton-X 100 solution in PBS with 2% of Normal Goat Serum (NGS, Jackson ImmunoResearch Laboratories). Afterwards, the brains were incubated for 3 days in the refrigerator (∼4°) on a shaker with the primary anti-synapsin antibody from mouse. A solution with 2% antibody, 2% NGS and 0.5% Triton-X 100 in PBS was used. After incubation the brains were rinsed five times for 10 min each in PBS. Then the secondary antibody, an anti-mouse antibody from goat with an Alexa Fluor 568 dye (4% in PBS with 1% NGS), was incubated for 2 days in the refrigerator on a shaker. The brains were then rinsed again three times in PBS for 10 min each, before they were dehydrated using an ethanol serial dilution. For that, they were rinsed for 10 min in every step: 30%, 50%, 70%, 90%, 95% ethanol in water and two times in 100% ethanol. The dehydrated brains were then cleared in methyl salicylate (M-2047; Sigma-Aldrich, Steinheim, Germany).

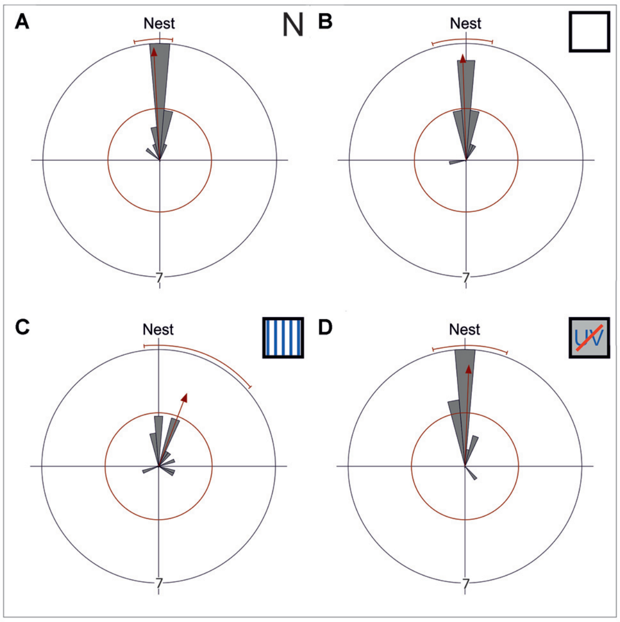

FIGURE 2 | Gaze directions during the longest stopping phases under different skylight conditions. Data are shown in gray and the corresponding statistics in red. The bins of the circular histogram include 10 degrees. The red circle indicates the critical value α = 0.05 of the Rayleigh uniformity test. The red arrow indicates the r-vector pointing towards the mean direction. If the length of the vector exceeds the red circle the data is directed ($p < 0.05$). When the data is directed, a red line indicates the 95% confidence interval. If the expected direction (Nest ≙ 180°) lies within the confidence intervals limits, the data is directed towards the nest entrance. The outer circle indicates tic 7. Each data point is contributed by one back turn of one ant. **(A)** The mean gaze direction of the longest stopping phase in pirouettes during learning walks under natural/no filter conditions (N) is directed towards the nest entrance (n = 15). **(B)** The same is true for the mean gaze direction of the longest stopping phase under control conditions (UV100; n = 15) and **(C)** under an artificial, fixed polarization pattern (P; n = 14). **(D)** Even when excluded from all celestial information (UVBS; n = 15) the ants were able to gaze towards the nest entrance during the longest stopping phases. The mean angle and the angular variance did not differ between the four groups. For statistical details see text.

Anterograde Tracings of Neuronal Projections from the Medulla

To determine the neuronal projections via the asot in *C. noda*, projection neurons of the dorsal and ventral medulla (ME) were fluorescently stained in ants reared in laboratory colonies. The tracings of neuronal projections from the ME were performed

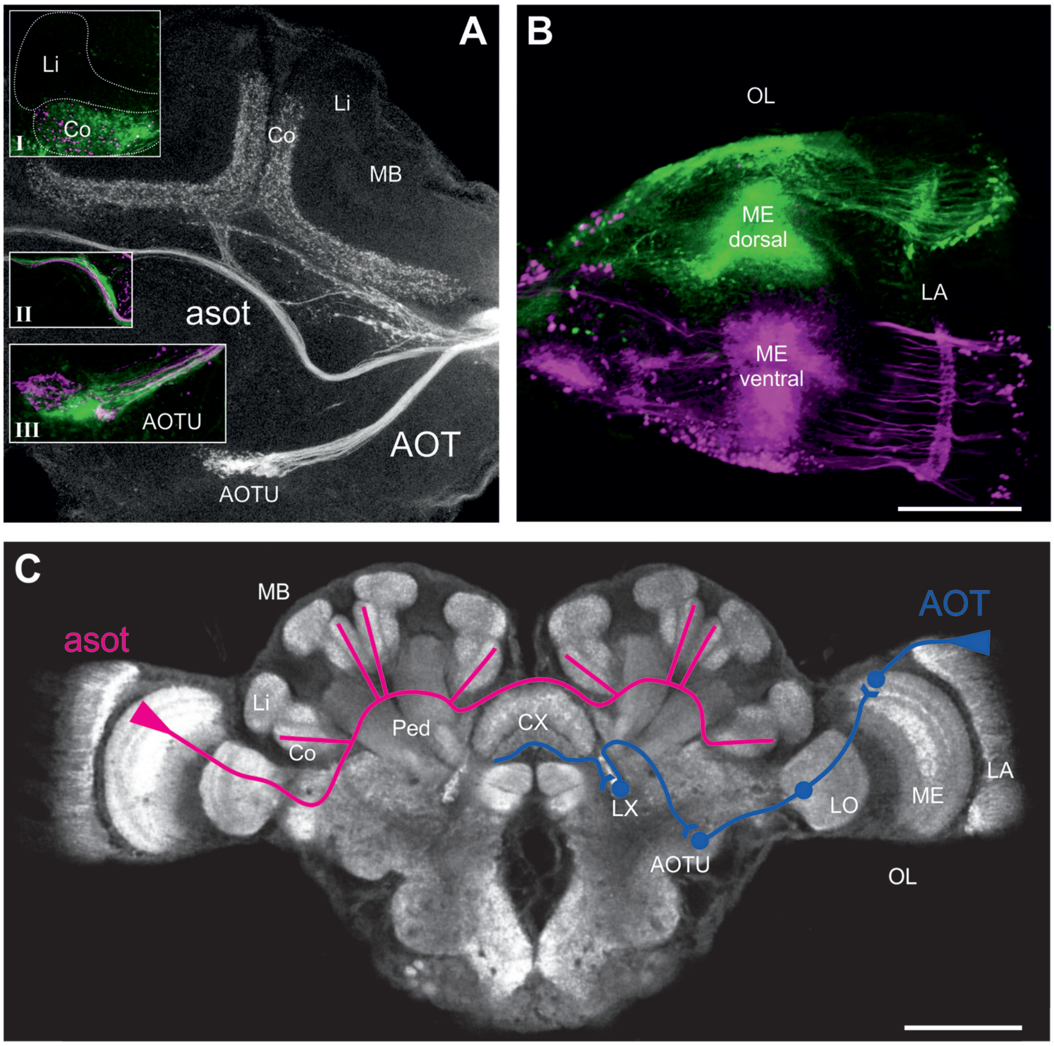

FIGURE 3 | Neuronal projections from the medualla (ME) via the anterior optical tract (AOT) and the anterior superior optic tract (asot) in the *Cataglyphis noda* brain. Anterograde tracings from focal dye injections the dorsal and ventral medulla (ME; microruby in magenta, Alexa 488 dextran in green, see under **B**): **(A)** Axon bundles from projection neurons in the medulla run anterior above the peduncle (Ped) and the central complex (CX) into the visual subregion of the mushroom body (MB) collar (Co) on both sides of the brain. Axonal projections from both the dorsal and the ventral ME run along the asot (inset **II**) into the Co. The most prominent input in the MB-calyx Co was found in injections into the dorsal ME (green) compared to those in the ventral ME (magenta) (inset **I**). Axonal projection from the ME also run into the anterior optical tubercle (AOTU) along the AOT. Z-projection from a stack of 27 images, 10x objective, 5 μm step size. Insets were taken with a 20x objective, 5 μm step size. **(B)** In the dorsal ME Dextran AlexaFloun488 (green) was injected using a glass capillary. In the ventral ME Dextran Tetramethylrhodamine (micro-Ruby) (magenta) was injected using a glass capillary. Images taken with a 10x objective, step size of 10 μm, stack of 19 images, zoom 2.65. The scale bar in **(B)**, also valid for **(A)**, is 100 μm. **(C)** Schematic depiction of the tracing of the asot (magenta) and the AOT (blue). The asot, as seen in the tracings in **(A)**, runs from the ME anterior above the peduncle and the CX into Co. The AOT (information combined with the one from Schmitt et al., 2016) runs from the dorsal rim of the lamina (LA) to the dorsal rim of the ME, and from there via the LO to the AOTU to be relayed further to the lateral complex (LX). The anterior CX pathway terminates in the lower half of the ellipsoid body (EB) of the CX (Schmitt et al., 2016). The confocal scan of the *C. noda* brain shows an anti-synapsin labeled brain, similar to the staining procedure used for the neuroanatomical analyses. The scale bar is 200 μm.

using similar methods as described in detail in Yilmaz et al. (2016). Ants were cooled and fixed with clay. A small window was cut in the head capsule, and the brain was rinsed with cooled ant ringer solution. Using a thin glass capillary, dextran tetramethylrhodamine (micro-Ruby, D-7162, Molecular Probes, Eugene, OR, USA) and Dextran AlexaFluor488 (D-22910, Molecular Probes, Eugene, OR, USA) were focally inserted in the dorsal and ventral medulla. The brain was then rinsed with ringer solution and the head capsule was covered with a thin piece of Parafilm to prevent the brain from drying out. The dyes were allowed to be transported by incubating the ants for 3 h at room temperature in a dark box with high humidity. Afterwards, the brains were dissected in cooled ringer solution and fixated in 4% formaldehyde in PBS overnight. The brains were rinsed five times in PBS for 10 min each before they were dehydrated using an ethanol serial dilution. For that, they were rinsed for 10 min, each step: 30%, 50%, 70%, 90%, 95% in water, and two times 100% ethanol. The dehydrated brains were then cleared and mounted in methyl salicylate. Finally, the brains were digitized in the confocal laser scanning microscope (see below) using a 20×- or 10×-objective and step sizes of 5 μm or 10 μm.

Data Analyses

High-Speed Video Analyses

The 4K-videos obtained from the experiments were converted into image stacks using the Free Video to JPG Converter (v. 5.0.99 build 823, DVDVideoSoft, DIGITAL WAVE LTD., London, UK). Subsequently, the pirouettes (tight back turns Fleischmann et al., 2017) performed by novices were analyzed frame by frame using the MATLAB (2015a, The MathWorks Inc., Natick, MA, USA) application DIGILITE (Jan Hemmi and Robert Parker, The Australian National University, Canberra, Australia). For this, the positions of the mandibles and the thorax were marked manually in each frame. Additionally, the position of the nest entrance and the north direction were marked. With these coordinates the gaze direction relative to the nest entrance of the ants during their back-turns was determined. The direction of the nest entrance was defined as 180°. Stopping phases during the pirouettes were defined as in Fleischmann et al. (2017), and the longest of these stopping phases was used to test the directedness of the back turns.

Neuroanatomical Analyses

For microscopic analyses, the brains that had been dissected and histochemically treated in our field laboratory were transferred to the University of Würzburg using a refrigerator unit (∼4°C). A confocal laser scanning microscope (Leica TCS SP2, Leica Microsystems GmbH, Wetzlar, Germany) was used for scanning the brains as image stacks at a step size of 5 μm. We used the 10×-objective for overviews with 2.5 optical zoom NA imm (for CX), the 20×-objective with 2.7 optical zoom NA imm for the MB calyx, and the 63×-objective with 2.0 optical zoom NA imm for detailed scans in the lip (Li) and collar (Co) of the MB calyx. Subsequently, the volumes of the different components of the CX (fan-shaped body (FB), ellipsoid body (EB), protocerebral bridge (PB), noduli (No)) and of the MB calyx (Li, Co) were analyzed using the 3D-reconstruction software Amira (Amira 6.0.0, FEI Company, Hilsboro, OR, USA). In addition, synaptic complexes (microglomeruli, MG) were quantified in the visual and olfactory subregions of the MB calyx (Li, Co) using a modified version of the protocol by Groh et al. (2012; for further details, see Rössler et al., 2017). The CX, MB and other major neuropils were easily distinguishable in anti-synapsin labeled whole mount brains (**Figure 3C**), and based on tracings (Yilmaz et al., 2016, **Figures 3A,B** for *C. noda*). MB-calyx MG were quantified by counting the anti-synapsin labeled synaptic boutons in a defined volume of 1000 μm^3. The MG density was then calculated by averaging multiple volumes of interest in the two subregions (three in the Co, four in the Li) as numbers of MG per μm^3 following the protocol by Groh et al. (2012) and Muenz et al. (2015). From these numbers the total number of MG per calyx subdivisions was estimated by multiplying the MG densities by the volume of the corresponding neuropil. The ants used in this experiment had a median thorax length of 4.24 mm, ranging from 3.18 mm to 5.58 mm. Thorax length correlates with body size (Vowles, 1954) and, therefore, also with total brain size (Wehner et al., 2007). Since we did not find a correlation between thorax length and the analyzed neuropils of interest (Spearman roh test ($\alpha = 0.05$): CX: $n_{CX} = 45$, $p_{CX} = 0.545$, $r_{CX} = 0.093$; MB: $n_{MB} = 43$, $p_{MB} = 0.058$, $r_{MB} = 0.291$), absolute volumes and MG numbers were used in this study. These results are coherent with results obtained using head width as a measure for body size in *C. fortis* (Stieb et al., 2010). As no major group-specific differences in thorax lengths were apparent (Supplementary Figure S1), comparisons were made without corrections for group bias in overall brain size.

Statistical Analyses

The gaze directions were grouped into 10°-bins as previously done by Fleischmann et al. (2017). The circular statistical software Oriana (Kovach Computing Services, Anglesey, UK) was used to check with the Rayleigh test whether the data was randomly distributed. If the gaze directions were directed ($\alpha = 0.05$), we calculated the 95% confidence interval to check whether the expected direction (nest entrance: 180°) was within the limits. The mean angle and the angular variance were compared between the groups using a Mardia-Watson-Wheeler multisample test ($\alpha = 0.05$).

In the neuroanatomical studies, the volume between the different groups (DD, UVBS, Dif, P, UV100) within each neuropil (CX, Co, Li) was compared using the Kruskal-Wallis-test ($\alpha = 0.05$). In cases when a difference between the groups occurred, a *post hoc* pairwise comparison between DD and the other groups was performed using a Mann-Whitney U-test with Bonferroni correction. A critical value of $\alpha = 0.05$ was used (after Bonferroni correction: $\alpha = 0.0125$).

RESULTS

Gaze Direction Analyses and Behavioral Observations Under Different Skylight Conditions

While initially leaving their nest under natural conditions (N), *C. noda* walked in small loops around their nest entrance, similar as shown earlier (Fleischmann et al., 2017). These learning walks were repeatedly interrupted by characteristic turns, so called voltes and pirouettes. During the latter, the ants performed multiple stopping phases ($n = 15$, 4 ± 1.75, median ± IQR) with the longest stopping phases directed towards the nest entrance (Rayleigh Uniformity Test: $Z_0 = 13.856$, $n = 15$, $p < 0.001$; 95% Confidence Interval (−/+) 167.9°/186.0°; Mean: 177.0°; **Figure 2A**). The gaze direction during the longest stopping phases was directed towards the nest entrance when the experimental setup was installed using a UV-light

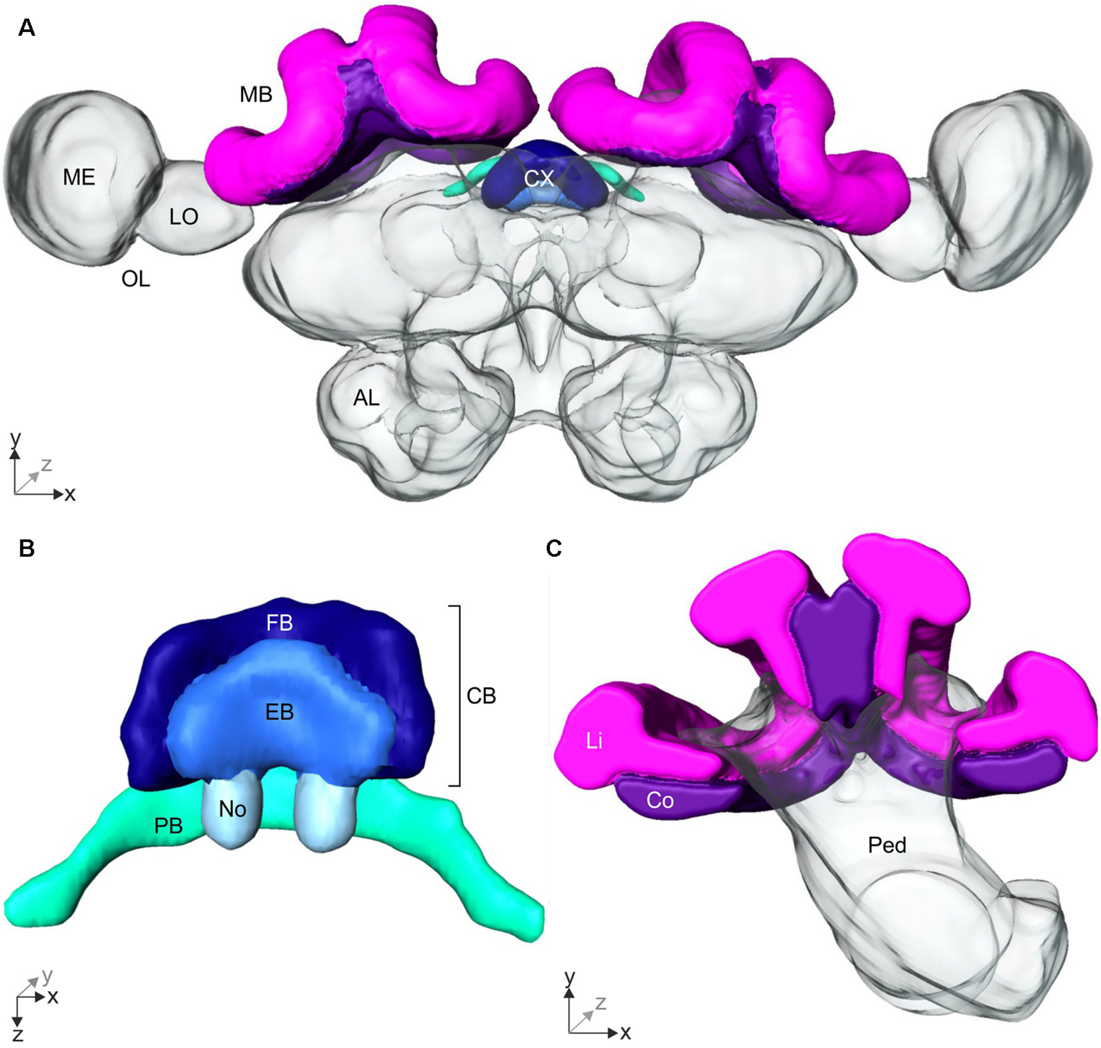

FIGURE 4 | 3D-reconstruction of the *Cataglyphis noda* brain. With the 3D-reconstruction software Amira the neuropils of the *C. noda* brain were manually reconstructed from the image stack obtained by the confocal laser scanning microscope. **(A)** 3D-reconstruction of a whole *C. noda* brain. To analyze the influence of celestial information during learning walks on neuroplasticity, the terminal stages of two visual pathways were reconstructed. The AOT transfers visual information, including polarization information, into the central complex (CX, shades of blue). The CX is located at the midline of the ant brain. Via the asot visual information is transferred to the mushroom body calyces (MB, magenta). Additionally, antennal lobes (AL), and optical lobes (OL) with the medulla (ME) and lobula (LO) are labeled. **(B)** The CX comprises several neuropils: The central body (CB, dark and light blue) is located most anterior. It consists of the large fan-shaped body (FB, dark blue) and the smaller ellipsoid body (EB, light blue), which is covered by the FB dorsally. Behind the CB, two globular neuropils, the noduli (No, pale blue) are located. Dorsally to that and slightly detached from the CB, the protocerebral bridge (PB, green) spans in a bridge-like shape between the mushroom bodies (MB). **(C)** The MB calyx includes the visual input region, the collar (Co, violet) and the olfactory input region, the lip (Li, magenta). They are located at the dorsal rim of the peduncle (Ped). Scale bars, **(A)** 200 μm; **(B,C)** 100 μm.

permeable filter as a control (UV100; Rayleigh Uniformity Test: $Z_0 = 12.306$, $n = 15$, $p < 0.001$; 95% Confidence Interval (−/+) 163.9°/192.55°; Mean: 178.2°; **Figure 2B**). When the natural skylight polarization pattern was altered to a linear one that did not change over the day (P) the overall structure of the walks remained unchanged and the gazes during the longest stopping phases were clearly directed towards the nest entrance (Rayleigh Uniformity Test: $Z_0 = 6.189$, $n = 14$, $p = 0.001$; 95% Confidence Interval (−/+) 173.1°/229.1; Mean: 201.1°; **Figure 2C**). One analyzed pirouette under P did not contain a stopping phase and therefore was not included in the circular statistics. After the learning walks had taken place for several days under this fixed polarization pattern, the polarization filter was rotated by either by 90° or in two steps of 45°. From visual observations we noticed that the sudden changes in the polarization pattern above the nest entrance seemed to increase the number of naïve ants performing learning walks shortly after the change took place (experimental day with stationary linear polarization pattern number of learning walks: $n = 71$ vs. experimental day with stepwise rotated (45°

every hour) linear polarization pattern number of learning walks: $n = 277$). When learning walks were performed under a diffused polarization pattern (Dif) no apparent changes in learning walk patterns compared to natural conditions could be observed. For the Dif conditions, further quantitative video analyses were not possible since we could not record through the diffusor. Nevertheless, more than 100 pirouettes, all directed towards the nest entrance, were observed during the three experimental days. However, even learning walks that were performed under the exclusion of any sky compass information by blocking UV-light, which is necessary for the ants to perceive the polarization pattern (Duelli and Wehner, 1973), and, at the same time, by excluding the position of the sun by using a sunshade (UVBS) were not altered in their overall structure compared to learning walks under natural conditions. The longest stopping phase of pirouettes under UVBS conditions was directed towards the nest entrance (Rayleigh Uniformity Test: $Z_0 = 11.406$, $n = 15$, $p < 0.001$; 95% Confidence Interval (−/+) 166.4°/200.0°; Mean: 183.2°; **Figure 2D**). The mean angle or the angular variance did not differ between all experimental groups (Mardia-Watson-Wheeler multi sample test: $W = 6.124$; $n_{\mathrm{N}} = 15$; $n_{\mathrm{UV100}} = 15$; $n_{\mathrm{P}} = 14$; $n_{\mathrm{UVBS}} = 15$; $p = 0.375$).

The AOT and asot in the *Cataglyphis* Brain

To investigate visual pathways to high-order integration centers in *C. noda* brains, we performed focal dye injections and anterograde neuronal tracings of neuronal projections from the dorsal and ventral medulla (ME; **Figure 3B**). This clearly revealed neuronal projections via the asot and via the AOT (**Figure 3A**). From 16 dye injected brains, three were successfully double stained (dorsal and ventral ME), three showed tracings from the dorsal ME only, and two from the ventral ME only. In all tracings, the asot projected from the ME anteriorly above the peduncle and the central complex (CX), bilaterally into the collar (Co) of the medial and lateral branches of the MBs (**Figures 3A,C**). Visual inspection of all tracings indicated that axonal projections via the asot from the dorsal ME were more prominent compared to the sparser projections and terminal branches from the ventral ME in the MB Co ($n = 8$; **Figure 3A**, inset I).

All tracings from the dorsal and ventral ME revealed projections to the anterior optic tubercle (AOTU) via the AOT (**Figures 3A,C**). The AOT was previously described in detail for *C. fortis* (Schmitt et al., 2016) by tracing projections only from the dorsal rim area of the lamina LA and ME. From there further stages are the lobula (LO), the AOTU, the lateral complex (LX) and finally the lower half of the EB of the CX (**Figure 3C**; for locust: Homberg et al., 2011; for *C. fortis*: Schmitt et al., 2016). Interestingly, our differential tracings from the dorsal and ventral ME revealed a clear pattern in the AOTU with a clear separation of ventral and dorsal projections in the upper unit of the AOTU and a mixed pattern in the lower part of the AOTU (**Figure 3A**, inset III).

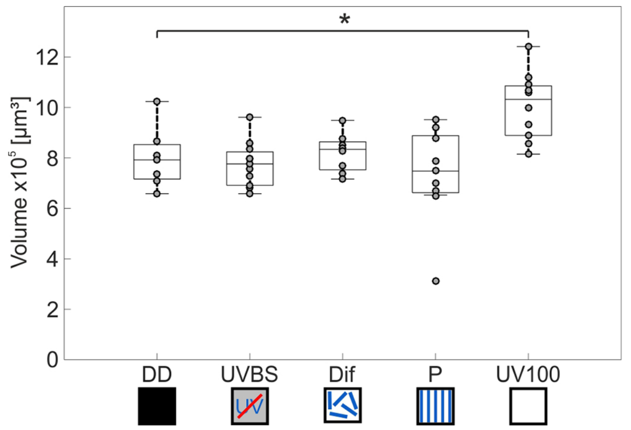

FIGURE 5 | Volume changes of the CX after 3 days of learning walks dependent on celestial information. The central line of each boxplot depicts the median of the data. The upper and lower limits of the boxes show the 25th and 75th percentiles, while the whiskers extend to the extreme data points without outliers. All data points (including outliers) are plotted as gray circles. A difference between the groups can be found using a Kruskal–Wallis test. With the Mann-Whitney U-test with a Bonferroni correction the data was *post hoc* compared to the DD group. The asterisk indicates that data is significantly different (after correction $p < 0.0125$) from the DD group. The central complex shows a volume increase compared to interior workers (DD; $n = 7$) only if the ants perceived the UV mediated natural polarization pattern that changes over the day (UV100; $n = 10$). If the polarization pattern was altered, either by diffusion (Dif; $n = 8$) or by a linear polarization filter (P; $n = 9$), no change in the volume of the CX occurred compared to DD. Similarly, when ants were excluded from any celestial information (UVBS; $n = 11$), no volume increase occurred. For statistical details and further explanations, see text.

Influence of Manipulated Skylight Input during Learning Walks on Neuronal Plasticity in the CX and MB

We investigated the influence of skylight manipulations during learning walks on neuronal changes in the terminal stages of the AOT and asot. The brains of ants that had participated in the behavior tests and had performed several days of learning walks under normal or altered skylight conditions were analyzed using 3D-reconstructions of the CX and MB (**Figure 4**), and quantifications of synaptic complexes in the MB. For comparison, brains of ants that had not yet performed learning walks (DD) were analyzed.

Volumetric Changes in the CX

The AOT transfers visual information into the CX (**Figure 3C**). The CX comprises several neuropils (**Figure 4B**): The central body (CB) is located most anterior and consists of the large FB and the smaller EB, which is covered by the FB dorsally. Behind the CB, two globular neuropils, the No, are located. Dorsally to that and slightly detached from the CB, the PB spans in a bridge-like shape between the MBs (**Figure 4B**). Comparing the CX of the ants that had previously participated in the behavioral studies (DD, UVBS, Dif, P, UV100), showed a statistically significant difference between their CX volumes (Kruskal–Wallis test: CX Volume: $\chi^2_4 = 16.38$; $n = 45$; $p = 0.0046$; **Figure 5**). Compared to the CX of interior workers (DD) the CX

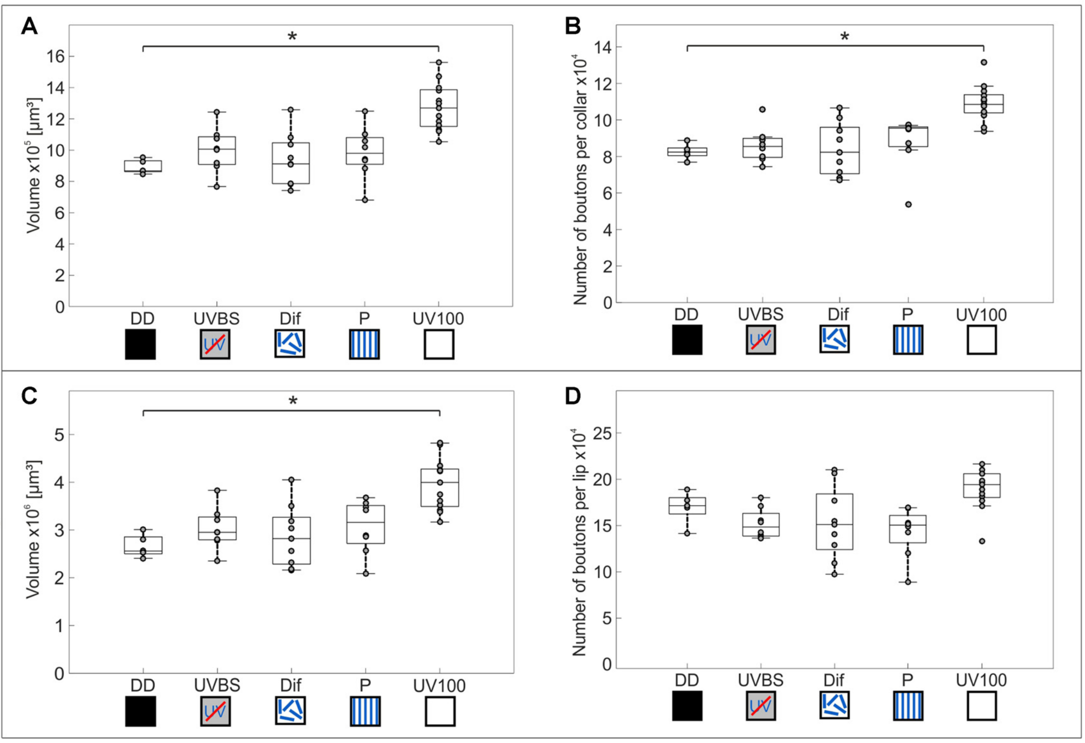

FIGURE 6 | Volume changes and changes in numbers of synaptic complexes in MB calyx subdivisions. The central line of each boxplot depicts the median of the data. The upper and lower limits of the boxes show the 25th and 75th percentiles, while the whiskers extend to the extreme data points without outliners. All data points (including outliers) are plotted as gray circles. To find a difference between the groups, a Kruskal–Wallis test was used ($\alpha = 0.05$). With the Mann-Whitney U-test with a Bonferroni correction the data was *post hoc* compared to the DD group if the Kruskal–Wallis test indicated a difference. The asterisk indicates data significantly different (after correction $p < 0.0125$) from the DD group. **(A)** In the MB-calyx Co, a significant volume increase compared to DD ($n = 5$) occurred only when the learning walks were conducted under the natural UV mediated changing polarization pattern (UV100; $n = 13$). **(B)** Similarly, the total number of synaptic boutons per calyx in the Co only increased when the learning walks were performed under UV100 compared to DD. **(C)** In the MB-calyx Li, a volume increase occurred only under UV100 conditions compared to DD, similar to the conditions in the Co. **(D)** However, in the Li no change in the total number of synaptic boutons per calyx occurred under any conditions. No significant differences in the MB occurred between DD and UVBS ($n = 8$), Dif ($n = 9$), or P ($n = 8$). For statistical details and further explanations, see text.

in brains of *C. noda* that had performed several learning walks under a naturally changing polarization pattern (UV100) showed a volumetric increase (Mann-Whitney U-test with Bonferroni correction: DD vs. UV100, $Z_4 = -2.6837$; $n_{DD} = 7$; $n_{UV100} = 10$; $p = 0.0073$). The volumetric increase in the CX was absent compared to DD when the ants performed their learning walks under restricted skylight conditions including an artificially fixed linear polarization pattern (P) (Mann-Whitney U-test with Bonferroni correction: DD vs. P, $Z_3 = 0.4234$; $n_{DD} = 7$; $n_P = 9$; $p = 0.6720$), a diffused polarization pattern (Dif) (Mann-Whitney U-test with Bonferroni correction: DD vs. Dif, $Z_2 = -0.7522$; $n_{DD} = 7$; $n_{Dif} = 8$; $p = 0.4519$), and without polarization pattern and information about the position of the sun (UVBS; Mann-Whitney U-test with Bonferroni correction: DD vs. UVBS, $Z_1 = 0.5434$; $n_{DD} = 7$; $n_{UVBS} = 11$; $p = 0.5869$). The same statistical relationships were found for the volume of the CB only, which includes the ellipsoid (EB) and the fan-shaped body (FB). When comparing the subunits (EB, FB, PB and No) individually, the same tendency was found, but was not statistically significant.

Volumetric Changes in the MB and Plasticity of Synaptic Complexes

Comparison of the volume and the numbers of synapsin labeled synaptic boutons in the MB calyx Co (**Figure 4C**) revealed a significant difference between the experimental groups of the behavior essay (Kruskal–Wallis test: Co Volume: $\chi^2_4 = 22.43$; $n = 43$; $p = 0.00016$; Co No. Synapses: $\chi^2_4 = 23.06$; $n = 43$; $p = 0.00012$; **Figures 6A,B**). Only ants that had performed several learning walks under a naturally changing skylight polarization pattern (UV100) showed an increase in the volume of the MB calyx Co and the estimated total number of synapses per calyx compared to ants that had not yet performed learning walks (DD; Mann-Whitney U-test with Bonferroni correction: Co Volume: DD vs. UV100, $Z_4 = -3.1543$; $n_{DD} = 5$; $n_{UV100} = 13$; $p = 0.0016$; Co No. Synapses: DD vs. UV100, $Z_4 = -3.1543$; $n_{DD} = 5$; $n_{UV100} = 13$; $p = 0.0016$). All groups that had performed learning walks under restricted skylight conditions did not show a significant increase compared to DD, neither in the volume nor in the total number of MG synaptic complexes per calyx in the MB calyx Co (Mann-Whitney

U-test with Bonferroni correction: CO Volume: DD vs. UVBS, $Z_1 = -1.5370$; $n_{DD} = 5$; $n_{UVBS} = 8$; $p = 0.1243$; DD vs. Dif, $Z_2 = -0.2667$; $n_{DD} = 5$; $n_{Dif} = 9$; $p = 0.7897$; DD vs. P, $Z_3 = -1.5370$; $n_{DD} = 5$; $n_P = 8$; $p = 0.1243$; Co No. Synapses: DD vs. UVBS, $Z_1 = -0.6587$; $n_{DD} = 5$; $n_{UVBS} = 8$; $p = 0.5101$; DD vs. Dif, $Z_2 = 0$; $n_{DD} = 5$; $n_{Dif} = 9$; $p = 1$; DD vs. P, $Z_3 = -1.8298$; $n_{DD} = 5$; $n_P = 8$; $p = 0.0673$). The volume of the Li also differed significantly between groups (Kruskal–Wallis test: Li volume: $\chi^2_4 = 20.08$; $n = 43$; $p = 0.00048$; **Figure 6C**). The volume was increased significantly compared to DD in ants that had performed several learning walks under UV100 conditions (Mann-Whitney U-test with Bonferroni correction: Li Volume: DD vs. UV100, $Z_4 = -3.1543$; $n_{DD} = 5$; $n_{UV100} = 13$; $p = 0.0016$). No difference in the Li volume occurred between DD and the other groups, (Mann-Whitney U-test with Bonferroni correction: Co Volume: DD vs. UVBS, $Z_1 = -1.2443$; $n_{DD} = 5$; $n_{UVBS} = 8$; $p = 0.2134$; DD vs. Dif, $Z_2 = -0.5333$; $n_{DD} = 5$; $n_{Dif} = 9$; $p = 0.7897$; DD vs. P, $Z_3 = -1.5370$; $n_{DD} = 5$; $n_P = 8$; $p = 0.1243$). In contrast to the visual MB subregion (Co), however, there was no significant difference compared to DD based on pair-wise comparison of the total number of MG synaptic complexes per calyx in the MB olfactory Li, despite the groups not coming from the same distribution (Kruskal–Wallis test: Li No. synaptic complexes: $\chi^2_4 = 15.81$; $n = 43$; $p = 0.0033$; Mann-Whitney U-test with Bonferroni correction: Li No. synaptic complexes: DD vs. UVBS, $Z_1 = 1.5370$; $n_{DD} = 5$; $n_{UVBS} = 8$; $p = 0.1243$; DD vs. Dif, $Z_2 = 0.9333$; $n_{DD} = 5$; $n_{Dif} = 9$; $p = 0.3506$; DD vs. P, $Z_3 = 1.9762$; $n_{DD} = 5$; $n_P = 8$; $p = 0.0481$; DD vs. UV100, $Z_4 = -1.9715$; $n_{DD} = 5$; $n_{UV100} = 13$; $p = 0.0487$; **Figure 6D**).

DISCUSSION

Celestial Information Is Not Necessary for the Look Back to the Nest Behavior

In the beginning of their foraging careers, *C. noda* perform learning walks that are repeatedly interrupted by turns with several stopping phases. The longest stopping phases are accurately directed towards the nest entrance (Fleischmann et al., 2017). It has previously been suggested that ants may use path integration information to align their back turns (Graham et al., 2010; Müller and Wehner, 2010). We used this conspicuous feature in the learning walks of *C. noda* as an easily quantifiable behavior readout in skylight manipulation experiments to ask whether celestial cues may serve as a reference system to align gaze direction. Our results demonstrate that neither an artificial (P) nor a diffused (Dif) polarization pattern disturbed the directedness of the longest stopping phases toward the nest entrance. Even with complete exclusion of the polarization pattern and the position of the sun (UVBS), the ants were still able to perform the look back to the nest entrance behavior. This strongly suggests that the celestial compass—providing the directional component of the path integration system during foraging (review: Wehner, 2003)—is not the system of reference used by ants to initially align the gaze direction during naïve learning walks. Our results underline the robustness and importance of the mechanism that is used to align the gaze direction during the longest stopping phases.

Possible Reference Systems for the Look Back to the Nest Behavior

As our results show that the celestial compass does not provide a reference system used during learning walks, other possibilities for the compass component of the path integrator have to be considered. A potential candidate could be the visual landmark panorama. Schultheiss et al. (2016) recently demonstrated that UV-light plays a crucial role for the use of the landmark panorama. However, our results show that *C. noda* was still able to look back to the nest entrance during learning walks under blocked UV-light spectrum (UVBS). Furthermore, the panorama information is not yet known or memorized in ants during naïve (first) learning walks and requires the completion of several learning walks (Fleischmann et al., 2016). The ants might also use nest odors to detect the direction of the nest. *C. fortis* were shown to use olfactory landmark cues near the nest (Steck et al., 2009). However, as the ants conduct their learning walks in increasing distances and in all compass (including upwind) directions away from the nest (Fleischmann et al., 2016), olfactory cues are not reliable during the entire learning walk sequences. The ants also walk cross wind in order to approach odor sources, in particular prey items during foraging (Wolf and Wehner, 2000; Buehlmann et al., 2014). This behavior has not become evident in learning walks, and as the ants perform pirouette-like turns all-around the nest entrance, cross wind orientation seems highly unlikely. Finally, the ants could use intrinsic (idiothetic) orientation mechanisms. Such mechanisms however, would be highly prone to cumulative errors (Müller and Wehner, 1994). An error in the gaze direction during the longest stopping phase of pirouettes would lead to a snapshot taken into the wrong direction. This could easily lead to serious errors in foragers, but also during learning walks with extended lengths. A more promising candidate for an initial reference system for the compass component of the path integrator during learning walks of *C. noda* is the geomagnetic field. This had already been suggested for the learning flights of bumblebees (Collett et al., 2013). Furthermore, *C. noda* was shown to learn magnetic landmarks (Buehlmann et al., 2012). Although the magnetic field strength, in these experiments, was far above the natural geomagnetic field, it appears likely that the ants possess a magnetic sense that could be used for the initial calibration of navigational information. A potential role of a magnetic sense has also been suggested for other ants (fire ants: Anderson and Vander Meer, 1993; leaf-cutter ants: Banks and Srygley, 2003; wood ants: Çamlitepe and Stradling, 1995; for a review see: Wajnberg et al., 2010). However, so far no use of the geomagnetic field for navigation, in particular as compass information for path integration, has been described in ants, neither for experienced foragers during their foraging runs, nor for learning walks in novices. Therefore, at this point the question regarding an initial reference system for the alignment of gaze directions to acquire and calibrate navigational information during learning walks has to remain open.

Visual Pathways in the *C. noda* Brain

To be used as navigational information, the visual information perceived by the ants during learning walks needs to be relayed to and processed in higher integration centers of the brain. Using anterograde tracing techniques, two prominent visual pathways become apparent in *C. noda*. Visual information from the ME is transferred bilaterally to the MB collars of the medial and lateral MB calyces, very similar to the projections found in other Hymenoptera (Gronenberg, 2001; Yilmaz et al., 2016). In *Drosophila* only a very small subset of visual neurons transfers information from the OL to the MB calyx (Vogt et al., 2016). This may suggest that this pathway is highly conserved across insects, but the number of neurons and their projection patterns are adapted to the visual ecology of individual species (Groh et al., 2014; Vogt et al., 2016; Yilmaz et al., 2016). One interesting feature in *C. noda* is that axonal projections from the dorsal ME appear more extensive compared to projections from dye injections into the ventral ME. This may indicate that the dorsal retina and celestial view aspects are more prominently represented in the MB calyx Co compared to terrestrial aspects from the lower part of the compound eye. More focal injections, also along the horizontal axis, are needed to further analyze this. In *C. fortis* the AOT was shown to house projections from the dorsal most regions of the medulla indicating that polarization information from the dorsal rim area of the eye is transferred via this pathway to the AOTU and the LX into the lower half of the EB of the CX (Schmitt et al., 2016), similar to the conditions found in locusts (Homberg et al., 2011). Our results show that also the ventral region of the medulla is relayed to the upper and lower part of the AOTU. Next we tested whether the high-order sensory integration centers (MB, CX) express neuroplasticity related to the quality of celestial information experienced during learning walks.

Natural Polarization Pattern Is Necessary for a Volume Increase in the CX

Although our manipulations of celestial information did not significantly alter the learning walk behavior, the restriction of skylight information interfered with neuroanatomical changes in the CX. A volume increase in the CX as compared to DD occurred only when the learning walks had been conducted under the full spectrum including UV-light and the naturally changing polarization pattern. Exposure to the full light spectrum including UV-light with an artificial, fixed polarization pattern (P) or without a usable polarization pattern (Dif) did not lead to a CX volume increase. In contrast, a volume increase in the CB of *Drosophila* occurs after the flies were exposed to UV-light (Barth and Heisenberg, 1997). However, in that case *Drosophila* did not perceive a natural light and polarization pattern. In *C. noda* the exclusion of UV-light, and thereby the reception of the polarization pattern during learning walks, prevented volumetric chances of the CX. It is not possible with the methods available to count synapses within subunits of the CX. Therefore, we only analyzed volumetric changes in the CX. Previous studies on large synaptic complexes (giant synapses, GS) in the lateral complex (LX) along the sky-compass pathway of *C. fortis* revealed a significant increase of GS numbers depending on exposure to the UV part of the light spectrum (Schmitt et al., 2016). Therefore, it seems likely that the volume increase in the CX is also due to an increase in the number of synapses along this pathway. This increase was found to be significant in the CB units, i.e., the input region of the CX. Within the CX, in particular the PB, the skylight polarization direction is represented in a map-like manner (Pfeiffer and Homberg, 2014), and it has been shown through computational investigation that the CX is able to store spatial information (Fiore et al., 2017). Whether the neuroanatomical changes we found in the CX are triggered by appropriate sensory exposure or following the formation of spatial memory is an interesting question that needs to be investigated in a more focused approach. The CX is also involved in higher order control of movement of the limbs (Strauss, 2002; Martin et al., 2015), landmark orientation, and angular path integration (Seelig and Jayaraman, 2015). All this makes the CX a well suited neuropil to link polarization information to other stimuli mediating directional information important for navigation, for example other terrestrial reference systems.

Sensory Experience of a Natural Polarization Pattern Is Necessary for an Increase in the Number of Synaptic Complexes in the Visual Subregions of the MB Calyx

Similar to the results just described for the CX it was only under exposure to the naturally changing UV polarization pattern that a volume increase was found in the MB-calyx of ants that had performed their learning walks. Kühn-Bühlmann and Wehner (2006) had previously shown an increase in the MB volume of experienced (aged) foragers compared to dark reared ants of age-controlled *Cataglyphis bicolor*. In our study, we focused on the transition phase between interior worker (DD) and forager. Our data suggests that a volume increase in the MBs occurs already during learning walks and that it is dependent on the presence of the natural polarization pattern (UV100). A net increase of MB synaptic complexes was found only in the visual input region. As the MB is a higher order integration center involved in learning and memory, this may indicate that the increase in MG numbers is related to visual experience. Computer simulations by Ardin et al. (2015) suggest that the large synaptic capacity of visual subregions in ant MBs are well suited for the storage of visual snapshots underling the potential role of the MBs for learning and memorizing panoramic landmark cues during learning walks. Studies by Stieb et al. (2010, 2012) have shown that the MB Co expresses light-induced and age-dependent changes in MG numbers in *C. fortis*. Stieb et al. (2010) also showed a volume increase in the Co after exposure to full spectrum light accompanied by a decrease in MG densities. Furthermore, studies in the honeybee (Hourcade et al., 2010) and leafcutter ants (Falibene et al., 2015) showed that the formation of stable long-term olfactory memory leads to an increase in the density and number of MG in the Li. In contrast to the laboratory and partly restrained conditions in these experiments, the ants used in our study were allowed to perform their natural behaviors in their natural habitat under natural or altered skylight conditions. Therefore, a mix of both effects—the first exposure to light and long-term memory formation following learning, might be expected in our experimental ants. As UV-light is crucial for learning terrestrial landmarks (Schultheiss et al., 2016), an increase in synaptic complexes could be expected in the presence of UV-light, even without a naturally changing polarization pattern (Dif). Our data shows that a volume increase in the Co was absent in ants that had performed their learning walks under the full light spectrum, but without a usable polarization pattern (Dif) or with an artificial, fixed polarization pattern (P). Only when ants perceived a full spectrum including UV light together with a naturally changing polarization pattern, an increase in the volume and number of MG occurred in the MB calyx Co. No such effect was seen in MB collar MG of honeybees after a fine color discrimination task (Sommerlandt et al., 2016) indicating that only certain parameter combinations may lead to measurable effects of structural synaptic plasticity. The increase in the estimated MG numbers in the MB-calyx Co indicates an outgrowth of new presynapses during learning walks under natural skylight—a process similar to what has been observed after the formation of long-term memory (Hourcade et al., 2010; Falibene et al., 2015).

Due to the prominent role of path integration, *Cataglyphis* have to calibrate their internal skylight compass to the solar ephemeris (the season- and place-specific course of the sun over the day) at the beginning of their foraging career (Wehner and Müller, 1993). A panoramic- and celestial snapshot based mechanisms based on long-term memory in the visual MBs might play a role in this initial calibration. Similarly, short term learning of celestial snapshots was recently suggested for sky-compass orientation in dung beetles (el Jundi et al., 2016). When the skylight polarization pattern, however, does not change over the day (P), is diffused (Dif), or is not available (UVBS), it would not make sense to take and store celestial snapshots. To store new celestial information and thereby fine tune an internal template of the solar ephemeris function makes only sense if the polarization pattern changes compared to a fixed reference system. This hypothesis is also backed up by our observation that the number of learning walks drastically increased when the linear polarization filter was rotated. Analyzing neuroanatomical changes in ants that have performed learning walks under such a systematically changed artificial polarization pattern would allow for a deeper insight into the correlation shown so far.

The present study represents a first step of probing potential effects of learning walks on neuroplasticity. We started this combined field and laboratory study by focusing on the terminal projection areas of two prominent visual pathways in the CX and MB. To obtain a more comprehensive understanding of the total extent of learning-walk induced neuroplasticity, future investigations will have to include more extensive neuroanatomical analyses of all major brain neuropils, for example analyzing their volume relationships, synapse densities (whenever feasible), also in relation to overall brain volumes—for example like it was done in recent volumetric analyses of brains in migratory and solitary locusts, or migratory and non-migratory moths (Ott and Rogers, 2010; de Vries et al., 2017). In the same line, as previous work in *Camponotus* ants (Yilmaz et al., 2016) and in *Drosophila* (Barth and Heisenberg, 1997) show that the optic lobes undergo plastic changes after artificial light exposure, future studies on learning-walk induced neuroplasticity in *Cataglyphis* ants should include neuropils peripheral to the CX and MB, like the optic lobes, the AOTU and the lateral complex.

CONCLUSION

Neither the polarization pattern, or other information from UV-light input, nor the position of the sun are necessary for *C. noda* to align their gaze directions during the longest stopping phase of pirouettes in learning walks. Thus, the celestial compass as part of the path integrator does not provide the ants with the reference system needed during naïve learning walks. However, although not being necessary for the accuracy of the look-back behavior, we show that proper perception of the natural polarization pattern that changes over the day is important for triggering neuroanatomical changes in the CX and MB calyx that take place during learning walks. In the MB-calyx Co, this volume increase is linked to an increase in the number of MG synaptic complexes indicating that plasticity related processes are triggered when the ants are confronted with a naturally perceived polarization pattern that changes over the day.

ETHICS STATEMENT

This study was carried out in accordance with the Greek and German laws.

AUTHOR CONTRIBUTIONS

RG, PNF, RW, WR conceived the study. WR and RW led the study. RG, PNF and KG collected and analyzed the data. RG and PNF drafted and WR and RW revised the manuscript. All authors approved the final version of the manuscript for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2017.00226/full#supplementary-material

REFERENCES

Anderson, J. B., and Vander Meer, R. K. (1993). Magnetic orientation in the fire ant, *Solenopsis invicta*. *Naturwissenschaften* 80, 568–570. doi: 10.1007/bf01149274

Ardin, P., Mangan, M., Wystrach, A., and Webb, B. (2015). How variation in head pitch could affect image matching algorithms for ant navigation. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 201, 585–597. doi: 10.1007/s00359-015-1005-8

Banks, A. N., and Srygley, R. B. (2003). Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethology* 109, 835–846. doi: 10.1046/j.0179-1613.2003.00927.x

Barth, M., and Heisenberg, M. (1997). Vision affects mushroom bodies and central complex in *Drosophila melanogaster*. *Learn. Mem.* 4, 219–229. doi: 10.1101/lm.4.2.219

Buehlmann, C., Graham, P., Hansson, B. S., and Knaden, M. (2014). Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. *Curr. Biol.* 24, 960–964. doi: 10.1016/j.cub.2014.02.056

Buehlmann, C., Hansson, B. S., and Knaden, M. (2012). Desert ants learn vibration and magnetic landmarks. *PLoS One* 7:e33117. doi: 10.1371/journal.pone.0033117

Çamlitepe, Y., and Stradling, D. J. (1995). Wood ants orient to magnetic fields. *Proc. R. Soc.* 261, 37–41. doi: 10.1098/rspb.1995.0114

Collett, T. S., Hempel de Ibarra, N., Riabinina, O., and Philippides, A. (2013). Coordinating compass-based and nest-based flight directions during bumblebee learning and return flights. *J. Exp. Biol.* 216, 1105–1113. doi: 10.1242/jeb.081463

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., et al. (2015). Exploratory behaviour of honeybees during orientation flights. *Anim. Behav.* 102, 45–57. doi: 10.1016/j.anbehav.2014.12.030

de Vries, L., Pfeiffer, K., Trebels, B., Adden, A. K., Green, K., Warrant, E., et al. (2017). Comparison of navigation-related brain regions in migratory versus non-migratory noctuid moths. *Front. Behav. Neurosci.* 11:158. doi: 10.3389/fnbeh.2017.00158

Duelli, P., and Wehner, R. (1973). The spectral sensitivity of polarized light orientation in *Cataglyphis bicolor* (Formicidae, Hymenoptera). *J. Comp. Physiol.* 86, 37–53.doi: 10.1007/BF00694476

el Jundi, B., Foster, J. J. J., Khaldy, L., Byrne, M. J. J., Dacke, M., and Baird, E. (2016). A snapshot-based mechanism for celestial orientation. *Curr. Biol.* 26, 1456–1462. doi: 10.1016/j.cub.2016.03.030

Falibene, A., Roces, F., and Rössler, W. (2015). Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. *Front. Behav. Neurosci.* 9:84. doi: 10.3389/fnbeh.2015.00084

Fiore, V. G., Kottler, B., Gu, X., and Hirth, F. (2017). *In silico* interrogation of insect central complex suggests computational roles for the ellipsoid body in spatial navigation. *Front. Behav. Neurosci.* 11:142. doi: 10.3389/fnbeh.2017.00142

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *J. Exp. Biol.* 219, 3137–3145. doi: 10.1242/jeb.140459

Fleischmann, P. N., Grob, R., Wehner, R., and Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *J. Exp. Biol.* 220, 2426–2435. doi: 10.1242/jeb.158147

Graham, P., Philippides, A., and Baddeley, B. (2010). Animal cognition: multi-modal interactions in ant learning. *Curr. Biol.* 20, R639–R640. doi: 10.1016/j.cub.2010.06.018

Groh, C., Kelber, C., Grübel, K., and Rössler, W. (2014). Density of mushroom body synaptic complexes limits intraspecies brain miniaturization in highly polymorphic leaf-cutting ant workers. *Proc. Biol. Sci.* 281:20140432. doi: 10.1098/rspb.2014.0432

Groh, C., Lu, Z., Meinertzhagen, I. A., and Rössler, W. (2012). Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. *J. Comp. Neurol.* 520, 3509–3527. doi: 10.1002/cne.23102

Gronenberg, W. (2001). Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *J. Comp. Neurol.* 435, 474–489. doi: 10.1002/cne.1045

Heinze, S., and Homberg, U. (2007). Maplike representation of celestial E-vector orientations in the brain of an insect. *Science* 315, 995–997. doi: 10.1126/science.1135531

Heinze, S., and Reppert, S. M. (2012). Anatomical basis of sun compass navigation I: the general layout of the monarch butterfly brain. *J. Comp. Neurol.* 520, 1599–1628. doi: 10.1002/cne.23054

Hempel de Ibarra, N., Philippides, A., Riabinina, O., and Collett, T. S. (2009). Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. *J. Exp. Biol.* 212, 3193–3204. doi: 10.1242/jeb.029751

Homberg, U., Heinze, S., Pfeiffer, K., Kinoshita, M., and el Jundi, B. (2011). Central neural coding of sky polarization in insects. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 366, 680–687. doi: 10.1098/rstb.2010.0199

Hourcade, B., Muenz, T. S., Sandoz, J.-C., Rössler, W., and Devaud, J.-M. (2010). Long-term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? *J. Neurosci.* 30, 6461–6465. doi: 10.1523/jneurosci.0841-10.2010

Ito, K., Shinomiya, K., Ito, M., Armstrong, J. D., Boyan, G., Hartenstein, V., et al. (2014). A systematic nomenclature for the insect brain. *Neuron* 81, 755–765. doi: 10.1016/j.neuron.2013.12.017

Kühn-Bühlmann, S., and Wehner, R. (2006). Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *J. Neurobiol.* 66, 511–521. doi: 10.1002/neu.20235

Labhart, T., and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* 47, 368–379. doi: 10.1002/(sici)1097-0029(19991215)47:6<368::aid-jemt2>3.0.co;2-q

Lehrer, M. (1993). Why do bees turn back and look? *J. Comp. Physiol. A* 172, 549–563. doi: 10.1007/bf00213678

Martin, J. P., Guo, P., Mu, L., Harley, C. M., and Ritzmann, R. E. (2015). Central-complex control of movement in the freely walking cockroach. *Curr. Biol.* 25, 2795–2803. doi: 10.1016/j.cub.2015.09.044

Menzel, R. (2001). Searching for the memory trace in a mini-brain, the honeybee. *Learn. Mem.* 8, 53–62. doi: 10.1101/lm.38801

Muenz, T. S., Groh, C., Maisonnasse, A., Le Conte, Y., Plettner, E., and Rössler, W. (2015). Neuronal plasticity in the mushroom body calyx during adult maturation in the honeybee and possible pheromonal influences. *Dev. Neurobiol.* 75, 1368–1384. doi: 10.1002/dneu.22290

Müller, M., and Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proc. Natl. Acad. Sci. U S A* 85, 5287–5290. doi: 10.1073/pnas.85.14.5287

Müller, M., and Wehner, R. (1994). The hidden spiral: systematic search and path integration in desert ants, *Cataglyphis fortis*. *J. Comp. Physiol. A* 175, 525–530. doi: 10.1007/bf00199474

Müller, M., and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Curr. Biol.* 20, 1368–1371. doi: 10.1016/j.cub.2010.06.035

Narendra, A., and Ramirez-Esquivel, F. (2017). Subtle changes in the landmark panorama disrupt visual navigation in a nocturnal bull ant. *Philos. Trans. R. Soc. B Biol. Sci.* 372:20160068. doi: 10.1098/rstb.2016.0068

Neuser, K., Triphan, T., Mronz, M., Poeck, B., and Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. *Nature* 453, 1244–1247. doi: 10.1038/nature07003

Opfinger, E. (1931). Über die Orientierung der Biene an der Futterquelle—die Bedeutung von Anflug und Orientierungsflug für den Lernvorgang bei Farb-, Form- und Ortsdressuren. *Z. Vgl. Physiol.* 15, 431–487. doi: 10.1007/BF00338108

Ott, S. R., and Rogers, S. M. (2010). Gregarious desert locusts have substantially larger brains with altered proportions compared with the solitarious phase. *Proc. Biol. Sci.* 277, 3087–3096. doi: 10.1098/rspb.2010.0694

Pfeiffer, K., and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. *Annu. Rev. Entomol.* 59, 165–184. doi: 10.1146/annurev-ento-011613-162031

Pfeffer, S. E., and Wittlinger, M. (2016). Optic flowodometry operates independently of stride integration in carried ants. *Science* 353, 1155–1157. doi: 10.1126/science.aaf9754

Philippides, A., de Ibarra, N. H., Riabinina, O., and Collett, T. S. (2013). Bumblebee calligraphy: the design and control of flight motifs in the learning and return flights of *Bombus terrestris*. *J. Exp. Biol.* 216, 1093–1104. doi: 10.1242/jeb.081455

Rössler, W., Spaethe, J., and Groh, C. (2017). Pitfalls of using confocal-microscopy based automated quantification of synaptic complexes in honeybee mushroom bodies (response to Peng and Yang 2016). *Sci. Rep.* 7:9786. doi: 10.1038/s41598-017-09967-8

Schmitt, F., Stieb, S. M., Wehner, R., and Rössler, W. (2016). Experience-related reorganization of giant synapses in the lateral complex: potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* 76, 390–404. doi: 10.1002/dneu.22322

Scholl, C., Kübert, N., Muenz, T. S., and Rössler, W. (2015). CaMKII knockdown affects both early and late phases of olfactory long-term memory in the honeybee. *J. Exp. Biol.* 218, 3788–3796. doi: 10.1242/jeb.124859

Scholl, C., Wang, Y., Krischke, M., Mueller, M. J., Amdam, G. V., and Rössler, W. (2014). Light exposure leads to reorganization of microglomeruli in the mushroom bodies and influences juvenile hormone levels in the honeybee. *Dev. Neurobiol.* 74, 1141–1153. doi: 10.1002/dneu.22195

Schultheiss, P., Wystrach, A., Schwarz, S., Tack, A., Delor, J., Nooten, S. S., et al. (2016). Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama. *Anim. Behav.* 115, 19–28. doi: 10.1016/j.anbehav.2016.02.027

Seelig, J. D., and Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. *Nature* 521, 186–191. doi: 10.1038/nature14446

Seid, M. A., and Wehner, R. (2009). Delayed axonal pruning in the ant brain: a study of developmental trajectories. *Dev. Neurobiol.* 69, 350–364. doi: 10.1002/dneu.20709

Sommerlandt, F. M. J., Spaethe, J., Rössler, W., and Dyer, A. G. (2016). Does fine color discrimination learning in free-flying honeybees change mushroom-body calyx neuroarchitecture? *PLoS One* 11:e0164386. doi: 10.1371/journal.pone.0164386

Steck, K., Hansson, B. S., and Knaden, M. (2009). Smells like home: desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Front. Zool.* 6:5. doi: 10.1186/1742-9994-6-5

Stieb, S. M., Hellwig, A., Wehner, R., and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* 72, 729–742. doi: 10.1002/dneu.20982

Stieb, S. M., Muenz, T. S., Wehner, R., and Rössler, W. (2010). Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Dev. Neurobiol.* 70, 408–423. doi: 10.1002/dneu.20785

Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Curr. Opin. Neurobiol.* 12, 633–638. doi: 10.1016/s0959-4388(02)00385-9

Stürzl, W., Zeil, J., Boeddeker, N., and Hemmi, J. M. (2016). How wasps acquire and use views for homing. *Curr. Biol.* 26, 470–482. doi: 10.1016/j.cub.2015.12.052

Vogt, K., Aso, Y., Hige, T., Knapek, S., Ichinose, T., Friedrich, A. B., et al. (2016). Direct neural pathways convey distinct visual information to *Drosophila* mushroom bodies. *Elife* 5:e14009. doi: 10.7554/eLife.14009

Vowles, D. M. (1954). The orientation of ants. I. The substitution of stimuli. *J. Exp. Biol.* 31, 341–355.

Wajnberg, E., Acosta-Avalos, D., Alves, O. C., de Oliveira, J. F., Srygley, R. B., and Esquivel, D. M. S. (2010). Magnetoreception in eusocial insects: an update. *J. R. Soc. Interface* 7, S207–S225. doi: 10.1098/rsif.2009.0526.focus

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. *J. Comp. Physiol. A* 189, 579–588. doi: 10.1007/s00359-003-0431-1

Wehner, R., Fukushi, T., and Isler, K. (2007). On being small: brain allometry in ants. *Brain Behav. Evol.* 69, 220–228. doi: 10.1159/000097057

Wehner, R., Meier, C., and Zollikofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecol. Entomol.* 29, 240–250. doi: 10.1111/j.0307-6946.2004.00591.x

Wehner, R., Michel, B., and Antonsen, P. (1996). Visual navigation in insects: coupling of egocentric and geocentric information. *J. Exp. Biol.* 199, 129–140.

Wehner, R., and Müller, M. (1993). How do ants acquire their celestial ephemeris function? *Naturwissenschaften* 80, 331–333. doi: 10.1007/bf01141909

Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. *Science* 312, 1965–1967. doi: 10.1126/science.1126912

Wolf, H., and Wehner, R. (2000). Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. *J. Exp. Biol.* 868, 857–868.

Yilmaz, A., Lindenberg, A., Albert, S., Grübel, K., Spaethe, J., Rössler, W., et al. (2016). Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant *Camponotus rufipes*. *Dev. Neurobiol.* 76, 1041–1057. doi: 10.1002/dneu.22374

Zeil, J. (1993). Orientation flights of solitary wasps (Cerceris; Sphecidae; Hymenoptera). *J. Comp. Physiol. A* 172, 189–205. doi: 10.1007/bf00189396

Zeil, J. (2012). Visual homing: an insect perspective. *Curr. Opin. Neurobiol.* 22, 285–293. doi: 10.1016/j.conb.2011.12.008

Zeil, J., Kelber, A., and Voss, R. (1996). Structure and function of learning flights in bees and wasps. *J. Exp. Biol.* 252, 245–252.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

The role of celestial compass information in *Cataglyphis* ants during learning walks and for neuroplasticity in the central complex and mushroom bodies

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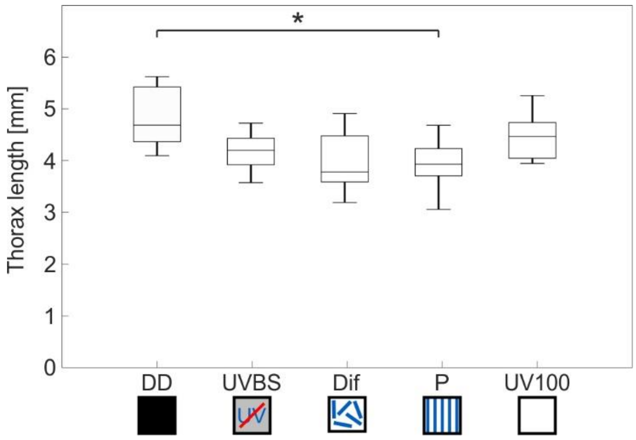
1 Supplementary Figure



Supplementary Figure 1. Comparison of the thorax length between the experimental groups. The central line of each boxplot depicts the median of the data. The upper and lower limits of the boxes show the 25th and 75th percentiles, while the whiskers extend to the extreme data points without outliers. A difference between the groups can be found using a Kruskal–Wallis test (χ_4^2=11.62; n=49; p=0.0240). The data was post-hoc compared to the DD group using the Mann-Whitney U-test with a Bonferroni correction. The asterisk indicates that data is significantly different (after correction p<0.0125) from the DD group. Only between DD and P the thorax length significantly differed (Mann-Whitney U-test with a Bonferroni correction: DD vs. P, Z_3=2.5404; n_{DD}=7; n_P=9; p=0.0118). No difference was found between the other groups compared to DD (DD vs. UVBS, Z_1=2.2642; n_{DD}=7; n_{UVBS}=11; p=0.0260; DD vs. Dif, Z_2= 2.1170; n_{DD}=7; n_{Dif}=9; p= 0.0418; DD vs. UV100, Z_4=2.5404; n_{DD}=7; n_{UV100}=13; p= 0.1779). Since no correlation between thorax length and the volume of the neuropils of interest was found, no correction for the allometric differences was used for the further analyses.

7 Manuscript VI: The Geomagnetic Field as a Compass Cue in *Cataglyphis* Ant Navigation

7 The Geomagnetic Field as a Compass Cue in *Cataglyphis* Ant Navigation

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Navigation is a challenge for migrating, foraging and homing animals. To calibrate their compass systems and learn landmark cues, navigating bees, wasps and ants perform well-structured learning flights or walks close to their nests. Learning walks in *Cataglyphis* ants comprise pirouettes with frequent stops to gaze back to the nest entrance. Although celestial cues provide the main directional information during foraging in *Cataglyphis*, they do not provide the compass for nest-centered views during learning walks. Here we show that the geomagnetic field serves as the directional cue enabling the ants to gaze accurately to their inconspicuous nest entrance during learning walks. Experimental rotation of the horizontal magnetic-field component changed the ants' gazes in a predictable manner, while field elimination or disarray resulted in randomly oriented gaze directions under unchanged natural skylight and panoramic conditions. This proves that information by the geomagnetic field supplies the ants' path integrator with directional compass information.

7.1 Introduction

The earth's magnetic field offers a stable reference system for navigation used across the animal kingdom (Goff et al. 1998; Guerra et al. 2014; Wiltschko and Wiltschko 1990; Warrant et al. 2016; Wiltschko and Wiltschko 1972). Migratory birds (Wiltschko and Wiltschko 1990; Wiltschko and Wiltschko 1972) and sea turtles (Goff et al. 1998) are famous for making use of geomagnetic information, especially its inclination, during their long-distance migration. Migratory insects also use the earth's magnetic field (monarch butterfly: Guerra et al. 2014; also suggested for bogong moth: Warrant et al. 2016). However, animals that do not travel over thousands of kilometers, still are in need of a directional reference system for their navigational tasks. *Cataglyphis* desert ants are prime examples for impressive navigational performances mainly based on celestial information (Wehner 2008). Thermophilic *Cataglyphis* ants inhabit hostile environments where they search over long distances for dispersed food items. The main navigational strategy is path integration using celestial cues to determine the direction (Wehner 2008) and a step counter to measure distances traveled (Wittlinger et al. 2006). In

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[§] These authors share a senior authorship addition, they use other navigational cues available like visual (Fleischmann et al. 2016) and olfactory (Steck et al. 2009) landmarks, wind direction (Müller and Wehner 2007), ground structure (Seidl and Wehner 2006) etc. It is still not known how naïve ants (novices) initially calibrate their celestial compass systems and how they acquire landmark and other information necessary for successful foraging and homing during their transition from the dark nest to outdoor foraging under bright sunlight. At the beginning of their foraging life, *Cataglyphis* ants perform so-called learning walks (Fleischmann et al. 2016; Fleischmann et al. 2017; Stieb et al. 2012; Wehner et al. 2004). Learning walks are explorative trips around the nest entrance during which the novices move slowly and observantly. Novices do not search for food until finishing this learning period during which they acquire landmark information (Fleischmann et al. 2016). Learning walks – like learning flights in flying hymenopterans (Zeil 2012) – are well structured (Fleischmann et al. 2017). *Cataglyphis noda* ants frequently include body rotations into their circuitous learning walks. Pirouettes are tight turns about the body axis during which the ants perform several stops (Fleischmann et al. 2017). During the longest stopping phases, the ants look back to the nest entrance, presumably to take snapshots of their homing direction (Fleischmann et al. 2017; Müller and Wehner 2010). It has been suggested that the ants use their path integrator to align their gaze directions (Fleischmann et al. 2017; Graham et al. 2010; Müller and Wehner 2010). Using the path integrator for this task is necessary as the nest entrance is invisible from the ant's perspective. During foraging the celestial compass provides the most prominent directional information for path integration (Wehner 2003), but it does not provide the ants with the necessary reference system during learning walks (Grob et al. 2017). Neither exclusion from the natural sky polarization pattern, the UV-light spectrum, the position of the sun nor the exclusion of all skylight cues did alter the accuracy of the gazes back to the nest entrance (Grob et al. 2017). Therefore, the geomagnetic field is a promising candidate to provide the necessary compass cue for the looks back to the nest entrance during learning walks.

7.2 Material and Methods

Animals and test site Two ant colonies of *Cataglyphis noda* (Brullé 1832) located in different clearings in the pine forest of Schinias National Park, Marathonas, Greece (coordinates: 38°08'N 24°01'E; geomagnetic field strength: $(46.1 \pm 0.2)\,\mu$T; horizontal component: $(26.6 \pm 0.2)\,\mu$T (`www.ngdc.noaa.gov/geomag-web/#igrfwmm`)) were used for the experiments during summers of 2016 (spiral experiment) and 2017 (Helmholtz coil experiment, fig. 7.1a). Before the experiments started all ants outside the nest were marked with car paint (Motip Lackstift Acryl, MOTIP DUPLI GmbH, Haßmersheim, Germany) for three consecutive days to ensure that only unmarked novices performing naïve learning walks were included in the experiments.

Camera setup Above the experimental area (i. e. above the natural nest entrance in the spiral experiment, or above the experimental table in the Helmholtz coil experiment, fig. 7.1a) two cameras were installed. A camcorder (HDR-CX330E, Sony Corporation, Minato, Japan) recorded the area nonstop during the experiments at 25 fps. A 4K-camcoder (HC-X1000, Panasonic Corporation, Kadoma, Japan) recorded learning walks of novices at 50 fps. Every time an unmarked ant left the nest entrance, the observer sitting next to the experimental setup

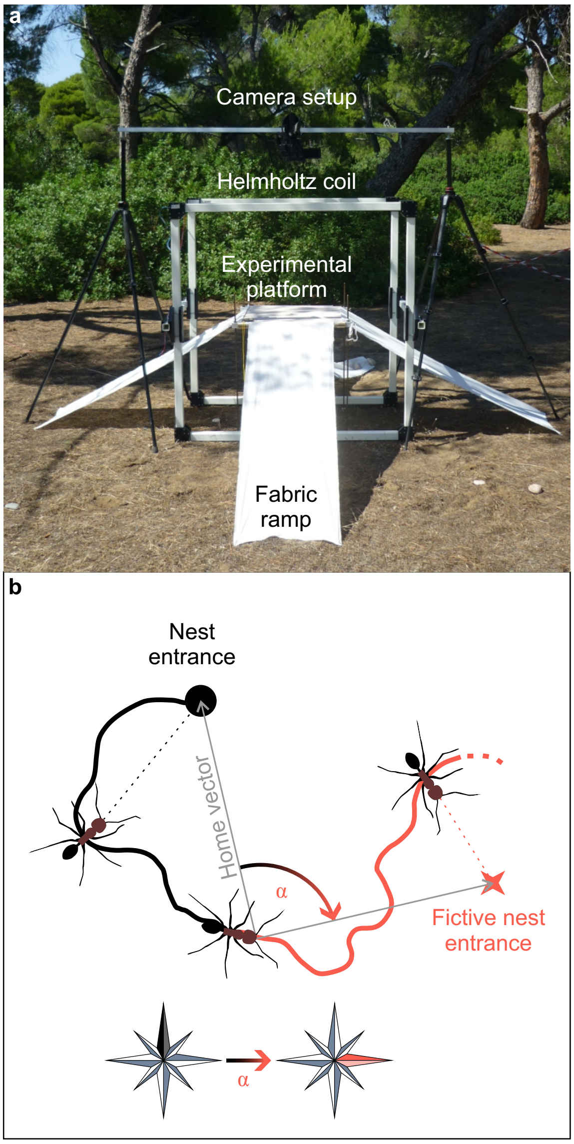

Figure 7.1: Experimental settings. **a**, Experimental setup (Helmholtz coil system, experimental platform, camera setup, fabric ramp) in the Greek pine forest. **b**, Proposed mechanism of integration of magnetic information into the path integrator during a learning walk after rotation of the horizontal component of the magnetic field. The black line represents the ant's path of a learning walk before the magnetic field is altered. Its gaze direction during the longest stopping phase (dashed line) of a pirouette is directed towards the nest entrance (black dot). When the horizontal component of the magnetic field is rotated (in the example: $\alpha = 90°$), the home vector of the ant is rotated as well. The ant continues its path (now shown in red) and eventually performs another pirouette. During the longest stopping phase the ant now looks to the fictive nest entrance (indicated by the red star).

started the recording of the 4K-camcoder using the Panasonic Image App (Version 10.9.2, Panasonic Corporation, Kadoma, Japan) on a Cat S60 smartphone (Caterpillar Inc., Peoria, USA) or a Xperia Z1 (Sony, Tokyo, Japan). High-speed recordings were stopped when the ant returned to the nest or left the experimental area (60 cm × 60 cm), i. e. fell of the platform or walked down one of the fabric ramps.

Experimental setup and procedure After three days of marking ants outside the nest, experimental trials were conducted for maximally three consecutive days, i. e. the learning walks performed were most likely early learning walks of novices. This was additionally ensured, because learning walks were restricted to the platform area and learning walks increase with experience (Fleischmann et al. 2016; Fleischmann et al. 2017; Wehner et al. 2004).

Electromagnetic spiral To disarray the magnetic field at the natural nest entrance a flat coil (diameter 70 cm, separation distance between the windings: 5 cm, diameter of the copper wire used: 1 mm) was installed around the nest entrance and covered with sand. The electromagnetic spiral was powered with three 4.5 V batteries (3R12, VARTA Consumer Batteries GmbH & Co. KGaA, Ellwangen, Germany) wired in parallel to confront ants with a radial magnetic field slightly stronger than the earth's magnetic field close to the ground. This altered magnetic field provided different directional information at any point on the spiral. Therefore, it was not suitable as a reliable reference system for the look-backs to the nest entrance during learning walks. As a control, ants were recorded during learning walks when the spiral was switched off.

Helmholtz coil system To confront ants with a precise magnetic field (controlled direction and strength) a Helmholtz coil system was used (HHS 5213-100, Schwarzbeck Mess-Elektronik, Schönau, Germany). Current was supplied to two coils with a customized DC power supply made by the Biocenter's electronic workshop. The current was constantly monitored with a multimeter (VC820-1, Voltcraft, Hirschau, Germany). Since homogeneity of the magnetic field is highest in the center of the coil system, ants had to perform their learning walks on a platform (60 cm × 60 cm, fig. 7.1a). For that reason, the natural nest entrance was covered with a cylindrical box (nest cover) with a tunnel (diameter: 3 cm) so that the ants could still leave the nest, but had to use an artificial nest entrance. The Helmholtz coil system and the camera setup were installed every morning and removed every evening. During the experiment, the tunnel of the nest cover was connected with the experimental table via a flexible tube (diameter: 3 cm). Ants left the tube through a hole (diameter: 3 cm) in the center of the elevated platform. They could leave the platform by walking on one of four fabric ramps (fig. 7.1a). Foragers learned quickly to use these ramps for outbound and inbound trips. When a novice performed a naïve learning walk, the Helmholtz coil was switched on after the ant had performed at least one pirouette. The recording was stopped, when the ant returned to the nest entrance or fell off the platform. Then the Helmholtz coil was also switched off.

Physical background of magnetic alterations The geomagnetic field can be altered by inducing an additional magnetic field with suitable current distributions. Once the current distribution is known, this additional magnetic field can be calculated with the Biot-Savart law. According to the superposition principle, the combined magnetic field is then found by vector addition of the two contributions.

In the case of the spiral, a flat coil setup, the resulting magnetic field is approximately radial in the plane above the spiral and mostly vertical in between the windings. On the whole, the total magnetic field is changing rapidly from one point to another which makes it unsuitable as a reference system for the ants. The current through the spiral was chosen to be slightly above the threshold for inducing movement of a magnetic compass needle in close proximity to the spiral. This procedure, together with a rough numerical estimation, ensured that the artificial field strength was in the same order of magnitude as the natural field strength.

The Helmholtz coil on the contrary generates a very homogenous magnetic field, which can still be used as a reference system. For reinforcement, elimination, and 180° rotation experiments, the coil axis was aligned parallel to the horizontal component of the natural magnetic field with the aid of a magnetic compass (Fluorescent Map Compass 3116, AceCamp GmbH, Offenbach, Germany). For the +90° and -90° rotation experiments, the coil axis was horizontally rotated about +45° and -45° with respect to the horizontal component of the natural magnetic field.

The coil used in the experiments had a rectangular cross-section with an electrical side length of 1.30 m (all technical details are given in the data sheet available online: `http://www.schwarzbeck.de/Datenblatt/K5213-100.pdf`). The coil separation was 0.71 m, which offers best uniformity along an axis through the coil center. The systematic error in field strength on the experimental platform (60 cm × 60 cm in 71 cm height) due to remaining field inhomogeneity in the Helmholtz coil was less than 10 %. In the reinforcement, elimination, and 180° rotation experiments, this results in a field strength variation of similar order. In the 90° rotation experiments, this corresponds to a directional variation of the total magnetic field of less than 4°. Compared to the spatial variations, the uncertainty in magnetic field strength due to the current measurement and coil alignment is negligible.

Data analysis The 4K-videos were converted into image stacks using the Free Video to JPG Converter (v. 5.0.99 build 823, DVDVideoSoft, DIGITAL WAVE LTD., London, UK). Pirouettes (full or partial tight turns about the ant's body axis(Fleischmann et al. 2017)) were analyzed manually frame by frame using the MATLAB (2015a, The MathWorks Inc., Natick, MA, USA) application DIGILITE (Jan Hemmi and Robert Parker, The Australian National University, Canberra, Australia). The positions of thorax and mandibles were marked in each frame. In addition, the nest entrance was marked. Using these coordinates the gaze directions of the ants could be determined relative to the nest. The direction of the nest was defined as 180°. Stopping phases during pirouettes were defined as it was done before (minimal duration: 100 ms) (Fleischmann et al. 2017; Grob et al. 2017). The longest stopping phase of each pirouette was used to compare gaze directions between experimental groups. In the spiral experiment, 15 pirouettes were analysed when the spiral was on and 15 pirouettes when the spiral was off. In all other experiments both the first pirouette of an ant on the experimental table and the first pirouette after the Helmholtz coil had been switched on were analysed ($n = 15$ per experiment). In the experiments where the horizontal component of the magnetic field was rotated (+90°, 180° and -90°, respectively) the fictive nest entrance position was calculated. Since it was different for every test ant (fig. 7.1b), it was determined individually for every ant. Data of the pirouette after the Helmholtz coil was switched on were analysed twice – once relative to the nest entrance and once relative to the fictive nest entrance position.

Statistics Circular statistics were performed using Oriana 4.02 (Kovach Computing Services, Anglesey, UK). Gaze directions during the longest stopping phases of pirouettes were grouped into 10°-bins. To check whether data were randomly distributed or directed, the Rayleigh test was used ($\alpha = 0.05$). If data were directed, the 95 % Confidence intervals were determined to check whether the expected direction (nest entrance or fictive nest entrance defined as 180°) was within these limits.

7.3 Results and Discussion

7.3.1 Search for the compass

To perform a look-back to the nest behavior, novices need to know the position of the nest entrance relatively to their own position. This is not trivial for the ants, since the navigational toolbox has yet to be filled with navigational information (Fleischmann et al. 2016). A likely mechanism to align the gaze direction is the path integrator, since its home vector pinpoints back to the nest entrance (Graham et al. 2010; Müller and Wehner 2010). If the path integrator is involved in guiding the gaze direction during pirouettes, the rotation of its directional component should lead to a predictable shift in the gaze direction during the longest stopping phase towards a fictive nest entrance (fig. 7.1b). Since celestial cues are not used as compass cue for the looks back to the nest entrance during the learning walks (Grob et al. 2017), the question remains which cue is used. A possible directional cue for path integration during this early learning behavior is the geomagnetic field. In contrast to skylight cues, the magnetic field is stable (i. e. has not to be calibrated like the solar ephemeris) and it is in principle available underground, i. e. inside the nest.

In order to test the potential role of the earth's magnetic field as the directional component of the path integrator during nest-centered views, we installed a subterranean electromagnetic spiral around the nest entrance to disarray the magnetic field available during learning walks. Novices that left the nest to perform naïve learning walks under natural conditions, fixated the nest entrance during the longest stopping phase (Rayleigh Uniformity Test: $Z_0 = 8.934$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 159.9°/205.7°; Mean: 182.8°; fig. 7.2a). When the current flow in the electromagnetic spiral was switched on, ants were no longer able to gaze back to the nest entrance (Rayleigh Uniformity Test: $Z_0 = 2.296$, $n = 15$, $p = 0.099$; Mean: 215.2°; fig. 7.2b). Importantly, the overall structure of the learning walks was not disturbed by this manipulation, but the results demonstrate that the gaze directions during the longest stopping phases in pirouettes were clearly altered. This indicates, that indeed the geomagnetic field could provide the directional reference system needed to align the gaze directions.

To confirm this by more precise manipulations, a Helmholtz coil setup was used to generate an accurately controlled magnetic field (fig. 7.1a). When the electromagnet was turned on during a learning walk to double the field strength of the horizontal field component, neither the current flow in the coil system, nor the strength of the horizontal component of the magnetic field (leading, at the same time, to an altered inclination) changed the gaze directions during the longest stopping phases in pirouettes (before alteration: Rayleigh Uniformity Test: $Z_0 = 10.855$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 157.8°/194.3°; Mean: 176.1°; after alteration: Rayleigh Uniformity Test: $Z_0 = 7.525$, $n = 15, p < 0.001$; 95 % Confidence Interval (−/+) 151.2°/204.6°; Mean: 177.9°; fig. 7.2c, d). However, in absence of the hor-

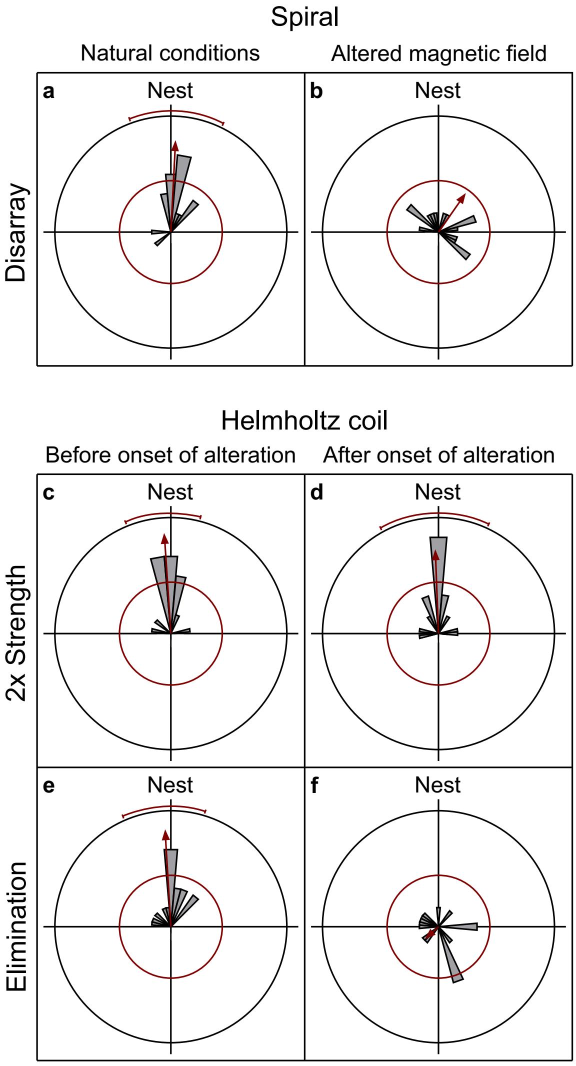

Figure 7.2: Effects of three alterations of the magnetic field on gaze directions. **a**, Natural conditions, i. e. electromagnetic spiral off (n = 15). **b**, Altered magnetic field, i. e. electromagnetic spiral on (n = 15). **c**, **d**, First pirouette before (**c**, geomagnetic field, i. e. Helmholtz coil off) and first pirouette after (**d**, double magnetic field strength, i. e. Helmholtz coil on) doubling the field strength of the horizontal component of the geomagnetic field during one learning walk (n = 15). **e**, **f**, First pirouette before (**e**, geomagnetic field, i. e. Helmholtz coil off) and first pirouette after (**f**, eliminated horizontal component, i. e. Helmholtz coil on) elimination of the horizontal component of the geomagnetic field during one learning walk (n = 15). Data show the gaze directions during the longest stopping phase of pirouettes relative to the nest entrance (labelled "nest"). Data are shown in grey: The bins comprise 10° and the black circle comprises 6 ants. Statistics are shown in red: The arrow indicates the r-vector which is significantly directed if it exceeds the circle indicating the significance level of the Rayleigh uniformity test ($\alpha = 0.05$). If data are directed, the arc indicates the 95 % confidence interval.

izontal component of the geomagnetic field (i. e. after elimination of the horizontal field component leading at the same time to an altered inclination), the ants' gazes during pirouettes were no longer directed to the nest entrance (before alteration: Rayleigh Uniformity Test: $Z_0 = 10.102$, $n = 15$,$p < 0.001$; 95 % Confidence Interval (−/+) 156.5°/196.7°; Mean: 176.6°; after alteration: Rayleigh Uniformity Test: $Z_0 = 0.283$, $n = 15$,$p < 0.283$; 95 %; Mean: 47.8°; fig. 7.2e, f). This shows, that the horizontal component of the geomagnetic field is a necessary and sufficient cue to gaze back to the nest entrance during learning walks, indicating that the geomagnetic field provides *C. noda* with the necessary reference system to gaze back towards the nest entrance during their learning walks. To test this hypothesis, we rotated the magnetic field perceived by the ants during learning walks using the Helmholtz coil setup (fig. 7.1a).

7.3.2 Directional input to the path integrator

Rotating the horizontal component of the magnetic field during a learning walk should lead to a likewise rotation of the home vector, if the directional information of the path integrator is provided by the direction and polarity of the magnetic field (fig. 7.1b). This should result in shifting the gaze direction of the longest stopping phase away from the nest entrance to a fictive nest entrance located at the end of this rotated home vector (fig. 7.1b). When ants left their nest starting their learning walks under natural conditions, they gazed very accurately and precisely back to their nest entrance (before alteration (+90°): Rayleigh Uniformity Test: $Z_0 = 11.531$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 169.9°/200.9°; Mean: 184.4°; fig. 7.3a; before alteration (180°): Rayleigh Uniformity Test: $Z_0 = 11.100$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 162.4°/197.7°; Mean: 180.0°; fig. 7.3c; before alteration (-90°): Rayleigh Uniformity Test: $Z_0 = 12.839$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 167.7°/193.1°; Mean: 180.4°; fig. 7.3e). As expected, a rotation of the horizontal component of the magnetic field by +90°, 180° or -90°, shifted the gaze direction during the longest stopping phase, and the gaze directions were no longer directed towards the nest entrance (after alteration relative to the nest entrance (+90°): Rayleigh Uniformity Test: $Z_0 = 5.019$, $n = 15$, $p = 0.005$; 95 % Confidence Interval (−/+) 235.4°/301.5°; Mean: 268.5°; fig. 7.3b; after alteration relative to the nest entrance (180°): Rayleigh Uniformity Test: $Z_0 = 1.051$, $n = 15$, $p = 0.356$; Mean: 289.6°; fig. 7.3d; after alteration relative to the nest entrance (-90°): Rayleigh Uniformity Test: $Z_0 = 1.942$, $n = 15$, $p = 0.144$; Mean: 122.9°; fig. 7.3f). However, the gaze directions tend to point to the direction in which the magnetic field was rotated. This trend is even significant in the +90°-rotation (fig. 7.3b). By determining the fictive nest entrance for each learning walk ($n = 15$ per experiment) and plotting the data from figs. 7.3b, d, f relative to the fictive instead of the real nest entrance, gaze directions during the longest stopping phase were accurately directed towards the fictive nest entrance (after alteration relative to the fictive nest entrance (+90°): Rayleigh Uniformity Test: $Z_0 = 8.038$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 141.6°/192.1°; Mean: 166.8°; fig. 7.3b'; after alteration relative to the fictive nest entrance (180°): Rayleigh Uniformity Test: $Z_0 = 6.672$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 146.9°/200.7°; Mean: 173.8°; fig. 7.3d'; after alteration relative to the fictive nest entrance (-90°): Rayleigh Uniformity Test: $Z_0 = 8.271$, $n = 15$, $p < 0.001$; 95 % Confidence Interval (−/+) 142.6°/191.9°; Mean: 167.3°; fig. 7.3f', Supplementary video). The results from experimental rotations of the horizontal component of the magnetic field demonstrate that the geomagnetic field provides the ants with the needed compass cue and that – as previously suggested (Graham et al. 2010; Müller

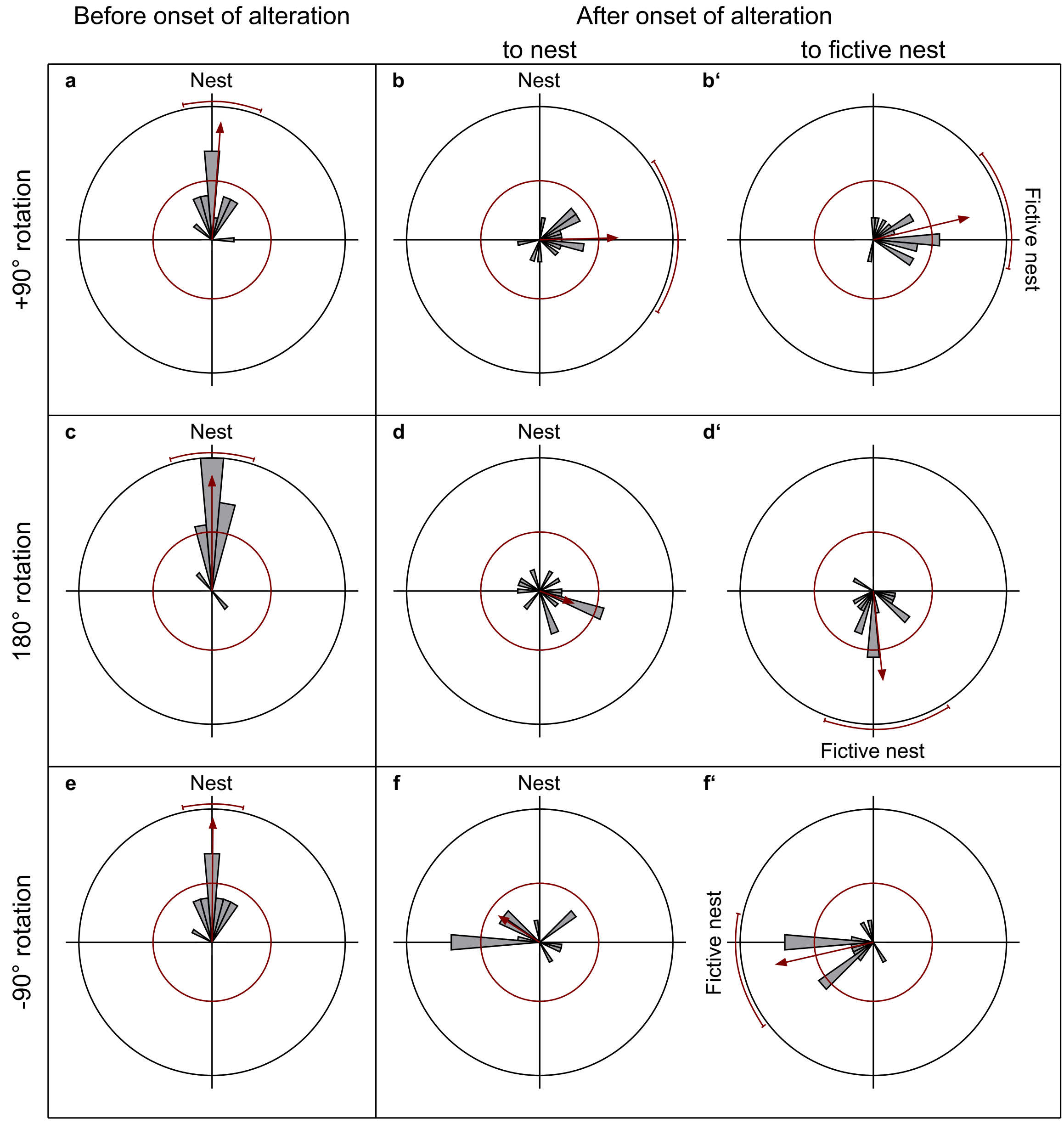

Figure 7.3: Rotation of the horizontal component of the magnetic field predictably changes gaze directions towards fictive nest entrance position. Gaze directions of first pirouettes before (**a**, **c**, **e** geomagnetic field, i. e. Helmholtz coil off) and of first pirouettes after (**b**, +90°; **c**, 180°; **d**, -90° rotated horizontal component of magnetic field, i. e. Helmholtz coil on) rotation during one learning walk (n = 15 for each rotation) relative to the nest entrance. **b**', **d**', **f**' Same data as in **b**, **d**, **f** relative to fictive nest entrance position. For explanations of data and statistics see fig. 7.2.

and Wehner 2010) – the path integrator indeed provides guidance for the back turns during learning walks in *Cataglyphis* ants. A previous study in leafcutter ants already suggested that the geomagnetic field might provide the ants with directional information during homing under certain conditions (Riveros and Srygley 2008). Until now the only hint that *Cataglyphis* ants might use magnetic cues was an experiment showing that they can learn magnetic abnormalities of high amplitudes as nest-defining cues (Buehlmann et al. 2012). Our present results clearly demonstrate that in contrast to magnetic orientation described for other ant species in experiments gained under skylight cue deprived conditions (Anderson and Vander Meer 1993; Banks and Srygley 2003; Camlitepe and Stradling 1995; Jander and Jander 1998) *C. noda* ants use the geomagnetic field as a main directional cue during their learning walks providing the necessary and sufficient compass information for this navigational task. Since *Cataglyphis* ants gradually learn visual panoramas during learning walks (Fleischmann et al. 2016), the geomagnetic field as a compass cue can be used as a geocentric reference system to learn the visual panorama during the stopping phases of pirouettes and during taking snapshots of the surrounding panorama (Fleischmann et al. 2017; Graham et al. 2010; Grob et al. 2017), similar as honeybees do when learning a feeder position (Collett and Baron 1994) or have to distinguish patterns (Frier et al. 1996). These nest-centred views from different directions and distances around the nest entrance provide the ants with a sufficient visual compass for successful navigation (Zeil et al. 2003). The importance of learning walks becomes clear as only ants that have performed learning walks around the nest entrance are able to use landmark guidance to find back to the nest entrance even from places they have not been bevor (chapter 5). Although the precise mechanism of snapshot memory formation is still unknown, investigations on neuronal plasticity of central visual neuropils found significant neuronal changes after the performance of learning walks under natural conditions (Grob et al. 2017; Kühn-Bühlmann and Wehner 2006). Furthermore, modelling work suggests that the large synaptic capacity of the mushroom bodies in the *Cataglyphis* brain is well suited for the storage of a large number of panoramic snapshots (Ardin et al. 2015).

The present results suggest that the ants use the horizontal field component as a polarity compass cue. This is also suggested for other invertebrates like honeybees (Lambinet et al. 2017a) and spiny lobsters (Lohmann et al. 1995). Although the conclusive experiment that *Cataglyphis* ants do not use an inclination compass has yet to be performed, e. g. by inverting the vertical component (Lambinet et al. 2017a), it appears very unlikely that *C. noda* uses an inclination compass as for example birds (Wiltschko and Wiltschko 1972) or turtles do (Light et al. 1993) during their long-distance migrations. To make use of the inclination gradient, animals have to move several kilometers, i. e. much farther than ants walk, in order to detect changes. If the ants were able to detect only a turned axis of the magnetic field, a bimodal distribution of gaze directions would be expected. The results, however (fig. 7.3), clearly show a unimodal distribution of gazes. Furthermore, ants (like other hymenopterans) do not possess a light-sensitive cryptochrome (Yuan et al. 2007), which presumably is used by birds (Hore and Mouritsen 2016) and some insects (Bazalova et al. 2016; Wajnberg et al. 2010) for magnetic-field inclination perception. Consequently, the search for a light-dependent compass mechanism in these species focused on radical pair formation (Hore and Mouritsen 2016) is very unlikely the mechanism used by *Cataglyphis* ants. A more promising candidate mechanism seems a magnetite-related cellular process as suggested for birds as a second, polarity sensitive magnetic sense (Nordmann et al. 2017) or for some insects (Wajnberg et al. 2010). Ferromagnetic material has been found in insects' antennae (Guerra et al. 2014) and in the abdomen (Lambinet et al. 2017b).

The remaining questions is why novices use the earth's magnetic field as a compass cue and why they switch later to their well-studied celestial compass system? Here we have shown that the ants use information provided by the geomagnetic field to direct the gaze directions accurately early in outdoor life during their learning walks. Besides providing a reference system for landmark learning, the geomagnetic field might also help *Cataglyphis* to calibrate the season- and place-specific course of the sun over the day (solar ephemeris) at the beginning of their foraging career, like birds do, when there is a cue conflict between these two reference systems (Wiltschko et al. 1998). This would make the geomagnetic field the initial compass guidance in *Cataglyphis* desert ants, before switching to their well-studied navigational toolkit (chapter 5), including e. g. the celestial compass and various landmark cues (Wehner 2003). Experienced *Cataglyphis* foragers do not seem to use the geomagnetic field as a directional cue anymore, if other navigational information, like the celestial compass or landmarks, are available. However, as our results show that the geomagnetic field provides a reliable directional cue during learning walks, the rational for this switch and whether the ants maintain using this navigational cue under certain conditions as experienced foragers has yet to be determined.

7.4 Outlook

The accurately directed stopping phases during pirouettes in *C. noda* learning walks provide us with a precise behavioral readout of the directional component of the path integrator. The rotation experiments using a Helmholtz coil setup demonstrate that the ants' behavioral responses after alteration of the horizontal component of the geomagnetic field is not just disturbed, but predictably directed towards the fictive nest entrance position. This will allow us to answer many follow up questions like why and when the ants switch the compass cue for the main directional input into the path integrator from learning walks to foraging. Furthermore, the question is whether experienced foragers rely on the same reference system as novices when re-learning the nest's surrounding after sudden changes of the landmark panorama. Furthermore, the underlying sensory and neuronal mechanisms of magnetic field perception in Hymenoptera are still unknown (Wajnberg et al. 2010). Using the longest stopping phases of the pirouettes in *C. noda* as a robust behavioral read out will provide a valuable tool for future manipulation experiments in the search for the physiological base of the ants' magnetic sense. It has previously been suggested in *Drosophila* (Gegear et al. 2008) and *Danaus* butterflies (Guerra et al. 2014), that the magnetic sense of these insects is light-dependent. However, this does not seem to be true for Hymenoptera. Honey bees (Lindauer and Martin 1968), bumble bees (Chittka et al. 1999) and several ant species (Anderson and Vander Meer 1993; Camlitepe and Stradling 1995; Jander and Jander 1998) are able to use magnetic cues in total darkness. This might allow *Cataglyphis* to use magnetic cues to orient themselves inside the darkness of their complex nest structures as do some underground mammals (Kimchi et al. 2004) and as it was suggested for bumblebees (Chittka et al. 1999) and for termites (Rickli and Leuthold 1988). In conclusion, our present results underline once more the diversity and complexity of the navigational system in *Cataglyphis* ants, where finely tuned interactions of navigational cues are used to ensure navigational success. The clear and prominent role of the geomagnetic field during learning walks in *Cataglyphis* provides a highly promising opportunity to investigate the mechanisms underlying a geomagnetic sense in Hymenoptera.

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Author Contributions

PNF, RG and VLM came up with the initial idea and design for the spiral experiment. All authors conceived the Helmholtz Coil experiment. WR and RW led the study, and WR was responsible for funding acquisition. PNF and RG conducted the experiments, and VLM helped to set them up and made all calculations necessary. PNF and RG analyzed the data, and prepared fig.s and supplementary video. All authors discussed the results. PNF and RG drafted the first version of the manuscript and WR and RW revised the manuscript. All authors approved the final version.

References

Anderson, J. B. and Vander Meer, R. K. (1993). Magnetic Orientation in the Fire Ant, *Solenopsis invicta*. *Naturwissenschaften*, 80(12):568–570.

Ardin, P., Mangan, M., Wystrach, A., and Webb, B. (2015). How variation in head pitch could affect image matching algorithms for ant navigation. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 201(6):585–597.

Banks, A. N. and Srygley, R. B. (2003). Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethology*, 109:835–846.

Bazalova, O., Kvicalova, M., Valkova, T., Slaby, P., Bartos, P., Netusil, R., Tomanova, K., Braeunig, P., Lee, H.-J., Sauman, I., Damulewicz, M., Provaznik, J., Pokorny, R., Dolezel, D., and Vacha, M. (2016). Cryptochrome 2 mediates directional magnetoreception in cockroaches. *Proceedings of the National Academy of Sciences*, 113(6):1660–1665.

Buehlmann, C., Hansson, B. S., and Knaden, M. (2012). Desert ants learn vibration and magnetic landmarks. *PLoS ONE*, 7(3):e33117.

Camlitepe, Y. and Stradling, D. J. (1995). Wood Ants Orient to Magnetic Fields. *Proceedings of the Royal Society B: Biological Sciences*, 261(1360):37–41.

Chittka, L., Williams, N. M., Rasmussen, H., and Thomson, J. D. (1999). Navigation without vision: bumblebee orientation in complete darkness. *Proceedings of the Royal Society B: Biological Sciences*, 266(1414):45–50.

Collett, T. S. and Baron, J. (1994). Biological compasses and the coordinate frame of landmark memories in honeybees. *Nature*, 368:137–140.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *The Journal of Experimental Biology*, 219:3137–3145.

Fleischmann, P. N., Grob, R., Wehner, R., and Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *The Journal of Experimental Biology*, 220:2426–2435.

Frier, H., Edwards, E., Smith, C., Neale, S., and Collett, T. (1996). Magnetic compass cues and visual pattern learning in honeybees. *The Journal of Experimental Biology*, 199:1353–1361.

Gegear, R. J., Casselman, A., Waddell, S., and Reppert, S. M. (2008). Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*. *Nature*, 454(7207):1014–8.

Goff, M., Salmon, M., and Lohmann, K. (1998). Hatchling sea turtles use surface waves to establish a magnetic compass direction. *Animal behaviour*, 55(1):69–77.

Graham, P., Philippides, A., and Baddeley, B. (2010). Animal cognition: multi-modal interactions in ant learning. *Current Biology*, 20(15):R639–R640.

Grob, R., Fleischmann, P. N., Grübel, K., Wehner, R., and Rössler, W. (2017). The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11:226.

Guerra, P. A., Gegear, R. J., and Reppert, S. M. (2014). A magnetic compass aids monarch butterfly migration. *Nature Communications*, 5:4164.

Hore, P. J. and Mouritsen, H. (2016). The Radical-Pair Mechanism of Magnetoreception. *Annual Review of Biophysics*, 45(1):299–344.

Jander, R. and Jander, U. (1998). The light and magnetic compass of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae). *Ethology*, 104(9):743–758.

Kimchi, T., Etienne, A. S., and Terkel, J. (2004). A subterranean mammal uses the magnetic compass for path integration. *Proceedings of the National Academy of Sciences*, 101(4):1105–1109.

Kühn-Bühlmann, S. and Wehner, R. (2006). Age-Dependent and Task-Related Volume Changes in the Mushroom Bodies of Visually Guided Desert Ants, Cataglyphis bicolor. *Journal of Neurobiology*, 66:511–521.

Lambinet, V., Hayden, M. E., Reid, C., and Gries, G. (2017a). Honey bees possess a polarity-sensitive magnetoreceptor. *Journal of Comparative Physiology A*, 203(12):1029–1036.

Lambinet, V., Hayden, M. E., Reigl, K., Gomis, S., and Gries, G. (2017b). Linking magnetite in the abdomen of honey bees to a magnetoreceptive function. *Proceedings of the Royal Society B*, 284:20162873.

Light, P., Salmon, M., and Lohmann, K. J. (1993). Geomagnetic Orientation of Loggerhead Sea Turtles: Evidence for an Inclination Compass. *Journal of Experimental Biology*, 182(1):1–10.

Lindauer, M. and Martin, H. (1968). Die Schwereorientierung der Bienen unter dem Einfluß des Erdmagnetfeldes. *Zeitschrift für Vergleichende Physiologie*, 60(3):219–243.

Lohmann, K., Pentcheff, N., Nevitt, G., Stetten, G., Zimmer-Faust, R., Jarrard, H., and Boles, L. (1995). Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *The Journal of Experimental bBology*, 198:2041–2048.

Müller, M. and Wehner, R. (2007). Wind and sky as compass cues in desert ant navigation. *Naturwissenschaften*, 94(7):589–594.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Current Biology*, 20(15):1368–1371.

Nordmann, G. C., Hochstoeger, T., and Keays, D. A. (2017). Unsolved mysteries: Magnetoreception—A sense without a receptor. *PLoS Biology*, 15(10):1–10.

Rickli, M. and Leuthold, R. H. (1988). Homing in Harvester Termites: Evidence of Magnetic Orientation. *Ethology*, 77(3):209–216.

Riveros, a. J. and Srygley, R. B. (2008). Do leafcutter ants, *Atta colombica*, orient their path-integrated home vector with a magnetic compass? *Animal Behaviour*, 75:1273–1281.

Seidl, T. and Wehner, R. (2006). Visual and tactile learning of ground structures in desert ants. *The Journal of Experimental Biology*, 209:3336–3344.

Steck, K., Hansson, B. S., and Knaden, M. (2009). Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Frontiers in Zoology*, 6:5.

Stieb, S. M., Hellwig, A., Wehner, R., and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 72(5):729–742.

Wajnberg, E., Acosta-Avalos, D., Alves, O. C., de Oliveira, J. F., Srygley, R. B., and Esquivel, D. M. S. (2010). Magnetoreception in eusocial insects: an update. *Journal of the Royal Society, Interface / the Royal Society*, 7:S207–S225.

Warrant, E., Frost, B., Green, K., Mouritsen, H., Dreyer, D., Adden, A., Brauburger, K., and Heinze, S. (2016). The Australian Bogong Moth Agrotis infusa: A Long-Distance Nocturnal Navigator. *Frontiers in Behavioral Neuroscience*, 10:77.

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. *Journal of Comparative pPysiology. A*, 189(8):579–88.

Wehner, R. (2008). The architecture of the desert ant's navigational toolkit (*Hymenoptera : Formicidae*). *Myrmecological News*, 12:85–96.

Wehner, R., Meier, C., and Zollikhofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecological Entomology*, 29:240–250.

Wiltschko, R. and Wiltschko, W. (1990). Zur Entwicklung der Sonnenkompaßorientierung bei jungen Brieftauben. *Journal für Ornithologie*, 131(1):1–19.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic Compass of European Robins. *Science*, 176:62–64.

Wiltschko, W., Wiltschko, R., Munro, U., and Ford, H. (1998). Magnetic versus celestial cues: Cue-conflict experiments with migrating silvereyes at dusk. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 182(4):521–529.

Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. *Science*, 312(5782):1965–1967.

Yuan, Q., Metterville, D., Briscoe, A. D., and Reppert, S. M. (2007). Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. *Molecular biology and evolution*, 24(4):948–55.

Zeil, J. (2012). Visual homing: an insect perspective. *Current Opinion in Neurobiology*, 22(2):285–293.

Zeil, J., Hofmann, M. I., and Chahl, J. S. (2003). Catchment areas of panoramic snapshots in outdoor scenes. *Journal of the Optical Society of America. A, Optics, image science, and vision*, 20(3):450–469.

8 General Discussion

Central place foragers are confronted with the challenge to find back to their nest after searching for food in the nest's surroundings. Experienced *Cataglyphis* foragers are superb navigators (for reviews: Ronacher, 2008; Wehner, 2003; Wehner, 2008; Wehner and Rössler, 2013). To return successfully to their nest after foraging over long distances in their hostile habitat, they use different navigational strategies. For path integration *Cataglyphis* ants combine celestial cues, i. e. mainly the position of the sun and the polarization pattern (e. g. Lebhardt and Ronacher, 2013; Lebhardt and Ronacher, 2015; Wehner and Müller, 2006), with idiothetic cues, i. e. a step counter (Wittlinger et al., 2006; Wittlinger et al., 2007), to build and constantly update their home vector (Müller and Wehner, 1988). For landmark guidance, they use any cue available in their environment; may it be a visual (e. g. Andel and Wehner, 2004; Wehner et al., 1996; Ziegler and Wehner, 1997), or an olfactory landmark (e. g. Buehlmann et al., 2015; Steck et al., 2009), a tactile structure on the ground (Seidl and Wehner, 2006), or even a vibrational or magnetic anomaly (Buehlmann et al., 2012). This impressive navigational toolkit is not available from the beginning on, but has to be filled with experience.

The present doctoral thesis aims at understanding how *Cataglyphis* ants calibrate their compass systems at the beginning of their foraging career, and how they acquire all information necessary for successful navigation later on as foragers.

When leaving the nest for the first time, *Cataglyphis* ants perform so-called learning walks which are similar to learning flights performed by wasps or bees (for reviews: Zeil, 2012; Zeil et al., 1996). These learning walks are well-structured excursions of short duration and length around the nest entrance during which the novices do not collect any food items (e. g. Stieb et al., 2012; Wehner et al., 2004). A crucial step was to unravel the spatial and temporal fine-structure and the ontogeny of *Cataglyphis'* learning walks (chapter 3 and 4). Following high resolution analyses of the fine structure of learning walks in different *Cataglyphis* species performed under natural conditions (chapter 3), learning walks were manipulated in various ways. Learning walks usually increase in duration and in length in all directions around the nest entrance. This natural sequence of subsequent walks was interrupted by restricting the space available around the nest entrance with a moat (chapter 5). The visual input during learning walks was changed by the setup of artificial landmarks around the nest entrance (chapter 3 & 4), or by manipulation of the celestial cues above the nest entrance (chapter 6). Since the manipulation of the celestial cues did not result in the expected behavioral changes, the earth's magnetic field at the nest entrance was altered using a flat coil or a Helmholtz coil (chapter 7). This opened up unique opportunities to investigate the consequences of of experimental manipulations with regard to the spatiotemporal characteristics of the learning walks performed under altered condition (chapter 3, 6, and 7) and with regard to the future foraging success (chapter 4 and 5). The starting point of the present doctoral thesis (chapter 2), i. e. the search for the earthbound reference system to calibrate the celestial compass systems of Cataglyphis, has to be revisited in the light of the results of the rather unexpected magnet manipulation experiments (chapter 7). These results indicate that the ants use the geomagnetic field as an earthbound reference system for memorizing the landmark panorama around their nest entrance for returning back to the nest.

All topics touched here will be discussed in more detail below. Furthermore, one crucial topic still open for discussion and further investigation is concerned with the problem of re-learning. How flexible can *Cataglyphis* ants re-learn? Do they use the same mechanisms for acquisition of navigational information as novices and for acquisition of (conflicting) information gained as already experienced foragers later on? And what happens on a neuronal level in the ant's brain when they learn something from scratch or re-learn something?

8.1 Spatiotemporal characteristics of *Cataglyphis'* learning walks

Many hymenopteran central place foragers perform learning walks or flights when leaving the nest at the beginning of their foraging careers or when they learn new important places like a feeder for example. Learning walks and flights share some common features (for a review: Zeil, 2012), but specific needs induced by different living conditions may cause important differences between learning walks or flights – even if the performers are closely related. For example, male bumblebees do not look back to the nest when leaving it in a way their female nestmates do, but when learning a feeder both males and females perform learning flights during which they fixate the flowers (Robert et al., 2017). Learning walks of *Cataglyphis* novices show both striking similarities and differences when compared across different Catagylphis species (Fleischmann et al., 2017). In the following paragraph the discussion will focus mainly on naïve learning walks of several *Cataglyphis* species.

As has been described for *C. bicolor* before (Wehner et al., 2004), also the learning walks of *C. fortis* (Fleischmann et al., 2016; Fleischmann et al., 2017; Stieb et al., 2012), *C. noda* (Fleischmann et al., 2017; Grob et al., 2017) and *C. aenescens* (Fleischmann et al., 2017) are well-structured. The ants slowly meander around their nest entrance and frequently include turns in their walk. Early walks are very short in distance and duration, but increase over time, i. e. with the increasing number of trips outside the nest (Fleischmann et al., 2016; Fleischmann et al., 2017; Wehner et al., 2004). Although the overall structures of learning walks in different *Cataglyphis* species are similar, there is one important difference between the learning walks of *Cataglyphis* inhabiting cluttered environments (*C. bicolor*, *C. noda* and *C. aenescens*) and those inhabiting barren saltpans without any obvious visual landmarks (*C. fortis*) (figure 8.1). Only the former include pirouettes, i. e. tight turns about the ant's body axis, in their learning walks (Fleischmann et al., 2017; Grob et al., 2017). They perform full and partial pirouettes as does another desert ant species living in the Namib desert (*Ocymyrmex robustior*: Müller and Wehner, 2010).

All *Cataglyphis* species investigated so far perform another type of turns, so-called voltes, during which they walk in a small circle (Fleischmann et al., 2017). The precise function of voltes is not yet known, but one potential function might be that voltes play a role in calibration of the celestial compass systems.

In contrast to voltes, pirouettes frequently include brief stopping phases during which the ants do not move (Fleischmann et al., 2017). The gaze direction during the longest stopping phase is accurately directed towards the nest entrance (Fleischmann et al., 2017).

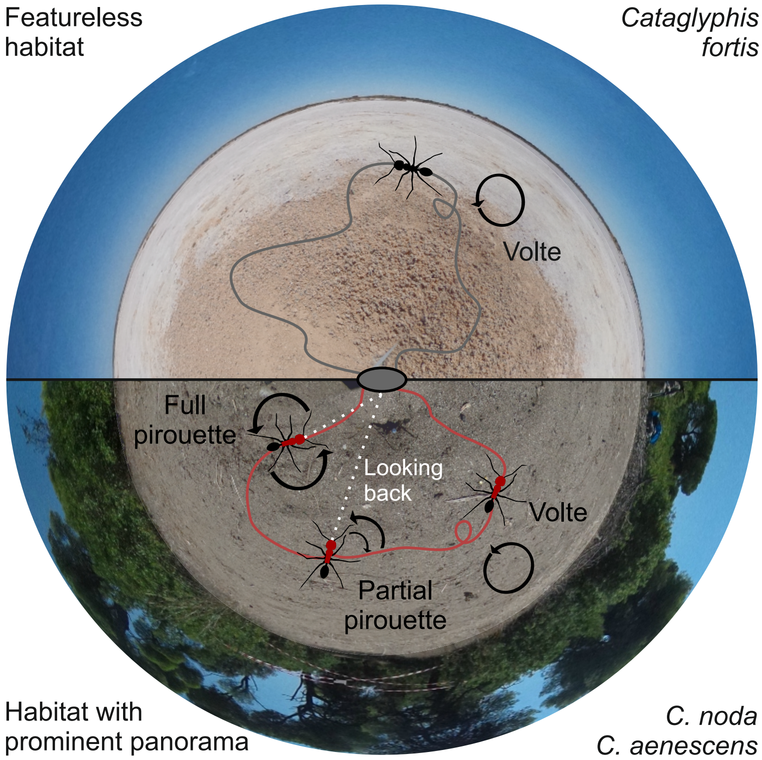

Figure 8.1: Learning-walk elements performed by different *Cataglyphis* species. *C. fortis* inhabiting featureless saltpans (upper half) performs voltes without distinct stopping phases during their learning walks. *C. noda* and *C. aenescens* inhabiting a cluttered environment (lower half) perform voltes without distinct stopping phases, as well as full and partial pirouettes during their learning walks. Pirouettes frequently include brief stops during which the ants look back to the nest entrance (indicated by the dashed lines). Figure: Pauline Fleischmann and Robin Grob 2017.

This directedness cannot be changed with altered skylight conditions – even if the ants do not get any information about the polarization pattern or the position of the sun, they are still able to look back to the nest entrance invisible from their perspective (Grob et al., 2017). Furthermore, ants performing (presumably re-)learning walks in experimental arenas inside a laboratory, i. e. without any view of the sky, gaze back to the nest entrance (personal observation) which indicates that celestial cues are not necessary to perform goal-oriented pirouettes. It has been proposed before that desert ants align their gaze directions towards the nest entrance using the path integrator (Fleischmann et al., 2017; Graham et al., 2010; Grob et al., 2017; Müller and Wehner, 2010). The path integrator gets its directional input from celestial cues during foraging (Lebhardt and Ronacher, 2013; Lebhardt and Ronacher, 2015; Wehner and Müller, 2006). Therefore, it was quite unexpected to find out that skylight cues were not needed in naïve ants to accurately gaze back to the nest entrance.

This finding has showed that ants must use another reference system for aligning their gaze directions (Grob et al., 2017). A promising possibility was the earth's magnetic field. The earth's magnetic field is relatively stable and, in principle, already available in the nest for interior workers. Ants leaving the nest for the first time might use this known reference system in order to acquire and calibrate new information as will be discussed in the next section. The magnetic manipulation experiments performed with a flat coil and a Helmholtz coil demonstrate that *C. noda* ants align their gaze directions during learning walks towards the nest entrance with the aid of the earth's magnetic field (chapter 7). When confronted with a disarrayed magnetic field or after the elimination of the horizontal field component, the novices' gaze directions were distributed randomly. If the horizontal field component was rotated, the ants' gaze directions during the longest stopping phases changed accordingly, i. e. towards the fictive nest entrance position. The magnetic manipulations were the first experimental paradigm which led to an alternation of learning walk elements. Only when the magnetic field was rotated, the ants' gaze directions changed. Neither setting up artificial landmarks on the saltpan (Fleischmann et al., 2017) nor changing the celestial cues above the nest entrance (Grob et al., 2017) changed the fine-structure of the learning walks or induced any changes in gaze directions. In contrast, the rotation of the horizontal field component shifted the gaze directions during the longest stopping phases of pirouettes in a predictable manner. This leads to the cconclusion that the geomagnetic field is the reference system needed for aligning the ants' gaze directions during learning-walk pirouettes (chapter 7). For the first time, it is shown that a geomagnetic compass cue is necessary and sufficient for accomplishing a specific navigational task in insects. An obvious follow-up question is whether the earth's magnetic field is used exclusively for aligning the gaze directions during first learning walks at the beginning of a forager career, or whether it is also used for other navigational tasks.

8.2 Search for the directional reference system(s)

The ants need directional reference systems for at least two different navigational tasks, i. e. an earthbound reference system to calibrate the solar ephemeris, and a reference frame for aligning their gaze directions towards a goal, e. g. the nest entrance, to memorize the landmark panorama for landmark guidance later on. How are these three navigational systems providing directional information, i. e. celestial cues, geomagnetic field and landmarks, connected to each other (figure 8.2)?

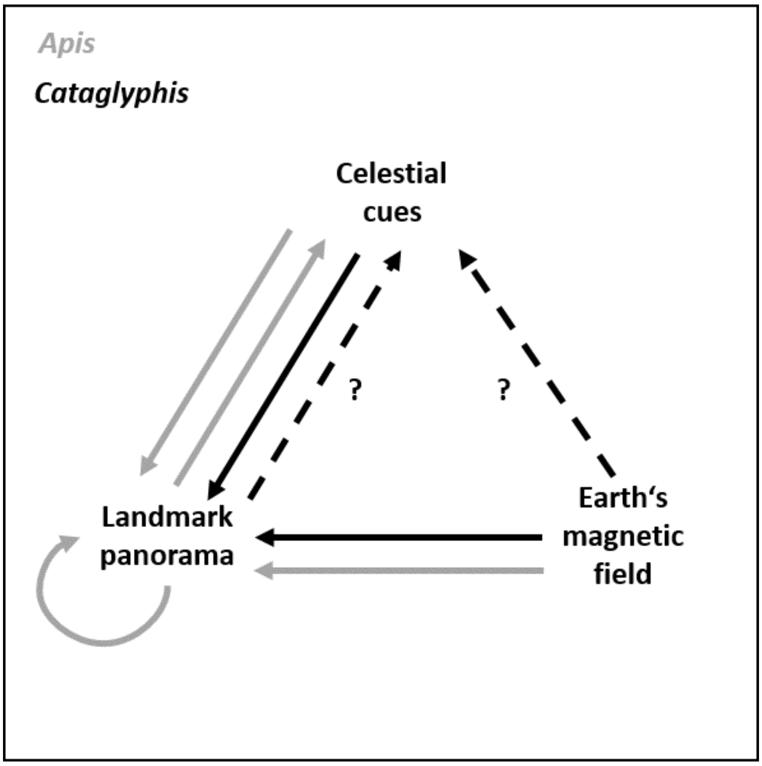

Figure 8.2: Relations between three navigational systems providing directional information. When an ant (bee) leaves its nest (hive) for the first time, many cues potentially helpful for navigation are available. Three of them are the earth's magnetic field, the visual panorama characterized by landmarks (horizon skyline), and celestial cues (i. a. sun, polarization pattern). Directional information given by one system is used as a reference system to acquire information provided by another system (shown as solid arrows). In *Cataglyphis* ants (black arrows) two links are known. (i) Novices use the earth's magnetic field to align their gaze directions to the nest entrance during the longest stopping phases of learning-walk pirouettes. (ii) Forager couple snapshots of landmarks to a celestial system of reference. In honey bees (Apis, grey arrows) four links are known. (i) Foragers use the earth's magnetic field to learn the position of a novel feeder. (ii) Foragers link landmarks to celestial compass cues. (iii) Landmarks are used to memorize the solar ephemeris. (iv) The panorama is used to align gaze directions towards certain goals. The question for *Cataglyphis* is which reference system they use to calibrate the solar ephemeris (dashed arrows). Do they use the landmark panorama, as honey bees do? This seems to be quite circuitous, because they would have to use the earth's magnetic field as a reference system to learn the landmarks in order to use the landmarks as a reference system for celestial cues. A more direct connection would be that the earth's magnetic field is the reference system for celestial cues. However, for this link, so far, there is no evidence, neither in ants nor in bees. For details see text.

In the last experiment of my PhD project, I showed that *C. noda* uses the earth's magnetic field to align their gaze directions to the nest (chapter 7). Similar results were obtained in honey bees when free-flying bees learned the position of a feeder (Collett and Baron, 1994), or had to discriminate patterns (Frier et al., 1996). However, in both experiments foraging bees only used magnetic information when visual cues were not available (Collett and Baron, 1994; Frier et al., 1996), whereas under natural conditions, *C. noda* ants fully rely on the magnetic field during their learning walks if all other cues are present or even in conflict (chapter 7). Taken together, these experiments show that the earth's magnetic field can be used (honey bees: feeder learning) or is even necessary and sufficient (desert ants: learning walks) to acquire, store and retrieve landmark information. Interestingly, honey bees trained in a tent under experimental magnetic conditions and tested in the same tent under natural magnetic conditions did not change their orientation, but kept the orientation which had been dictated by the artificial magnetic field during training. This indicates that the bees used panoramic information available during training and testing (Collett and Baron, 1994). Hence, also the panorama can be used by bees to align gaze directions towards certain goals. Another remarkable aspect is, that the bees learning a novel feeder rely on magnetic information to align their gaze directions, but later on they use other cues which they must have learned before (the panorama within the tent in this case). This will be discussed in more detail below.

In the first experiment of my PhD project, I tried to determine whether the horizon, i.e the visual skyline, provides the earthbound reference system to calibrate the celestial compass systems in *C. fortis* (chapter 2). Since the crucial experiment with the rotating channel could not be performed, this question remains open. However, in honey bees it has been convincingly shown that landmarks are used to calibrate the solar ephemeris (Dyer, 1987; Dyer and Gould, 1981; Towne and Moscrip, 2008; for a review: Dyer, 1996). This indicates that foraging honey bees can use the landmark panorama to memorize the daily course of the sun. On cloudy days, they forage and communicate according to the (invisible) position of the sun by determining its position from their memory with respect to the panorama (Dyer and Gould, 1981). However, the relation between landmarks and celestial cues is not a one-way but a bilateral relation. Celestial cues are also used to distinguish ambiguous landmark situations. Bees trained to one of four possible feeder positions relative to a single landmark choose the correct position under full-sky conditions, but when the sky is cloudy they cannot find the correct position (Dickinson, 1994). Desert ants (*C. fortis*) also store landmark information in relation to celestial cues, but the ants can decouple their snapshots from this framework under certain conditions (Åkesson and Wehner, 2002).

The picture of the relations between the earth's magnetic field, the landmark panorama and celestial cues is at this point already very complex. However, so far, no direct link between the geomagnetic field as an earthbound reference system and the celestial compasses has been detected in ants or bees. The process of using the earth's magnetic field to acquire all landmark information and to then use the panorama to calibrate the celestial compass systems seems to be quite complicated. This process appears especially unlikely, because *Cataglyphis* ants use up to three days (Fleischmann et al., 2016) of their short life outside the nest (Schmid-Hempel and Schmid-Hempel, 1984) to perform learning walks during which they learn the landmarks. This raises the question of when should they have the time to make the second step of this sketched learning process? A further argument may be that the landmark panorama in the natural habitat of *C. fortis* , i. e. at the saltpans, is very uniform making it probably very difficult to distinguish different directions. That is the reason why path integration using celestial cues to determine the direction is especially important for ants living in such featureless environments (for a review: Cheng et al., 2014). So far it is not known whether *C. fortis* ants can make use of the earth's magnetic field in a way comparable to their relatives *C. noda* . The task of using the rather featureless saltpan panorama as a reference system seems very demanding. Honeybees, for example, already fail to realize that their reference system has changed after experimental displacement to a twin landscape (Towne and Kirchner, 1998). For these reasons, it might well be the case that there exists a link between the geomagnetic field and the celestial cues not yet discovered in *C. fortis* ants. Since *C. fortis* ants do not perform any pirouettes with gazes directed to the nest entrance (Fleischmann et al., 2017), a behavioral readout is not yet available. Maybe a closer investigation of the voltes of *C. fortis* (and other *Cataglyphis* species) will help to discover the potential link between earth's magnetic field and celestial cues.

With that said, the rotating channel experiment (chapter 2), in the worst case, would have not led to the results expected, i. e. that the horizon offers the earthbound reference system for calibration of the solar ephemeris. The *C. fortis* ants in the channel may have accessed another reference system, namely the earth's magnetic field. Whether this is really the case and whether passive movement in the channel would not interfere with the ants' calibration process remains speculative at this point. One may assume that the ants would have needed at least the arena in addition to the channel in order to perform learning walks. Since the width of the linear channel was only 12cm, the performance of learning walks was almost impossible (chapter 2). Already the confinement to an area of 30cm width restricted the learning walks of ants (*C. noda*) enormously. *C. noda* ants that were trained in the linear moat setup could not learn the panorama of the pine trees in a way that they could use that information to successfully return to the nest when being tested (chapter 5). This indicates that the spatial restriction of learning walks has negative consequences for the foragers. Furthermore, when an arena (diameter: 60cm) was installed at the starting point of the channel, *C. fortis* ants performed a considerable number of learning walks including voltes (chapter 2). A more detailed analysis of the voltes, the conspicuous turns without goal-centered stopping phases in learning walks (Fleischmann et al., 2017), might potentially be helpful in the future when investigating the relations of the earthbound reference system and the calibration of the solar ephemeris in *C. fortis* .

8.3 Learning from scratch versus re-learning

The experiments presented in my PhD thesis mainly investigated learning walks of naïve ants, so-called novices (or newcomers in chapter 4). A reasonable number of studies have investigated re-learning walks (e. g. Jayatilaka, 2014; Müller, 1984; Müller and Wehner, 2010; Narendra and Ramirez-Esquivel, 2017) of pre-experienced animals. One advantage of re-learning experiments is that the investigation of re-learning behavior of already experienced animals is more easily accessible than the learning of novices, because less monitoring is necessary. A downside of these experiments is that previous experiences of the test animals are not known. This means that groups of re-learners often are much more heterogeneous than those of novices learning from scratch (see chapter 4). However, from a biological point of view learning and re-learning experiments in novices and foragers, respectively, may lead to completely different outcomes. Therefore, it is worthwhile to look more closely at that type of learning that is investigated. This rises the crucial questions whether animals at different stages of experience that are confronted with similar tasks perform the same learning procedure with regard to how they acquire the information to be learned (e. g. structure of learning walks), with regard to how flexible they learn new information (i. e. can they learn anything at any time?) and with regard to the neuronal basis (i. e. is the brain plastic enough to store new information at any time).

In the following, it will be discussed to what extend naïve learning walks and re-learning walks may be similar or dissimilar. Before getting deeper into the discussion, one note concerning the terminology should be added. In the following, "learning (in novices)" only covers the situation that naïve animals learn something from scratch, e. g. novices leaving the nest for the first time acquire information about the nest's surroundings. In contrast "re-learning" covers many different situations. Three examples of different types of re-learning are: (i) learning in another context, e. g. experienced foragers may learn a new feeder, (ii) learning a new aspect in a former known environment, e. g. learning a new landmark at the nest entrance which has been introduced experimentally, or (iii) learning a completely new environment after relocation, e. g. a naturally or experimentally induced move of the colony. It is very likely that for the test animals and their brains these slight differences in experimental design cause huge differences in the learning process. As will be shown for some examples, these differences in experimental settings may be responsible for different (and sometimes even contradictory) results.

Any change in a familiar landmark panorama, i. e. both adding new landmarks (e. g. Fleischmann et al., 2016; Müller and Wehner, 2010) as well as removing familiar landmarks (e. g. Narendra and Ramirez-Esquivel, 2017), induces re-learning in ants. The question is whether naïve learning walks and re-learning walks have the same structure and the same outcome, i. e. whether the learning process is organized in the same way, and whether information is acquired in the same way. Concerning the first part of the question, findings have shown is that *C. fortis* ' naïve learning walks and re-learning walks are organized similarly in terms of spatiotemporal development. The landmark-learning paradigm with novices (Fleischmann et al., 2016) had already been performed in the 1980ies with experienced foragers as part of a diploma thesis (Müller, 1984). When comparing the ontogeny of the naïve learning walks and the re-learning walks, there is no obvious difference noticeable between the general structures of learning walks in novices and re-learning walks in foragers. In both cases, ants' paths increase with experience, and ants eventually start foraging after the completion of several learning walks. The same is true for the performance at the test field – when putting the paths of test ants next to each other it is not possible to decide whether it is a path of an ant tested in the 1980es or thirty years later. Both novices and re-learners only find the position of the fictive nest entrance after performing several learning walks, and only foragers approach the position of the fictive nest entrance directly (Fleischmann et al., 2016; Müller, 1984). Although the ants' path at the nest entrance and on the test field seem to be similar, different learning processes may underlie the behavior. Whether the fine-structure of learning-walk elements and home searches when being tested is also similar remains to be tested. One possibility to detect potential differences may be to track the ants with high-speed cameras during their walks (Häfner, 2016; Risse et al., 2017).

Since the accurately directed gazes during stopping phases in learning-walk pirouettes of *C. noda* offer an easily quantifiable behavior readout, the learning walks of these ants may be very useful to the comparison of learning procedures of novices and re-learners. For example one might ask whether, when, and how *C. noda* ants switch from using the magnetic field as a reference system to using celestial cues. Honey bees, for example, use the magnetic field to align their gaze directions when learning a feeder, but keep their direction relative to the surrounding panorama as experienced foragers, even if the magnetic field is changed (Collett and Baron, 1994). *C. noda* ants look back to a new feeder when they leave it for the first time (personal observation) as do honeybees (Lehrer, 1991). To investigate the question whether *C. noda* foragers use the magnetic field to align gaze directions during learning a feeder position, they could be trained to a feeder placed in a Helmholz coil. Do these foragers use the same reference system to align their gazes to the feeder as novices do when learning the nest's surrounding?

Another possibility to compare the performance of novices and experienced foragers is the repetition of the Helmholtz coil experiment (chapter 7) with experienced foragers. Since foragers learned to use the experimental setup rapidly and were obviously not disturbed by the ongoing experimentally induced changes of the magnetic field (personal observation), artificial landmarks have to be installed in addition to the Helmholtz coil. These landmarks will induce re-learning walks (e. g. Müller and Wehner, 2010), but the crucial question is whether the experienced foragers will again use the magnetic field to align their gaze directions. Most likely, foragers will use another reference system as experienced honeybees do, like for example celestial cues (Dickinson, 1994) or other visual cues like the panorama (Collett and Baron, 1994; Fry and Wehner, 2002) while ignoring the magnetic field. Depending on the outcome, i. e. in case the hypothesis is confirmed experimentally, the question remains whether experienced foragers will never use the magnetic field as directional cue again, and whether they can switch back to using the magnetic field under certain conditions, or whether the magnetic field will continue to run in parallel to being used as a directional cue under certain conditions.

To test these possibilities an experiment investigating the third type of re-learning introduced above by means of replacing a colony to a completely new site would be needed. All foragers would have to be marked for three days and then the colony could be excavated and released at another clearing to test whether a completely new environment will induce the same learning walks in experienced foragers and novices.

8.4 Closing remarks and outlook

The present PhD thesis underlines once again that *Cataglyphis* ants are with good reason famous insect model organisms for navigation. During the transition from interior to exterior worker, *Cataglyphis* ants pass through an impressive learning period characterized by drastic behavioral and neuronal changes. The key results of my PhD thesis are summarized in the following paragraph.

During the rapid change from living in complete darkness to foraging with mainly visual guiding, *Cataglyphis* ants acquire an immense variety of information, especially visual information like the solar ephemeris (chapter 2) and the landmark panorama surrounding the nest. Furthermore, they switch flexibly between navigational strategies, i. e. path integration using different compass systems like celestial cues or the magnetic field, and landmark guidance. The well-structured learning walks (chapter 3) are crucial for the ants to become successful foragers as has been shown in several experiments. Novices necessarily have to perform learning walks to acquire landmark information around the nest to pinpoint the nest's position as foragers (chapter 4). Furthermore, ants need enough space to perform learning walks, because restricting the space around the nest entrance during learning walks reduces their success of finding back to the nest. They mainly rely on path integration at the beginning of their foraging career, but with experience gained over time, i. e. with an increasing number of feeder visits, the importance of landmark information increases and guides the ants back to the nest (chapter 5). Manipulating the celestial input during learning walks, does not change the behavioral performance, but has drastic consequences on the neuronal level (chapter 6). In contrast to manipulations of celestial cues, the learning-walk behavior is clearly influenced in a predictable manner by altering the magnetic field during learning walks (chapter 7).

The findings presented here lead to a number of exciting follow-up research questions. Future experiments have to cover a wide range of research techniques from different disciplines to get a deeper understanding of how navigational information is acquired, stored, retrieved, used and re-adjusted. Three key topics for follow-up experiments are:

- Initial calibration of the solar ephemeris: What is the earthbound reference system needed for the calibration during learning walks – the panorama, the earth's magnetic field or other factors? Which role do the conspicuous voltes performed by all *Cataglyphis* species investigated so far play during learning walks? Are they used for the calibration of celestial compass systems? Furthermore, how and where is the solar ephemeris stored in the brain?

- Behavioral switches during transition phases: When, why and how flexible do *Cataglyphis* ants switch between different compass systems or navigational strategies? Where and how in the brain is decided which information is used predominantly in a particular situation?

- The magnet compass in *Cataglyphis*: Is the earth's magnetic field only used for aligning gaze directions during learning walks in novices, or is it also used for other navigational tasks later on in foraging life? Where are the magnet receptors located, and which mechanism enables an ant to perceive the magnetic field, especially the polarity of its horizontal component? Where and how is directional information provided by the geomagnetic field processed in the brain and how is it integrated into other sensory modalities comprising the navigational toolkit of *Cataglyphis* ants?

The research approaches outlined above prove that *Cataglyphis'* learning walks are not only crucial for the ants to become successful foragers, but also for biologists that are curious to find out how such small animals like an ants accomplish these astonishing navigational achievements.

Bibliography

Åkesson, S. and Wehner, R. (2002). Visual navigation in desert ants *Cataglyphis fortis*: are snapshots coupled to a celestial system of reference? *The Journal of Experimental Biology*, 205:1971–1978.

Andel, D. and Wehner, R. (2004). Path integration in desert ants, *Cataglyphis*: How to make a homing ant run away from home. *Proceedings of the Royal Society of London B: Biological Sciences*, 271(1547):1485–1489.

Anderson, J. B. and Vander Meer, R. K. (1993). Magnetic Orientation in the Fire Ant, *Solenopsis invicta*. *Naturwissenschaften*, 80(12):568–570.

Ardin, P., Mangan, M., Wystrach, A., and Webb, B. (2015). How variation in head pitch could affect image matching algorithms for ant navigation. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, 201(6):585–597.

Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., and Webb, B. (2016). Using an insect mushroom body circuit to encode route memory in complex natural environments. *PLOS Computational Biology*, 12(2):e1004683.

Baird, E., Byrne, M. J., Smolka, J., Warrant, E. J., and Dacke, M. (2012). The dung beetle dance: An orientation behaviour? *PLoS ONE*, 7(1):e30211.

Banks, A. N. and Srygley, R. B. (2003). Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethology*, 109:835–846.

Barth, M. and Heisenberg, M. (1997). Vision Affects Mushroom Bodies and Central Complex in *Drosophila melanogaster*. *Learning & Memory*, 4:219–229.

Bazalova, O., Kvicalova, M., Valkova, T., Slaby, P., Bartos, P., Netusil, R., Tomanova, K., Braeunig, P., Lee, H.-J., Sauman, I., Damulewicz, M., Provaznik, J., Pokorny, R., Dolezel, D., and Vacha, M. (2016). Cryptochrome 2 mediates directional magnetoreception in cockroaches. *Proceedings of the National Academy of Sciences*, 113(6):1660–1665.

Becker, L. (1958). Untersuchungen über das Heimfindevermögen der Bienen. *Z. vergl. Physiol.*, 41(1):1–25.

Bisch-Knaden, S. and Wehner, R. (2001). Egocentric information helps desert ants to navigate around familiar obstacles. *The Journal of Experimental Biology*, 204(24):4177–4184.

Bisch-Knaden, S. and Wehner, R. (2003a). Landmark memories are more robust when acquired at the nest site than en route: experiments in desert ants. *Naturwissenschaften*, 90:127–130.

Bisch-Knaden, S. and Wehner, R. (2003b). Local vectors in desert ants: Context-dependent landmark learning during outbound and homebound runs. *Journal of Comparative Physiology A*, 189:181–187.

Bregy, P., Sommer, S., and Wehner, R. (2008). Nest-mark orientation versus vector navigation in desert ants. *The Journal of Experimental Biology*, 211:1868–1873.

Buehlmann, C., Graham, P., Hansson, B. S., and Knaden, M. (2014). Desert ants locate food by combining high sensitivity to food odors with extensive crosswind runs. *Current Biology*, 24(9):960–964.

Buehlmann, C., Graham, P., Hansson, B. S., and Knaden, M. (2015). Desert ants use olfactory scenes for navigation. *Animal Behaviour*, 106:99–105.

Buehlmann, C., Hansson, B. S., and Knaden, M. (2012). Desert ants learn vibration and magnetic landmarks. *PLoS ONE*, 7(3):e33117.

Camlitepe, Y. and Stradling, D. J. (1995). Wood Ants Orient to Magnetic Fields. *Proceedings of the Royal Society B: Biological Sciences*, 261(1360):37–41.

Capaldi, E. A. and Dyer, F. C. (1999). The role of orientation flights on homing performance in honeybees. *The Journal of Experimental Biology*, 202:1655–1666.

Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M., and Riley, J. R. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature*, 403:537–540.

Cheng, K., Schultheiss, P., Schwarz, S., Wystrach, A., and Wehner, R. (2014). Beginnings of a synthetic approach to desert ant navigation. *Behavioural Processes*, 102:51–61.

Chittka, L., Williams, N. M., Rasmussen, H., and Thomson, J. D. (1999). Navigation without vision: bumblebee orientation in complete darkness. *Proceedings of the Royal Society B: Biological Sciences*, 266(1414):45–50.

Christian, M. (2015). *Erfahrungsabhängiges Lernen von Landmarken bei der Wüstenameise Cataglyphis fortis*. Bachelor thesis, Universität Würzburg.

Collett, M. (2010). How desert ants use a visual landmark for guidance along a habitual route. *Proceedings of the National Academy of Sciences*, 107(25):11638–11643.

Collett, M. and Collett, T. S. (2000). How do insects use path integration for their navigation? *Biological Cybernetics*, 83(3):245–259.

Collett, M., Collett, T. S., Bisch, S., and Wehner, R. (1998). Local and global vectors in desert ant navigation. *Nature*, 394:269–272.

Collett, T. S. (1998). Rapid navigational learning in insects with a short lifespan. *Connection Science*, 10(3-4):255–270.

Collett, T. S. and Baron, J. (1994). Biological compasses and the coordinate frame of landmark memories in honeybees. *Nature*, 368:137–140.

Collett, T. S., Collett, M., and Wehner, R. (2001). The guidance of desert ants by extended landmarks. *The Journal of Experimental Biology*, 204:1635–1639.

Collett, T. S., de Ibarra, N. H., Riabinina, O., and Philippides, A. (2013). Coordinating compass-based and nest-based flight directions during bumblebee learning and return flights. *The Journal of Experimental Biology*, 216(6):1105–1113.

Collett, T. S., Dillmann, E., Giger, A., and Wehner, R. (1992). Visual landmarks and route following in desert ants. *Journal of Comparative Physiology A*, 170:435–442.

de Vries, L., Pfeiffer, K., Trebels, B., Adden, A. K., Green, K., Warrant, E., and Heinze, S. (2017). Comparison of Navigation-Related Brain Regions in Migratory versus Non-Migratory Noctuid Moths. *Frontiers in Behavioral Neuroscience*, 11:158.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H., Chamkhi, H., Greggers, U., and Menzel, R. (2015). Exploratory behaviour of honeybees during orientation flights. *Animal Behaviour*, 102:45–57.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P. B., Nguyen, H., Chamkhi, H., Meyer, H., Singh, P. K., Manz, G., Greggers, U., and Menzel, R. (2016). Honeybees Learn Landscape Features during Exploratory Orientation Flights. *Current Biology*, 26(20):2800–2804.

Dickinson, J. A. (1994). Bees link local landmarks with celestial compass cues. *Naturwissenschaften*, 81(10):465–467.

Dillier, F.-X. and Wehner, R. (2004). Spatio-temporal patterns of colony distribution in monodomous and polydomous species of North African desert ants, genus *Cataglyphis*. *Insectes Sociaux*, 51(2):186–196.

Duelli, P. and Wehner, R. (1973). The Spectral Sensitivity of Polarized Light Orientation in *Cataglyphis bicolor* (Formicidae, Hymenoptera). *Journal of Comparative Physiology*, 86(1):37–53.

Dyer, F. (1996). Spatial memory and navigation by honeybees on the scale of the foraging range. *The Journal of Experimental Biology*, 199:147–154.

Dyer, F. C. (1987). Memory and sun compensation by honey bees. *Journal of Comparative Physiology A*, 160(5):621–633.

Dyer, F. C. and Dickinson, J. a. (1994). Development of sun compensation by honeybees: how partially experienced bees estimate the sun's course. *Proceedings of the National Academy of Sciences*, 91(May):4471–4474.

Dyer, F. C. and Gould, J. L. (1981). Honey bee orientation: a backup system for cloudy days. *Science*, 214(4524):1041–1042.

el Jundi, B., Foster, J. J., Khaldy, L., Byrne, M. J., Dacke, M., and Baird, E. (2016). A Snapshot-Based Mechanism for Celestial Orientation. *Current Biology*, 26(11):1456–1462.

Falibene, A., Roces, F., and Rössler, W. (2015). Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. *Frontiers in Behavioral Neuroscience*, 9:84.

Fiore, V. G., Kottler, B., Gu, X., and Hirth, F. (2017). In silico Interrogation of Insect Central Complex Suggests Computational Roles for the Ellipsoid Body in Spatial Navigation. *Frontiers in Behavioral Neuroscience*, 11:142.

Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., and Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *The Journal of Experimental Biology*, 219:3137–3145.

Fleischmann, P. N., Grob, R., Wehner, R., and Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *The Journal of Experimental Biology*, 220:2426–2435.

Freas, C. A. and Cheng, K. (2017). Learning and time-dependent cue choice in the desert ant, *Melophorus bagoti*. *Ethology*, pages 1–13.

Freas, C. A., Narendra, A., and Cheng, K. (2017). Compass cues used by a nocturnal bull ant, Myrmecia midas. *The Journal of Experimental Biology*, 220:1578–1585.

Frier, H., Edwards, E., Smith, C., Neale, S., and Collett, T. (1996). Magnetic compass cues and visual pattern learning in honeybees. *The Journal of Experimental Biology*, 199:1353–1361.

Fry, S. N. and Wehner, R. (2002). Honey bees store landmarks in an egocentric frame of reference. *Journal of Comparative Physiology - A*, 187(12):1009–1016.

Gegear, R. J., Casselman, A., Waddell, S., and Reppert, S. M. (2008). Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*. *Nature*, 454(7207):1014–8.

Goff, M., Salmon, M., and Lohmann, K. (1998). Hatchling sea turtles use surface waves to establish a magnetic compass direction. *Animal behaviour*, 55(1):69–77.

Gould, J. L. (1980). Sun compensation by bees. *Science*, 207(4430):545–547.

Graham, P. (2010). Insect Navigation. In Breed, M. and Moore, J., editors, *Encyclopedia of Animal Behaviour*, volume 2, pages 167–175. Oxford Academic Press.

Graham, P. and Philippides, A. (2017). Vision for navigation: What can we learn from ants? *Arthropod Structure and Development*, 46(5):718–722.

Graham, P., Philippides, A., and Baddeley, B. (2010). Animal cognition: multi-modal interactions in ant learning. *Current Biology*, 20(15):R639–R640.

Grob, R. (2016). *Experience-dependent behavioural changes and neuronal plasticity in visual pathways of Cataglyphis desert ants*. Master thesis, Universität Würzburg.

Grob, R., Fleischmann, P. N., Grübel, K., Wehner, R., and Rössler, W. (2017). The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11:226.

Groh, C., Kelber, C., Grübel, K., and Rössler, W. (2014). Density of mushroom body synaptic complexes limits intraspecies brain miniaturization in highly polymorphic leaf-cutting ant workers. *Proceedings of the Royal Society B: Biological Sciences*, 281:20140432.

Groh, C., Lu, Z., Meinertzhagen, I. a., and Rössler, W. (2012). Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee Apis mellifera. *The Journal of Comparative Neurology*, 520(15):3509–3527.

Gronenberg, W. (2001). Subdivisions of hymenopteran mushroom body calyces by their afferent supply. *Journal of Comparative Neurology*, 435(4):474–489.

Guerra, P. A., Gegear, R. J., and Reppert, S. M. (2014). A magnetic compass aids monarch butterfly migration. *Nature Communications*, 5:4164.

Häfner, B. (2016). *Experience-dependent navigation in desert ants: comparing different methods of data recording*. Bachelor thesis, Universität Würzburg.

Harkness, R. D. (1977). The carrying of ants (*Cataglyphis bicolor* Fab.) by others of the same nest. *Journal of Zoology: Proceedings of the Zoological Society of London*, 183:419–430.

Harris, R. A., Hempel de Ibarra, N., Graham, P., and Collett, T. S. (2005). Ant Navigation: Priming of visual route memories. *Nature*, 438:302.

Heinze, S. and Homberg, U. (2007). Maplike Representation of Celestial E-Vector Orientations in the Brain of an Insect. *Science*, 315:995–997.

Heinze, S. and Reppert, S. M. (2012). Anatomical basis of sun compass navigation I: the general layout of the monarch butterfly brain. *The Journal of Comparative Neurology*, 520(8):1599–1628.

Hempel de Ibarra, N., Philippides, A., Riabinina, O., and Collett, T. S. (2009). Preferred viewing directions of bumblebees (*Bombus terrestris* L.) when learning and approaching their nest site. *The Journal of Experimental Biology*, 212:3193–3204.

Hölldobler, B. and Wilson, E. (1990). *The Ants*. Havard University Press, Cambridge, Massachusetts.

Homberg, U., Heinze, S., Pfeiffer, K., Kinoshita, M., and el Jundi, B. (2011). Central neural coding of sky polarization in insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1565):680–687.

Hore, P. J. and Mouritsen, H. (2016). The Radical-Pair Mechanism of Magnetoreception. *Annual Review of Biophysics*, 45(1):299–344.

Hourcade, B., Münz, T. S., Sandoz, J.-C. C., Rössler, W., and Devaud, J. M. (2010). Long-term memory leads to synaptic reorganization in the mushroom bodies: A memory trace in the insect brain? *The Journal of Neuroscience*, 30(18):6461–6465.

Huber, R. and Knaden, M. (2015). Egocentric and geocentric navigation during extremely long foraging paths of desert ants. *Journal of Comparative Physiology A*, 201(6):609–616.

Ito, K., Shinomiya, K., Ito, M., Armstrong, J., Boyan, G., Hartenstein, V., Harzsch, S., Heisenberg, M., Homberg, U., Jenett, A., Keshishian, H., Restifo, L., Rössler, W., Simpson, J., Strausfeld, N., Strauss, R., and Vosshall, L. (2014). A Systematic Nomenclature for the Insect Brain. *Neuron*, 81(4):755–765.

Jander, R. and Jander, U. (1998). The light and magnetic compass of the weaver ant, *Oecophylla smaragdina* (Hymenoptera: Formicidae). *Ethology*, 104(9):743–758.

Jayatilaka, P. (2014). *Individual foraging careers of the Jack Jumper ant, Myrmecia croslandi*. Doctoral thesis, Australian National University.

Judd, S. P. D. and Collett, T. S. (1998). Multiple stored views and landmark guidance in ants. *Nature*, 392:710–714.

Kimchi, T., Etienne, A. S., and Terkel, J. (2004). A subterranean mammal uses the magnetic compass for path integration. *Proceedings of the National Academy of Sciences*, 101(4):1105–1109.

Knaden, M. and Wehner, R. (2004). Path integration in desert ants controls aggressiveness. *Science*, 305:60.

Knaden, M. and Wehner, R. (2005). Nest mark orientation in desert ants *Cataglyphis*: What does it do to the path integrator? *Animal Behaviour*, 70:1349–1354.

Knaden, M. and Wehner, R. (2006). Ant navigation: resetting the path integrator. *The Journal of Experimental Biology*, 209(1):26–31.

Kohler, M. and Wehner, R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: How do they interact with path-integration vectors? *Neurobiology of Learning and Memory*, 83:1–12.

Kühn-Bühlmann, S. and Wehner, R. (2006). Age-Dependent and Task-Related Volume Changes in the Mushroom Bodies of Visually Guided Desert Ants, Cataglyphis bicolor. *Journal of Neurobiology*, 66:511–521.

Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microscopy research and technique*, 47(6):368–379.

Lambinet, V., Hayden, M. E., Reid, C., and Gries, G. (2017a). Honey bees possess a polarity-sensitive magnetoreceptor. *Journal of Comparative Physiology A*, 203(12):1029–1036.

Lambinet, V., Hayden, M. E., Reigl, K., Gomis, S., and Gries, G. (2017b). Linking magnetite in the abdomen of honey bees to a magnetoreceptive function. *Proceedings of the Royal Society B*, 284:20162873.

Lebhardt, F. and Ronacher, B. (2014). Interactions of the polarization and the sun compass in path integration of desert ants. *Journal of Comparative Physiology. A*, 200:711–720.

Lebhardt, F. and Ronacher, B. (2015). Transfer of directional information between the polarization compass and the sun compass in desert ants. *Journal of Comparative Physiology A*, 201(6):599–608.

Legge, E. L. G., Wystrach, A., Spetch, M. L., and Cheng, K. (2014). Combining sky and earth: desert ants (*Melophorus bagoti*) show weighted integration of celestial and terrestrial cues. *The Journal of Experimental Biology*, 217:4159–4166.

Lehrer, M. (1991). Bees which turn back and look. *Naturwissenschaften*, 78:274–276.

Lehrer, M. (1993). Why do bees turn back and look? *Journal of Comparative Physiology A*, 172(5):549–563.

Light, P., Salmon, M., and Lohmann, K. J. (1993). Geomagnetic Orientation of Loggerhead Sea Turtles: Evidence for an Inclination Compass. *Journal of Experimental Biology*, 182(1):1–10.

Lindauer, M. (1957). Sonnenorientierung der Bienen unter der Äquatorsonne und zur Nachtzeit. *Die Naturwissenschaften*, 44(1):1–6.

Lindauer, M. and Martin, H. (1968). Die Schwereorientierung der Bienen unter dem Einfluß des Erdmagnetfeldes. *Zeitschrift für Vergleichende Physiologie*, 60(3):219–243.

Lohmann, K., Pentcheff, N., Nevitt, G., Stetten, G., Zimmer-Faust, R., Jarrard, H., and Boles, L. (1995). Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *The Journal of Experimental bBology*, 198:2041–2048.

Marschewski, S. (2017). *Manipulation of Cataglyphis learning walks with a rotating disc*. Bachelor thesis, Universität Würzburg.

Martin, J. P., Guo, P., Mu, L., Harley, C. M., and Ritzmann, R. E. (2015). Central-complex control of movement in the freely walking cockroach. *Current Biology*, 25(21):2795–2803.

McMeeking, R. M., Arzt, E., and Wehner, R. (2012). Cataglyphis desert ants improve their mobility by raising the gaster. *Journal of Theoretical Biology*, 297:17–25.

Menzel, R. (2001). Searching for the memory trace in a mini-brain, the honeybee. *Learning & Memory*, 8(2):53–62.

Merkle, T., Knaden, M., and Wehner, R. (2006). Uncertainty about nest position influences systematic search strategies in desert ants. *The Journal of Experimental Biology*, 209:3545–3549.

Merkle, T. and Wehner, R. (2008). Landmark cues can change the motivational state of desert ant foragers. *Journal of Comparative Physiology A*, 194(4):395–403.

Merkle, T. and Wehner, R. (2010). Desert ants use foraging distance to adapt the nest search to the uncertainty of the path integrator. *Behavioral Ecology*, 21(2):349–355.

Muenz, T. S., Groh, C., Maisonnasse, A., Le Conte, Y., Plettner, E., and Rössler, W. (2015). Neuronal plasticity in the mushroom body calyx during adult maturation in the honeybee and possible pheromonal influences. *Developmental Neurobiology*, 75:1368–1384.

Müller, M. (1984). *Interokularer Transfer bei der Wüstenameise Cataglyphis fortis (Formicidae, Hymenoptera).* Diplomarbeit, Universität Zürich.

Müller, M. (1989). *Mechanismus der Wegintegration bei Cataglyphis fortis (Hymenoptera, Insecta).* Phd thesis, Universität Zürich.

Müller, M. and Wehner, R. (1988). Path integration in desert ants, Cataglyphis fortis. *Proceedings of the National Academy of Sciences*, 85(14):5287–5290.

Müller, M. and Wehner, R. (1994). The hidden spiral: Systematic search and path integration in desert ants, *Cataglyphis fortis*. *Journal of Comparative Physiology A*, 175:525–530.

Müller, M. and Wehner, R. (2007). Wind and sky as compass cues in desert ant navigation. *Naturwissenschaften*, 94(7):589–594.

Müller, M. and Wehner, R. (2010). Path integration provides a scaffold for landmark learning in desert ants. *Current Biology*, 20(15):1368–1371.

Muser, B., Sommer, S., Wolf, H., and Wehner, R. (2005). Foraging ecology of the thermophilic Australian desert ant, *Melophorus bagoti*. *Australian Journal of Zoology*, 53(5):301–311.

Narendra, A., Gourmaud, S., and Zeil, J. (2013). Mapping the navigational knowledge of individually foraging ants, *Myrmecia croslandi*. *Proceedings of the Royal Society B*, 280:20130683.

Narendra, A. and Ramirez-Esquivel, F. (2017). Subtle changes in the landmark panorama disrupt visual navigation in a nocturnal bull ant. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372:20160068.

Narendra, A., Si, A., Sulikowski, D., and Cheng, K. (2007). Learning, retention and coding of nest-associated visual cues by the Australian desert ant, *Melophorus bagoti*. *Behavioral Ecology and Sociobiology*, 61(10):1543–1553.

Neuser, K., Triphan, T., Mronz, M., Poeck, B., and Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. *Nature*, 453(7199):1244–1247.

Nicholson, D., Judd, S. P. D., Cartwright, B. A., and Collett, T. S. (1999). Learning walks and landmark guidance in wood ants (*Formica rufa*). *The Journal of Experimental Biology*, 202:1831–1838.

Nordmann, G. C., Hochstoeger, T., and Keays, D. A. (2017). Unsolved mysteries: Magnetoreception—A sense without a receptor. *PLoS Biology*, 15(10):1–10.

Nørgaard, T., Gagnon, Y. L., and Warrant, E. J. (2012). Nocturnal Homing: Learning Walks in a Wandering Spider? *PLoS ONE*, 7(11):e49263.

Opfinger, E. (1931). Über die Orientierung der Biene an der Futterquelle - Die Bedeutung von Anflug und Orientierungsflug für den Lernvorgang bei Farb-, Form- und Ortsdressuren. *Zeitschrift für vergleichende Physiologie*, 15(3):431–487.

Osborne, J. L., Smith, A., Clark, S. J., Reynolds, D. R., Barron, M. C., Lim, K. S., and Reynolds, A. M. (2013). The ontogeny of bumblebee flight trajectories: From naïve explorers to experienced foragers. *PLoS ONE*, 8(11):e78681.

Ott, S. R. and Rogers, S. M. (2010). Gregarious desert locusts have substantially larger brains with altered proportions compared with the solitarious phase. *Proceedings of the Royal Society B: Biological Sciences*, 277(1697):3087–3096.

Peckham, G. W. and Peckham, E. G. (1898). On the instincts and habits of the solitary wasps.

Petrov, I. Z. (1993). Some remarks on the foraging strategy in *Cataglyphis aenescens* Nyl. (Hymenoptera, Formicidae). *Tiscia (Szeged)*, 27:23–28.

Pfeffer, S. E., Bolek, S., Wolf, H., and Wittlinger, M. (2015). Nest and food search behaviour in desert ants, *Cataglyphis*: A critical comparison. *Animal Cognition*, 18(4):885–894.

Pfeffer, S. E. and Wittlinger, M. (2016). Optic flowodometry operates independently of stride integration in carried ants. *Science*, 353(6304):1155–1157.

Pfeiffer, K. and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. *Annual review of entomology*, 59:165–84.

Philippides, A., de Ibarra, N. H., Riabinina, O., and Collett, T. S. (2013). Bumblebee calligraphy: The design and control of flight motifs in the learning and return flights of *Bombus terrestris*. *The Journal of Experimental Biology*, 216:1093–1104.

Riabinina, O., de Ibarra, N. H., Philippides, A., and Collett, T. S. (2014). Head movements and the optic flow generated during the learning flights of bumblebees. *The Journal of Experimental bBology*, 217(Pt 15):2633–42.

Rickli, M. and Leuthold, R. H. (1988). Homing in Harvester Termites: Evidence of Magnetic Orientation. *Ethology*, 77(3):209–216.

Risse, B., Mangan, M., Del Pero, L., and Webb, B. (2017). Visual Tracking of Small Animals in Cluttered Natural Environments Using a Freely Moving Camera. *Workshop on Visual Wildlife Monitoring*, pages 2840–2849.

Riveros, A. J., Esquivel, D. M., Wajnberg, E., and Srygley, R. B. (2014). Do leaf-cutter ants *Atta colombica* obtain their magnetic sensors from soil? *Behavioral Ecology and Sociobiology*, 68(1):55–62.

Riveros, a. J. and Srygley, R. B. (2008). Do leafcutter ants, *Atta colombica*, orient their path-integrated home vector with a magnetic compass? *Animal Behaviour*, 75:1273–1281.

Robert, T., Frasnelli, E., Collett, T. S., and de Ibarra, N. H. (2017). Male bumblebees perform learning flights on leaving a flower but not when leaving their nest. *The Journal of Experimental Biology*, 220:930–937.

Ronacher, B. (2008). Path integration as the basic navigation mechanism of the desert ant, *Cataglyphis fortis* (Forel, 1902) (Hymenoptera: Formicidae). *Myrmecological News*, 11(August):55–62.

Ronacher, B. and Wehner, R. (1995). Desert ants *Cataglyphis fortis* use self-induced optic flow to measure distances travelled. *Journal of Comparative Physiology A*, 177:21–27.

Rössler, W., Spaethe, J., and Groh (2017). Pitfalls of using confocal-microscopy based automated quantification of synaptic complexes in honeybee mushroom bodies (response to Peng and Yang 2016). *Scientific Reports*, 7:9786.

Schmid-Hempel, P. and Schmid-Hempel, R. (1984). Life fureation and turnover of foragers in the ant *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Insectes Sociaux*, 31(1):345–360.

Schmitt, F., Stieb, S. M., Wehner, R., and Rössler, W. (2016). Experience-related reorganization of giant synapses in the lateral complex: Potential role in plasticity of the sky-compass pathway in the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 76(4):390–404.

Scholl, C., Kübert, N., Muenz, T. S., and Rössler, W. (2015). CaMKII knockdown affects both early and late phases of olfactory long-term memory in the honeybee. *Journal of Experimental Biology*, 218(23):3788–3796.

Scholl, C., Wang, Y., Krischke, M., Mueller, M. J., Amdam, G. V., and Rössler, W. (2014). Light exposure leads to reorganization of microglomeruli in the mushroom bodies and influences juvenile hormone levels in the honeybee. *Developmental Neurobiology*, 74(11):1141–1153.

Schultheiss, P. and Cheng, K. (2011). Finding the nest: Inbound searching behaviour in the Australian desert ant, *Melophorus bagoti*. *Animal Behaviour*, 81(5):1031–1038.

Schultheiss, P., Wystrach, A., Schwarz, S., Tack, A., Delor, J., Nooten, S. S., Bibost, A. L., Freas, C. A., and Cheng, K. (2016). Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama. *Animal Behaviour*, 115:19–28.

Schwarz, S., Wystrach, A., and Cheng, K. (2017). Ants' navigation in an unfamiliar environment is influenced by their experience of a familiar route. *Scientific Reports*, 7(1):14161.

Seelig, J. D. and Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. *Nature*, 521(7551):186–191.

Seid, M. A. and Wehner, R. (2009). Delayed axonal pruning in the ant brain: A study of developmental trajectories. *Developmental Neurobiology*, 69(6):350–364.

Seidl, T. and Wehner, R. (2006). Visual and tactile learning of ground structures in desert ants. *The Journal of Experimental Biology*, 209:3336–3344.

Sommer, S. and Wehner, R. (2012). Leg allometry in ants: extreme long-leggedness in thermophilic species. *Arthropod Structure & Development*, 41(1):71–77.

Sommerlandt, F. M., Spaethe, J., Rössler, W., and Dyer, A. G. (2016). Does fine color discrimination learning in free-flying honeybees change mushroom-body calyx neuroarchitecture? *PLoS ONE*, 11(10):e0164386.

Steck, K., Hansson, B. S., and Knaden, M. (2009). Smells like home: Desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Frontiers in Zoology*, 6:5.

Stieb, S. M., Hellwig, A., Wehner, R., and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 72(5):729–742.

Stieb, S. M., Münz, T. S., Wehner, R., and Rössler, W. (2010). Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Developmental Neurobiology*, 70(6):408–423.

Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Current Opinion in Neurobiology*, 12:633–638.

Stürzl, W., Zeil, J., Boeddeker, N., and Hemmi, J. M. (2016). How wasps acquire and use views for homing. *Current Biology*, 26:470–482.

Tinbergen, N. (1932). Über die Orientierung des Bienenwolfes. *Z. Vergl. Physiol.*, 16:305–334.

Towne, W. F. and Kirchner, W. H. (1998). Honey bees fail to update their solar ephemerides after a displacement. *Naturwissenschaften*, 85(9):459–463.

Towne, W. F. and Moscrip, H. (2008). The connection between landscapes and the solar ephemeris in honeybees. *Journal of Experimental Biology*, 211(23):3729–3736.

Vogt, K., Aso, Y., Hige, T., Knapek, S., Ichinose, T., Friedrich, A. B., Turner, G. C., and Rubin, G. M. (2016). Direct neural pathways convey distinct visual information to *Drosophila* mushroom bodies. *eLIFE*, 5:e14009.

Vollbehr, J. (1975). Zur Orientierung junger Honigbienen bei Ihrem 1. Orientierungsflug. *Zool. Jb. Physiol.*, 79:33–69.

von Frisch, K. (1949). Die Polarisation des Himmelslichtes als orientierender Faktor bei den Tänzen der Bienen. *Experientia*, 5(4):142–148.

Vowles, B. Y. D. M. (1953). The orientation of ants I. The substitution of stimuli. *Jorunal of Experimental Biology*, 31(3):341–355.

Wagner, W. (1907). *Psycho-biologische Untersuchungen an Hummeln*. E. Schweizerbartsche Verlagsbuchhandlung, Stuttgart.

Wajnberg, E., Acosta-Avalos, D., Alves, O. C., de Oliveira, J. F., Srygley, R. B., and Esquivel, D. M. S. (2010). Magnetoreception in eusocial insects: an update. *Journal of the Royal Society, Interface / the Royal Society*, 7:S207–S225.

Warrant, E., Frost, B., Green, K., Mouritsen, H., Dreyer, D., Adden, A., Brauburger, K., and Heinze, S. (2016). The Australian Bogong Moth Agrotis infusa: A Long-Distance Nocturnal Navigator. *Frontiers in Behavioral Neuroscience*, 10:77.

Wehner, R. (1982). Himmelsnavigation bei Insekten. Neurophysiologie und Verhalten. *Neujahrsbl Naturf Ges Zürich*, 184:1–132.

Wehner, R. (1983). Taxonomie, Funktionsmorphologie und Zoogeographie der saharischen Wüstenameise *Cataglyphis* fortis (Forel 1902) stat. nov. *Senckenbergiana biol.*, 64:89–132.

Wehner, R. (1987a). 'Matched filters' - neural models of the external world. *Journal of Comparative Physiology A*, 161(4):511–531.

Wehner, R. (1987b). Spatial Organization of Foraging Behavior in Individually Searching Desert Ants, *Cataglyphis* (Sahara Desert) and *Ocymyrmex* (Namib Desert). In Pasteels, J. M. and Deneubourg, J.-L., editors, *From Individual to Collective Behavior in Social Insects*, pages 15–42. Birkhäuser Verlag, Basel Bostom.

Wehner, R. (1990). On the brink of introducing sensory ecology: Felix Santschi (1872-1940) - Tabib-en-Neml. *Behavioral Ecology and Sociobiology*, 27:295–306.

Wehner, R. (1992). Arthropods. In Papi, F., editor, *Animal Homing*, pages 45–144. Chapman and Hall, London.

Wehner, R. (1994). The polarization-vision project: Championing organismic biology. In Schildberger, K. and Elsner, N., editors, *Neural Basis of Behavioural Adaptations*, pages 103–143. Gustav Fischer Verlag, Stuttgart Jena New York.

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. *Journal of Comparative pPysiology. A*, 189(8):579–88.

Wehner, R. (2008a). The architecture of the desert ant's navigational toolkit (*Hymenoptera : Formicidae*). *Myrmecological News*, 12:85–96.

Wehner, R. (2008b). The desert ant' s navigational toolkit: Procedural rather than positional knowledge. *Journal of The Institute of Navigation*, 55(2):101–114.

Wehner, R. (2014). Polarization Vision: A Discovery Story. In Horváth, G., editor, *Polarized Light and Polarization Vision in Animal Sciences*, pages 3–25. Springer Berlin Heidelberg.

Wehner, R., Boyer, M., Loertscher, F., Sommer, S., and Menzi, U. (2006). Ant navigation: One-way routes rather than maps. *Current Biology*, 16(1):75–79.

Wehner, R., Cheng, K., and Cruse, H. (2014). Visual navigation strategies in insects: lessons from desert ants. In Werner, J. S. and Chalupa, L. M., editors, *The new visual neurosciences*, pages 1153–1163. MIT Press, Cambridge.

Wehner, R., Fukushi, T., and Isler, K. (2007). On being small: Brain allometry in ants. *Brain, Behavior and Evolution*, 69(3):220–228.

Wehner, R., Harkness, R. D., and Schmid-Hempel, P. (1983). *Foraging Strategies in Individually Searching Ants Cataglyphis bicolor (Hymenoptera: Formicida)*. Gustav Fischer Verlag, Stuttgart New York.

Wehner, R. and Labhart, T. (2006). Polarisation Vision. In Warrant, E. J. and Nilson, D. E., editors, *Invertebrate Vision*, pages 291–347. Cambridge University Press, Cambridge.

Wehner, R. and Lanfranconi, B. (1981). What do the ants know about the rotation of the sky? *Nature*, 293(5835):731–733.

Wehner, R., Meier, C., and Zollikhofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecological Entomology*, 29:240–250.

Wehner, R., Michel, B., and Antonsen, P. (1996). Visual navigation in insects: Coupling of egocentric and geocentric information. *The Journal of Experimental Biology*, 199:129–140.

Wehner, R. and Müller, M. (1993). How do ants acquire their celestial ephemeris function? *Naturwissenschaften*, 80:331–333.

Wehner, R. and Müller, M. (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proceedings of the National Academy of Sciences*, 103(33):12575–12579.

Wehner, R. and Müller, M. (2010). Piloting in desert ants: Pinpointing the goal by discrete landmarks. *The Journal of Experimental Biology*, 213:4174–4179.

Wehner, R. and Räber, F. (1979). Visual spatial memory in desert ants, *Cataglyphis bicolor* (Hymenoptera: Formicidae). *Experentia*, 35(September):1569–1571.

Wehner, R. and Rössler, W. (2013). Bounded plasticity in the desert ant's navigational tool kit. In Menzel, R. and Benjamin, P. R., editors, *Invertebrate Learning and Memory*, pages 514–529. Academic Press, San Diego.

Wehner, R. and Srinivasan, M. V. (1981). Searching behaviour of desert ants, genus *Cataglyphis*. *Journal of Comparative Physiology A*, 142:315–338.

Wehner, R. and Wehner, S. (1990). Insect navigation: use of maps or Ariadne's thread? *Ethology Ecology & Evolution*, 2(1):27–48.

Wehner, R. and Wehner, S. (2011). Parallel evolution of thermophilia: Daily and seasonal foraging patterns of heat-adapted desert ants: *Cataglyphis* and *Ocymyrmex* species. *Physiological Entomology*, 36(3):271–281.

Wiltschko, R. and Wiltschko, W. (1990). Zur Entwicklung der Sonnenkompaßorientierung bei jungen Brieftauben. *Journal für Ornithologie*, 131(1):1–19.

Wiltschko, W. and Wiltschko, R. (1972). Magnetic Compass of European Robins. *Science*, 176:62–64.

Wiltschko, W., Wiltschko, R., Munro, U., and Ford, H. (1998). Magnetic versus celestial cues: Cue-conflict experiments with migrating silvereyes at dusk. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 182(4):521–529.

Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. *Science*, 312(5782):1965–1967.

Wittlinger, M., Wehner, R., and Wolf, H. (2007). The desert ant odometer: a stride integrator that accounts for stride length and walking speed. *The Journal of Experimental Biology*, 210:198–207.

Wolf, H. and Wehner, R. (2000). Pinpointing food sources: olfactory and anemotactic orientation in desert ants, *Cataglyphis fortis*. *The Journal of Experimental Biology*, 203:857–868.

Wystrach, a., Beugnon, G., and Cheng, K. (2012). Ants might use different view-matching strategies on and off the route. *Journal of Experimental Biology*, 215(1):44–55.

Wystrach, A., Dewar, A., Philippides, A., and Graham, P. (2015a). How do field of view and resolution affect the information content of panoramic scenes for visual navigation? A computational investigation. *Journal of Comparative Physiology A*, 202(2):87–95.

Wystrach, A., Mangan, M., and Webb, B. (2015b). Optimal cue integration in ants. *Proceedings of the Royal Society B: Biological Sciences*, 282(1816):20151484.

Wystrach, A., Philippides, A., Aurejac, A., Cheng, K., and Graham, P. (2014). Visual scanning behaviours and their role in the navigation of the Australian desert ant *Melophorus bagoti*. *Journal of Comparative Physiology A*, 200(7):615–626.

Yilmaz, A., Lindenberg, A., Albert, S., Grübel, K., Spaethe, J., Rössler, W., and Groh, C. (2016). Age-related and light-induced plasticity in opsin gene expression and in primary and secondary visual centers of the nectar-feeding ant Camponotus rufipes. *Developmental Neurobiology*, pages 1041–1057.

Yuan, Q., Metterville, D., Briscoe, A. D., and Reppert, S. M. (2007). Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. *Molecular biology and evolution*, 24(4):948–55.

Zeil, J. (1993a). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) I. Description of flight. *Journal of Comparative Physiology A*, 172:189–205.

Zeil, J. (1993b). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera) II. Similarities between orientation and return flights and the use of motion parallax. *Journal of Comparative Physiology A*, 172:207–222.

Zeil, J. (2012). Visual homing: an insect perspective. *Current Opinion in Neurobiology*, 22(2):285–293.

Zeil, J., Hofmann, M. I., and Chahl, J. S. (2003). Catchment areas of panoramic snapshots in outdoor scenes. *Journal of the Optical Society of America. A, Optics, image science, and vision*, 20(3):450–469.

Zeil, J., Kelber, A., and Voss, R. (1996). Structure and function of learning flights in bees and wasps. *The Journal of Experimntal Biology*, 199:245–252.

Ziegler, P. E. and Wehner, R. (1997). Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. *Journal of Comparative Physiology A*, 181:13–20.

Zollikofer, C., Wehner, R., and Fukushi, T. (1995). Optical scaling in conspecific *Cataglyphis ants*. *The Journal of Experimental Biology*, 198:1637–1646.

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Curriculum vitae with list of publications

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Appendix

Affidavit

I hereby confirm that my thesis entitled “Starting foraging life: Early calibration and daily use of the navigational system in *Cataglyphis* ants” is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Place, date Signature

“Dissertation Based on Several Published Manuscripts“

Statement of individual author contributions and of legal second publication rights

(If required please use more than one sheet)

Publication (unpublished manuscript): Fleischmann, P. N., Rössler, W., & Wehner, R. What is the earthbound reference system for the daily time compensation of the celestial compass in *Cataglyphis*?

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	RW	WR	PNF		
Methods Development	RW	WR	PNF		
Data Collection	PNF				
Data Analysis and Interpretation	PNF	RW	WR		
Manuscript Writing					
Writing of Introduction	PNF	RW	WR		
Writing of Materials & Methods	PNF	RW	WR		
Writing of Discussion	PNF	RW	WR		
Writing of First Draft	PNF				

Explanations (if applicable):

Publication (complete reference): Fleischmann, P. N., Grob, R., Wehner, R., & Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *Journal of Experimental Biology*, *220*(13), 2426-2435

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	PNF	WR	RG	RW	
Methods Development	PNF	RG	WR		
Data Collection	PNF	RG			
Data Analysis and Interpretation	PNF	RG	WR	RW	
Manuscript Writing					
Writing of Introduction	PNF	WR	RW	RG	
Writing of Materials & Methods	PNF	WR	RW	RG	
Writing of Discussion	PNF	WR	RW	RG	
Writing of First Draft	PNF				

Explanations (if applicable):

Publication (complete reference): Fleischmann, P. N., Christian, M., Müller, V. L., Rössler, W., & Wehner, R. (2016). Ontogeny of learning walks and the acquisition of landmark information in desert ants, *Cataglyphis fortis*. *Journal of Experimental Biology*, *219*(19), 3137-3145.

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	RW	PNF			
Methods Development	PNF	VLM	MC	RW	
Data Collection	PNF	MC			

Data Analysis and Interpretation	PNF	MC	VLM	RW	WR
Manuscript Writing					
Writing of Introduction	PNF	RW	WR	VLM	MC
Writing of Materials & Methods	PNF	RW	WR	VLM	MC
Writing of Discussion	PNF	RW	WR	VLM	MC
Writing of First Draft	PNF				

Explanations (if applicable):

Publication (unpublished manuscript): Fleischmann, P. N., Rössler, W., Wehner, R. Early in foraging life: spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*.

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	PNF	RW	WR		
Methods Development	PNF				
Data Collection	PNF				
Data Analysis and Interpretation	PNF	RW	WR		
Manuscript Writing					
Writing of Introduction	PNF	RW	WR		
Writing of Materials & Methods	PNF	RW	WR		
Writing of Discussion	PNF	RW	WR		
Writing of First Draft	PNF				

Explanations (if applicable):

Publication (complete reference): Grob, R*, Fleischmann, P. N*, Grübel, K., Wehner, R., Rössler, W. The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11, 226.

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	PNF	WR	RG	RW	
Methods Development	RG	PNF	WR	KG	
Data Collection	RG	PNF	KG		
Data Analysis and Interpretation	RG*	PNF*	WR	RW	KG
Manuscript Writing					
Writing of Introduction	RG*	PNF*	WR	RW	
Writing of Materials & Methods	RG*	PNF*	WR	RW	
Writing of Discussion	RG*	PNF*	WR	RW	
Writing of First Draft	RG*	PNF*			

Explanations (if applicable):

*These authors have contributed equally to this work.

Publication (unpublished manuscript): Fleischmann, P. N*, Grob, R*, Müller, V. M., Wehner, R.†, Rössler, W.† The Geomagnetic Field as a Compass Cue in *Cataglyphis* Ant Navigation

Participated in	**Author Initials,** Responsibility decreasing from left to right				
Study Design	PNF*	RG*	WR†	RW†	VLM
Methods Development	PNF*	RG*	VLM	WR	
Data Collection	PNF*	RG*			

Data Analysis and Interpretation	PNF*	RG*	WR†	RW†	VLM
Manuscript Writing					
Writing of Introduction	PNF*	RG*	WR†	RW†	VLM
Writing of Materials & Methods	PNF*	RG*	VLM	WR†	RW†
Writing of Discussion	PNF*	RG*	WR†	RW†	VLM
Writing of First Draft	PNF*	RG*			

Explanations (if applicable):

* These authors have contributed equally to this work.

† These authors share a senior authorship.

The doctoral researcher confirms that she/he has obtained permission from both the publishers and the co-authors for legal second publication.

The doctoral researcher and the primary supervisor confirm the correctness of the above mentioned assessment.

Pauline Nikola Fleischmann

Doctoral Researcher's Name Date Place Signature

Wolfgang Rössler 15.01.2018 Würzburg

Primary Supervisor's Name Date Place Signature

Prof. Dr. Wolfgang Rössler

“Dissertation Based on Several Published Manuscripts“

Statement of individual author contributions to figures/tables/chapters included in the manuscripts

(If required please use more than one sheet)

Publication (unpublished manuscript): Fleischmann, P. N., Rössler, W., & Wehner, R. What is the earthbound reference system for the daily time compensation of the celestial compass in *Cataglyphis*?

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	PNF				
2	PNF				
3	PNF				
T1	PNF				

Explanations (if applicable):

Publication (complete reference): Fleischmann, P. N., Grob, R., Wehner, R., & Rössler, W. (2017). Species-specific differences in the fine structure of learning walk elements in *Cataglyphis* ants. *Journal of Experimental Biology*, *220*(13), 2426-2435

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	PNF	RG			
2	PNF	RG			
3	PNF	RG			
4	PNF	RG			
5	PNF	RG			
6	PNF	RG			

Explanations (if applicable):

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Figure	**Author Initials,** Responsibility decreasing from left to right				
1	PNF				
2	PNF	MC			
3	PNF	MC	VLM		
4	PNF	MC			
5	PNF	MC			
S1	PNF	MC			
S2	PNF	MC			
S3	PNF	MC			
S4	PNF	MC	VLM		
S5	PNF	MC	VLM		

Explanations (if applicable):

Publication (unpublished manuscript): Fleischmann, P. N., Rössler, W., Wehner, R. Early in foraging life: spatial and temporal aspects of landmark learning in the ant *Cataglyphis noda*.

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	PNF				
2	PNF				
3	PNF				
4	PNF				
5	PNF				
6	PNF				
7	PNF				
8	PNF				
T1	PNF				

Explanations (if applicable):

Publication (complete reference): Grob, R*, Fleischmann, P. N*, Grübel, K., Wehner, R., Rössler, W. The Role of Celestial Compass Information in *Cataglyphis* Ants during Learning Walks and for Neuroplasticity in the Central Complex and Mushroom Bodies. *Frontiers in Behavioral Neuroscience*, 11, 226.

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	RG*	PNF*			
2	RG*	PNF*			
3a, b	WR	KG			
3c	RG*	PNF*	WR		
4	RG*	PNF*			
5	RG*	PNF*			
6	RG*	PNF*			
T1	RG*	PNF*			

Explanations (if applicable):

* These authors have contributed equally to this work.

Publication (unpublished manuscript): Fleischmann, P. N*, Grob, R*, Müller, V. M., Wehner, R.†, Rössler, W.† The Geomagnetic Field as a Compass Cue in *Cataglyphis* Ant Navigation

Figure	**Author Initials,** Responsibility decreasing from left to right				
1	PNF*	RG*			
2	PNF*	RG*			
3	PNF*	RG*			

Explanations (if applicable):

* These authors have contributed equally to this work.

† These authors share a senior authorship.

I also confirm my primary supervisor’s acceptance.

Pauline Nikola Fleischmann		Würzburg	
Doctoral Researcher’s Name	Date	Place	Signature