Flexible use of multiple cues: Multimodal navigation in the desert ant *Cataglyphis*

Dissertation

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. Importance of being able to navigate efficiently

Being able to find the way efficiently through the world is a requirement for most animal species, including humans. In our everyday life we are faced with the challenge to orientate towards places of interest and thus a sophisticated sense of orientation is advantageous. When we return to a familiar place we are able to target the goal by having knowledge about the rough direction and distance we have to travel and, in addition, we are capable of learning an essential location by remembering some key features of the surrounding world. Initially, direction and distance information are primarily used, however, when we become familiar with the environment we start to benefit from environmental orientation cues. For instance, when I move towards my house I do not only have information about the rough direction and distance but also about additional environmental cues that are useful for successful orientation. The giant and unique tower in the city centre that is next to my house is useful to take as a visual landmark aiming for during homing. At closer range, the bakery and the emanating smell of coffee and fresh pastries next to my door is an additional orientation cue that guides me home accurately.

When we look a little bit further we have numerous even more fascinating examples of outstanding navigation skills in animals. For instance, homing pigeons find back to their home loft even when in unfamiliar terrain, procellariiform seabirds forage for food covering huge distances over the ocean and manage finally to return to their nesting site, and salmons return from the ocean to their natal river to spawn ((DeBose and Nevitt, 2008) and references therein).

As shown in these examples, it is obvious that sophisticated navigational skills are crucial when moving from A to B. For the survival, animals must have the capability to find back home or to a rewarding feeding place. They move across different scales and different navigational performances are used depending on lifestyle, habitat, sensory limitations and behavioural context.

1.2. What can we learn from insects?

Insects show an enormously rich repertoire of behaviour even though equipped with relatively limited neural hardware (see table 1 in (Huston and Jayaraman, 2011); for honey bees see (Srinivasan, 2010) and box 1 in (Chittka and Niven, 2009)). Their complex but often robust and stereotype behaviour can be studied at different levels for which reason insects are powerful research objects in neuroethology. The combination of behavioural studies with a range of techniques such as neuroanatomy or in vivo neurophysiology provides us with insights into the neural basis of sensori-motor integration (see e.g. (Comer and Robertson, 2001; Huston and Jayaraman, 2011)). In addition to recent methodological advances that provide information about neural circuits or even single neurons, studying insects in their natural environment tells us about the ecological relevance of the generated behaviour and is thus important for a holistic understanding.

Early studies already report on the insects' sophisticated spatial behaviour. Santschi showed a century ago for the first time the use of the sun as a compass cue in ants (Santschi, 1911), Tinbergen's famous digger wasp experiment revealed the use of landmarks for pinpointing the nest position (Tinbergen, 1951) and Von Frisch explored the well-known waggle dance in honeybees that is used to inform other bees of the location of a rewarding feeding site (von Frisch, 1967). When it comes to spatial cognition and navigation that is a well-defined and straightforward behaviour resulting in animals moving from A to B, social insects play a key role, in particular hymenopteran social insects such as honeybees or ants (Graham, 2010). Honeybees for instance travel several kilometres when visiting a flower and find back to their hive again (Pahl et al., 2011). The fact that such small insects housing only tiny brains have highly sophisticated navigational skills has impressed scientists from different fields like animal behaviour, cognitive psychology, neuroscience and robotics for decades and ants have proven to be a particular successful model in studying spatial behaviour (Figure 1).

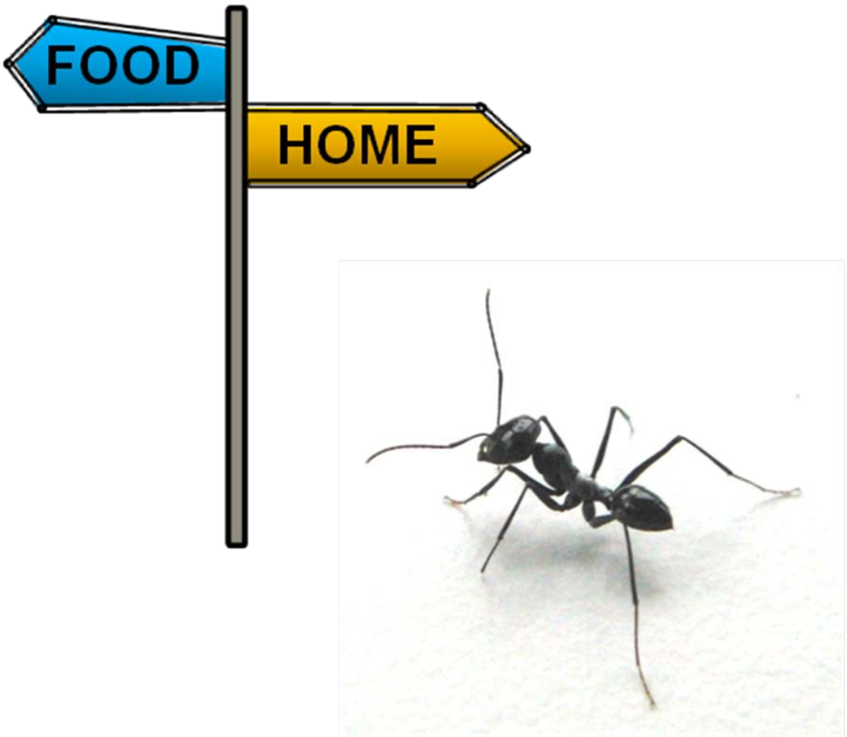

Figure 1: *Cataglyphis fortis* desert ant equipped with remarkable navigational skills.

Ants provide a unique tool for unraveling the mechanisms of navigation since their behaviour is easily studied under lab conditions but also in complex natural environments (see e.g. (Wystrach and Graham, 2012)). Sterile foragers of social insects forage for food and do little else during their lifetime, i.e. they are specialized for foraging and thus navigation. Ants, as social insects in general, have noteworthy learning skills (for honeybees see e.g. (Sandoz, 2011)) such that innate navigational mechanisms can be complemented by learnt cues. By combining different research fields such as behavioural studies (for reviews see e.g. (Cheng 2012; Cheng et al., 2009; Wehner, 2003; Wehner, 2008; Wehner, 2009; Wehner, 2012)), functional neuroanatomy (see e.g. (Stieb et al., 2011a)) and computational modeling (see e.g. (Baddeley et al., 2012; Baddeley et al., 2011; Garnier et al., 2013; Lambrinos et al., 2000)) we start to understand how spatial cognition and behaviour emerge from the interaction between brain, body and environment.

1.2.1. Why study *Cataglyphis* desert ants?

In the present thesis and the included manuscripts I to IV, two desert ant species from the genus *Cataglyphis* were investigated. While the Saharan salt pans, the natural habitat of *Cataglyphis fortis* (Figure 2, left), are only sparsely overgrown and often devoid of any vegetation, the coastal steppe-like environment in Turkey, that is the habitat of *Cataglyphis noda* (Figure 2, right), is characterized by arrays of grass tussocks and trees.

Figure 2: *Cataglyphis fortis* (left) and *Cataglyphis noda* (right) forager.

Despite their diverse ecological niches, *Cataglyphis* species share numerous features in common. As diurnal scavengers they have adapted to their hostile and harsh environment (Figure 3) by physiological, morphological and behavioural specializations.

Figure 3: Salt pan in Tunisia that is the natural habitat of the desert ant *Cataglyphis fortis* (left) and a *C. fortis* forager caught by a robber fly (right).

Thermophilic *Cataglyphis* ants reach their physiological thermal limit during foraging under extremely high temperatures with surface temperatures of over 50º Celsius measured at the surface (Wehner et al., 1992; Wehner and Wehner, 2011). Heat shock proteins that are still produced at rather high temperatures enable *Cataglyphis* ants to tolerate such high temperatures (Gehring and Wehner, 1995). A morphological adaptation of the heat tolerant desert ants are their long legs (Sommer and Wehner, 2012) as they allow fast running (i.e. reducing the time being exposed to high surface temperatures) with walking speeds up to 1 m/s (Wehner, 1983). When foraging for food, mainly dead arthropods succumbed to the heat, the ants travel huge distances ((Wehner, 1983; Wehner, 1987); for foraging behaviour see also manuscript IV). Contrary to many other ant species, *Cataglyphis* ants do not use pheromone trails for navigation. The unpredictable food distribution and the high surface temperature account for the fact that the ants do neither orientate along shared odour trails nor do they show recruitment behaviour (Ruano et al., 2000). They rather forage individually for food (Wehner, 1983).

Desert ants have become a model system for navigation with a focus on visually-guided navigation. Individually foraging ants can be studied throughout their lifetime as a forager within the entire spatial range of their natural environment (Wehner et al., 2004). They are central-place foragers and thus highly motivated to return to their home base when foraging by using innate navigational mechanisms but also by learning about key features from their environment (for an overview of the navigational mechanisms see 1.4.). Another strand of *Cataglyphis* research is the study of neural plasticity. For instance, the transition from interior workers to foragers navigating successfully through the desert comes along with volume changes and structural synaptic plasticity in the ants' mushroom bodies (Kuhn-Buhlmann and Wehner, 2006; Seid and Wehner, 2008; Stieb et al., 2011a; Stieb et al., 2010).

1.3. Sense of smell in insects: From sensory input to motor output

Insects have to deal with multiple sensory modalities, which provide them with information about essential things of the world. A sensory system that is vital for survival is olfaction. The sense of smell is important for finding food, mates or oviposition sites. Insects need to extract behaviourally relevant olfactory information from a complex chemical world, and transform and integrate the information such that a behaviourally adequate motor output is generated.

For a long time *Cataglyphis* ants were considered to be exclusively visually-guided navigators. Recent findings, however, reveal that the ants' excellent sense of smell is also part of their navigational toolkit (Steck, 2012). Since I focus on odour-mediated orientation and foraging, this section of my thesis aims at giving a brief overview of olfaction from perception to behaviour with a focus on olfactory-guided orientation and navigation in insects, and ants in particular (for reviews of insect olfaction see e.g. (Dacks et al., 2009; Hansson and Stensmyr, 2011; Martin et al., 2011; Sachse and Krieger, 2011; Vosshall, 2000)) .

1.3.1. Perception and processing of olfactory information

Insects detect volatile chemical compounds with their head appendages (antenna and palps) that differ greatly in shape but share the same function among insects (Schneider, 1964). Olfactory sensilla covering the distal part of the antenna house the dendrites of olfactory sensory neurons (OSNs) (Zacharuk, 1980). After having passed the porous wall of the sensilla, the usually hydrophobic odour molecules are supposed to be transported by odour-binding proteins (OBPs) through the sensillum lymph to the receptor binding sites (Vogt and Riddiford, 1981). Each OSN expresses one olfactory receptor (OR) along with the co-receptor Orco (Larsson et al., 2004; Vosshall and Hansson, 2011). Metabotropic odorant receptors (Buck and Axel, 1991) have a unique topology in insects (Benton et al., 2006) and characteristic affinities to odour molecules. The selectivity ranges from receptors highly specific to single odorants to broadly tuned ones (see e.g. (Carey et al., 2010; Hallem and Carlson, 2006; Stensmyr et al., 2012; Stensmyr et al., 2003; Todd and Baker, 1993)). However, in general the olfactory system is tuned to ecological relevant chemical compounds (Linz et al., 2013). The number of odorant receptors differs greatly among insects. Hymenoptera have with up to 200-400 olfactory receptors (Robertson et al., 2010; Wurm et al., 2011; Zhou et al., 2012) many more ORs than for instance the vinegar fly *Drosophila melanogaster* has (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999). The high number in ants (Wurm et al., 2011; Zhou et al., 2012) highlights the importance of olfaction in the ants' complex social system including highly sophisticated chemical communication and orientation (Hoelldobler and Wilson, 1990). Furthermore, insects also have ionotropic receptors (IRs) forming ion channels (Benton et al., 2009). OSNs expressing the same receptor type and thus responding to the same odour molecules typically converge in a single glomerulus within the antennal lobe (AL) that is the first odour-processing centre in the insect brain (see Figure 4; (Gao et al., 2000; Vosshall et al., 2000)). In the case of social Hymenoptera, the axons of the OSNs form sensory tracts that innervate different clusters of glomeruli (Kelber et al., 2010; Kirschner et al., 2006; Stieb et al., 2011b; Zube et al., 2008). In the AL, odour information is coded by characteristic spatiotemporal activity patterns of glomeruli in a combinatorial manner (Galizia and Menzel, 2001; Hallem and Carlson, 2006; Knaden et al., 2012; Sachse et al., 1999). A network of local interneurons (LNs) shapes the output from the AL (Wilson, 2008) that goes via projection neurons (PNs) further to higher brain centres like the lateral horn (LH) and the mushroom bodies (MB) (Figure 4; (Distler and Boeckh, 1996; Hildebrand and Shepherd, 1997; Tolbert and Hildebrand, 1981)). In the higher brain centre further processing and integration with other sensory modalities take place (Gronenberg, 1999; Gronenberg, 2001; Gronenberg and Holldobler, 1999; Gronenberg and Lopez-Riquelme, 2004). The lateral horn is involved in innate behaviour (Datta et al., 2008; Luo et al., 2010; Tanaka et al., 2004) whereas the mushroom bodies are needed for learning and memory (Heisenberg, 2003; Menzel et al., 1996; Menzel and Muller, 1996; Zars, 2000; Zars et al., 2000).

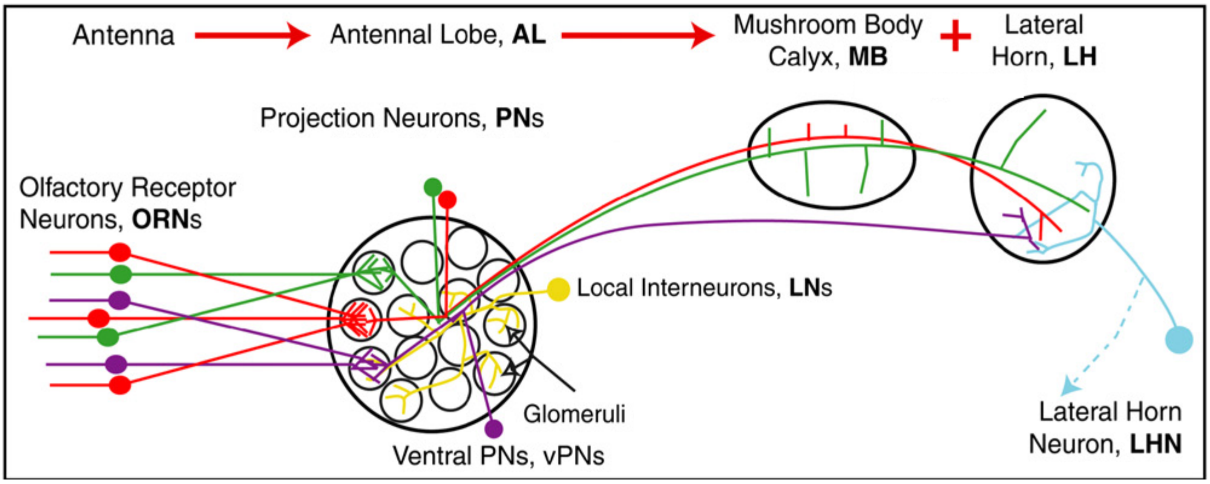

Figure 4: Olfactory system in *Drosophila melanogaster* (modified from (Jefferis et al., 2007)).

1.3.2. Odour-mediated behaviour

The processing of sensory input eventually results in motor output, i.e. perception and processing of olfactory information lead to an odour-mediated behaviour.

1.3.2.1. The insects' chemical world

Insects exhibit innate responses to many odours that are biologically important (see e.g. (Martin and Hildebrand, 2010)). A well-studied class of chemical signals are pheromones that are used for intraspecific communication (Wyatt, 2003). Sex pheromones e.g. allow insects to localize a mating partner, whereas various ant species travel between the nest and a plentiful feeding site by following a pheromone trail deposited by foragers shuttling between the nest and the food source. Social insects have a highly sophisticated chemical communication system including sexual communication, nest mate recognition, caste discrimination, alarm and recruitment behaviour and trail pheromone system (Hoelldobler and Wilson, 1990).

Apart from pheromones emitted by conspecifics, volatiles produced by various objects in the environment are also available as cues, e.g. volatiles emitted by a host plant attract insects for oviposition while the smell of a flower or a feeding site guides them to a food source (for hawkmoths see e.g. (Riffell, 2012)). Moreover, environmental odours available in the insects' habitat can be used as landmarks for navigation (Steck et al., 2009). In the present thesis, I investigate the navigation and orientation behaviour of ants responding to this kind of odour information, i.e. environmental odours either innately attractive or learnt. We have learnt that *Cataglyphis* ants are able to learn and use olfactory information when homing (Steck et al., 2009). However, the role of olfactory information during natural foraging and homing and the interplay with the basal mechanisms of path integration is not known in desert ants (see manuscripts II to IV).

Typically, insects do not perceive single odours but behavioural relevant stimuli are mostly mixtures of volatiles within a chemical complex world (see e.g. (Riffell, 2012; Riffell et al., 2009a)). Although insects usually receive olfactory information as complex blends, they also respond to single odours. Numerous odours generate an innate behaviour, i.e. they either elicit attraction or repulsion (Dekker et al., 2006; Knaden et al., 2012; Stensmyr et al., 2012; Stensmyr et al., 2003).

Moreover, apart from the innate odour-mediated behaviour insects also show learnt behaviours. For example, hawkmoths learn to feed from certain flowers by olfactory conditioning (Riffell et al., 2008b; Riffell et al., 2013) or honeybees learn odours from feeding sites and are able to return to them by using the learnt olfactory information (Reinhard et al., 2004). A well-known experiment for odour learning in honeybees is the proboscis extension reflex learning paradigm (PER) in which bees learn to associate an odour with sucrose solution as reward (Bitterman et al., 1983). Likewise, in a few studies with ants it is reported that they are capable to learn associations between olfactory information and a food stimulus by classical conditioning (Dupuy et al., 2006; Guerrieri and d'Ettorre, 2010; Roces, 1990; Roces, 1994). Desert ants, furthermore, are capable to learn innately meaningless odours as nest landmarks in order to find back home ((Steck et al., 2009); see also 1.4.3. and manuscript I). In the case of the olfactory landmarks used by desert ants, innately meaningless odour information has to fulfil the condition that it is reliable and robust, i.e. the olfactory cues must be stable in time and space such that it is worth to learn them as a place-specific landmark.

1.3.2.2. Olfaction used for navigation: How do insects use odour plumes?

A simple form of olfactory-driven behaviour is chemotaxis, which is a directed movement along an odour gradient (Gomez-Marin et al., 2010; Gunn and Fraenkel, 1961). Tethered *Drosophila* flies for example tend to turn towards the antenna that is exposed to a higher odour concentration (Borst and Heisenberg, 1982). In nature, however, odour dispersion depends on various factors such as wind condition, habitat structure or size of the odour source (Carde and Willis, 2008; Riffell et al., 2008a). Volatile chemical compounds emitted by an odour source are usually dispersed, mixed, and diluted by air movements and form a filamentous odour plume (Murlis et al., 1992; Murlis et al., 2000; Riffell et al., 2008a). Odour plumes are complex patchy distributions of spatiotemporal fluctuating odour packages interspersed with clean air (Figure 5).

Figure 5: Odour plume visualised with smoke at our field site in Tunisia.

The fine-scale structure of the odour plume is crucial for the resultant behaviour (Mafra-Neto and Carde, 1994). In flying (Kennedy et al., 1980; Kennedy et al., 1981; Vickers and Baker, 1994; Willis and Baker, 1984) and walking moths (Kramer, 1992) intermittent rather than homogenous odour information elicit plume following, whereas *Drosophila* flies fly upwind within both kind of plumes (Budick and Dickinson, 2006).

One line of research is the study of insects orienting towards an odour source by moving upwind along the odour plume. Most of the research how insects use odour plumes has been done with flying insects (see below). When it comes to walking insects and how they discover and use odour plumes only little is known (for cockroaches see (Willis and Avondet, 2005); for desert ants see (Wolf and Wehner, 2000) and manuscripts II to IV). A common strategy in flying insects is to follow the odour plume upwind to the source when approaching resources (Budick and Dickinson, 2006; Carde, 1996; Carde and Willis, 2008; Chow and Frye, 2009; Murlis et al., 1992). Changing wind or the habitat structure may cause the odour plume to meander such that a straight upwind movement does not always guide the insect eventually to the odour source. In flying moths, optomotor anemotaxis enables them to determine upwind heading (Baker et al., 1984; Kennedy and Marsh, 1974), however, since simply heading straight upwind may guide the insects occasionally out of the plume moths complement this strategy with casting behaviour (Kennedy, 1983; Kuenen and Carde, 1994) resulting in upwind zigzag flights (Willis and Arbas, 1991). For flying insects a visual feedback is used for stabilizing an upwind flight (Budick et al., 2007; Reiser et al., 2004), i.e. optic flow provides information about flight speed (Carde and Willis, 2008). Plume tracking is enhanced in the presence of visual cues (Fadamiro et al., 1998; Frye et al., 2003), where the crossmodal interaction works because attractive odours enhance the gain of optomotor responses during flight (Chow and Frye, 2008) and it is therefore easier to track a spatial odour gradient through more precise flight (Duistermars and Frye, 2010).

Insects not only need to be able to follow an odour plume up to the source but must initially find the plume by screening the environment for attractive odours emanating from resources of interest. While we have gained an advanced understanding of odour plumes and how flying insects use them, less is known about the discovery of odour plumes. Different models predict optimal courses considering wind conditions resulting in animals moving preferrerably crosswind, upwind or downwind (Dusenbery, 1989; Dusenbery, 1990; Sabelis and Schippers, 1984). However, behavioural data is rare.

In contrast to vinegar flies and hawkmoths that orientate towards attractive odour sources, ants perform sophisticated navigation. Although desert ants do not follow pheromone trials olfactory information is used for navigation. The navigational toolkit of the desert ants including odour-mediated navigation is summarized in 1.4. (see also manuscripts).

1.4. Current understanding of ant navigation

For a long time, desert ants were considered to navigate exclusively by visual guidance (for reviews of desert ant navigation see e.g. (Cheng 2012; Cheng et al., 2009; Graham, 2010; Wehner, 2008; Wehner, 2009)). However, ants have to deal with multiple sources of information, i.e. they interact with their environment using various sensory modalities. Desert ants primarily rely on path integration, an innate navigational mechanism that continuously informs the ants about their position relative to the nest (Collett and Collett, 2000). As they become experienced they in addition rely on learnt information from visual cues (Zeil, 2012). Recent findings reveal that the ants' navigational toolkit is even more diverse and that they use a large diversity of information sources for navigation (tactile information, (Seidl and Wehner, 2006); thermal information, (Kleineidam et al., 2007); magnetic and vibrational information, manuscript I). Furthermore, we have recently learnt that the desert ants' excellent sense of smell is also part of their navigational repertoire ((Steck, 2012) and references therein; see also manuscripts from this thesis). Hence, the ants' navigational system is extremely diverse and flexible and functions rather in a multimodal than unimodal fashion. For example, homing ants benefit from combining visual and olfactory cues, i.e. ants learn a multimodal landmark much faster than a unimodal one (Steck et al., 2011). Despite being equipped with all these sophisticated navigation skills, ants occasionally miss the target. Therefore, the navigational mechanisms are complemented by a systematic search behaviour which allows the ants to find the nest when the entrance is missed (Wehner and Srinivasan, 1981).

In this part I will give a brief overview over the ants' navigational mechanisms focusing on path integration, landmark guidance (visual and olfactory) and systematic search. For further information see also the manuscripts included in this thesis.

1.4.1. Path integration

Desert ants continuously perform path integration and are thus always informed about their position relative to the nest, i.e. they are capable to return home along the beeline at every point of their often rather circuitous journey (Figure 6). Path integration takes into account the ants' walking distances and directions, integrate this information, and provides the ants with a vector that is pointing back to the starting point (Collett and Collett, 2000; Muller and Wehner, 1988; Wehner and Srinivasan, 2003). The directional information is derived from a celestial compass, mainly from the polarized light pattern in the sky (Wehner and Muller, 2006). A step integrator provides the ants with information about the distance travelled (Wittlinger et al., 2006; Wittlinger et al., 2007).

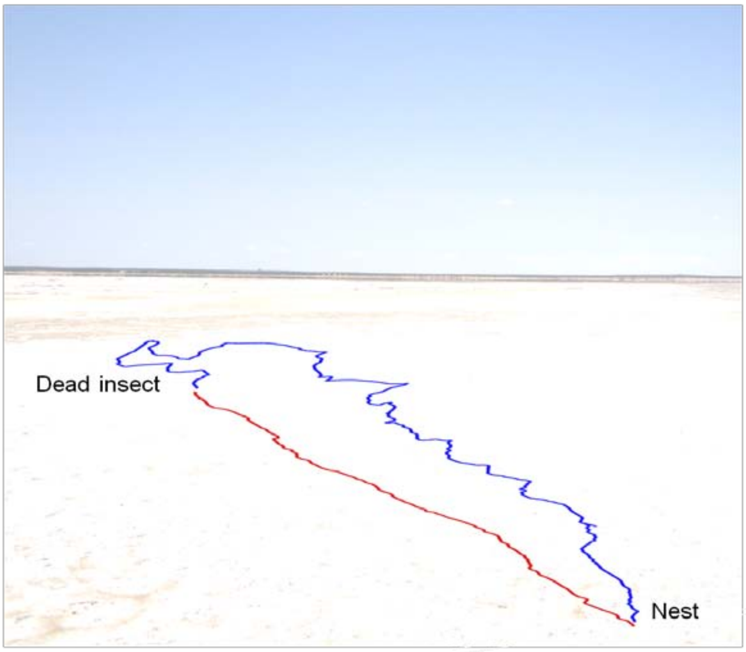

Figure 6: Path integration: Foodwards (blue) and homewards (red) path of a *C. fortis* forager.

Path integration is essential on early foraging trips and in unfamiliar terrain but is prone to cumulative errors such that the ants may not arrive exactly at the target they are aiming for (Merkle et al., 2006; Sommer and Wehner, 2004). Therefore ants complement this strategy by learning reliable environmental cues at key locations and/or by performing a systematic search strategy (see below). However, even when using other navigation mechanisms path integration keeps running as a backup system (Andel and Wehner, 2004; Knaden and Wehner, 2005).

1.4.2. Visually-guided navigation

Ants learn and use visual cues in order to locate a goal (Knaden and Wehner, 2005; Wehner and Raeber, 1979) or follow a route (Kohler and Wehner, 2005; Mangan and Webb, 2012). When learning and using key features from the world, ants need to extract reliable and robust information from the complex environment, form stable memories and recall them when needed. By matching currently perceived views with the ones stored at the goal location or when pointing in the correct direction, ants are able to move towards a target using familiar visual cues (Durier et al., 2003; Graham et al., 2004). Ants that inhabit environments that are rich in visual cues have to extract reliable and robust information from a complex scene. It seems that wide-field panoramic views provide reliable visual information for orientation (Graham and Cheng, 2009a; Graham and Cheng, 2009b). Hence, the entire familiar view rather than recognition of single objects is crucial for visual navigation in ants with low-resolution vision (Wystrach et al., 2011).

1.4.3. Olfactory-guided navigation

Cataglyphis ants are able to learn and use environmental odours presented at the nest entrance as landmarks when homing, i.e. the inconspicuous nest entrance is pinpointed with a higher accuracy in the presence of the learnt olfactory cue than without odour or in the presence of a non-training odour (Steck et al., 2009). Although tested ants do not show the same search accuracy when the learnt odour is presented against an olfactory background, the ants still recognize and use a learnt odour within the odour blend (Steck et al., 2009). This, apart from the capability to distinguish different odours, is crucial for landmark navigation since the chemical background of the ants' habitat may change over time and may not be as robust and reliable as the olfactory landmark itself. Moreover, ants are even able to locate the nest entrance within an olfactory scene composed of multiple odours each with individual spatial relationships to the nest (Steck et al., 2010). Hence, in addition to the ability to orient by means of visual cues, *C. fortis* foragers show remarkable olfactory-driven navigation. Interestingly, visual and olfactory cues are learnt equally fast (Steck et al., 2011).

1.4.4. Systematic search behaviour

Searching for the nest may become necessary when the other navigational mechanisms like path integration and landmark guidance are not available or not sufficient to bring the ant back into the nest. In such situations, ants engage in a systematic search (Muller and Wehner, 1994; Schultheiss and Cheng, 2011; Wehner and Srinivasan, 1981). The search pattern consists of searching loops with increasing size centred on the starting point of the search, the point where the nest is supposed to be. The uncertainty about the nest position determines the spread of the search (Merkle et al., 2006). Uncertainty depends on the foraging distance (Merkle and Wehner, 2010) and the visual complexity of the habitat (Schultheiss et al., 2013).

2. AIMS AND OBJECTIVES OF THE THESIS

Being equipped with a sophisticated navigational toolkit is of great importance for *Cataglyphis* desert ants that inhabit harsh and food-impoverished environments. Ants readily learn and use a multitude of sensory cues for finding their way through the desert. In the first manuscript I investigate how ants learn and use multiple cues for locating the nest entrance. I deal with the question which sensory modalities can be part of the navigational toolkit. While most investigations on desert ant navigation have focused on path integration and visually-guided navigation (Graham, 2010), we have only recently learnt that the sense of smell is also part of the ants' navigational toolkit (Steck, 2012). Together with these new findings questions about how ants might use odour information derived from the environment for locating resources of interest have started to become of particular interest. In the manuscripts II and IV, I tackle the question if ants, either searching for food or heading back home, orientate by using odour information derived from the environment. How do ants use volatiles for locating resources of interests such as food and the nest? Finally, when we consider that ants make use of multiple cues for navigation the question also arises how ants process input of multiple sensory modalities. For generating adequate behaviour output, ants need to weigh multiple cues flexibly. Manuscript II and III deal with this issue and describe how ants deal with vector and olfactory information depending on whether they are on the inbound or outbound journey of their foraging path.

3. OVERVIEW OF MANUSCRIPTS

3.1. Manuscript I

Desert Ants Learn Vibration and Magnetic Landmarks

Cornelia Buehlmann, Bill S. Hansson & Markus Knaden

PLoS ONE, 2012, 7(3): e33117

In the first manuscript we describe the flexibility of the ants' navigational system. On the way back home, desert ants are able to locate the nest entrance by using magnetic and vibrational landmarks. *Cataglyphis noda* ants are able to use cues for navigation that are usually sensed in a different context. Since the magnetic field usually might provide rather directional than positional information, while vibration sensing so far has been shown to be involved in social behaviour, any existence of magnetic or vibration nest landmarks is unlikely. However, it seems that *Cataglyphis* ants that have adapted to extremely harsh habitats use sensory input from whatever modality is available in order to navigate efficiently.

Built on an idea conceived by all three authors.
Experimental design: CB (50%), MK (50%)
Performance of experiments: CB (100%)
Data analyses: CB (95%), MK (5%)
Manuscript writing: CB (50%), MK (30%), BSH (20%)

3.2. Manuscript II

Path Integration Controls Nest-Plume Following in Desert Ants

Cornelia Buehlmann, Bill S. Hansson & Markus Knaden

Current Biology, 2012, 22(7): 645-649

The experiments of manuscript II reveal that homing *Cataglyphis* ants pinpoint the nest entrance by moving upwind along the odour plume emanating from the nest. However, they only respond to the plume when the path integrator indicates that they are close to home. This is important insofar as ants do not discriminate between the plume of their own and a foreign nest but pass neighbouring colonies during homing. We identified CO_2 as one component of the nest plume that alone is sufficient to provoke plume following when available in an adequate concentration. Therefore, although the cues that induce plume following are not nest specific, path integration might assure that homing *C. fortis* ants do not by chance follow the wrong nest plume and become killed within a foreign nest.

Built on an idea conceived by all three authors.
Experimental design: CB (50%), MK (50%)
Performance of experiments: CB (85%), MK (15%)
Data analyses: CB (80%), MK (20%)
Manuscript writing: CB (50%), MK (30%), BSH (20%)

3.3. Manuscript III

Flexible weighing of olfactory and vector information in the desert ant *Cataglyphis fortis*

Cornelia Buehlmann, Bill S. Hansson & Markus Knaden

Biology Letters, 2013, 9: 20130070

In the third manuscript we show that the weighing of path integration and plume following is context dependent. While homing ants only respond to nest odours when they are close to home, these results show that foraging ants mostly respond to food odours. Although foraging ants aiming for food are equipped with path-integration information that guides them back to a learnt feeding site, they respond to food odours also when being still far away from that feeder position. Hence, we report here that *C. fortis* ants that rely strongly on path integration retained the needed flexibility.

Built on an idea conceived by all three authors.
Experimental design: CB (50%), MK (50%)
Performance of experiments: CB (100%)
Data analyses: CB (100%)
Manuscript writing: CB (50%), MK (30%), BSH (20%)

3.4. Manuscript IV

Screening the desert for food: Olfactory-driven foraging strategies in desert ants

Cornelia Buehlmann, Bill S. Hansson & Markus Knaden

In preparation for Science

In manuscript IV we present evidence that desert ants are extremely efficient in localizing even tiny food items far away from their nests. This efficiency is accomplished by a combination of high sensitivity towards a key odorant (linoleic acid) emitted by dead arthropods and far reaching crosswind paths during which large areas of the desert are screened for food-derived odour plumes.

Built on an idea conceived by all three authors.
Experimental design: CB (35%), MK (30%), BSH (35%)
Performance of experiments: CB (70%), MK (30%)
Data analyses: CB (85%), MK (15%)
Manuscript writing: CB (60%), MK (20%), BSH (20%)

4. MANUSCRIPT I – DESERT ANTS LEARN VIBRATION AND MAGNETIC LANDMARKS

Ant picture taken by Elisa Badeke

OPEN ACCESS Freely available online

PLoS one

Desert Ants Learn Vibration and Magnetic Landmarks

Cornelia Buehlmann, Bill S. Hansson[9], **Markus Knaden***[9]

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Abstract

The desert ants *Cataglyphis* navigate not only by path integration but also by using visual and olfactory landmarks to pinpoint the nest entrance. Here we show that *Cataglyphis noda* can additionally use magnetic and vibrational landmarks as nest-defining cues. The magnetic field may typically provide directional rather than positional information, and vibrational signals so far have been shown to be involved in social behavior. Thus it remains questionable if magnetic and vibration landmarks are usually provided by the ants' habitat as nest-defining cues. However, our results point to the flexibility of the ants' navigational system, which even makes use of cues that are probably most often sensed in a different context.

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Introduction

Ants are equipped with sophisticated navigational skills (for reviews see [1,2,3,4]). Multiple orientation cues are available in the ants' environment that can be used to return to the nest. The individually foraging desert ants of the genus *Cataglyphis* perform path integration during foraging that takes into account the ants' walking distances and directions and continuously provides the ants with a home vector that points back to the nest entrance, a tiny hole in the desert ground [5,6]. Compass information (mainly based on polarized skylight [7], but also on the position of the sun and even on wind direction [8]) provides the ants with directional information while a step integrator informs them about the distances covered [9]. Since path integration is error prone [10,11], *Cataglyphis* ants also use visual [12,13], olfactory [14,15,16] and tactile landmarks [17] to pinpoint their nest. In studies with other ant species gravity [18] and the earth's magnetic field [19,20] have been reported to provide directional information. Thermal radiation has been shown to be perceived and used in leaf-cutting ants for relocation of brood and fungus [21,22] while vibrational signals are used for communication about food sources or buried nest mates [23,24].

Taken together, ants have access to a large variety of potential cues. In the present account we provide evidence that *Cataglyphis* ants use this diversity of information sources for navigation. Although probably neither magnetic nor vibrational landmarks are provided by the ants' habitat as nest-defining cues, *C. noda* foragers were able to associate a magnetic landmark and a local vibration with the nest entrance.

Results and Discussion

We trained and tested ants in a channel with either a magnetic, vibrational, visual, or olfactory nest-defining landmark (Figure 1) and compared the nest-search performances of these ants with those of ants that either were trained and tested without landmark (control ants) or naïve ants that experienced the landmark in the test situation for the first time. To investigate whether the ants relied on landmarks or on path-integration, we established a conflict between these two sources of information (see Figure 1B and also Material and Methods). Control ants (training and test without landmark) searched near the nest position defined by the path integrator (Figures 2 and 3). The same was true for naïve ants that experienced the landmark in the test channel for the first time (Figures 2 and 3), indicating that the landmarks were not innately attractive to the ants. However, ants that were trained with a landmark as a nest-defining cue and later tested with this cue focused their search at the landmark (Figures 2 and 3). Hence, our results suggest that *C. noda* foragers were able to learn and use all provided cues – be they magnetic, vibrational, visual or olfactory information – in order to locate the nest position.

It is well known that ants orientate by using visual and olfactory cues (see above), but debate continues on whether and how they use the magnetic sense for orientation. In studies dealing with the magnetic sense of ants a change of the magnetic field's polarity resulted in disturbed homing behavior [19,20]. However, navigation by using the magnetic field as a compass does not seem to be the primary mechanism in ant navigation [25]. Our data suggest that apart from using magnetic cues for compass information *Cataglyphis* ants can learn and use a magnetic landmark as a nest-defining landmark. The use of positional information derived from local anomalies of the earth's magnetic field has been shown also for other animals, e.g. for sea turtles, birds, and spiny lobsters [26,27]. Furthermore bees can be trained to visit a feeder that is equipped with a changed magnetic field [28]. However, it remains questionable whether any natural magnetic anomalies exist that on a scale of a few meters could help ants to localize their nest entrance. Furthermore our finding does

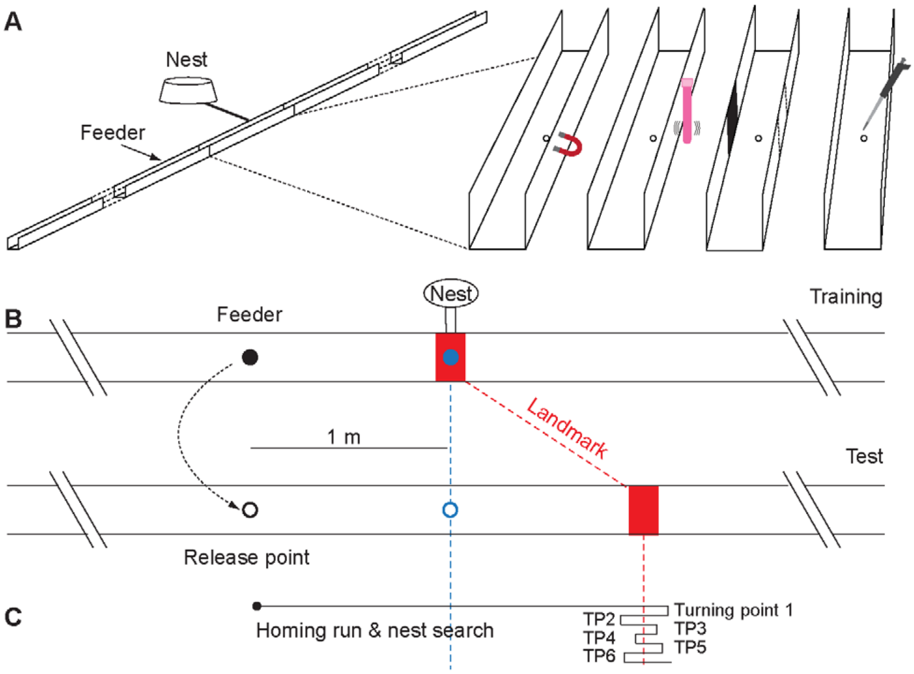

Figure 1. Experimental procedure. (A) The ants' nest was connected with a tube to the training channel where the ants were trained to visit a feeder 1 m away from the nest entrance that was marked with either a magnetic, vibrational, visual, olfactory or no landmark. For size and shape of the solenoid, and for the application of the massaging rod next to the channel see Material and Methods. (B) Trained ants were displaced from the feeder of the training channel into the parallel test channel (displacement shown by dashed arrow) where the homing runs and nest searches of the tested ants were tracked and recorded. Blue filled circle, nest entrance; black filled circle, feeder; black empty circle, release point; blue empty circle, fictive nest position, red rectangle, landmark; blue dashed line, nest position as defined by path integration, red dashed line, nest position as defined by landmark. Nest-to-feeder distance, 1 m; landmark was 1 m behind fictive nest position in test channel. (C) Exemplar homing run and nest search. We analyzed the first six turning points (TP1–TP6) after the ants had crossed the nest-defining cue for the first time.
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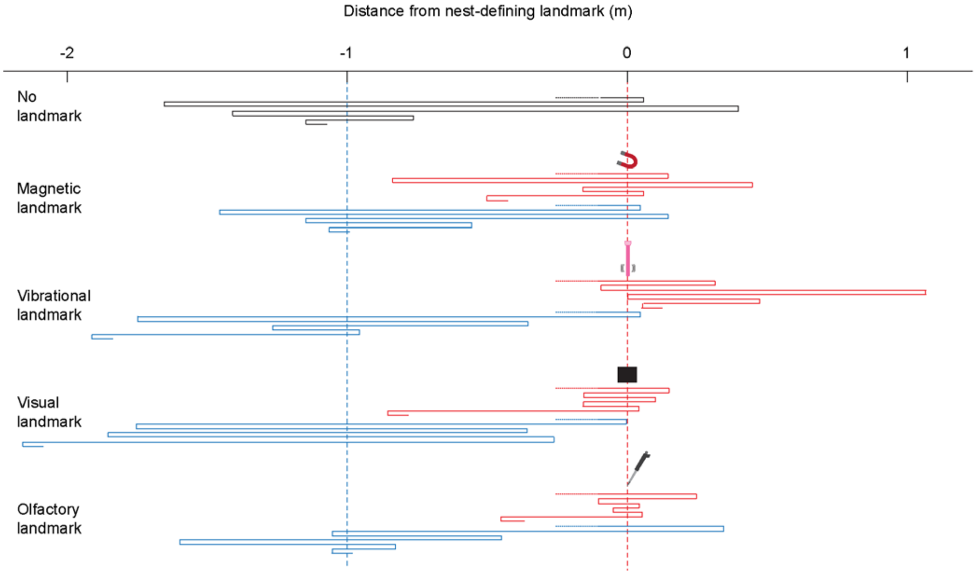

Figure 2. Individual test runs of homing ants. Schematic nest searches of ants trained and tested with a nest-defining landmark that was either a magnetic, vibrational, visual or olfactory cue (red), control ants trained and tested without landmark (black) or naïve ants that experienced the landmark in the test for the first time (blue). Blue dashed line, nest position as defined by path integration; red dashed line, nest position as defined by landmark; point of release for each homing run at position -2 m from nest-defining cue. The first six turning points after the ants had passed the landmark for the first time were analyzed for their median position.
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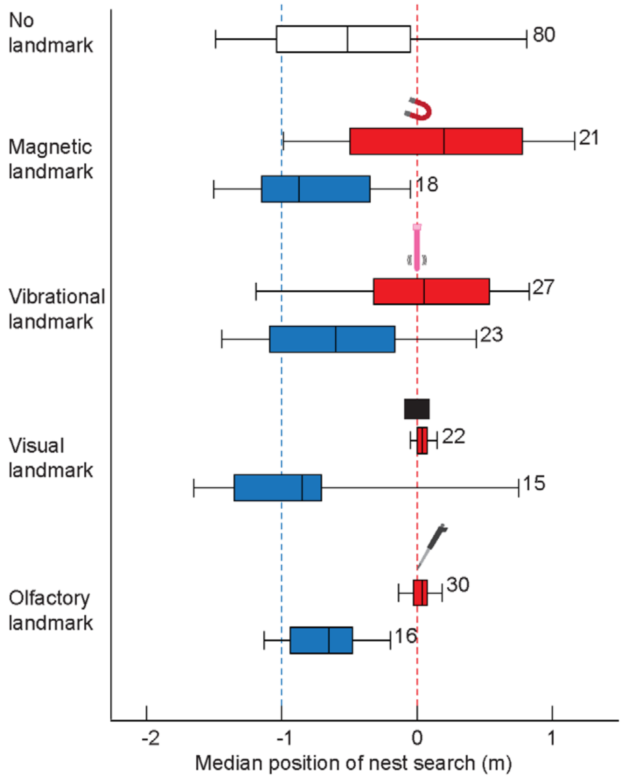

Figure 3. Ants learn magnetic, vibrational, visual and olfactory landmarks. Box plot representation of the medians of the first six turning points of ants that were trained and tested with a landmark (red boxes), control ants trained and tested without a landmark (white box), and naïve ants that experienced the landmark during the test for the first time (blue boxes). Blue dashed line, nest position as defined by path integration; red dashed line, nest position as defined by landmark. Box plots show median, interquartile range and whiskers indicating the 90th and 10th percentiles. Kruskal-Wallis test and Dunn's multiple comparison tests were performed for selected pairs: control (training and test without landmark) versus naïve ants (landmark only during test), for each landmark type $P>0.05$; ants trained and tested with the landmark versus naïve ants, for each landmark type $P<0.05$; ants trained and tested with the landmark versus control ants, for each landmark type $P<0.05$. Numbers depict sample sizes.
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not necessarily suggest any specialized magnetic-sensitive organ in *Cataglyphis*, as the strong change of the magnetic field induced by the solenoids (see Material and Methods) could potentially have led to an unspecific change of neuronal activity that later was associated with the nest entrance. While the existence of a magnetic sense in ants is still under discussion, the use of vibrational signals is well investigated. Ants are very sensitive to vibration [23,24]. However, our finding that *Cataglyphis* learns vibrational nest-defining landmarks is surprising. Buried leaf-cutting ants call for help via vibrations that are sensed by nest mates through several centimeters of nest material [24]; hence, it is within the realm of possibility that *Cataglyphis* foragers can sense the whole community below the nest entrance. However, it remains an open question if vibrational landmarks exist and are used by these ants in their natural habitat as nest-defining cues.

Our findings highlight the flexibility of the ants' navigation system. Not only can they associate visual and olfactory cues with the nest entrance, but they can apparently also learn magnetic and vibrational cues that are typically sensed in a completely different context.

Materials and Methods

Field Site and Ant Species

The experiments with the desert ants *Cataglyphis noda* (Brullé, 1832) were performed between mid June and July 2011 in the ants' natural habitat. The field site was located in Çirali, Turkey (36°25′N, 30°29′E). No specific permits were required for the described field studies.

Experimental Procedure

Training Procedure. The ants' nest was covered with a bucket and connected to a U-shaped linear channel (cross section, total length: 19.5 m, width: 7 cm, height: 7 cm) so that the ants could enter the training channel by a tiny hole in the channel floor (Figure 1A). We trained *C. noda* foragers in this channel to visit a feeder 1 m upwind of the nest entrance that was marked with one of the following nest-defining cues (Figure 1A).

Magnetic landmark: As a magnetic landmark we used two solenoids (circular nickel-coated neodymium magnets, 5 mm in diameter and 10 mm high, volume: 393 mm^3, NdFeB magnet in N45) placed adjacent to the nest entrance on the outer walls of the aluminum channel. They caused a 180° reversal in the polarity and an increase in the intensity of the magnetic field (maximal intensity of 21000000 nT measured within the channel, compared to the earth's magnetic field strength of 41000 nT).

Vibrational landmark: We put a massaging rod into the ground outside of the channel next to the position of the nest entrance. Apart from the vibrations within the channel we could not measure any changes in the magnetic field in the presence of the rod. In order to exclude that the ants learned minor magnetic effects rather than vibrational effects of the rod, we in addition trained and tested ants in the presence of a vibrating rod that was placed close to the nest but had no contact to the ground (i.e. did not generate vibrations). The nest-search performances of the tested ants did not differ from those of control ants that were trained and tested without the rod (Mann-Whitney test, $P>0.05$, data not shown).

Visual landmark: Two pieces of black cardboard (each 10 cm×7 cm) that were placed adjacent to the nest entrance on the inner walls of the channel were used as a visual landmark.

Olfactory landmark: As an olfactory landmark we dropped dilute methyl salicylate (1:50 in hexane) directly at the nest entrance on the channel floor (see also [14]). Due to evaporation we renewed the olfactory landmark every 15 min.

Test Procedure. Trained ants were captured at the feeder and together with a food crumb were released into an aligned test channel so that they were still equipped with the path-integration vector that guided them to the fictive nest position (Figure 1B). The nest-defining cue that was presented in the training channel was placed 1 m behind the nest position as defined by path integration. The conflict between path integration and landmark information allowed us to investigate whether the ants were relying on landmark information or on path-integration information. When an ant does not reach the nest entrance after it has run off its path-integration vector it starts a systematic nest search [29,30]. Within the linear test channel this systematic nest search is reduced to one dimension and is characterized by the turning points [31]. We tracked and recorded the turning points (TP) of the homing ants by aligning a measuring tape along the channel outer wall.

Analyses and Statistics

The first six turning points after the ants had crossed the nest-defining cue for the first time were analyzed for their median position (Figure 1C). Analyses had to be restricted to the first six turning points, because many tested ants managed to leave the channel afterwards. The non-parametric Kruskal-Wallis test with Dunn's multiple comparison tests and the Mann-Whitney test were performed throughout the analyses with the statistic software GraphPad Instat (version 3.06). We only analyzed ants that took a food crumb and crossed the landmark position within the first 11 turning points.

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Author Contributions

Conceived and designed the experiments: CB MK BSH. Performed the experiments: CB. Analyzed the data: CB MK. Wrote the paper: CB MK BSH.

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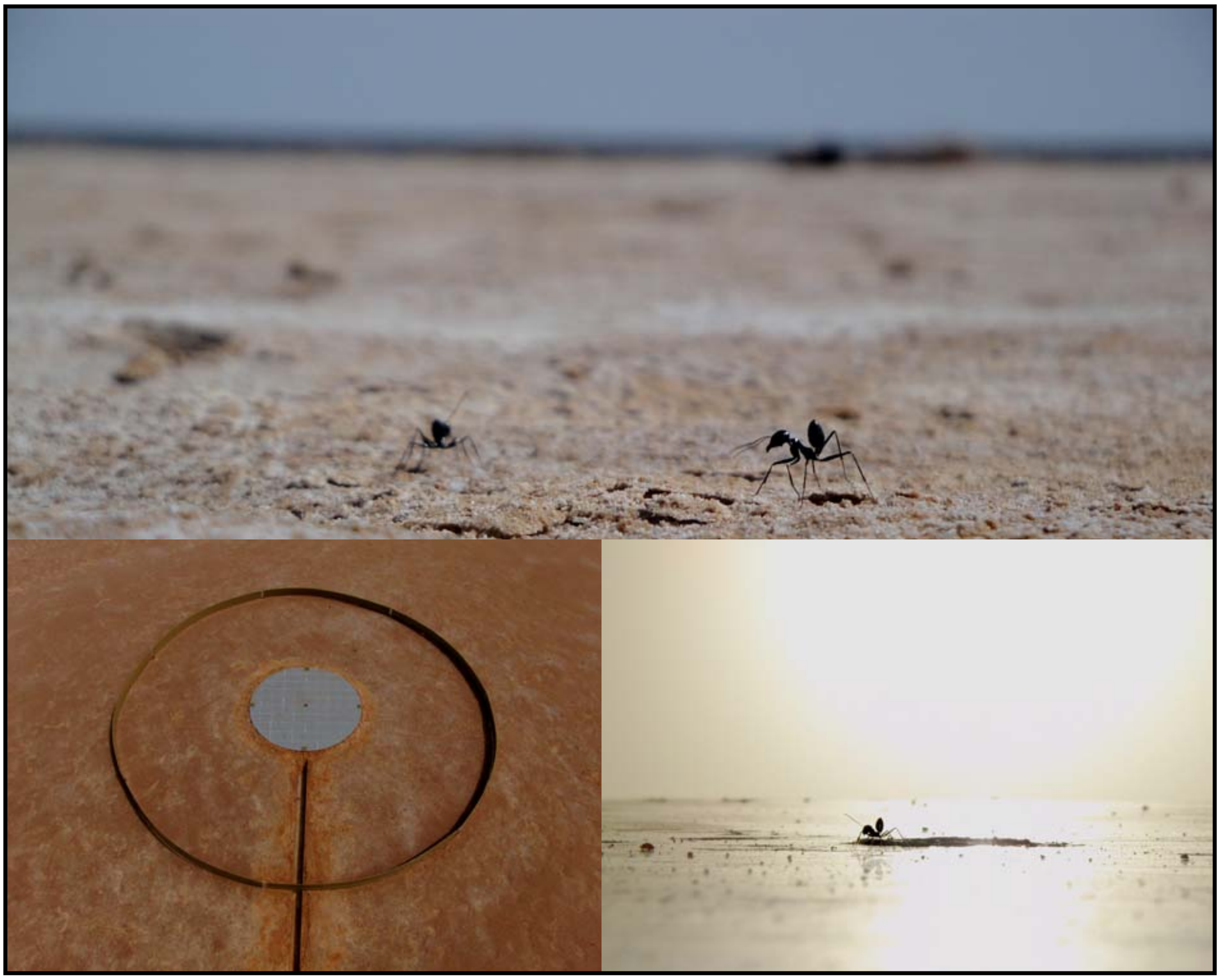
5. MANUSCRIPT II – PATH INTEGRATION CONTROLS NEST-PLUME FOLLOWING IN DESERT ANTS



Picture at the bottom right taken by Markus Knaden

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Report

Path Integration Controls Nest-Plume Following in Desert Ants

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Summary

The desert ant *Cataglyphis fortis* is equipped with sophisticated navigational skills for returning to its nest after foraging [1, 2]. The ant's primary means for long-distance navigation is path integration, which provides a continuous readout of the ant's approximate distance [3] and direction [4] from the nest [5]. The nest is pinpointed with the aid of visual [6–8] and olfactory landmarks [9–11]. Similar landmark cues help ants locate familiar food sites. Ants on their outward trip will position themselves so that they can move upwind using odor cues to find food [12]. Here we show that homing ants also move upwind along nest-derived odor plumes to approach their nest. The ants only respond to odor plumes if the state of their path integrator tells them that they are near the nest. This influence of path integration is important because we could experimentally provoke ants to follow odor plumes from a foreign, conspecific nest and enter that nest. We identified CO_2 as one nest-plume component that can by itself induce plume following in homing ants. Taken together, the results suggest that path-integration information enables ants to avoid entering the wrong nest, where they would inevitably be killed by resident ants.

Results and Discussion

Foraging *Cataglyphis fortis* ants preferably approach their nest from downwind during the final few centimeters of homing (Figure 1A), suggesting nest-derived plume-following behavior (for visualization of the nest-derived plume see Movie S1 available online). When approaching the nest from downwind, ants pinpointed the nest entrance either straight or on a counterturning walking track (Movie S2).

In order to systematically compare ants that approach the nest from downwind and upwind, we trained ants to a feeder and displaced them downwind or upwind, respectively (Figure 1B; for details, see Experimental Procedures). Following their path-integration vector, we saw that homing ants reached a fictive nest position that was either downwind or upwind of the nest entrance. When starting their nest search upwind of the nest entrance, ants usually exhibited characteristic loops, whereas ants approaching the nest from downwind tended to pinpoint the nest entrance on a rather straight route (Figure 1B; for example runs, see Figure 1Biv).

Are the plumes that the ants follow nest-specific, or do ants also follow plumes of foreign conspecific colonies? We covered two nests with circular arenas that allowed the ants to leave and enter their nests via a central opening (Figure 2A; for details, see Experimental Procedures). To exclude any nest-defining cues other than nest odor, we installed circular barriers (height, 0.1 m; Ø: 3.4 m) surrounding the arenas. A U-shaped aluminum channel (length, 2 m) pointing away from the arena was dug into the ground and led the ants under the barrier toward the feeder. After about 30 min, the ants learned to enter the channel and pinpoint the feeder. Homing ants had to pass along the channel, climb onto the arena via a sand ramp, and locate the nest entrance in the center of the arena. The visually identical arena setups allowed us to transfer ants from the feeder of their own setup to a setup connected either with a foreign nest or with no nest (no-nest control). Homing ants were thus tested in a familiar visual surrounding but with their own nest-derived plume, a foreign plume, or no plume. Whereas only 16% of the ants ended up at the fictive nest entrance in the control, 71% (73%) of the ants pinpointed the nest after the first contact with their own (foreign) nest-derived plume (Figure 2B). Thus, plume-following behavior was not restricted to the plume of the home nest. Ants approached the nest upwind with a zig-zag movement (see Movie S2) that resembled the movement of flying insects following plumes [13–17]. Plume-following moths, for instance, exhibit movements in a zig-zag fashion to relocate the odor plume when it is lost [17].

We next asked whether we could elicit plume following in *C. fortis* with a single plume component. Increased CO_2 concentrations have been reported within the nests of leaf-cutting ants [18], and CO_2 has been shown to be involved in the social behavior of different ant species (e.g., the localization of buried nest-mates) [19, 20]. By measuring the CO_2 concentrations, we found increased levels at nest sites (median 352 ppm; $n = 18$) compared to control sites (279 ppm; $n = 13$; Mann-Whitney test, $p < 0.001$; for details, see Experimental Procedures). When we tested ants in the absence of any natural nest odor (no-nest control) but provided them with an artificial CO_2 plume that mimicked the concentration emanating from a nest, the presence of CO_2 was sufficient to induce plume-following behavior in 62% of the homing ants (Figure 2B). When the CO_2 concentration was increased to about twice the values measured at the nest sites, it was no longer attractive (data not shown). CO_2 was probably not the only volatile emanating from the nest. However, its high volatility and its diffusion characteristics (due to its weight, CO_2 usually accumulates at ground level) predestine this molecule as a nest cue for homing ants.

Our results suggest that nest-derived plumes are not nest-specific and that CO_2 is one of the active compounds inducing plume-following behavior in homing ants. Trusting a single cue that is not nest-specific is dangerous. As is true for other ants [21, 22], *C. fortis* reacts extremely aggressively toward non-nest-mates that enter the nest vicinity [23, 24]. In order to quantify the costs of following the wrong plume, we—by displacement of homing ants to foreign nests—encouraged homing foragers to enter a foreign nest. This procedure resulted in the death of 13 out of 27 displaced ants, whereas the remaining 14 ants, which also entered the foreign nest, were able to escape after initial contact with resident ants

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(for details, see Experimental Procedures). Following foreign nest-derived plumes was thus shown to be dangerous and behavior that ants would likely avoid.

Consequently, we asked whether *C. fortis* foragers experienced foreign nest-derived plumes under natural conditions, and, if they did, whether such contacts inevitably caused the death of the forager. Alternatively, the ants might have developed a counter-strategy that allowed them to home successfully. Using GPS, we tracked foraging ants in their natural habitat. Two out of 20 ants crossed the nest-derived plumes of neighboring nests during their homing efforts (Figure 3A; for details, see Experimental Procedures) but did not respond to the plumes. Foragers came into contact with plumes from foreign nests but responded with a counter-strategy that circumvented the fatal error of entering the wrong nest. There were two major differences between the arena experiments and the freely foraging ants. The first was that the state of the path integrator corresponded to a position close to home in the arena experiments and to a position far from home for the freely foraging ants. The second difference was that the visual surroundings were the same for the foreign and natal nests in the arena experiments but differed in the freely foraging ants.

We next tested whether the state of the path integrator helped the ants to distinguish their own nest from a foreign nest. We performed an experiment in which ants were equipped with path-integration vectors of variable lengths when exposed to the plume of their own nest. We trained the ants in the open field to feeders that were placed at different distances (Figure 3B; for details, see Experimental Procedures), captured the ants at the feeder and released them close to their home nest. Although displaced from the feeder to a position close to the nest entrance, ants that entered the nest-derived plume still had path-integration vectors of different lengths available. All ants that—at the time of plume contact—had run off their path-integration vector responded to the plume (Figure 3Bi). When the ants still were equipped with a long vector, few entered the nest; in other words, the majority ignored the nest-derived plume and relied instead on the vector information (Figures 3Bii and 3Biii). Therefore, ants seemed to follow the nest-derived plume only when the home vector indicated that they were close to home. The inclusion of path integration might ensure that homing ants do not follow the wrong nest-derived plumes, which would likely be fatal (see above).

Subsocial shield bugs (*Parastrachia japonensis*) face similar navigational tasks as the ants mentioned above. Like *C. fortis* foragers, the bugs use path integration to find their way back

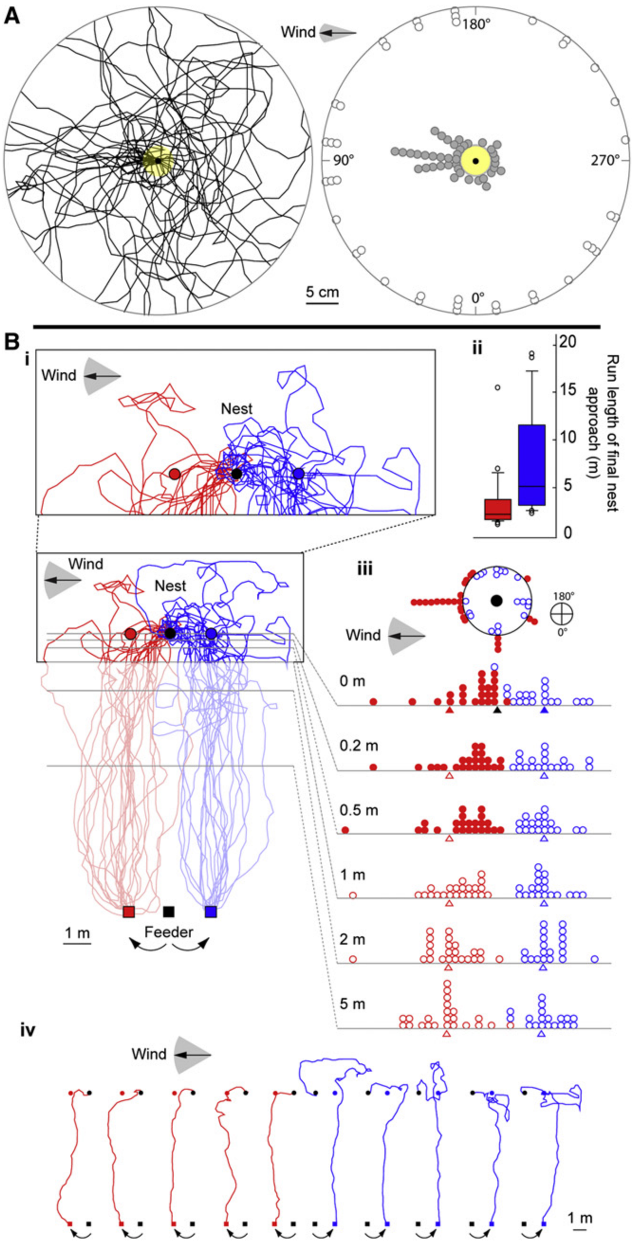

Figure 1. Homing Ants Follow Nest-Derived Odor Plumes

(A) Final nest approach during natural foraging (n = 40 ants). Homing trajectories (left) and angular distribution (right) of ants (gray circles; arranged in 15° sectors) approaching their nest (filled black circle). Wind direction, ranging from 75° to 105°. At outer circle (Ø 25 cm), ants are not aligned with plume (gray empty circles; Rayleigh test, $p > 0.05$, mean vector, 50°; length of mean vector, 0.1). At inner circle (Ø, 2.5 cm; shown by yellow filled area), ants are aligned with plume (gray filled circles; Rayleigh test, $p = 0.001$; mean vector, 98°; length of mean vector, 0.4). For visualization of nest-derived plume, see Movie S1.

(B) Nest approach after displacement.

(Bi) Homing trajectories of ants approaching the nest (black circle) from downwind (red paths, n = 23 ants) or upwind (blue paths, n = 19 ants). Nest-to-feeder distance was 10 m. Curved arrows depict the displacement. Red (blue) square depicts release point after 1.5 m downwind (or upwind) displacement from feeder. Red (blue) circle indicates path-integration (PI) defined nest position after downwind (upwind) displacement.

(Bii) Box plot representation of median and interquartile ranges of run lengths of final parts of the homing runs (within the black rectangle in Bi); whiskers, 90th and 10th percentiles; circles, outliers. Approaches from downwind were significantly shorter (Mann-Whitney test, $p < 0.001$).

(Biii) Statistical analysis of homing paths. Black arrowhead depicts nest position. Filled red (blue) arrowhead depicts fictive nest position of ants displaced downwind (upwind). Open arrowheads indicate paths as defined by path integration. Circles depict positions where the ants crossed the gray lines shown in (Bi). Filled circles depict positions where the ants deviated from the path as defined by path integration (Wilcoxon signed rank test, each $p < 0.05$). Open circles depict positions where ants did not deviate from path as defined by path integration ($p > 0.05$). Inset figure shows circular distribution of ants entering a circle (radius, 0.2 m) surrounding the nest entrance (filled red circles, data significantly directed, Rayleigh test, $p < 0.001$, mean vector, 82°, length of mean vector, 0.6; open blue circles, data not directed, $p > 0.05$; mean vector, 204°; length of mean vector, 0.2).

(Biv) Example runs of ants approaching the nest from downwind (in red) and upwind (in blue), respectively (paths also shown in Bi).

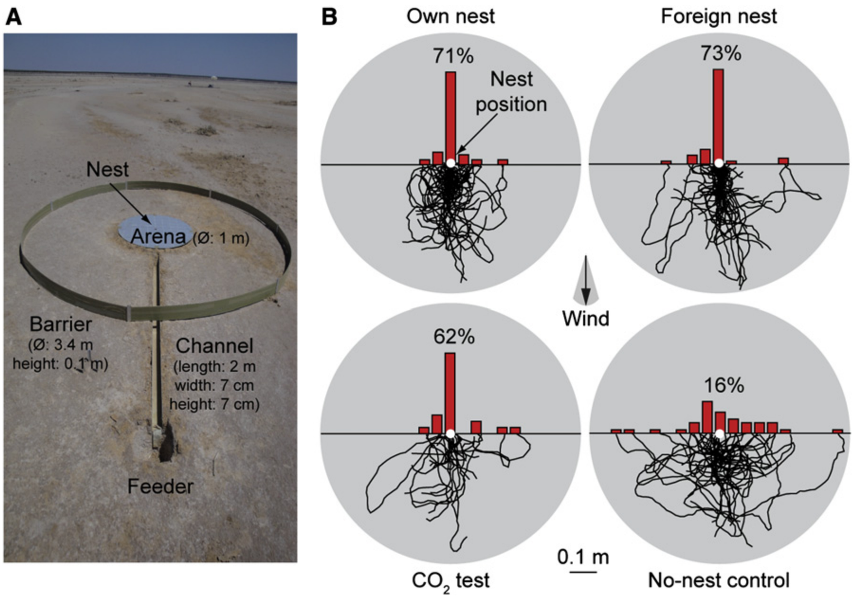

Figure 2. Plume Following Is Not Nest-Specific and Can Be Induced by CO_2

(A) Experimental setup: circular arena with central opening (3 cm) placed on top of the nest and a surrounding barrier to exclude remote nest-defining visual cues. Aluminum channel dug into the ground leads ants to an artificial feeder. Ants leave the nest by the central opening, pass the channel, and get cookie crumbs at the feeder.

(B) Nest approach of ants captured at the feeder and released either at the same feeder (own nest), at a setup connected to another nest (foreign nest), at a setup not connected to any nest (no-nest control), or at a setup not connected to any nest but equipped with a CO_2 plume (CO_2 test). Gray circles depict circular arenas (Ø: 1 m). Black lines show paths from 2 s before the first plume contact until the ants reached either the nest position (white circle) or a fictive line orthogonal to the wind direction crossing the central opening (own nest, n = 52, median distance when passing the nest: 0 cm; foreign nest, n = 44, median distance: 1 cm; no-nest control, n = 37, median distance: 7 cm; CO_2 test, n = 21, median distance: 2 cm). Runs were normalized to wind direction. Histograms depict line crossings in 5 cm bins. Numbers above bars depict percentage of ants that crossed the line at the nest position. No-nest control differed significantly from conditions in which ants were tested either with own nest, foreign nest, or CO_2 plume (Kruskal-Wallis test and Dunn's multiple comparison tests, $p < 0.05$). There was no difference between the latter three test conditions ($p > 0.05$). For details, see Experimental Procedures. For example run, see Movie S2.

to the nest, which they pinpoint using chemical cues [25]. In contrast to *C. fortis* ants, the bugs react to the chemical cues from their own nest even when the path integrator tells them that they are far away from home. However, because the bugs are able to discriminate their own from foreign nests by chemical cues, they do not run the risk of entering the wrong nest [26].

In addition to chemical cues, ants use visual landmarks to pinpoint the nest entrance [6, 7]. Similar to our results for nest-odor cues, ants ignore nest-defining visual cues when the path-integration vector indicates that they have not yet arrived in the vicinity of the nest-entrance hole [26]. Our results suggest that, apart from informing foragers about their position relative to the nest, path integration seems to play a crucial role in preventing foragers from being killed by following ambiguous nest cues.

Experimental Procedures

Experimental Site and Ant Species

Field experiments with the desert ant *C. fortis* were performed during early June and mid-August 2010 in a flat salt pan near the village of Menzel Chaker (Tunisia).

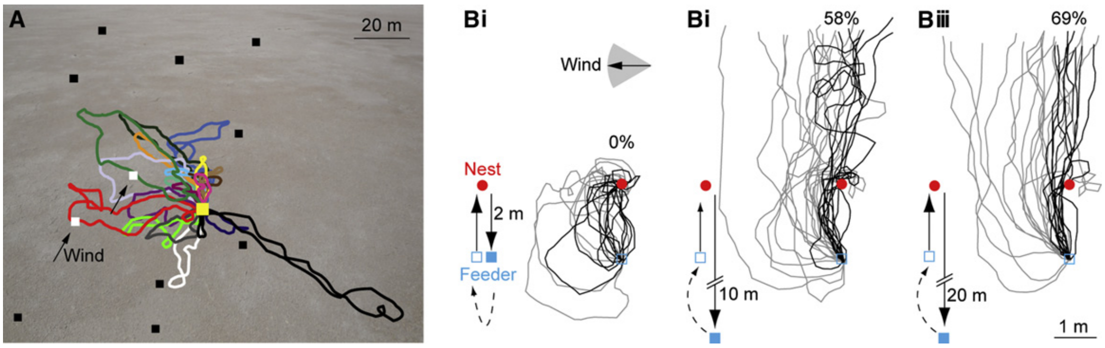

Figure 3. Path Integration Controls Plume-Following Behavior

(A) Foraging runs of 20 ants from a single nest (yellow square) tracked with GPS. Different colors represent different foraging runs. Black and white squares indicate foreign nests whose plumes were (white) or were not (black) crossed by tracked foragers. Crossing the nest plume was assumed to have occurred when an ant passed the nest less than 0.3 m downwind. The mean minimum distance between neighboring nests was 23 m; the mean maximum foraging distance was 28 m.

(B) Experimental paradigm in which we set path integration in conflict with the nest-derived plume. Ants trained from nest (red circle) to feeder (blue square; nest-to-feeder distance; Bi, 2 m; Bii, 10 m; Biii, 20 m) were displaced from feeder (dashed arrows) and released along training route 2 m away from the nest (release point, open blue square). Ants followed their path-integration vector (black arrows) and reached the nest-derived plume with a home-vector length of 0 m (Bi), 8 m (Bii), or 18 m (Biii). Black trajectories depict ants that passed the nest in less than 0.3 m distance in downwind direction (Bi, n = 12 ants; Bii, n = 13 ants; Biii, n = 13 ants). Gray trajectories depict ants that passed the nest more than 0.3 m distance in a downwind direction (Bi, n = 9 ants; Bii, n = 17 ants; Biii, n = 15 ants). Numbers above trajectories depict the percentage of ants that passed but rejected the plume and followed the path-integration vector (chi-square test for trend, $p < 0.05$).

Experiments

Ants Follow the Plume of Their Nest

We first video-recorded the final part of the ants' homing runs in the vicinity of a nest entrance during natural foraging. A thin thread attached to a needle and placed close to the nest entrance continuously informed us about wind direction. Recorded traces were transferred to paper and digitized using Graph Click (version 3.0). A circle (Ø: 1 m; 45° segments) painted on the ground served as a reference. The positions of the homing ants when crossing two fictive circles with radii of 25 cm and 2.5 cm were recorded and the angular distributions were analyzed using Oriana 3 (used throughout the analyses of the circular data). The directionality of the data was examined using the Rayleigh test.

We next trained foraging ants to visit an artificial feeder containing cookie crumbs at a distance of 10 m in the open and flat salt pan; the nest-to-feeder direction was orthogonal to the wind direction (see Figure 1B). After at least half a day of training, we captured ants at the feeder and displaced them 1.5 m either downwind or upwind of the feeder. We recorded homing runs on paper until the ants entered the nest. A grid (mesh width, 1 m) on the ground served as a reference. Ants displaced in such a way still had their path-integration vector available and reached a fictive nest position that was located downwind or upwind of the nest, respectively. Here and everywhere else in the following experiments, the ants were tested individually and only once. Homing traces were digitized and the run lengths of final parts of the traces (i.e., within the black rectangle in Figure 1Bi) were calculated by Gedit Graphics Editor and Run Analyzer [27]. Run lengths of upwind approaches were compared with those of downwind approaches using the Mann-Whitney test (GraphPad Instat, version 3.06, was used throughout the statistical analyses of the noncircular data). The positions where the ants (displaced either upwind or downwind) crossed horizontal lines at 5 m, 2 m, 1 m, 0.5 m, 0.2 m, and 0 m away from the fictive nest positions were measured in order to see whether the ants' homing paths differed from the path of the vector course (see gray lines in Figure 1Bi). The Wilcoxon signed rank test was performed to analyze whether the data differed from 0 (direct line between release point and fictive nest position). In addition, the angular distribution of the final nest approach was measured at 0.2 m from the nest and the directionality of the data was examined using the Rayleigh test.

Foreign Nest-Derived Plumes Induce Plume-Following Behavior

An aluminum arena (Ø: 1 m) was placed on the flat desert ground on top of the nest entrance so that ants were forced to leave and enter the nest solely through a central hole in the arena (Ø: 0.03 m). The arena established a homogeneous and defined area around the nest entrance and excluded any nest-defining cues other than nest odor. An aluminum channel (U-shaped cross-section; length: 2 m, width: 0.07 m, height: 0.07 m) dug into the ground led the ants to a feeder containing cookie crumbs (see Figure 2A). Within an hour, all foraging ants of a nest had learned this procedure. The arena was surrounded by a 0.1 m high circular barrier (Ø: 3.4 m) to prevent ants from using remote nest-defining visual cues. A thin thread attached to a needle and placed close to the nest entrance provided continuous information about wind direction. Smoke (produced by an air flow tester, Draeger Safety AG, Luebeck, Germany) released from the central hole of the arena allowed us to visualize the nest-derived plume and confirmed that the wind close to the nest entrance was not turbulent in the presence of the surrounding barrier, i.e., the plume was straight (see Movie S1). Based on the visualization with smoke, we defined the odor plume as a straight line originating at the nest. Preliminary observations of ants tested in the arena that was connected to their own nest revealed that homing ants almost always made immediate turns toward the nest when they passed the nest downwind in less than 0.3 m distance, whereas they did not do so when passing the nest farther away. This suggests that the functional reach of the potential plume lies around 0.3 m. Consequently, we assumed a nest-derived plume of 0.3 m length in subsequent experiments. Identical setups at different sites allowed us to video-record the homing performances of ants returning from the feeder to either their own nest, to a foreign nest, or to a no-nest control. In order to suppress any contact with resident ants during testing, a net covered the nest entrance. The testing of ants under different conditions was randomized. The video-recorded homing paths were recorded on paper from shortly before the ants had their first contact with the odor plume (as defined by the filmed position of the thin thread) and lasting until they either reached the nest position or crossed the fictive line as shown in Figure 2B. Because the nest entrance on the arena was visually inconspicuous homing ants did not always reach the nest directly but missed the entrance by few centimeters. At that time, the path-integration vector was run off and the ants started a nest search until they finally reached the nest plume [28, 29]. A grid (mesh width, 0.1 m) painted on the arena served as a reference. Run directions were normalized to wind direction. The absolute values of the distances between crossing positions and nest entrance were analyzed using the Kruskal-Wallis test with Dunn's multiple comparison tests.

Risk of Following the Plume of a Foreign Nest

In an additional experiment, we let the ants return to the foreign nest but used no net to cover the entrance hole, i.e., the ants were not prevented from entering the foreign nest. We marked the ants at the feeder with a color dot on their gaster, displaced them to a foreign nest, tracked the number of test ants entering the nest, and observed the response of resident ants. We counted ants that were directly attacked and pulled deeper into the nest. Test ants that did not reappear at the surface after 2 hr were considered dead.

CO_2 Triggers Plume-Following Behavior

Carbon dioxide released from nests was measured either 2 cm downwind of the nest entrances at ground level or at the same height in a nest-free area (using a Telaire 7001 CO_2 measurement device).

In the absence of any nest, using the same arena setup as described previously, we provided ants with an artificial CO_2 plume that mimicked the concentration emanating from a nest. CO_2 was released out of the central opening of the arena (around 500 ppm measured 2 cm downwind of the opening) using a tube connected to a CO_2 bottle.

State of the Path-Integration Vector Controls the Response to the Nest Odor

Natural foraging runs: All *C. fortis* nests in an area of approximately 100 m × 100 m were localized with a GPS measurement device (GARMIN eTrex Vista HCx). Foraging runs of 20 ants of a nest located centrally were GPS-tracked, i.e., one of us carried the GPS device with an activated path-recording function and followed foraging ants at a distance of 2 m. Due to the flatness of the area, the GPS always received input from at least 12 satellites, resulting in an accuracy of about 2 m. When ants passed close to the vicinity of neighboring nests, we immediately measured the minimum distance between the path and the nest entrance and recorded the actual wind direction. GPS-recorded foraging runs were analyzed graphically, and mean minimal distances between neighboring nests (n = 12) and maximal foraging distances of ants (n = 20) were measured.

Conflict of path integration vectors and plume-following behavior: Foraging ants were trained from the nest to a feeder located 2 m, 10 m, or 20 m away from the nest with the nest-to-feeder direction being orthogonal to wind direction (Figure 3B). After at least half a day of training, we captured ants at the feeder and released them along the training route 2 m away from the nest (in the 2 m training paradigm, the ants were captured and released at the feeder). When entering the plume, the ants' home-vector length was either 0 m (training distance: 2 m), 8 m (training distance: 10 m), or 18 m (training distance: 20 m). Homing runs of ants were recorded on paper until the ants either entered the nest or overshot the nest entrance for more than 4 m; runs were then digitized. A grid (mesh width, 1 m) on the ground served as a reference. Only ants that passed the nest downwind (i.e., on the side of the nest-derived plume) were recorded, and we only considered ants that crossed the plume within the functional reach of 0.3 m in the analyses. The number of ants that entered the nest after contact with the odor plume was counted, and the data were analyzed with a chi-square test for trends.

Supplemental Information

Supplemental Information includes two movies and can be found with this article online at doi:10.1016/j.cub.2012.02.029.

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6. MANUSCRIPT III – FLEXIBLE WEIGHING OF OLFACTORY AND VECTOR INFORMATION IN THE DESERT ANT *CATAGLYPHIS FORTIS*

Picture at the bottom taken by Kathrin Steck

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Animal behaviour

Flexible weighing of olfactory and vector information in the desert ant *Cataglyphis fortis*

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Desert ants, *Cataglyphis fortis*, are equipped with remarkable skills that enable them to navigate efficiently. When travelling between the nest and a previously visited feeding site, they perform path integration (PI), but pinpoint the nest or feeder by following odour plumes. Homing ants respond to nest plumes only when the path integrator indicates that they are near home. This is crucial, as homing ants often pass through plumes emanating from foreign nests and do not discriminate between the plume of their own and that of a foreign nest, but should absolutely avoid entering a wrong nest. Their behaviour towards food odours differs greatly. Here, we show that in ants on the way to food, olfactory information outweighs PI information. Although PI guides ants back to a learned feeder, the ants respond to food odours independently of whether or not they are close to the learned feeding site. This ability is beneficial, as new food sources—unlike foreign nests—never pose a threat but enable ants to shorten distances travelled while foraging. While it has been shown that navigating *C. fortis* ants rely strongly on PI, we report here that the ants retained the necessary flexibility in the use of PI.

1. Introduction

Desert ants have a remarkable navigational toolkit [1,2]. Path integration (PI) is performed continuously and takes into account walking distance and direction; moreover, it provides ants with a homeward vector pointing back to the starting point of their journey that is the nest [3]. PI is essential on early foraging trips but is prone to cumulative errors [4]. Therefore, experienced ants complement this strategy with landmark navigation, i.e. the use of place-specific olfactory and visual cues [5,6]. Once homing ants have got close to the nest, they eventually follow the odour plume emanating from the nest in order to pinpoint the entrance accurately [7].

Ants use these navigational strategies not only for localizing the nest, but also for returning to a familiar feeding site. PI guides them towards the known feeder, which is eventually pinpointed by its odour plume ([8] and references therein). In contrast to homing ants that compute a homeward vector from a current location to their home, ants heading for a familiar feeder first have to retrieve the coordinates from the feeder as recorded on previous visits. However, as in homeward vectors, information about direction and distance are not only encoded [8], but also integrated [9] in foodward vectors. Hence, PI is involved in both foodward and homeward runs.

In *Cataglyphis fortis*, it is known that PI is the predominant navigational strategy [1,2]. Furthermore, it was shown recently that homing ants are attracted to nest odours only when close to home, i.e. when their PI vector is run off (see figure 1*a*,*b* and also [7]). Here, we ask whether this dominance of

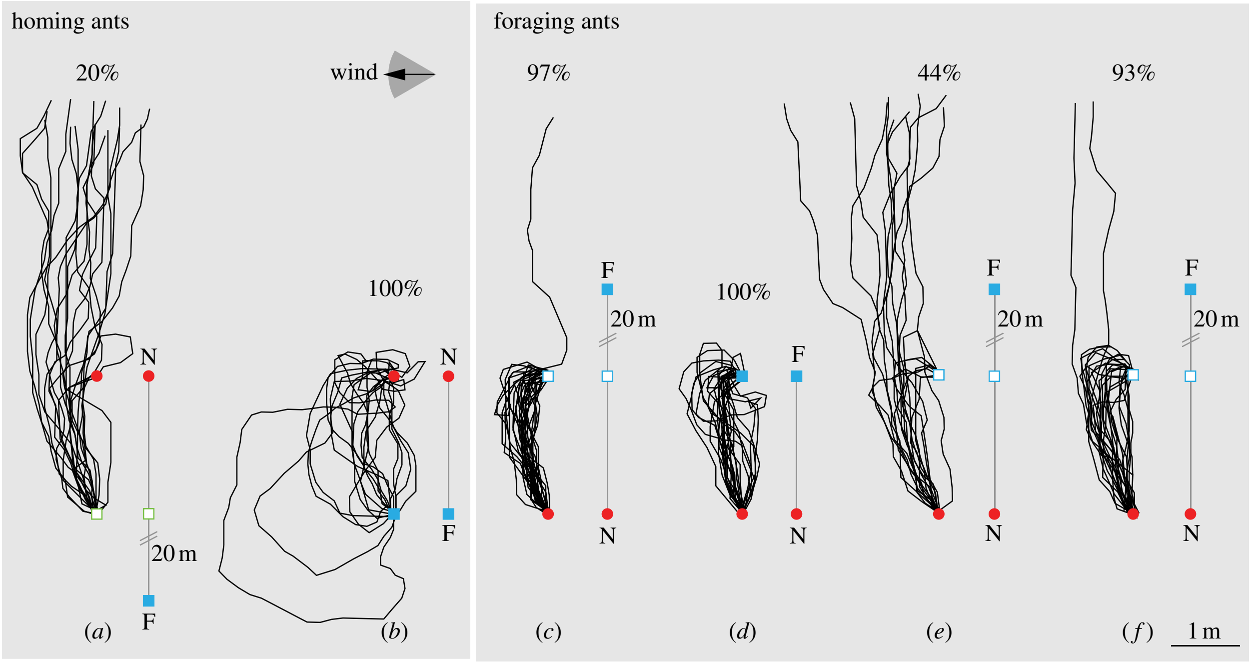

Figure 1. Olfactory and vector information in ants on the way home (*a*,*b*) and on the way to a familiar feeder (*c*–*f*). Ants were trained from the nest (red circle) to a feeder (filled blue square; nest-to-feeder distance, 20 m; except (*b*) and (*d*), 2-m control training paradigm). Homing ants (*a*) were captured at the feeder and released along the route 2 m away from the nest (green square). They still had a long PI vector when they encountered the nest plume and did not respond to the nest odour ($n = 20$ ants), whereas control ants (*b*) had run off their homeward vector when encountering the nest plume and directly followed the plume into the nest ($n = 18$ ants). (*a*,*b*) Adapted from Buehlmann *et al*. [7]. During tests with foraging ants on the way to the feeder, the odour source was placed 2 m away from the nest (empty blue square). Ants with a training distance of 20 m still had a long PI vector when they encountered the food plume ((*c*), $n = 30$ ants; (*e*), $n = 18$ ants; (*f*), $n = 27$ ants), but control ants in (*d*) had run off their foodward vector by the time they had reached the food odour ($n = 25$ ants). The feeder contained either cookie crumbs during both training and test (*a*–*d*), cookies during training but a dead cricket during test (*e*) or dead crickets during training and test (*f*). Numbers above trajectories depict the percentage of ants that followed the plume and neglected the PI vector. Raw data for the ant trajectories are stored at DRYAD (doi:10.5061/dryad.d1jk8).

vector over olfactory information is obligatory. Do ants on the way to a familiar feeding site only respond to the food odour when they are close to the expected feeder position?

2. Material and methods

(a) Experimental site and ant species

Experiments were performed in the natural habitat of *C. fortis* during summer 2012 in a dry salt pan near the village of Menzel Chaker, Tunisia.

(b) Experimental procedures

Ants were trained to a stable feeder (Petri dish containing cookie crumbs ad libitum) located 20 m away from the nest, with the nest-to-feeder direction being orthogonal to wind direction (figure 1*c*). In the 2-m control paradigm, both training and test distance of the feeder were 2 m from the nest (figure 1*d*). After at least half a day of training (approximately 15 foraging runs), we recorded the foraging trajectories of ants that left the nest heading for the learned feeder, while we placed identical cookie crumbs along the training route 2 m away from the nest (in the 2-m training paradigm there was no displacement of the feeder). Therefore, when encountering the food plume, test ants still had a long PI vector pointing to the feeder they had visited previously, while control ants had run off their vector completely. In an additional paradigm, we presented a small dead sun-dried cricket along the route to ants that were trained as before with cookies (figure 1*e*). In the final experiment, we trained and tested the ants with dead crickets (figure 1*f*). All ants were tested only once. Foraging runs were recorded on paper until the ants either reached the test feeder or overshot the feeder position for more than 4 m. A grid (mesh width, 1 m) on the ground served as a reference. The runs were digitized using Graph Click (v. 3.0). Only ants that passed the odour source downwind (i.e. on the side of the food-derived plume) were analysed and, as we do not know the functional reach of the different plumes, we analysed only those ants that crossed the plume within 1 m from the source. We conducted all experiments under similar wind conditions. The number of ants that pinpointed the source after contact with the odour plume was counted. Raw data for the ant trajectories are stored at DRYAD (doi:10.5061/dryad.d1jk8).

(c) Statistics

Data were analysed using Fisher's exact test performed with the statistic software GRAPHPAD INSTAT (v. 3.06). The *p*-values were adjusted by the Bonferroni correction.

3. Results

In a previous study [7], we showed that homing desert ants follow the nest plume only when they have run off the homeward vector (summarized in figure 1*a*,*b*; (*a*) versus (*b*), $p < 0.05$; see also [7]). Here, we test whether ants on the way to a familiar feeding site also do not respond to a food odour when they are still far away from the expected feeder location. Ants on the journey to a stable feeder (filled with cookie crumbs) 20 m away encountered the expected odour plume already along the route 18 m before the familiar feeder position was reached. Twenty-nine out of 30 ants followed the plume (figure 1*c*; (*c*) versus (*d*), $p > 0.05$). Because ants that have crossed the position upwind did not respond to the stimulus (see the electronic supplementary material, figure S1), we can exclude stimuli other than olfactory cues. Hence, unlike in homing ants, when they are on the way to food, olfactory input outweighs vector information.

Are ants at the start of a food vector primed to encounter the odour of a particular food? To approach this question, we trained ants as before but tested them with the odour of a single dead cricket by placing it at the test position described above. Here, only 10 out of 18 ants followed the plume, whereas the rest followed the PI vector to the feeder they had learnt (figure 1*e*; (*e*) versus (*c*,*d*), $p < 0.05$ each). But when ants were trained and tested with crickets 25 out of 27 ants followed the plume of a single cricket at 2 m from the nest (figure 1*f*; (*f*) versus (*c*), $p > 0.05$), indicating that the minute concentration present in the odour plume of a dead insect was sufficient for interruption of the journey, once the ants have experienced this odour before.

4. Discussion

We showed recently, not only that homing ants prefer to approach the nest from downwind in order to follow the odour plume, but also that ants follow the nest plume only when the path integrator tells them that they are close to home ([7]; summarized in figure 1*a*,*b*). Here, we provide evidence that this weighing of olfactory and vector information is not obligatory. Ants that are trained to a stable feeder pinpoint it using PI [8]. However, when they encounter a food plume, even though they may not have run off their foodward vector, they follow the food plume up to the food (figure 1*c*,*f*).

The difference between the responses to food and nest odours (figure 1*a*,*c*) could have been explained by the strong smell of cookies, which is probably more intense than the smell of a nest. However, this seems unlikely given that ants also responded to the odour of a single, small sun-dried cricket (figure 1*f*). We rather explain the different weighing in terms of functional benefits for the ants. Interestingly, ants followed the plume of a dead cricket more often when they had been trained to this kind of food (figure 1*e*,*f*). Hence, the interruption of a journey towards food is weaker if the food is common but has a different odour from what is expected, i.e. at the start of a foodward vector ants are primed to encounter the odour of a particular food. In wood ants and bees, it has been shown that the decision between choosing foodward or homeward journey is regulated by the animals' feeding state [10,11]. We show that whether or not ants respond to a familiar odour plume depends not only on their behavioural state, being on the way to the nest or feeder, but also on the ants' previous food-finding experiences and nutritional value of the food item (a dry cricket could have been less attractive in those experiments at that time than the cookies).

Our finding suggests that ants use PI and plume following as needed. The higher flexibility in ants returning to food compared with ants aiming for the nest is also found in the ants' searching pattern when the target is not encountered. Ants that do not encounter the nest after having run off the homeward vector centre their search on the expected nest location [12]. Although PI is involved in both homeward and foodward runs [9], the endpoint of a foodward journey is less strongly determined. When food is not encountered at the familiar position, the search strategy depends on their previous experience of food abundance and reliability [8]. Under natural conditions, without having experienced a plentiful feeder over a long time, ants tend to search beyond the expected food site [8,13].

As *C. fortis* does not discriminate the plume of its own nest from that of foreign ones, but encounters foreign nest plumes repeatedly when homing, it is essential for the survival to strictly follow the PI vector and respond to a nest plume only when the nest is almost reached and the value of the PI vector is close to zero. If not, the ant would risk following the wrong plume and being killed in a foreign nest. However, by responding to food plumes also when still far away from the food source they have learnt and still equipped with the foodward vector pointing at a learned food source, foraging ants retain the flexibility needed to adjust their behaviour and localize new food sources that might be situated closer to the nest. This allows the ants to reduce foraging distances as well as the time spent outside the nest that is beneficial in the harsh desert environment. One question which this study leaves open, but which will be answered elsewhere, is whether homing ants ignore all odours when their home vector is active or whether they ignore nest odours, but can still respond to food odours.

While it has been shown that *C. fortis* relies strongly on PI, we report here that the ants retained the necessary flexibility when performing PI by weighting vector and olfactory information depending of their behavioural state.

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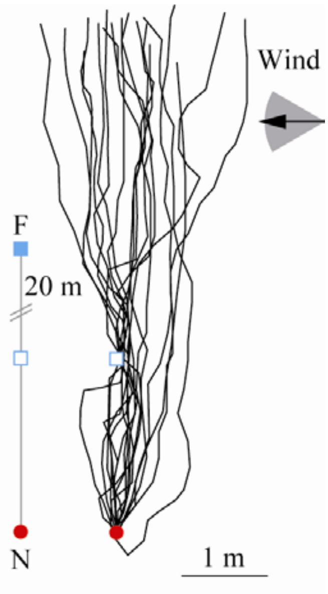
Supplementary Figure

Additional data for experimental condition shown in figure 1c. Ants trained to a feeder (blue square; petri dish filled with cookie crumbs) 20 m away from the nest (red circle) with nest-to-feeder direction being orthogonal to wind were tested when food was given prematurely on their foraging journey. Lines show trajectories from ants that passed the position upwind without encountering the food plume (n = 18 ants). All ants followed their foodward vector and ignored the introduced stimulus. Since they did not respond to it without encountering the odour plume we conclude that the positive responses shown in figure 1 are provoked by olfactory cues.



7. MANUSCRIPT IV – SCREENING THE DESERT FOR FOOD: OLFACTORY-DRIVEN FORAGING STRATEGIES IN DESERT ANTS

Pictures taken by Markus Knaden

Screening the desert for food: Olfactory-driven foraging strategies in desert ants

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Abstract

Cataglyphis fortis ants, known for their impressive homing capabilities, travel huge distances in search for food in the hostile and food-impoverished salt pans of Tunisia. Here we show that the ants localize tiny dead insects – i.e. their main food source – within a very short time span, even when the food items are placed more than 300 m away from the closest nest. We furthermore show that food is localized exclusively by the sense of smell. We identify linoleic acid as a key compound involved in food detection. Because of its low volatility linoleic acid constitutes an ideal food indicator under the hot desert conditions. The efficiency of the search is accomplished by far reaching crosswind runs during which large areas of the salt-pan habitat are screened for food-derived odour plumes. Hence, in order to survive the harsh environment, *Cataglyphis* has not only developed impressive homing capabilities but have also evolved a sophisticated strategy to localize food as fast as possible.

Introduction

Desert ants feeding on dead arthropods have the challenge to forage for food items that are distributed unpredictably in space and time in the food-impoverished terrain of Saharan salt pans (Wehner, 1983). The thermophilic diurnal scavengers of the genus *Cataglyphis* do not lay pheromone trails, but are rather equipped with a sophisticated personal navigational toolkit (Wehner, 2003). The ants primarily rely on path integration when in unfamiliar terrain and as they become experienced they in addition rely on learnt information from visual (Graham, 2010; Wehner, 2008; Wehner, 2009) and olfactory cues (Buehlmann et al., 2012; Steck, 2012). While most studies focused on navigational mechanisms when targeting a familiar place, be it the nest or a learnt feeding site, less is known about the ants' strategies to efficiently localize sparse natural food sources. Individual foragers tend to search for food within restricted sectors (Wehner, 1987), i.e. ants keep searching for food in areas where they initially found food successfully. Furthermore, desert ants that encounter a plentiful and reliable feeding site will return to it (Schultheiss and Cheng, 2012; Wolf et al., 2012). However, usually food items occur scattered in an unpredictable way (Wehner et al., 1983). Since desert ants reach their physiological thermal limit during foraging (Cerda et al., 1998;

Lenoir et al., 2009; Wehner et al., 1992) efficient foraging strategies that optimize the rate of encountering food items within short time are essential for survival.

In the present account we show that *Cataglyphis* ants are extremely efficient in localizing even tiny food items far away from their nests. We furthermore show that this efficiency is accomplished by a combination of high sensitivity towards a key odorant (linoleic acid) emitted by dead insects and far reaching crosswind runs during which large areas of the salt-pan habitat are screened for food-derived odour plumes.

Results

In order to investigate the food-localization capabilities of *Cataglyphis* we placed individual dead crickets (size, 5mm) at different distances (5 to 300 m; for distribution see Figure 1A) to the salt pan border where almost all ant nests are situated. We then measured the time until the ants discovered the food item. Irrespective of the distance to the nests, the food items were discovered on average in less than 3 minutes ((1), t = 2.4 min (median), n = 15 food items; (2) t = 2.9 min, n = 15 food items; (3) t = 2.4 min, n = 31; food items; Kruskal-Wallis test and Dunn's multiple comparison tests show no significant differences between groups). How do ants searching for food manage to detect such tiny food items distributed unpredictably in time and space that efficiently? The localization of dead arthropods was exclusively olfactory driven since foraging ants only targeted the food item when they passed the food downwind, i.e. encountered the odour plume emanating from the insect (Figure 1B). Ants that passed the food item upwind did not approach it although they occasionally passed it by only a few centimetres. Hence, the role of vision for food detection can be neglected. Furthermore, foraging ants were highly sensitive to food items. In order to analyse the functional reach of the food plume, we used a stick with a dead insect attached to it (or with no insect as a control) and placed it upwind of foraging ants at varying distances. Although the number of ants responding to the plume decreased with increasing distance to the food item (distances were categorized in three groups: < 2 m, within 2 to 4 m, > 4 m), still 40% of the ants detected the insect at a distance of more than 4 m (Chi-square test for trends, $p < 0.05$; < 2 m, 79% followed plume, n = 38 ants; 2 – 4 m, 53%, n = 40 ants; > 4 m, 40%, n = 15 ants; control, 0%, n = 13 ants). The maximum distance over which an ant turned directly into the plume, followed it and removed the insect was 5.9 m. We conclude that ants detect food by olfaction over several meters distance.

We next followed freely foraging ants with a handheld GPS device. On search for food the ants covered distances of up to 1259 m (median walking distance, 341 m; maximal distance away from nest, 356 m; Figure 1C). 53% of the foraging ants successfully found a food item. While following the ants with GPS we observed individuals that displayed far reaching crosswind runs (as the wind direction could be deducted from moving sand particles and bent ant antennae). In order to quantify this so far unknown behaviour we simultaneously tracked foraging ants with a GPS measurement device and video recorded the wind direction with the help of a vane. We afterwards analyzed the fine structure of the ants' trajectories in relation to the actual wind direction. As we observed before, the analysis revealed extensive crosswind walks (Figure 2) that were only interrupted by short upwind movements. From our observation these upwind movements usually pinpointed some kind of organic material that was probed by the ant and either refused or picked up and brought back to the nest. We conclude that the ants do not search randomly for food but rather maximize the probability to discover food plumes by performing extensive crosswind walks.

What are the chemical compounds that elicit plume following and facilitate the localization of food items? We analyzed the smell of dead insects (see GC-MS spectra in Supplementary Figure) and beside many unidentified components we found several so-called necromones, i.e. odorants that are known to occur in dead animal material (Gordon, 1983; Haskins and Haskins, 1974; Visscher, 1983; Wilson et al., 1958). In order to test whether any of these compounds was sufficient to provoke plume following in foraging ants, we established a behavioural paradigm for testing freely foraging ants in the field. We followed foraging ants until they switched to the characteristic crosswind runs and then presented an odorant in a position so that the ants would pass the plume about 1 m downwind of the source. Odorants that resulted in interruption of crosswind runs and led to plume following towards the source were defined as attractive. We found that not only the entire body extracts of dead insects but also single identified compounds provoked plume following. In total, six out of 15 tested odours were attractive and elicited plume following in food-searching ants (Figure 3). While 5 of the 6 attractive odorants attracted only half of the tested ants linoleic acid turned out to be the most attractive odorant. The attractiveness of linoleic acid remained even when we reduced the odorant amount in the source from the usually applied concentration (2×10^{-2}) down to a 10^{-5} dilution (Figure 3). We conclude that linoleic acid is a key odorant used by the ants to detect tiny food items over long distances.

Having shown that ants localize their food items over long distances and that linoleic acid is an active compound used to pinpoint the food we next asked whether homing ants (i.e. ants that have found and grabbed a food item already) become attracted by linoleic acid, also. When we presented linoleic acid to ants that already headed home with a dead insect packed in their mandibles, more than 50% of the ants still responded and followed the odorant plume (12 out of 22 homing ants). When analysing the food items that were carried by the ants, we found that ants still responded to the compound when their food item was small, but neglected it when the food item was significantly bigger (median weight of items of responding ants, 4×10^{-4} g; median weight of items of non-responding ants, 4×10^{-3}g; $p < 0.05$, Mann-Whitney test). Hence, whether or not homing ants responded to linoleic acid depended on the size of their actual food item.

Discussion

When an arthropod in the Tunisian salt pan succumbs to the heat it takes on average less than 3 minutes until it becomes detected by a *Cataglyphis* forager (Figure 1B). How are desert ants able to detect the tiny dead arthropods that are scattered in a spatiotemporally unpredictable manner so efficiently? Dead arthropods provide chemical information that the ants are extremely sensitive to, and they can, using olfactory cues only, detect food items from far away (Figure 1C, D). Detecting and orienting towards a resource of interest by means of olfaction is widespread in flying insects like flies and moths when searching for food, mating partners or hosts. The insects approach the target by following an odour plume against the wind up to the source once having encountered the plume (Budick and Dickinson, 2006; Carde, 1996; Carde and Willis, 2008; Murlis et al., 1992). While we have gained an advanced understanding of odour plumes and how flying insects use them, less is known in walking insects (cockroaches (Willis and Avondet, 2005), desert ants (Buehlmann et al., 2012; Wolf and Wehner, 2000)). We, therefore, looked for potential behaviourally active compounds emanating from dead insects, i.e. the natural food source of the desert ants, and identified beside other compounds several fatty acids including linoleic acid (Supplementary Figure). These so-called necromones have been described to induce necrophoric or necrophobic behaviour in insects. Social insects such as ants or honey bees remove corpses of dead nestmates in order to minimize the contagion risk (Gordon, 1983; Haskins and Haskins, 1974; Visscher, 1983; Wilson et al., 1958), while isopods, cockroaches or springtails avoid shelters containing dead conspecifics (Nilsson and Bengtsson, 2004a; Nilsson and Bengtsson, 2004b; Rollo et al., 1994; Yao et al., 2009). When testing foraging ants with the full blend emitted by dead insects or with individual components of the blend the ants were attracted by the full blend but also by some of the single compounds (Figure 3A), i.e. single food-related compounds were sufficient to induce odour-mediated behaviour resulting in ants following the plume to the source. It has been shown that odour blends usually are more attractive than their individual compounds (Riffell et al., 2009a; Riffell et al., 2009b; Webster et al., 2010). It was therefore surprising that the ants displayed the strongest response to the individual component linoleic acid. We conclude that linoleic acid plays a key role in the detection of food items in the rather food-impoverished and hostile habitat (Figure 3A). As it is a stable long-chained fatty acid with low volatility, linoleic acid might present a weak but persistent stimulus provided by a dead insect under the thermal conditions of the saltpan. By having evolved such a high sensitivity to this specific compound (Figure 3B) *Cataglyphis fortis* might have increased the functional reach of the weak odour plume. It will be interesting to investigate if linoleic acid is indeed detected by and processed in a dedicated neural line of the ant olfactory system as has been observed for highly important odours in other insects (Stensmyr et al., 2012).

However, pinpointing food by plume following requires that the ant initially reaches the functional space of the odour plume. By travelling long distances through the desert (the maximum distance of more than 1200 m described here (Figure 2) is much longer than any *Cataglyphis* run reported before (Wehner, 1983; Wehner, 1987)) the ants probably reach their physiological thermal limit and risk death by desiccation. Therefore, instead of performing random search patterns the ants should screen the environment for food plumes systematically. It has been shown for *Drosophila* flies (Zanen et al., 1994) and albatrosses (Nevitt et al., 2008), that both optimize their search behaviour by adapting flight headings according to wind conditions. Our analysis of the fine structure of the ants' foraging paths revealed that ant foragers indeed display a similar systematic plume search by performing extensive crosswind movements (Figure 2). The combination of a very high sensitivity towards odours emitted by dead insects (Figure 1D) and far reaching crosswind runs, during which large areas of the salt-pan habitat are screened for food-derived odour plumes, enables the ants to screen the salt pan for food with an impressive efficiency.

During walking ants do not only screen for food plumes, but perform path integration in order to find the way back to the nest (Muller and Wehner, 1988). We recently showed that homing *C. fortis* ants pinpoint the nest entrance by plume following, but follow the nest plume only when the path integrator indicates that the ant is close to home (Buehlmann et al., 2012), i.e. in this situation vector information outweighs olfactory information. Next, we therefore asked whether homing ants avoid responding to any kind of odours or only selectively neglect nest odours when passing by a neighbouring nest. Is the identified food odour linoleic acid a stimulus that is strong enough to outweigh path integration in homing ants? When we exposed linoleic acid to ants that already headed home with a dead insect packed in their mandibles, the food odour still evoked plume following in numerous ants (see Figure 3C). Analyses of the food items that were carried revealed that the size of the carried dead insect governed the ant's willingness to respond to the plume. Ants carrying large items neglected the plume and continued homing, while ants with small items followed the plume. We do not know whether the ants actively decided to follow the plume or not based on an evaluation of the value of the food item, or whether large items smelled stronger and hence masked the linoleic acid plume. However, regardless of the causation, this behaviour together with the ants' high sensitivity to food odour and their systematic plume search are very likely part of the secret behind the success of this *Cataglyphis* species in the harsh environment of Tunisian salt pans.

Material and Methods

Experimental site and ant species

Field experiments with *Cataglyphis fortis* were conducted during summer 2012 in a dry salt pan near the village of Menzel Chaker, Tunisia.

Odour collection and chemical analyses

In order to identify potential food odours we collected and analyzed the smell of dead insects (approximately 8 small crickets with a body length of 0.5 cm) either by body extraction or headspace volatile collections. For extraction dead crickets were put in 1 ml hexane for 10 min and the supernatant was stored a few days at -20ºC until the GC-MS analysis. In order to get a broad overview about potential odours emitted by dead arthropods we, in addition, collected headspace volatiles. Approximately 8 dead crickets were placed into a glas vial (100 ml) and by means of Super Q adsorbent filters (30 mg; Alltech, Deerfield, IL, USA) headspace odours were collected at room temperature for 8h (0.4 l min^{-1}) using a vacuum pump. Adsorbed volatiles were desorbed by eluting filters with 300 µl dichloromethane (DCM, 99%; Roth). Samples were stored at -20ºC for a few days until analysis. In order to get more desert-like conditions we, furthermore, collected headspace volatiles in an oven at 40 ºC for 1 h by means of Solid Phase Micro Extraction (SPME) fibres (100µm PDMS; Supelco 57341U). All samples were analysed by using gas chromatography (GC; Agilent 6890N) in combination with mass spectrometry (MS; Agilent MS 5975B). The GC was equipped with a non-polar HP5 column (30 m × 250µm × 0.25 µm) with helium as a carrier gas (1.1 ml min^{-1} flow rate). Temperature program was 40°C for 2 min, rising to 300°C (SPME: 280°C) at 10°C min^{-1}, held for 10 min. Selected compounds were identified by their mass spectra in a National Institute of Standards and Technology library search (NIST). Those compounds that could be identified in dead insects by at least one of our sample procedure were later used for behavioural experiments.

Behavioural experiments

A) Recording of natural foraging paths:

The ants' foraging paths from three neighbouring colonies were tracked with a GPS measurement device (GARMIN eTrex 30), i.e. one of us carried the GPS device with an activated path-recording function and followed foraging ants at a distance of about 2 m. For several foraging runs we recorded the wind direction simultaneously by video recording a small wind vane moving over a 360º scheme. GPS data were transferred using EasyGPS. Tracks were plotted in GPSVisualizer. Walking distances of individual ants were calculated in EasyGPS and maximal foraging distance was measured. Foraging success was calculated by dividing the number of ants that successfully found a food item by the total number of tracked foraging runs. We excluded here individuals for which it was not possible to identify whether or not the ant forager had grabbed a food item. Several paths were analysed in more detail considering wind direction. Only successful foraging runs that were tracked in one-second intervals and for which we had the time available where the ant found the food item were taken for the fine-scale analyses. The walking direction at every single tracking point was calculated using trigonometry functions. From the corresponding wind-video recordings we extracted wind direction for all the analysed data points and calculated the differences between walking and wind direction for all tracking points. Circular data were plotted in PAST (version 2.17b) as rose diagrams with abundances proportional to area. We defined following sectors: Downwind (0º ± 30º), upwind (180º ± 30º) and crosswind (90º ± 30º and 270º ± 30º). The remaining values in between were considered as undefined and excluded for the percentage calculations of upwind, downwind and crosswind parts. For the graphical analyses of the individual runs, each one-second interval was colour coded corresponding to its direction relative to the wind. Further, circular data were analysed using Oriana 4. Directionality of the data was examined using the Rayleigh test and mean vector (μ) and length of mean vector (r) were determined.

B) Detection of dead arthropods:

In order to measure how efficiently ants detect dead arthropods, we placed single dead sun-dried crickets (body length, 5 mm) randomly distributed at different distances to the salt lake border (5-300 m) where almost all ant nests were located. We measured the time until the food item was discovered. By changing positions continuously ants could not localize a food item by returning to a learnt feeding site. We compared the discovery times within different areas of the salt pan (i.e. different distances from the border-located nests) by the non-parametric Kruskal-Wallis test with Dunn's multiple comparison tests performed with GraphPad Instat (version 3.06).

By studying the ants' final approach to food items we investigated the role of olfaction in food finding. We selected an area on the salt crust in the flat and open salt pan where numerous ants were foraging. Since ants searching for food and homing ants heading back to the nest could easily be distinguished by their walking behaviour we could ensure that we only tested food-searching ants. A small wind vane continuously informed us about wind direction. We followed individual ants until they switched to the characteristic crosswind runs and then presented the dead insect to individual foraging ants in a way that they passed it either upwind or downwind. We recorded the ants' paths on paper (a grid (mesh width, 1 m) on the ground of the salt pan served as a reference) until the ants either reached the insect or passed it for a few meters.. Walking traces were digitised using Graph Click (version 3.0) and run lengths were calculated by Gedit Graphics Editor and Run Analyser (Khan et al., 2012).

Additionally, we measured the response of food-searching ants when they encountered the food plume at different distances to the odour source in order to get an idea about the functional reach of the odour plume. Again, we tested foraging ants that performed crosswind paths so that we could present the dead insect in a way that the ants encountered the odour plume of the insect. As a control we tested ants without any insect. We marked the position at which the ants encountered the plume and measured the distance to the given odour source. Ants either reached the insect by following the plume or passed the plume without response to it. For analyses, the distances were categorized in three groups (< 2 m, within 2 to 4 m, > 4 m) and the relative frequency of ants following the plume to the source was calculated. The data were analyzed with a chi-square test for trends in GraphPad Instat.

C) Response to food odours:

Body extracts of dead insects and single compounds identified in the blend of dead insects were tested behaviourally. Following odorants were selected (CAS numbers in brackets): indole (120-72-9), nonanal (124-19-6), decanal (112-31-2), palmitic acid (57-10-3), oleic acid (112-80-1), linoleic acid (60-33-3), stearic acid (57-11-4), propionic acid (79-09-4), isovaleric acid (503-74-2), active valeric acid (116-53-0), 3-octen-2-one (18402-82-9), 2,3-butanediol (513-85-9), (E)-2-octenal (2548-87-0) and octacosane (630-02-4). Methyl salicylate (119-36-8) was used as an additional odorant not present in dead arthropods. To deliver the odorant stimulus, 20 µl of diluted compound (1:50 in hexane) was dropped on a piece of filter paper. The paper was placed in an eppendorf tube, which was attached to the tip of an aluminium stick. A thread fixed at the tube informed us continuously about wind direction. As a control ants were tested with the solvent only. Linoleic acid that elicited the strongest response was further applied in different concentrations ranging from 10^{-7} to 10^{-4} dilutions. We again followed ants until they switched to the characteristic crosswind runs and then presented the odorant in a position so that the ants would pass the plume about 1 m downwind of the source. Again, the relative frequency of ants following the plume to the source was calculated for each tested odorant. Fisher's exact test was used to test responses to odorants against the response to the solvent control using GraphPad Instat.

In a further experiment we were interested in the response of homing ants to linoleic acid. Therefore, homing ants carrying a food item were tested in the same way as for foraging ants. After the ants either responded or not to the odorant plume we caught the ants in order to collect their food item. The food items were weighted and the weights of items of responding and non-responding ants were tested using the Mann-Whitney test.

Acknowledgements

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Figure 1: *C. fortis* localizes food items within short time and from far away by its sense of smell. A) Tunisian salt pan inhabited by the desert ant *Cataglyphis fortis*. Top layer of the habitat ground consists either of organic material (1), sand (2), or salt crust (3). Colonies (shown as ‘x’, same colour coding as shown in C) are exclusively situated along the border of the salt pan. **B)** Olfactory-guided food approach. Trajectories of ants (n = 60 ants) passing a food item either downwind (red: positive responses, black: negative responses) or upwind (grey). **C)** Foraging paths of ants from three neighbouring nests (blue, n = 17 ants; red, n = 20 ants; green, n = 14 ants). Walking distances range from 31 to 1259 m with a median distance of 341 m. Maximal distance away from nest, 356 m.

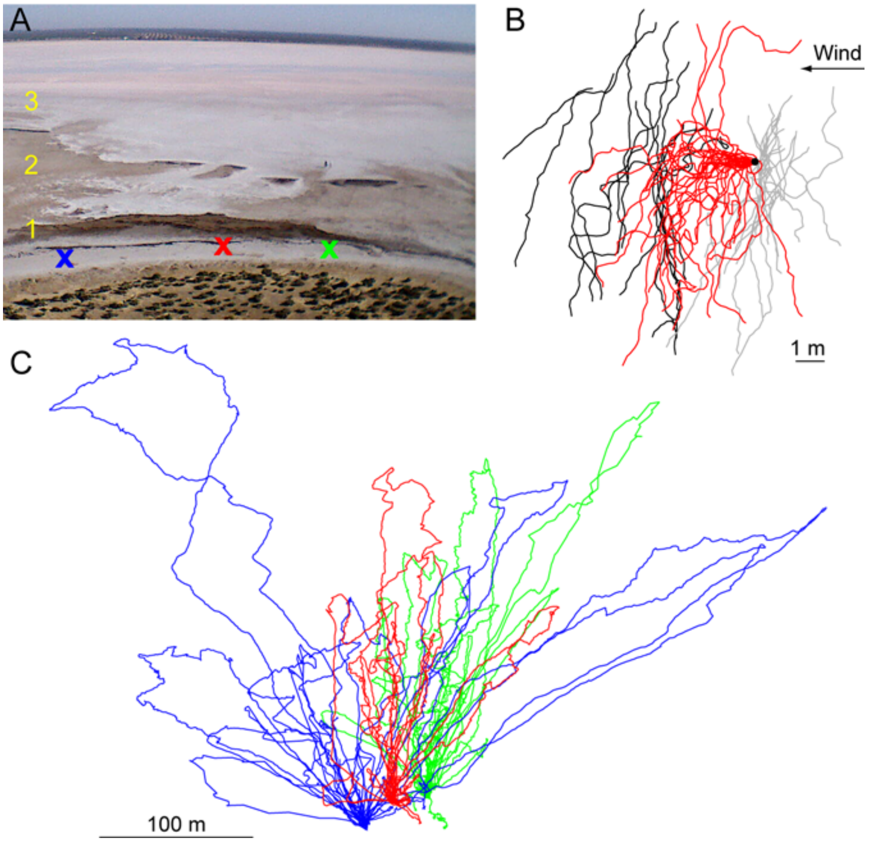


Figure 2: Structure of foraging paths: Extensive crosswind path combined with upwind plume following. A) Fine-scale analyses of 3 trajectories relative to the wind. Analyses are based on differences between walking and actual wind direction throughout the path (tracking intervals, 1 sec). Colour coding: yellow, downwind (difference is 0º ± 30º); red, upwind (180º ± 30º); blue, crosswind (90º ± 30º and 270º ± 30º); white, remaining values . Wind direction is shown as mean direction ± circular standard deviation (i, 261º ± 10º; ii, 277º ± 9º; iii, 258º ± 10º). N = nest; F = food item. **B)** Counts from entire runs shown in B plotted as rose diagrams. Colour coding as described above. Abundances are proportional to area. For calculations of percentages the values of unspecific directions (i.e. directions between upwind and crosswind or downwind and crosswind runs) were excluded. Data are directed (Rayleigh test, $p < 0.05$ each). Mean vector (first value) and length of mean vector (second value) for each run: i: 110º, 0.4; ii: 96º, 0.7; iii: 120º, 0.8.

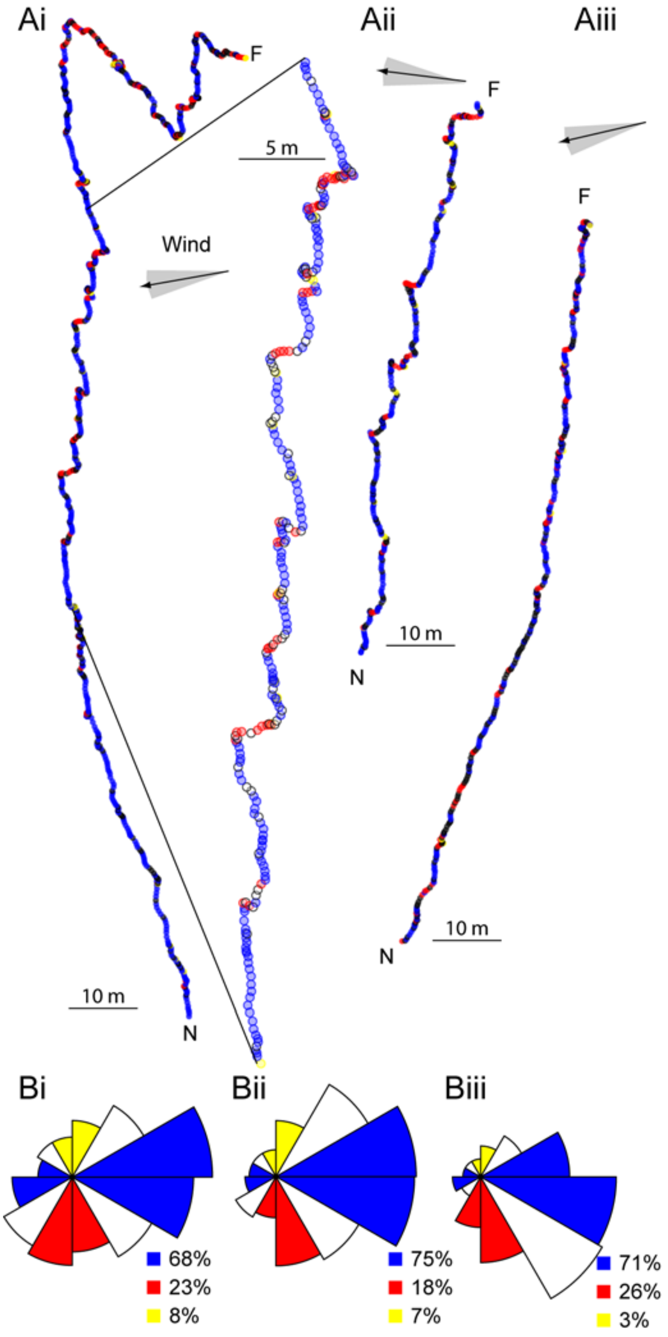
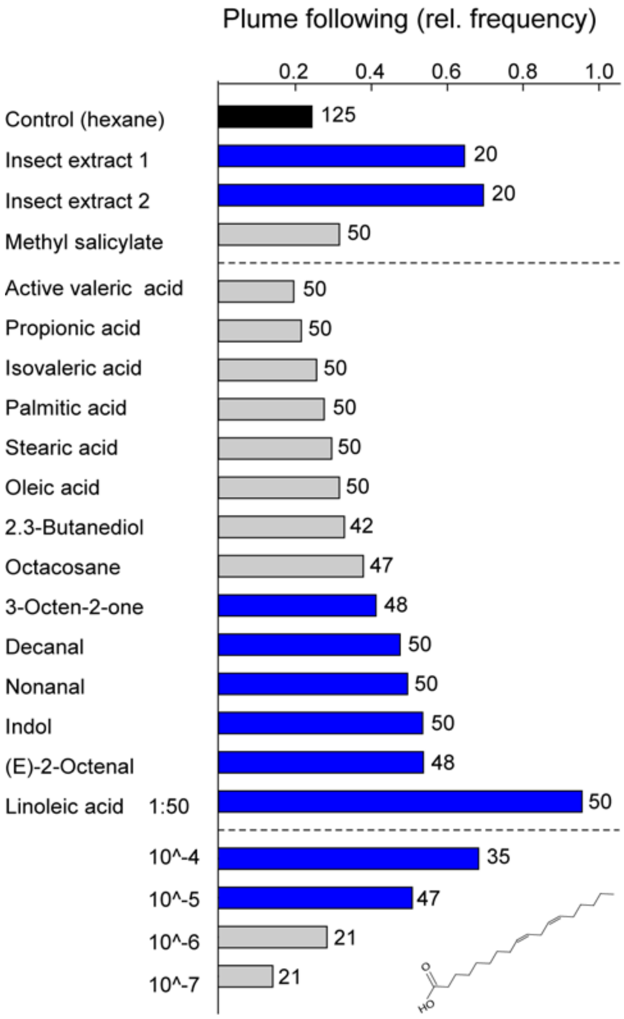
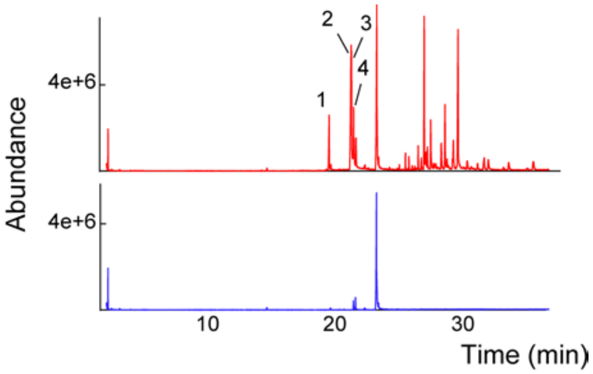


Figure 3: Odorants used for detection of food items: Linoleic acid plays a key role in food detection. Plume following in foraging ants in response to insect extracts and single identified food odorants (dissolved 1:50 in hexane; linoleic acid tested at different concentrations). Frequency is calculated relative to total number of tested ants for each odorant. Sample sizes are shown next to the bars. Fisher's exact tests revealed differences between tested odorants/extracts and solvent control: $p < 0.05$ (blue), $p > 0.05$ (grey).



Supplementary Figure: Smell of dead insects. Example of a gaschromatographic profile of a body extraction of dead crickets (in red) with selected identified compounds chosen for behavioural experiments shown in Figure 3: (1) palmitic acid, (2) linoleic acid, (3) oleic acid and (4) stearic acid and the solvent control (hexane; in blue).



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8. GENERAL DISCUSSION

The main thing an ant forager is doing during its lifetime is to collect food and bring it back to its colony. However, although this seems to be a rather simple and straightforward task, it demands sophisticated spatial skills from an animal that possesses only a tiny brain. When we consider the small size of an ant and the rather huge distances it travels (individual walking distances of up to 1300 m; see manuscript IV), it becomes obvious that an ant needs to have an excellent navigational toolkit in order to perform this tricky task without getting lost and succumb to the desert heat.

In the present thesis, I show that the navigational toolkit of *Cataglyphis* desert ants is extremely flexible, for instance ants readily learn and use sensory input from whatever modality available (manuscript I). Most investigations on desert ant navigation have focused on path integration and visually-guided navigation (Graham, 2010) and we have only recently learnt that the sense of smell is part of the ants' navigational toolkit (Steck, 2012). Due to the fact that desert ants were considered to navigate mainly by means of vision, the ants' ability to learn and use experimentally-provided odours for locating the nest added a new aspect to the field of ant navigation. The question how ants might use odour information derived from the environment for locating resources of interest is of particular interest. Manuscripts II and IV describe that individually foraging *Cataglyphis* ants – either aiming for food or the nest – target the goal by moving upwind along the odour plume emanating from the target of interest and that single compounds of the odour plume are sufficient to elicit this behaviour. However, being able to pinpoint the target by using odour information requires the ability to locate it initially. Contrary to homing ants aiming for a target that has a well-defined position, food-searching ants aim for food that is scattered randomly. Hence, depending on the kind of target they are aiming for – either the nest or food – different navigational strategies are required. Manuscript IV reveals that foraging ants screen the desert efficiently for food by combining a high sensitivity towards food odours and extensive crosswind walks. Moreover, depending on the behavioural context, the sensory input needs to be weighed differently, i.e. an ant should not always go for an attractive odour. While it is mostly beneficial to follow an attractive food plume an ant should absolutely avoid following a nest plume of a neighbouring colony although it smells like the own home. Experiments with ants either heading home or to a plentiful and reliable feeding site reveal that there is a flexible interaction between olfactory information emanating from the source they are aiming for and vector information provided by the continuously running path integrator (see manuscript II and III). Hence, while travelling through the hostile desert, *Cataglyphis* ants use a multitude of cues for navigation with high flexibility.

In the following sections I will discuss the findings of this thesis.

8.1. Multimodal navigation: Multiple cues used for pinpointing the nest

Path integration continuously provides the ants with a home vector pointing towards the starting point of their journey that usually is a rather inconspicuous nest entrance (Wehner and Srinivasan, 2003). Although an ant continuously monitors and integrates its motions and turns in order to have information about its position relative to the starting point, it may not always pinpoint the target accurately, because path integration is based on internal rather than external information and, therefore, is prone to cumulative errors (Merkle et al., 2006; Sommer and Wehner, 2004). Therefore, this mechanism is complemented by the use of further cues that are available in the habitat, i.e. once the path-integration vector has brought an ant close to its nest the entrance is pinpointed by using nest-defining cues. We have an advanced understanding of visually-guided navigation (Zeil, 2012) and we have also learnt recently that desert ants are able to learn and use olfactory cues for pinpointing the nest (Steck, 2012). However, we know little about other sensory modalities that are used for navigation. The findings described in manuscript I add new aspects to the current knowledge of multimodal navigation in insects.

We found that the desert ants' navigational toolkit is extremely flexible with the ants making use of whatever cue is available in order to navigate efficiently (Buehlmann et al., 2012a). We provide the first evidence that *Cataglyphis* ants are able to acquire and use magnetic information for locating the nest (Buehlmann et al., 2012a). The use of the earth's geomagnetic field for spatial behaviour is widespread among animals, e.g. in birds (Wiltschko and Wiltschko, 2005), sea turtles (Lohmann et al., 2012), spiny lobsters (Boles and Lohmann, 2003) and also insects (Riveros and Srygley, 2010; Wajnberg et al., 2010). Although numerous studies in ants reveal that a change of the magnetic field's polarity disturbs the orientation behaviour ((Anderson and Vandermeer, 1993; Banks and Srygley, 2003; Camlitepe and Stradling, 1995; Riveros and Srygley, 2008; Sandoval et al., 2012); but see also (Klotz et al., 1997; Rosengren and Fortelius, 1986)), we have only little knowledge regarding how ants might use magnetic information for navigation. In our experimental paradigm described in the first manuscript, *Cataglyphis noda* ants learnt to shuttle back and forth within an aluminium channel between the nest and a feeding site while the local change of the magnetic field provoked by small solenoids placed at the nest entrance provided information that could be used as a nest landmark (Buehlmann et al., 2012a). The solenoids provoked both a reverse of the field's polarity and a strong increase in the intensity. Hence, the ants could use one or both of these cues for locating the nest. However, it has to be taken into account that the rather strong increase in magnetic intensity may have caused neural activity other than usually happening under more natural magnetic conditions. Similarly, other studies report that animals can be trained to associate a local anomaly of the magnetic field with food and use this information for locating a feeding site (honeybees, (Walker and Bitterman, 1989); pigeons, (Thalau et al., 2007)). The earth's magnetic field is omnipresent and thus provides reliable information that can be used for orientation. When we look at a larger scale, the magnetic field provides compass information but also positional information. For instance, young loggerhead sea turtles perform extensive transoceanic migrations and are able to determine the swimming direction by using local signatures of the magnetic field along their migratory route (Lohmann et al., 2001). These distinct 'landmarks' along their route differ regarding the intensity and inclination of the earth's magnetic field and differences in both of these parameters can be detected by loggerheads (Lohmann and Lohmann, 1994; Lohmann and Lohmann, 1996) and thus be used as positional information. Whether distinct magnetic signatures acting as potential landmarks also exist at the scale of a few meters in the ants' natural habitat remains to be tested.

We were further able to show the ants' ability to learn and use a vibrational landmark for locating the nest (Buehlmann et al., 2012a). Typically, vibrational signals are known to be used in rather different contexts (for reviews see (Cocroft, 2005; Hill, 2001)). Vibration through the substrate is common for communication, e.g. in predator-prey interactions, recruitment to food or mate choice in numerous animals. For instance, leaf-cutting ants make use of vibrational signals to locate buried nest mates (Markl, 1965). Ants stridulate whenever prevented from moving freely and elicit digging behaviour in conspecifics. Also, they recruit workers to a rewarding food source by vibrational information (Roces et al., 1993). Thus, it is not surprising that desert ants can learn to use vibrational cues as positional information for locating the nest.

The results from the first manuscript suggest that when magnetic and vibrational cues are available, ants use the initially meaningless cues as landmark information for locating the nest. Ants may not only need to detect these modalities but they further need to distinguish different intensities since magnetic but also vibrational signals are omnipresent in the environment. Being explored under rather artificial conditions, the relevance of this behaviour for the animal is still questionable. However, although we do not know, whether ants make use of these modalities during foraging and homing through the natural habitat, our results highlight the flexible use of multiple cues for navigation in *Cataglyphis* desert ants.

It is impressive how ants deal with a multitude of cues when it comes to navigation. It seems that any reliable information provided by local landmarks is readily learnt in order to move successfully through the desert environment. Thermal radiation (Kleineidam et al., 2007), ground structure (Seidl and Wehner, 2006) and/or gravity (Vowles, 1954) are further cues that have been reported to be used by ants for navigation. Apart from our newly discovered cues that the ants used for locating the nest, *C. noda* ants, like the well-investigated *Cataglyphis fortis*, also located the nest by using either visual or olfactory cues (Buehlmann et al., 2012a). Knowledge regarding visually-guided navigation when targeting a goal or following a habitual route in insects is quite advanced (Zeil, 2012). Moreover, by combining recordings of visual scenes from the ants' perspective with spatial behaviour we start to get a holistic understanding of navigation by the use of visual cues (Wystrach and Graham, 2012). When it comes to olfactory-guided navigation in individually foraging desert ants, however, we know only little. The role of the sense of smell in freely navigating ants will be discussed in the following paragraphs based on the results presented in the manuscripts II to IV.

8.2. Odour sources of interest in the environment of a desert ant: Aiming for a target by following the odour plume to the source

The navigational toolkit of *Cataglyphis* ants is extremely flexible, for instance ants use sensory input from whatever modality available (see previous paragraph and (Buehlmann et al., 2012a)). One of these modalities is olfaction. Olfactory information is known to be essential for most animals, and especially for insects. Recent studies with *Cataglyphis fortis* provide us with knowledge about the ants' capability to learn and use olfactory information (Steck, 2012). Homing ants approaching the nest are able to pinpoint it by using a learnt odour (Steck et al., 2009) or they are even able to locate the nest entrance after having learnt an olfactory scene composed of multiple odours each with individual spatial relationships to the nest (Steck et al., 2010). Hence, although desert ants do not orientate along pheromone trails they still use their excellent sense of smell for navigation. Equipped with the knowledge that desert ants are able to learn and use experimentally provided odours for navigation (see also (Buehlmann et al., 2012a)) we tackled the question if ants also make use of natural odour sources in their everyday life. Since the main task of an ant forager is to collect food and bring it back to the colony, it is essential for it to find either food or the home efficiently. Do ants searching for food or heading back home orientate by using odour information derived from the environment? How do ants use volatiles for locating resources of interests?

Desert ants prefer to approach a learnt feeding site by positioning themselves in such a way that they are able to approach it by using the odour plume emanating from the feeder (Wolf and Wehner, 2000). When it comes to natural foraging and localization of dead arthropods that are scattered randomly, the question arises how ants deal with this challenging task. On average a food item lays in the desert for less than 3 minutes before it gets picked up by an ant (see manuscript IV). How do foraging ants manage to detect tiny food items distributed randomly that efficiently? The results presented in manuscript IV show that dead arthropods provide chemical information that can be used by ants for food detection. *Cataglyphis fortis* ants detect tiny dead arthropods from far away exclusively by the sense of smell (see manuscript IV). Following an odour plume up to the source is a widespread orientation strategy in many animals for locating resources of interest (Murlis et al., 1992; Vickers, 2000). For instance, vinegar flies and hawkmoths tend to head upwind along the odour plume when approaching a feeding site, mating partner or a host (see introduction). While we have an advanced understanding of odour plumes and how flying insects use them, we know only little in walking insects (cockroaches, (Willis and Avondet, 2005); desert ants, (Wolf and Wehner, 2000)). Therefore, the present findings add new and important aspects to our knowledge about plume following in walking insects. Another strand of research dealing with odour plumes investigates robots that have chemical sensing capabilities (Ando et al., 2013; Ishida et al., 2012; Lytridis et al., 2001; Willis, 2005). Hence, studying plume-following behaviour in insects is of broader interest. Numerous of the identified and behaviourally tested chemical compounds from dead arthropods are fatty acids, often described as so-called necromones, inducing necrophoric or necrophobic behaviour in insects. Social insects such as ants or honey bees remove corpses of dead nest mates in order to minimize the contagion risk (Gordon, 1983; Haskins and Haskins, 1974; Visscher, 1983; Wilson et al., 1958), while isopods, cockroaches or springtails avoid shelters containing dead conspecifics (Nilsson and Bengtsson, 2004a; Nilsson and Bengtsson, 2004b; Rollo et al., 1994; Yao et al., 2009). A recent study further revealed that absence of chemical compounds associated with life rather than an increase in decomposition products (fatty acids) in dead animals triggers necrophoresis (Choe et al., 2009). Food-searching ants were not only attracted by the body extracts of dead insects but also by several single odorants identified within the blend of dead insects. Hence, single food-related compounds were sufficient to induce odour-mediated behaviour resulting in ants following the plume up to the source (for details see manuscript IV). Animals usually do not perceive single odours but behavioural relevant stimuli are typically mixtures of volatiles (see e.g. (Riffell, 2012; Riffell et al., 2009a)). Although single compounds of an attractive blend can be sufficient to elicit either attraction or aversion (see e.g. (Knaden et al., 2012)), they do not always elicit a response (Riffell et al., 2009a). Single compounds of an attractive mixture can even evoke aversion behaviour when presented as single odorants (Webster et al., 2010). Among our identified and tested odorants that provoked plume following in food-searching ants linoleic acid seems to play a key role for the detection of food items (see manuscript IV). Linoleic acid is a stable long-chained fatty acid with low volatility and thus seems to be an ideal attractant under the thermal conditions of the salt lake because of its persistence.

Localization of food is obviously important for an ant and we show that detection of food items by using the sense of smell is quite efficient. Once having encountered a food item, an ant has the challenge to find back home. The second manuscript reveals that homing *C. fortis* ants prefer to approach their nest by following the nest-derived odour plume either rather straight or on a counterturning walking track (Buehlmann et al., 2012b). In moth it is known that they combine zigzagging through an odour plume while heading upwind to the odour source and wide casting movements to search for the odour plume whenever they lose it (David et al., 1983; Kennedy and Marsh, 1974). Zigzag movements when approaching an odour source are further shown in many more animals like for instance cockroaches (Willis and Avondet, 2005), bees (Martin, 1965), dogs (Gibbons, 1986), rats (Khan et al., 2012), birds (DeBose and Nevitt, 2008) and also humans (Porter et al., 2007) (see also Figure 7).

Figure 7: A dog (left) and human (right) using odour information for finding the way through the environment. Scent trail in yellow; track in red (from (Porter et al., 2007); dog path originally from (Gibbons, 1986)).

Ants encountering the nest plume enter the nest faster than ants deprived of olfactory information. Hence, ants seem to profit from nest-derived odours when approaching their nest in terms of entering the nest as quickly as possible. Since the path-integration derived home vector may guide homing ants not exactly to the nest entrance, complementation of the navigational toolkit with olfactory-driven behaviour should be beneficial for ants in the hostile and rather featureless environment of the desert. Surprisingly, homing ants were not only attracted by the own nest odour but also by the odour plume emanating from a foreign colony (Buehlmann et al., 2012b). Therefore, nest plumes do not seem to be nest specific but rather share key odorants that are sufficient to elicit attraction. We could identify carbon dioxide (CO_2) as a component of the nest plume that was sufficient to induce plume following in homing ants when presented in an adequate concentration (Buehlmann et al., 2012b). The role of CO_2 in insect orientation has been studied in various species, e.g. blood-feeding insects benefit from it while locating their host and herbivorous insects use it to steer towards plants (for reviews see (Guerenstein and Hildebrand, 2008; Jones, 2013)). Its behavioural function differs among insects according to the animals' lifestyle. While CO_2 elicits host-seeking behaviour in mosquitoes (Dekker et al., 2005; Gillies, 1980) it evokes aversive behaviour in walking vinegar flies (Faucher et al., 2006; Suh et al., 2004; Turner and Ray, 2009). An ant nest, like colonies from social insects in general, accumulates CO_2 that is a product of respiration and degradation of organic matter, i.e. ants encounter CO_2 concentrations far above the atmospheric value within the nest (ants, (Kleineidam and Roces, 2000); honeybees, (Seeley, 1974)). Early studies with ants revealed that CO_2 is involved in social behaviour, e.g. in localization of buried nest mates (Hangartner, 1969; Wilson, 1962). In manuscript II we provide evidence that *Cataglyphis* ants make use of the emanating CO_2 and follow the plume up to the source when approaching the nest (Buehlmann et al., 2012b). As CO_2 emanates from every nest, nest-plume following is not nest-specific. Along with CO_2 emanating from the nest there may be further volatiles not yet identified. In addition to the volatile chemical compounds emanating from the nest that form an odour plume, there may also be some less volatile chemical compounds that only function in the closest vicinity of the nest entrance. Cuticular hydrocarbons that are used in ants for discrimination between nest mates and non-nest mates (Sturgis and Gordon, 2012) have been described in the nest and at the nest entrance of some ant species (Cammaerts and Cammaerts, 2000; Grasso et al., 2005; Lenoir et al., 2009). It is reported that the nest mound containing hydrocarbons can attract homing ants and serve as home-range marking (Hangartner et al., 1970; Sturgis et al., 2011). However, hydrocarbons are only volatile at a very short range of about one centimetre (Brandstaetter et al., 2008). Hence, although hydrocarbons can facilitate the homing performance in ants, this is only likely in the very close vicinity of the nest entrance. Considering the fact that despite its low volatility linoleic acid works as an efficient food attractant in the desert, it would also be worth investigating the role of less volatile hydrocarbons in the nest vicinity of *Cataglyphis* desert ants.

When investigating the chemical world of *Cataglyphis* ants and its behavioural relevance it is worth to have a look also at the olfactory system of these ants that have adapted to the rather hostile desert environment. While other *Cataglyphis* species inhabit nutritionally much richer low-shrub semi-deserts, *C. fortis* is the only *Cataglyphis* species that inhabits the food-impoverished salt pans in North Africa. The ants have adjusted their foraging strategies to the harsh surrounding resulting in high-speed foraging covering huge foraging areas (see manuscript IV and (Wehner, 1983; Wehner, 1987)) and high nest-site stabilities (Dillier and Wehner, 2004). The hostile environment of *C. fortis* ants might also have led to adaptations in the olfactory system. Stieb and co-workers revealed recently that the antennal lobe of *C. fortis* foragers contains about 200 glomeruli, which is less than found in other *Cataglyphis* species. However, contrary to all other *Cataglyphis* species investigated, *C. fortis* has a single enlarged glomerulus (Stieb et al., 2011b). The size of a glomerulus is largely determined by the number of OSN axons terminating in it (Dekker et al., 2006), i.e. an enlarged glomerulus indicates an over representation of OSN binding sites necessary for a particular odorant that could have a particular relevance for the animal. Macroglomeruli have mainly been investigated in male moths responding to pheromones emitted by females (Dacks et al., 2009). In non-sexual ants they are so far known only in leaf-cutting ants (Kelber et al., 2009; Kleineidam et al., 2005) and involved in the detection and processing of trail pheromones (Kuebler et al., 2010). The function of the enlarged glomerulus in *C. fortis* foragers, for which neither sex nor trail pheromones are relevant, remains unknown. Being equipped with the current knowledge about the chemical world of *C. fortis* ants, the question arises whether this adaptation in the ants' olfactory system enables them to perceive either a nest or food odour more efficiently. The key odorants that pop up are carbon dioxide for finding home and linoleic acid for locating food (see manuscripts II and IV). Since the position of the enlarged glomerulus in *C. fortis* close to the antennal nerve entrance is not in agreement with the position of the CO_2 glomerulus found in other insects (see for instance (Guerenstein et al., 2004)) linoleic acid is a promising candidate to be tested.

8.3. Locating odour plumes of interest

Imagine the situation of an ant forager leaving the nest for foraging. It is a sunny summer day, the sun heats up the desert ground up to over 50 degrees, predators like robber flies, spiders or lizards are waiting for an ant meal and the tiny dead arthropods succumbed to the heat are unpredictably placed somewhere in the hostile salt pan up to a few hundred meters away from the little ant standing at the nest entrance. This is the challenging task a foraging ant has to deal with every time it leaves the nest. Locating a food item by using odour information emanating from the object of interest might be very helpful at close range (see previous paragraph and also manuscript II and IV) but it requires that the ant first reaches the functional space of the odour plume.

C. fortis foragers travel large distances through the desert when searching for food and cover distances up to 1300 m, by that reaching places that are more than 350 m away from the nest (see manuscript IV). It seems that the hostile and food-impoverished habitat demands even longer foraging journeys than observed so far (Buehlmann et al., 2012b; Wehner, 1983; Wehner, 1987). Foragers perform extensive crosswind movements with upwind headings in between (manuscript IV). The combination of far reaching crosswind paths and a high sensitivity towards food odours emitted by dead arthropods (ants can detect a dead insect placed up to 6 m upwind; see manuscript IV) indicates that foraging ants screen huge areas for food. Taking into account the ants' average walking distance (for simplification we assume that the food item is encountered in the middle of the path), the functional reach of a food-odour plume and the percentage of crosswind movements we get an approximate area of 700 square metre that one ant screens in a single foraging run. When we extrapolate this to 10 foraging runs we get an area in the size range of a football field that the ants screen for food sources. Although we also need to consider that foraging ants occasionally cross certain areas more than once it highlights the impressive food search of the ants in the rather food-impoverished salt lake. Hence, instead of performing random search patterns the ants seem to screen the environment for food plumes systematically. In *Drosophila* it has been shown that the flies optimize their search behaviour by adapting flight headings according to wind conditions in order to maximize the chance to encounter odour plumes (Zanen et al., 1994). Under steady wind condition flies screen the environment for odour plumes by flying crosswind, while under changing wind upwind flights are preferred. This behaviour is supported by mathematical models that predict optimal foraging trajectories considering wind conditions resulting in animals moving preferably crosswind, upwind or downwind depending on wind conditions (Dusenbery, 1989; Dusenbery, 1990; Sabelis and Schippers, 1984). Navigation by means of olfaction is known in animals over a range of spatial scales (for review see e.g. (DeBose and Nevitt, 2008)). When looking at a larger scale, wandering albatrosses perform olfactory-guided search when foraging over huge areas of open ocean for sparsely distributed food (Nevitt, 1999; Nevitt, 2008). Corresponding to our findings in desert ants, the birds perform long crosswind flights that enhance the chance to encounter odour plumes (Nevitt et al., 2008), especially plumes of dimethyl sulphide released by phytoplankton that is used as a foraging cue to localize adequate feeding sites (Nevitt and Bonadonna, 2005; Nevitt et al., 1995).

Once having grabbed a food item an ant is occasionally up to over 300 meters away from the nest that was the starting point of its journey (manuscript IV). In order to eventually pinpoint the nest by following the odour plume the ant initially needs to reach the vicinity of the nest. Contrary to ants aiming for food that is scattered randomly, homing ants aim for a target with a well-defined position. On leaving their nest ants continuously monitor their direction and distance from the nest and can use this information to compute a vector from their current location back to the nest (Wehner and Srinivasan, 2003). Hence, by means of path integration and occasionally the addition of visual cues an ant reaches the nest vicinity, where it can pinpoint the entrance by using olfactory information.

8.4. Multimodal interactions: Is it always worth to follow an attractive odour plume?

During navigation, ants need to process input from multiple modalities. For example, they perform path integration, are guided by visual scenes and use their sense of smell for orientation (see introduction and manuscripts). When we consider the ant’s multimodal navigational toolkit and the different navigational tasks it is dealing with, such as finding food or home, the question arises if the ant’s spatial behaviour can be explained by hardwired behavioural responses provoked by environmental stimuli. Should a homing ant e.g. always go for the smell of home and a food-searching ant always for the smell of food once encountered?

An ant having detected a food item and starting its way home will most probably pass neighbouring colonies that smells like the own nest (see Figure 8 and (Buehlmann et al., 2012b)). How does an ant tackle this problematic task?

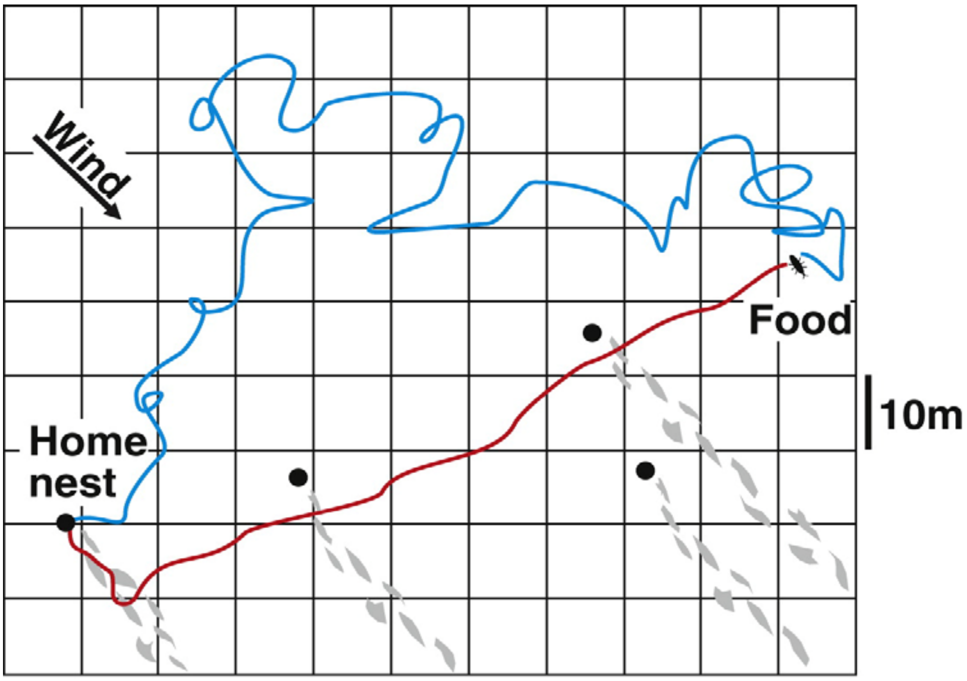

Figure 8: A homing ant encounters nest plumes from foreign colonies (black circles) that smell like the own nest on its way back home. Foodward path, blue; homeward path, red (From (Willis, 2012)).

Ending up in the wrong nest should absolutely be avoided, as following the wrong nest plume is usually lethal. In the case of entering a foreign nest, the risk of getting attacked and killed by the resident ants is very high, e.g. every second forager encouraged to enter a foreign colony did not survive (Buehlmann et al., 2012b). Hence, although *C. fortis* does not seem to discriminate between the odour plume of its own and a foreign nest, the ants can efficiently discriminate between nest mates and non-nest mates. As also shown in other ant species

(Scharf et al., 2011; Stuart, 1991), *C. fortis* foragers show a high level of aggression against non-nest mates, especially in the vicinity of the nest for defending the colony (Knaden and Wehner, 2003; Knaden and Wehner, 2004). It thus seems that trusting a single cue that is not nest specific is rather risky. How does an ant control its behaviour output when it smells an attractive nest odour that emanates either from the own or a foreign colony? Although the ants in our displacement experiments followed foreign nest plumes and entered the nest, freely foraging ants seem to use a counter strategy in order to avoid ending up in the wrong nest and risk getting killed. As the freely walking ants passed the foreign plumes when their own nest was still far away, we hypothesized that their path-integration vector circumvented any response to the foreign plumes. The findings of the second manuscript reveal that homing ants respond to nest odours only when they are close to home. This is crucial, as homing ants often pass through plumes emanating from foreign nests and do not discriminate between the odour plume of their own nest and that of a foreign nest (Buehlmann et al., 2012b). Hence, although the cues provoking plume following do not seem to be nest specific, path integration assures that homing ants do not by chance follow the wrong nest plume and become killed within a foreign nest (Buehlmann et al., 2012b). Shield bugs also perform path integration and locate the burrow by chemical cues. However, in these insects passing the burrow and encountering the burrow plume is sufficient to stop the homing run independent of the state of the path integrator (Hironaka et al., 2007). This behaviour is not critical, since these bugs do distinguish between the own and a foreign home odour.

Our results provide the first evidence of plume following close to the ant's nest and its dependence on the ant's path-integration system. Similarly, other studies have documented this kind of relationship for vision and path integration. Homing *C. fortis* ants only follow a visual nest landmark once they have run off most of their path-integration vector (Bregy et al., 2008). In this ‘final state’ of the home vector nest-defining landmarks can override vector information (Knaden and Wehner, 2005; Sassi and Wehner, 1997) but they never reset it to zero state (Andel and Wehner, 2004; Knaden and Wehner, 2005). Resetting of the path integrator only occurs when the ant enters its nest (Knaden and Wehner, 2006). Path integration and visual learning interact indirectly as ants can use path integration to guide specific learning walks (Muller and Wehner, 2010; Nicholson et al., 1999), which facilitates the learning of visual information. Similarly, ants using path integration in unfamiliar terrain will take consistent and straight paths, thus simplifying the learning of visual information. Thus path integration initially provides a scaffold for landmark learning (Collett et al., 2001; Muller and Wehner, 2010), however, visual cues can later be retrieved independently of path integration (Kohler and Wehner, 2005). It remains to be investigated if ants use the path integration state as a cognitive context during processing of olfactory information or if path integration generates different movements for home vectors of different lengths and this mediates an implicit interaction between cues only at the level of the behavioural output. Furthermore, cue weighing also depends strongly on the habitat an ant species has evolved in or the surrounding a colony has settled down in. When information from path integration and visual landmarks is experimentally set in conflict, ants from visually rich environments tend to rely on landmarks, whereas ants from visually poor environments weigh information from the path integrator higher (Buehlmann et al., 2011; Cheng et al., 2012; Narendra, 2007). Hence, at the behavioural level, cue use has been adapted to the ants' habitat and behavioural ecology.

These results about the interaction of path integration with either olfactory or visual nest cues provide evidence that the path integrator prevents homing ants from following ambiguous nest cues. Although foragers usually do not have stable feeding sites which are worth to return to, in the case of an experimentally provided rich feeding site, ants do return to the position of the rewarding feeding site (Schultheiss and Cheng, 2012; Wolf et al., 2012) by means of path integration. Hence, ants either heading home or targeting a familiar feeding site are equipped with a path-integration vector that points towards the target that is eventually approached by following the odour plume. We have learnt that homing *C. fortis* ants strongly rely on the path-integration vector and only respond to a nest plume when the home vector is run off, i.e. vector information outweighs olfactory information (Buehlmann et al., 2012b). What about attractive food odours that an ant encounters on the way back to the familiar feeding site? The results of manuscript III show that the behaviour of foraging ants heading for a familiar feeding site differs greatly from homing ants. In foraging ants, olfactory information outweighs path-integration information (Buehlmann et al., 2013), which is beneficial as new food sources – unlike foreign nests – never pose a threat but enable ants to shorten distances travelled while foraging. Furthermore, whether or not a plume dominates path integration does not only depend on the context, whether an ant is homing or foraging, but also on previous food experiences of the ant, i.e. ants followed the food plume more willingly, when they had been trained to this kind of food (Buehlmann et al., 2013).

These novel findings add new aspects to our understanding of the interaction of multiple cues. Although using the same navigational mechanisms – path integration and plume following – the ants weigh them flexibly depending on the context. Hence, *Cataglyphis* ants that rely strongly on path integration when travelling through the desert retained the necessary flexibility in the use of path integration. We next asked whether homing ants that do not react to nest odours from neighbouring nests shut down their sense of smell completely until they have reached the vicinity of their nest. Do they avoid responding to any kind of odours or only selectively neglect nest odours when passing by a neighbouring nest? Taking advantage of the highly attractive food odour linoleic acid that provoked plume following in almost all tested food-searching ants (see manuscript IV), we approached this issue. When we exposed ants that already headed home with a dead insect packed in their mandibles to linoleic acid, the food odour still evoked plume following in some ants but not in others (see manuscript IV). Analyses of the food items that were carried revealed that the dead arthropods of the ants that were still responding to linoleic acid were significantly smaller than those of non-responding ants (see manuscript IV). Hence, whether or not homing ants responded to linoleic acid depended on the food item they already had found. However, we do not know, whether the ants actively decided to follow the plume or not based on an evaluation of the value of the food item, or whether large items smelled stronger and, hence, masked the provided plume. Taken together, these findings provide evidence that ants deal with a multitude of information in an impressively flexible manner.

There are other interesting interactions when ants use multimodal information. For instance, the acquisition of bimodal cues (visual and olfactory cues presented together) is much faster than the learning of a single cue (Steck et al., 2011). Bimodal landmarks are first learnt as their individual components but later stored as a unit. Therefore, although the presence of a second sensory cue enhances the learning performance of a unimodal cue initially, the components of the bimodal cue are fused together after several training trials and ants will no longer respond to either of the components presented alone. When it comes to the integration of multimodal cues, we have a better understanding in vinegar flies and hawkmoths (for review see e.g. (Frye, 2010)). Several studies have revealed that multimodal integration enhances performance, for instance in perception (Chow and Frye, 2008) and learning (Guo and Guo, 2005). For flying insects visual feedback is needed for stabilizing an upwind flight (Budick et al., 2007; Reiser et al., 2004), thus plume tracking is enhanced in the presence of visual cues (Chow and Frye, 2008; Duistermars and Frye, 2008; Fadamiro et al., 1998; Frye et al., 2003). While less is known for walking insects, my findings suggest that multimodal interaction in ants is a frequent phenomenon, enabling them to forage efficiently in their harsh habitat.

8.5. Concluding remarks

Cataglyphis ants are impressive navigators equipped with a navigational toolkit using cues from multiple sensory modalities. Moreover, ants deal with different modalities in a rather flexible manner. Questions how ants might use odour information derived from the environment for locating resources of interests have gained interest and added new aspects to the field of ant navigation and animal navigation in general. Hence, *Cataglyphis* ants are not anymore a model system just for studying visual navigation but rather for studying navigation by means of multiple cues. The powerful statement “I think that every animal we look at is a more competent, more robust, more flexible, more miniaturized and a more energy-, material-, sensor- and computation-efficient agent than anything we have ever built," from Jochen Zeil and his following conclusion "So would anyone need more justification for how fundamentally important, intellectually challenging and promising it is to conduct research into the navigational abilities of insects?" (in (Gross, 2012)) points at the great opportunity we have by studying insects.

Picture taken by Markus Knaden

9. SUMMARY

Navigation is a straightforward but tricky task for animals moving from A to B. When returning back home or to a rewarding feeding site they move over a wide range of distances, equipped with different navigational skills depending on lifestyle, habitat, sensory limitations and behavioural context.

Desert ants have become a powerful model system for unravelling the mechanisms of navigation. When travelling through their hostile and food-impoverished desert environment individually foraging ants primarily rely on path integration, an innate navigational mechanism that continuously informs the ants about their position relative to the nest. While becoming experienced the ants in addition rely on learnt information from visual cues. Recent findings reveal that the ants' navigational toolkit is even more diverse and that they use a diversity of sensory modalities for navigation. In the present thesis I show that *Cataglyphis noda* ants readily learn and use magnetic and vibrational cues for pinpointing the nest. Although it remains questionable whether these cues exist in the ants' foraging range as nest-defining landmarks it highlights the ants' flexible use of multiple cues for navigation. Another modality used for navigation is olfaction. The sense of smell is crucial for insects in order to locate food, mates or hosts. Although *Cataglyphis* ants do not orientate along odour trails, they are able to use experimentally provided olfactory cues as landmarks. Due to this new finding the questions of whether and how ants might use environmentally derived olfactory information has gained particular interest. Is odour information in the environment available and can be used by the ants for navigation? In the present thesis I and my coworkers reveal that *Cataglyphis fortis* is extremely efficient in localizing tiny food items that are spatiotemporally unpredictably scattered in the salt-pan habitat. Far-reaching crosswind walks combined with a high sensitivity towards food odours enable the ants to detect dead arthropods within short time and from far away exclusively by means of olfaction. Numerous of the identified and behaviourally tested odours from dead arthropods are known as so-called necromones and our results suggest that linoleic acid is a key compound guiding the ants to the source. Due to being a stable long-chained fatty acid, linoleic acid has low volatility and, hence, seems to be an ideal attractant under the thermal conditions of the salt lake. When screening the desert for food odours, ants cover huge distances and by that occasionally reach places that are more than 350 metres away from the nest. Contrary to food-searching ants homing ants target a goal with a well-defined position – the nest. Path integration enables the ants to return to the close vicinity of the nest. We reveal that homing ants finally pinpoint their nest by following a nest-produced odour plume up to the nest entrance and that carbon dioxide, which we identified in the nest plume, is sufficient to induce this behaviour. Hence, when

pinpointing the nest or a food item, *Cataglyphis* ants benefit from odour information derived from the resource of interest.

Taking all these findings into account ants have to deal with a multitude of cues that provide information about essential things of the world. When travelling through the environment, the ants need to process input from different modalities, i.e. ants perform path integration, are guided by visual scenes and use their excellent sense of smell for orientation. We show that homing ants do only respond to the nest odour when the path integrator indicates that they are close to home. This is crucial as ants do not distinguish between their own and a foreign nest plume, but still repeatedly pass neighbouring colonies during homing. Path integration prevents the ants from following the plume of a foreign nest, in which they would inevitably be killed. Is this dominance of the path integrator hard wired? We provide evidence that the weighing of path integration and plume following is flexible and context dependent. Ants trained to a stable feeder navigate towards this feeder by path integration. However, even when the path integrator indicates that the feeder is still far away most ants respond to food plumes. This is beneficial because new food sources – unlike a foreign nest – never pose a threat but enable ants to shorten foraging distances. Hence, *Cataglyphis fortis* strongly relies on path integration when travelling through the rather featureless environment but retains the necessary flexibility to respond to cues from other modalities.

It seems that the evolutionary pressures of their harsh environment has pushed the ants to use whatever sensory modality available in order to navigate as efficiently and safely as possible towards different resources.

10. ZUSAMMENFASSUNG

Die Fähigkeit, sich erfolgreich orientieren zu können, ist für Tiere von grösster Wichtigkeit. Gerade soziale Insekten, müssen in der Lage sein, zurück zu ihrem Nest oder zu einer Futterstelle zu finden

Wüstenameisen haben ein faszinierendes Navigationssystem, welches es ihnen ermöglicht, sich effizient und zielsicher durch die karge Wüstenlandschaft zu bewegen. Daher sind sie bedeutende Modellorganismen in der Navigationsforschung geworden. Wegintegration ist der basale Navigationsmechanismus, der die Ameisen fortwährend über ihre Position relativ zum Nest informiert. Dadurch ist es ihnen möglich, auf direktem Weg zum Nest zurück zu kehren, sobald sie ein Futterstück gefunden haben. Während die Tiere auch nach langen Ausläufen von ihrem Wegintegrator zur Nähe des Nestes geleitet werden, nutzen sie für die letztendliche Anpeilung des Nesteinganges visuelle Landmarken. Neben Wegintegrator und visuellen Landmarken, verfügen die Ameisen jedoch über weitere Navigationsstrategien. Einige davon bearbeite ich in der vorliegenden Arbeit. Ich zeige, dass *Cataglyphis noda* in der Lage ist, magnetische und vibrierende Landmarken zu lernen und zum Heimkehren zu benutzen. Auch wenn offen bleibt, ob dies zur Nestlokalisation auch im natürlichen Habitat genutzt wird, zeigen unsere Ergebnisse, wie flexibel und vielseitig die Navigationsmechanismen der Ameisen sind. Auch olfaktorische Reize liefern Informationen, welche zur Navigation benutzt werden können. Der Geruchssinn ist nicht nur für die Orientierung bei Ameisen wichtig, sondern für Insekten im Allgemeinen von zentraler Bedeutung, um z. B. Futter, Partner oder eine Wirtspflanze aufzuspüren. Obwohl *Cataglyphis* sich nicht – wie die meisten anderen Ameisenarten – mit Hilfe von selbstgelegten Duftspuren orientiert, konnte vor kurzem gezeigt werden, dass die Tiere in der Lage sind, einen Duftstoff mit dem Nesteingang zu assoziieren und diesen als Orientierungshilfe zu benutzen. Ausgestattet mit diesem Wissen, wurden Fragen zur natürlichen Vorkommnis und Bedeutung von Düften besonders interessant. Welche Rolle spielt die geruchsgesteuerte Orientierung bei Ameisen, die entweder auf der Suche nach Futter, oder auf dem Rückweg zum Nest sind? In dieser Doktorarbeit wird gezeigt, dass Ameisen der Art *C. fortis* tote Insekten, welche unvorhersehbar im kargen Habitat verstreut sind, extrem effizient lokalisieren. Während der Futtersuche, legen die Ameisen extrem lange Distanzen zurück und erreichen Positionen, die über 350 m vom Nest entfernt sind. Grosse Abschnitte der Suchläufe verlaufen rechtwinklig zum Wind. Kombiniert mit einer hohen Sensitivität für Futterdüfte ermöglichen solche Läufe es den Ameisen, die Wüste ausschliesslich mit dem Geruchsinn effizient nach Futter abzusuchen. Viele der identifizierten Düfte von toten Insekten sind sogenannte Nekromone. Aufgrund meiner Untersuchungen konnte ich zeigen, dass Linolsäure eine besondere Bedeutung hat. Diese langkettige und stabile Fettsäure ist ausserordentlich attraktiv für futtersuchende Ameisen. Sie weist eine geringe Volatilität auf und scheint damit ein ideales Lockmittel in der heissen Wüstenlandschaft zu sein.

Im Gegensatz zu futtersuchenden Tieren, welche zufällig gestreute Futterstücke auffinden müssen, peilen heimlaufende Tiere das Nest an. Wir zeigen hier, dass der Wegintegrator die Ameisen zwar in die unmittelbare Nähe des Nests zurück bringt, sie den Nesteingang aber letztendlich durch die Nestduftfahne anpeilen. Der Nestgeruch wird genutzt, um den Nesteingang effizient zu orten. Innerhalb des Nestgeruchs konnte ich CO_2 als für das Heimkehrverhalten ausreichende Komponente identifizieren.

Unsere Resultate zeigen, dass *Cataglyphis* Ameisen mit einer Vielzahl an sensorischen Reizen umgehen müssen. Der Wegintegrator liefert kontinuierlich Information über Distanz und Richtung relativ zum Ausgangspunkt, Ameisen müssen visuellen Input verarbeiten, und geruchliche Reize liefern ebenfalls Informationen, welche zur Navigation genutzt werden können. Wie die einzelnen Reize von den Ameisen gewertet werden ist situationsabhängig. Ich konnte zeigen, dass heimkehrende Ameisen erst auf einen attraktiven Nestduft antworten, wenn der Wegintegrator abgelaufen ist. Dies ist überlebenswichtig, da Ameisen nicht zwischen dem Nestduft ihres eigenen und eines fremden Nests unterscheiden, sie aber vermehrt an fremden Kolonien vorbeilaufen. Der Wegintegrator verhindert somit, dass heimlaufende Ameisen einer Duftfahne von einem fremden Nest folgen und in einem Nachbarsnest enden (wo sie angegriffen und getötet würden). Ist diese Dominanz des Wegintegrators gegenüber von olfaktorischer Information fest verankert? In einer weiteren Studie zeige ich, dass die Gewichtung von Vektor- und Geruchsinformation flexibel und situationsbedingt ist. Ameisen die zu einer verlässlichen Futterstelle trainiert werden, nähern sich dieser Position durch Wegintegration an. Obwohl auch hier der Wegintegrator sie zurück zum angestrebten Ziel bringt, antworten futtersuchende Tiere hier unterwegs trotzdem auf Futterdüfte (werten in dem Fall also Duftinformation höher als die Information des Wegintegrators). Dies ist auch vorteilshaft, da Futter, im Gegensatz zu einer fremden Kolonie, keine Gefahr darstellt. Wir können somit zeigen, dass *C. fortis* Ameisen, die hauptsächlich Wegintegration durchführen, die nötige Flexibilität beibehalten haben, um auf andere sensorische Reize kontextabhängig zu reagieren.

Es scheint, dass es in der kargen und rauen Wüstenlandschaft für die Ameisen überlebenswichtig ist, alle vorhanden sensorischen Reize zu benutzen, um sich so effizient wie möglich orientieren zu können.

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12. DECLARATION OF INDEPENDENT ASSIGNMENT

I declare in accordance with the conferral of the degree of doctor from the School of Biology and Pharmacy of Friedrich Schiller University Jena that the submitted thesis was written only with the assistance and literature cited in the text.

People who assisted in experiments, data analysis and writing of the manuscripts are listed as co-authors of the respective manuscripts. I was not assisted by a consultant for doctorate theses.

The thesis has not been previously submitted whether to the Friedrich Schiller University, Jena or to any other university.

Jena, 23rd April 2013

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