Local homing of the bumblebee, Bombus terrestris

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Local homing of the bumblebee, *Bombus terrestris*

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Erklärung

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Anne Lobecke

Contents

Summary

Bumblebees have been fascinating me since I was a child, running through my parent's garden and following those fluffy, buzzing, flying creatures. I always wanted to know where the bumblebees live, where they come from and return to. But foremost, how they know and recall where they live. My parents, back then able to answer almost all my 'scientific' questions, could just reply with a shrug of their shoulders. This question did not let me off for years and is one of the reasons for the present thesis.

Although bumblebees are no model organism like honeybees, they become more and more common in behavioural experiments. This might be due to some advantages bumblebees have towards their close relatives, at least when it comes to rearing the animals and designing experiments. These advantages will be mentioned in the introduction, but one essential attribute of bumblebees is the inconspicuousness of their nest entrances, which, regarding their homing ability, raise the questions I had as a child: How do bumblebees, as central place foragers, learn where they live and what helps them to find back to this specific place? I must admit that, even years later, I still have never seen a ground-nesting bee such as *Bombus terrestris* (Linnaeus, 1758) actually nest in the ground. Nevertheless, I observed bumblebees nesting behind wood panels of a barn, underneath eaves, in an old, partly hollowed tree and even behind the wainscot of a porch. All places which might have not been easy to recognise.

The present thesis focuses on questions regarding the homing ability of bumblebees, although not in a natural, but an indoor environment. Most previous experiments were field studies, where the circumstances were hardly or not at all controllable by the experimenter. Even indoor experiments often had

additional cues besides the nest hole, which are known to serve as landmarks and might help insects in finding back to a specific place. The novelty in our experiments was the addition of seven dummy holes besides the nest hole connected to the bumblebees' hive, therefore creating an ambiguity of the flight arena and making it hard – if not impossible – for the bees to select the 'correct' hole. As a substitute, we provided the insects with two distinct cylinders, which could serve as landmarks but were the only cue we deliberately provided and could control.

The thesis is composed of three parts, each covering a different, but related main question in the context of local homing in bumblebees.

The **first part** concentrates on the first departure flight – called outbound flight – of naïve bumblebees in a new environment. Our goal was to obtain high-speed camera recordings of the bumblebees' flight manoeuvres, which are assumed to be specially adapted for the environmental situation, therefore displaying a learning behaviour. The analysis of the flights was supposed to reveal a specific flight structure which helps the bees gather information and memorise the surroundings of the nest hole and its precise location. Besides a high variability, outbound flights seem to consist of three different flight phases, which we defined by the flight height of the bee and its distance to the nest hole, respectively. We hypothesised, that the first and second sections of the departure are the most relevant in learning the nest hole and its vicinity.

The **second part** attends the first return flight – called inbound flight – of the still homing-naïve bumblebees which did one departure flight before in the same environment. Again, flights were recorded and analysed regarding mostly the same parameters as in first outbound flights. Since homing algorithms and previous studies suggested a specific behaviour, we compared the flights and the homing success of our bees and discussed several problems and differences to former experiments. Like outbound flights, returns were characterised by a high inter-individual variability. Furthermore, the first inbound flights were less directed to the nest hole, i.e. far not straight on trajectories and consisted of a more loop-like structure than expected. Although the cylinders provided distinct geometrical information, bumblebees did not always approach the nest hole but made ‘homing errors’, i.e. landed at dummy holes on their first return.

The development of outbound and inbound flights and a probable learning process displayed in the flight structure are topics of the **third part**. Since learning and later recalling the nest hole position is essential for central place foragers, there might be a development or even learning process in the flights of bumblebees with increased experience. Therefore, we recorded flight series of the bumblebees which had performed a single outbound and inbound flight before. To make suggestions not only on the colony level, we additionally marked each bumblebee and were able to observe possible inter-individual differences. Besides the parameters we observed for the single flights of part one and two, we also analysed parameters such as duration and velocity of the flight because they might be affected by a learning process. Flight series of outbound and inbound flights were still characterised by a high variability, a loop-like structure and ‘homing errors’. A displacement of the cylinders to a dummy hole showed that not all bumblebees relied on them as directional cues to find back to the nest hole.

Originally, the project was supposed to consist of three separated parts. During the data analyses of part two and three we faced afore mentioned results, which were not explicable with common homing algorithms or behavioural observations in previous studies. Since these findings might be explained by the bumblebees’ nature and the specific experiment’s conditions for both first returns and flight series, we merged both parts and summarised our findings in a combined discussion, where we gave explanatory approaches to integrate the observed behaviour into the biological context.

Chapter 1

Introduction

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1.1 Navigation

The art of navigation fascinates mankind since its early development in India, Egypt and Lebanon, 6000 years ago. As children we wanted to travel to continents far away, explore mystical islands and discover new territories and their cultures. Navigation still charms us with its mesmerisation in adulthood. But in this context navigation is only the genus of a set of techniques, such as celestial navigation, to be used in sea travel and long-distance routes overland. Much longer than for 6000 years, navigation is essential for animals as well. Birds, fish, mammals and insects use navigation for migratory routes, foraging trips and to find mating partners. The utilisation of senses is diverse and ranges from smell and taste via magnetic field detection and sound detections, such as echolocation through to vision.

Visual navigation might be the easiest comprehensible kind of navigation since humans orientate most commonly solely relying on their visual sense. Not only have sailors used the stars at night or beacons such as salient coastlines during the day to navigate. Most of us might be familiar with the scenario, where we could not find back to a specific place, be it a schoolfriend's family home or our car in a large car park. If we did not memorise the number of the floor or the parking lot, we might be desperately lost, although we probably can remember the appearance of our car. But what do searching for a specific house, getting lost at a car park and the navigation of animals have in common? For both, humans and animals, navigation is not restricted to take place on a scale of hundreds of kilometres or metres. During the so-called large-scale navigation, the navigating agent integrates different cues, such as the direction of the goal determined by, for instance, using a compass, and the length of the route. Sometimes, landmarks, such as trees, mountains or buildings are used to navigate in a relative direction to them. Small-scale navigation, in contrast, takes place within a few metres around a target structure and is used to define its exact position in space, e.g. the location of a food source or even our car in a parking structure. Both large-scale and small-scale navigation and the applied strategies are used by most animals and humans in their daily life. Since the current thesis will solely focus on small-scale navigation, the digression about large-scale navigation studies will be very brief. Small-scale navigation is especially common in animals, such as insects, which must return to a feeding site or a nest location. Naturally, insects also use large-scale navigation to find the overall direction of a previously visited food site or a mating ground (Collett and Collett, 2002; Dyer, 1996; Wehner, 1996, 1999; Wolf, 2011). But if it comes to pinpoint a small goal location within several metres or centimetres, small-scale navigation techniques are used (Collett and Collett, 2002; Collett et al., 2006; Wehner et al., 1996; Wolf, 2011; Zeil et al., 2009).

There is a difference between navigation at a food source and at the nest, in form of the location's relevance. An insect normally has one nest in its entire life, where it must return to on a frequent basis. Feeding sites, such as flower patches, are loosely scattered and much more conspicuous than most nest holes (Benitez-Vieyra et al., 2007; Gumbert and Kunze, 2001; Johnson et al., 2003). The flower patch position, thus, is suggested to be less important to remember for a foraging insect (Robert et al., 2018). Returning to a nest location is, however, essential for some social and solitary bee species and other hymenopterans, respectively, because they must provide their offspring with food such as nectar and pollen.

Back to our automobile in the car park: To facilitate remembering its location, some car parks offer colour codes for different floors or areas and provide every parking lot with a specific number. Nevertheless, even without this help, we might find cues, which help us finding back to our car later. We could, for instance, remember the cars next to our own automobile. Although this is not a very reliable cue since this car could have left the car park when we return. Another approach is to remember structures of the car park in the close vicinity: A broken window, a concrete pile with graffiti art on it or the number of parking lots between our car and the stairway. This behaviour is very similar to what is known from insect navigation on a small spatial scale, the so-called 'local homing'. To learn the spatial location of their target, e.g. a food source or their nest, insects use visual memories, acquired during their departure flights or walks from the goal location. Since flying insects are confronted with the need to navigate in 3-dimensional space, they rely on visual information not only provided by different views of the scenery surrounding the location (Collett and Collett, 2002; Collett et al., 2006; Zeil et al., 2009), but especially on visual information generated by the displacement of images on their retina, resulting from structured movements (Dittmar et al., 2010; Egelhaaf, 2009; Egelhaaf et al., 2012; Gibson, 1950, 1979; Srinivasan, 1993).

These peculiar movements, the so-called 'learning flights', could be observed when an insect leaves its nest hole. Since insects cannot use stereopsis due to their small interocular distance, they must use these peculiar movements during their flight to gather depth information about the vicinity of their nest. In contrast to rotational movements, translational movements provide depth information via 'optic flow' (Egelhaaf et al., 2012). Therefore, learning flights, analysed in previous studies, contain a saccadic structure, where translational and rotational movements are often separated from each other (Boeddeker et al., 2010, 2015; Braun et al., 2010, 2012; Geurten et al., 2010; Schilstra and van Hateren, 1999).

Other characteristic flight patters have been observed in wasps and bees during their first departure flights from their nests, during which the insects are assumed to gather relevant information about the surroundings of this specific place (Collett and Lehrer, 1993; Stürzl et al., 2016; Zeil, 1993; Collett, 1996; Zeil et al., 2007, 2009; Braun et al., 2010; Dittmar et al., 2010; Lehrer and Collett, 1994; Lehrer, 1991, 1993; Philippides et al., 2013; Hempel de Ibarra et al., 2009; Collett et al., 2013; Riabinina et al., 2014; Robert et al., 2018; Becker, 1958; Tinbergen, 1932). Furthermore, it is known that navigating insects use salient objects, such as trees, as landmarks, which serve as decision points (Collett, 1996; Collett and Barron, 1995; Collett and Lehrer, 1993; Collett et al., 1996; Collett and Rees, 1997; Fry and Wehner, 2005; Menzel, 2009) or may provide local information with respect to a map-like representation of the vicinity (Menzel et al., 2005; Cruse and Wehner, 2011). Many studies in the context of landmark learning concentrate on large-scale navigation (honeybees: (Capaldi and Dyer, 1999; Capaldi et al., 2000; Menzel, 2009; Menzel et al., 2005) bumblebees(Osborne et al., 2013; Woodgate et al., 2016)). Nevertheless, it is still discussed, which environmental information or features are learned, stored and later used for finding back to the goal location.

Moreover, most studies, concentrating on learning flights in insects, did not concentrate on a development of flights, although, a progression of learning and the consequences for the spatio-temporal organisation of such flights are expected due to observations in, for instance, wasps and honeybees (e.g. Lehrer (1991, 1993); Zeil (1993)). The peculiar movements during the departure, such as arcs (especially wasps) and the so-called 'turn-back-and-look' behaviour, where honeybees turn around when leaving their hive or a food source and face towards the goal location while backing away from it, decrease in their manifestation over several consecutive flights. Nevertheless, experiments with individually marked bumblebees and, therefore, the possibility to systematically record consecutive flight series of departure and return flights and analyse a probable learning progress, are rare (Robert et al., 2018). Just as little do previous studies deliberately exclude directional cues from their set-ups, probably because their experiments pursued another research question than understanding mechanisms of local homing (e.g. Hempel de Ibarra et al. (2009); Robert et al. (2018)). As a novelty in experiments to investigate homing performance, we added seven dummy holes in the floor of the flight arena to create an ambiguous experimental situation for the bumblebees. Furthermore, we eliminated all directional cues, besides two salient cylinders next to the hole, connected to the bumblebees' nest (for further experimental details, see Materials and methods in Chapter 2).

The present thesis investigates several issues of local homing with individually marked, initially naïve bumblebees in an artificial environment. We want to analyse the process and ontogeny of learning. How are the peculiar flight manoeuvres in bumblebees designed and how do they develop with experience when the bees are more familiar with their environment? Which information in the nest hole's vicinity is relevant and learned and, finally, used on the return flight to guide the insect back to its home location? Are two salient cylinders, which might provide the only directional information, a reliable cue for the bumblebees? We further ask, which behavioural strategies are used to acquire the information and how experience and, thus, a learning process, might affect these strategies.

We hypothesise, that bumblebees use a specific movement strategy in the close vicinity of its nest hole to determine the available information to a large extent. Since bumblebees must be flexible in their learning behaviour, due to an unpredictable nest hole environment, the gained goal-centred dynamical visual information and the corresponding behaviour are assumed to be interrelated. The bumblebees should use the cylinders as directional cues and use them to be guided back to their nest hole. After several flights, bumblebees should have become familiar with the static environment and the peculiar flight manoeuvres, suggested to be relevant for the acquisition of spatio-temporal information, should decrease in their manifestation. Therefore, learning and homing section of the flight might change, respectively.

If observations and data analyses came to other conclusions than we expected from previous studies, this thesis gives explanatory approaches to fit the observed behaviour into the behavioural context.

1.2 The bumblebee *Bombus terrestris*

Bombus terrestris (Linnaeus, 1758), the buff-tailed bumblebee, is one of over 250 known bumblebee species in the world and the most common bumblebee species in Europe (Rasmont et al., 2008). *B. terrestris* is a eusocial insect, which means that bees of this species live in colonies, including one egg-laying queen and 300 - 400 individuals. All workers are females and derive from the queen, which is the only reproducing individual during most of the time in the life cycle of a bumblebee's hive. Drones, the male individuals, are born from unfertilised eggs and only fit the role of mating within the colony.

Given the complex systems of mating and sex determination, females in a bumblebee hive are closer related to their sisters than to their own daughters, regarding the number of genes they have in common. A consequence of this phenomenon is that all female workers in a hive of *B. terrestris* care for the eggs laid by the queen and mostly remove eggs of other workers. Moreover, this system, combined with social castes in a colony, ensure that workers defend the colony, raise the queen's offspring and forage for its food. This need to return to the hive after a foraging trip might be one aspect why the bumblebees' popularity in science increased during the last years, at least in experiments in the context of navigation and learning, especially when a series of consecutive trips away from and back to the nest is needed for the experimental design.

Although the aspect above is also true for other hymenopterans, such as the honeybee as a common model-insect, bumblebees have even further advantages towards their close relatives. One aspect of a bumblebee's life, which makes this species particularly interesting in experiments of homing ability in flying insects, is the fact that queens of *B. terrestris* use holes in the ground, such as abandoned mouse burrows, for the construction of their hives. Although this bumblebee species had been observed by using tree stumps, wood panelling and eaves for hive construction. One common feature of all these nesting places is the inconspicuousness of the nest entrance. Since bumblebees are born inside the hive, their initial behaviour when leaving the nest for the first time is, thus, to make a learning flight. From previous experiments in our group – done by Laura Dittmar, Marcel Mertes and Norbert Boeddeker – we know, that bumblebees perform such flights in an indoor experimental set-up as well. In contrast to honeybees, which usually have a very conspicuous, specially marked hive box and must be trained on a reward providing feeder, bumblebees urge to perform a learning flight at their nest hole is an innate behaviour.

Besides the smaller and less regulated colonies, and therefore a much easier separating and handling of individual animals, bumblebee breeding is also commercialised, making them available all year long. In nature, all bumblebee workers and the queen die at the end of the summer, only new queens survive the winter, but they must still hibernate. This general disadvantage of bumblebees is compensated by the fact, that our experiments were conducted indoors in a laboratory environment, where experiments were principally possible all year round.

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Chapter 2

Taking a goal-centred dynamic snapshot as a possibility for local homing in initially naïve bumblebees

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2.1 Abstract

It is essential for central place foragers, such as bumblebees, to return reliably to their nest. Bumblebees, leaving their inconspicuous nest hole for the first time need to gather and learn sufficient information about their surroundings to allow them to return to their nest at the end of their trip, instead of just flying away to forage. Therefore, we assume an intrinsic learning programme that manifests itself in the flight structure immediately after leaving the nest for the first time. In this study, we recorded and analysed the first outbound flight of individually marked naïve bumblebees in an indoor environment. We found characteristic loop-like features in the flight pattern that appear to be necessary for the bees to acquire environmental information and might be relevant for finding the nest hole after a foraging trip. Despite common features in their spatiotemporal organisation, first departure flights from the nest are characterised by a high level of variability in their loop-like flight structure across animals. Changes in turn direction of body orientation, for example, are distributed evenly across the entire area used for the flights without any systematic relationship to the nest location. By considering the common flight motifs and this variability, we came to the hypothesis that a kind of dynamic snapshot is taken during the early phase of departure flights centred at the nest location. The quality of this snapshot is hypothesised to be 'tested' during the later phases of the departure flights concerning its usefulness for local homing.

2.2 Introduction

The necessity of finding a route between the nest and a feeding site characterises a bumblebee's everyday life as well as that of other hymenopterans. Bumblebees hatch inside their nest. When they leave it to forage for the first time, they are completely naïve and unfamiliar with its surroundings. In contrast to the hive of commercially bred honeybees which is often coloured, the nest holes of bumblebees are inconspicuous and hard to find for humans, which makes it even more impressive that bumblebees find the nest entrance after returning from a foraging trip. To accomplish this challenging task, the insect is required to gather sufficient information about the surroundings of the nest hole, suggesting an intrinsic learning program. This learning program should manifest itself in the flight structure of the departure flights immediately after leaving the nest for the first time. However, such a program cannot be expected to be entirely static and stereotyped, as it needs to be adjusted somehow to the specific environmental situation. This situation is unpredictable for the bee when leaving the nest hole for the first time and may differ a lot, for instance, when the nest entrance is oriented horizontally or vertically, or the vegetation next to it is tightly cluttered or, alternatively, only loosely scattered. The learning program might also differ in detail between individual bumblebees from the same hive. However, common flight characteristics, which help all individuals gathering information, should be detectable by analysing the flights.

Characteristic flight patterns, commonly interpreted as learning flights, have been observed in bees and wasps when they are unfamiliar with the surroundings of a relevant place. They then perform peculiar flight sequences after leaving this place, which have been concluded to help the gathering of visual information about the environment near this place. Previous studies describe such learning flights as distinct and relatively stereotyped movement patterns with several common flight motifs. Flight manoeuvres of increasing arcs are characteristic for social wasps (Collett and Lehrer, 1993; Stürzl et al., 2016; Zeil, 1993). Thereby, the insects continually gain height and turn in such a way towards a pivoting point that they keep the retinal image of the goal in the ventral part of the fronto-lateral visual field (Collett and Zeil, 1996; Zeil et al., 2007, 2009). Similar flight patterns were also described for honeybees when leaving a profitable food source. Most of these departure flights contain a high amount of translational movement and a backing away from the target structure, while facing it for a large proportion of time (Braun et al., 2010; Dittmar et al., 2010; Lehrer and Collett, 1994). This behaviour, often termed turn-back-and-look behaviour, was first described by Lehrer (Lehrer, 1991, 1993) for honeybees as part of an efficient navigation system. Bumblebee departure flights from their nest hole show a loop-like structure which differs from the arcing pattern of social wasps and honeybees (Philippides et al., 2013). Bumblebees, rather than performing a turn-back-and-look behaviour, make small excursions away from the nest and then fly back towards the nest region and look at it (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013). These movement patterns might be part of an efficient navigation system in bumblebees that allows the insects to gather, learn and later retrieve the information in the vicinity of their nest relevant for finding the way back to it.

Navigation in hymenopterans takes place on a wide range of spatial scales (Collett and Collett, 2002; Wolf, 2011). Insects determine the direction to the goal (e.g. by using the sun compass) and the length of the segments of locomotion during large-scale navigation (within hundreds of metres or even kilometres). The length of the segments of locomotion might be provided by visual odometry in flying insects or by counting steps in ants (Collett and Collett, 2002; Collett et al., 2006a; Wittlinger et al., 2006; Wolf, 2011). However, route-finding during large-scale navigation may also be based, depending on the habitat and species, on other visual information, such as characteristic views along the different routes to the goal location (Collett et al., 2003; Freas et al., 2017; Narendra et al., 2013; Osborne et al., 2013; Towne et al., 2017; Woodgate et al., 2016). Small-scale navigation or local homing of hymenopterans is assumed to rely mainly on visual cues, such as the spatial constellation of conspicuous objects close to the goal or the skyline of the panorama surrounding it (e.g. (Buehlmann et al., 2016; Collett and Collett, 2002; Collett et al., 2006a,b; Freas et al., 2017; Towne et al., 2017; Zeil et al., 2009). Another visual cue exploited is optic flow: Since stereopsis is not feasible for insects in the spatial range relevant for local homing, they rely largely on visual information from retinal image displacements generated by their structured movements (Dittmar et al., 2010; Egelhaaf, 2009; Egelhaaf et al., 2012; Gibson, 1950, 1979; Srinivasan, 1993)). Translational movement causes close target structures, such as the nest hole at departure and objects close to it, to shift further across the retina than objects further away (Stürzl and Zeil, 2007), which provides the insect with depth information (Lehrer and Collett, 1994). The location of the nest hole in relation to surrounding environmental features, such as vegetation, might, thus, be gathered and memorised in this way (Dittmar et al., 2010; Zeil and Wittmann, 1993).

Despite all these studies, the flight manoeuvres that are essential to find a way back to a specific place are not yet entirely clear. Furthermore, it is still an open question whether the insects learn during the entire first departure flight or only during specific parts of it, for example, when passing the place primarily in translational movement or at the end of an arc. Here, we address these still unresolved problems by analysing the spatio-temporal characteristics of departure flights of naïve bumblebees (*Bombus terrestris*, Linnaeus 1758) after they leave their nest for the first time. Considering that returning safely and fast to the nest is essential for bumblebees, our analysis will rest on the assumption that learning behaviour is the outcome of dynamic interactions between innate behavioural learning routines and visual information about the environment, which is actively shaped by just this behaviour as a consequence of the closed action-perception loop. The intrinsic learning program is expected to manifest itself, at least in a given environment, by a flight strategy with clearly invariant behavioural motifs. Therefore, we searched for invariants across animals in the spatio-temporal characteristics of the flight pattern that allow us to pinpoint the intrinsic behavioural program.

Several studies on local homing concentrated on the organisation of departure flights of bumblebees in semi-natural settings (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014). Since the rich environmental information in such environments can hardly be controlled by the experimenter, we carried out our analysis under laboratory conditions, where the environment is controlled by the experimenter. This indoor setting also implicates that the experimental set-up is restricted in space. Our analyses, thus, focus on small-scale navigation, i.e. on how the animals localise their inconspicuous nest hole when they are already close to it. Complementary studies concentrated on large-scale navigation in honeybees and bumblebees by using radar techniques and analysed on a much larger, but coarser spatial scale how the animals manage to get to the vicinity of their nest (Capaldi and Dyer, 1999; Capaldi et al., 2000; Osborne et al., 2013; Woodgate et al., 2016). Our analyses focus explicitly on small-scale navigation in a local-homing task. For the first time, we recorded, in a systematic way, consecutive sequences of outbound and inbound flights of individually marked bumblebees that have been initially naïve regarding the visual environment of their nest entrance, allowing us to analyse the process and ontogeny of learning. In contrast to several other studies investigating bumblebee flight trajectories (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014), we used two high-speed cameras instead of one to get three-dimensional (3D) data. On this basis, we could reconstruct flight organisation in 3D. The present paper is the first of a series which analyse the entire progression of learning and the consequences for the spatio-temporal organisation of successful return flights to the nest after foraging trips. We conclude the analysed flights to be successful departure flights during which the bumblebees learn sufficient information about their environment, because most of the flights were followed by a return flight which ended at the nest hole connected to the hive (Lobecke et al., in prep.). In this first paper of the series, we focus on the very first outbound flights of bumblebees that are entirely naïve regarding the specific environment in which they forage and attempt to answer the following questions: In which way is the intrinsic behavioural program affected by the specific spatial layout of the surroundings of the nest entrance? How stereotyped is the innate learning strategy and how variable and interindividually different may the behaviour be while still ensuring homing success?

2.3 Materials and methods

2.3.1 Animals and experimental set-up

We obtained commercial bumblebee hives of *Bombus terrestris* (Linnaeus, 1758), containing only a few individuals, from Koppert (Berkel en Rodenrijs, The Netherlands). The beehive was kept within a cubic Perspex box (each side measuring 30 cm) covered with black cloth in a room with a 12/12 h light-dark cycle. A Perspex tunnel connected the nest box to another box of the same size, where the animals were free to fly and had access to an artificial feeder. In the first day after their arrival, the bees had the possibility to learn how to use the artificial feeder filled with a commercial sucrose solution from Koppert, which was one of five feeders used later in the experiments. After one or two days, the feeder was removed for most of the time and only returned to prevent the animals from starving during phases where no experiments were performed. The bumblebees had access to pollen, put directly into the nest box, ad libitum. Another tunnel section from the Perspex tunnel between the boxes led the bumblebees to a PVC tube (inner diameter 20 mm) connected to a hole in the floor of the test arena (Fig. 2.1 A).

The behavioural analysis was performed in an octagonal test arena with an inner diameter of 95 cm, which was placed on a table (Fig. 2.1 B). Each wall segment was 60 cm high and 40 cm wide. The floor of the arena was covered with a red artificial grass carpet (Kunstgras Wereld, Antwerpen, Belgium) to add structure to the ground, but no distinct cues, ensuring a stable flight performance by the bumblebees. Eight holes (3 cm in diameter) were drilled into the arena floor, each placed orthogonally to one of the wall segments at a distance of 22 cm (Fig. 2.1 B).

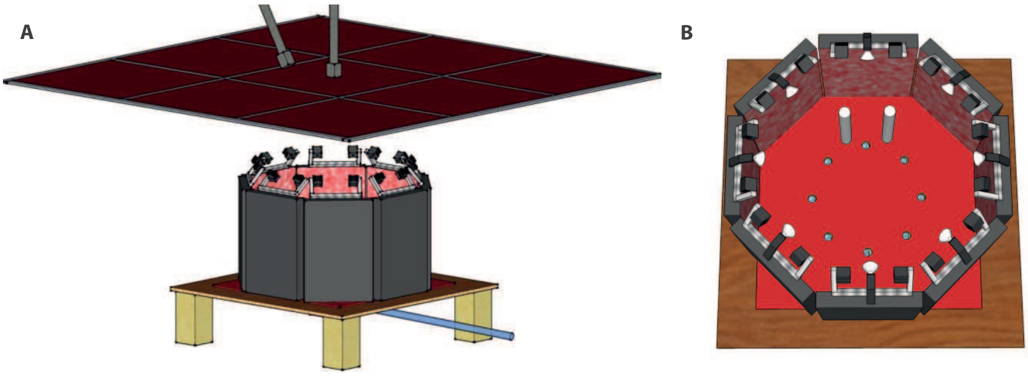

Figure 2.1: **Experimental set-up.** (A) Flight arena seen from the side. Red acrylic glass plate construction above the table with the flight arena. Grey structures above the glass plate construction are the high-speed cameras. (B) Top view into the flight arena with eight holes, two cylinders next to the hole connected to the nest; the other holes were closed a few centimetres below the arena floor. The light set-up consisted of 16 red LEDs (indicated by the grey boxes close to the left and right of each panel of the octagonal arena wall) and eight white LEDs (indicated in white in the centre of each arena panel). The LEDs were mounted on the upper edges of the arena walls.

Throughout the different experiments, only one of the eight holes was connected to the nest. The bumblebees could enter the arena via the PVC tube and started their flights from the nest hole connected. Two white cylinders were placed 10 cm from that hole to indicate its connection to the nest. Apart from these cylinders, the nest hole could not be distinguished visually from the other holes. Regarding the holes in the floor, the arena was symmetrical and provided an ambiguous situation for the experiments. A red acrylic glass plate, 3 m * 3 m, was mounted 40 cm above the arena (Fig. 2.1 A). Only light between 650 and 800 nm could pass through the acrylic glass. Therefore, the bumblebees, able to see light only up to 640 nm (Skorupski et al., 2007), were prevented from seeing the ceiling of the room and the cameras, which were placed above the glass plate (Fig. 2.1 A). Eight white and 16 red LED lamps were positioned symmetrically with respect to the arena centre on top of its walls to provide sufficient light for the camera recordings (Fig. 2.1 B). The luminance at the bottom of the arena varied between 100 and 200 cd/m2. The bumblebees could leave the octagonal test arena and go into a large indoor flight room via the 40-cm gap between the arena walls and the acrylic glass plate. Bumblebees had access to feeders placed on a table in a corner of the flight room. The bees could forage at those feeders, which provided commercial sugar solution, and fly back to the hive. This ready-made solution was mixed with water at a ratio of 3:1. Beige curtains separated the flight area containing the test arena from the rest of the room. Ten fluorescent lamps (Biolux 965, Osram, Germany) illuminated the room (55 – 100 cd/m2). We used Biolux (Osram, Germany) light with a spectrum between 400 and 700 nm to create as natural spectral lighting conditions as possible.

2.3.2 Recording procedure

Bumblebees could be separated by removable doors in the tunnel system, so that only one bee at a time could enter the flight arena. Their outbound and inbound flights were recorded with two high-speed cameras. These cameras (Falcon2 4M, Teledyne DALSA, Inc.) were placed above the acrylic glass plate (Fig. 2.1 A) and recorded the flights of the bumblebees at 148 fps, with an exposure time of 1/1000 s and a spatial resolution of 2048 * 2048 px. The optical axis of the top camera pointed straight down. The optical axis of the second camera was 45° to the vertical.

We recorded continuously for several hours on a hard disk array using the software Marathon Pro (GS Vitec, Germany). Relevant sequences of outbound and inbound flights were stored as 8-bit jpeg images for the flight analyses. Sequences without relevant flights, i.e. where bumblebees just cross the recording area between the upper walls and the acrylic glass plate construction, were discarded. A webcam (AXIS M10 Network Camera) was placed above the feeding table to monitor whether bumblebees were foraging during the experiments.

2.3.3 Training and test procedure

The bumblebees entered the test arena through one of the nest holes in the arena floor. Only one of eight nest holes was connected to the nest during the experiments. We started the recordings immediately we detected the bumblebee at the nest hole. During the training procedure, the two cylinders were placed next to the hole which was connected to the nest and their positions were not changed during the first departing and return flights of each bee recorded. Bumblebees were able to forage at the feeding table during their flights in the flight room. After stopping a recording session at the end of one day, the end of the PVC tube leading to the arena was cleaned with 70 % ethanol to remove potential odour cues placed by the bees. The space available for the bumblebees' outbound and inbound flights was restricted in such an arena. As an advantage of this restricted space, the bumblebees were forced to do the major part of their departure flights in an area which is entirely viewed by the cameras. Consequently, the flights recorded contain no gaps provoked by an open space set-up, i.e. when the bumblebees were not restricted by any walls and could leave the recording area during the first seconds of the flight. Nevertheless, the flight structure obtained under these conditions does not differ in any obvious way from the departure flights obtained in other studies under different environmental conditions (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014).

2.3.4 Data analysis

The image sequences from both cameras were analysed with the custom-built software ivTrace ((Lindemann, 2005) https://opensource.cit-ec.de/projects/ivtools), where the position of the bee and the orientation of its body length axis were determined automatically. Additionally, ivTrace calculated the body orientation (yaw angle) from the top camera images. In some cases, ivTrace had problems to track the elliptical form of the bumblebee's body, and the yaw angle could not be determined automatically. This could happen when a bee crossed one of the nest holes or one of the edges between the arena wall segments. Then, the software could only partially distinguish the bee from the dark background.

In cases in which the automatic tracking procedure failed, the body position of the bee and the orientation of its body long axis were determined manually. The Camera Calibration Toolbox for MATLAB (Jean-Yves Bouguet) was used for the camera calibration and the 3D stereo triangulation. A checkerboard pattern (5 cm per square) was used for the calibration. We determined the difference between recordings by the camera and the calculation. The average position error for the top and the side camera were 0.11 and 0.09 px, respectively. The time-series of body orientation angles of the bees was filtered using a Gaussian filter with a window length of 1.35 ms for some aspects of the analyses. In addition to the yaw angle of the bees' body orientation, several other parameters, for example, height over ground and retinal position of the nest hole, were analysed and compared to characterise the spatio-temporal structure of the flights.

The analysis is based on 21 first departure flights of 21 initially naïve bees with a total duration of 835 s. The three trajectories shown in Figure 2 are selected samples aimed to visualise the range of variability of the first flight across bumblebees.

2.4 Results

This study is based on the assumption that the spatio-temporal organisation of outbound flights of bumblebees after leaving the nest hole for the first time is the outcome of dynamic interactions between innate behavioural learning routines and visual information about the environment. This information is actively shaped by the innate behaviour due to the closed action-perception loop. The astonishing feat that a single departure flight in an unpredictable environment is sufficient for the initially naïve insects to return to their home location is worth investigating in a systematic way. That bumblebees and other hymenopterans gather relevant information about the environment on their departure flights from their nests is plausible as they perform peculiar flight sequences, and the departure flights decrease in duration and complexity with experience (Lehrer, 1991, 1993).

Here, we analyse for the first time systematically what is special about the structure of the first departure flight of naïve bumblebees, interindividually and compared to other flying hymenopterans. Are there invariant motifs in the flight manoeuvres which might be necessary for learning the location of the nest hole? We took a closer look at the flight structure of initially naïve bumblebees in an indoor test arena to find this out.

2.4.1 Description of the overall flight structure

We observed a broadly similar flight pattern in bumblebees as described for social wasps and honeybees (cf. Introduction): The flights, starting from the nest hole, increased in height and distance to the starting point over time, as shown for three example flights in Figure 2.2.

In contrast to the arcs of social wasps and the backing away from the nest hole of honeybees, the bumblebees performed loop-like excursions away from the nest and then flew back towards the nest region, a performance that is reflected in fluctuations of flight height and distance to the nest hole (Fig. 2.3).

This flight characteristic and particularly the characteristic variations in distance to the nest appear to be in accordance with what has been described for bumblebees under outdoor conditions, although these studies did not monitor the height of the animals (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014). Bumblebees faced towards the nest region in large parts of the loops (Fig. 2.4), as do wasps and honeybees for most of the time during the initial sections of their departure flights from the nest hole (Collett and Lehrer, 1993; Stürzl et al., 2016; Zeil, 1993).

After spending some time close to the nest hole, the bumblebees extended their departure flights towards the centre of the arena, where more space is available for their flights. The area between the nest hole and the closest arena wall was mostly avoided by the bumblebees. This suggests that they familiarise themselves with the immediate surroundings of the nest hole during this early part of the flight and take information about the spatial layout of the environment into account in shaping their flights. The bumblebees flew close to the ground most of the time during this initial flight section, with an altitude roughly below 100 mm. After some time, they increased height and distance to the nest hole in loop-like flight patterns covering large parts of the horizontal extent of the flight arena, including the nest hole region.

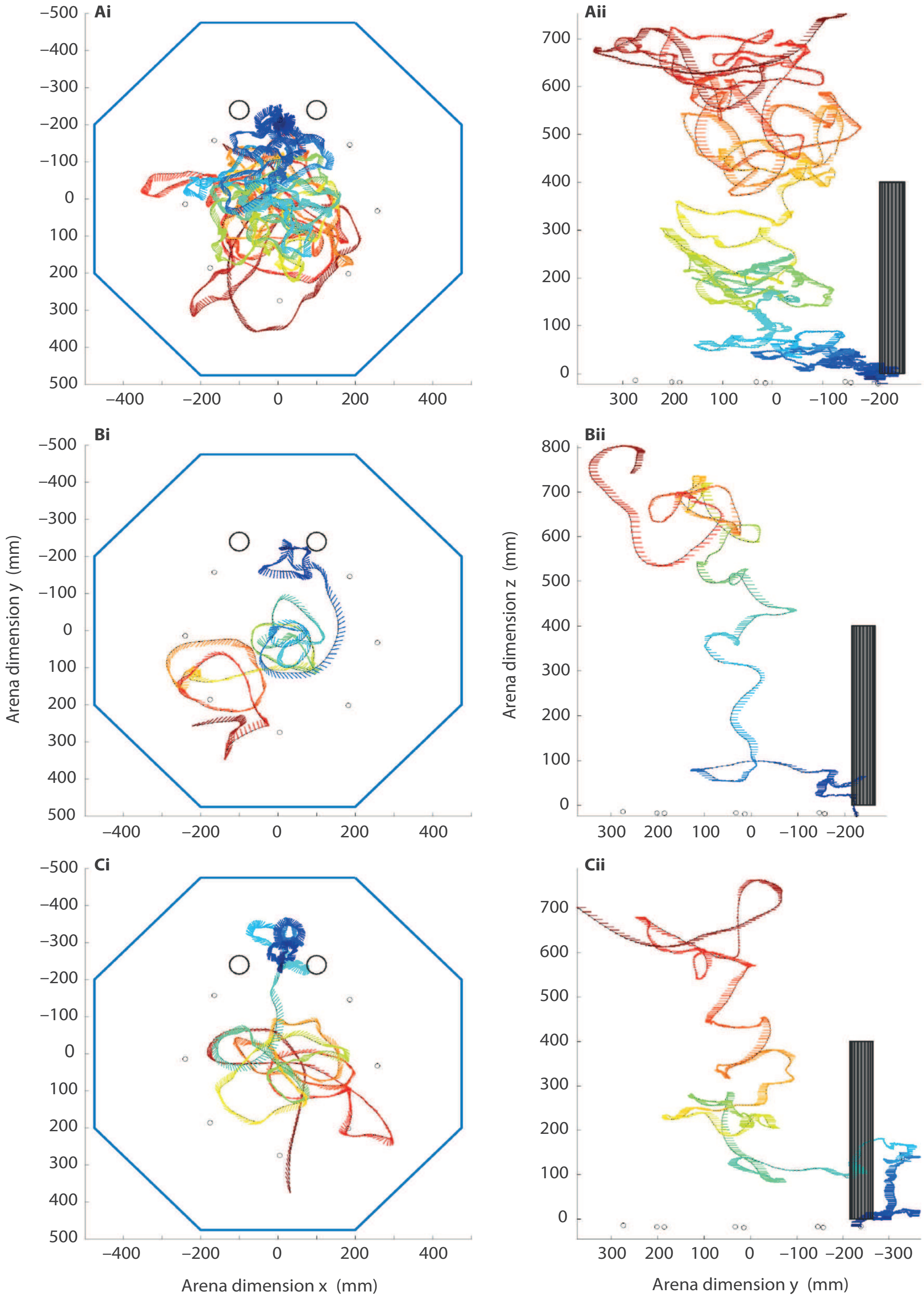

Figure 2.2: **Flight trajectories of the first flights of three different bumblebees seen from above and from one side.** Three example trajectories out of the 21 first departure flights analysed. Grey circles in the top view (Ai–Ci) and grey rectangles in the side view (Aii–Cii) indicate cylinders; coloured lines indicate the orientation of the bee's body-length axis every 20.27 ms; end of lines marks head position; and sequence of head positions defines trajectory. Trajectories are colour coded with time: dark blue indicates beginning of flight and dark red indicates end of flight. Axes scales are given in mm. Coordinates '0.0' represent the centre of the flight arena

Figure 2.3: **Time course of altitude and distance to the nest hole.** Data are shown for initial segments of the same three departure flights as shown in Fig. 2.

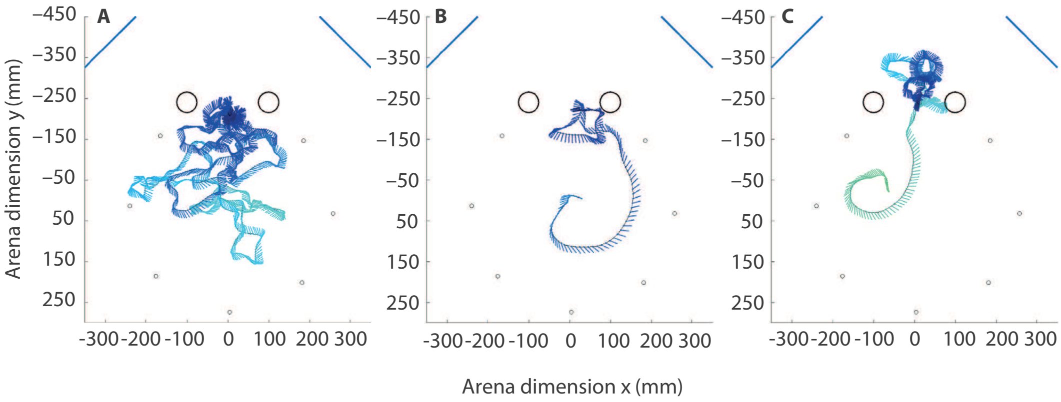

Figure 2.4: **First phase of departure flights.** Top view of initial segments of the same three departure flights as shown in Fig. 2 for flight height above ground below 100 mm (seen from above). Coloured lines show the orientation of the bee's body-length axis every 20.27 ms; end of lines marks head position; and sequence of head positions defines trajectory. Flight trajectories colour coded with time as in Fig. 2. The flight examples show many segments of translational movement.

When the bumblebees reached the height of the cylinders' upper edge at 400 mm, they mostly circled around at this altitude, using the entire arena space. These observations and previous studies suggest that the learning of the nest hole location and its immediate environment occurs during the initial phase of the departure flights. Therefore, we decided to divide the flights into three different phases:

- Phase 1 represents the flight sections below 100 mm above ground level of the arena. This phase may include fluctuations in altitude where the bee's altitude exceeds 100 mm, but then returns to an altitude of less than 100 mm.
- Phase 2 includes the flight sections between 100 and 400 mm altitude, excluding the flight sections where altitudes exceeded 100 mm (contained in phase 1) and including brief flight sections where the bee's altitude exceeds 400 mm, but then returns to an altitude of less than 400 mm.
- Phase 3 contains flight sections exceeding 400 mm altitude, i.e. 400 to 800 mm. Fluctuations which belong to phase 2 were excluded. Flight manoeuvres above a height of 800 mm were excluded from the analyses.

The exact height thresholds defining the three flight phases do not represent altitudes that correspond to any marked changes in flight style and might, to some extent, be arbitrary. Rather, the transitions between flight phases appear to be somehow smooth. However, we ensured that the conclusions we will draw from our experiments are independent of the specific classification into the three flight phases.

2.4.2 Leaving direction from the nest hole

When bumblebees leave their nest hole for the first time, they do not know anything about its specific surroundings. This means that they cannot know in which direction to head for their search for potential feeding sites. Accordingly, the direction of the first departure from the nest hole should be arbitrary, unless the tube leading the bee to the nest hole was in some way asymmetric. Therefore, we analysed whether potential tube asymmetries affected the leaving direction of bees from the nest hole. This was done by subdividing the arena floor around the nest hole into eight 45°-segments and counting the bees entering each segment after leaving the nest hole. The number of 21 initial departure flights available did not allow for a finer segment size as a basis for statistical testing. Only the segment entered first was counted, independent of the segment where the bumblebee started its flight. A Chi^2 test showed no significant deviation from a uniform distribution at a significance level of $p = 0.05$ and, thus, no evidence that the tube properties influence the bumblebees' direction of departure in any strong way.

A similar result was obtained for the direction of take-off around the nest location (Chi^2 test, $p = 0.05$ significance level). These results suggest that the asymmetry in the flight pattern of the population of outbound flights (see next paragraph) is largely independent of the asymmetries in the tube system that leads the bees to the nest hole. Accordingly, the asymmetry in the overall flight pattern of all bees tested was probably caused by the spatial layout of the test arena (i.e. location of cylinders and walls of the arena).

2.4.3 Asymmetry of flight around nest hole

The bumblebees were confronted with an initially unpredictable environmental situation in our experimental set-up, including unequal distances to the eight wall segments of the arena and the two cylinders which we positioned next to the nest hole. As long as the bees did not consider any environmental information when shaping their flights, the overall distribution of flight paths across bumblebees should be symmetrical around the nest hole, because they do not have any reason to prefer one direction, although individual flights might be asymmetric just by chance. Hence, as soon as asymmetries in the overall flight patterns across flights can be detected, spatial information about the surroundings of the nest hole is used by the bees to organise their flights.

The bumblebees' flights shifted towards the centre of the arena after an initial flight phase close to the nest hole, as Figures 2 and 4 illustrate. We scrutinised the flight trajectories in two ways to find out when after flight onset spatial information is employed by the bees: We first divided the arena conceptually by a horizontal line crossing the nest hole to test whether the closest wall influenced the shape of the bumblebees' flights. This line served as a symmetry line for the flight pattern. The range closer to the wall was defined as range 1 and the one towards the centre of the arena as range 2 (Fig. 2.5 A). We expected the bees to spend more time of their flight in range 2, which is the direction to the centre of the arena where more space is available. The time point when the bees started spending more time in range 2 rather than in range 1 is interpreted as the time point when the spatial layout of the arena plays a role in shaping the flights. On average, the bumblebees never tended to spend more time (over 50 %) of their flights in range 1 rather than in range 2. After seven seconds of the flight, they spent an average of more than 75 % of their flight in range 2, the direction to the centre of the arena (Fig. 2.5 B). A binomial test showed a significant deviation from a uniform distribution ($p = 0.001$).

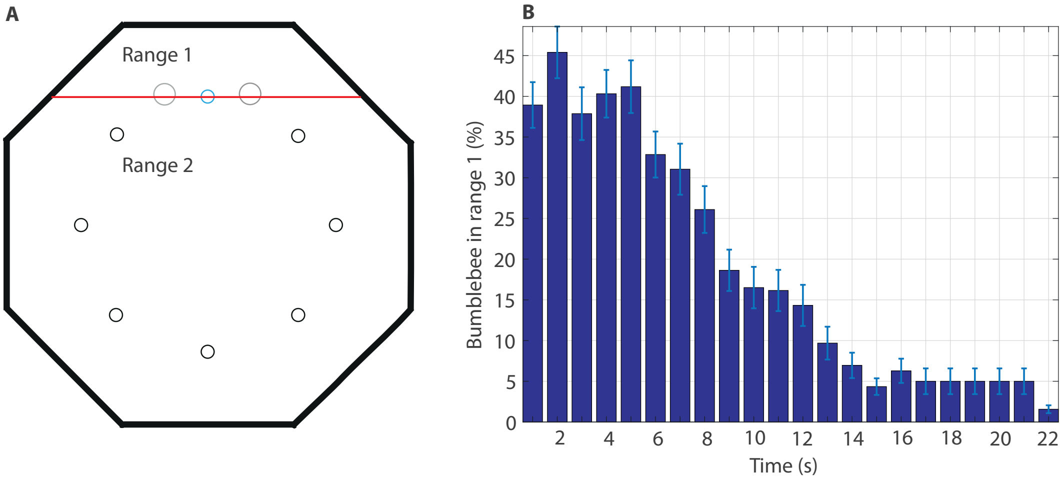

Figure 2.5: **Asymmetry of flight around nest hole.** (A) Arena divided into range 1 and range 2 (red line). Black circles: 'dummy' nest holes, blue circle: connected nest hole, grey circles: cylinders. (B) Percentage of time bumblebees spent in range 1 as a function of time. Time was binned in 1 s intervals. Dark blue bars: mean across bees, light blue bars: standard deviation. N=21 first departure flights.

We carried out further experiments to test directly whether this shift of the flight trajectories towards the centre of the arena is a consequence of the unequal distances to the eight wall segments. In these experiments, we closed all eight peripheral nest holes and opened one nest hole in the centre of the arena, so that all wall segments were at the same distance to the nest hole and the flight structure should not depend on the arena architecture. Now, both ranges covered the same size of the arena: Range 1 was above the horizontal line crossing the nest hole in the centre, while range 2 was beneath it. Although individual flights observed under this condition (n = 8) were still asymmetrical and tended to cover one range of the arena, the outbound flights generally show no preference of one range over the other (data not shown, binomial test, p = 0.05).

Another observation during these control experiments was that individual bees, after they started flying into a given range of the arena, stuck to it until they reached the height of the cylinders (400 nm), and then tended to use the whole arena for the last flight phase before leaving the arena. However, both ranges were chosen with the same likelihood across bees.

We used the same flight data to test whether and after what time interval the two cylinders close to the nest hole shaped the flight trajectories. Two conceptually perpendicular lines across the arena divided the space into four segments, of which two include a cylinder (Fig. 2.6 A). The analyses showed that the bumblebees avoided the segments containing the cylinders during most of their flight time (over 50 %). They spent an average of more than 75 % of their flight time in range 2 after eight seconds (Fig. 2.6 B). A binomial test showed a significant deviation from a uniform distribution (p = 0.001). These results, thus, reveal that after leaving the nest for the first time, the innate learning routines of bumblebees are modified immediately or, at the latest, after a few seconds, by spatial information about the specific surroundings, probably extracted from the retinal image changes actively generated by the behavioural routines.

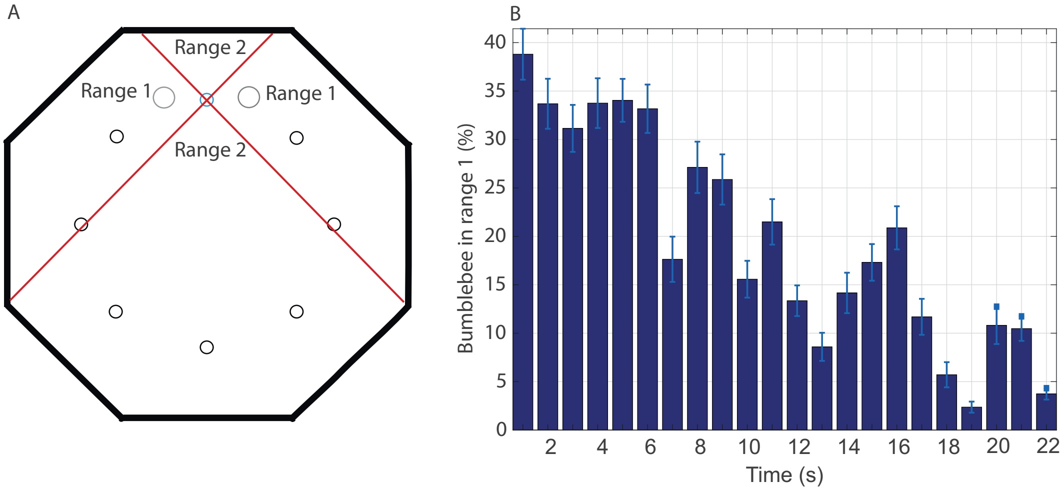

Figure 2.6: **Asymmetry of flight around nest hole in respect to cylinders.** (A) Arena divided into ranges 1 and ranges 2 (red lines). Black circles: ‘dummy’ nest holes, blue circle: connected nest hole, grey circles: cylinders. (B) Percentage of time bumblebees spent in range 1 as a function of time. Time was binned in 1 s intervals. Dark blue bars: mean across bees, light blue bars: standard deviation. N = 21 first departure flights.

2.4.4 Turn-back-and-look behaviour – retinal position of the nest hole

Honeybees perform a so-called turn-back-and-look behaviour, where the bees turn around immediately after leaving the hive and face its entrance during the initial sections of the departure flight (Lehrer, 1991, 1993). Similarly, social wasps keep the retinal image of the target in the ventral part of the fronto-lateral visual field during the initial phase of departure flights (Collett and Lehrer, 1993; Collett and Zeil, 1996; Zeil et al., 2007, 2009). Nevertheless, fixation of the nest hole has been reported to be rather inaccurate, since the image of the nest hole is kept within a rather extended retinal area after the insect has gained distance from the nest (Zeil, 1993). These studies suggest that it might be useful, if not essential, for hymenopterans to look with the frontal part of their visual field at the nest hole and its surroundings at least in the initial sections of the first outbound flight.

A histogram of the retinal nest-hole position was determined to assess whether this also holds for bumblebees, i.e. whether they keep the retinal image of the nest hole in a specific range of the visual field during significant parts of the initial phase of the outbound flights. Figure 2.7 A shows that the nest hole is kept broadly in the frontal visual field between -60° and 60° across the bees tested for most of the time. However, there seems to be no distinct region of the eye where the bumblebees fixated their nest hole. Rather, bees tended to look roughly towards the nest hole and its neighbouring regions for most of the time during the initial phase of outbound flights. This characteristic does not hold if bees gained height during the subsequent flight phases. A Chi^2 test in phase 2 (significance level of $p = 0.05$) showed no significant deviation from a uniform distribution (Fig. 2.7 B). Furthermore, the retinal image of the nest hole was in the rear part of the eye for more time than it was in the frontal visual field in phase 3 (Fig. 2.7 C). This might be a consequence of the structure of flight trajectories in the octagonal arena: Bumblebees used more space of the arena and tended to fly in increasing loops at higher altitudes. The time intervals where the bees face the nest hole region are, therefore, shorter

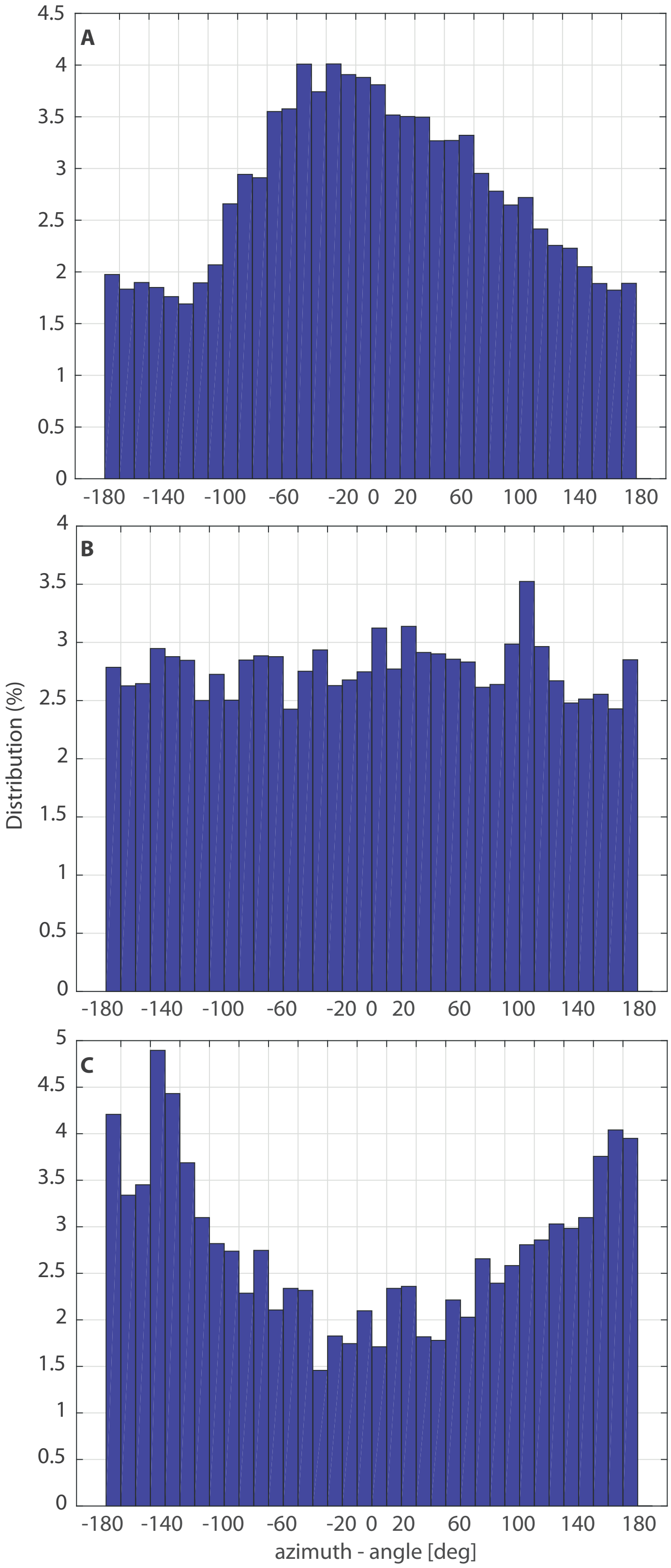

Figure 2.7: **Histogram of the retinal nest hole position.**(A) Flight phase 1 (below a height of 100 mm): the nest hole is kept broadly in the frontal visual field between –60 deg and +60 deg across the bees tested for most of the time of a departure flight. (B) Flight phase 2 (height between 100 mm and 400 mm): no distinct region of the eye where the bumblebees fixated their nest hole. (C) Flight phase 3 (above a height of 400 mm): the retinal image of the nest hole was in the rear part of the eye for more time than it was in the frontal visual field; over 75 % in –180 deg to –60 deg and 60 deg to 180 deg but less than 25 % in the region between –60 deg and +60 deg. Red dashed lines indicate means of retinal position. N = 21 first departure flights.

than the time where the nest whole is seen roughly in the lateral regions and the rear part of the visual field. Furthermore, the nest hole might play a minor or no role at heights above 400 mm. Therefore, the corresponding data cannot be interpreted as an active looking away from the nest hole.

We had a closer look at the first sections of the outbound flights, since the fixation of the nest hole in a broad frontal retinal area plays a significant role in the initial phase. Zeil et al. (Zeil et al., 2009) observed that fixation periods in wasps occur during translations past the nest entrance, mostly during the arcs, where the wasps tend to pivot around the nest entrance (Boeddeker et al., 2010; Zeil et al., 2009). We looked for locations in the flight arena where the bumblebees kept the nest region in the frontal visual field between $-25°$ and 25° to find out whether this is also a characteristic of bumblebees' first outbound flights.

These locations are distributed throughout the whole area covered by the flight trajectories and do not correspond to distinct locations in the arena relative to the nest hole (Fig. 2.8 A-C). The duration of the flight sections when the bumblebees faced the nest region varies for the individual bees as well as across bees, and covers a broad range of time intervals (Fig. 2.8 D).

Durations between 0 and 65 ms might be explained by a full rotation or loop flown by the bumblebee where the nest location crossed the insect's retina inevitably between $-25°$ and 25°. The other large portion of data covers a range between 165 and 550 ms, and we conclude them to be fixations of the nest region in the frontal visual field. We found no systematic relation between the locations of these fixations and the nest region: The flight sections where the bumblebees kept the nest hole between $-25°$ and 25° in their frontal visual field are distributed evenly across the entire area of the flights (Fig. 2.8 E).

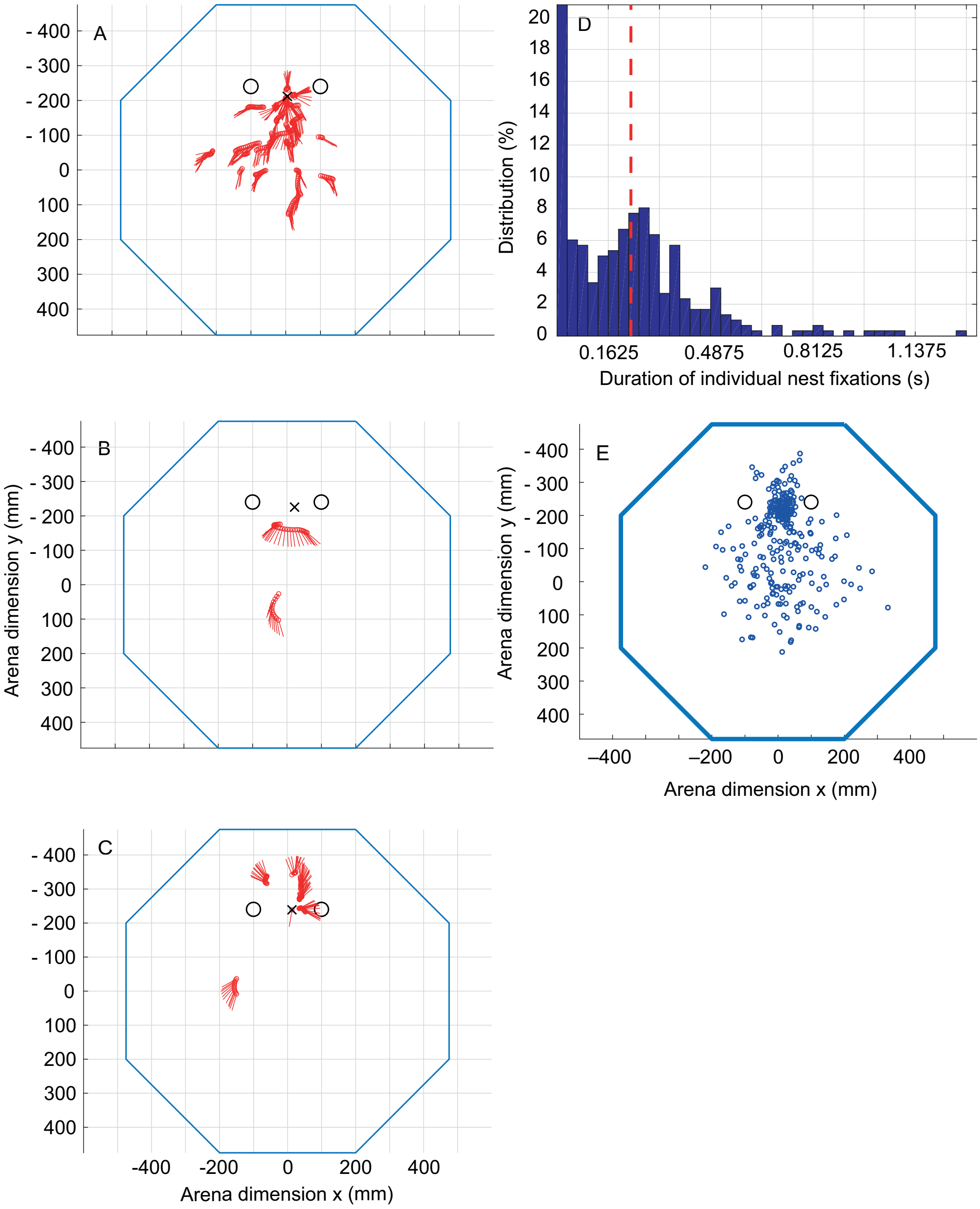

Figure 2.8: **Locations in flight arena where bees fixate nest hole with frontal part of their visual field and duration of fixations.** (A–C) Locations and durations of nest fixations during first outbound flights (same as shown in Fig. 2.2). The position (red dots) and orientation (red lines) of the bumblebee in the arena when the nest hole is in the frontal visual field (between –25 deg and +25 deg) is plotted. Time between consecutive dots is 20.27 ms. Crosses indicate the nest hole; circles indicate the cylinder; arena walls are shown in blue. (D) Duration of individual nest fixations in seconds for all bumblebees in flight phase 1 (below 100 mm). N=21 first departure flights. (E) Locations in flight arena where bees fixate nest hole with frontal part of their visual field. Blue circles show the centre of each individual fixation section for all bumblebees in flight phase 1 (below 100 mm). Black circles indicate cylinders. N=21 first departure flights.

2.4.5 Sideward and forward components of flight

Flying insects, such as bees, perform a saccadic flight and gaze strategy to separate rapid head and body saccades from largely translational intersaccadic locomotion (Boeddeker et al., 2010, 2015; Braun et al., 2010, 2012; Geurten et al., 2010; Schilstra and van Hateren, 1999). This strategy facilitates access to spatial information from the resulting optic flow (Egelhaaf et al., 2012), because only translational optic flow is distance-dependent and contains spatial information.

A sequence of pure translational and pure rotational movements in one flight segment, therefore, might be expected for outbound flights of bumblebees as well. Although there are clear indications in our data for such a saccadic flight strategy (Fig. 2.9 A), the spatial resolution of our video footage was not sufficient, given the chubby shape of bumblebees and the relatively large area that had to be filmed, to address the temporal fine structure of the bees' gaze strategy precisely at the level of body orientation and, especially, not at the level of head orientation. This issue will be tackled in detail in a forthcoming study (Doussot et al., in prep.).

Translational movements can be either forward/backward, sideward or a combination of both (diagonal) without changes in the yaw angle of the body orientation. We determined the proportion of either of these components of translational movements to characterise the overall flight characteristic after leaving the nest hole and, especially, to what extent the bees performed sideward versus forward/backward movements. Flight sections where sideward components are prevalent are particularly relevant when spatial information is extracted from the retinal image flow in the frontal visual field, whereas forward or backward movement facilitates the extraction of spatial information in the lateral field.

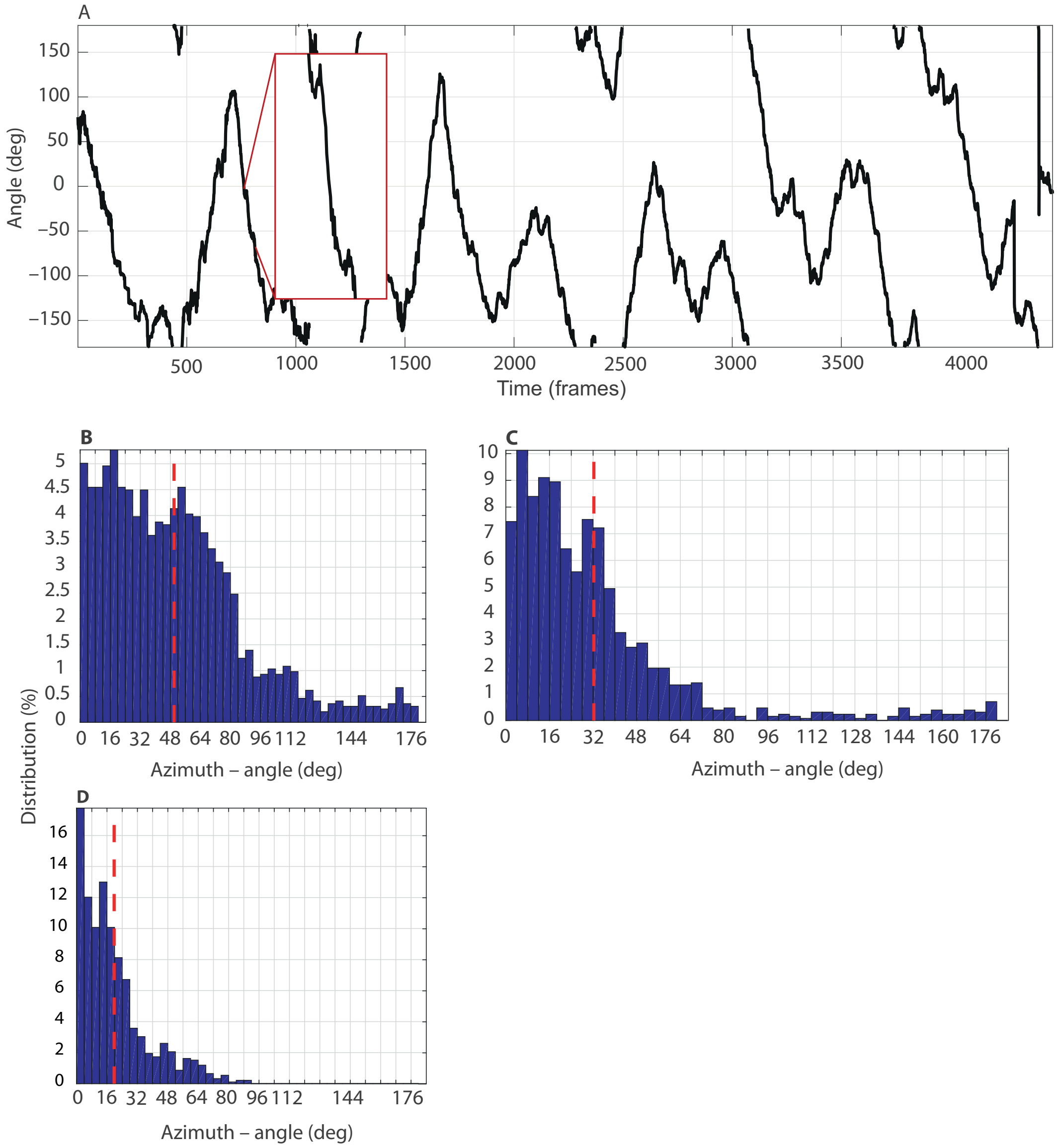

Figure 2.9: **Saccadic flight structure.** (A) Time course of orientation of body-length axis of an example bumblebee on its first departure flight. The red rectangle shows an inset of the orientation to highlight the characteristic saccadic flight structure in an enlarged fashion. (B) Sideward and forward components of flight: distribution of direction of the translational component of motion relative to the orientation of the flight trajectory for all bumblebees for flight phase 1 (B), flight phase 2 (C) and flight phase 3 (D). The angle was determined from the ratio between the forward and sideward components of translation. The average angle is shown in red (dashed lines: 50 deg, 32 deg and 20 deg in B, C and D, respectively). An angle of 0 deg corresponds to pure forward movement and an angle of 90 deg represents pure sideward movement.

Sideward translational components predominated in the flight pattern in the first flight phase, while forward or backward movements were less prominent. This characteristic is specific for the initial phase of departure flights, as the proportion of sideward motion in later phases decreases over time and forward movements dominate the overall translatory flight component (Fig. 2.9 B-D). Flight manoeuvres with large sideways translational components close to a goal location are also known for honeybees (Braun et al., 2012; Dittmar et al., 2010) and hoverflies (Geurten et al., 2010). These sideways movements can be used by the insects to extract relative motion cues to estimate their distance to targets, such as the nest hole, which seems to be relevant in the early learning phase (Dittmar et al., 2010). These observations suggest that the sideward components during the initial phase of departure flights of bumblebees might play a role in gathering depth information in the close vicinity of the nest hole.

2.4.6 Changes in turn-direction (CTD) of the body

Not only translational movements play a role in an insect's flight. Bumblebees perform loop-like excursions from and back to the nest hole during departure flights. Therefore, apart from translational flight sections, the flights show rotations of the bees' body length axis (yaw rotations). The CTD might be particularly relevant, as they indicate decision points in flight behaviour. Such CTD for social wasps are generated at the end of the arcs characterising their departure flights and have been concluded to be elicited whenever the retinal image of the nest entrance moves to a lateral position in the visual field (Collett and Lehrer, 1993; Zeil, 1993; Zeil et al., 1996, 2007, 2009). The CTD, thus, lead to a correction of the accumulating retinal position error of the nest entrance (Zeil, 1993).

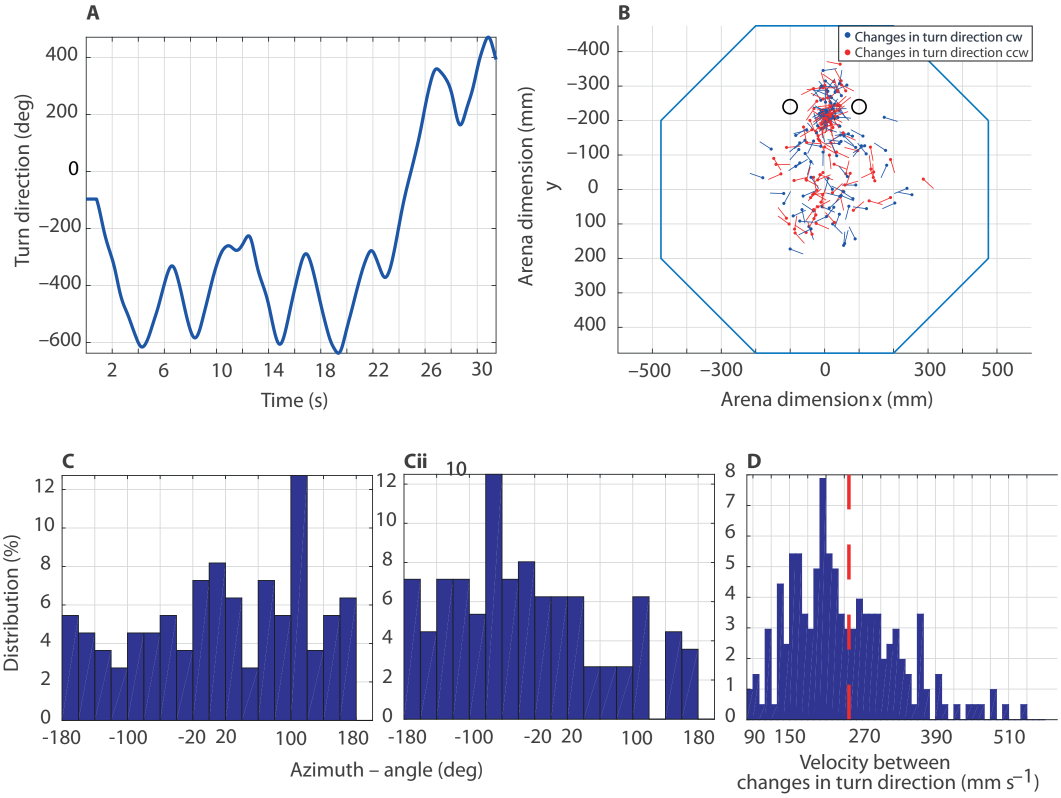

Figure 2.10: **Changes in turn direction of body orientation.** (A) Turn direction of body orientation of a bumblebee's first departure flight as a function of time. (B) Locations of changes in turn direction from clockwise to counter-clockwise and vice versa. Clockwise and counter-clockwise turns for the first departure flight of all bumblebees in phase 1 (below 100 mm). Black circles indicate cylinders. N=21 first departure flights. Bumblebee 'architecture': filled circle, head; line, orientation of body-length axis. (C) Retinal position of the nest at clockwise and counter-clockwise changes in turn direction (CTD) for the first outbound flight below 100 mm, (Ci) clockwise CTD, number of CTD is 106, (Cii) counter-clockwise CTD, number of CTD is 107. N = 21 first departure flights. (D) Flight velocity between CTD for the first outbound flight, below 100 mm, number of CTD is 195. Dashed line indicates mean of velocity. N = 21 first departure flights.

Inspired by these observations, we took a closer look at the CTD of body orientation of bumblebees. The bees' body orientation shows an alternating sequence of clockwise and counter-clockwise rotations (Fig. 2.10 A). We analysed whether the reversals of turning direction are generated in specific spatial regions in the arena relative to the nest hole to get hints as to what environmental cues (i.e. the cylinders, the edges between arena walls or the nest hole itself) might trigger these changes. The locations where the bees perform CTD seem to be randomly distributed across the entire flight area during the initial phase of departure flights (Fig. 2.10 B).

Nevertheless, we observed a tendency for more clockwise CTD when the nest hole was on the right side of the bee and more counter-clockwise CTD when the nest hole was on the left side (Fig. 2.10 Ci and 2.10 Cii). This linking might reflect attempts of the bee to keep the nest hole region in the frontal visual field, performing a body rotation towards the nest when it leaves the fronto-lateral field. These attempts are performed in a similar, though not as precise a way as has been concluded for wasps (Zeil, 1993; Zeil et al., 1996, 2007, 2009). This flight pattern disappears during later flight phases where the nest hole region might only play a minor or no role in shaping the flight (data not shown).

Zeil (Zeil, 1993) described a surprisingly constant rate of the CTD for wasps. We observed an average period of 1.6 s for the overall flight for bumblebees. Furthermore, we did not find any specific differences in the frequency of CTD for the different flight phases. Since the distance covered by the bee between CTD increased with altitude, the flight velocity during the turns increased accordingly (Fig. 2.10 D). This shows that bumblebees in our experiments seemed to have a specific frequency range in which they performed the CTD. However, this range did not appear to be much affected by the bees' position in the arena. Rather, a CTD seemed to be initiated after a broadly constant time interval rather than a specific flight distance.

2.5 Discussion

Bumblebee foragers are confronted with unfamiliar and largely unpredictable surroundings of their nest hole on their first outbound trip. Therefore, they need to gather sufficient information about these surroundings before they leave the vicinity of the nest hole to be able to find it again after a foraging trip. This implies a kind of innate learning program that controls, at least, the learning behaviour after a forager bee leaves the nest hole for the first time. The diversity of environments, however, makes it essential for the assumed innate learning program to be flexible to adjust it to the specific surroundings.

Previous studies propose that insects take some kind of panoramic information from the target location after leaving their nest. What information about the environment is stored and recalled on the return flights is still, to a large extent, an open question, as there is evidence for a wide range of possibilities. Representations about the environment might be based on a panoramic retinotopic snapshot of brightness values (Kollmeier et al., 2007) or of local motion values ('motion snapshot'; (Dittmar et al., 2010)). It might also be based on a more parsimonious representation, such as the skyline of the horizon (Baddeley et al., 2011; Basten and Mallot, 2010; Graham and Cheng, 2009; Kollmeier et al., 2007; Philippides et al., 2011; Wystrach et al., 2011).

The information stored at the goal location is assumed to be compared in an appropriate way with the corresponding environmental information taken during the return flights to the nest. One way to accomplish this is to determine the similarity of retinotopic representations of the environment and to move in a way that increases the similarity (Cartwright and Collett, 1987; Vardy and Möller, 2005; Zeil et al., 2009). Another possibility is not to store the information on a retinotopic basis, but to determine an average landmark vector. The average landmark vector is just the sum of vectors representing, for instance, the average brightness across the elevation at each azimuthal position, or of the vectors pointing to 'landmarks' identified in the retinal image. Landmarks might be simple environmental features, such as trees. The goal direction during the return flight is determined according to this scheme at any location as a difference between the average landmark vector previously determined at the goal location and the vector determined at the current location (Lambrinos et al., 2000). This kind of mechanism could be shown in model simulations to be sufficient to account, within a catchment area, for local homing, i.e. for the return of the agent back to its goal (Lambrinos et al., 2000; Mölller, 2000; Stürzl and Mallot, 2006). The size and shape of the catchment area depends on both the environment and the local homing mechanism. Note that all these mechanisms referred to above only account for local homing, i.e. finding the location of a virtually invisible goal within the vicinity defined by its catchment area.

Local homing only represents one phase, though an important one, of the navigation behaviour of bumblebees, and many ant species. Navigation takes place on a large range of spatial scales, as has already been addressed in the Introduction, and, accordingly, a variety of mechanisms of route-learning partly combined with odometry have been proposed that help the animals to find their routes back into the immediate vicinity of the goal, where the local homing mechanisms take over (e.g. Baddeley et al. (2012); Capaldi and Dyer (1999); Capaldi et al. (2000); Collett et al. (2014); Dewar et al. (2014); Knaden and Graham (2016); Müller and Wehner (2010); Müller et al. (2018); Narendra et al. (2013); Osborne et al. (2013); Woodgate et al. (2016).

All the models mentioned for the explanation of local homing in insects, i.e. their final approach to their goal completing a longer excursion, have in common that the information that is later used for returning to the goal is goal-centred, i.e. gathered locally at the goal location. These explanatory models, although they can explain local homing, seem to be somehow in disagreement with the concept of learning flights, where the insect is thought not to gather the relevant information just at the goal location, but during the entire initial flight section (e.g. corresponding to our flight phase 1) after leaving the nest hole.

On the one hand, it might be plausible that insects learn during the entire initial section of departure flights from the goal, because of the animal's heading direction during such flights: Wasps (Collett and Lehrer, 1993; Stürzl et al., 2016; Zeil, 1993), honeybees (Dittmar et al., 2010, 2011; Lehrer, 1991, 1993; Lehrer and Collett, 1994) and bumblebees (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014) tend to orientate towards the goal location, such as the nest hole or a food source, for quite some time during the initial sections of their departure flights.

On the other hand, why should insects spend energy and time to perform a complex sequence of movements to gather information near their goal if one single goal-centred panoramic is already sufficient for a successful return? This issue is further accentuated not only by the high degree of interindividual variability in the individual flight patterns of bumblebees as characterised here, but also between consecutive outbound flights of individual bees (Lobecke et al., in prep.), although there are obvious differences between different hymenopteran species in this regard (wasps: (Collett and Lehrer, 1993; Zeil, 1993), honeybees: (Lehrer and Collett, 1994).

The variability of the initial phase of outbound flights across bumblebees was investigated systematically in the present study: Although the overall flight structures differ tremendously between individuals, there are still common behavioural motifs in almost all outbound flights. Bumblebees leave the nest hole and spend the initial sections of departure close to the goal. They also roughly keep the nest hole region in their frontal visual field during periods in this initial section of the departure flights. Although the corresponding flight sections reveal a consistent spatial relationship to the nest hole and its vicinity, they are broadly spread in space in individual flights.

After some time, the bees increase height and distance to their nest hole by performing loop-like manoeuvres. Thereby, the overall flight trajectories shift towards the centre of the flight arena. Although we cannot exclude that the spatio-temporal details of the initial phase of departure flights may depend on the specifics of the experimental set-up (e.g. its ground texture), our findings are in accordance with what has been described for bumblebees in other experimental settings, including semi-natural surroundings (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014).

The retinal location of the nest hole during the initial sections of departure flights might also play a role as a kind of trigger in bumblebees for changing the turn-direction, as has been proposed for solitary wasps (Stürzl et al., 2016; Zeil, 1993). However, the pattern of locations of changes in flight direction is highly variable in bumblebees: These locations may be almost anywhere in the flight arena covered by the flight trajectories. Additionally, the fine structure of the flights does not reveal obvious similarities between different bumblebees' flight manoeuvres. Since the environment was kept constant in our experiments, this high variability can hardly be explained by the flexibility needed for an innate behavioural learning program and the adaptivity of individuals to specific unpredictable environmental situations.

The spatio-temporal characteristics of departure flights and, especially, the non-existence of a consistent pattern in their fine structure and the great interindividual variability led us to a new hypothesis regarding the functional significance of the departure flights. We hypothesise that bumblebees gather information only during the very initial section of the flights, while they are still very close to the goal. In this section, it is suggested they determine a dynamic representation of the surroundings as seen from a very small region around the goal ('goal-centred dynamic snapshot') that determines the catchment area in the vicinity of the nest hole.

The later flight sections of phase 1 of the departure flights (according to our classification explained in Results) are then hypothesised to be employed to probe the quality and usefulness of this goal-centred information and the catchment area around the nest location. This means that the bumblebee moves in a loop-like manner to somehow 'simulate' a return flight to the nest after the foraging trip to find out whether the information provided by the goal-centred dynamic snapshot is sufficient to eventually find the nest hole again. This behaviour might, thus, also be understood as an information gathering process, however, not information about the nest hole location itself, but rather the reliability of the goal-centred dynamic snapshot and the validity of the catchment area. A similar hypothesis has been proposed by Stürzl and Zeil (Stürzl and Zeil, 2007). They suggested that the behaviour during acquisition of a visual representation of the environment around the goal might reflect a need for 'quality assurance' and the insects, therefore, may continuously check by moving and comparing whether the representation they have acquired is robust and informative enough for a successful return.

According to the hypothesis above, the initially naïve insects at the very beginning of their first outbound flight might gather information about the surroundings of the nest entrance only very locally, i.e. from a nest-centred perspective, rather than during the entire phase 1 of the departure flights. Still, they might not take only a kind of stationary panoramic snapshot, as is usually assumed in local homing models (see above). Bumblebees are assumed to have to move in the close vicinity of the nest hole: They need to turn around to get panoramic information about the environment. These rotations should be interspersed with brief translational flight intervals (e.g. intersaccadic intervals) if the animal also needs to extract information about the spatial layout of the environment from the perspective of the nest hole. All this information might then be combined into a goal-centred representation of the behaviourally relevant environmental information. This information may then be employed as a basis of some local homing mechanism (see above). Further experiments are required which focus on the very initial phase of the departure flights while the bees move very close to the nest location; a high spatial resolution is then required to allow us to resolve both body and head orientation in greater detail. This detailed analysis in not yet possible on this basis, since our current analysis covered the entire departure flights.

Overall, our hypotheses suggest that – in accordance with the common local homing models (see above) – goal-centred information is sufficient to guide the insect back to its home location on the return flights. If this were correct, the interindividual variability observed in the overall flight patterns would not be deleterious, because most of this part of the departure flights was not a component of a learning routine, but would just serve to probe the catchment area. This can, in principle, be done either systematically or by a somehow random procedure. This issue needs to be tested in further modelling analyses. In any case, as a consequence of such a scheme, the variability of departure flights is probably not to be the outcome of some kind of noise originating at any information processing stage in the nervous system, but part of a strategy probing the usefulness of the information acquired before at the goal location.

Upcoming studies investigating the initial learning behaviour in hymenopterans must be designed in a way to test whether the phases after the initial sections of departure flights serve as a measure of the reliability of the catchment area using goal-centred dynamic information about the goal environment, actively gathered very close to the goal location.

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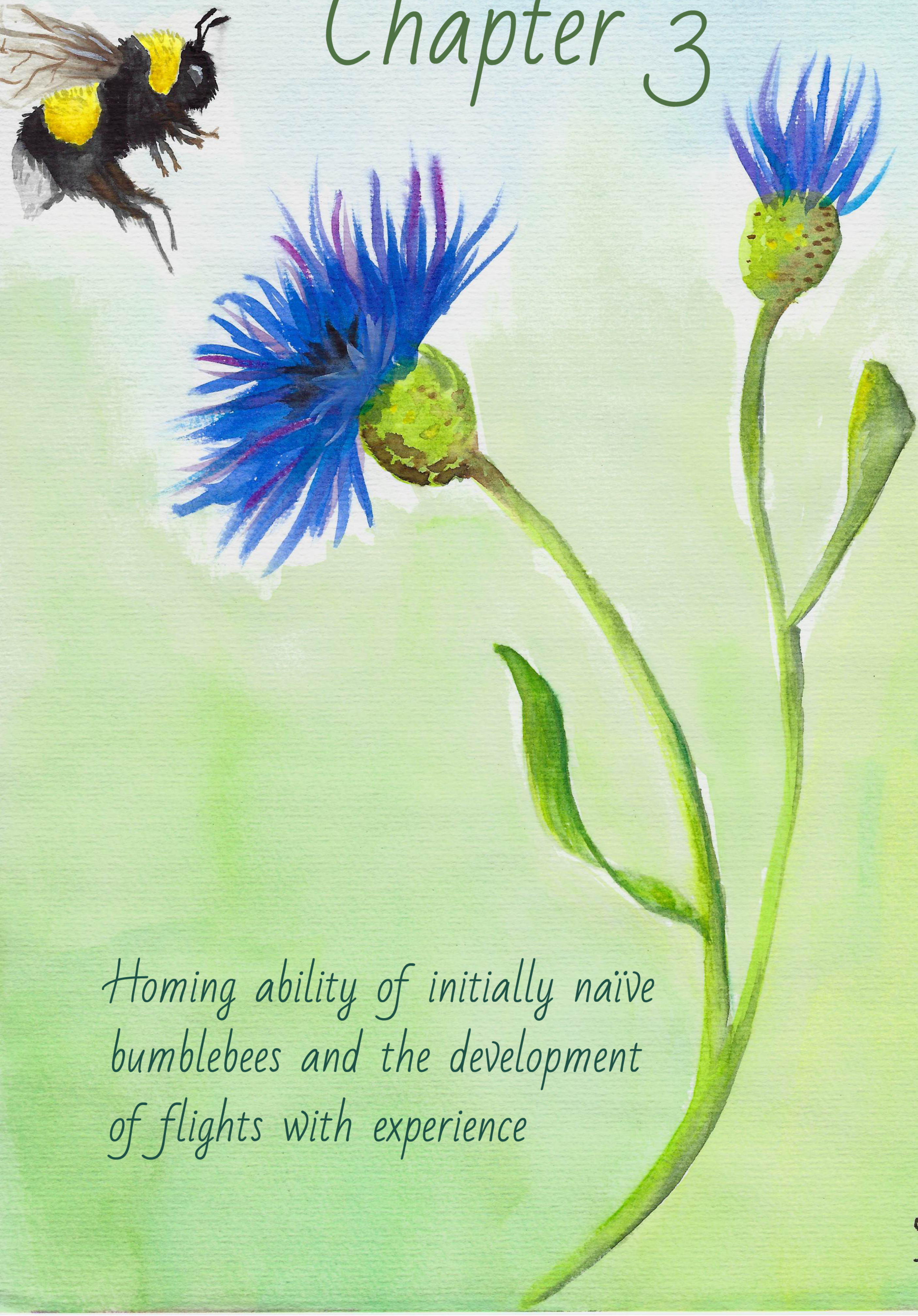
Chapter 3



Homing ability of initially naïve bumblebees and the development of flights with experience

Mai 2018

3.1 Introduction

How central place foragers, such as bumblebees, find back to their home location after a foraging trip is still, to a large extent, an open question. Many explanatory approaches have in common that they assume the insects to recall spatio-temporal information as previously seen at the goal location and to compare this memorized information in some way with the information derived from the current visual input. There is evidence for a wide range of possible mechanisms.

One class of hypothesised mechanisms are part of models relying/based on snapshot-like spatio-temporal information. They accomplish homing by determining the similarity of retinotopic representations of the environment at the current and the home location and moving in a way which increases the similarity of these representations (e.g. Vardy and Möller (2005); Zeil et al. (2003); Stürzl et al. (2008); Cheung et al. (2008); Murray and Zeil (2017)).

Another class of possible mechanisms determines an average vector, which either represents the sum of unit vectors pointing, for instance, to distinguished landmarks in the environment, such as trees ('average landmark vector') or the centre of mass of the intensity values averaged along the elevation of the retinal image ('average skyline vector'). The goal direction is then determined at any location during the return as the difference between this vector, i.e. the average landmark vector or the average skyline vector, computed at the goal location and at the current position (e.g. Cartwright and Collett (1983, 1987); Lambrinos et al. (2000); Mölller (2000); Hafner (2001); Stürzl and Mallot (2006); Mangan and Webb (2009); Basten and Mallot (2010); Yu et al. (2012); Müller et al. (2018)). Depending on the characteristics of the environment, these homing algorithms could be shown in model simulations to be sufficient for returning to the home location at least from within a certain area of the environment around the goal, i.e. the catchment area.

All these local homing models have in common that the essential information to find back to the goal after an excursion, is gathered locally at the goal location ('goal-centred information') before leaving it the first time. However, if this kind of mechanism relying on goal-centred information were the basis of local homing behaviour of insects, it might be a waste of time and energy - at least from the perspective of cost-benefit calculations - that flying hymenopterans perform their characteristic complex flight manoeuvres, usually termed learning flights, after departure from their nest or a profitable food source. Despite species-dependent differences, these flight manoeuvres are characterised by a loop- and/or zigzag-like pattern, where the insects tend to face in the direction of the goal or prominent landmarks for most sections of the departure flights (e.g. Lehrer (1991); Collett and Lehrer (1993); Zeil et al. (2009); Boeddeker et al. (2010); Müller and Wehner (2010); Collett et al. (2013); Philippides et al. (2013)).

Given such complex departure flights, more elaborate homing mechanisms have been proposed: For social wasps it has been assumed that multiple snapshots might be taken at the end of arcs the animal is flying during the departure flights (Zeil, 1993a; Stürzl et al., 2016). The corresponding changes of turning direction have been proposed to be initiated whenever the retinal image of the nest hole or a prominent landmark moves to a lateral position in the visual field (Collett and Lehrer, 1993; Zeil, 1993a; Zeil et al., 1996, 2007, 2009).

Since we did not find in our investigation of first outbound flights of initially naïve bumblebees a correlation between these changes of turn direction (CTD) and the bumblebees' position in our flight arena nor the retinal position of the nest hole (Lobecke et al., 2018), we do not consider a multiple snapshot mechanism a plausible hypothesis to account for bumblebee homing behaviour.

Rather, we hypothesised the outbound flights to consist of several phases each with different functions for the learning progress: Bumblebees are assumed to gather information only during the very initial section of the flight, while they are still close to the nest hole. In this section, they are suggested to gather goal-centred information of the surroundings that determines the catchment area near the nest hole. The later and usually much longer part of a first flight phase, where the bumblebees still fly relatively close to the ground, might then serve to test the reliability of the gathered information ('probing phase' of outbound flights), because initially naïve bees have no a priori knowledge about the size and shape of the catchment area around their nest hole. The loop-like structure of the probing phase might, thus, somehow 'simulate" a return flight to the nest. If this hypothesis were correct, it could also explain the high level of variability in the loop-like flight structure of the bumblebees' departure flights at least under our experimental conditions.

If the information used for the return flight were goal-centred as assumed by our goal-centred dynamic snapshot hypothesis (Lobecke et al., 2018), bumblebees are expected to return on an almost direct course to their goal, i.e. by decreasing their distance to the goal location continuously. However, such relatively direct return flights have not been described consistently for bumblebees, so far. Rather, the appearance of return flights to the goal location is very variable, possibly depending, at least to some extent, on the various experimental conditions employed in the different studies. For instance, some of these studies have been performed outdoors or in green houses where, apart from the landmarks in the vicinity of the goal, unspecified additional visual cues were inevitably present, such as direction cues provided by the sun, the polarisation pattern of the sky or the characteristics of the skyline structure (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014; Robert et al., 2018). Such additional cues may have been used by the bees, apart from the dedicated landmarks close to the nest hole, to control their return flights and, thus, might have affected the flights' directedness.

To study the significance of visual landmarks for the homing ability of homing-naïve bumblebees we did our best to deliberately exclude all directional cues inside our flight arena, except of two salient cylinders, placed close to the nest hole. Moreover, we did not want the nest hole to act itself as a conspicuous visual cue, in contrast to other studies (e.g. Robert et al. (2018)). Since it is not possible to make the nest hole completely invisible, at least from a close distance, we introduced seven additional, visually identical and symmetrically placed (but blind ending) holes in the arena floor ('dummy holes'). This measure created an ambiguity for the bumblebees, which can only be resolved by using the cylinders close to the hole connected to the nest as homing guides (for details see Material and methods and (Lobecke et al., 2018)). If the cylinders serve as landmarks, they could, at least in principle, guide the bumblebees in a faultless way to the nest hole.

Once insects have returned successfully to their home location after their first outbound trip, one might expect the next outbound flights to become shorter in time and less complex, because the animals may have gained experience about the nest surroundings. This expectation is suggested by previous studies, at least for wasps and honeybees: the insect's consecutive departures become straighter and include less or, after several flights, even no turn-back-and-look behaviour (Zeil et al., 1996; Lehrer, 1991, 1993; Collett and Lehrer, 1993).

Here we aim to analyse outbound flight series, especially the learning and the probing phase, and raise the question, whether there are significant changes in their duration and spatio-temporal structure. However, not only outbound flights might change as a consequence of increasing experience. Improvements as a consequence of learning might also be reflected in a shorter duration of return flights and a higher velocity or a more directed approach to the nest hole. To the best of our knowledge, there is no evidence from previous studies regarding the return flight performance over sequences of subsequent flights of individual bees.

Since the seven additional holes ('dummy holes' that are not connected to the nest) create an ambiguity in the flight arena with respect to the connected eighth nest hole, bumblebees in our experiments could make homing errors. This experimental design differs, to the best of our knowledge, from the design of all other homing studies published so far where just one nest hole existed (e.g. Tinbergen (1932); Becker (1958); Collett and Lehrer (1993); Zeil (1993a,b); Brünnert et al. (1994); Hempel de Ibarra et al. (2009); Collett et al. (2013); Philippides et al. (2013); Riabinina et al. (2014); Stürzl et al. (2016); Robert et al. (2018)).

The possibility to 'fail' allowed us to assess homing performance also by analysis to what extent the bees made errors by confounding the connected hole with one of the others as well as potential improvements of performance with experience on a population as well as on an individual basis. Do the bumblebees land at dummy holes on their first return? And if so, does the probability to do so ('error probability') change over several flights? Do bumblebees, returning to a dummy hole on their first inbound flight, improve their return in a way, that they land at the connected nest hole on one of their subsequent returns or do they still approach a dummy hole after several flights? Moreover, if bees make an error, which dummy hole do they approach and does this give a hint to the cues they use on their return? Do bumblebees, which reached the connected nest hole on their first inbound flights, never land at a dummy hole on subsequent returns? These questions will also be addressed in the current study.

Due to the fact that the connected nest hole is indicated by the two landmark cylinders, the question arises of what may happen when the cylinders, as salient landmarks, are displaced to a dummy hole after the bee had the opportunity to experience their functional relevance during a series of out- and inbound flights. Does such a displacement affect the outbound or inbound flights?

Although only a single nest hole was available in previous studies, honeybees and social wasps were observed, after displacement of the nest or the landmarks, to show an additional learning flight on their departure only after they had difficulties in finding the target location on their previous return (Wolf, 1926, 1927; Becker, 1958; Van Iersel and van der Assem, 1964; Zeil, 1993a; Brünnert et al., 1994; Zeil et al., 1996). Hence, if the cylinders are the cue guiding the insects' return to the connected nest hole, we hypothesise the bumblebees to rely on the landmark position after several flights and to be miss-led to a dummy hole after displacement of the landmarks. We further assess, whether the change of the cylinder position affects the subsequent outbound flight.

To address these questions, we recorded and analysed 3-dimensional trajectories of 20 initially homing naïve, individually marked bumblebees to show their homing ability on their first inbound flight in an ambiguous situation with regard to potential nest holes, with two landmarks presenting the only unambiguous cue for the correct hole, as well as the potential development of subsequent out- and inbound flights with increased experience. A 3-dimensional approach based on two cameras was used, because our analysis of the first outbound flights of homing naïve bumblebees revealed the important role of height in structuring their flight behaviour (Lobecke et al., 2018).

3.2 Materials and methods

The behavioural analyses are based on experiments done in 2014 – 2016 in an experimental set-up already described in Lobecke et al. (Lobecke et al., 2018). We here summarise only those methodological aspects that are necessary to understand the analysis of the current study. For details we refer to Lobecke et al. (Lobecke et al., 2018).

3.2.1 Animals and experimental setup

The experiments were done with commercial bumblebee hives of *Bombus terrestris* from Koppert (Berkel en Rodenrijs, The Netherlands), kept in a Perspex box connected via a PVC tube to a hole in the floor of the test arena. This octagonal test arena (height and diameter) was placed on a table covered with a red artificial grass carpet (Kunstgras Wereld, Antwerpen, NL). Eight holes (30 mm in diameter) - each placed at a distance of 100 mm orthogonal to the centre of a wall segment - were drilled into the arena floor (cf. chapter 2, fig. 2.1). Throughout the different experiments, only one of the eight holes was connected to the nest, and the bees started their flights at this hole. Regarding the holes in the floor, the arena was symmetrical and provided an ambiguous situation for the bees. Furthermore, the connected nest hole could only be distinguished visually from the other (dummy) holes by two white cylinders (400 mm high), placed at a distance of 100 mm at each side of the nest hole. For a further test with five additional homing-naïve bumblebees all seven dummy holes were covered by the same artificial grass carpet, which covered the arena floor. Only the nest hole remained accessible.

During the experiments the bumblebees were allowed to leave the illuminated arena into the flight room through a 400-mm gap between the arena walls and a red acrylic glass construction, mounted above the arena. In a corner of the flight room bees were able to forage at artificial feeders, filled with commercial sugar solution (Koppert (Berkel en Rodenrijs, The Netherlands) mixed with water in a ratio 3:1.

3.2.2 Recording procedure

To ensure that only one bumblebee at a time could enter the arena, bumblebees were separated by removable doors in the tunnel system leading to the arena. Their outbound and inbound flights were recorded with two high-speed cameras (Falcon2 4M, Teledyne DALSA, Inc.) from above the acrylic glass construction at 148 fps and a resolution of 2048*2048 px. The top camera's optical axis pointed straight down, while the second camera's optical axis was 45° to the vertical. Using the software Marathon Pro (GS Vitec) we recorded continuously for several hours, starting with the bumblebee entering the flight arena. Relevant sequences of outbound and inbound flights were stored as 8-bit jpeg images for the flight analyses. Recording intervals without relevant flights were deleted.

3.2.3 Training and test procedure

Relevant recording intervals started, when the bees entered the flight arena from the nest hole or the flight room outside the arena, respectively. Bumblebees were individually marked with acrylic paint on their thorax either after the first outbound flight or the first inbound flight. They were able to enter the test arena several times a day on consecutive days, so that interruptions longer than 12 hours were mostly excluded. After several outbound and inbound flights, the cylinders where displaced: We changed their position from the nest hole, i.e. the hole connected to the tunnel system and the hive, to the dummy hole opposite to the correct one.

3.2.4 Data analysis

The flight recordings from both cameras were analysed with the custom-built software ivTrace ((Lindemann, 2005) https://opensource.cit-ec.de/projects/ivtools) where the position of the bee and the orientation of its body length axis were determined automatically. Additionally, ivTrace calculated the body yaw angle from the top camera. Besides the yaw angle of the bees' body several other parameters, i.e. height over ground and retinal position of the nest hole, were analysed and compared to characterise the flights' spatio-temporal structure. The data of the entire first inbound flight were stored from the time point the bumblebee entered the arena. For analysis of consecutive flights and of the development of the outbound and inbound flights we grouped the flights into those outbound or, respectively, inbound flights before the cylinders were displaced and those that included the outbound or inbound flights with the cylinders at their new position.

3.2.5 Naming of flight phases

Since the phase of the inbound flights close to the arena floor, i.e. below a height of 100 mm, which eventually terminates by selecting a hole, is clearly distinguished from the beginning of the flight, the here called 'entry phase' into the flight arena, it will be called 'homing phase' in the following. The bumblebees' height fluctuates during the homing phase and sometimes exceeds 100 mm for a short time. These fluctuations in height are included into the homing phase, thus applying the same criterion as has been done for the 1st phase of the departing flights characterised in our previous paper (Lobecke et al., 2018). To pay special attention to the phase immediately prior landing at a hole, we defined this 'pre-landing phase' as the third section of the homing flight, where the bumblebees stay constantly below a height of 100 mm and finally reach the nest hole or a dummy hole.

Since our analyses in the previous paper (Lobecke et al., 2018) suggest that bees with a flight height above 100 mm might be mainly interested in leaving the arena and not in further gathering information about the nest hole location.

Therefore, we decided to merge phase 2 and 3, used in Lobecke et al., 2018, to a ‘leaving phase’. Furthermore, we hypothesised phase 1 below 100 mm to be composed of a ‘learning phase’ and a subsequent ‘probing phase’, which includes fluctuations over 100 mm (see (Lobecke et al., 2018)).

Phases of outbound and inbound flight are now comparable to each other, due to their height and the inclusion or exclusion of fluctuations, respectively. As discussed in Lobecke et al. (Lobecke et al., 2018), the height threshold does not represent altitudes that correspond to any obvious changes in flight style and the transitions between the phases appear to be smooth. All conclusions we drew from our experiments were virtually independent of the specific classification into the flight phases.

3.3 Results

We tested the hypothesis suggested by the performance of current homing algorithms (see Introduction, chapter 3) that bees might return on a relatively direct trajectory back to their nest hole based on goal-centred visual information gathered during their departure. Even in an experimental set-up where only two cylinders provide visual cues that distinguish the nest hole in an otherwise ambiguous flight arena, such kind of homing algorithm should at least, in principle, be sufficient for a targeted homing. Since bumblebees had the possibility to land at dummy holes on their return flight rather than at the nest hole, we additionally analysed the ‘homing errors” and associated landing preferences. Then we analysed the potential development of consecutive outbound and inbound flights. We tested the hypothesis that flights become shorter and less complex with experience and that homing errors may decrease. Finally, we investigated the consequences of changing the cylinders’ position and, thus, of a displacement of potentially landmark cues.

3.3.1 Homing performance of homing-naïve bumblebees during their first inbound flight

We recorded and analysed 20 first inbound flights of 20 homing-naïve bumblebees. Since all of them performed a previous first outbound flight, we assumed that they had gathered information about the surroundings of the nest hole allowing them to find their way back. 15 first inbound flights where successful, i.e. ended at the nest hole. Five bumblebees did not end their initial inbound flight at the nest hole, but at one of the two neighbouring dummy holes (fig. 3.1). This overall performance is very unlikely to be the result of random search by the bees, which would be expected in a fully ambiguous situation with eight identical holes without any visual cues distinguishing one of them. Thus, we conclude from these results - despite the errors the bees obviously made - that the cylinders are used by the bees as distinguishing cue of the nest hole and that the relevant information is gathered during the first outbound flight allowing the bumblebees to find back to the nest hole vicinity. However, this information does not appear to be sufficient for a successful return in all cases.

Even the successful bumblebees do not appear to return to their nest along relatively straight and targeted trajectories, but usually spent much time during the return by flying tortuous loops often covering large parts of the arena apart from the regions close to its walls. Like the first outbound flights (Lobecke et al., 2018), the first inbound flights are characterised by a high amount of variability. This variability together with the loop-like and zigzagging behaviour appears to be somehow reminiscent of search behaviour, which appears to be superimposed on the information-based homing behaviour. We concentrate, in the following analysis on the common features, which characterise the first inbound flights of homing-naïve bumblebees and might be essential for a successful return to a goal location.

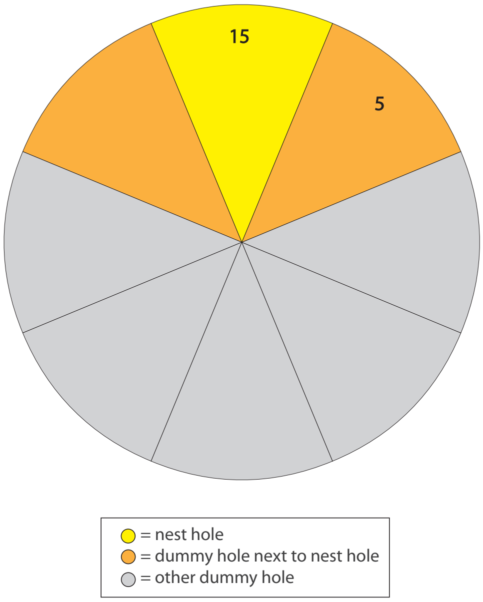


Figure 3.1: **'Homing error' of first inbound flight.** 'Homing error' and landing preference for first inbound flights of initially naïve bumblebees (N = 20). The segments of the circle display the sections of the nest hole (yellow), the dummy holes neighbouring the nest hole (orange) and other dummy holes (grey), respectively. Note: The large circle stands for the flight arena and not a normal pie chart.

Overall flight structure

Similar to the first outbound flights (Lobecke et al., 2018), the first inbound flights show a high amount of variability, although all flights have a complex structure of irregular loops in common (three example flights are shown in fig. 3.2). Even relatively short inbound flights are far from revealing a continuous decrease of distance to the nest hole (fig. 3.2 A). The third bumblebee (figure 3.2 Ci, Cii) ended its flight at a dummy hole, even after an extended time of searching.

Characteristic of all 20 first inbound flights is the fast decrease in height after the insect enters the arena (fig. 3.2 Aii - Cii). Sometimes the bumblebees gain some height again afterwards, but they mostly do not increase their height above the height of the two cylinders (400 mm) indicating the connected nest hole. Close to the ground level they cover almost all space, at least inside the ring of the nest and dummy holes (fig. 3.2 Ai - Ci). If they get closer to the arena wall, what occurs only rarely, they do this as part of a loop around a hole or a cylinder. In any case, the bumblebees do not steadily approach the nest hole, neither in height nor with respect to their 2-dimensional distance to the nest hole. Rather, these two parameters fluctuate considerably with amplitudes of up to several centimetres and variable period lengths in the range of several seconds (fig. 3.3). Fluctuations in the 2-dimensional distance range between 1 and 2 seconds; on average, the bees have a distance peak every 1.25 seconds (± 0.25 s). The height fluctuates in a range between 1 and 1.8 seconds, with an average of 1.4 seconds (± 0.21 s).

For a better comparison, the initial 13 seconds of the inbound flights of the three example bees from fig. 3.2 are shown in fig. 3.3 Ai - Aiii at the same time scale (which required to omit large parts of the longer flights of the 2nd and 3rd bee, which are shown in fig. 3.3 Bi and Bii).

Apart from the differences in flight duration and in the height and distance fluctuations, the fast decrease in height during the first few seconds appears to be independent of the overall duration of the flights. This fast decrease is a common feature across bees and, as mentioned above, is reflected in the duration of the different phases of the first inbound flight of all tested 20 bumblebees (fig. 3.4).

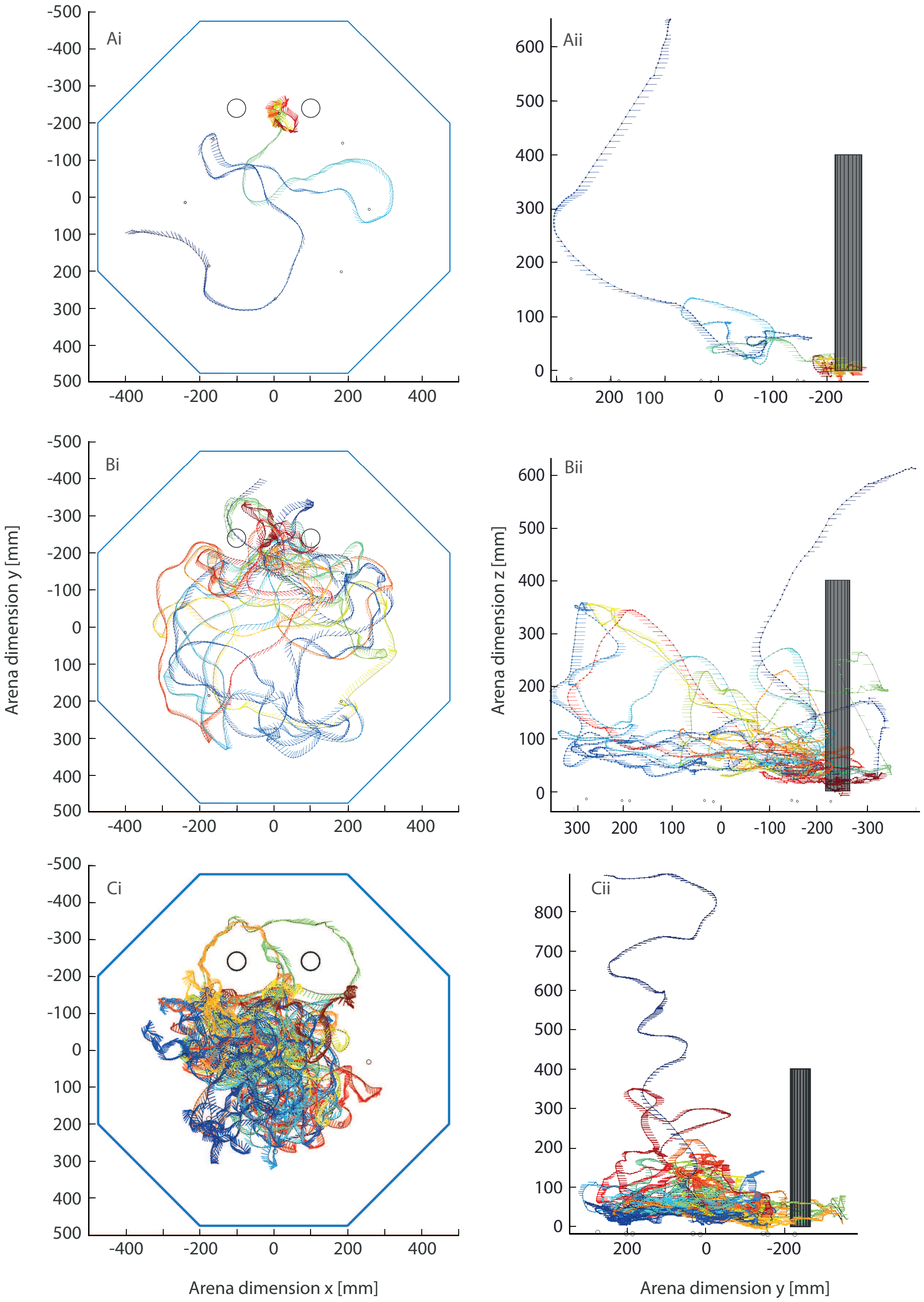
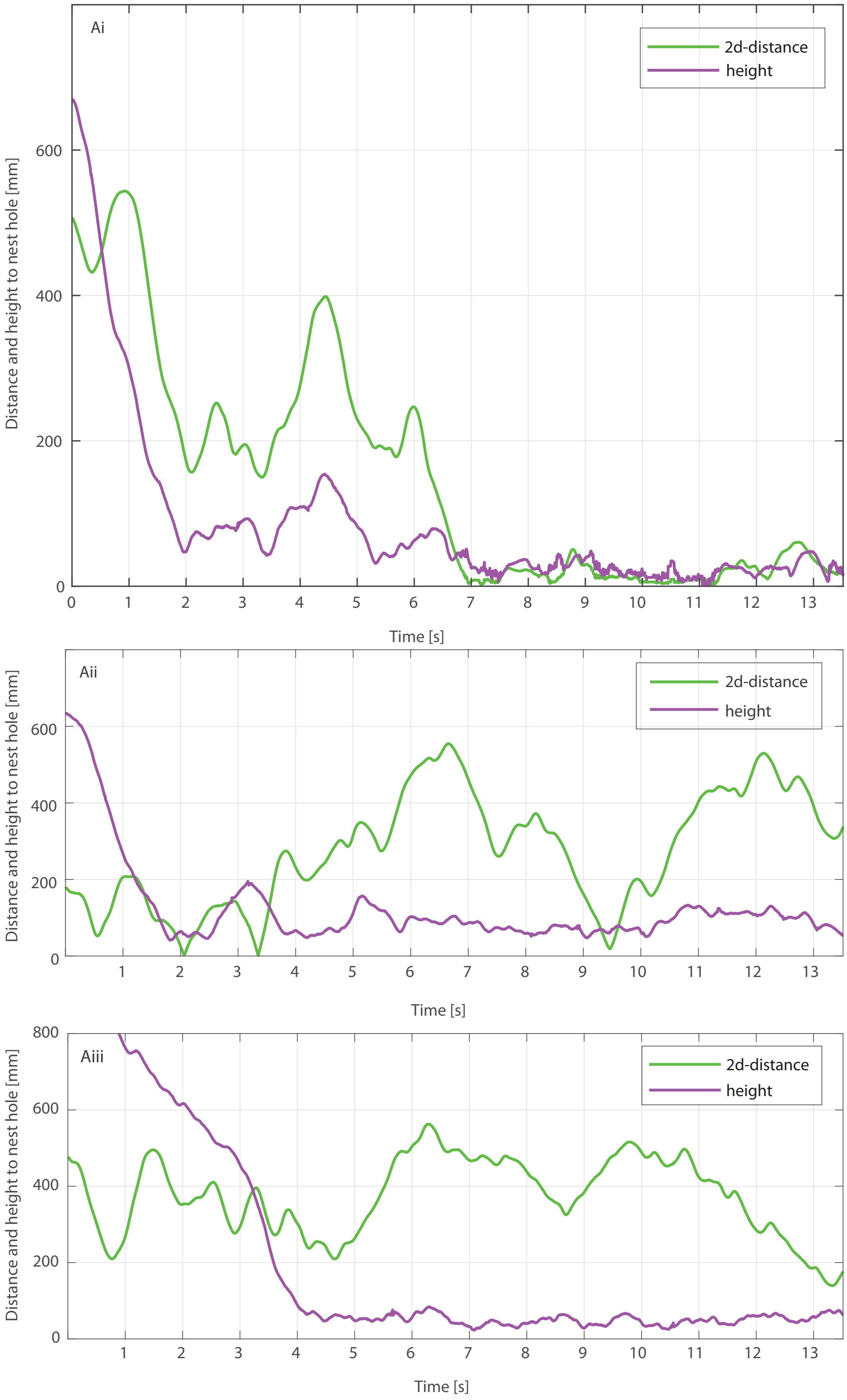


Figure 3.2: **Flight trajectories of first inbound flights seen from above and from one side.** Three example trajectories out of the 20 first inbound flights analysed. Black circles (top view: Ai-Ci) and grey rectangles (side view: Aii – Cii): cylinders; small circles: Nest hole (between cylinders) and dummy holes; coloured lines indicate the orientation of the bee's body long axis every 20.27 ms; end of lines mark head position; sequence of head positions defines trajectory. Trajectories colour-coded with time: Dark blue beginning of flight, dark red end of flight. Coordinate (0;0) represents the centre of the flight arena at floor level.


Ai
2d-distance
height
Distance and height to nest hole [mm]
0
200
400
600
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Time [s]
Aii
2d-distance
height
Distance and height to nest hole [mm]
0
200
400
600
1
2
3
4
5
6
7
8
9
10
11
12
13
Time [s]
Aiii
2d-distance
height
Distance and height to nest hole [mm]
0
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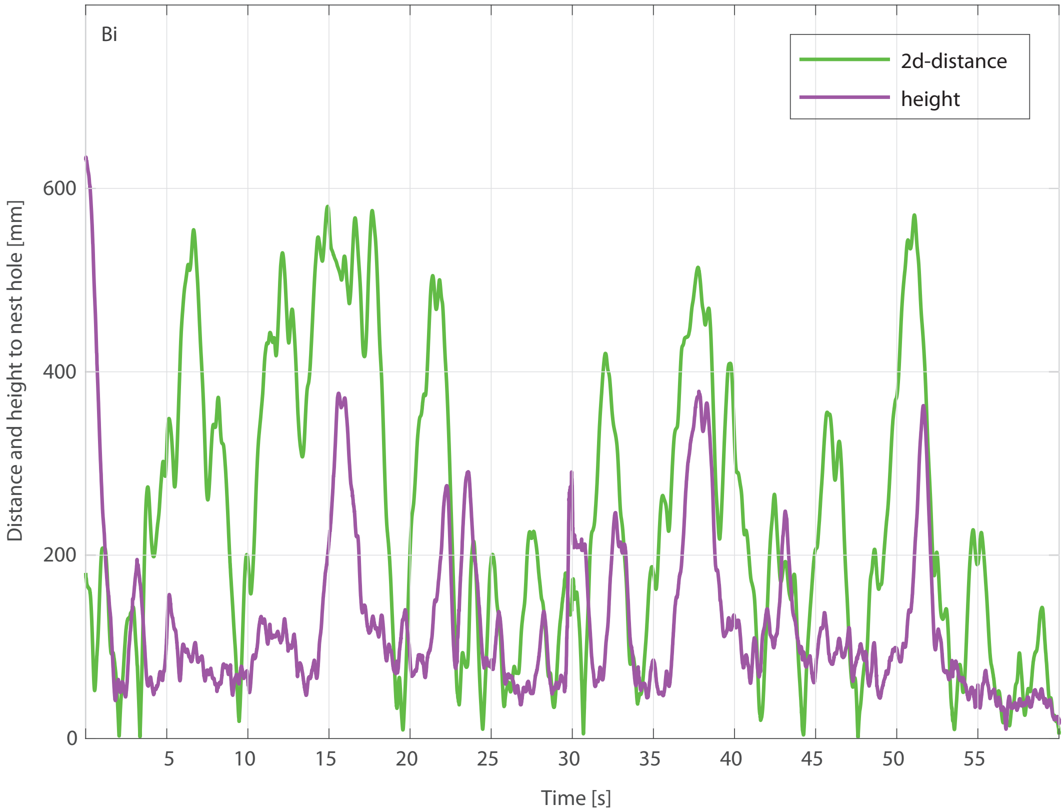
Time [s]


Bi
2d-distance
height
Distance and height to nest hole [mm]
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200
400
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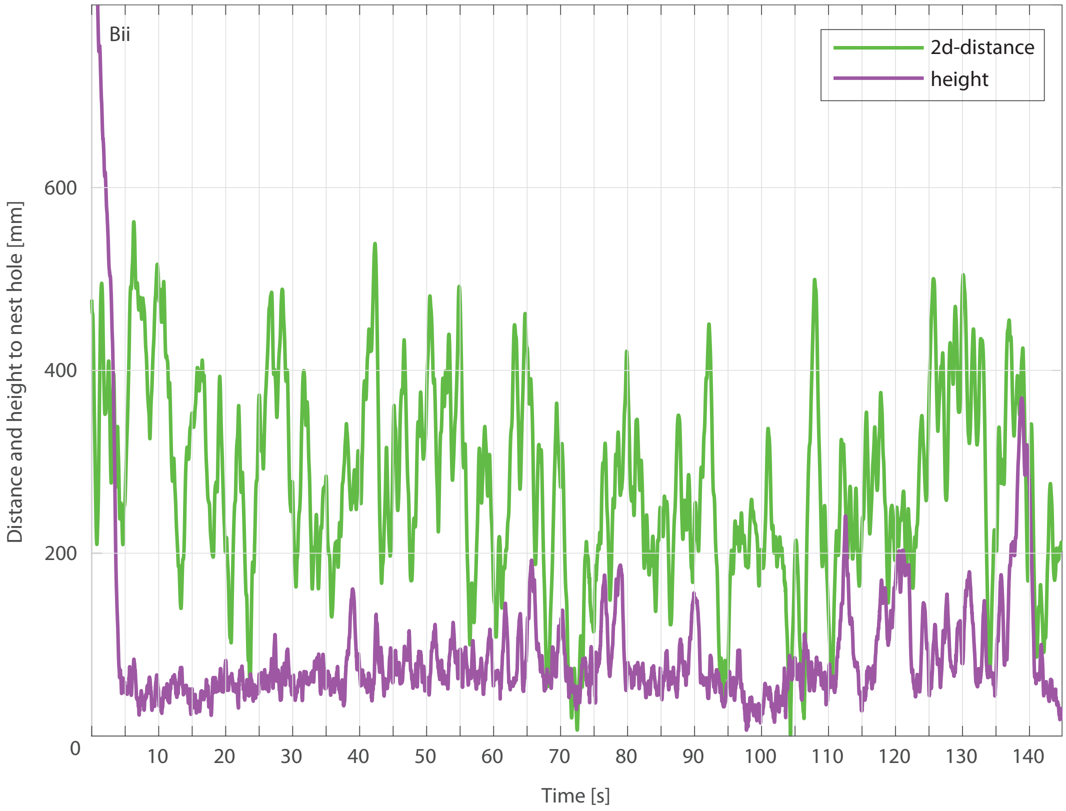
Time [s]


Bii
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Distance and height to nest hole [mm]
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80
90
100
110
120
130
140
Time [s]

Flight duration

The average duration of the entire first inbound flight is 43 seconds but characterised by a high amount of variability. As expected from the bumblebees' trajectories (fig. 3.2) and the time-dependent height of the bumblebees (fig. 3.3), the duration of the arena entry phase of the flights (i.e. above 100 mm) is, on average, relatively short with 4 seconds (fig. 3.4). It is additionally the phase with the lowest variation, 25th and 75th percentiles at 1.7 and 6.1 seconds, respectively. The duration of this phase is suggested to be independent of the overall length of the entire flight, due to its low variability. In contrast, the duration of the homing phase has a median value of 18 seconds (25th and 75th percentiles at 4.7 and 54.6 seconds) and represents the largest part of the entire inbound flight and has the highest variation. The pre-landing phase's median value is 9 seconds (25th and 75th percentiles at 4.3 and 13.1 seconds; fig. 3.4).

To test, whether the inbound flights get straighter and less variable, if the situation is not as ambiguous as with the eight potential nest holes in the test arena, we performed the same experiment in our octagonal arena, but closed the seven dummy holes and made them invisible. In this control situation, we tested five initially naïve bumblebees and recorded their first outbound and inbound flight. The duration for the first inbound flights is 23 seconds on average. Since this duration is 20 seconds less than the average of the first inbound flights in the arena with eight potential nest holes, this difference might be caused by the ambiguity provided by the presence of dummy holes. Nevertheless, the high variability of the flight durations as well as of the time-dependent fluctuations of the bees' height and 2-dimensional distance to the nest hole reflect the enormous variability between individual flights even under conditions that are unambiguous with respect to the nest hole situation. Since also with just one nest hole open, the first inbound flights reveal loops and distance variations to a similar degree as when all holes were kept open (fig. 3.5), we can conclude that bees usually do not approach a single goal in a direct way, i.e. by steadily reducing the distance to it.

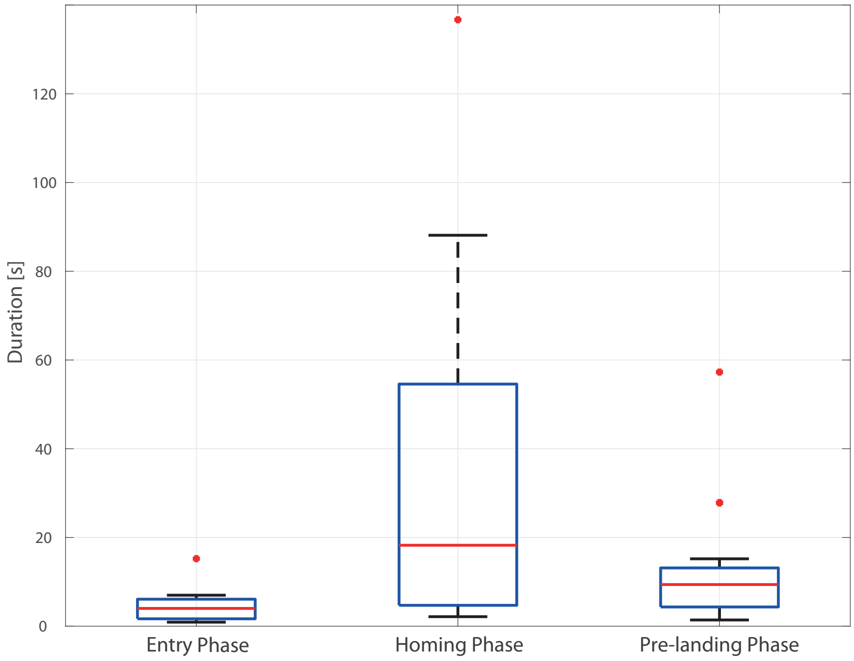


Figure 3.4: **Flight duration.** Flight duration of the first inbound flight of initially naïve bumblebees (N = 20). Central mark (red): median, edges of box (blue): 25th and 27th percentiles, whiskers (black): most extreme data points, red dots: outliers.

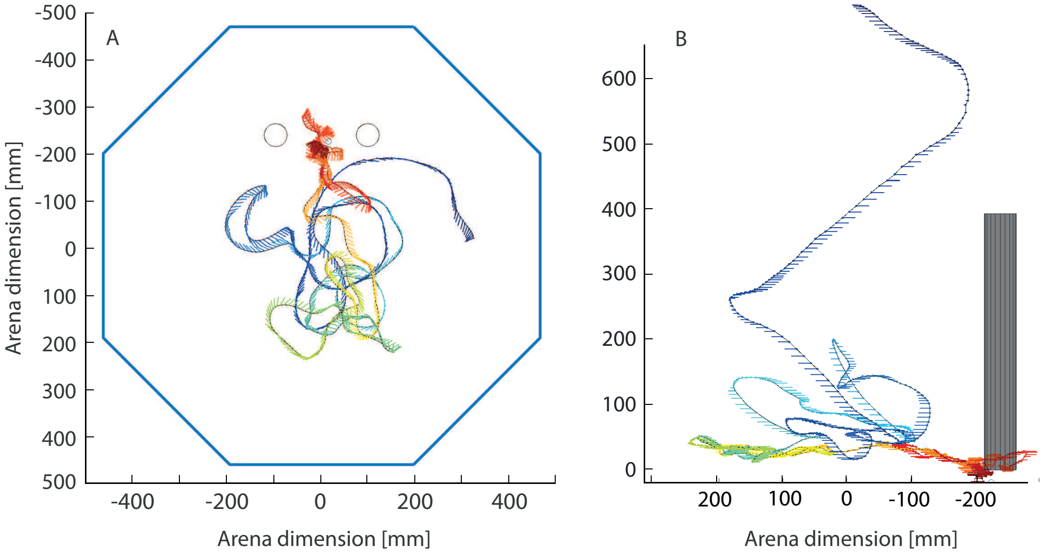
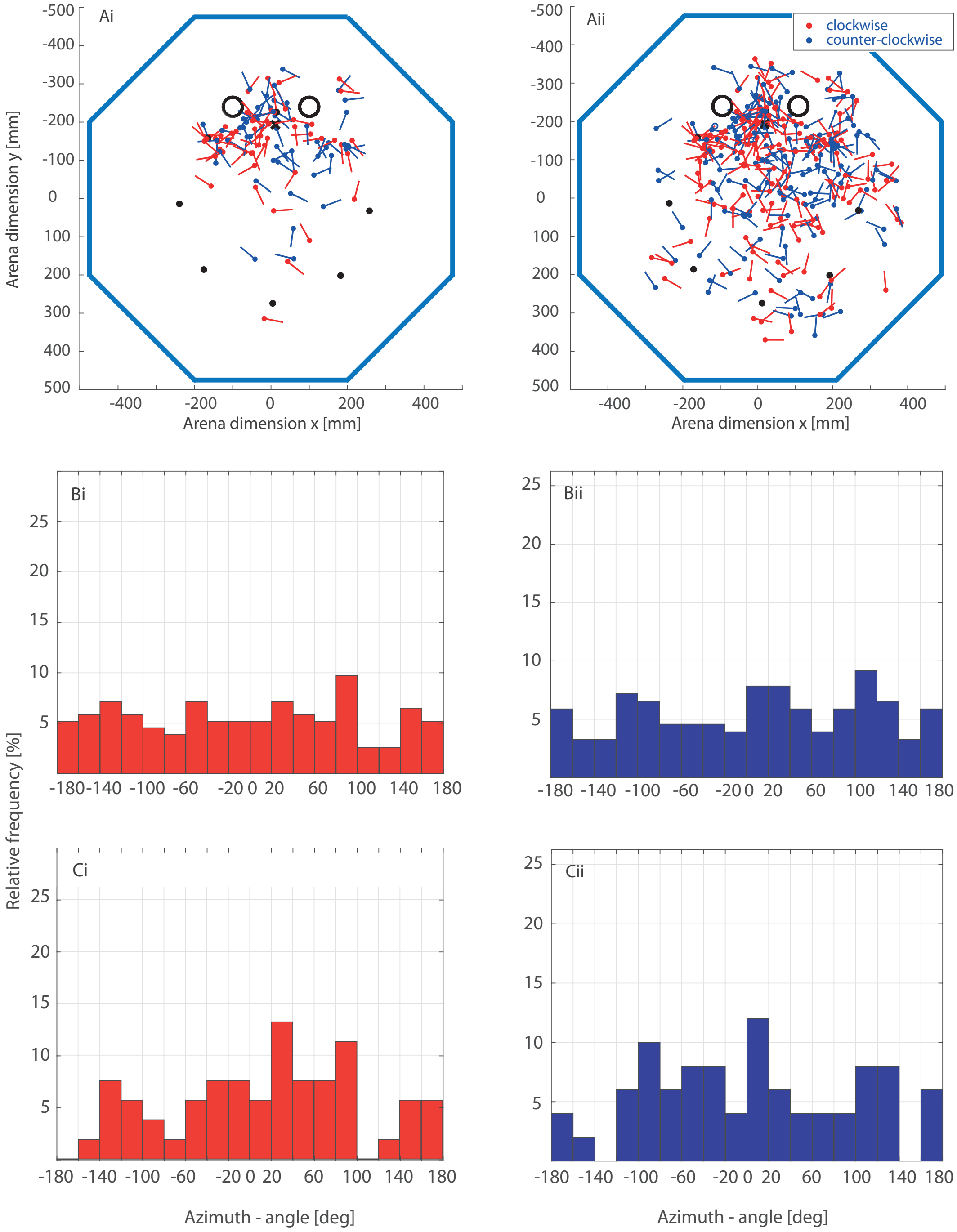


Figure 3.5: **Flight trajectory of first inbound flight without dummy holes.** One example trajectory out of the five first inbound flights analysed with the dummy holes covered with floor texture. Black circles in the top view (Ai) and grey rectangles in the side view (Aii): cylinders; circle in top view: Nest hole (between cylinders) and dummy holes; coloured lines indicate the orientation of the bee's body long axis every 20.27 ms; end of lines mark head position; sequence of head positions defines trajectory. Trajectory colour-coded with time: Dark blue beginning of flight, dark red end of flight. Coordinate (0;0) represents the centre of the flight arena at floor level.

Changes of turn direction (CTD)

To analyse, whether the high amount of variability is also reflected at a more detailed level in the flight structure, we assessed the CTD from clockwise to counter-clockwise and *vice versa*, which might be decision points in the bees' flight behaviour. A CTD might be triggered, for instance, in specific sections of the arena by a critical retinal position of the nest hole or of a cylinder, as has been suggested in previous studies on wasps (see Introduction). Therefore, we analysed where in the flight arena CTD were initiated. The analysis was done separately for inbound flights that ended either at the nest or at a dummy hole, because the locations at which CTD are initiated might have been affected in different ways depending on the hole at which the return flight terminates. Figure 3.6 Ai shows the horizontal projection of locations of clockwise and counter-clockwise CTD during the entire homing phase for first inbound flights ending at the nest hole ($N = 15$). Bumblebees perform CTD almost everywhere in the arena. As observed in the example trajectories in figure 3.1 bumblebees only avoided the area of the arena close to the wall. Most CTD are performed near the nest hole between the cylinders. During the pre-landing phase (fig. 3.6 Aii) bumblebees perform their CTD mostly in front of the cylinders and in the vicinity of the nest hole.

Bumblebees do not preferentially change their turn direction when the nest hole is perceived in a specific region of the eye during the homing phase and, in particular, not when it is seen in the frontal visual field (fig. 3.6 Bi, Bii), although there might be a weak tendency for a fixation peak during the pre-landing phase (fig. 3.6 Ci, Cii).


Ai
Aii
clockwise
counter-clockwise
Arena dimension y [mm]
Arena dimension x [mm]
-500
-400
-300
-200
-100
0
100
200
300
400
500
Bi
Bii
Ci
Cii
Relative frequency [%]
Azimuth - angle [deg]
-180
-140
-100
-60
-20
0
20
60
100
140
180
5
10
15
20
25

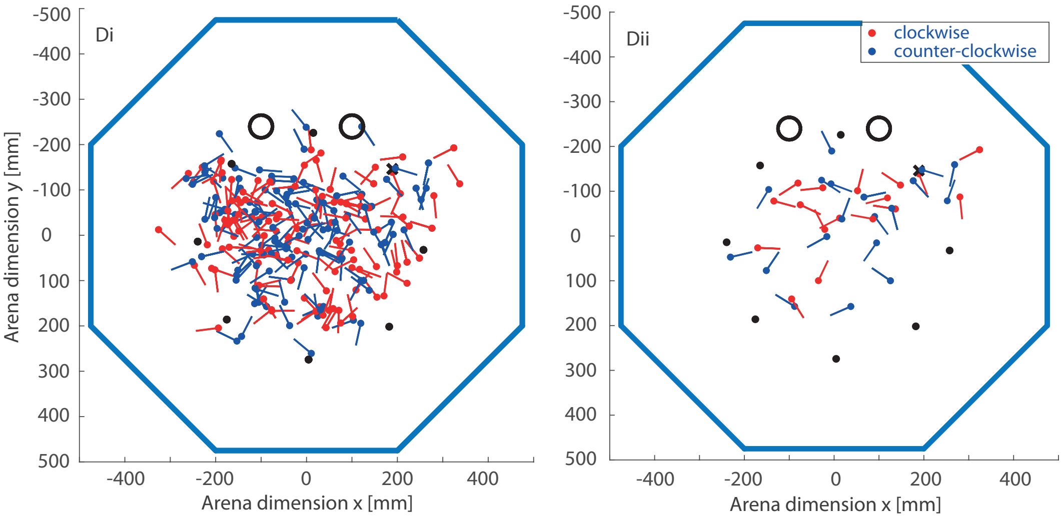


Figure 3.6: **Changes in turn-direction (CTD) of body orientation during homing and pre-landing phase.** A: locations of CTD from counter-clockwise to clockwise (red) and *vice versa* (blue) for the first inbound flight of all bumblebees in i: homing phase, ii: pre-landing phase. Black circles: cylinders, n = 20 first inbound flights; bumblebee 'architecture": Filled circle = head, line = orientation of body long axis. B: retinal position of the nest at instance of clockwise and counter-clockwise CTD for the first inbound flight during homing phase, i: clockwise, number of CTD =154, ii: counter-clockwise, number of CTD = 153, n = 20 first inbound flights. C: retinal position of the nest at CTD for the first inbound flight during pre-landing phase, i: clockwise, number of CTD = 53, ii: counter-clockwise, number of CTD = 50, n = 20 first inbound flights. D: CTD during the first incorrect inbound flight (N = 5). Di: homing phase; Dii: pre-landing phase.

Figure 3.6 Di and Dii shows the locations of clockwise and counter-clockwise CTD in the arena for first inbound flights ending at a dummy hole (N = 5). Bumblebees landed at a dummy hole close to the right cylinder. During such first inbound flights the bumblebees perform their CTD more in the centre of the arena and less in the vicinity of the nest hole (homing phase), than do bumblebees during flights that terminate at the nest hole. They perform almost no CTD in the close vicinity of the nest hole during the pre-landing phase, which might be expected as they land at a dummy hole and the nest hole may be of no further interest during the pre-landing phase.

Retinal position of the nest hole during inbound flights

Since most bumblebees end their first inbound flight at the nest hole, they learned relevant information about its location during the preceding departure flight. Hence, the cylinders close to the nest hole are expected to play a decisive role in controlling the first inbound flight, even if this is not obvious from the spatial distribution of CTD. Therefore, we scrutinize the distribution of the retinal position of the nest hole during the entire first inbound flight and not only at the CTD.

If the inbound flight primarily serves the need to lead the insect back to the nest hole by using the spatio-temporal representation of the nest surroundings acquired during the initial outbound flight, the retinal position of the nest hole region might play a role in controlling the inbound flights (see also Introduction). At least during the last part of inbound flights an influence of the retinal position of the nest hole region on the bees' yaw orientation is expected, given that the bees fly mainly forward before landing.

In the analyses we differentiated between inbound flights ending at the nest hole and inbound flights ending at a dummy hole. Figure 3.7 A shows the retinal position of the nest hole during the entire homing phase for the correct inbound flights (N = 15). No obvious peak could be observed in the retinal position of the nest hole region. During the pre-landing phase the retinal position of the nest hole region differs obviously: Bumblebees keep the nest hole region within a range of 80° in the frontal visual field of their eyes, not perfectly centred about the frontal midline but just slightly shifted to the right. Bumblebees, which eventually chose a dummy hole, probably might have assumed it to be the nest hole and, therefore, this dummy hole might have been kept in the frontal visual field. To test this hypothesis, we analysed the retinal position of both the nest hole and the approached dummy hole during these flights. As assumed, the nest hole location seems to play a minor or no specific role during the entire homing phase as well as the pre-landing phase, respectively; at least the bumblebees did not keep it in the frontal visual field (Fig. 3.7 Bi and Bii).

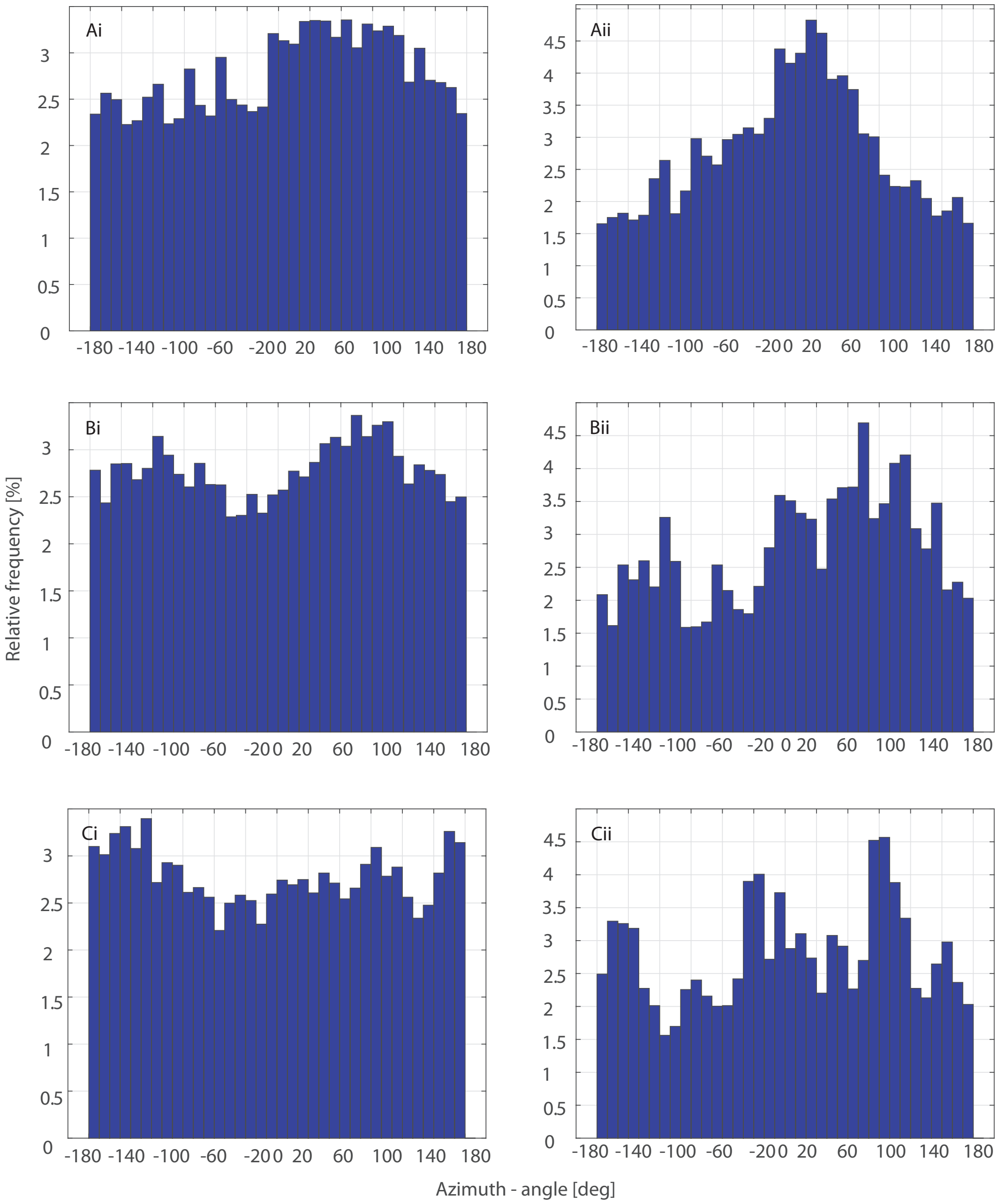
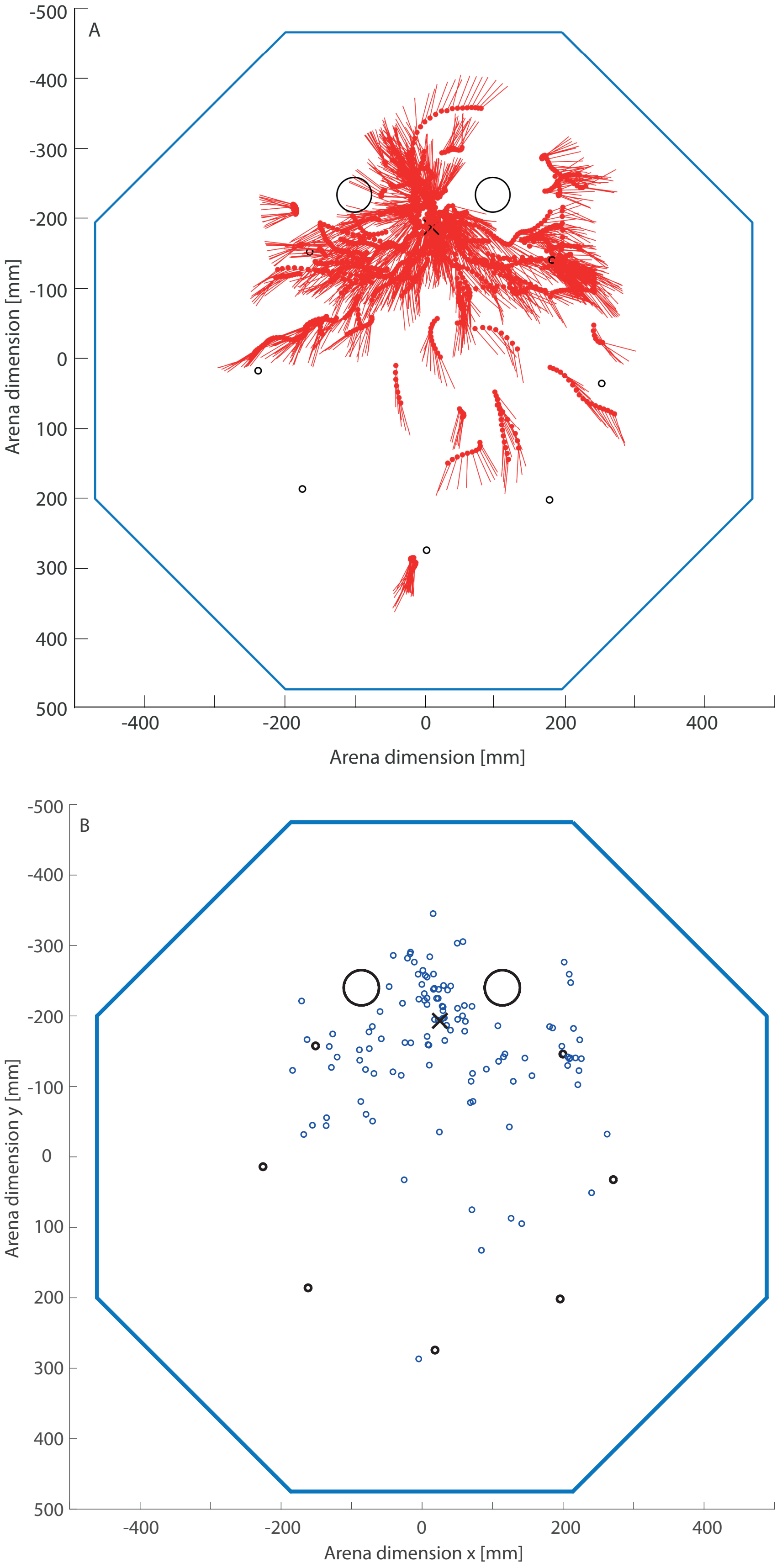
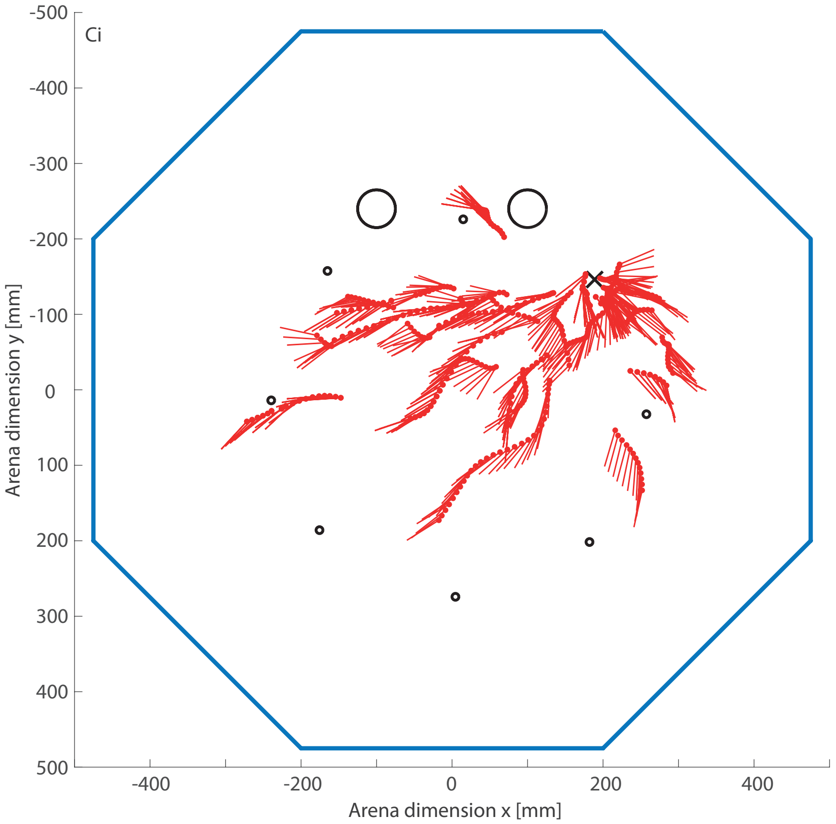


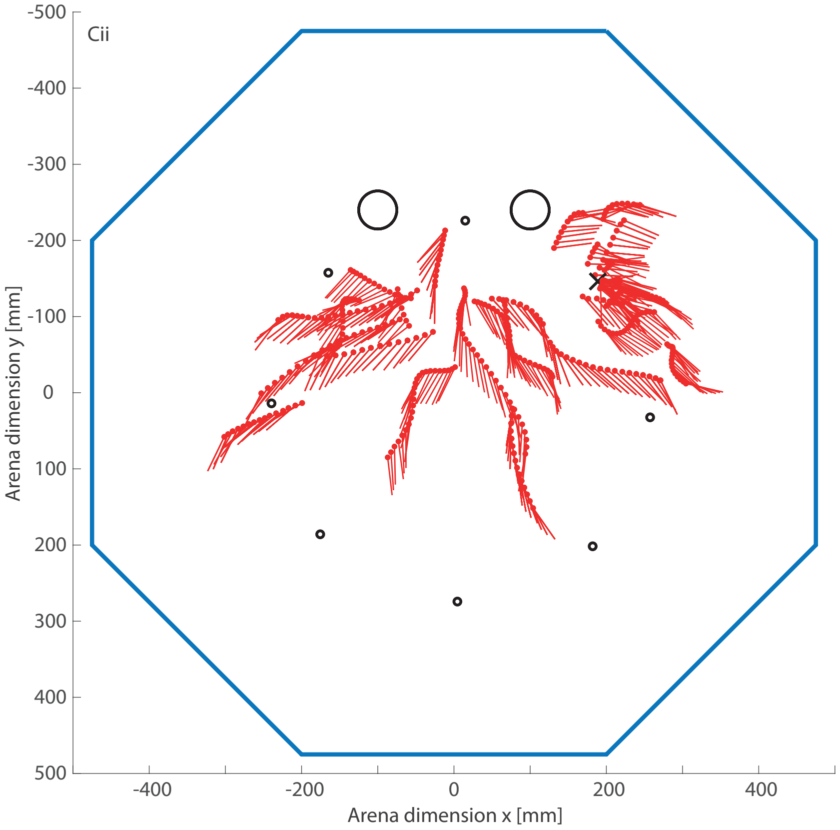
Figure 3.7: **Histogram of the retinal nest hole position.** A: retinal position of nest hole during correct (i.e. ending at nest hole) inbound flights (N = 15) during i: homing phase and ii: pre-landing phase. B: Retinal position of correct nest hole during incorrect (i.e. ending at a dummy hole) first inbound flights (N = 5), i: homing phase, ii: pre-landing phase. C: Retinal position of dummy hole during incorrect first inbound flights (N = 5), i: homing phase, ii: pre-landing phase.

However, in contrast to our expectations based on the finding that the nest hole was kept in the fronto-lateral visual field during the pre-landing phase of successful inbound flights (fig. 3.7 Aii), the bumblebees do not show a strong preference to keep the dummy hole in a specific retinal region during the pre-landing phase although they eventually land at it (fig. 3.7 Cii).

Where in the flight arena do the bumblebees keep the nest region in the frontal visual field between $-25°$ and $25°$? Do they fixate the nest hole location at characteristic places in the arena during their first inbound flights? Nest hole fixations occur mostly during the homing phase and the pre-landing phase (fig. 3.8 A). Just after entering the flight arena before they descend to the ground, they rarely or even never keep the nest hole location in the frontal visual field between $-25°$ and $25°$. The arena locations of the middle of the flight sections, where the bees fixated the nest hole region are plotted for all first inbound flights ending at the nest hole ($N = 15$) in figure 3.8 B. To exclude points where the nest hole area is perceived in the frontal visual field for only a very short time, e.g. when sweeping across the retina during a rotation of the bumblebee, we defined the minimal length of 10 frames for a nest fixation sequence, i.e. ≈ 67 ms. The locations of nest fixation sequences are distributed across the entire extent of the arena that is covered by flight trajectories, although they are most frequent in the nest hole vicinity during the pre-landing phase of return flights. We additionally did this analysis with first inbound flights ending at a dummy hole and plotted the locations of the fixations for the dummy hole at which the bees ended their flight and for the nest hole, respectively (fig. 3.8 Ci, ii). Fig. 3.8 Di and Dii show the middle of these fixation parts in the trajectory, which are not very concentrated at a specific arena region.


A
-500
-400
-300
-200
-100
0
100
200
300
400
500
Arena dimension [mm]
-400
-200
0
200
400
Arena dimension [mm]
B
-500
-400
-300
-200
-100
0
100
200
300
400
500
Arena dimension y [mm]
-400
-200
0
200
400
Arena dimension x [mm]


Ci
-500
-400
-300
-200
-100
0
100
200
300
400
500
Arena dimension y [mm]
-400
-200
0
200
400
Arena dimension x [mm]


Cii
-500
-400
-300
-200
-100
0
100
200
300
400
500
Arena dimension y [mm]
-400
-200
0
200
400
Arena dimension x [mm]

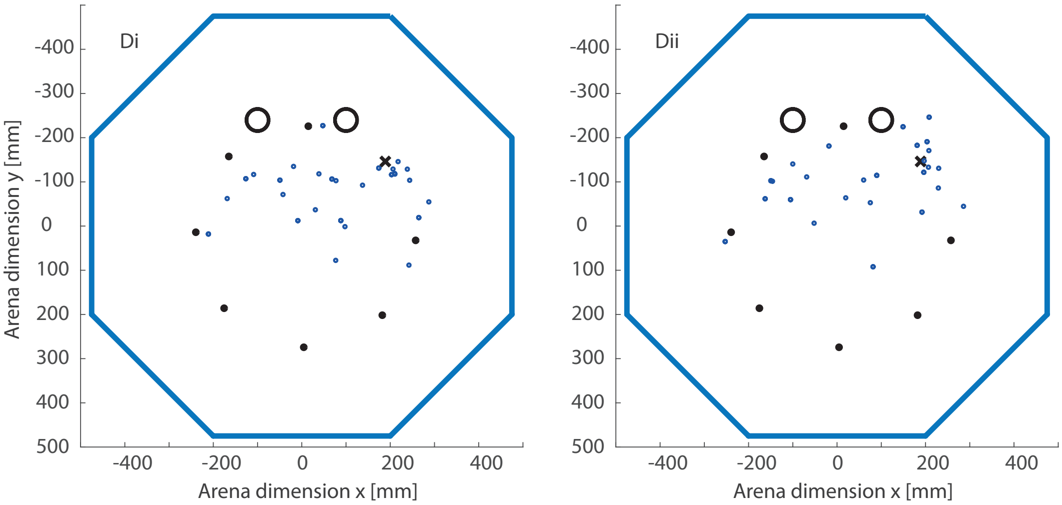
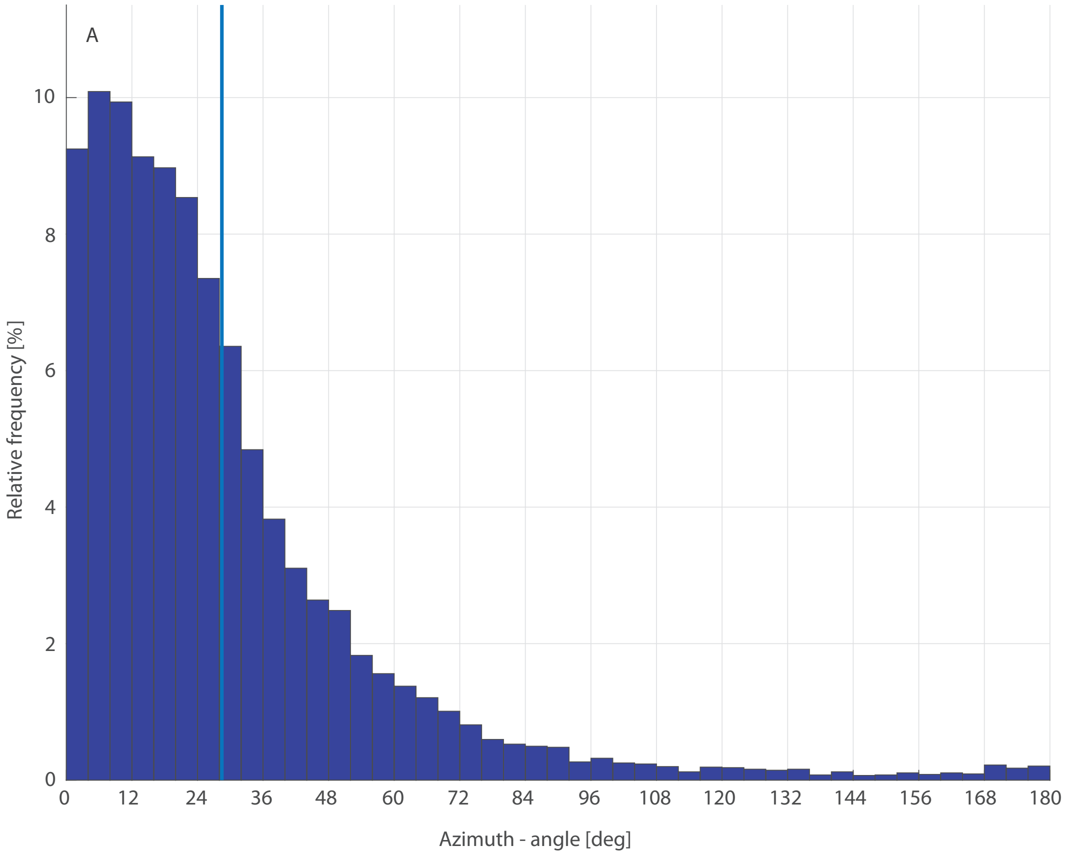
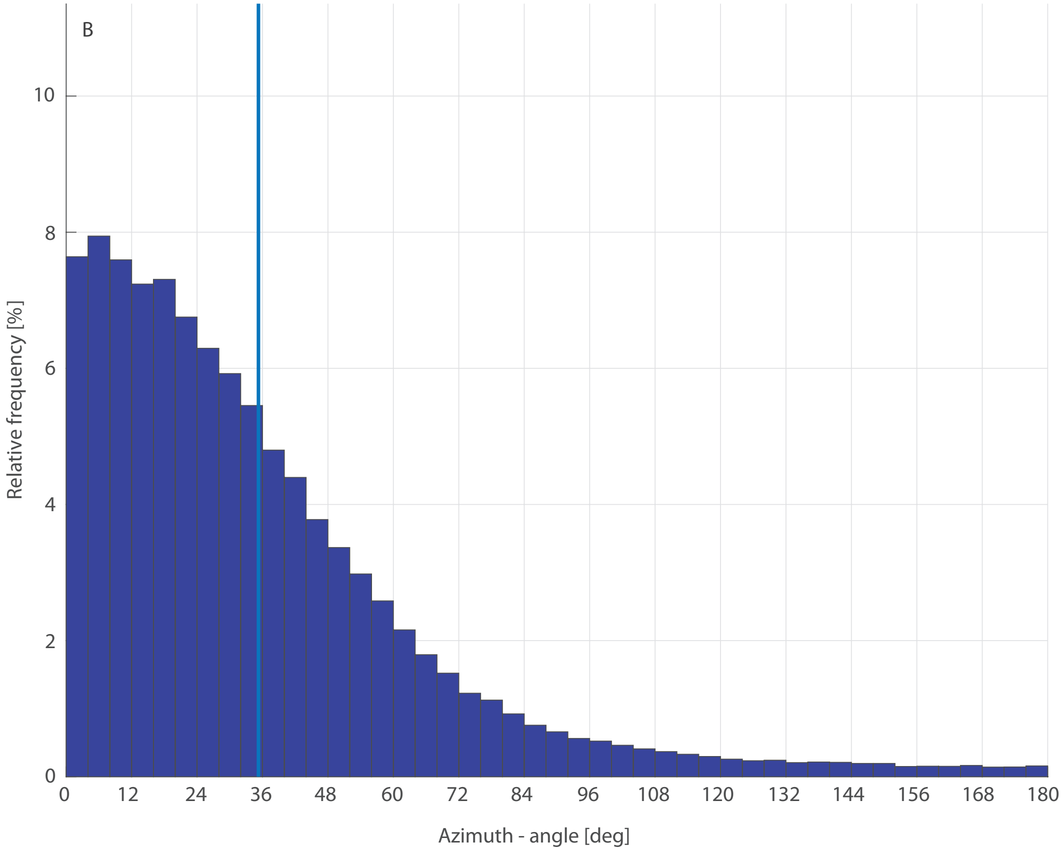


Figure 3.8: **Locations in flight arena where bees fixate nest hole within frontal part of their visual field.** A: locations of nest fixations during pre-landing phase for all bumblebees with a correct first inbound flight (N = 15). The position (red dots) and orientation (red lines) of the bumblebee in the arena when the nest hole ('X') is in the frontal visual field (between −25° and 25°) is plotted. Time between consecutive dots: 20.27 ms. X: landing hole, O: cylinder, blue: arena walls. B: Middle of locations in the flight arena where bees fixate nest hole with frontal part of their visual field during correct inbound flights. Blue circles show the centre of each individual fixation section for all bumblebees (N = 15) during pre-landing phase. C: Nest fixations during incorrect inbound flights (N = 5), pre-landing phase, bees facing i: the dummy hole they landed at ('X') , ii: the nest hole. D: centre of each individual fixation section for bumblebees (N = 5) during pre-landing phase of incorrect inbound flights. i: facing the dummy hole ('X'), ii: facing the nest hole.

Sideward and forward components of flight

Flying insects have been shown in previous studies to perform a saccadic flight and gaze strategy to separate rapid head and body saccades from largely translational intersaccadic locomotion (Boeddeker et al., 2010, 2015; Braun et al., 2010, 2012; Geurten et al., 2010; Schilstra and van Hateren, 1999). Only translational optic flow is distance-dependent and, thus, contains spatial information. Consequently, the saccadic flight and gaze strategy allows to gather spatial information from the resulting optic flow during intersaccadic intervals (Egelhaaf et al., 2012). Spatial information can be extracted best in the viewing direction orthogonal to the translational movement. Hence, during sideward flight the extraction of spatial information from the retinal image flow is easiest in the frontal visual field and during forwards and backwards motion in the lateral visual field.


A
Relative frequency [%]
0
2
4
6
8
10
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
Azimuth - angle [deg]


B
Relative frequency [%]
0
2
4
6
8
10
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
180
Azimuth - angle [deg]

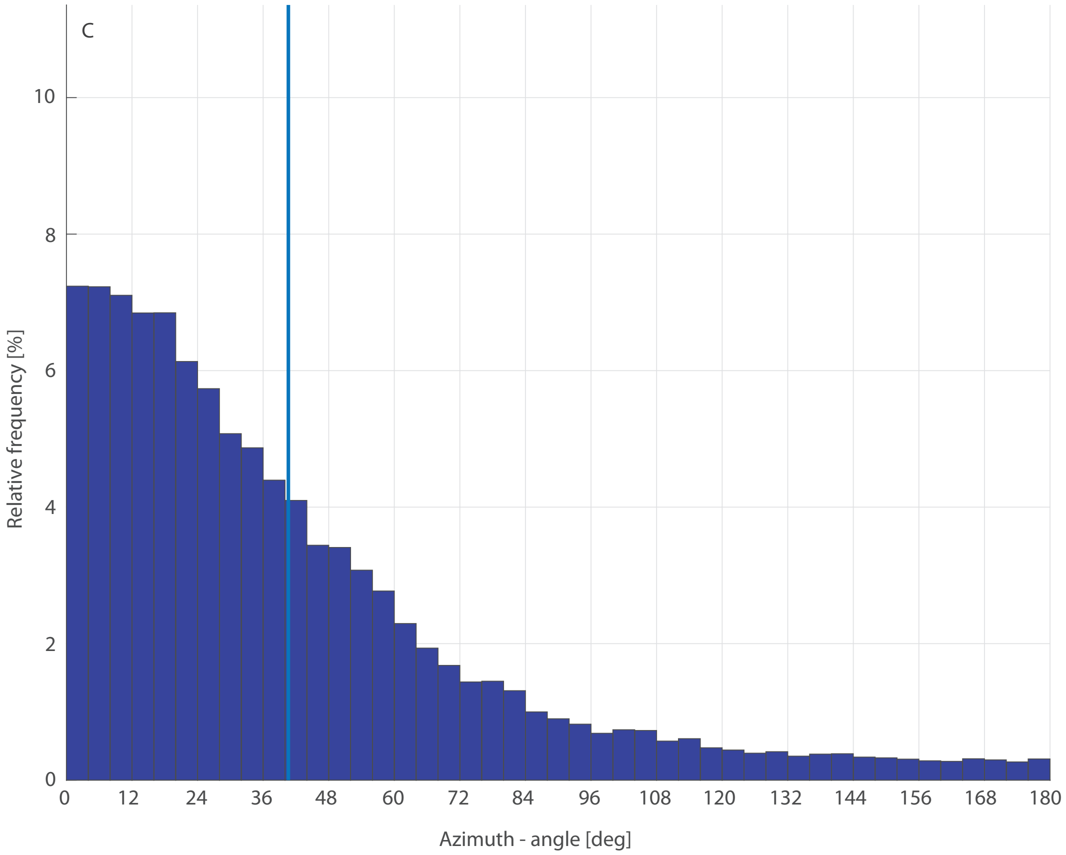


Figure 3.9: **Translational movements.** Sideward and forward/backward components of flight: distribution of direction of the translational component of motion relative to the orientation of the flight trajectory for all bumblebees and all flight phases during first inbound flights. A: entry phase, B: homing phase, C: pre-landing phase. The angle was determined by calculating the angle between the orientation of the body yaw axis and the flight direction. The average angle is shown in blue (line: 28°, 35° and 40°). An angle of 0° corresponds to pure forward movement and an angle of 90° represents pure sideward movement to the right or left, respectively.

The spatial resolution of our recordings was not sufficient to address the temporal fine structure of the bees' gaze strategy precisely at the level of body orientation and, especially, not at the level of head orientation. Nevertheless, there are clear indications in our data for a saccadic flight strategy (Fig. 3.9 A). We could estimate the relation of sideward and forward/backward movement of the bumblebees by calculating the angle between the orientation of the body yaw axis and the flight direction. The translational movement of the bumblebees is dominated by almost pure forward movement (angle between body orientation and flight direction 0° – 12°) during all phases of the first inbound flight (fig. 3.9 B – D). Nevertheless, the amount of almost pure sideward movement (angle between body orientation and flight direction 84°

– 96°) increases from the entry phase to the homing phase and even more in the pre-landing phase. Hence, bumblebees show more sideward movements when they get closer to the ground and, in particular, to the goal. Hence, we conclude the generation of more sideward movement, when the bees are close to the ground and one of the holes, to be part of the returning behaviour of bumblebees. As mentioned above, translational movement is necessary for insects to gain depth information via optic flow. This behaviour, thus, might be essential for the bumblebees to distinguish the hole and the cylinders from the arena background and to measure the remaining distance to its goal.

3.3.2 Development of outbound and inbound flights with potentially increasing experience

To test the hypothesis that there might be some learning progress after initially homing-naïve bumblebees left their nest hole and returned to it several times, we analysed series of consecutive outbound and inbound flights both with respect to homing errors, i.e. whether they landed at the nest hole or a dummy hole, as well as with respect to a range of flight parameters characterising the spatio-temporal profile of the flights.

Homing error

Since bumblebees did not always approach the nest hole, but one of the dummy holes on their return flight, the information given by the two white cylinders next to the nest hole seem to be insufficient for a reliable and goal-directed return. After we analysed the homing error of the first inbound flight for homing-naïve bumblebees (fig. 3.10 A), we additionally analysed it for all subsequent 116 inbound flights, before the cylinder arrangement was changed. 102 of these flights ended at the nest hole, ten at the two dummy holes next to it. Four flights ended at other dummy holes (fig. 3.10 B). Since, on the whole, 88 % of the flights ended at the nest hole, it is suggested that the bees may have become slightly better in finding the nest hole with more experience as compared with the homing performance recorded for the 1st inbound flight (see above). Eleven bumblebees (out of 20) never ended their inbound flights at a dummy hole, but for most (seven) of them only a relatively short sequence of flights, i.e. less than six flights, could be recorded.

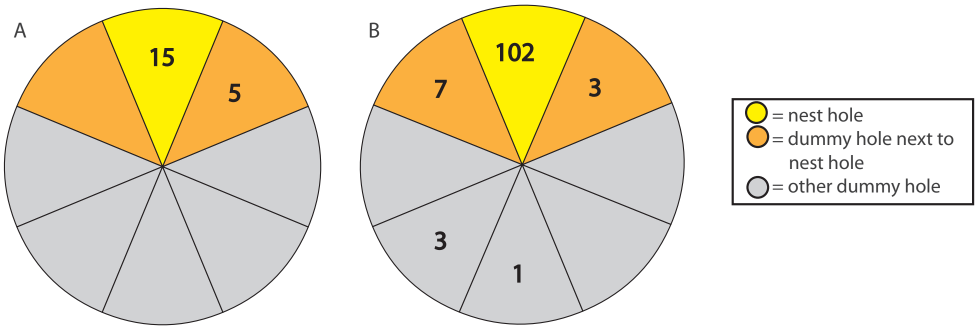
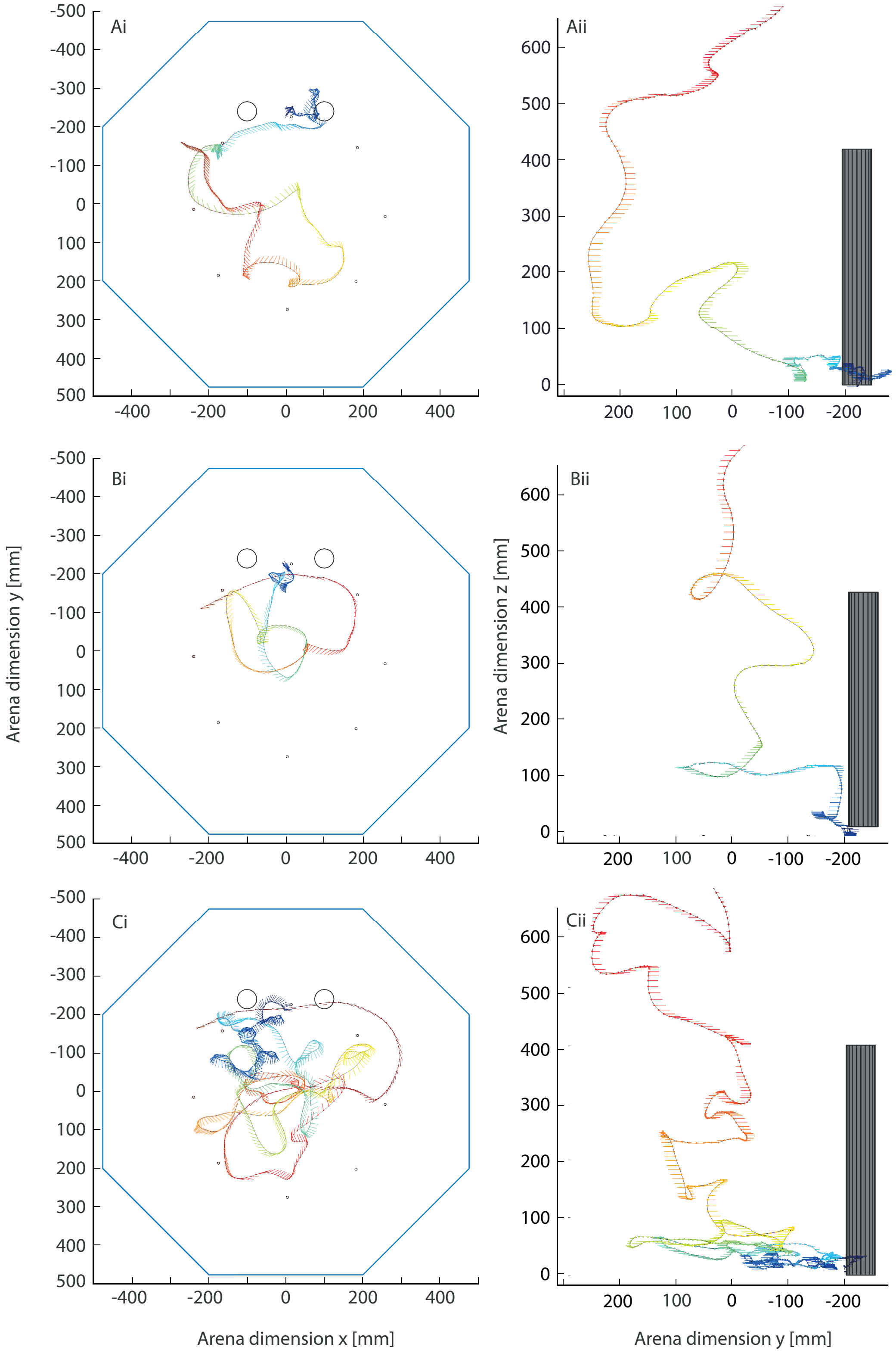


Figure 3.10: **'Homing error', flight series of 1st sequence.** 'Homing error' and landing preference for inbound flights of the 1st sequence (before cylinder displacement) for 20 bumblebees, n = 136 flights. The segments of the circle display the sections of the nest hole and dummy holes, respectively. A: landings after the first inbound flight n = 20, B: landings after all other inbound flights of the 1st sequence, n = 116.

Nevertheless, the homing performance was very variable for individual bees, but especially across bees. Two bumblebees, for example, ended only their first inbound flight at a dummy hole, and always landed at the nest hole afterwards. Other bumblebees ended their consecutive inbound flights several times at a dummy hole, but in-between also at the nest hole, so that their success in finding the nest hole seems to be somehow random. Other bees always used the same dummy hole for their landing, e.g. the dummy hole next to the nest hole, when they made an error in-between correct return flights, while other bees used different dummy holes. Still, all inbound sequences, where bumblebees used dummy holes more than once, were mixed with landings at the nest hole: No bumblebee landed always at a dummy hole. Taken all these data together we conclude, that most bumblebees are well able to find the nest hole marked by the cylinders despite the great variability in homing performance of individual bees as well as inter-individually. Moreover, despite the large variability there might be an overall tendency for an improvement in homing performance when comparing the first inbound flights with the subsequent ones.

Overall flight structure



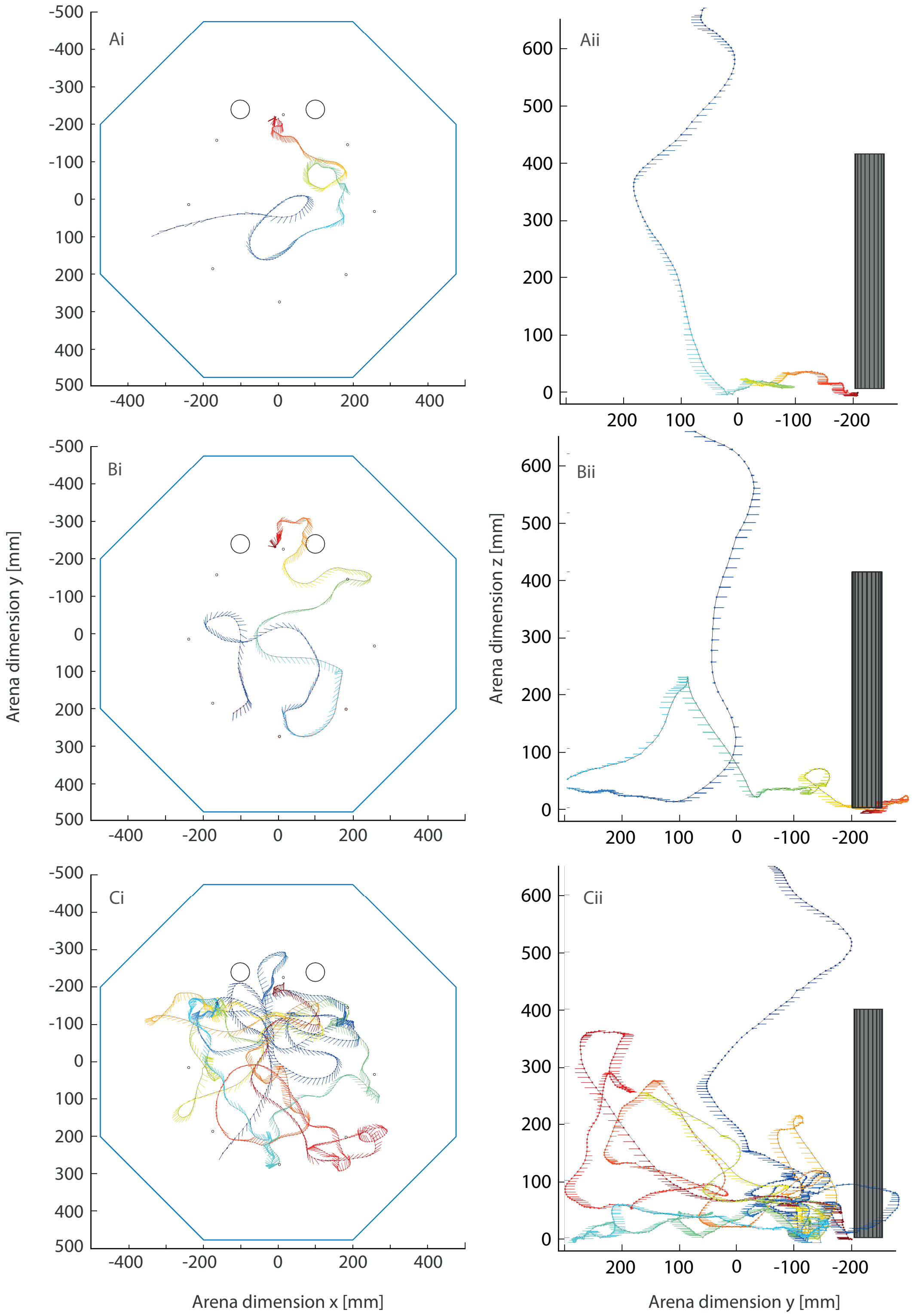
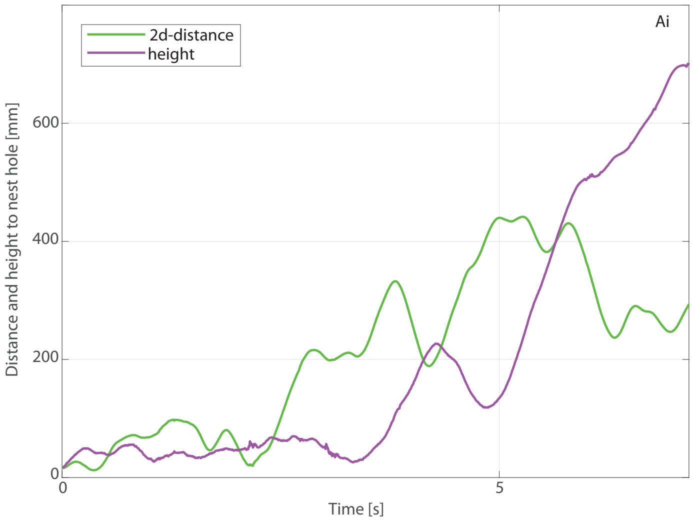
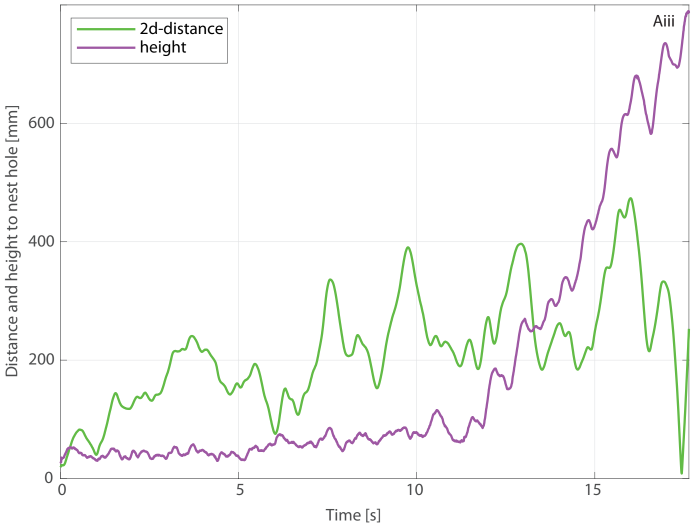


Figure 3.11: **Flight trajectories of last outbound and inbound flights of 1st sequence.** Three example trajectories of last outbound (A-C) and inbound (D-F) flights. Specifications see figure 3.2


Ai
2d-distance
height
Distance and height to nest hole [mm]
0
200
400
600
0
5
Time [s]


Aii
2d-distance
height
Distance and height to nest hole [mm]
0
200
400
600
0
5
Time [s]


Aiii
2d-distance
height
Distance and height to nest hole [mm]
0
200
400
600
0
5
10
15
Time [s]

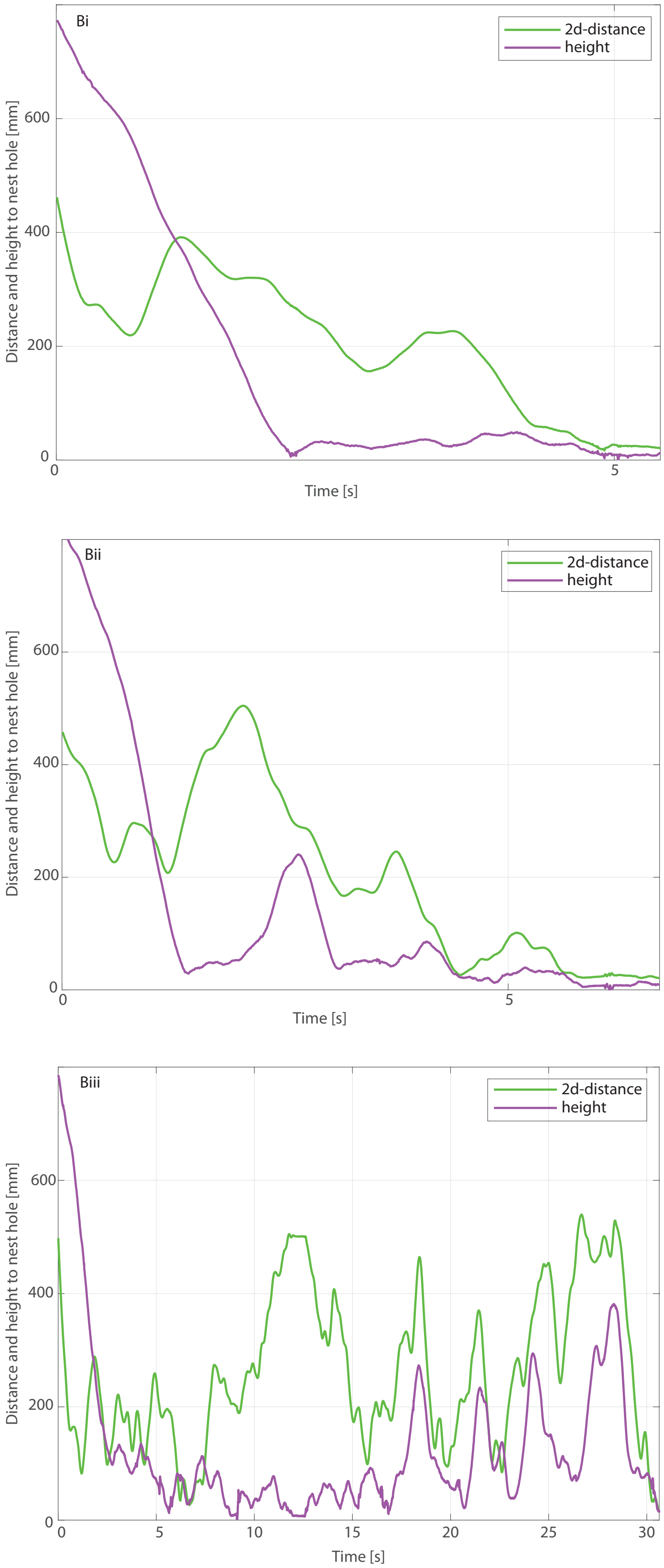
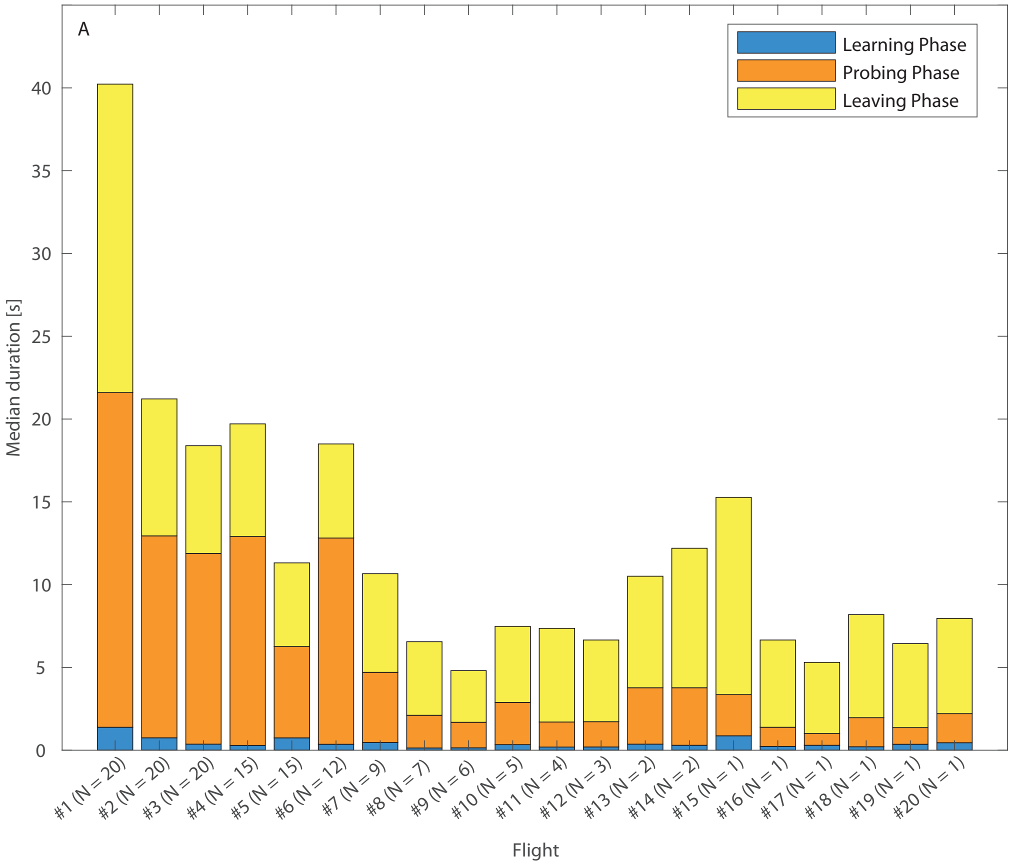


Figure 3.12: **Time course of altitude and distance of the bumblebee to the nest hole, last flights, 1st sequence.** The same three outbound (A) and inbound (B) flights as shown in Fig. 3.11. Flights are shown in their entire length, time scales (linear) on x-axes vary.

Flight trajectories of the bumblebees tested do not show a large development over time (fig. 3.11). Although the flight route becomes shorter and less complex, outbound and inbound flights are still characterised by a loop-like structure and far away from straight flights. Furthermore, the diverse behaviour of the bees is visible in the profile of height and 2-dimensional distance to the nest hole (fig. 3.12). Shown are the last flights of the 1st sequence, i.e. before the cylinders were displaced. Bumblebees did not have the same experience at this time, but all did at least three outbound or inbound flight.

Flight duration

Given the hypothesis, that outbound and probably inbound flights get shorter when the bees gain experience during consecutive flights, we analysed the development of flight duration for inbound and outbound flight series. On average across bees, consecutive outbound and inbound flights show a decrease in duration (figure 3.13 A and 3.14 A) and, thus, suggest a development of the flight due to an increasing experience. Nevertheless, series of single bumblebees may differ much from this development on an individual basis: Some bees' flight duration continually decreases over the flights, while other bees reveal a considerable variation in their flight duration. Examples of this variability are shown in fig. 3.13 Bi-iii. We found this variability especially for inbound flights (fig. 3.14 Bi-iii), where the flight duration between flights with or without a homing error does not vary in a significant way.



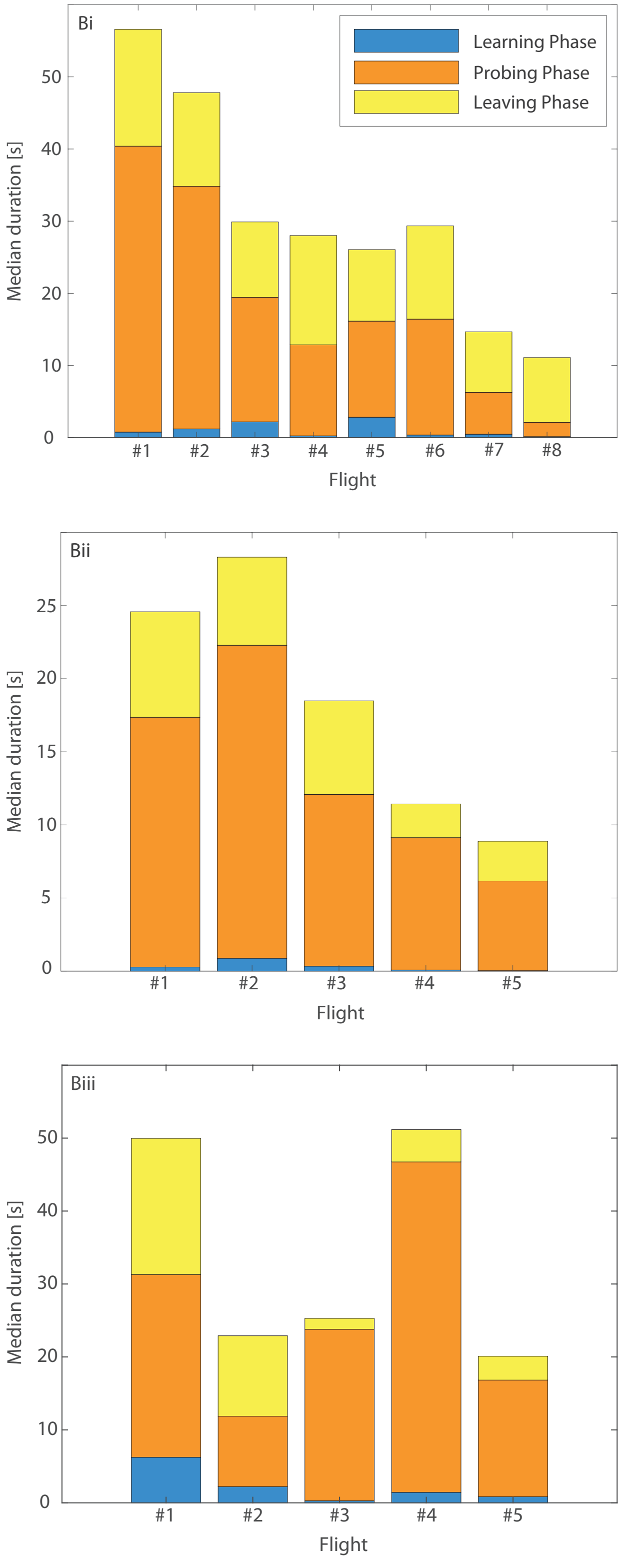
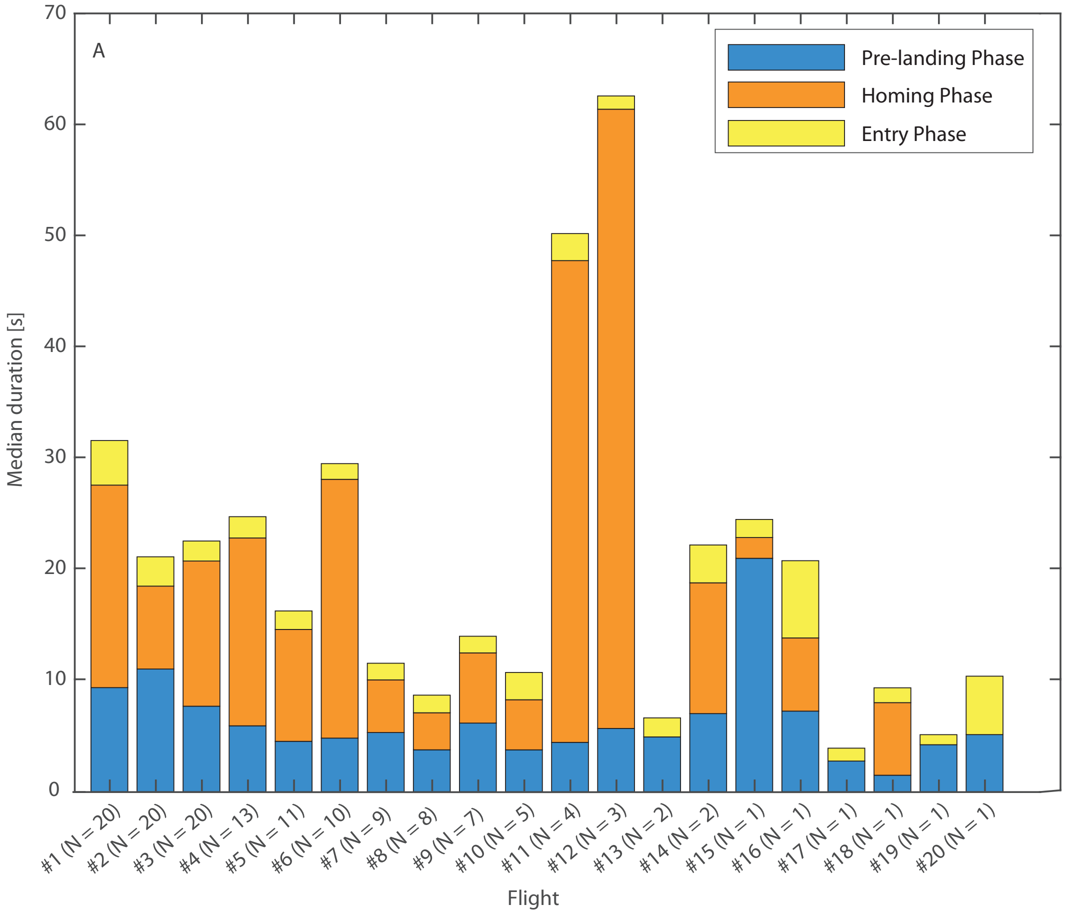


Figure 3.13: **Flight duration, outbound flight series, 1st sequence.** Median of flight duration of all outbound flights during the 1st sequence for 20 bumblebees. Blue: learning phase, orange: probing phase, yellow: leaving phase, N = number of bumblebees. Note that the number of bees per flight decreases over time. A: outbound flights, Bi – iii: examples of bumblebees' individual flight duration development during 1st sequence.


A
Median duration [s]
0
10
20
30
40
50
60
70
Pre-landing Phase
Homing Phase
Entry Phase
#1 (N = 20)
#2 (N = 20)
#3 (N = 20)
#4 (N = 13)
#5 (N = 11)
#6 (N = 10)
#7 (N = 9)
#8 (N = 8)
#9 (N = 7)
#10 (N = 5)
#11 (N = 4)
#12 (N = 3)
#13 (N = 2)
#14 (N = 2)
#15 (N = 1)
#16 (N = 1)
#17 (N = 1)
#18 (N = 1)
#19 (N = 1)
#20 (N = 1)
Flight

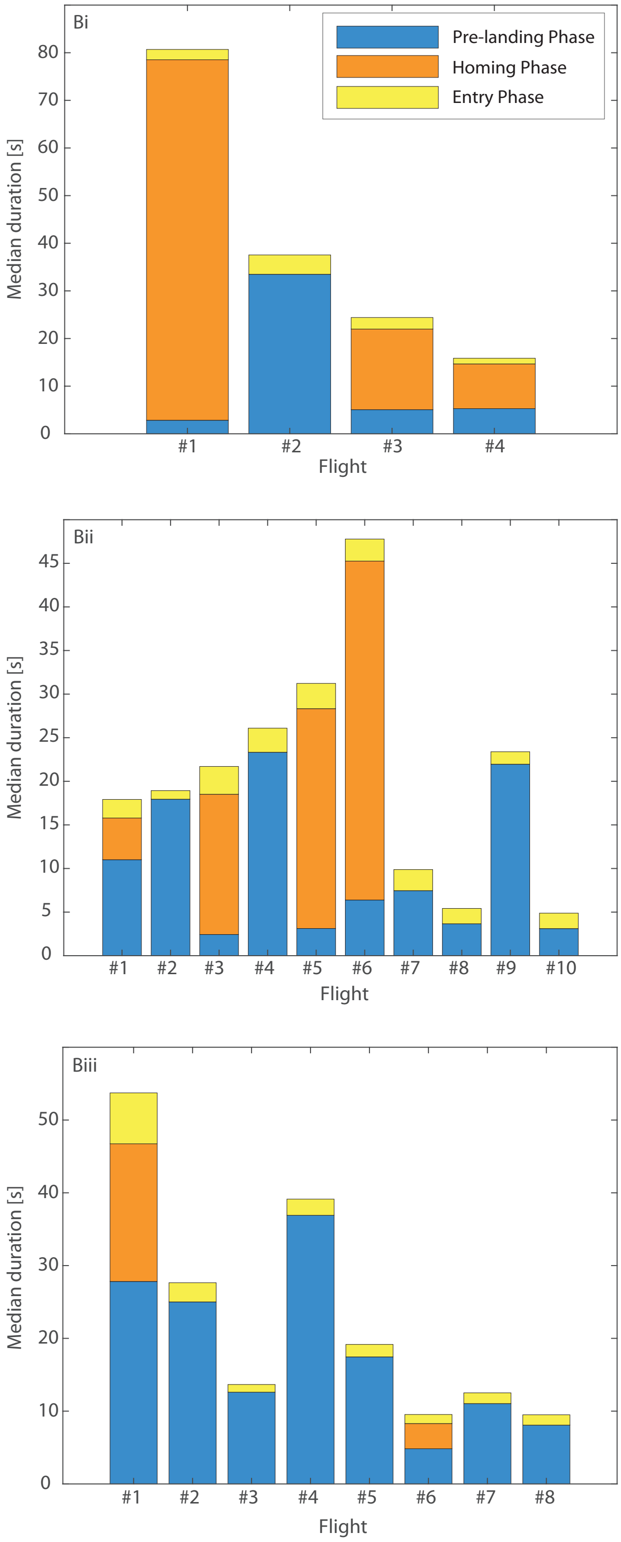
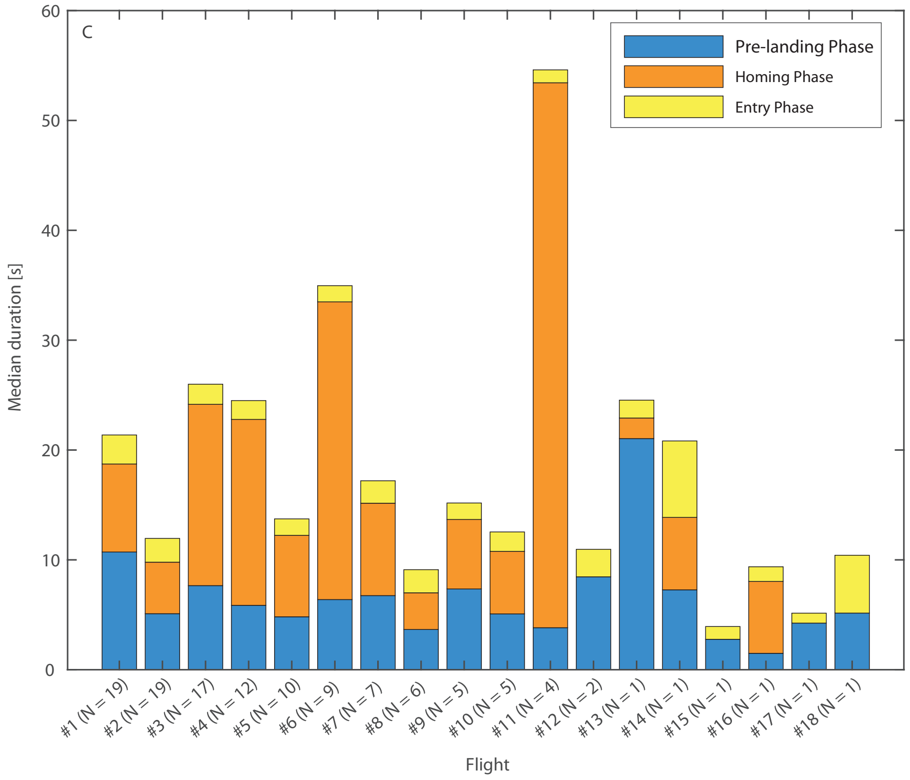
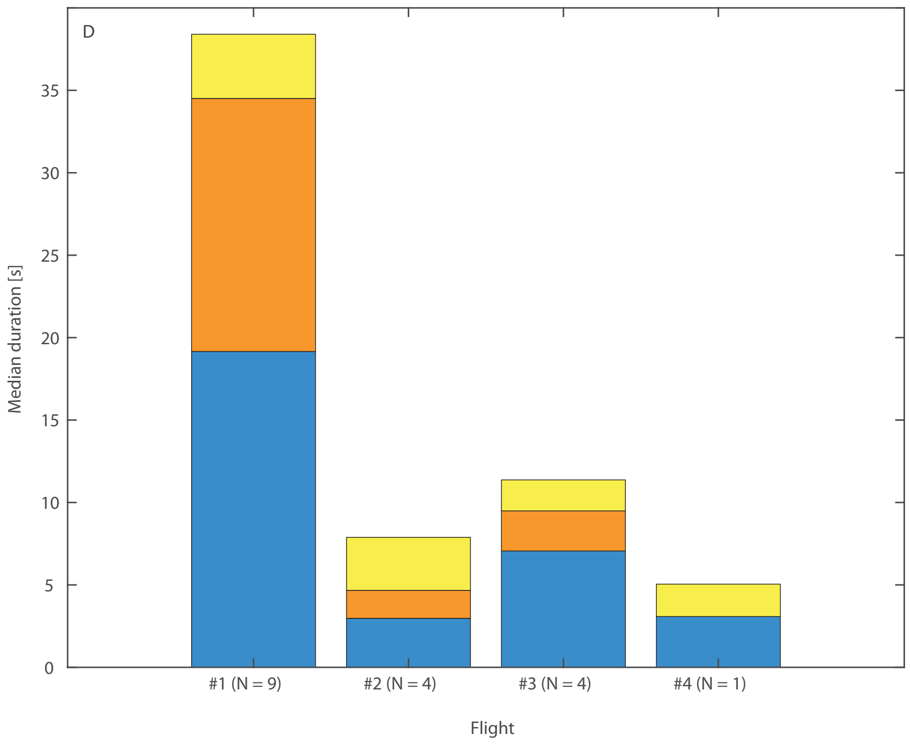


Figure 3.14: **Flight duration, inbound flight series, 1st sequence.** Median of flight duration of all inbound flights during the 1st sequence for 20 bumblebees. Blue: pre-landing phase, orange: homing phase, yellow: entry phase, N = number of bumblebees. Note, that the number of bees per flight decreases over time. A: inbound flights, Bi – iii: examples of bumblebees' individual flight duration development during 1st sequence, a missing homing phase (orange) means that the bumblebee flew without fluctuations from a height above 100 mm to a height below 100 mm. C: 'correct' inbound flights, D: 'incorrect' inbound flights.


C
Median duration [s]
60
50
40
30
20
10
0
Pre-landing Phase
Homing Phase
Entry Phase
#1 (N = 19)
#2 (N = 19)
#3 (N = 17)
#4 (N = 12)
#5 (N = 10)
#6 (N = 9)
#7 (N = 7)
#8 (N = 6)
#9 (N = 5)
#10 (N = 5)
#11 (N = 4)
#12 (N = 2)
#13 (N = 1)
#14 (N = 1)
#15 (N = 1)
#16 (N = 1)
#17 (N = 1)
#18 (N = 1)
Flight


D
Median duration [s]
35
30
25
20
15
10
5
0
#1 (N = 9)
#2 (N = 4)
#3 (N = 4)
#4 (N = 1)
Flight

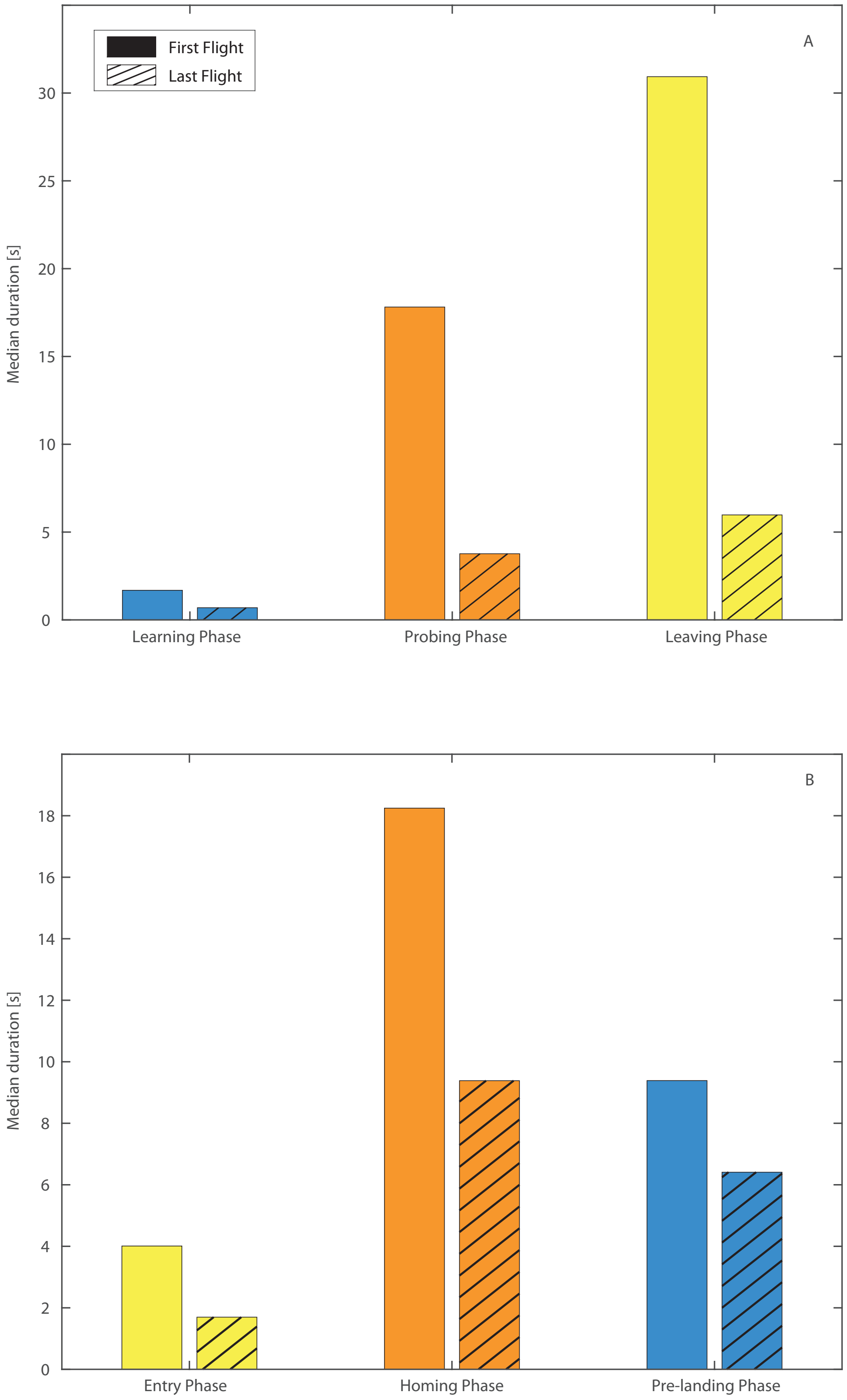


Figure 3.15: **Flight duration of different phases, outbound flight series, first and last flight of 1st sequence.** Duration of flights during the 1st sequence for 20 bumblebees, divided into all flight phases. A: outbound flights, B: inbound flights. Blue: Learning/Pre-landing phase, orange: probing/homing phase, yellow: leaving/entry phase; non-hatched bar: first flight of 1st sequence, hatched bar: last flight of 1st sequence. N = 20.

By analysing all flight phases for the first and the last flight before changing the position of the cylinders, we could better see the difference between bumblebees when they were homing-naïve and when they had gained some experience. Since the duration of the learning and probing phase has decreased after several flights (fig. 3.15 A), we conclude that the bumblebees need less time to learn and specially to probe the goal-centred information when they are more familiar with the nest hole surroundings. Additionally, also the duration of the leaving phase decreases, suggesting that bumblebees need also less time to find a way out of the arena. During inbound flights, flight duration of the homing and the pre-landing phase decreased between the first and last flight before shifting the position of the landmarks, again hinting at a learning process (fig. 3.15 B).

Flight velocity

Gained experience and an increasing familiarisation with the environment might manifest itself not only in the flight duration, but also in flight velocity. To assess whether flight velocity is affected by the bumblebees' experience, we analysed its development for outbound and inbound flights over time. We divided the flights into phases below and above 100 mm as in the flight duration analysis. Inbound flights are on average always flown at a higher velocity than outbound flights, at least flights at a similar state of experience. On average, there seems to be a trend in becoming faster for outbound as well as inbound flights (fig. 3.16 A, B). This trend is more pronounced in phases of outbound flights rather than inbound flight phases (fig. 17 A, B). However, this tendency is rather weak and not significant because of the high variability on an individual level. Hence, flight velocity seems to be less affected by the bee's experience than the flight duration, indicating a shorter flight route.

Moreover, we found no obvious difference in flight velocity between inbound flights that ended at the nest hole and returns which ended at a dummy hole (fig. 3.18 A, B).

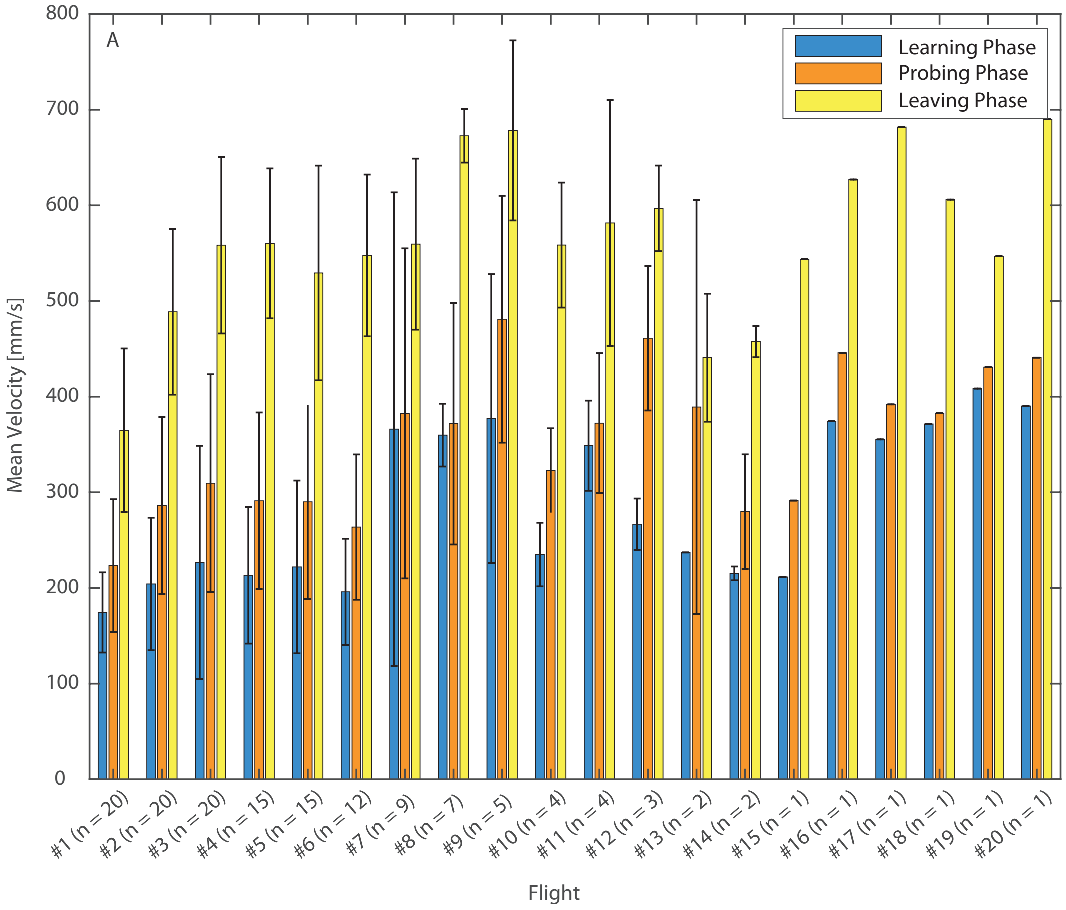
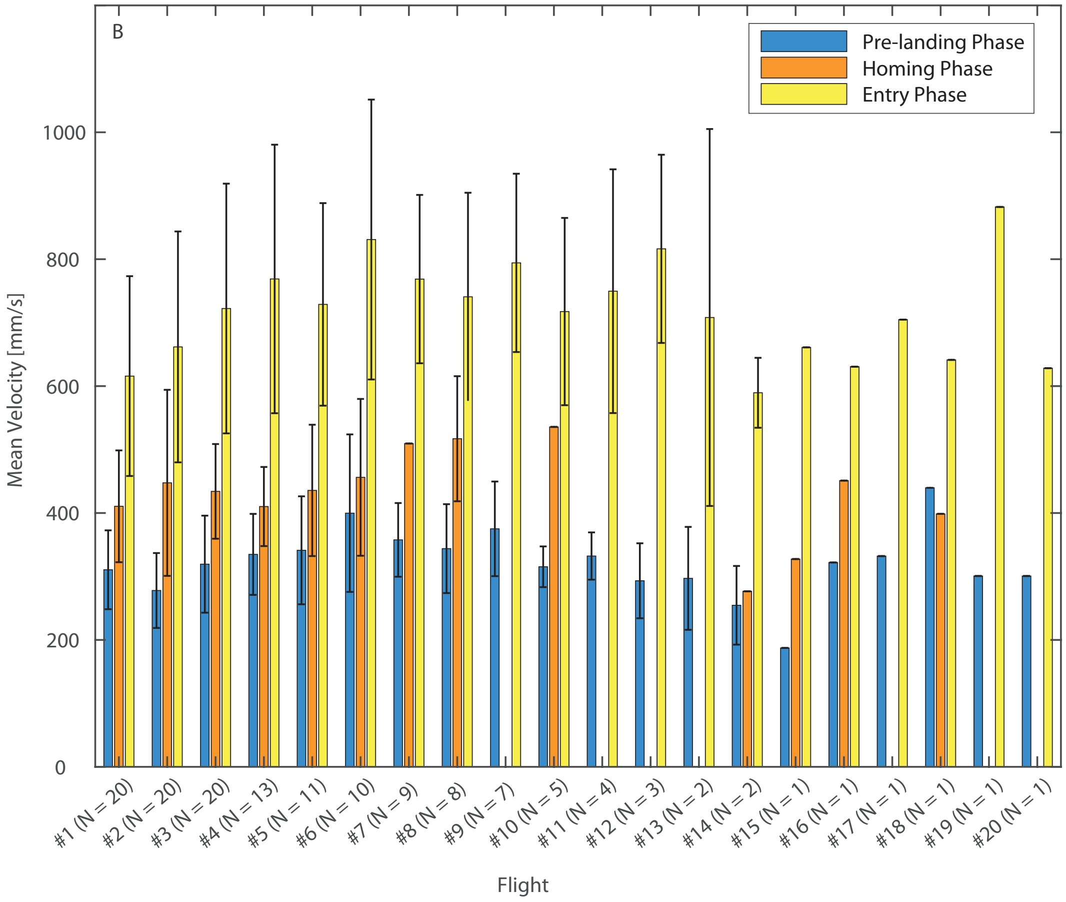


Figure 3.16: **Flight velocity, flight series of 1st sequence.** Mean and standard deviation of velocity of all flights during the 1st sequence for 20 bumblebees. Blue: learning phase, orange: probing phase, yellow: leaving phase, N = number of bumblebees. Note, that the number of bees per flight decreases over time. A: All outbound flights, B: all inbound flights.


B
Pre-landing Phase
Homing Phase
Entry Phase
Mean Velocity [mm/s]
0
200
400
600
800
1000
#1 (N = 20)
#2 (N = 20)
#3 (N = 20)
#4 (N = 13)
#5 (N = 11)
#6 (N = 10)
#7 (N = 9)
#8 (N = 8)
#9 (N = 7)
#10 (N = 5)
#11 (N = 4)
#12 (N = 3)
#13 (N = 2)
#14 (N = 2)
#15 (N = 1)
#16 (N = 1)
#17 (N = 1)
#18 (N = 1)
#19 (N = 1)
#20 (N = 1)
Flight

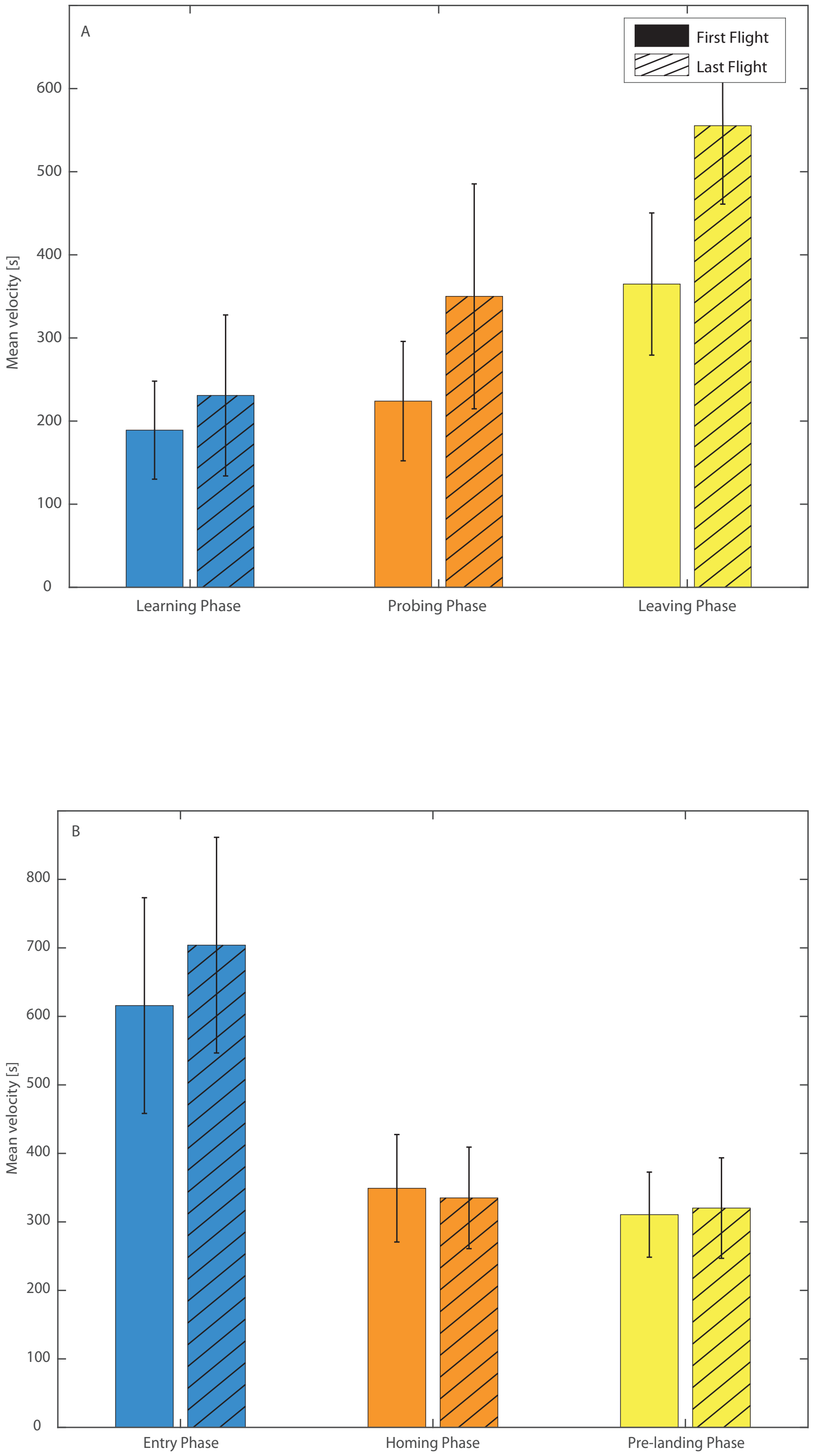


Figure 3.17: **Flight velocity, development of 1st sequence.** Mean flight velocity for first and last flights of the 1st sequence during different phases of A: outbound flights and B: inbound flights. Blue: Learning/Pre-landing phase, orange: probing/homing phase, yellow: leaving/entry phase; non-hatched bar: first flight of 1st sequence, hatched bar: last flight of 1st sequence.

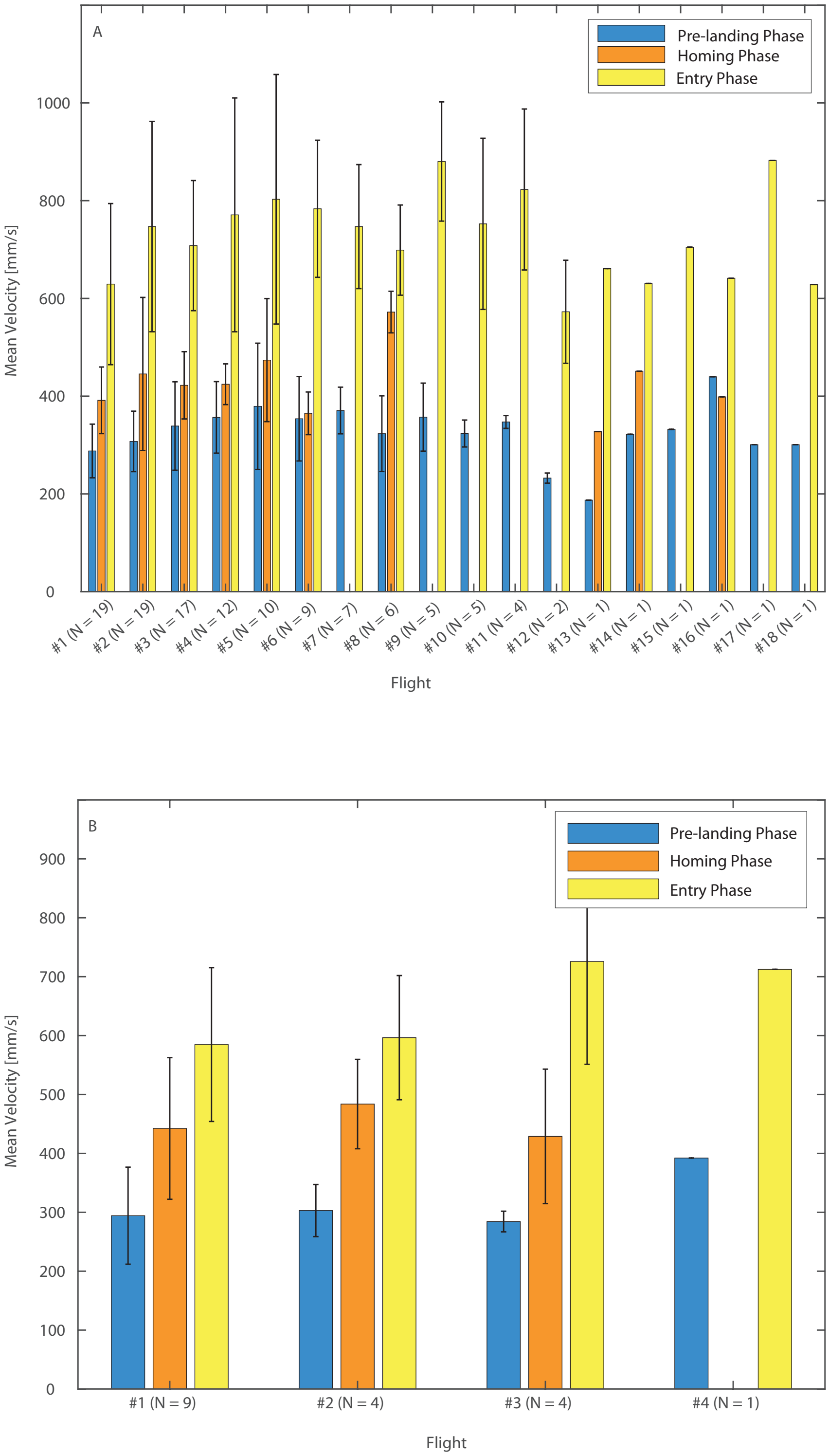


Figure 3.18: **Flight velocity, flight series of 1st sequence, divided into ’correct’ and ’incorrect’.** Mean and standard deviation of velocity of all flights during the 1st sequence for 20 bumblebees. Blue: learning phase, orange: probing phase, yellow: leaving phase, N = number of bumblebees. Note, that the number of bees per flight decreases over time. A: ‘correct” inbound flights, B: ’incorrect’ inbound flights

Relation between outbound and inbound flight duration

Given that bumblebees and other central place foraging insects use the departure from a goal location to gather spatio-temporal information about the vicinity of this location, which later helps them to find back to this place, one might assume that the duration of the outbound flight is somehow related to the duration of the inbound flight. One may argue in two somehow contrasting ways. On the one hand, a longer outbound flight might suggest that bumblebees spend more time during departure with learning and probably probing the goal-centred information and might then have less difficulties in finding back, i.e. leading to a shorter subsequent inbound flight. *Vice versa*, a shorter outbound flight, where the insect spends less time for learning and probing might entail a longer inbound flight because of the bumblebee's difficulties to find the target location. On the other hand, just the opposite correlation may also be conceivable: A longer outbound might be a consequence of difficulties in learning a complex nest hole surrounding. Then the return is likely to be a challenge and may require a relatively long time. For experienced insects, on the other hand, a rather short outbound flight might be sufficient for a short, goal-directed inbound flight.

To analyse a possible relationship between outbound and inbound flights, we plotted the duration of the homing and pre-landing phase of each inbound flight against the duration of the learning and probing phase of the preceding outbound flight for all initially homing-naïve bumblebees, for which a consecutive sequence of at least five flight pairs could be recorded.

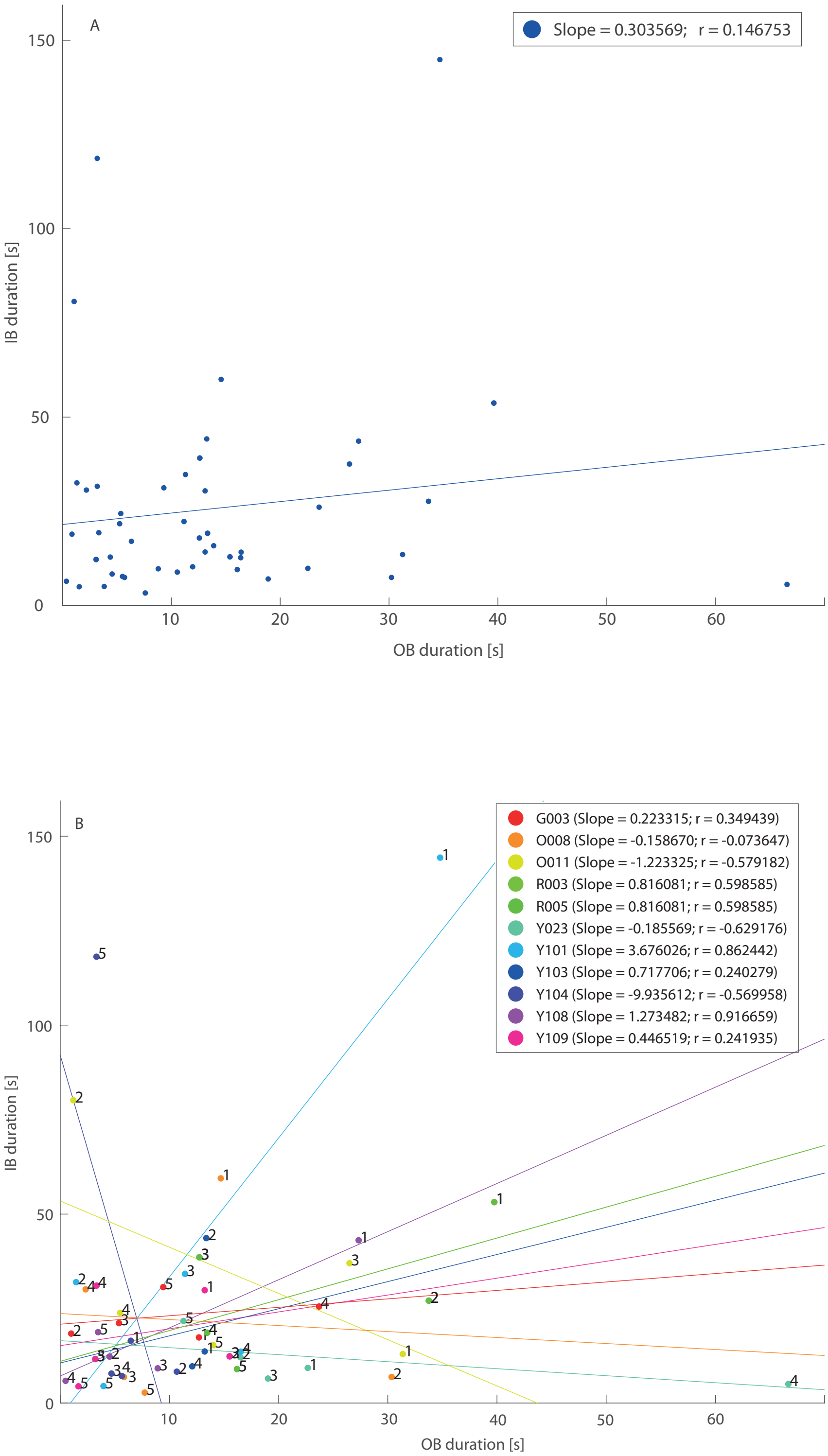


Figure 3.19: **Correlation between duration of outbound and inbound flights.** Duration of outbound flight (x-axis) plotted against duration of inbound flight (y-axis). Plotted are the bumblebees which made at least five pairable outbound and inbound flights; analysed are the first five pairs of flights, flight duration for sections below 100 mm, i.e. initial and probing phase (outbound), homing and pre-landing phase (inbound). A: tendency for all bees, B: tendencies for individual bees.

There seems to be no correlation between the duration of the outbound and the subsequent inbound flight when the data of all bees are collectively taken into account (slope of regression line: 0.3, regression coefficient: ≈ 0.15 (fig. 3.19 A)). The regression coefficient close to zero reflects the high variation of the data. Moreover, correlations still vary a lot on an inter-individual level: Some bumblebees seem to have longer inbound flights, when the preceding outbound flight was long and shorter inbound flights, when the preceding outbound flight was short. But other bumblebees have shorter inbound flights when the preceding departure was long and *vice versa*. For other bees, no significant correlation is obvious at all; accordingly, the slopes of the regression lines vary between ≈ -10 and ≈ 3.7. The regression coefficient is never closer to -1 than ≈ -0.63 and only two regression lines show a coefficient of ≈ 0.9, reflecting the high inter-individual variation across bees and, thus, do not allow us to draw any consistent conclusion (fig. 3.10 B).

A correlation between flight duration is also conceivable the other way round: A long inbound flight, where a bumblebee had difficulties in finding the connected nest hole, might provoke a longer outbound flight to better learn and probe the goal-centred information. If we plot the duration of the learning and probing phase of outbound flights against the duration of the homing and pre-landing phase of the respective preceding inbound flight, the slope of the regression line is ≈ 0.04 when data of all bees are taken into account (regression coefficient: ≈ 0.06, fig. 3.20 A). Again, there is a high variability inter-individually with the slope of the regression lines varying between ≈ -0.9 and ≈ 0.8 (fig. 3.20 B). The regression coefficients never closer to -1 than ≈ -0.48 or to 1 than ≈ 0.73 indicate that there is no consistent relationship between the duration of outbound and preceding inbound flights.

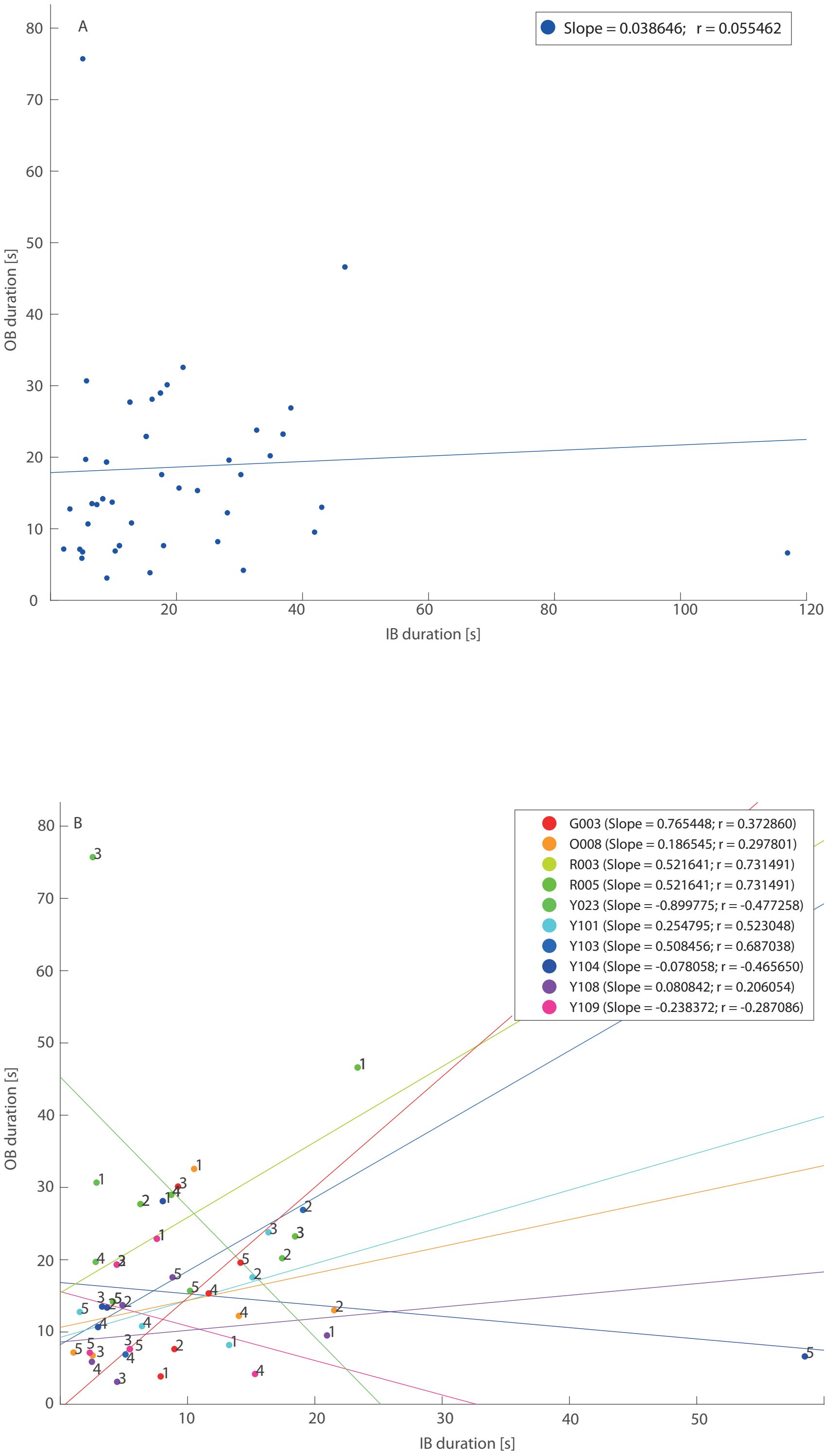


Figure 3.20: **Correlation between duration of inbound and outbound flights.** Duration of inbound flight (x-axis) plotted against duration of outbound flight (y-axis). Plotted are the bumblebees which made at least five pairable outbound and inbound flights; analysed are the first five pairs of flights, flight duration for sections below 100 mm, i.e. initial and probing phase (outbound), homing and pre-landing phase (inbound). A: tendency for all bees, B: tendencies for individual bees.

Retinal position of nest hole

Previous studies suggest that keeping the nest hole in a frontal retinal position might be essential or, at least, useful for insects during their initial section of the departure flight (Lehrer, 1991, 1993; Collett and Lehrer, 1993; Collett, 1996; Zeil et al., 2007, 2009). Since we found that bumblebees in our experiments keep the nest hole in a broad frontal region of their visual field but do this only during the probing phase of outbound flights (Lobecke et al., 2018) and the pre-landing phase of their first inbound flights, the development of this parameter across consecutive outbound and inbound flights might be worth analysing.

We compared the retinal position of the nest hole during the first outbound and inbound flight with that of the corresponding last flights, before the cylinder landmarks were displaced from the nest hole to a dummy hole. The slight trend, to keep the nest hole region in the frontal part of the visual field during the probing and pre-landing phase does not change much with experience and is still present for the last flight of the analysed sequence (fig. 3.21 Ai, ii and Bi, ii). For all other phases of outbound and inbound flights we found no evidence for looking at the nest hole by the frontal part of the eye (e.g. learning phase of outbound flights, fig. 3.21 Ci, Cii). The data suggest, that the nest hole does not play an important role in shaping the flights apart from those phases prior to landing or just after departure. In any case, there is no obvious change in nest hole fixation in neither outbound nor inbound flights with increasing experience.

As for the first outbound and inbound flight, we analysed where in the flight arena the bumblebees keep the nest hole in the frontal visual field between $-25°$ and $25°$ for both the series of outbound and inbound flights. These flight sections might shorten over time or might occur at different positions in the arena when the bees have become familiar with the nest hole surroundings.

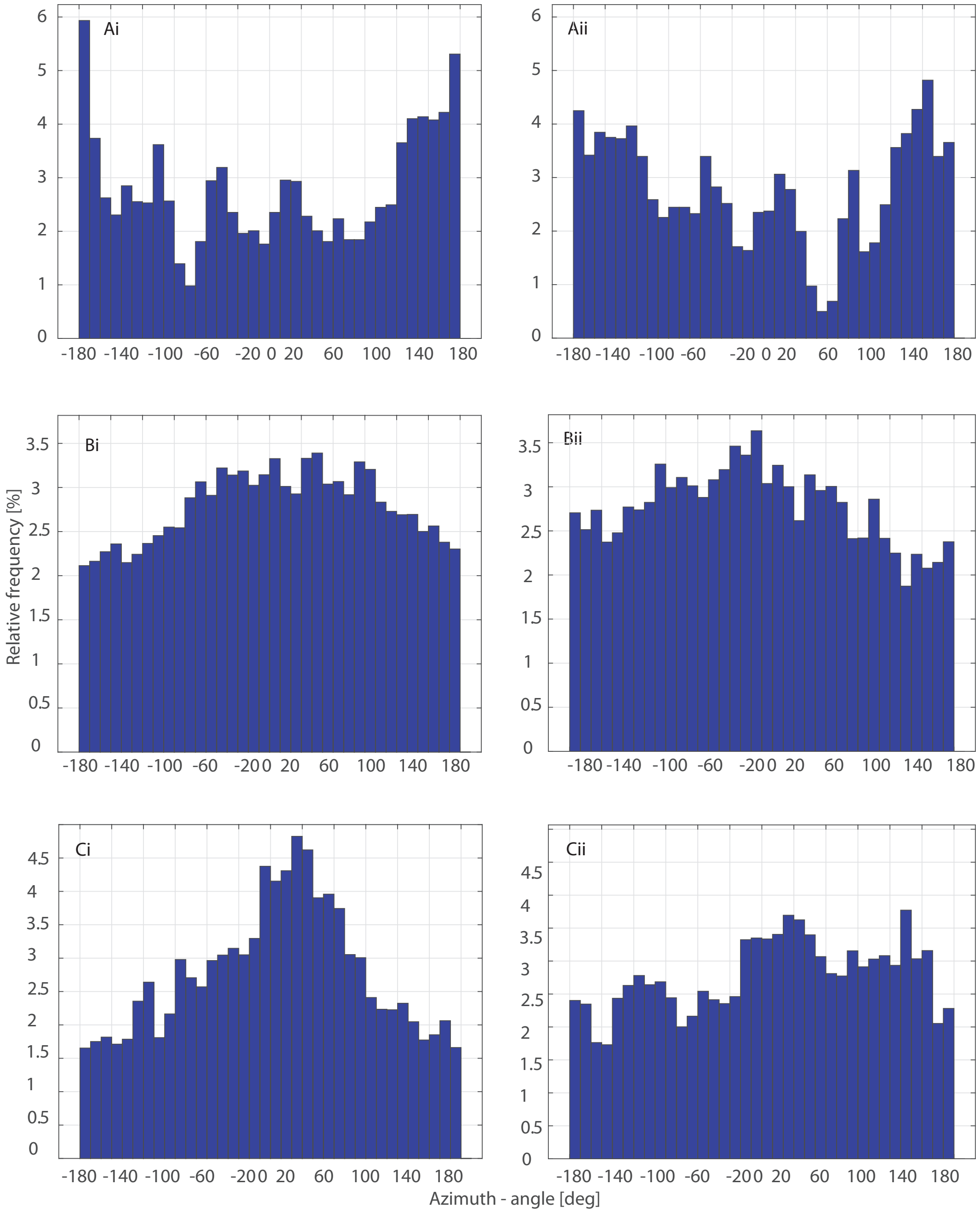


Figure 3.21: **Histogram of the retinal nest hole position.** A: Retinal position of nest hole during learning phase of outbound flights (N = 20), i: first flight and ii: last flight. B: Retinal position of nest hole during probing phase of outbound flights (N = 20), i: first flight and ii: last flight. C: Retinal position of nest hole during pre-landing phase of correct first inbound flights (N = 15), i: first flight, ii: last flight. Analyses of retinal position of the nest hole or the chosen dummy hole of incorrect inbound flights did not reveal any obvious tendency.

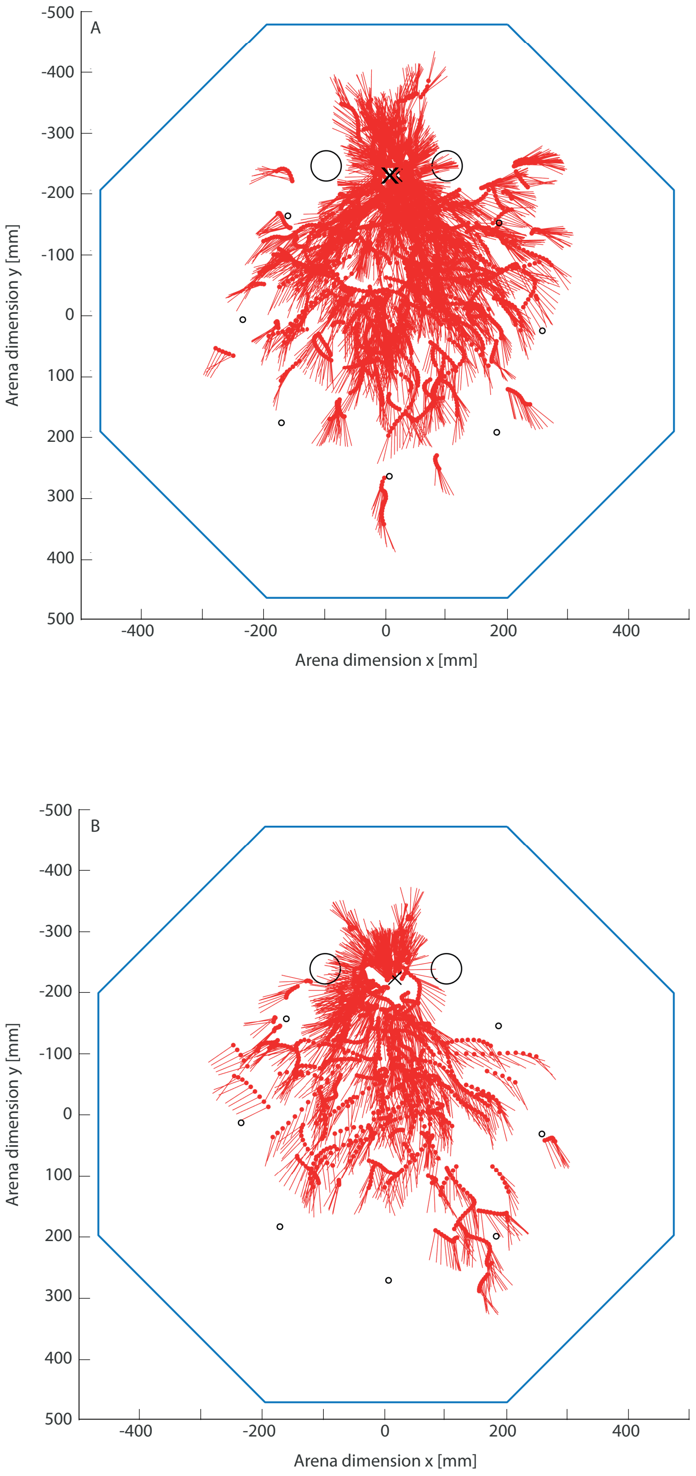


Figure 3.22: **Locations of nest fixation, outbound.** Locations in flight arena where bees fixate nest hole with frontal part of their visual field during the probing phase of first and last outbound flight of the 1st sequence (N = 20). The position (red dots) and orientation (red lines) of the bumblebee in the arena when the nest hole is in the frontal visual field (between $-25°$ and $25°$) is plotted. Time between consecutive dots: 20.27 ms. A: first flight, B: last flight, X: nest hole, o: dummy holes, O: cylinder.

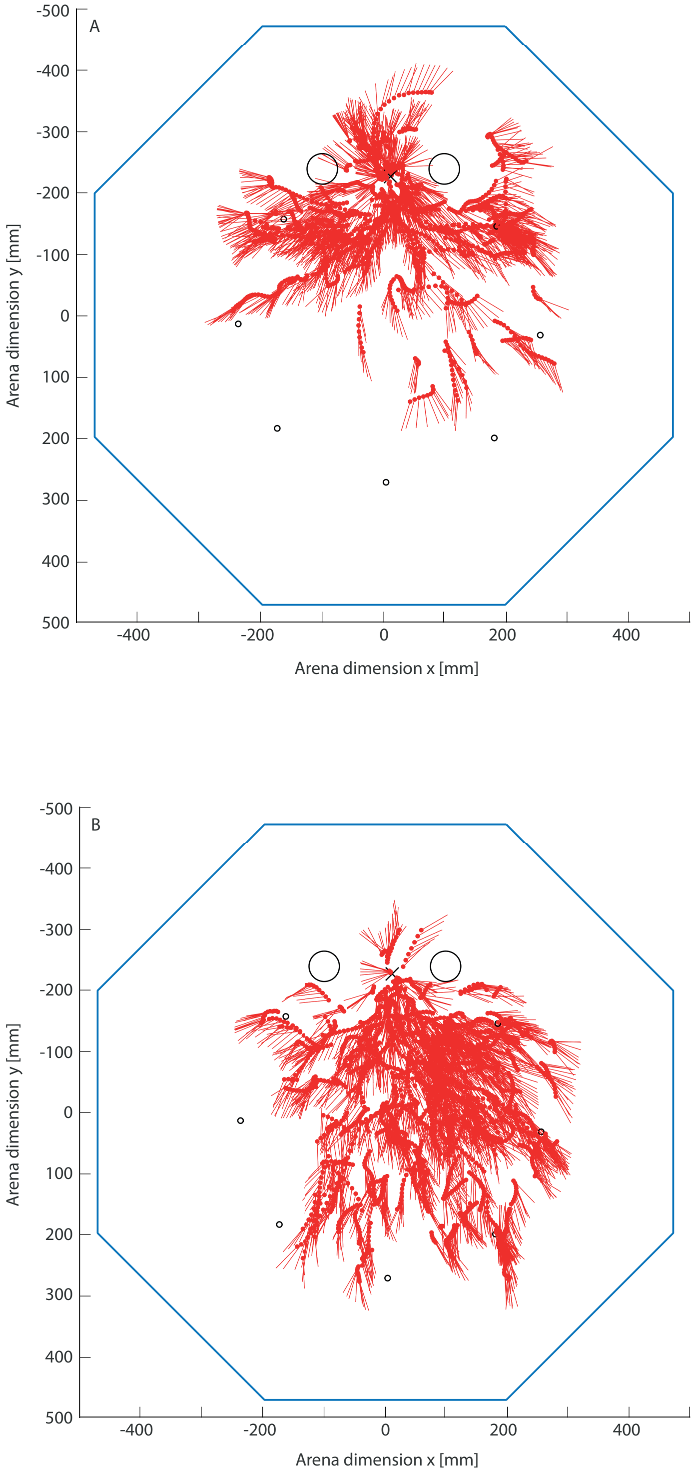


Figure 3.23: **Locations of nest fixation, inbound.** Locations in flight arena where bees fixate nest hole with frontal part of their visual field during the pre-landing phase of first and last inbound flight of the 1st sequence (N = 20). The position (red dots) and orientation (red lines) of the bumblebee in the arena when the nest hole is in the frontal visual field (between $-25°$ and $25°$) is plotted. Time between consecutive dots: 20.27 ms. A: first flight, B: last flight, X: nest hole, o: dummy holes, O: cylinder.

However, our data reveal that the sections of the trajectories, where the bumblebees keep the nest hole in the frontal visual field, do not change much over time, i.e. with increasing experience of the bees (fig. 3.22 A and B). Only for the inbound flight series a slight difference between first and last flight of the sequence can be seen: While during the first inbound flight, bumblebees fixate the nest hole and its two neighbouring dummy holes at locations concentrated around these positions, the fixate locations widely spread over the entire horizontal extent of the arena during the last inbound flight (fig. 3.23 A and B).

Translational movements

Since translational movements provide spatial information via optic flow (Egelhaaf et al., 2012), they might be essential in the process of information gathering during learning and return flights. Therefore, we analysed the development of translational movements during the flight series of outbound and inbound flights.

As suggested in Lobecke et al. (Lobecke et al., 2018), the distribution of translational movements during the learning phase of the outbound flight is relatively flat, because of the proportional high amount of backward movements. This trend seems to be almost independent from the state of the bumblebees' experience (fig. 3.24 Ai, ii). However, there is a slight trend of decreased forward movement with experience (cf. i and ii of fig. 3.24 B and i and ii of fig. 3.25 A, B): the distribution of movements broadens for the last flight of the sequence and while most movements of the first flight are on average forward (between 0° and 25°), the last flights show the same amount of movements between 0° and 48° (outbound) or 0° and 36° (inbound), respectively. This trend is, thus, seen in the average movement angle (blue line in fig. 3.24 and 3.25). For the inbound flights, the amount of backward movements increases with experience (fig. 3.25 A, B).

The distribution of translational movements for the probing phase of the outbound flights is a descriptive evidence for the loop-like structure of the bumblebees' flights, being in contrast to the arc-like structure of wasps (Collett and Lehrer, 1993; Stürzl et al., 2016; Zeil, 1993a) and the ‘turn-back-and-look” behaviour of honeybees (Lehrer, 1991, 1993). However, there is still a large variability across bumblebees for all flight phases, independent of their state of experience. Why bees show less forward movement during the last flights of the sequence, is an open question.

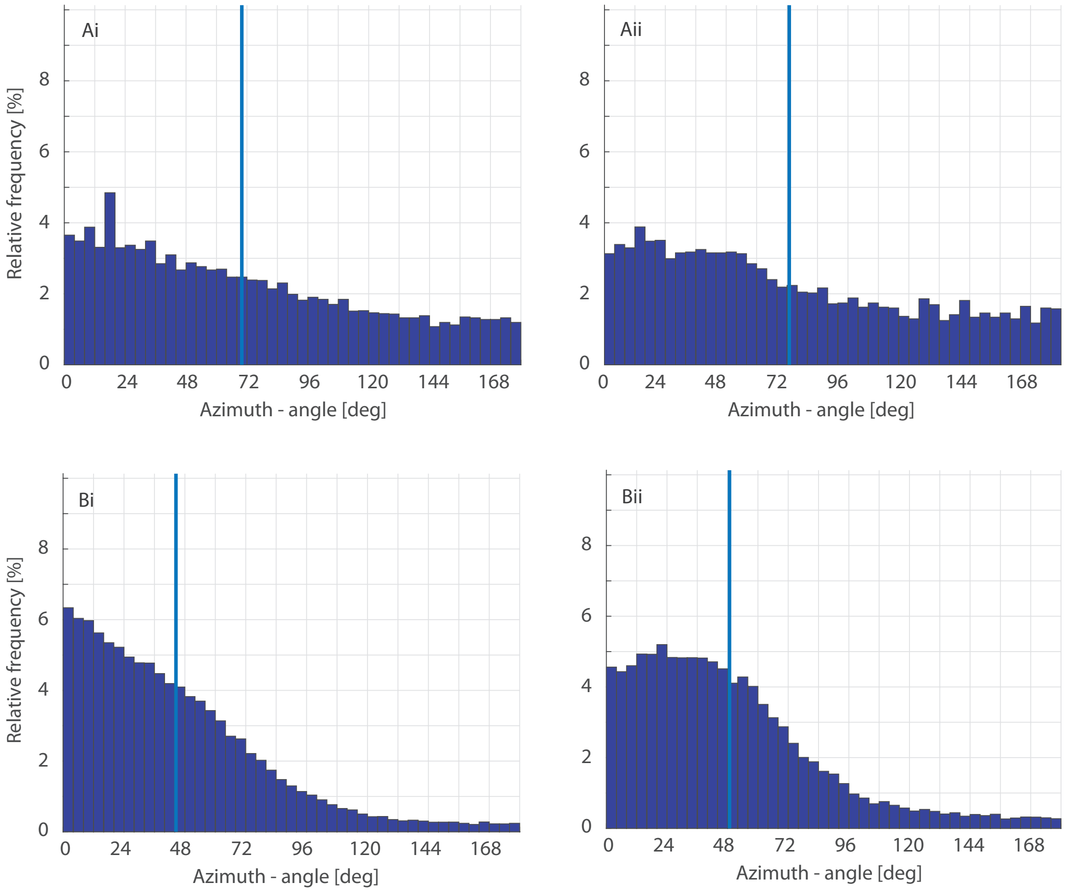


Figure 3.24: **Development of translational movements during outbound flights.** Sideward and forward/backward components of flight: Relative frequency of direction of the translational component of motion relative to the direction of flight for all bumblebees (N = 20) during outbound flights. A: initial phase, B: probing phase, i: first flight, ii: last flight. The angle was determined from the ratio between the directions of the forward and the sideward component of translation. Blue line: average angle (Ai: 70°, Aii: 73°, Bi: 44°, Bii: 49°). An angle of 0° corresponds to pure forward movement and an angle of 90° represents pure sideward movement to the right or left, respectively.

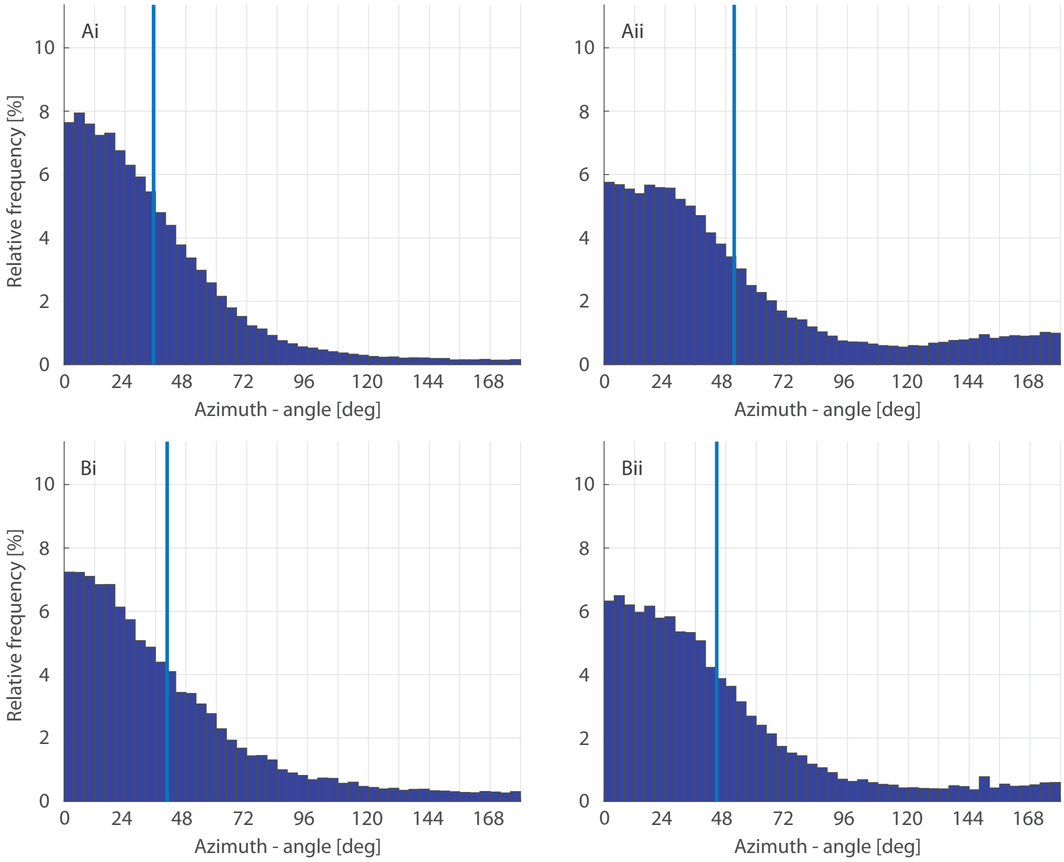


Figure 3.25: **Development of translational movements during inbound flights.** Sideward and forward/backward components of flight: Relative frequency of direction of the translational component of motion relative to the direction of flight for all bumblebees (N = 20) during inbound flights. A: homing phase, B: pre-landing phase, i: first flight, ii: last flight. The angle was determined from the ratio between the directions of the forward and the sideward component of translation. Blue line: average angle (Ai: 35°, Aii: 51°, Bi: 40°, Bii: 44°). An angle of 0° corresponds to pure forward movement and an angle of 90° represents pure sideward movement to the right or left, respectively.

3.3.3 Consequences of cylinder displacement

In the previous experiments we could provide strong evidence that the two cylinders placed close to the nest hole are used by the bees as cues to find the nest when returning to it after a foraging trip. This conclusion holds despite the homing errors that can be observed and the complex spatio-temporal structure and variability of inbound flights. To test whether the cylinders and their provision of distinct geometrical information are the reliable cue for the bumblebees to find back to their nest hole, we changed the cylinder arrangement.

After several flights with the cylinders next to the nest hole they were displaced to the opposing dummy hole in experiments with nine of the bumblebees. The displacement was accomplished while the bees were in the nest. We analysed the landing preference and the duration of outbound and inbound flights after cylinder displacement. If the bees recognise a modification of the nest hole surroundings on their subsequent departure, we might expect a longer outbound flight due to learning and probing the new situation. If such a modification is not recognised on the departure, bumblebees might have difficulties on their return finding the connected nest hole; if they rely exclusively on the cylinders as cues, they are then expected to land at the dummy hole now marked by the cylinders.

Homing error

On the first inbound flight after the cylinders had been displaced five of nine bees landed still at the nest hole which was now no longer marked by the cylinders. Two bumblebees landed at a dummy hole next to the nest hole and only two landed at the opposing dummy hole, which was now marked by the cylinders (fig. 3.26). If all 69 inbound flights are considered that were obtained after displacement of the cylinders, 40, i.e. 58 % of the inbound flights ended at the nest hole. 29, i.e. 42 % of these flights ended at a dummy hole. Only 45 %, i.e. 13 flights of these 29 flights ended at the opposing dummy hole now marked by the cylinders. The other flights ended at dummy holes next to the nest hole or next to the opposing dummy hole.

This variability in landing preferences is also reflected in the behaviour of individual bees after displacement of the cylinders. Some bumblebees landed only once at a dummy hole and after that always at the nest hole, although the cylinders were placed near the opposing hole. Others landed several times at a dummy hole before they landed at the nest hole. One bumblebee never found its way back to the nest hole and always ended its flight at the opposing, now cylinder-marked hole or at one of the dummy holes next to it.

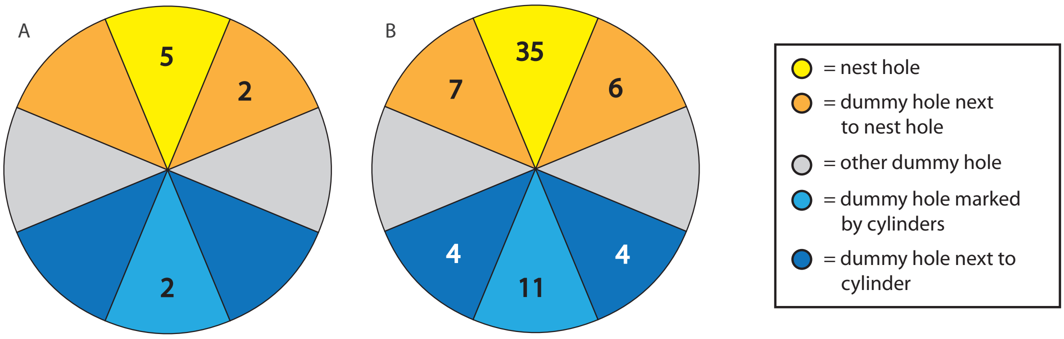


Figure 3.26: **'Homing error', 2nd sequence.** 'Homing error' and landing preference for inbound flights of the 2nd sequence (after cylinder displacement) for nine bumblebees, n = 69 flights. The segments of the circle display the sections of the nest hole and dummy holes, respectively. A: landings after the first inbound flight n = 9, B: landings after all other inbound flights of the 2nd sequence, n = 60. Note: The large circle stands for the flight arena and not a normal pie chart.

These results suggest that some of the bumblebees seem to use and rely on the cylinders as landmarks and, thus, landed at the cylinder-marked nest hole before and at the opposing dummy hole after the displacement of the cylinders. However, other bees performed as if the cylinders had no functional significance at all. Taken together, we have to conclude that bumblebees are likely to use additional cues to rely on, although we did our best when designing the flight arena to omit such cues. Which additional cues the bees may use to find back to the nest hole either on their first inbound or, at least, on their 2nd flight after displacement of the landmarks, could not be unravelled in our experiments. Further studies with changed landmark arrangements and systematically introducing additional cues, such as odour cues, are necessary to clarify this issue (see also Discussion).

Flight duration after cylinder displacement

Difficulties in finding back to the nest hole, besides a lack of motivation to return to the hive, might manifest themselves in the duration of the inbound flights after the cylinders were displaced. Moreover, if the bumblebees recognised the modification of the surroundings of the nest hole already on their first departure after cylinder displacement, we suggest the flight duration to increase, compared to the last flight before displacement. We found the flight duration only slightly affected by the cylinder displacement: The first outbound flights after the cylinder position was changed is longer than the outbounds before. However, the second outbound flights are, on average, even longer than the first ones (fig. 3.27 A). Mainly the probing phase has been found to be affected. This finding suggests that bumblebees probe the new goal-centred information longer probably due to the modification of cylinder position. Nevertheless, the increase in flight duration is small and still very variable among bumblebees. The first inbound flight after cylinder displacement is, on average, longer than the last flight before displacement (fig. 3.27 B). Mostly affected is the homing phase, where the bumblebees might have difficulties in finding the nest hole.

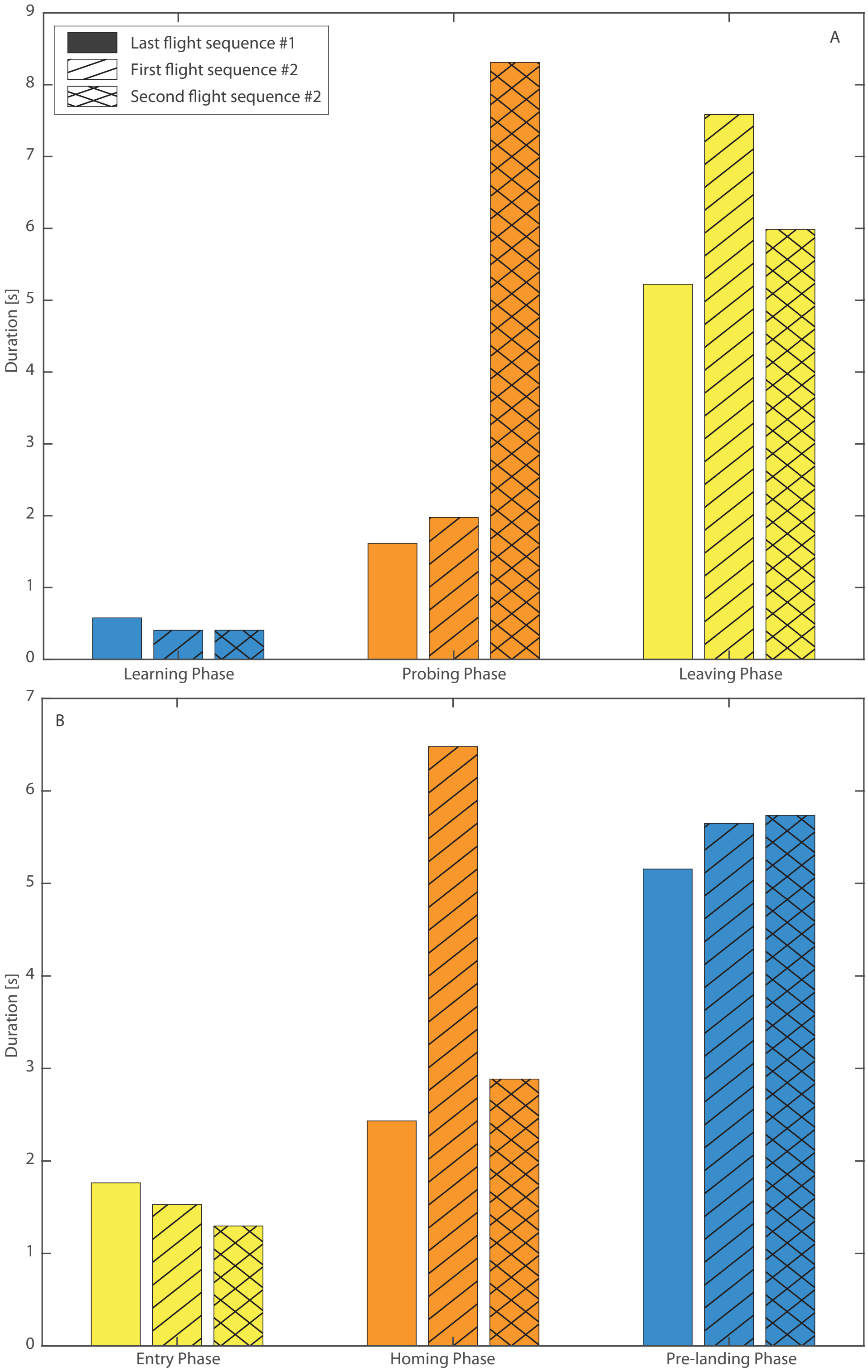


Figure 3.27: **Flight duration of different phases of flights before (1st sequence) and after (2nd sequence) cylinder displacement.** Median of flight duration of flights during the 2nd sequence for eight bumblebees, divided into all flight phases. A: outbound flights, B: inbound flights. Blue: Learning/Pre-landing phase, orange: probing/homing phase, yellow: leaving/entry phase; non-hatched bar: last flight of 1st sequence, hatched bar: first flight of 2nd sequence, cross-hatched bar: second flight of 2nd sequence, N = 8.

Flight velocity after cylinder displacement

Although we did not find a strong development of the flight velocity during consecutive flights before the cylinders were displaced, we analysed whether this is similar after displacement. A decrease in flight velocity might be hypothesized when the bumblebees are confronted with significant modifications in their otherwise familiar surroundings. Nevertheless, the velocity of outbound and inbound flights is not significantly affected by the cylinder displacement (fig. 3.28).

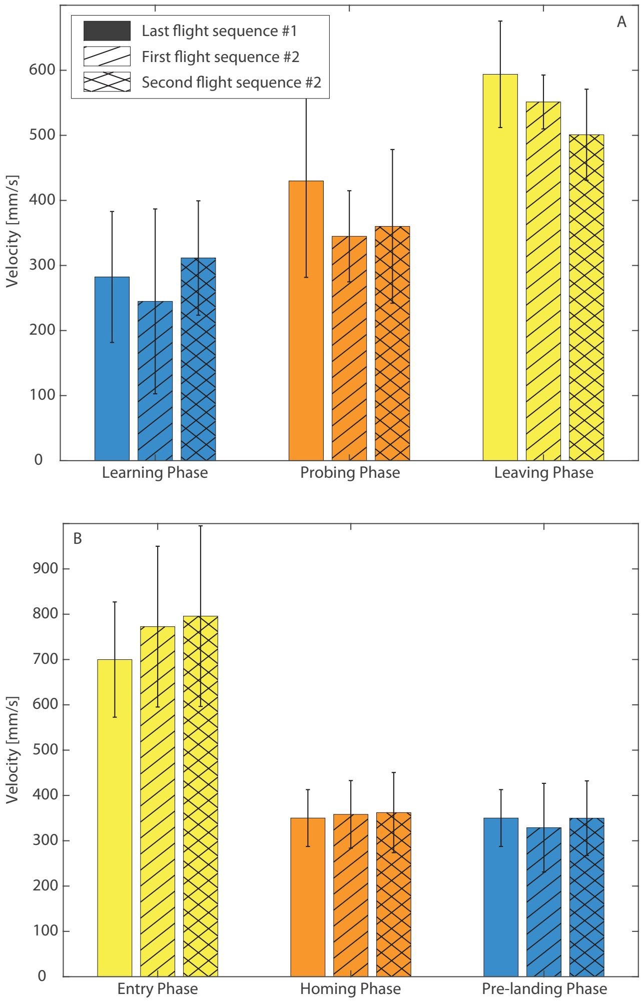


Figure 3.28: **Flight velocity of different phases of flights before (1st sequence) and after (2nd sequence) cylinder displacement.** Mean of flight velocity with standard deviation of flights during the 2nd sequence for eight bumblebees, divided into all flight phases. A: outbound flights, B: inbound flights. Blue: Learning/Pre-landing phase, orange: probing/homing phase, yellow: leaving/entry phase; non-hatched bar: last flight of 1st sequence, hatched bar: first flight of 2nd sequence, cross-hatched bar : second flight of 2nd sequence, N = 8.

3.4 Discussion

We analysed the homing ability of 20 individually marked homing-naïve bumblebees. First inbound flights were found to be highly variable in their spatio-temporal flight structure. Since the first outbound flights of naïve bumblebees were also very complex and variable (Lobecke et al., 2018) and retracing of departure flight paths on the subsequent return has been discussed in several studies (Zeil, 1993b; Stürzl et al., 2016), we will discuss the degree of similarity of first outbound and subsequent inbound flight based on our experimental data.

To characterise a potential learning process during subsequent outbound flights, we furthermore recorded a series of consecutive outbound and inbound flights after the bumblebees' initial flights and analysed the development of several flight parameters. The series showed a reduction in flight duration, but other parameters such as flight velocity or retinal position of the nest hole seem to be less or not at all affected by the experience of bumblebees gained after several flights. Despite the overall reduced duration of the flights, the did not get much straighter with increasing experience and were, in their majority, still characterised by a complex and variable loop-like structure. Moreover, at best a weak tendency could be observed for a reduction in the homing errors that bees made on their return to the nest hole. Again, the performance of individual bees as well as inter-individually was found to be highly variable. After displacing the landmark cylinders that distinguished the nest hole from identically looking dummy holes during the initial sequence of outbound and inbound flights only a minority of bees searched at the dummy hole that was now marked by the cylinders. Again, homing performance was highly variable.

3.4.1 Overall flight structure of first inbound flights of homing-naïve bumblebees

If a bumblebee worker manages to return to her inconspicuous nest hole after the first foraging trip, she obviously gathered during the preceding departure flight sufficient information about the nest's surroundings. This first return flight of a homing-naïve bee might be the most difficult one in her foraging life, because she had only one departure, i.e. learning flight before. Many popular models of local homing are based on algorithms based on a panoramic representation of the visual environment gathered at the goal location ('goal-centred information'). Model simulations and experiments with robot platforms could indeed show that such homing mechanisms may lead an agent back to its goal location at least from within a limited spatial range, i.e. the goal's catchment area (Lambrinos et al., 2000; Mölller, 2000; Stürzl and Mallot, 2006; Stürzl and Zeil, 2007). Usually the trajectory of locomotion during return flights is relatively direct, i.e. the distance between the agent and the goal continually decreases.

Our analysed flights of 20 homing-naïve bumblebees returning to their nest hole in an octagonal flight arena appear to be much more complex in space and time. Almost none of the inbound flights follows a relatively direct trajectory, i.e. the 3-dimensional distance to the nest location did not decrease continuously, but rather usually fluctuates considerably revealing a complex loop-like structure. Despite the variability in the overall duration and the complex spatio-temporal profile of the flights, we could observe one prominent invariant feature across all return flights, i.e. a fast decrease of flight height immediately after the bumblebees entered the arena from above. The duration of this fast descent was relatively independent of the duration of the subsequent highly variable homing phase.

3.4.2 Comparison of first outbound and subsequent inbound flight

In our experiments, trajectories of first outbound (cf. (Lobecke et al., 2018) and inbound flights of bumblebees show similarities in their complexity and their loop-like structures. Moreover, in both outbound and inbound flights, the changes in turn direction are performed almost along the entire trajectory and are mostly concentrated near the nest hole during the learning and prelanding phase, respectively. Also those parts of the first phase of outbound flights and of the homing phase of inbound flights, where the bees view the nest environment with their frontal visual field, are scattered in a similar way in the flight arena. Last but not least, during most of the first phase of the outbound flights as well as of the homing phase of inbound flights the bumblebees fly relatively close to the arena floor, thus constraining the perspective from which the environment is seen in a similar way.

For a further comparison between the first outbound and first inbound flights we analysed several additional flight parameters (see tables 3.1 and 3.2). Given the large variation of all parameters, it is hard to draw any firm conclusions about their similarity or difference. Only the flight velocity seems to be a parameter, which differs in a consistent way: The first inbound flights are flown with a higher velocity, on average, than the preceding departure flights.

Flight	Duration [s]	Velocity [mm/s]
1st OB	40 (29, 65)	284 ± 63
1st IB	32 (11, 77)	391 ± 80

Table 3.1: **Comparison of flight parameters 1.1** Parameters (2nd column: median (25th and 75th percentiles), 3rd column: mean + standard deviation, cf. figures) of entire first outbound and entire first inbound flight for all bumblebees (N = 20; OB: outbound, IB: inbound).

Flight	Number of CTD	Frequency of CTD [1/s]	Number of lnf	Duration of lnf [ms]
1st OB	27.1	1.11 ± 1.54	24.3	286 ± 182
1st OB	30.95	1.17 ± 1.27	30.7	238 ± 149

Table 3.2: **Comparison of flight parameters 1.2** Parameters (5th column: mean + standard deviation, cf. figures) of entire first outbound and entire first inbound flight for all bumblebees (N = 20; lnf: locations of nest fixation, CTD: Change of turn direction; OB: outbound, IB: inbound).

In general, our data does not provide any evidence that bumblebees retrace the outbound flights by their inbound flights. None of the flight characteristics that might help during local homing, such the positions in the arena, where the bees orient towards the nest hole, match between outbound and inbound flights. Retracing of flight paths has been discussed in previous studies on solitary wasps. Although solitary wasps do not retrace exactly the paths of the preceding departure flight (Zeil, 1993b; Stürzl et al., 2016), they face into similar directions during departure and return, and their body orientations depend on their position relative to the nest or feeder environment in a similar way (Zeil, 1993b; Collett and Barron, 1995). Moreover, the flight direction and the retinal position of close landmarks were similar during the return and the preceding departure flights (Zeil, 1993b). Stürzl et al. (Stürzl et al., 2016) concluded that returning wasps fly through a sequence of orientations and locations in reverse order in which they had been flown during departure flights. Also honeybees' return flights share some characteristics with their preceding departure flights: gaze changes were shown to be fast and saccadic, indicating no pronounced differences in lateral movements between both flight types (Boeddeker et al., 2010).

Likewise, bumblebees' departure and return flights differed only slightly in their saccadic yaw velocities (Boeddeker et al., 2015). Nevertheless, these similarities cannot be interpreted as indications for a retracing of departure flight paths.

3.4.3 Is there a learning progress in consecutive outbound and inbound flights?

Spatio-temporal information about the goal location is assumed to gathered during outbound flights and to help in finding back to the location on the bee's subsequent returns. In previous studies, insects were observed to do more than one learning flight (Lehrer, 1993) and to re-learn every first flight of a day (Brünnert et al., 1994; Zeil, 1993a) or after the nest surroundings have changed (Van Iersel and van der Assem, 1964; Zeil, 1993a). Nevertheless, the decreasing amount of learning flights over time, such as the 'turn-back-and-look" behaviour of honeybees (Lehrer, 1991), suggest a progress in learning (Lehrer, 1991, 1993; Zeil et al., 1996). Such learning process might be reflected in different parameters. Here we analysed whether parameters, such as flight duration and velocity, but also the error rate in finding the nest hole, change over the time course of several consecutive outbound and inbound flights.

We already mentioned the high variability in outbound and inbound flights, because of which we could not observe a consistent learning progress among all flight parameters. Nevertheless, we found a development in some flight parameters that reflect most likely a consequence of experience of the bees: Outbound as well as inbound flights show a decrease in their overall duration over several flights across the population of tested bees. Yet, both flight types are characterised by a high variation on an individual basis. Some bees continuously decrease the duration of their flights, while others show no consistent decrease in flight duration. Anyway, we suggest the decreasing flight duration to be an effect of the learning process when bees become more familiar with the environment.

In contrast to the expectation that bumblebees might fly faster when they are familiar with the spatial circumstances of the nest vicinity, we found no significant evidence for such a development in flight velocity. On average, all phases of outbound and inbound flights are flown with a slightly increased velocity over the sequence of consecutive flights, but this increase is only marginal and goes along with a high variability across bumblebees. This suggests, that a decrease of the bumblebees' flight route is an effect of increased experience.

All other flight parameters showed no obvious development over time. Robert et al. (Robert et al., 2018) showed that the fixation rates of the nest hole decreased over subsequent flights, whereas we could not observe any obvious change in the retinal position of the nest region between flights of homing-naïve and potentially experienced bees. Therefore, we suggest other flight parameters to be mostly independent of the bumblebees' experience under our experimental conditions.

3.4.4 Relevance of landmarks

It is known from previous studies that hymenopteran insects use landmarks for navigation, either to use its position information, possibly with respect to a map-like representation of the surroundings (Gould, 1987; Menzel et al., 2005; Cruse and Wehner, 2011) or as a decision point for subsequent actions (Collett, 1996; Collett and Barron, 1995; Collett, 1996; Collett and Lehrer, 1993; Collett and Rees, 1997; Fry and Wehner, 2005; Menzel, 2009). As a novelty in analyses of homing performance, bumblebees in our experiments were confronted not just with their nest hole, but seven blind-ending dummy (nest) holes. As a consequence, they frequently made homing errors, although two salient cylinders provided – at least geometrically – distinct information about the nest hole position.

Nevertheless, the landing preference was not random: All bumblebees landed at the nest hole or at one of the two dummy holes next to it. Not a single recorded flight ended at the dummy holes perpendicular to the nest hole, suggesting that the cylinders play a relevant role in the bumblebees' homing behaviour. Furthermore, at the end of the first inbound flight, 75 % of bumblebees landed at the nest hole, suggesting that most of them learned its position already during the previous first outbound flight. Likewise, 96 % of consecutive flights ended at the nest hole marked by the cylinders or at one of the two dummy holes next to it and, thus, to the cylinders. This suggests the two salient cylinders to help in finding back to the goal location.

After the cylinders were displaced close to the dummy hole opposing the nest hole, surprisingly only few bumblebees seemed to rely on the position of the cylinders: then only 22 % of the first inbound flights ended at the dummy hole now marked by the cylinders. This value is surprisingly small, given that 75 % of homing-naïve bumblebees approached the nest hole on their first inbound flight while it was marked by the cylinders (see above) and if we assume they rely on the cylinders as distinct markers of the position of the nest entrance. Furthermore, only 28 % of consecutive inbound flights after cylinder displacement were cylinder-related approaches, i.e. that they end at the dummy hole marked by the cylinders or the two dummy holes next to it.

Hence, other cues might be relevant in our setup to guide the bees, although we did our best to eliminate all such cues.

That bumblebees never landed at dummy holes perpendicular to the nest hole or - after cylinder displacement - perpendicular to the dummy hole marked by the cylinders, might suggest another possible reason for such a high 'homing error'. Bumblebees might not recognise or learn that the nest hole is located between the two cylinders but still link at least one cylinder to the nest hole position. A confusion of the cylinder's direction in regard to the nest hole (left or right of it) might then occur on the bumblebee's return. Therefore, the inbound flight might end at the dummy hole to the right or to the left of the nest hole because bumblebees remember only one cylinder next to their target location.

The landing preference was very variable before and after the cylinders were displaced. Although some individuals show a learning progress when they landed at the nest hole after landing at a dummy hole, we did not expect the bumblebees to make as many homing errors even after several departures and return flights. Even after several flights bees were far away from landing constantly at the nest hole and there was no obvious decrease in the 'homing error' over time. Nevertheless, the cylinders can be concluded to be a relevant cue for the bumblebees, but to what extent a bee relies on this information appears to be variable and might depend on external and internal cues beyond our control (see below).

3.4.5 Possible reasons for spatio-temporally complex local homing behaviour

Previously described departure and, especially, return flights of hymenopterans vary a lot in the appearance of their trajectories. There are pronounced differences between species (see Introduction, chapter 3). Even bumblebees' flights vary tremendously. The complex spatio-temporal structure of first inbound flights of homing-naïve bumblebees does not match the somehow obvious assumption that a foraging insect might return to its home location on a preferably direct and, therefore, fast path. At least after several consecutive flights, be it inbound or outbound, one might assume a straighter flight path relative to the nest after either leaving the nest hole or entering it, respectively. Although flights in our experiments show a slight tendency of a decreased route length, suggested by a shorter flight duration but only marginal increased flight velocity, but especially inbound flight were still loopier in their spatio-temporal structure after several flights than expected.

There might be several – not mutually exclusive – potential reasons for the complex loop-like and across individuals highly variable flights, though sections of the flights can be shown to be unlikely. Part of this variability, at least across different studies on bumblebee homing behaviour, might be a consequence of the different experimental conditions the insects encountered, e.g. a different visual cue available in addition to the cylinders usually employed to mark the nest hole.

The landmarks marking the nest hole were not sufficiently strong cues

The homing result of the inbound flights is not random but ends in a large majority of cases at the nest hole or at least at a dummy hole next to it. Therefore, at least the last section of the first inbound flight ('pre-landing phase') suggests a clear association between cylinder position and nest hole location. Hence, the cylinders play a prominent role in guiding the bumblebees back to their goal location and, therefore, their position and appearance might be important to learn during the previous outbound flight. Nevertheless, the bumblebees may see the cylinders only relatively late during their inbound flight, but not immediately after first entering the flight arena from the foraging room. With the maximal distance between bumblebee - at the upper edge of the farthest arena wall - and the cylinders relevant in our recordings being 100 cm, each cylinder subtended a visual angle of 2.86° in width and 43.6° in height. When the bumblebee is in the centre of the arena close to its floor, each cylinder subtended a visual angle of 7.15° in width and 53.14° in height.

Since previous studies on single object resolution in *B. terrestris* revealed that bees can detect objects of a minimum visual angle between 2.3° and 7°, depending on the body size of the bee (Spaethe and Chittka, 2003; Dyer et al., 2008; Wertlen et al., 2008; Chakravarthi et al., 2016), we conclude that bumblebees, at the latest, are able to perceive the cylinders in the middle of the arena, independent of the bees' height over ground. The cylinders' homogeneous white texture should then easily be discriminated against the textured background wall. Honeybees could be shown to recognise and use even camouflaged landmarks, i.e. landmarks covered with the same texture as the background (Dittmar et al., 2011) and motion sensitive visual neurons in the bumblebee brain, when stimulated by optic flow corresponding to that experienced during learning flights, could be shown respond to landmark cylinders irrespective of their texture and even if they were camouflaged and could be detected only on the basis of relative motion cues (Mertes et al., 2014). Hence, the loop-like and across bees highly variable search behaviour observed before the bees entered a potential nest hole is most likely not a consequence of an inconspicuousness of the cylinders.

Conspicuousness of the nest hole

Robert and colleagues (Robert et al., 2018), for instance, marked a single nest hole with a surrounding purple ring and, thus, made it particularly conspicuous and, probably, easier to find for the bumblebees; this was done deliberately, because these experiments pursued another research question than understanding mechanisms of local homing. In our analysis we wanted to test the homing ability during first inbound flights of homing-naïve bumblebees in a situation, where the nest hole is as inconspicuous as possible, and the two white-surfaced cylinders represent the only visual cues that provide unambiguous information about the nest hole location.

To prevent the nest hole, which might still be visible for the bumblebees from several centimetres, from being a distinct target, we created an – except for the cylinders – ambiguous symmetric situation by adding seven dummy holes. To analyse the potential influence of the ambiguities introduced by these additional nest holes, we tested five additional homing-naïve bees on their first inbound flight, with all seven dummy holes closed (see Material and Methods). Although the resulting five flights were slightly shorter than in the reference situation with eight nest holes, they were still loopy and did not reflect a directed approach to the nest hole marked by the cylinders. Therefore, we suggest the many possible nest holes in our arena not to be the main reason for the complex spatio-temporal structure of the inbound flights.

Directional cues in addition to landmarks indicating the nest hole

In (more) natural settings, other visual cues might provide directional information, such as the sun, the polarisation pattern of the sky or the overall panorama of the scenery surrounding the nest location at some distance (Collett et al., 2013; Hempel de Ibarra et al., 2009; Philippides et al., 2013; Riabinina et al., 2014; Robert et al., 2018). Bumblebees tend to use, for instance, the compass direction, if available, to align the direction in which they face the nest during return flights with their preferred direction during learning flights (Hempel de Ibarra et al., 2009).

Since this directional information might help the insects during local homing to guide the animal to the goal location, more information provided by such cues might thus lead to a straighter return than an experimental situation providing less directional cues. We deliberately eliminated directional cues in our experimental design. Nevertheless, we must qualify the remote possibility, that bumblebees may have used the direction from which they entered the test arena when returning from the foraging room as a directional cue.

Indeed, we find a slight preference of the entering bees for three of the eight arena wall segments, but this bias was not statistically significant. However, even with a significant bias, its influence on the homing behaviour would likely to be negligible, because, in contrast to visually salient directional cues, the entering direction is not discernable anymore after the bee is inside the test arena. Hence, other information, such as directional cues, which are usually present in a natural complex environment and in most studies on bumblebee homing behaviour (see above), but were deliberately avoided in our experimental set-up, might be important for shaping the return flights. Although the cylinders alone might give a geometrically distinct information about the nest hole position, this information might have been insufficient for the bees because its innate learning program might be designed for a much more complex environment and the integration of multiple cues. This hypothesis might explain the higher complexity of outbound flights as well.

Motivational state of the bumblebees

One further important factor which should be considered when making assumptions about the homing ability of bumblebees is the internal state of the insect: We cannot exclude the possibility that returning bumblebees in our experiment do not have the primary intention to return to the nest hole during the entire recorded flight. This might be a general problem with indoor experiments, where the reward (sugar solution in our experiment) is often given *ad libitum* and the innate pressure to forage and provide the hive with food might be not a primary issue for the insect. Furthermore, the bees' behaviour could also be driven by the need to explore the environment, depending on their behavioural trait. This might be also a reason for undirected departures and returns or approaches to an allegedly incorrect nest hole.

Since the from previous studies to some extent divergent behaviour of bumblebees in return flights in our experiments might be caused by a combination of parts of the possibilities mentioned above, we suggest further experiments on the homing ability of bumblebees to vary the number and quality of visual cues provided as well as to reduce the profitability of the food reward to potentially modify the motivation of the animals.

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Conclusion

This last section draws a general conclusion from the present thesis, mentions problems during the experiments and gives an outlook for further experiments concerning local homing.

The present combination of studies shows how complex the learning and homing behaviour of bumblebees might be – even in a laboratory environment, where most features of the experimental design are controlled by the experimenter. Although the results of this project are not contradictory to previous studies regarding small-scale navigation in hymenopterans, they are not as explicit and coherent as expected – for instance from computational model analyses. The possible reasons, why bumblebees in our experiments showed a more complex and less purposeful behaviour are diverse, and most of them are mentioned in the discussion of the previous chapter. It is not possible to investigate and prove all possible reasons for the sparsely goal-directed behaviour with the present data. Nevertheless, some aspects should be considered in further experiments in local homing ability in bumblebees and other hymenopterans. As mentioned above, the deliberate exclusion of external directional cues is important for experimental design in small-scale navigation. If directional cues are provided, they should be used and controlled in a systematic way to prove their role in learning and homing behaviour.

Another important factor in behavioural experiments is the availability of food, such as sugar solution and pollen we used. Hymenopterans must provide their own or the queen's offspring with food on a regular basis. If the storage chambers of a nest are full, there is no or at least less need to forage. In contrast to the more organised hives of honeybees, the structure of a bumblebee nest is kind of messy and, due to the organisation of honeypots and the ceiling being made of bee wax above it, harder to control by the experimenter. However, food availability should be limited in some way – at least the amount of sugar solution bumblebees could collect – to prevent a lack of motivation, e.g. reflected in 'overeaten' bees, which prefer to rest instead of quickly return to the hive. Bumblebees in our experiments were able to forage sugar solution at a feeding table in the flight room. My impression has been, that only bumblebees which used this artificial feeder or were provided with a food reward after their trip, were motivated enough to enter the flight arena unforced and in a consistent way several times a day.

The internal state of the bumblebees, which is highly important to consider in behavioural experiments, is mostly uncontrollable. The internal state is not to be confused with the bee's motivation to forage. The latter arises from the need to gather food and its availability for the hive, whereas the internal state might be attributed to the division of labour in the colony: Some bumblebees – determined by their size and not their age as in honeybees – are responsible for foraging flights, while other bees explore, defend the colony or take care of the brood. In experiments concentrating on learning and homing behaviour, the division of labour should be taken into consideration, to prevent bumblebees from being forced to do a task they are not intended to do and, therefore, cannot fulfil in an appropriate manner. If these factors are not considered during the design process and the implementation of an experiment, the behaviour of the insects might be misinterpreted.

Another factor is the behavioural trait, which might be expressed in different behaviour of individual bees in similar situations. We could, besides our estimate of stored sugar solution in the hive and the resulting motivation of the bees, not prove whether a bumblebee, landing at a dummy hole, 'failed' its return flight or is just an exploring bumblebee, mainly driven by the need to explore or even plain curiosity.

To ensure a high quality of studies, further experiments in small-scale navigation should consider these factors which might influence the insects' behaviour and, thus, the results of an experiment.

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Dr. Olivier Bertrand hat mir über meine Anfangsabneigung gegen Matlab

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