ROUTE FIDELITY AND NAVIGATION TASK TRAINING IN DESERT ANT *CATAGLYPHIS FORTIS*

MASTERS THESIS BY

MORGAN OBERWEISER

EXTERNAL SUPERVISOR:

DR. BILL S. HANSSON

INTERNAL SUPERVISOR:

DR. NIELS J. DINGEMANSE

DATE OF SUBMISSION:

4 FEBRUARY 2022

ABSTRACT

The desert ant *Cataglyphis fortis* has many adaptations which help it to survive in its harsh and featureless environment. These ants have an especially developed sense of memory and behavioral flexibility in learning. These skills may contribute to their impressive navigational abilities which utilize path integration and the processing of sensory cues. In this study, route fidelity and task training were examined in order to further explore these ants' neuronal and behavioral abilities. Route fidelity, as seen in some other desert ant species, requires strong recall from long-term memory. It also indicates idiosyncrasy, which is a type of repeated route often examined in desert ant species but not yet shown in *C. fortis.* Task training within a standardized maze is a very useful tool for studying an organism's ability to learn and especially for manipulating the sensory information which might affect their learning ability. I found that *C. fortis* exhibit high route fidelity, and also are successful in learning a difficult navigation task from our new methodology. Further studies are described which would augment these findings into a more complete study on desert ant navigation.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 BACKGROUND

The desert habitat is uncommonly harsh and offers many unique challenges to the organisms that make a home there. Most desert animals have developed several behavioral adaptations which reduce their exposure to the unforgiving landscape and any predators they may face in such unprotected areas. The ant species *Cataglyphis fortis* Forel 1902 exhibits many such adaptations to streamline their meandering foraging runs and long-distance homing trails, thereby decreasing the time spent outside of the safety of their subterranean nest. They have an incredible capacity for memory and neuronal plasticity, which allows for high levels of behavioral flexibility in learning, and benefits them as they navigate their high-stakes environment (Rössler 2019, Stieb *et al.* 2011).

In contrast with most other ant species, *C. fortis* foragers do not use trail pheromones for navigation, mainly because their food sources are usually small and able to be exploited in one trip, and because the extreme temperatures of their environment seem to discourage the use of chemical trails (Knaden & Graham 2016). In their nearly featureless desert environment, these ants have instead developed a straightforward system for calculating nestward routes from long-distance foraging sites through path integration (Heinze *et al.* 2018, Müller & Wehner 1988). Path integration (PI) is common and well-studied in many systems including mammals, birds, and insects (Etienne & Jeffery 2004, Mittelstaedt & Mittelstaedt 1982, Heinze *et al.* 2018). Specifically in *C. fortis,* this method of navigation requires an individual to constantly monitor its position relative to a starting point by continuously computing directional information (via a "sky compass," the polarized light pattern in the sky) and distance information (via step integration, where the individual keeps track of the number of steps it's taking) (Knaden & Graham 2016, Wehner *et al.* 2014). This system is so finely-tuned that *C. fortis* individuals can even accurately measure ground distance in three dimensions when travelling on undulating terrain, for example (Grah *et al.* 2005).

Foraging desert ants will embark on a rambling search for a suitable food source, and once one has been identified they will make an impressively straight line back to their

home nest, using PI as well as any available sensory cues to guide them. Some different desert ant species, such as the Australian *Melophorus bagoti* and the European *Cataglyphis velox,* have an easier time retaining route memories because they inhabit environments with sparse tufts of vegetation to act as conspicuous visual landmarks (Wystrach et al. 2010). With the obstacles in their environment creating the potential for any number of specific paths between the nest and the food source, these ants exhibit what are called idiosyncratic routes. Idiosyncrasy is observed when ants create arbitrary, individual-specific routes between their nest and food source- more specifically, these idiosyncratic routes differ more strongly between individuals than they do within individuals (Mangan & Webb 2012, Kohler & Wehner 2005). Therefore, there are two facets to idiosyncrasy: route fidelity within individuals, and variation of routes between individuals. Neither of these have been clearly demonstrated in *C. fortis.*

C. fortis inhabit an arid salt pan environment with very few physical landmarks, so they are not known to use distinctive routes in the same way as the species mentioned above. In fact, several studies have pitted the navigational skills of *C. fortis* against some of these species, and concluded that they differ from *M. bagoti* in that they rely on their internal PI vector rather than the visual landmarks in their vicinity, and therefore learn visually-guided navigation tasks at slower pace and with a lower level of accuracy (Schwarz & Cheng 2010, Buehlmann *et al.* 2011). That is not to say, however, that *C. fortis* are incapable of displaying idiosyncrasy in their foraging routes. *C. fortis* are still known to take individualized routes during their foraging behavior, for example, in cases where an abundant source of food requires several trips to and from the nest (Buehlmann *et al.* 2015). Interestingly, these habitual routes in *C. fortis* seem to be mainly guided not by visual cues, but by olfactory landmarks (Buehlmann *et al.* 2015).

The route fidelity aspect of idiosyncrasy is especially dependent on memory (Mangan & Webb 2012). It is shown in *C. velox* that idiosyncratic routes are stored in long-term memory, and that individuals can retain memories of multiple routes at a single time (Mangan & Webb 2012, Sommer *et al.* 2007). Therefore, the detection of route fidelity in any taxa would not only be a first step toward verifying idiosyncrasy, but also an indication of a highly impressive level of memory. Since idiosyncrasy as an adaptation

serves to protect individuals from environmental dangers by shortening their time outside of the nest, this feat of memory would definitely be considered as an example of the neuronal and behavioral plasticity which make desert ants so successful in their harsh environment (Rössler 2019).

Another consequence of such neuroplasticity and behavioral flexibility can be seen in desert ants' ability for learning. For decades, *C. fortis* and other desert ant species have been utilized in experiments where they have been trained to complete navigation tasks, both in their natural environment and in artificial maze structures (Wehner 2019). Several recent experiments with *Cataglyphis niger* show impressive learning ability in amazingly complex mazes (Saar *et al.* 2017, Bega *et al.* 2019, Saar *et al.* 2020). Such mazes can be used to offer a standardized environment in which many individuals can encounter the same stimuli and cues. This is especially interesting as a way of testing how the animals utilize sensory cues throughout their navigation process.

In addition to path integration, desert ants often rely on geocentric cues perceived in their environment while they are navigating to and from their nest. In the case of *C. fortis,* these cues can be visual (physical landmarks), olfactory (odors emanating from food, nest, or other surroundings), and even tactile (ground structure) (Knaden & Graham 2016). So far, visual cues have been the main study focus when looking into ant navigation, as they are most easy to detect and manipulate (Bisch-Knaden & Wehner 2001, Zeil 2012). However, it has become apparent that olfaction plays a bigger role than originally assumed in *C. fortis* navigation (Buehlmann *et al.* 2013, Huber & Buehlmann *et al.* 2012, Knaden 2017, Steck *et al.* 2009, Steck 2012). For example, Buehlmann et al. found evidence that desert ants take olfactory "scenes" into account during foraging runs, and can use these chemical cues for navigation in the absence of other directional information (2015). During nest-searching at the end of a foraging trip, individuals are able to find their nest entrance much more precisely when using bimodal cues (consisting of visual and olfactory information) than when using unimodal cues (Steck *et al.* 2011). *C. fortis* also uses multimodal cue processing while performing learning walks as new foragers (Vega Vermehren *et al.* 2020). In general, the more sensory input an animal is able to perceive and process, the more successful it will be at completing its task (Arleo

& Rondi-Reig 2007). For example, comparisons of unimodal and bimodal sensory integration in both humans (Shams & Seitz 2008) and *Drosophila* (Guo 2005) show that learning skills and task completion are enhanced when bimodal information is available. It stands to reason that in *C. fortis,* the processing of bimodal cues should benefit individuals not only in nest entrance searching and learning walks, but throughout all navigational processes.

1.2 AIM AND OBJECTIVES

The overall aim of this study was to better understand the navigational abilities of *C. fortis,* especially in terms of route fidelity (as a precursor to demonstration of idiosyncrasy) and the ability to learn difficult tasks (as a precursor to in-depth study of the effects of cue modality on route learning). This was explored by introducing ants to a maze-like channel with two decision points, which was used to assess their navigational performance in several different experiments.

Objective 1 was to test the route fidelity of *C. fortis* ants by recording direction decisions of individuals throughout a set of 10 homing runs. These navigation decisions were used to generate a route fidelity score, which was compared with data generated from two different simulations. *I hypothesized that* C. fortis *individuals would have significantly higher route fidelity scores than the simulated individuals.*

> I confirmed that *C. fortis* individuals do show route fidelity compared to both a completely random simulation and a weighted simulation which takes into account the ants' preferred route types.

Objective 2 was to test the ability of *C. fortis* to complete a difficult navigation task after training. In this case, the difficult navigation task involved performing a route that was found to be the least favored in the maze setup- Left then Right, or LR. *I hypothesized that after LR training,* C. fortis *individuals would show a significant increase in the number of individuals performing an LR route.*

> I confirmed that *C. fortis* individuals do significantly increase in their completion of

6

the difficult navigation task after receiving training to do so.

In addition to the above objectives, this study was also used as an opportunity to test new methodology in the study of the navigation of *C. fortis* ants. The experimental setup with is described below was developed completely by the experiments, and has never before been used with any ant species. After several missed field seasons due in part to the COVID-19 pandemic situation, it was extremely valuable to test the experimental methods described below in order for future research to be planned by the Odor Behavior Group at the Max Planck Institute for Chemical Ecology.

2. MATERIALS AND METHODS

2.1 STUDY SITE AND SPECIES

Behavioral experiments were performed on *Cataglyphis fortis* populations living in the arid salt pans of the Tunisian desert (Fig. 1). This field site is very well-known and has been heavily used for ant navigation research for over 50 years (Wehner 2019). The field site is located just east the village of Menzel Chaker (34º96'N, 10º41'E). It is located on the geographical feature of a salt lake which dries up in the summer, leaving a bare and featureless plain covered in salt deposits. Ant nests, which look like slight humps raising out of the ground with a hole around 2 cm wide in the middle, could be found distributed sparsely around the salt pan. Singular nests were often reused, but only after a period of 3 weeks had passed, ensuring that all members of the colony were naïve and had no prior training from past experiments (Fleischmann *et al.* 2016).
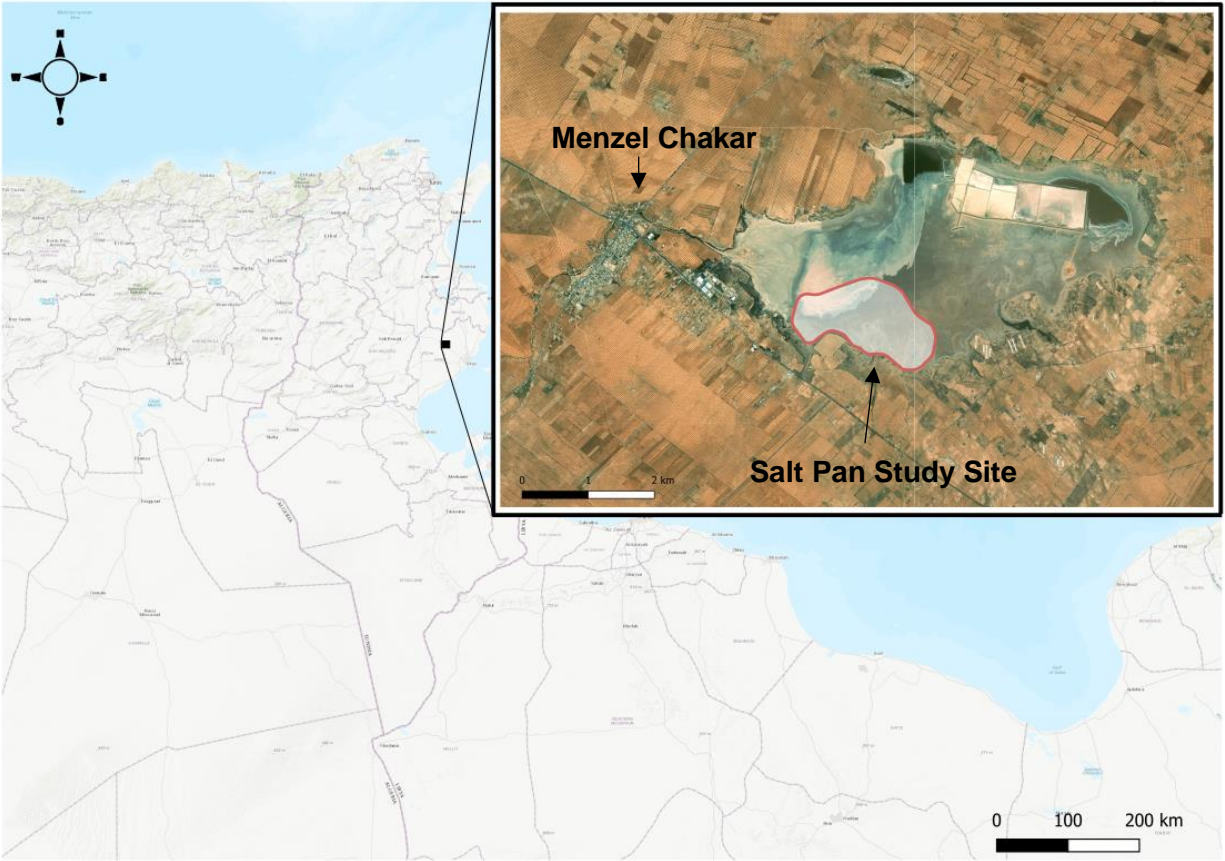
Figure 1: Map depicting the study site. The inset satellite image was not taken in the summer season, as water deposits can still be seen. During the field season, the area was instead completely dried up with salt deposits left behind. The white shaded area with a red border denotes the area of the salt pan in which experiments were run. To the west of the salt flat can be seen the Tunisian village of Menzel Chakar. Map created with QGIS version 3.18.1 (QGIS.org) using basemaps "ESRI World Topo" and "ESRI Satellite," and compiled using Inkscape version 1.0 (https://inkscape.org).

2.2 NEST AND CHANNEL SETUP

Once a sufficient nest was selected, it was enclosed with a plastic ring which was ~20 cm in height and treated with a fluoropolymer resin to keep the ants from escaping. The plastic ring was connected to the training channel with a short plastic tube, creating a passage with a diameter of 2 cm through which the ants could enter the channel. The

tube was also covered with dirt in order to create the illusion that it was a safe underground tunnel. The training channel was constructed of aluminum channeling 7 cm wide and 7 cm tall, including two wider diamond-shaped areas (Fig. 2). Fine sand was glued to the bottom of the channeling in order to facilitate the ants' walking, and brown packing tape lined the inside walls in order to dull reflections of sunlight and discourage escape by smoothing the surface. The channel was a total of 500 cm long, with the nest entrance located 70 cm from one end and the feeder at the other.
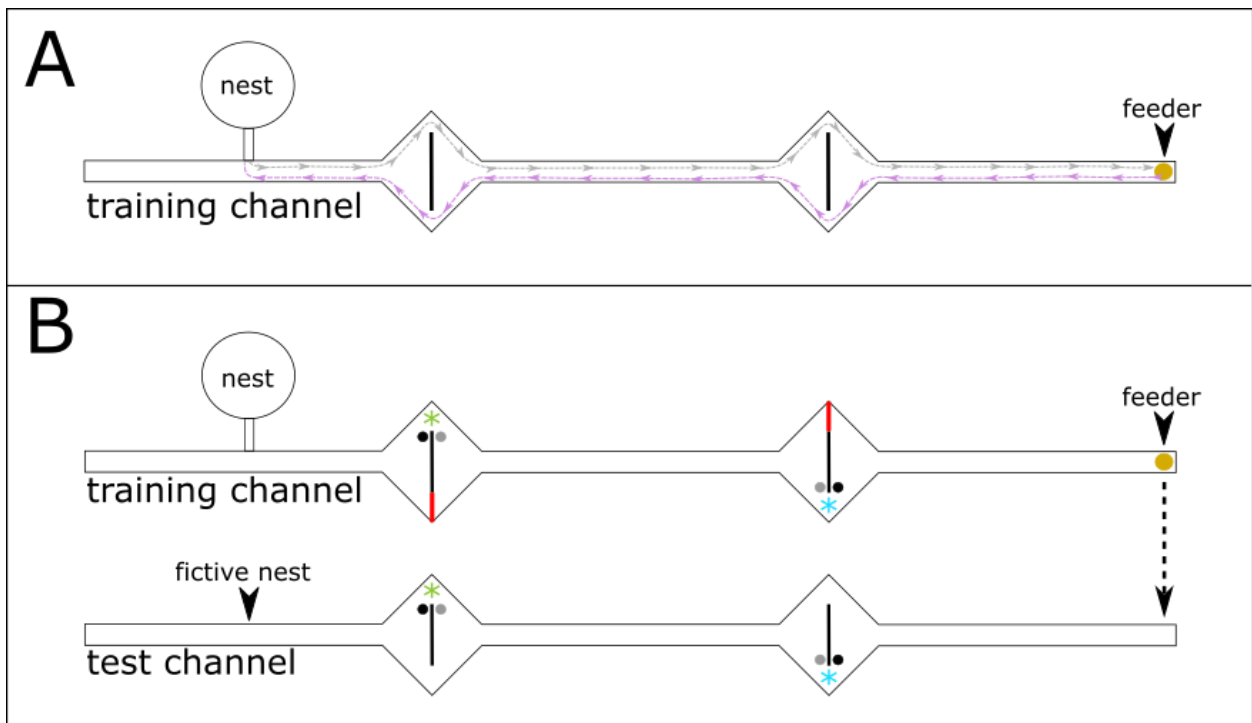

Figure 2: Schematics of channel setups. **A:** Setup for Experiment #1, which only included a training channel. The dotted arrows show an example route that an individual might take, with the grey arrows indicating the outbound path and the purple arrows indicating the inbound path. For the majority of our experiments, only the inbound path is analyzed, as it represents homing behavior. The path shown would be notated as LLLL, as from the travelling ant's point of view. **B:** Setup for Experiment #2, including a training channel and a test channel. The red portion of the divider indicates where the passageways were closed in Treatments #2-#6. The visual cues are indicated by the circles within the diamond, with black circles indicating larger plastic cylinders and grey circles indicating

smaller plastic cylinders. The olfactory cues are indicated by the asterisks within the diamonds, with the green asterisk indicating where indole was deposited and the blue asterisk indicating where methyl salicylate was deposited. The dotted arrow indicates where experimental individuals were displaced from the training channel to the test channel.

2.3 FEEDER TRAINING

Once the training channel was connected to the nest, a feeder was placed at the far end of the channel. The food prize consisted of small ~2x2 mm pieces of a locally available biscuit cookie. The food was placed in a large pile at the feeder site, and at the onset of the feeder training a trail of food crumbs was lead from the nest entrance to the feeder. Ants were allowed ~10-20 mins to acclimate to the channel and discover the food source before the experiments were started in earnest. After the conclusion of the feeder training, a nest was engaged in only one of the two following behavioral experiments:

2.4 EXPERIMENT #1: ROUTE FIDELITY

Once the ants had successfully identified the feeder and begun foraging, aluminum dividers (covered with brown packing tape like the channel walls) were placed in the diamonds, creating two passageways (L and R, always notated from the travelling ant's point of view) through which the subjects could pass. For this experiment, both passages remained open at all times (Fig. 2A).

As the ants continued foraging, individuals were collected at the feeder and painted with a unique combination of two paint dots on their gasters (Fig. 3). The paint consisted of nail polish diluted with acetone, and was completely safe for the animals and did not affect their foraging behavior. After being painted, the ants were allowed a longer stretch of time, around 3 hours, to really acclimate to the channel and learn the route to the feeder and back to the nest. During this time the feeder was constantly monitored and replenished so that the subjects didn't run out of food.

Figure 3: Depicts how subjects were marked for individual identification in Experiment #1, with two dots of paint on their gaster. The individual shown was identified as "WP" : "white pink"

After the long training period, observations began. One individual was identified by its color code while exiting the nest. This individual was followed through at least 10 complete consecutive foraging runs, with each direction decision sequence noted. For example, an individual which turned L then R on its outbound run, and R then L on its inbound run, would have completed the run LRRL.

2.4.1 RF SCORE CALCULATION

After the collection of the sequence of at least 10 foraging runs, a route fidelity (RF) score was calculated for each individual. Only the two directions chosen during the incoming trip from the feeder to the nest were used for the calculation, as we are most interested in the decisions made while an ant is homing. This resulted in the data for each individual consisting of ten consecutive direction decisions, as can be seen in the examples in Table 1. The proportions of each of the four possible routes were calculated, with *LL* being the proportion of times the ant made took a Left-Left route, *RR* being the proportion of times the ant made took a Right-Right route, *LR* being the proportion of times the ant took a Left-Right route, and *RL* being the proportion of

times the ant took a Right-Left route. These proportions were entered into the following formula in order to calculate the RF score:

$$(LL^2 + RR^2 + LR^2 + RL^2) * 4 = RF\ score$$

This formula assigns a weight to each route based on the number of times it was taken by an individual. The scores fall on a scale from 1 – 4, with 1 being the least consistent (all 4 routes taken equally) and 4 being the most consistent (only one route taken). The weighting system based on squares ensures that an individual is rewarded with more points for showing fidelity to a particular route, and the higher fidelity to a single route results in the highest score. Notice individuals 2 and 3 in Table 1: though they both took a LL route 80% of the time, individual 3 also showed some fidelity to the RR route whereas individual 2 took two differing routes. This resulted in individual 3 having a higher RF score.

Table 1: Sequence of routes taken by 4 example individuals, and their resulting RF scores. The scores range from 1.36 (the lowest score possible in our experiment, since with 10 trials it was impossible to take each route equally) and 4 (the highest possible score showing total route fidelity.

Individual											RF Score
1	LR	LL	LR	LL	LR	LL	LL	RR	RL	LR	1.36
2	LL	LL	LL	LL	LL	LL	RR	LL	LL	RL	2.64
3	LL	LL	RR	RR	LL	LL	LL	LL	LL	LL	2.72
4	LL	LL	LL	LL	LL	LL	LL	LL	LL	LL	4

2.5 EXPERIMENT #2: LR TRAINING

Once the ants had successfully identified the feeder and begun foraging, aluminum dividers (covered with brown packing tape like the channel walls) were placed in the diamonds. For Treatment #1, both of the passages created by the divider remained open during the training. For Treatments #2-#6, the R then L passages were closed, forcing the individuals to always take a LR route through the channel (Fig. 2B).

For Treatments #3-#6, sensory cues were also added at this time. Visual cues consisted of black plastic cylinders, 3 cm in diameter, with heights of either 5 cm (large) or 2 cm (small). Two different sizes were used so that the animals would be able to differentiate between the two separate obstacle points. The cylinders were placed just beside the passageways on either side of the divider. Olfactory cues consisted of either 2µL methyl salicylate (1:50 in hexane) or 2µL diluted indole (1:50 in hexane). These solutions have been previously shown to be distinguishable by individuals, but do not innately attract or repel them (Buehlmann et al. 2015). Two different chemicals were used so that the animals would be able to differentiate between the two separate obstacle points. When olfactory cues were used, the solutions were deposited directly onto the floor of the channel in the middle of the passageway, and reapplied every 10 minutes to ensure that the sensory experience remained strong. This method of olfactory cue placement was adapted from previous work by Huber & Knaden (2017). The specific combinations in which visual and olfactory cues were used in each treatment can be seen in Table 2. The treatments are labelled with four +/-, as for example ++ -+. The first two labels indicate if visual, then olfactory cues were used during the training period, and the last two labels indicate if visual, then olfactory cues were used during the test. So the treatment ++ -+ employed both visual and olfactory cues during training but had only olfactory cues during testing.

Table 1: Setup for training and testing in each treatment. It is indicated which passages were open, and which sensory cues were present. Schematics of these treatments can be found in the Supplementary Material, Section 8.1.

Treatment	Training channel conditions			Test channel conditions		
	Passages	Visual cues	Olfactory cues	Passages	Visual cues	Olfactory cues
1. Control / baseline	Both open	X	X	Both open	X	X
2. -- --	Only LR open	X	X	Both open	X	X
3. ++ ++	Only LR open	✓	✓	Both open	✓	✓
4. ++ --	Only LR open	✓	✓	Both open	X	X
5. ++ -+	Only LR open	✓	✓	Both open	X	✓
6. +- +-	Only LR open	✓	X	Both open	✓	X

Once the training channel was properly prepared with the appropriate divider and cue combination, an extended training period of around 3 hours was begun, during which the ants acclimated to the channel and learned the route to the feeder and back to the nest. During this time the feeder was constantly monitored and replenished so that the subjects didn't run out of food.

In the meantime, a test channel was built parallel to the training channel. The test channel was identical to the training channel in construction, though it didn't contain a feeder or a passage to the nest. The point on the channel that correlates to the nest entrance in the training channel is referred to as the "fictive nest," as, based on their path integrator, individuals who are placed into the channel will believe that the nest will be found there. In all treatments, the dividers placed into the test channel had both passageways open. In Treatments #3, #5, and #6, cues were also included in the test channel. The combinations of cues used in each treatment can be seen in Table 2.

After the long training period, experiments began. An individual holding a cookie crumb in its mandibles was removed from the training channel at the feeder using a falcon tube. The individual was then deposited at the equivalent location in the test channel, and a timer was started. As the focal individual made its way toward the fictive nest, its direction decisions were noted. The timer was stopped as the individual passed through the second passageway. After the run was completed the individual was painted (so that it wasn't accidentally tested twice) and returned to the nest. In the case that the individual took longer than 2 minutes to reach the second passageway or dropped its cookie, the trial was abandoned and the ant was replaced back to the nest

2.6 STATISTICAL ANALYSIS AND DATA GRAPHICS

Statistical analyses and the creation of graphics were performed in R version 3.6.1 (R Core Team, 2021) via the RStudio program (RStudio Core Team, 2019). Data graphics were created in R using the packages "ggplot2" (Wickham 2016) and "RColorBrewer" (Neuwirth 2014).

2.6.1 RF SCORE SIMULATIONS

For comparison against the collected idiosyncrasy scores, two simulated datasets were created with RF scores calculated from sequences of 10 direction decisions for 10,000 individuals. Simulation #1 represented a completely random route choice, with each route having a 25% chance of being selected for each run. However, we have seen that the ants greatly prefer to take symmetrical routes (LL and RR) to asymmetrical routes (LR and RL). Therefore, Simulation #2 included weights to the selection of the routes which were pulled from the control data gathered in Experiment #2. In the generation of the 10-run sequence for each individual, there was a 50% chance of a RR route, a 40% chance of a LL route, a 5% chance of a LR route, and a 5% chance of a RL route.

16

The simulations were created and run in R version 3.6.1 (R Core Team, 2021) via the RStudio program (RStudio Core Team, 2019). The R code used to create these simulations can be found in the Supplementary Material, Section 8.2.

The average RF scores were compared between the collected and simulated datasets using a Welch's two sample t-test. In order to avoid oversignificance, tests were also run with smaller simulated datasets equal to the experimental dataset (n=23) and the results came to the same conclusion. Therefore, the larger simulated datasets were retained for purposes of more effective visual comparison.

2.6.2 EFFECTS OF LR TRAINING

In experiment #2, the only datasets with enough power for statistical analysis were Treatment #1 (control) and Treatment #2 (LR training with no cues). In order to determine whether the LR training (without cues) had an effect on the ants' route choices, a Pearson's Chi-squared test with Yates' continuity correction was used.

3. RESULTS

3.1 EXPERIMENT #1: ROUTE FIDELITY

A sample size of 23 individuals were tested for route fidelity, and their RF scores had a mean of 3.091521 (sd = 0.9002814). In Simulation #1, where the selection of routes was completely random, scores had a mean of 1.299 (sd = 0.2300322). In Simulation #2, where the selection of routes was weighted (50% RR, 40% LL, 5% LR, and 5% RL), scores had a mean of 1.89 (sd = 0.3796878). As a reminder of the scoring system, the RF scores range from 1 to 4 with 1 indicating complete randomness and 4 indicating complete route fidelity. The observed data are compared with both simulations in Figure 4. The t-test used to compare the means of the observed data and both simulations indicated that they were significantly different in both cases (p <0.0001 for both tests).

Density of observed (blue) vs simulated (grey) RF scores

Simulation #1 (25:25:25:25)
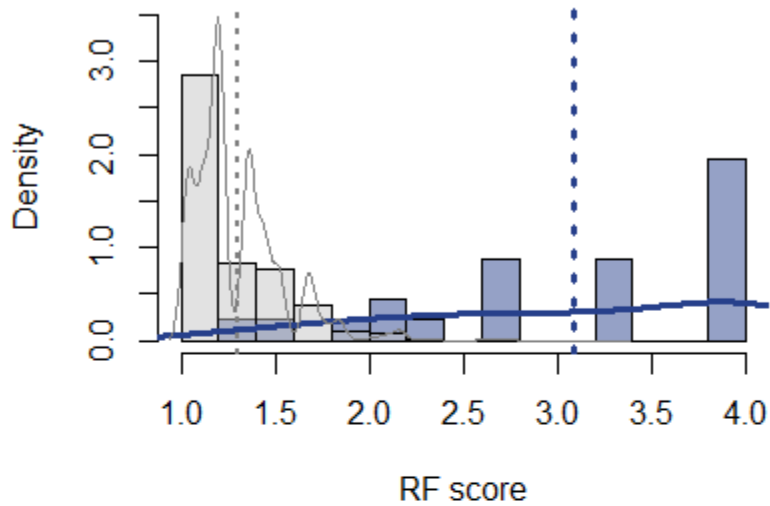
Simulation #2 (50:40:5:5)
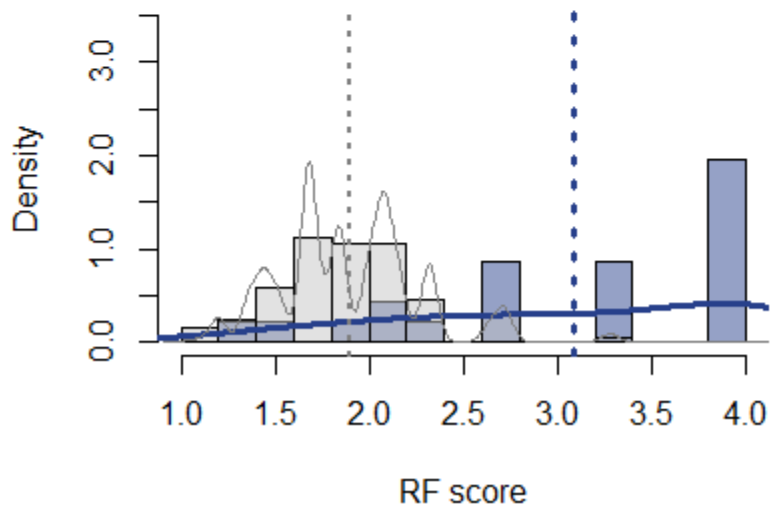
Figure 4: Density plot comparing the observed and simulated RF scores. Blue bars indicate the observed data while grey bars indicate simulated data. Dotted lines indicate mean values. The simulations differ in that in Simulation #1 all routes were assigned at

equal rates, while in Simulation #2 the routes were weighted according to the ants' choice preferences (50% RR, 40% LL, 5% LR, 5% RL). An RF score of 1 indicates no preference for any of the four possible routes, while a score of 4 indicates complete fidelity to a single route.

3.2 EXPERIMENT #2: LR TRAINING

In Experiment #2, only Treatments #1-#3 had the power to be statistically analyzed. Treatments #4-#6 will be discussed in Section 4.2.1. The routes taken in Treatments #1-#3 can be seen in Figure 5. After training with both passages open (Treatment #1, ctrl), 4.48% of individuals later took a LR route, while 4.48% took RL, 41.79% took LL, and 49.25% took RR (n=67) in the test situation. When ants were trained with only LR passage open and with no additional cues (Treatment #2, ----), 24.71% of individuals later took a LR route, while 15.29% took RL, 17.64% took LL, and 42.35% took RR (n=85) in a test channel where all passages were open. The same training and test but with added bimodal (visual-olfactory) cues (Treatment #3, ++++), resulted in 34.43% of individuals taking a LR route, while 6.56% took RL, 9.84% took LL, and 49.18% took RR (n=61).
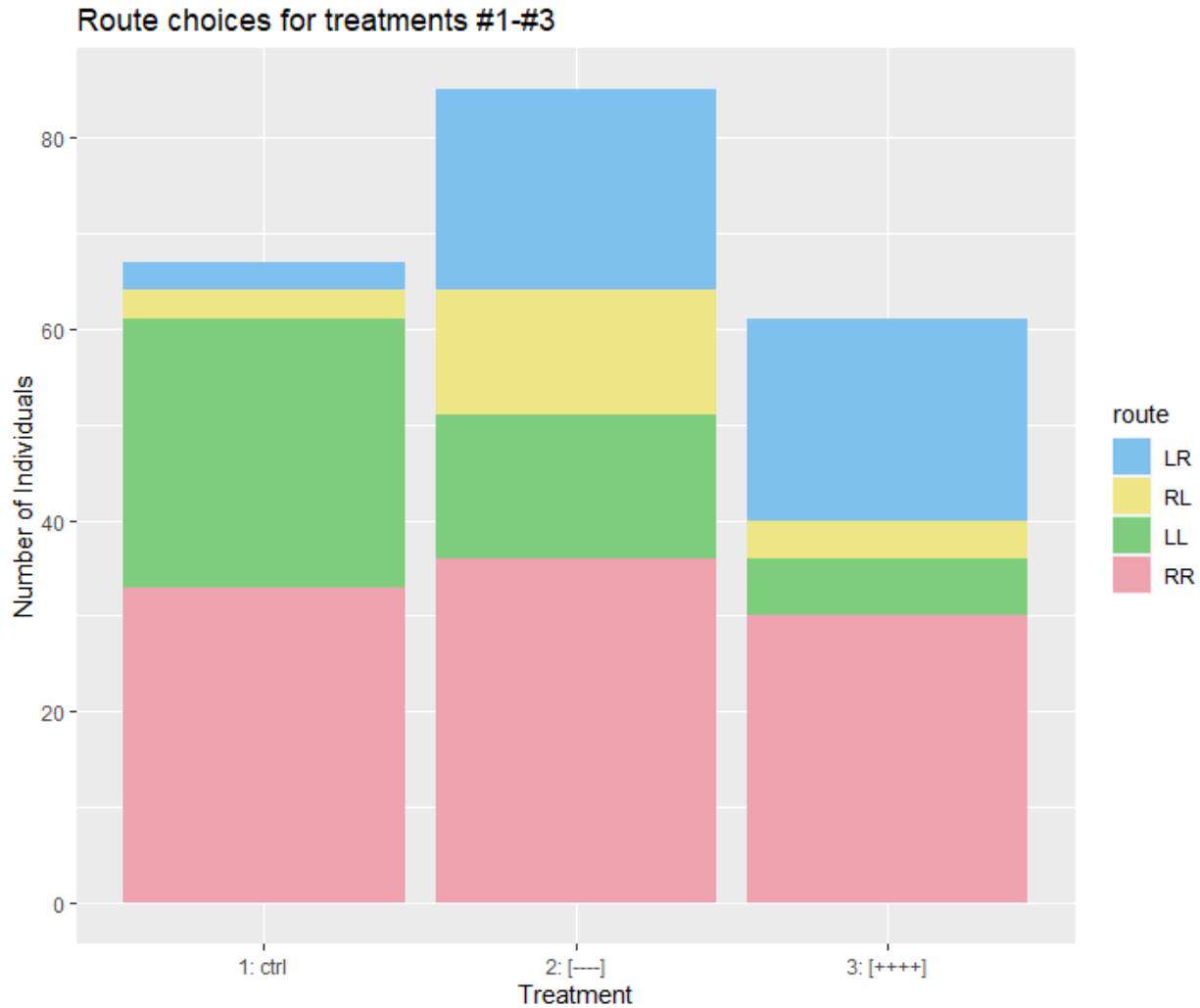
Figure 5: The route choices taken by individuals in Treatments #1-#3. In Treatments #2 and #3, the blue portion of the bar representing the LR route is of special note because this is the route that the individuals are being trained to use.

In comparing the control treatment (Treatment #1) with the basic LR training (Treatment #2), it was shown by a Pearson's chi squared test that there is a significant relationship between the training level of the ants (trained vs. not trained) and the number of individuals which performed an LR route ($p < 0.01$). Therefore, the training of the ants by the placement of the dividers in Treatment #2 successfully taught a difficult navigation task.

However, in comparing the basic LR training with no cues (Treatment #2) with LR training with both visual and olfactory cues (Treatment #3), it was shown by a Pearson's chi squared test that there was no significant relationship between the presence of cues and the number of individuals which performed an LR route ($p = 1$). Therefore, there is no evidence to support that the presence of sensory cues helps ants to more successfully learn a difficult navigation task.

4. DISCUSSION

4.1 LIMITATIONS

This study contained severe limitations in sample sizes and statistical power for nearly every experiment. The experiments suffered from an extremely hot summer with measured temperatures reaching up to 54ºC, which resulted in decreased foraging motivation of the ants. For example, in several instances many hours were devoted to training a nest of animals, but once the time for testing came about it was too hot and the exhausted ants stopped foraging.

The field trip was also unexpectedly shortened from 10 weeks to 6 weeks due to the COVID-19 pandemic situation. Considering that the first two weeks or so were used to adjust the experimental plans to real practice and learn the peculiarities of the study subject, less than half of the planned experiments were able to be carried out

Therefore, it is best to regard this study as preliminary work in support of future experiments that will test the inclination of *C. fortis* to complete idiosyncrasy routes, as well as how this desert ant species utilizes visual and olfactory sensory information in the completion of navigation tasks.

This study was also used as a means to test new methodologies. The channel mazes as described in this report were developed from scratch by the experimenters and have not been employed with *C. fortis* previously. The tests run during this experiment will facilitate future experiments run by the Odor Behavior Group of the Max Planck Institute for Chemical Ecology.

4.2 EXPERIMENT #1: ROUTE FIDELITY

The route fidelity tests were completed on a sample of 23 individual ants. Each individual was observed through 10 complete foraging runs, and their route decisions were notated. Route decisions consisted of the directions that ants decided to take through two different passageways in the maze channel. These route decisions were then used to calculate

the RF score, which describes route fidelity on a scale from 1 to 4, 1 being completely evenly divided between the four possible routes and 4 being completely faithful to one route. The tested individuals earned a mean RF score of 3.09, with almost 40% of the ants scoring a perfect 4. Therefore, it is quite clear from the beginning that the majority of these ants prefer to complete routes that are highly faithful to one route type.

Two simulations were created against which to test the observed data. In the first simulation, each of the four route possibilities (LL, RR, LR, RL) were pulled at equal rates to make up the ten run set for each individual. This simulation represents a scenario in which ants had absolutely no route preference; they would have no preference for any particular route type, nor any preference in the sequence of their routes throughout the ten run set. This is not very likely in nature, but it is interesting to see in comparison just how non-random the observed ants' routes really are. The individuals from Simulation #1 earned a mean RF score of 1.30, with almost 15% of the ants scoring the lowest possible score, 1.04 (a score of 1 was not possible in this particular scenario because with 10 runs it was impossible for individuals to take each route an equal number of times). So clearly, an ant that has no preference for their route will generally achieve a very low RF score. When compared against the observed individuals, the Simulation #1 unsurprisingly generated a significantly dissimilar mean.

In contrast with the simulation described above, ants in the wild will not generally take any route at random. Based on the control treatment performed on 67 individuals as a part of Experiment #2, I found that *C. fortis* individuals greatly preferred to take symmetrical routes (LL, RR) rather than asymmetrical routes (LR, RL). In this particular control experiment, the RR route was taken by around 50%, LL was taken by about 40%, LR was taken by about 5%, and RL was taken by about 5% of the individuals. It should be noted as well that the control experiment from Experiment #2 had an almost identical setup as that used to test the ants' route fidelity; the only difference was that the individuals were displaced from a training channel to a test channel before being tested. Otherwise, the placement of the dividers and absence of cues matched between these two scenarios.

This leads to Simulation #2, which takes in to account the ants' route preferences when generating the navigation data for each artificial individual. Therefore, RR had a 50% chance, LL had a 40% chance, LR had a 5% chance, and RL had a 5% chance of being pulled to make up the 10 run set for each individual. This simulation represents a scenario in which ants show some route preferences; they have a shown preference for particulars routes (in this case symmetrical ones), but still don't show any preference in the sequence of their routes throughout the ten run set. These individuals from Simulation #2 earned a mean RF score of 1.89, with less than 1% of the ants scoring the lowest possible score, 1.04, and also less than 1% of the ants scoring a perfect 4. This simulation shows a much more realistic spread than simulation #1, but still differs in comparison with the observed data. When means were compared statistically, Simulation #2 also showed a significantly different mean.

Based on the fact that both simulations differ significantly in RF score from the observed data, we can conclude that *C. fortis* individuals in the wild do tend to show notable route fidelity. Seeing as the food source used in the experiment was highly abundant and reliable, it certainly benefitted the subjects to memorize a particular route and maximize their time in collecting as many resources as possible. This ability to recall and retrace favored routes would be useful to ants in the wild in cases where they found particular food sites that they would want to repeatedly exploit. This wouldn't be too uncommon in their environment even though it is harsh- many of the nests observed in the field site were within a reasonable distance of a more vegetative landscape which would provide both plant food resources and a higher abundance of insect carrion.

4.2.1 ADDITIONAL OBSERVATIONS

The internal stability of individual's routes were also considered. The outbound route was compared against the inbound route, and it was seen that individuals were more likely to have routes that were somehow symmetrical. Routes fell into 3 categories: stable, inverted, and unstable. If an individual used a stable route, they performed the same direction choice on both the outbound and inbound runs (e.g. LL LL). If an individual used an inverted route, their outbound route was the opposite of their

inbound route (e.g. LL RR). In this case they didn't make the same direction decision, but rather took the same physical pathway during both trips. If an individual used an unstable route, there was no similarity between their outbound and inbound paths (e.g. LR LL). Out of a total of 255 foraging runs made by 23 individuals, 53.33% were stable, 21.57% were inverted, and 25.1% were unstable (Fig. 6).
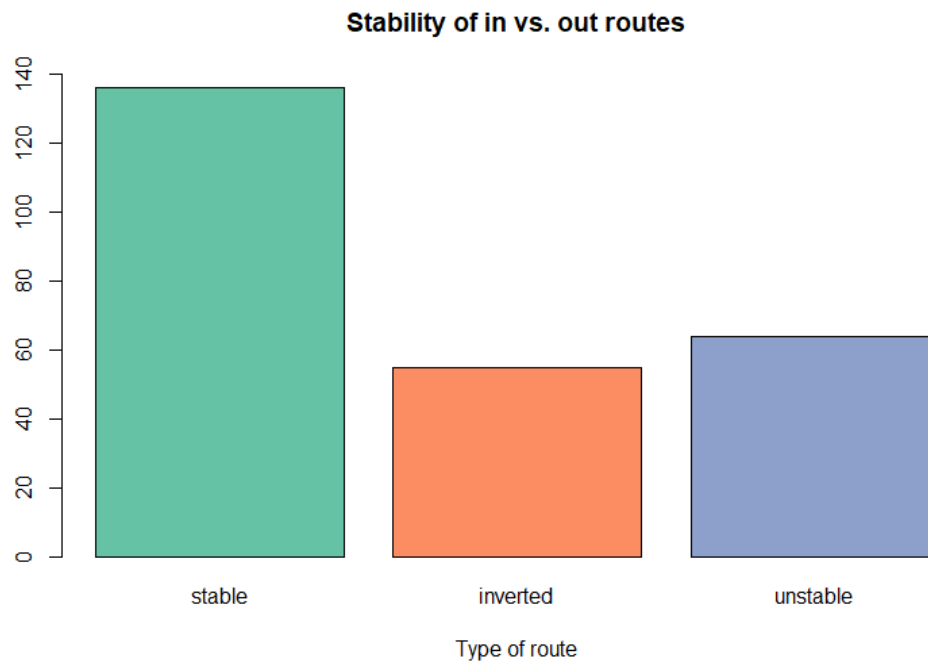

Figure 6: Bar chart describing the type of route taken over 255 trips made by 23 individuals.

4.2.2 Future Experiments

These findings on route fidelity may serve as one half of a more complete study on idiosyncrasy in *C. fortis* ants. The concept of idiosyncrasy has two requirements: route fidelity and variation between individuals. Therefore, the ideal counterpart to this study would be an experiment to test for variation between individuals in a foraging situation. It was not so possible to test for variation between individuals in our setup, because the subjects only had two binary choices to make, and therefore there were only four possible route outcomes. Not to mention that there were clearly biases to route preference in our particular assay, with symmetrical routes being more common than asymmetrical routes,

for example. This effectively reduces our realistic homing route choices to only two, RR and LL. Perhaps a more complicated maze, such as those used in Saar *et al.* 2017, Bega *et al.* 2019, and Saar *et al.* 2020, would offer subjects a much larger variety of different routes which could then be differentiated as individualized. Alternatively, routes could also be recorded in the natural habitat and compared between individuals as in Mangan & Webb 2012. Understanding whether or not *C. fortis* exhibits some sense of idiosyncrasy in its route formation retention would offer a more complete view of their model navigation system, as well as allow for more meaningful comparisons with other desert ant species such as *Melophorus bagoti* and *Cataglyphis velox.*

4.3 EXPERIMENT #2: LR TRAINING

Navigation task training is quite a common method of testing learning skills in *C. fortis.* My training setup included a maze where individuals had to pass through two passageways on their way to the feeder and return through another two passageways on their way back to the nest. However, only the homing path, or the two decisions made on the trip from the feeder back to the nest, were considered for this experiment. The homing path was prioritized because apart from path integration generally many navigation strategies aren't thought to be utilized during foraging, as the individual is only searching for a food source throughout the landscape. During homing is when the toolbox of memory, path integration, and landmark recognition are utilized, so that the subject can find its way back to where it started.

During Treatment #1, the control treatment, ants were observed through the maze with no manipulation- my only intent was to record their natural preferences inside the experimental setup, with every route option available to them at all times. It was found that by instinctive preference, 49.25% of the individuals took a RR route, 41.79% took a LL route, 4.48% took a LR route, and 4.48% took a RL route (as a sidenote, these data are those referenced in the creation of Simulation #2 in the route fidelity experiment, section 2.6.1). By this control experiment we can surmise that the ants greatly prefer to take symmetrical routes like LL and RR, rather than asymmetrical routes such as LR or RL.

Both asymmetrical routes had the same number or occurrences, but I arbitrarily chose LR as the baseline for our training regimen. Since it was so unpopular in the control experiment, I deemed it a difficult navigation task, and subsequently aimed to train a larger percentage of individuals to take this particular route. Training involved closing first the right passageway and second the left passageway, thereby forcing the animals to always take a LR route during the training period.

Treatment #2 consisted of only this training regimen, with no added sensory cues (----). Then when the animals were tested, both passageways were opened, and I was able to see if each individual retained their LR training or reverted to a more natural (typically symmetrical) route. Of the 85 individuals tested in treatment #2, 42.35% individuals took a RR route, 17.64% took LL, 15.29% took RL, and 24.71% took the LR route. So between the control experiment and Treatment #2, the number of ants taking the LR route increased from 4.48% to 24.71% - this was deemed significant when analyzed with a x^2 test. Therefore, the LR training in fact had success in training a higher number of animals to complete a navigation task that they would normally find unfavorable.

In Treatment #3, visual and olfactory cues were added to see if their mere presence would train a larger proportion of ants to successfully complete the LR training (++++). Visual cues in the form of plastic cylinders were added just beside the left then right passageways (the ones kept open during training). Olfactory cues were also added right in the middle of the left then right passageways (the ones kept open during training). These olfactory cues, indole in the diamond closest to the nest and methyl salicylate in the diamond closest to the feeder, have been previously shown to be discernable to *C. fortis* ants, but do not innately attract the animals (Buehlmann *et al.* 2015). Contrary to expectations, the presence of sensory information didn't have a significant effect on the ants' ability to successfully complete the training. Of the 61 individuals tested in Treatment #3, 49.18% took a RR route, 9.84% took LL, 6.56% took RL, and 34.43% took LR. Although the number of individuals taking the LR route increased from 24.71% in Treatment #2 (no cues) to 34.43% when cues were provided, this was not enough of an increase to be considered statistically significant. There was, however, a bit of a disparity

in sample sizes between the two treatments, so perhaps with some further sampling the increase may gain some significance.

4.3.1 ADDITIONAL OBSERVATIONS

Although they weren't analyzed statistically due to small sample sizes, the trends seen in Treatments #4 - #6 will be discussed here.

In Treatment #4, ants were trained with both visual and olfactory cues and then tested with no cues (++--). This resulted in 31.82% of individuals taking a LR route during the test, while 0% took RL, 13.64% took LL, and 54.55% took RR (n=22). The LR route was still taken by 31.82% of individuals, similar to the 34.43% when cues were present for both training and testing (++++), but greater than the 24.7% when no cues were used (----). This comparison of Treatments #2, #3, and #4 can be seen in Figure 7. This seems to indicate that the presence of cues did not hold so much importance to the individuals during the test. The training augmented with visual and olfactory cues seemed sufficient to successfully train the animals that they still performed in similar numbers when the cues were removed during the test.
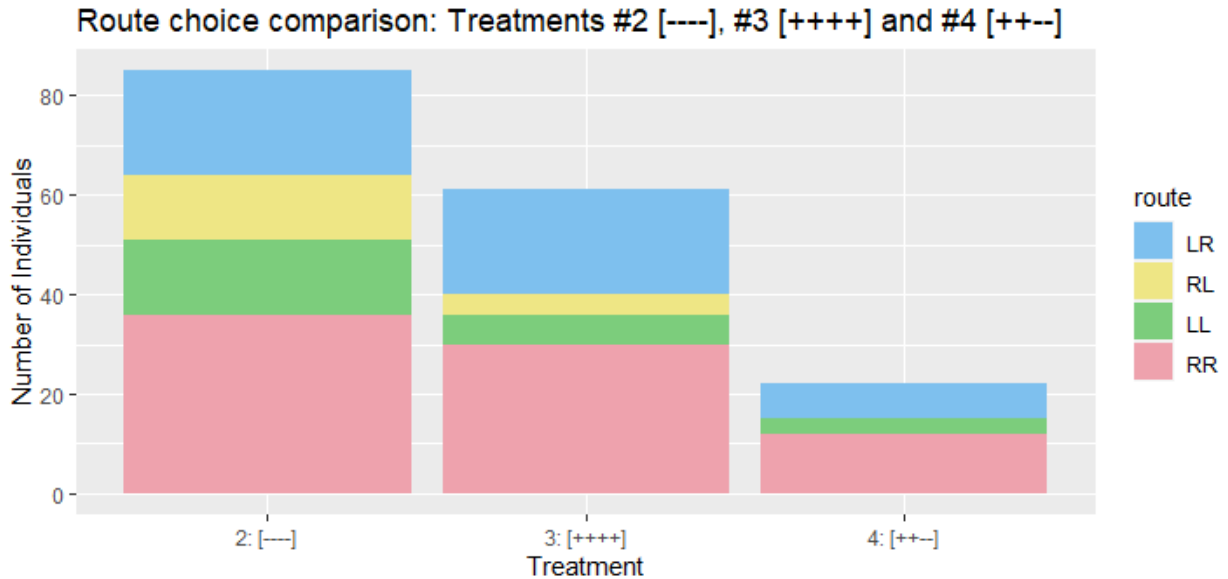
Figure 7: A comparison of route choices taken by subjects in treatments #2 [----], #3 [++++], and treatment #4 [++--]

Of particular note is Treatment #5, in which the ants were trained with both visual and olfactory cues, and were tested with only olfactory cues (++-+). In this trial 10% of the individuals took a LR route during testing, while 15% took RL, 15% took LL, and 60% took RR (n=20). This experiment was designed to test the effect of cue modality by training with bimodal cues then testing with a unimodal cue. Though the sample size is very small, the percentage of ants that took a LR route (15%) seems to be much smaller than when ants are offered bimodal cues in both training and testing (34.43%), and also smaller than when no cues were offered at all during both training and testing (24.7%). This comparison of Treatments #2, #3, and #5 can be seen in Figure 8. This indicates that the change of cue modality from bimodal to unimodal rather confused the individuals in the test channel, making them perform even more poorly not only than the trial which included bimodal cues, but also than the trial in which no cues are present at all.

30
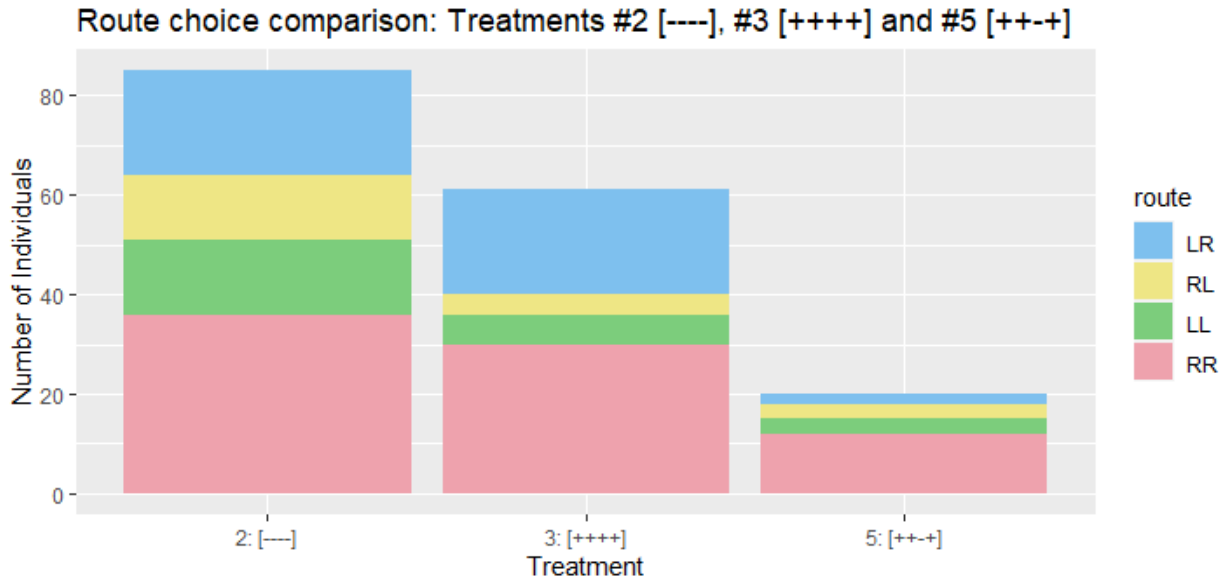
Figure 8: A comparison of route choices taken by subjects in treatment #2, treatment #3 [++++] and treatment #5 [++-+]

Treatment #6 was also designed to explore the effectiveness of bimodal vs. unimodal cues. In this treatment, ants were trained and tested only with visual cues to the LR route (+-+-). 0% of individuals took a LR route after this training, while 20% took RL, 30% took LL, and 50% took RR (n=10). Again, we must refrain from making any assumptions because the sample size was only 10 individuals, but it does seem interesting that not a single ant took the LR route that they were trained on. Figure 9 shows a comparison of this trial along with Treatments #2 and #3.
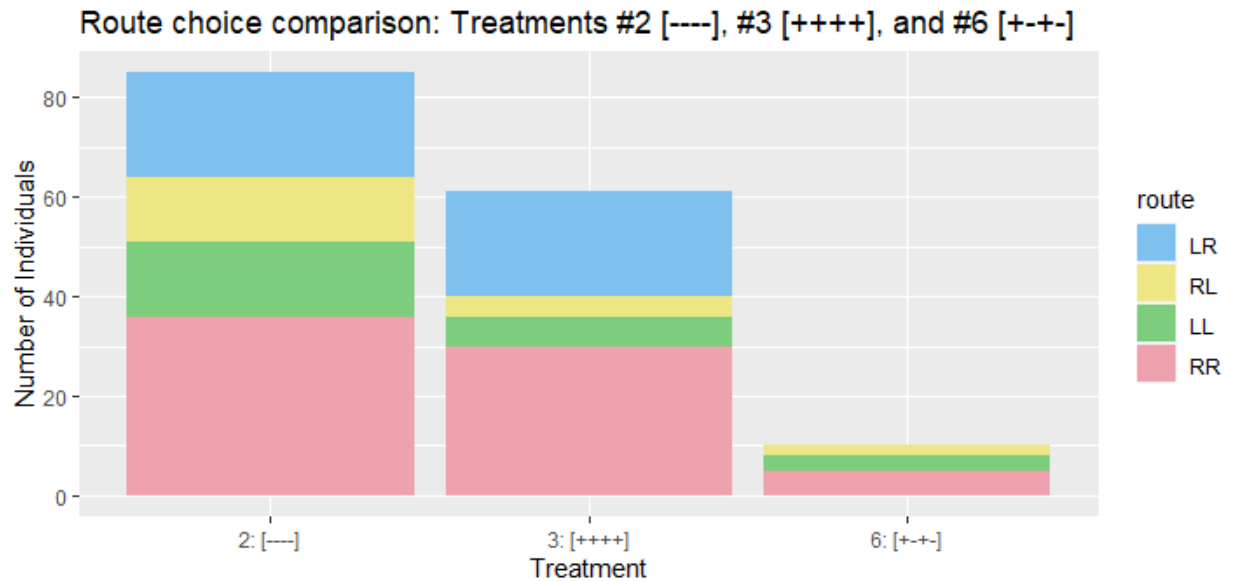
Figure 9: A comparison of route choices taken by subjects in Treatment #2, Treatment #3 [++++] and Treatment #6 [++-+]

4.3.2 FUTURE EXPERIMENTS

This study on the effectiveness of LR training in *C. fortis* would serve as a valuable preliminary exploration for a larger study on the effects of cues (and especially cue modality) on route learning. As introduced in section 4.2.1, several of the treatments in which cues were added in combination didn't have sufficient sample sizes for analysis. In future studies, each of the possible cue combinations should be further explored to further our understanding of how this sensory input affects route learning in *C. fortis.* It will be especially interesting to further test whether the presence of two cues at once (bimodal) has any different effect on the ants' learning abilities than the presence of only one sensory cue (unimodal).

5. CONCLUSION

The desert ant species *Cataglyphis fortis* is well-studied in its navigational abilities. These ants have an incredibly sharp memory and a great potential for learning; only a few of the many adaptations which help them to survive in their brutal habitat. Although the studies on *C. fortis* are plentiful, there are still some gaps in our knowledge about how exactly they retain and learn routes. In this study I tested wild *C. fortis* individuals in their route fidelity, as well as their ability to learn and complete a difficult navigation task.

The study in route fidelity generated a route fidelity score (RF score) for several individuals based on 10 consecutive route decisions. These RF scores were compared against two sets of simulated data, each giving a different weight to the possible route choices. In the case of both simulations, it was evident that *C. fortis* individuals have a significant propensity for high route fidelity.

In the second experiment, ants were trained to make the most unfavorable route, LR, while foraging through a maze channel. I found that LR training did have success in teaching a significant proportion of individuals to complete this navigation task. Visual and olfactory cues were also added and considered as supplements to this training.

These findings show promise on their own in clarifying some aspects of *C. fortis* behavior. However, they are perhaps most useful if considered preliminary work for a larger study on the navigation of these desert ants. The route fidelity experiment can be implemented as the first half of a confirmation toward idiosyncrasy in *C. fortis.* The study on navigation task training serves as a great first step and control exploration for a larger study in which all combinations of visual and olfactory cues are considered in order to work out exactly how cues, and especially the modality of cues, affect navigation decisions.

6. ACKNOWLEDGEMENTS

7. BIBLIOGRAPHY

Arleo, A. & Rondi-Reig, L. (2007). Multimodal sensory integration and concurrent navigation strategies for spatial cognition in real and artificial organisms. *Journal of Integrative Neuroscience*, 06, 327–366.

Bega, D., Samocha, Y., Yitzhak, N., Saar, M., Subach, A. & Scharf, I. (2019). The effect of maze complexity on maze-solving time in a desert ant. *Behavioural Processes*, 166, 103893.

Bisch-Knaden, S. & WehnerR. (2001). Egocentric information helps desert ants to navigate around familiar obstacles. *Journal of Experimental Biology*, 204, 4177–4184.

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

Buehlmann, C., Hansson, B.S. & Knaden, M. (2013). Flexible weighing of olfactory and vector information in the desert ant Cataglyphis fortis. *Biology Letters*, 9, 20130070.

Buehlmann, C., Hansson, Bill S. & Knaden, M. (2012). Path Integration Controls Nest-Plume Following in Desert Ants. *Current Biology*, 22, 645–649.

Buhlmann, C., Cheng, K. & Wehner, R. (2011). Vector-based and landmark-guided navigation in desert ants inhabiting landmark-free and landmark-rich environments. *Journal of Experimental Biology*, 214, 2845–2853.

Etienne, A.S. & Jeffery, K.J. (2004). Path integration in mammals. *Hippocampus*, 14, 180–192.

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

Forel, A. (1902). Les fourmis du Sahara algérien récoltées par M. le Professeur A. Lameere et le Dr. A. Diehl. *Annales de la Société entomologique de Belgique*, 46.

Grah, G., Wehner, R. & Ronacher, B. (2005). Path integration in a three-dimensional maze: ground distance estimation keeps desert ants Cataglyphis fortis on course. *Journal of Experimental Biology*, 208, 4005–4011.

Guo, J. (2005). Crossmodal Interactions Between Olfactory and Visual Learning in Drosophila. *Science*, 309, 307–310.

Heinze, S., Narendra, A. & Cheung, A. (2018). Principles of Insect Path Integration. *Current Biology*, 28, R1043–R1058.

Huber, R. & Knaden, M. (2017). Homing Ants Get Confused When Nest Cues Are Also Route Cues. *Current Biology*, 27, 3706-3710.e2.

Knaden, M. & Graham, P. (2016). The Sensory Ecology of Ant Navigation: From Natural Environments to Neural Mechanisms. *Annual Review of Entomology*, 61, 63–76.

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

Mangan, M. & Webb, B. (2012). Spontaneous formation of multiple routes in individual desert ants (Cataglyphis velox). *Behavioral Ecology*, 23, 944–954.

Mittelstaedt, H. & Mittelstaedt, M.-L. . (1982). Homing by Path Integration. *Avian Navigation. Proceedings in Life Sciences*, 290–297.

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

Neuwirth, E. (2014). RColorBrewer: ColorBrewer Palettes. R package version 1.1-2. Available at: https://CRAN.R-project.org/package=RColorBrewer/

QGIS.org (2022). QGIS Geographic Information System. QGIS Association. Available at: http://qgis.org/

R Core Team (2019). R: A Language and Environment for Statistical Computing. Vienna, Austria. Available at: http://www.R-project.org/

RStudio Team (2019). RStudio: Integrated Development Environment for R. RStudio, Inc., Boston MA. Available at: http://www.rstudio.com/

Rössler, W. (2019). Neuroplasticity in desert ants (Hymenoptera: Formicidae) - importance for the ontogeny of navigation. *Myrmecol. News*, 29, 1–20.

Saar, M., Gilad, T., Kilon-Kallner, T., Rosenfeld, A., Subach, A. & Scharf, I. (2017). The interplay between maze complexity, colony size, learning and memory in ants while solving a maze: A test at the colony level. *PLOS ONE*, 12, e0183753.

Saar, M., Hershkovitz, D., Amano, O., Bega, D., Subach, A. & Scharf, I. (2020). The effect of food preference, landmarks, and maze shift on maze-solving time in desert ants. *Behaviour*, 157, 629–665.

Schwarz, S. & Cheng, K. (2010). Visual associative learning in two desert ant species. *Behavioral Ecology and Sociobiology*, 64, 2033–2041.

Shams, L. & Seitz, A.R. (2008). Benefits of multisensory learning. *Trends in Cognitive Sciences*, 12, 411–417.

Sommer, S., von Beeren, C. & Wehner, R. (2007). Multiroute memories in desert ants. *Proceedings of the National Academy of Sciences*, 105, 317–322.

Steck, K. (2012). Just follow your nose: homing by olfactory cues in ants. *Current Opinion in Neurobiology*, 22, 231–235.

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

Steck, K., Hansson, B.S. & Knaden, M. (2011). Desert ants benefit from combining visual and olfactory landmarks. *Journal of Experimental Biology*, 214, 1307–1312.

Stieb, S.M., Hellwig, A., Wehner, R. & Rössler, W. (2012). Visual experience affects

both behavioral and neuronal aspects in the individual life history of the desert ant Cataglyphis fortis. *Developmental Neurobiology*, 72, 729–742.

Vega Vermehren, J.A., Buehlmann, C., Fernandes, A.S.D. & Graham, P. (2020). Multimodal influences on learning walks in desert ants (Cataglyphis fortis). *Journal of Comparative Physiology A*, 206, 701–709.

Wehner, R. (2019). The Cataglyphis Mahrèsienne: 50 years of Cataglyphis research at Mahrès. *Journal of Comparative Physiology A*, 205, 641–659.

Wehner, R., Cheng, K. & Cruse, H. (2014). Visual navigation strategies in insects: lessons from desert ants. In: *The New Visual Neurosciences.* The Mit Press, Cambridge, Massachusetts, pp. 1153–1163.

Wickahm, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag New York.

Wystrach, A., Schwarz, S., Schultheiss, P., Beugnon, G. & Cheng, K. (2010). Views, landmarks, and routes: how do desert ants negotiate an obstacle course? *Journal of Comparative Physiology A*, 197, 167–179.

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

8. SUPPLEMENTARY MATERIALS
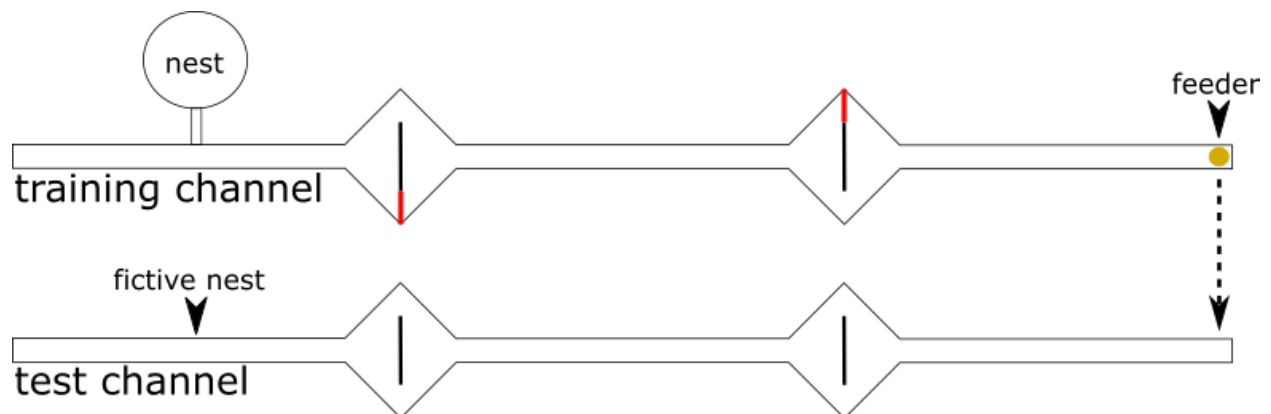## 8.1 LR TRAINING TREATMENT SCHEMATICS

The following material includes schematics of the channel setups used in the LR training portion of this study (Section 2.5)
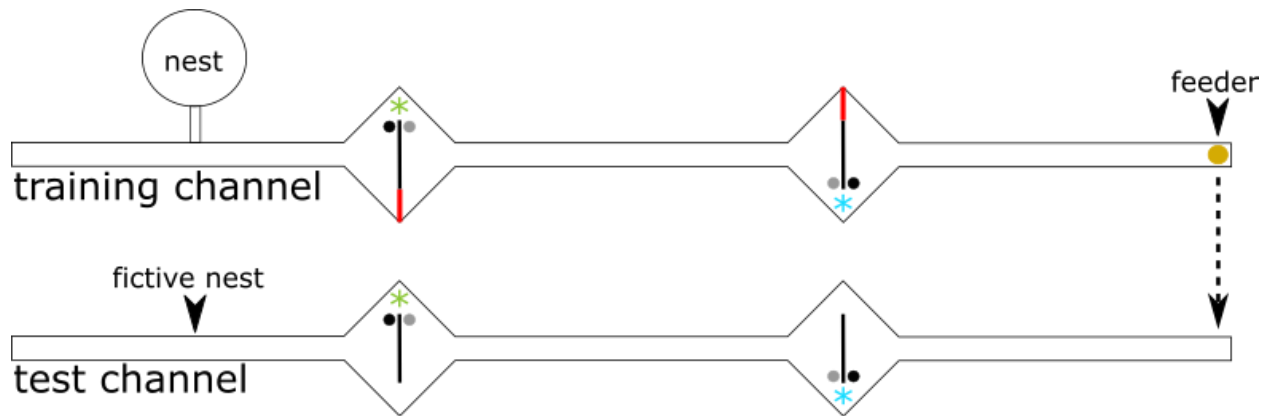
TREATMENT #1: ctrl
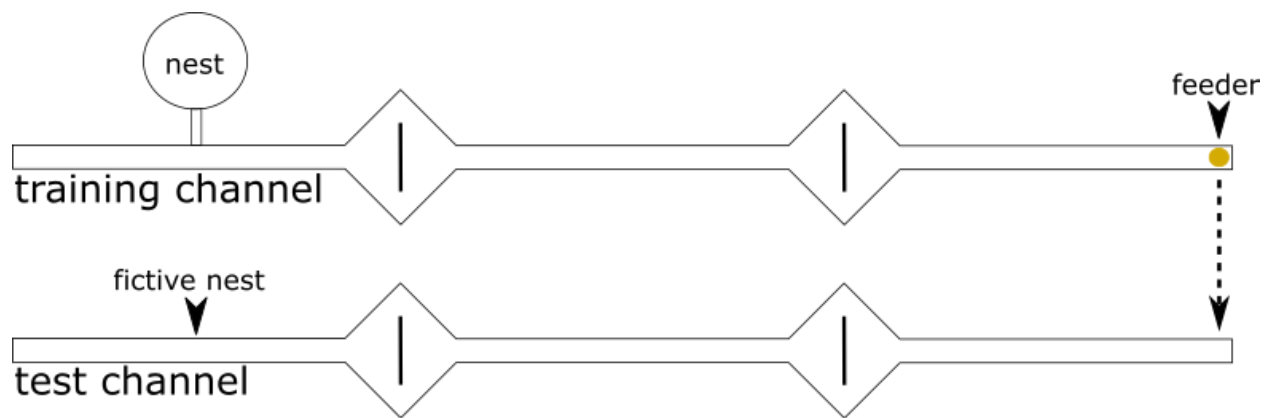
In this treatment passageways were open both ways during both training and testing. No cues were present at any time during the trials.
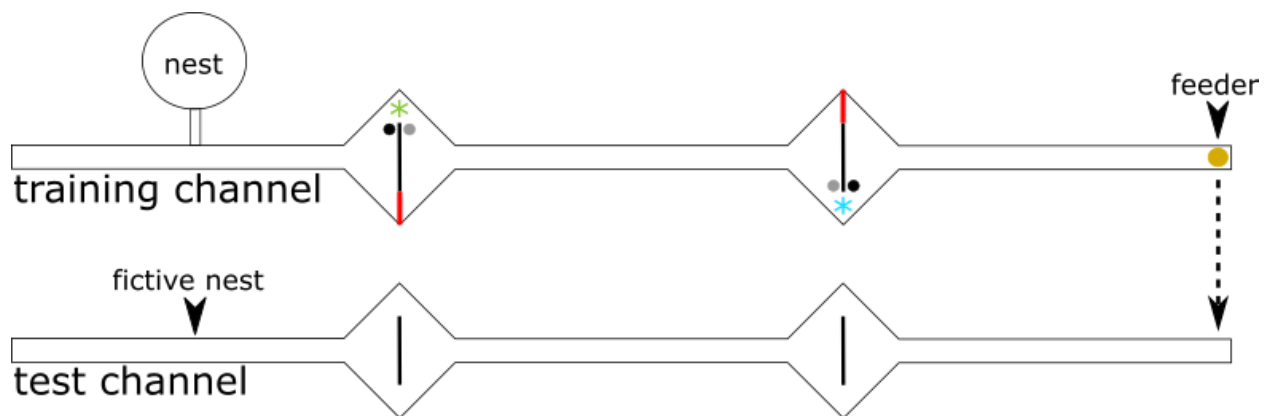
TREATMENT #2: [----]

In Treatment #2 the red passageways were closed during training, constituting LR training. No cues were present at any time during the trials.

TREATMENT #3: [++++]

In Treatment #3 the red passageways were closed during training, constituting LR training. Both visual (large/small plastic cylinders, denoted by black/grey circles) and olfactory (indole/methyl salicylate, denoted by green/blue asterisks) were present during both the training and testing portions of the trials.
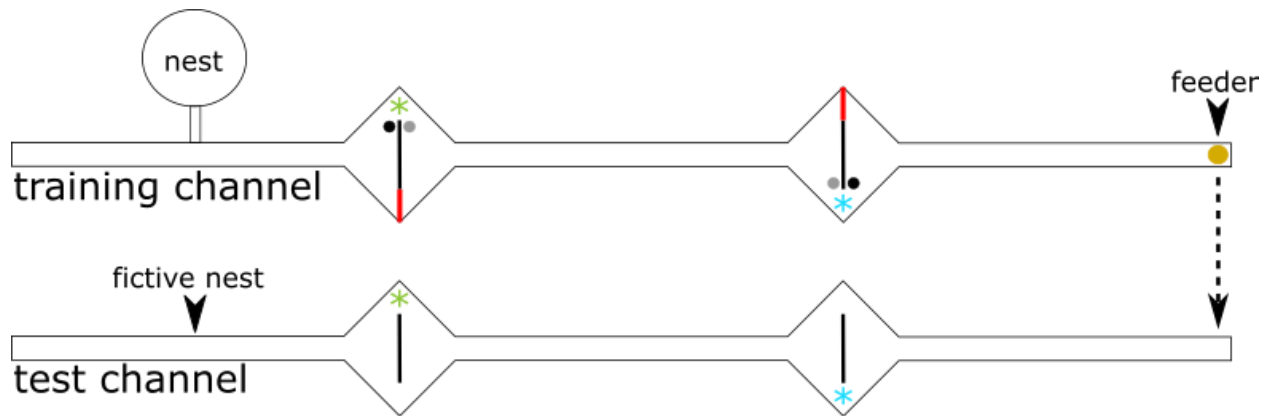
TREATMENT #4: [++--]

In Treatment #4 the red passageways were closed during training, constituting LR training. Both visual (large/small plastic cylinders, denoted by black/grey circles) and

olfactory (indole/methyl salicylate, denoted by green/blue asterisks) were present during the training portion of the trials, but no cues were employed during testing.

TREATMENT #5: [++-+]

In Treatment #5 the red passageways were closed during training, constituting LR training. Both visual (large/small plastic cylinders, denoted by black/grey circles) and olfactory (indole/methyl salicylate, denoted by green/blue asterisks) were present during the training portion of the trials, but only olfactory cues were employed during testing.

TREATMENT #6: [+-+-]
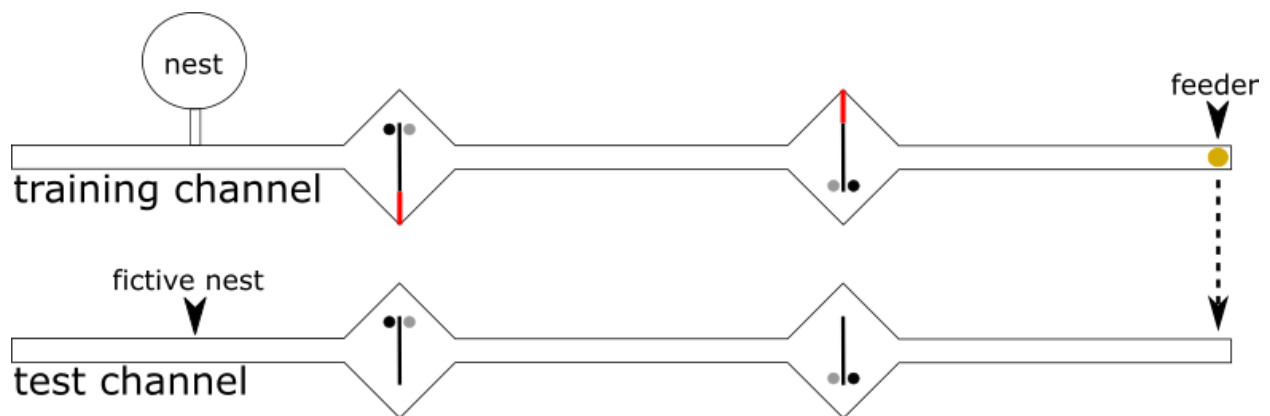
In Treatment #6 the red passageways were closed during training, constituting LR training. Only visual cues (large/small plastic cylinders, denoted by black/grey circles) were present during both the training and testing portions of the trials.

8.2 R CODE FOR SIMULATIONS

The following material includes the R code used to create the simulations in the route fidelity section of this study (section 2.6.1)

```
### SIMULATION #1 – equal probability for each route

#first, make a vector for the route choices:
#LL = 25%
#RR = 25%
#RL = 25%
#LR = 25%
sim1 <- c("LL","RR","RL","LR")

#second, create a vector of 10,000 to store the RF scores
sim1data <- rep(1,10000)

#finally, a loop to generate a sample of 10 routes and then
calculate the RF scores for each individual
for (i in 1:10000) {
   samp1 <- sample(sim1,size=10,replace=TRUE)
   score1 <- ((length(samp1[samp1=="LL"])/10)^2 +
             (length(samp1[samp1=="RR"])/10)^2 +
             (length(samp1[samp1=="LR"])/10)^2 +
             (length(samp1[samp1=="RL"])/10)^2)*4
   sim1data[i] <- score1
}

#the vector "sim1data" now contains 10,000 simulated RF scores
based on the criteria for simulation #1




### SIMULATION #2 – weighted probability for each route based on
ants' natural preferences

#first, make a weighted vector for the route choices:
#LL = 40% (8/20)
#RR = 50% (10/20)
#RL = 5% (1/20)
#LR = 5% (1/20)
```

```
sim2 <- c("LL","LL","LL","LL","LL","LL","LL","LL",
          "RR","RR","RR","RR","RR","RR","RR","RR","RR","RR",
          "RL",
          "LR")

#second, create a vector of 10,000 to store the RF scores
sim2data <- rep(1,10000)



#finally, a loop to generate a sample of 10 routes and then
calculate the RF scores for each individual
for (i in 1:10000) {
   samp2 <- sample(sim2,size=10,replace=TRUE)
   score2 <- ((length(samp2[samp2=="LL"])/10)^2 +
              (length(samp2[samp2=="RR"])/10)^2 +
              (length(samp2[samp2=="LR"])/10)^2 +
              (length(samp2[samp2=="RL"])/10)^2)*4
   sim2data[i] <- score2
}

#the vector "sim2data" now contains 10,000 simulated RF scores
based on the criteria for simulation #2
```