Homeward Navigation in *Pogonomyrmex occidentalis* Harvester Ants

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ABSTRACT

The purpose of this study was to determine the relative importance of known sources of navigational information for invertebrates in *Pogonomyrmex occidentalis* ants. The seemingly simple question of how animals find their way home leads to the study of complicated interactions between sensory abilities of animals and their potential for solving problems. Complex animal behavior can be difficult to study and explain because scientists cannot make inferences about the thoughts or motives behind a given behavior, but only analyze concrete observations. To address this problem, simple animals can be used as model systems. Understanding what processes underlie common behaviors in small-brained creatures provides insight about the evolution of complex behavior. This type of “bottom up” study of navigation contributes to the overall understanding of spatial cognition and may help explain more complicated navigation behaviors such as map-reading and complex route memorization. This study analyzes the relative importance of polarized light patterns, landmarks, and panoramic cues on the ability of *Pogonomyrmex* ants to orient homeward during foraging. When an ant is displaced from its original location to a new spot around the nest, it initially walks in the direction its nest would have been, had the ant not been moved. However, when a wall was placed behind the nest while an ant was navigating homeward, it became disoriented, walked in random directions, and took more time to find its nest. This result suggests that the *Pogonomyrmex* rely on panoramic images of scenery, rather than the other tested navigation methods.

I. INTRODUCTION

Complex problems can arise when animals are trying to find their way “home” to their nest or den after foraging. Consequently, animals need neural mechanisms, however simple or sophisticated, to resolve those problems. Information from landmarks, memory of the route travelled, the position of the sun or other forms of compasses may be used in homeward orientation. Birds and mammals may use higher-order neural processes in generating cognitive maps for calculating movements (Murray et al., 2006). However, many invertebrates, including ants, find their way home using essentially the same information available to vertebrates. Generally, these invertebrates are not credited with the capacity to use cognition, in the forms of thinking, reasoning, or the use of cognitive maps (Wystrach and Graham 2012; Graham 2010). My thesis is a study of the homeward orientation of harvester ants, *Pogonomyrmex occidentalis*, and the results open the door to considering whether animals such as ants might also use cognitive mechanisms in orientation.

The study of cognition, often defined as the ability of an animal to contemplate the past, predict the future, and act based on that information (Breed and Moore, 2012), is a major focus of research in biology and psychology. One specific and ongoing challenge associated with this field of study is the question of how to identify higher-level cognitive abilities. It is unscientific to make inferences about the mental processes or neural mechanisms behind observed behavior and only permissible to draw conclusions from observable evidence. This problem is referred to as ‘Morgan’s Cannon,’ named for the 19th century British psychologist C. Lloyd Morgan (Morgan 1903; Wystrach and Graham 2012). Morgan (1903) states that animal activity should not be interpreted as evidence of higher-level cognition if it can be otherwise explained in terms

of processes that stand lower on the scale of evolution and development. Morgan's Cannon makes it quite difficult to develop definitions of higher and lower psychological processes.

The lack of an objective understanding of what constitutes higher-level cognition forces researchers to consider other evidence to formulate their conclusions about observed patterns. One tool in the field of cognition in both humans and other animals is the principle of parsimony, taking an evolutionary perspective. Understanding how certain cognitive adaptations evolved from basic and phylogenetically widespread processes is crucial to grasping those cognitive adaptations as a whole (Wystrach and Graham 2012). This 'bottom-up' approach to spatial cognition can provide information about how complex behaviors might, to some extent, be explained by simple and widespread mental processes. Such an approach can also reveal how two seemingly different sophisticated behaviors could be explained by the same basic mental pattern.

Navigation is an excellent model behavior for the comparative study of spatial cognition for several reasons. It is ubiquitous in the animal kingdom; most animals must be able to navigate to important locations. It is a clearly defined spatial behavior easily quantified and analyzed; the goal of the animal is to get from point A to point B. Simple creatures navigate within familiar environments, which entails several cognitive processes. The animal must be able to extract relevant information from its environment, organize that information into basic memories, and then convert those memories into spatial decisions. From a bottom-up perspective, simple and widespread spatial mechanisms could also be underlying factors in complex navigational behaviors, such as route learning, or even map-reading in humans.

Eusocial insects, those that are characterized by cooperative brood care and clearly defined jobs, and ants specifically, are widely used as a model for navigation research. They are

an outstanding example of how small brains can be capable of remarkably efficient navigation in complex environments (Wystrach and Graham 2012; Wehner 2003) and they are particularly easy to study. Individual foraging specialists leave the nest to find food and then must navigate back. Their jobs are so clearly defined that it is a simple matter to assign motivation to their behavior, and these foragers are readily observable outdoors (Wystrach and Graham 2012). The fact that social insects act as an effective model for navigation studies has led to a substantial wealth of knowledge about their cognitive abilities; the methods employed by different species of social insects are well documented and well understood.

II. BACKGROUND

Insects have a navigational toolkit that consists of a path integrator (a navigational method allows for an ongoing estimate of the distance and direction of the goal location), a compass (often based on polarized light patterns in the sky), landmark recognition, “snapshot” memories of the landscape, and strategies to interlink these information sources to generate rich and seemingly complex navigation behavior (Dias and Breed 2008; Wehner 2003; Fukushi and Wehner 2004; Wystrach and Graham 2012; Graham 2010; Fukushi 2001). The use of olfactory information and pheromone trails is also important in homing for ants (Morgan 2009). A thorough understanding of every component of the navigational toolkit facilitates research that explores how insects integrate incoming information. Multiple types of information are available to ant workers for navigation, but certain sources will receive priority according to specific needs, circumstances, and environments (Dias and Breed 2008; Fukushi and Wehner 2004; Wystrach and Graham 2012). For instance, Dias and Breed (2008) identified polarized light as a primary cue for nest-bound navigation in *Formica podzolica* in the initial stages of orientation in an unfamiliar context, while supporting the claims made by Fukushi (2001) and Fukushi and

Wehner (2004) about the critical importance of panoramic cues. These studies demonstrate that ants may use different types of information during different stages of the navigational process. Additionally, certain environments may be devoid of important information such as landmarks or celestial information, requiring ants to rely on other available sources.

Path integration. Ants can use path integration to organize information about orientation and length of journey segments to maintain an ongoing estimate of the distance and direction of the starting point (Graham 2010; Wehner 2003). Path integration allows the ant to make several stops and changes of direction on the outward journey, and still be able to return directly home rather than having to retrace its steps. Animals using path integration need a mechanism for measuring the angles of the turns they make throughout the journey as well as some sort of internal odometer to track distance traveled. Most insects rely on the sun as a celestial compass to measure the angles of the turns made during a foraging trip (Graham 2010). The method used to measure distance travelled depends upon the type of locomotion used by a given species, and there is evidence that ants measure distance travelled by somehow “counting” steps, a tool referred to as a step integrator (Wittlinger et al., 2006; Graham 2010).

Compass orientation. Insects can use celestial information as a compass. The sun’s position in the sky, given the time of day, and polarization patterns created by scattering of sunlight in the upper atmosphere are both commonly used celestial cues (Wehner 2003; Graham 2010; Dias and Breed 2008). Animals that must navigate at night can use star patterns, a phenomenon widely researched in migrating birds (Able and Able 1995; Able 1991). There is evidence that some species of ants, as well as other animals, rely on the earth’s magnetic field as a compass (Riveros and Srygley, 2008; Able and Able, 1995; Diego-Rasilla et al., 2008).

Landmark and snapshot memories. Ants, and insects in general, use landmarks and “snapshot” memories of landscapes in navigation in addition to polarized light. Landmarks are helpful in defining a location, and learning how a goal location (such as a nest entrance) relates to the surrounding landmarks thus reduces the risk that cumulative errors in path integration will result in an individual missing the goal location entirely (Graham 2010). Nearby landmarks are useful for defining locations, but not for navigating to them. Wystrach et al. (2011) found that small displacements in prominent landmarks did not affect ants’ paths during experiments. Instead, evidence suggests that navigation is based on scene recognition that does not depend on the extraction and identification of specific landmarks (Fukushi and Wehner 2004; Fukushi 2001; Wystrach et al. 2011 Graham and Cheng 2009).

Ants and other insects memorize “snapshots” of their surroundings from the perspective of a future goal location. An ant navigating back to the goal location after an excursion, moves so as to decrease the discrepancy between the “snapshot” landscape around the goal and the current image of the landscape (Fukushi 2001). Ants can learn visual patterns in retinotopic coordinates (Wehner and Raber 1979; Judd and Collett 1998; Fukushi and Wehner 2004) and link those visual memories to an earth-based system of reference (Wehner et al 1996). They store a number of views of the same location from different vantage points and link different landmark memories by local vectors (Collett et al. 1998; Fukushi and Wehner 2004; Nicholson et al. 1999), which guides them from one visual signpost to the catchment area of the next (Wehner et al. 1996). Ants are able to follow learned routes simply by comparing site-based views, and can do so independently of path integration. For example, some *Cataglyphis* ants are able to navigate through a cluttered environment back to their nest, regardless of their starting point (Fukushi and

Wehner 2004; Wehner et al. 1996). Fukushi (2001) and Fukushi and Wehner (2004) go so far as argue that some ants rely almost exclusively on this type of landscape information.

My aim in this paper is to explore the relative roles of these navigational methods in the local species of *Pogonomyrmex occidentalis* harvester ants in Boulder, Colorado.

Pogonomyrmex occidentalis harvesters were chosen because they are particularly easy to observe for this type of study. They are relatively large and have distinct sand mounds on top of their nest making them easy to find. Their foraging behavior makes their goal location obvious; once they acquire food, they navigate directly back toward the nest (Dias and Breed 2008). Because the ant's target is known, an observer can measure the accuracy with which the ant is walking back to the nest. Measurements can then be compared to determine the ants' ability to navigate under various treatments.

To address the relative importance of known insect navigation techniques, I designed a series of experiments that examined the ant's use of each type of information independently. A series of initial displacement experiments were performed to determine how ants behave when displaced from their original location within their foraging range. Do they orient in the direction of their real nest or in the direction their nest would have been, had the ant not been moved, termed the 'fictive nest direction' (Fukushi, 2001)? Do they become disoriented and move in random directions? The importance of local landmarks was also assessed as a part of the first experiment by adding more prominent landmarks, in the form of two traffic cones, near the nest. I hypothesized that greater levels of displacement around the nest would result in greater levels of disorientation.

I then designed treatments that isolated specific navigational input (landmark, polarized light compass, snapshot) by making each one, in turn, unavailable to the ants. I measured the

ant's initial movement direction after each manipulation, the number of search-loops and returns to the site to which they were displaced (the 'drop site'), and the amount of time it took each ant to reach the nest. If these measures significantly changed relative to controls, the hypothesis that a specific input is used in orientation was supported. I developed a detailed picture of how harvester ants orient to their nests by sequentially testing the effects of displacement, landmarks, compass, and snapshot orientation.

III. METHODS

To answer the question of how *Pogonomyrmex* ants orient to their nest while foraging, I examined five different colonies, all within the same field on the East campus of the University of Colorado [$+40^{\circ} 0' 42.89''$, $-105^{\circ} 14' 59.82''$], between July and October 2012. I chose which nest to use during a trial based upon which had the highest level of ant activity, and used each nest an equal number of times during the course of the experiments.

1. Displacement Experiments

My initial experiments examined how *Pogonomyrmex occidentalis* ants behave when displaced within their foraging range, away from a foraging location. Ants were baited with cookie crumbs 2 m away from the nest. Once an ant had a firm grasp on a crumb, it was picked up using forceps. The use of food was important because (i) it facilitated the capture of individuals and (ii) it triggered homeward orientation by the ants (Dias and Breed, 2008). Captured ants were displaced at various degrees (15° , 30° , 60° , and 90°), using the vector from the ant's initial location to the nest as 0° , along an arc with a 2 m radius. The displacement changed the ant's angular orientation but not its distance from the nest (Fig. 1).

For the controls, I performed $10\ 0^{\circ}$ displacement trials, where the ant was picked up, held for 10 seconds, then placed at its original location. I also conducted five trials for each of the 15° ,

30°, 60°, and 90° displacements. I used a large metal protractor that measured the angle with the nest entrance acting as the vertex to measure the angles of displacement and to insure that the ant was kept at 2 m from their nest at all times. These experiments were modeled after Dias and Breed (2008), with the exception that the ants were always held 2m from the nest and were transported along a circle. Dias and Breed (2008) transported the ants along an axis that transected the nest, or laterally – along a line drawn at a 90° angle to the original line.

Figure 1. (Left) The diagram on the left is a representation of the experimental layout. The ants were baited and captured 2m from the nest, then moved x° , which could be 0°, 15°, 30°, 60°, or 90°, to another location 2m from the nest. **(Right)** The diagram on the right demonstrates the meaning of the real nest direction (a) and the fictive nest direction (b). After the ant is moved, the real nest direction is the actual direction the nest is in relation to the new location. The fictive nest direction is the direction the nest would have been had the ant never been moved.

Literature on ant orientation identifies the fictive nest as the direction in which the nest would have been had the ant not been displaced, and the real nest as the actual direction of the nest (Fig. 1 (right); Fukushi, 2001). The direction of the fictive and the real nest were recorded for every trial from the location to which the ant was displaced. Directions were recorded in compass degrees. In addition, I recorded (i) the initial direction in which the ant walked and (ii) the distance walked in that direction, (iii) direction and distance from the nest after three

minutes, (iv) the number of times the ant returned to the location to which it was displaced, (v) the number of search loops it made, and (vi) the time it took for each ant to find its way back to the nest. These values were recorded based upon the assumption that an ant that is more disoriented will take significantly more time to reach the nest, will perform more searching loops, and will take an initial direction that is further away from the direction of the real nest (measured in degrees).

The purpose of the initial displacement experiments was to determine if the methods used by *Pogonomyrmex occidentalis* ants during navigation enabled them to remain oriented after displacement, resulting in initial movement in the homeward direction. Alternatively, if the methods used by the ants are not able to account for changes in a starting location, the ants would walk in the direction of the fictive nest and become disoriented. Such displacements could also result in complete disorientation, which would be evidenced by random initial directions. Using different degrees of displacement also allows for an analysis of how the initial direction could change according to the level of disorientation. In other words, *are ants always able to navigate in the direction of the real/fictive nest, regardless of their position around the nest?*

In order to assess the role of nearby landmark recognition, I placed two standard orange traffic cones one meter from the nest, one on either side, and conducted the same set of trials as described above, with the sole difference being that the traffic cones were present nearby throughout the process. These cones were left near the nest for three days to allow the ants time to acclimate before the experiments resumed. Five trials were conducted for each displacement. The use of traffic cones was conceptualized following Tinbergen (1951)'s groundbreaking results with digger wasps. He found that wasps used terrestrial landmarks to provide a stable

reference with which to define a location. My hypothesis was that the traffic cones would provide a stronger landmark cue than was present in the relatively uniform landscape of the field.

In summary, the goal of the displacement experiments was to determine (a) if there was a trend in the initial direction taken by the ant, either in the direction of the real or fictive nest, in trials with and without traffic cones, and (b & c) if the degree of displacement or the presence of prominent landmarks affect the level of disorientation in the ants (measured by the number of search loops and returns to the drop site, as well as amount of time taken to reach the nest).

1a. Statistical Tests for The Relationship Between Fictive Nest Direction, Real Nest Direction, and The Initial Direction of the Ants

I used the statistical analysis software, *R*, to test my hypotheses about the effects of the displacement or the presence of more prominent landmarks on general orientation. To assess if there was a relationship between the initial direction taken by the ants and the fictive nest direction, I performed a linear regression between the initial direction and the fictive nest direction for trials with and without traffic cones. If the regression of the initial and fictive nest directions was significant and did not vary greatly at any degree of displacement, I could conclude that the ants initially walk in the direction of the fictive nest, rather than in the direction of the real nest or in random directions.

I also conducted a two-way ANOVA to analyze the effect of displacement on initial direction, as well as the effect of the traffic cones. I calculated the difference between the fictive nest direction and the initial direction and then used these values for the ANOVA. The results of this two-way ANOVA would demonstrate if the ants' ability to navigate in the fictive nest direction is at all inhibited by increased levels of displacement. Additionally, if the ANOVA showed a significant effect of the traffic cone treatment, I could conclude that landmarks play a role in the orientation and navigation of *Pogonomyrmex occidentalis*.

1b. Statistical Tests for the Effect of Displacement and Presence or Absence of Traffic Cones on Search Loops and Returns to the Site to Which the Ant was Displaced (“The Drop Site”)

For the analysis of search loop behavior, I counted how many times an ant crossed its own prior path during the trial, as well as the number of times the ant returned to the location to which it was displaced (which I termed the “drop site”). A two-way ANOVA for each set of data analyzed the influence of the degrees of displacement and the effects of the presence of traffic cones during the trials. I hypothesized that the number of search loops and returns to the drop site should increase with degrees of displacement. Further, if the ants were relying on landmarks to navigate and the cones acted as more effective landmarks, then ants would use fewer loops and returns to the drop site during trials that included the cones.

1c. Statistical Tests for the Effect of Displacement and Traffic Cones on the Overall Time Needed to Return to Their Nest

I also examined the amount of time it took the ants to return to the nest after being displaced. A two-way ANOVA showed the effect of the displacement and the presence or absence of traffic cones on the overall time needed by the ants to return to their nest with the crumb. I hypothesized that increased time needed to reach the nest indicated greater levels of disorientation, so time would increase with increased displacement. Additionally, if landmarks were key to navigation, and the cones were more prominent in the landscape, then the amount of time needed to reach the nest would be decreased when the cones were present.

1d. Statistical Tests for the Effect of Displacement Traffic Cones on the Distance Traveled in the Initial Direction

A two-way ANOVA was used to analyze if there was an effect of the traffic cones or displacement on the distance an ant walked in its initial direction. This analysis aimed to provide insight on how an ant would react when receiving contradictory navigational data. For instance, the ant may be able to tell that it is experiencing the landscape or skylight patterns at a different

perspective than expected, may be at a different location in relation to a given landmark than expected, or may have walked further than expected before arriving at the nest. If the ant abandons its initial course to initiate search loops, it is a sign that it has perceived the existence of incompatible navigational information, and is acting accordingly.

2. Exploring Information Sources for Navigation

My focus then turned to analyzing known sources of information for navigation and orientation in ants to test the relative importance of each. Specifically, I tested for the use of nearby landmarks, polarized light, and more distant landmarks. To do this, I used methods similar to those described above.

For each treatment, I baited and captured an ant 2m away from the nest entrance, held it for 10 seconds, and released it at the location of capture, (identical to the process used for the displacement trials at 0°). The ants were not displaced around the nest. The data collected from these trials were analyzed along with data collected during displacement experiments, but only for those trials in which the ants were not moved from their original location. Treatments explored the effects of landmarks, polarized light patterns, and “snapshot” memories of larger scenery. Each treatment was designed to isolate one information source and make it unavailable to the ants. I hypothesized that, if the ants initially walked in random directions, made more search loops and returns to the drop site, and took significantly more time to navigate to the nest during a specific treatment, then the information source that was obscured to the ants must play a significant role in their ability to navigate.

2a. Moving the Traffic Cones

To further explore the importance of landmarks, I conducted five trials in which the ants were not displaced, but cones present in previous trials were moved. The ants were again baited

2m away from the nest and held for 10 seconds. During this time, I moved the cones to other locations around the nest. The ant was then released, and their journey home was tracked as before. If the ant showed signs of disorientation, this would suggest that it was using information about the location of landmarks relative to its nest to navigate home after foraging.

2b. Polarized Light Filter

To test the hypothesis that polarized light has an effect on the ants' ability to navigate back to their nest from 2 m away, I repeated the process of baiting, capturing, and releasing the ant at the same location, but then tracked their journey home while covering them with a polarized light filter (which was a 0.4 m x 0.4 m square). I was careful to keep the filter half a meter from the ground with the ant always in the middle of the shadow it cast, a method modeled after Dias and Breed (2008). I conducted five of these trials, and if ants demonstrated significant signs of disorientation in comparison to other treatments, this evidence was taken to support the hypothesis that ants use polarized light to set compass bearings in order to orient themselves.

2c. Wall Blocking Panoramic View

I repeated the process of baiting, capturing, releasing, and tracking subjects, but this time placed a 1 m x 1.5 m cardboard wall behind the nest to test the importance of distant, large-scale landmarks. This method was modeled after Fukushi (2001), which also examined the importance of panoramic landmarks for ant navigation. The wall blocked the ants' view of the entire landscape behind their nest as they navigated home. Any observed disorientation during these trials would support the hypothesis that ants use larger scale and more distant panoramas in order to orient during foraging.

2d. Statistical Analysis

I used a linear regression of the relationship between the fictive and initial directions for each treatment to test if ants initially walked in the direction of the fictive nest. I used one-way ANOVAs to analyze data for the difference between the initial direction and the fictive nest direction, frequency of search loops and returns to drop site, and time taken to reach nest. Each ANOVA included the 0° displacement data from the previous displacement trials with and without the traffic cone as a control.

IV. RESULTS

1. Displacement Experiments

1a. The Relationship Between Fictive Nest Direction, Real Nest Direction, and The Initial Direction of the Ants

During the displacement experiments, I observed that the ants appeared to initially walk in the direction of the fictive nest in the trials with and without traffic cones, regardless of the degree of displacement. I conducted a linear regression between the fictive nest direction and the subject's initial direction for trials with and without traffic cones to test this observation. Both regressions were highly significant (**Fig. 2**). The ants would initially walk in the direction of the fictive nest, rather than in the direction of the real nest. The consistency and accuracy with which the ants walked in the fictive nest direction provides evidence that route memorization and pheromone trails are not crucial to *Pogonomymex* navigation.

Figure 2. Relationship between the direction of the fictive nest (in degrees) and the initial direction. Trials with no cones yielded a slope of 0.9219, an R-squared value of 0.98, and a p-value of 2e-16. Trials with cones yielded a slope of 0.998058, an R-squared value of 1.00, and a p-value of 2e-16. These results indicate that the fictive nest and initial directions are so strongly correlated that they are nearly the same.

A two-way ANOVA was used to assess the effect of displacement and the presence of traffic cones on the initial direction of the subject. The results indicated that neither the presence of traffic cones nor the degree to which an ant was displaced significantly affected the relationship between initial and fictive nest directions (**Table 1; Fig. 3**). This means that, regardless of the presence of traffic cones or the degree of displacement, the ants initially walked in the fictive nest direction. A Tukey post-hoc test showed that there was no significant difference between any treatments or degrees of displacement (**Fig. 4**).

Table 1	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	1	88.4	88.408	0.9682	0.3304 ns
Degrees	4	381.8	95.453	1.0453	0.3949 ns
Treatment:Degrees	4	421.9	105.481	1.1551	0.3432 ns
Residuals	45	4109.2	91.316		

Table 1. The table above presents the results of a two-way ANOVA analyzing the effects of displacement and presence of traffic cone on the difference between initial and fictive nest directions during displacement trials. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 3. Difference between initial and fictive nest directions for trials with and without traffic cones. The larger bar for no modification at 15 degrees of displacement represents the effect of a single, large outlier. The smaller, darker bar at that location represents the mean difference between, excluding the outlier.

Figure 4. A Tukey post-hoc test revealed that there was no significant difference between either of the treatments or any of the degrees of displacement. Displacement groups under the same line are not significantly different from one another.

This pattern can be visualized by standardizing the data so that the real nest direction for every trial is 0. I did this by finding the difference between the real nest direction and the initial direction of the subject for each trial, assigning a value of 0 as the real nest direction for every trial, then assigning the absolute value of the difference between real nest and initial directions as the adjusted initial direction. This information also supports the interpretation that the initial direction correlates with the fictive nest direction, regardless of the amount of displacement (**Fig. 5**).

Figure 5. This figure shows the initial directions of ants in the absence of traffic cones (red dots) and in the presence of traffic cones (blue dots). The arrow labeled “RN” points in the direction of the real nest (all of which were standardized to point to the top of the chart). The arrow labeled “FN” stands for the fictive nest direction and was standardized by finding the absolute value of the difference between the original real and fictive nest directions. As the ants are displaced at a greater number of degrees around the circle, the initial directions tend to be in the direction of the fictive nest, even as it becomes farther away from the direction of the real nest.

1b. Statistical Tests for the Effect of Displacement and Presence or Absence of Traffic Cones on Search Loops and Returns to the Site to Which the Ant was Displaced (“The Drop Site”)

Using the number of times each subject crossed its own path during a trial, as well as the number of times the ant returned to the original drop site, two-way ANOVAs were performed to analyze the influence of both the number of degrees of subject displacement, and the presence or absence of traffic cones (**Fig. 6; Table 2 & 3**). The number of times the ant crossed their own path is an indicator of the number of search loops made by the ant, and therefore the ant’s level of disorientation. The number of times the ant returned to the drop site was also used as an indicator of disorientation. The number of search loops made, as well as the number of returns to the drop site, increased with greater levels of displacement. The number of search loops was not affected by the presence or absence of the traffic cones, nor was the frequency of returns to the drop site. Following the two-way ANOVA, a Tukey post-hoc test was performed to analyze which degrees of displacement resulted in significant differences for the number of searching loops or the number of returns to the drop site made by the ants (**Fig. 7 & 8**).

Table 2	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	1	2.97	2.970	0.8655	0.3572 ns
Degrees	4	363.54	90.885	26.4886	>0.001 ***
Treatment:Degrees	4	9.92	2.481	0.7232	0.5807 ns
Residuals	45	154.40	3.431		

Table 2. . The above table presents the results of a two-way ANOVA that analyzed the relative effect of the presence or absence of a traffic cone and displacement around the nest on the number of searching loops made by an ant. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Table 3	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	1	1.227	1.2273	1.5341	0.2219 ns
Degrees	4	68.175	17.0437	21.3047	>0.001 ***
Treatment:Degrees	4	0.525	0.1312	0.1641	0.9555 ns
Residuals	45	36.000	0.8000		

Table 3. The above table presents the results of a two-way ANOVA that analyzed the relative effect of the presence or absence of a traffic cone and displacement around the nest on the number of returns to the drop site made by an ant. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 6. (Left) Search Loops. Number of search loops performed by the ants increased significantly with increased displacement, but was not significantly affected by the presence of traffic cones. **(Right)** Drop Site. The number of times the ants returned to the drop site increased significantly, but was not affected significantly by the presence of traffic cones.

Figure 7. Tukey HSD post hoc examining search loops across displacements. Groups that lay under the same line with identical letters are not significantly different from one another, while groups that are labeled under different letters do have statistically significant differences in the number of search loops performed at that level of displacement ($p < 0.05$).

Figure 8. Tukey HSD post hoc examining search loops across displacements. Groups that lay under the same line with identical letters are not significantly different from one another, while groups that are labeled under different letters do have statistically significant differences in the number of search loops performed at that level of displacement ($p < 0.05$).

1c. Statistical Tests for the Effect of Displacement and Presence or Absence of Traffic Cones on the Overall Time Needed by an Ant to Return to Its Nest After Displacement

Another two-way ANOVA was used to analyze the effect of displacement and the presence of traffic cones on the amount of time taken by an ant to reach its nest (sec). The analysis revealed that the traffic cones had no significant effect on the amount of time needed to return to the nest ($df=1$, $F\text{-value}=0.26$, $p\text{-value}=0.60$) but the effect of displacement was significant ($df=4$, $F\text{-value}=11.96$ $p\text{-value}=7.42e-07$). This, combined with the data that demonstrated the effect of displacement on the frequency of search loops and returning to the drop site, and the fact that the initial direction is usually that of the fictive nest direction, provides significant evidence that ants become more disoriented with greater displacement (in degrees) from their foraging location (**Fig. 9; Table 4**).

Table 4	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	1	21602	21602	0.245	0.6231 ns
Degrees	4	3906462	976615	11.0744	p>0.001 ***
Treatment:Degrees	4	33831	8458	0.0959	0.9833 ns
Residuals	45	3968410	88197		

Table 4. The above table presents the results of a two-way ANOVA that analyzed the relative effect of the presence or absence of a traffic cone and displacement around the nest on the amount of time needed to reach the nest by ants during displacement trials. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 9. Time needed to reach nest. Multiple two-way ANOVAs demonstrated that ants spent more time trying to find their nest as they were displaced to locations farther away from their original foraging site. The above graphs show this trend in time increasing with number of degrees displaced, along with the trend that showed that ants also needed to conduct more searching loops (left), which included more returns to the drop site (right), regardless of the presence of traffic cones. The data points on the left increase in size according to the number of search loops that ant performed and the data points on the right increase in size according to an increasing number of returns to the drop site.

A Tukey post hoc test was used to analyze for significant differences within the data (**Fig.**

10).

Figure 10. Result of a Tukey HSD post hoc test that analyzed the time (in seconds) needed to reach the nest for different degrees of displacement. Groups that lie under the same line with identical letters are not significantly different from one another, while groups that are labeled under different letters do have statistically significant differences ($p < 0.05$).

Id. Statistical Tests for the Effect of Displacement and Presence or Absence of Traffic Cones on the Distance Traveled in the Initial Direction

A final two-way ANOVA and Tukey HSD test was performed to analyze if there was any effect of the traffic cones or displacement on the distance the ants traveled in their initial direction during the displacement trials. Given that there is evidence that the level of disorientation increases with greater levels of displacement, the results of this analysis provide insight on the ants' behavior when experiencing greater levels of disorientation. The two-way ANOVA showed that the degrees of displacement had a significant effect, while the presence of the traffic cones did not. Ants that were displaced greater distances, and were more disoriented, walked a shorter distance in the initial direction (**Table 5; Fig. 11**).

Table 5	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	1	383	383	0.1604	0.690697 ns
Degrees	4	50049	12512.2	5.239	p>0.01 **
Treatment:Degrees	4	5639	1409.8	0.5903	0.671386 ns
Residuals	45	107473	2388.3		

Table 5. The above table presents the results of a two-way ANOVA that analyzed the relative effect of the presence or absence of a traffic cone and displacement around the nest on the distance travelled in the initial direction by ants during displacement trials. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 11. Ants walked in the initial direction a significantly shorter distance with greater degrees of displacement. Although the difference was significant, the R-value for the two-way ANOVA is small, demonstrating that only 21.14% of the variation could be explained.

The two-way ANOVA was followed up with a Tukey HSD test, which showed that the only significant differences between degrees of displacement was between 0 degrees and 15 degrees, and 0 degrees and 90 degrees (**Fig. 12**). These results are somewhat consistent with what was expected, although one would also expect 0 degrees to be significantly different from 30 and 60 degrees as well. The possibility that the 15 degree displacement group was

significantly different, while other, larger displacements were not could be attributed to the fact that there was a large outlier within the 15 degree displacement group.

Figure 12. A Tukey HSD post-hoc test showed that there were significant differences between 0 and 15 degree displacements, and 0 and 30 degree displacements. Groups under a line with the same letter label are not significantly different, but groups labeled with different letters are significantly different from one another ($p > 0.05$).

2. Exploring Navigation Techniques

Once I found that the ants were initially walking in the direction of the fictive nest, the next step was to isolate each known information source to assess the relative importance of each. Doing so could also explain why the ants walked in the direction of the fictive nest so consistently, and what source of information they were using to do so.

2a. Analysis of Initial Direction for Each Treatment

As with the displacement experiments, regression analysis established the relationship between the initial direction and the fictive nest direction. The relationship between the two was strongly significant for the trials involving moving of the cones and involving polarized light

filter, but there was no significant relationship between the two in the trials that blocked the landscape view (**Fig. 13**).

Figure 13. Relationship between the initial and real nest direction taken by ants. The first two graphs show that ants initially walked in the direction of the real nest for trials with moving traffic cones (left) and for trials with a polarized light filter (middle). For trials with the moving cones, a regression analysis yielded a regression line with the equation $y=1.01x-3.78$, with a p-value of $4.21e-7$ and an R-squared value of 1.00. The regression analysis for polarized light filter trials yielded the equation, $y=0.99x-0.84$, with a p-value of $1.39e-7$ and an R-squared value of 1.00. The regression between the initial direction and the real nest direction for trials with the wall present was not significant (right).

As with the displacement experiments, the relationship between the initial, fictive nest, and real nest directions can be demonstrated by standardizing the data to be displayed on a chart (**Fig. 14**).

Figure 14. Chart showing the initial directions of ants during trials with moving traffic cones, the polarized light filter, and the wall. The above diagram demonstrates the initial directions of ant treated with moving traffic cones (orange), polarized light filter (red), and a cardboard wall (green). This shows that, like in the displacement trials, ants initially walked in the direction of the real nest in all but the trials that included the wall. With the wall, the ants walked in more random directions.

To analyze if the difference between the treatments was significant, an ANOVA was conducted using the difference between the initial and real nest directions for each treatment (**Table 6; Fig. 15**). The ANOVA revealed that the wall was the only treatment that differed significantly from the others.

Table 6	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	4	23898	5974.6	11.005	P<0.001 ***
Residuals	28	15201	542.9		

Table 6. This table shows the results of the ANOVA that analyzed the difference between the initial direction of the ants and the direction of the real nest across all of the treatments. The results show that the difference between the initial and real nest directions does not vary significantly across treatments, except for those that included the wall. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 15. This is a graphical representation of the table above. The relationship between initial and real nest directions is not significantly different between any of the treatments, except for the wall.

2b. Analysis of The Number of Times Ants Crossed Their Own Path or Returned to Drop Site Across Treatments

For trials with or without the traffic cone at 0° displacement, trials with moving cones, or a polarized light filter, ants did not make any search loops or returns to their drop site. For trials with the wall, however, ants did display this behavior. For trials with the wall, ants crossed their own paths an average of 9.38 times/trial (SD = 4.93, N = 8) and returned to the drop site an average of 3.25 times/trial (SD = 2.55, N = 8).

2c. Analysis of Time Needed to Reach Nest Across Treatments

An ANOVA showed that the time needed to reach the nest by the ants was significantly affected by treatments. A Tukey HSD a posteriori test showed that the ants required significantly

more time to reach their nest when the wall was present than with all other treatments (**Table 7; Fig. 16**).

Table 7	Df	Sum of Squares	Mean Squares	F-value	P-value
Treatment	4	5860433	1465108	15.282	p<0.001 ***
Residuals	28	2684451	95873		

Table 7. The above table presents the results of a one-way ANOVA that analyzed the relative effect of each treatment on the time needed to return to the nest by the ants. (ns = not significant, * p-value>0.05, ** p-value>0.01, *** p-value>0.001)

Figure 16. Time needed by an ant to reach the nest for various treatments does not differ significantly across treatments, with the exception of those where the wall is present. When the wall is present, the ants need significantly more time to reach their nest during trials.

V. DISCUSSION

From the results, I conclude that panoramic landscape cues, or ‘snapshot’ memories, are crucial for homeward navigation in the western harvester ant, *Pogonomyrmex occidentalis*.

Harvester ant workers do not adjust their orientation when polarized light input is disrupted or when landmarks in the vicinity of their nest are moved. A displaced worker ant initially moves

in the direction of the fictive nest, suggesting their course is set by compass or broader landscape (snapshot) cues. After a search phase, they then readjust their orientation to eventually reach their nest. Blocking the ant's snapshot view of the landscape disrupts their ability to reorient after displacement. This result is consistent with studies of various other ant species. For example, Fukushi (2001) concluded that the ant *Formica japonica* relies on the distant skyline as a navigational guide mark. However, the result of the present experiment contrasts with those obtained for other species, such as *Cataglyphis bicolor* that relies more strongly on a polarized light compass for navigation (Fent 1986), or *Atta colombica*, that uses the earth's magnetic field as a reference to orient while using path integration to navigate home (Riveros and Srygley 2008).

The effect of displacements, and the trend for ants to initially walk in the direction of the fictive nest rather than in the direction of the real nest, was not significantly affected by the degree of displacement or by the presence of traffic cones. However, with larger displacements, the fictive nest direction was made further from the real nest direction, and the ants therefore became more disoriented. I found strong correlations between the number of degrees of displacement and the number of search loops, returns to drop site, and time required to reach the nest. The ants are not completely disoriented by such displacements, since they did not walk in random directions, and were instead simply unable to account for the changes caused by the displacements during the homeward orientation.

These experiments did not specifically test the effects of olfactory cues in homeward navigation for *Pogonomyrmex occidentalis* ants, and such cues cannot be ruled out. However, the consistency with which ants initially walked in the direction of the fictive nest, regardless of displacement, as well as the trend for the ants to perform more search loops, returns to the drop

site, and to need more time to reach the nest, cannot be easily explained by reliance on pheromone trails or scents emanating from the nest. While the role of olfactory information could be greater during the final stages of searching for the nest, there is no reason to believe that olfactory cues are a primary tool during early navigation. Similarly, magnetic cues were not specifically addressed, but the significant levels of disorientation that resulted from the wall treatment relative to the other treatments would suggest that magnetic cues do not play a significant role in providing either compass or landscape information in the homeward navigation of *Pogonomyrmex occidentalis*. Dias and Breed (2008) reached a similar conclusion regarding magnetic cues when *F. podzolica* ants from their experiments lost vector-based orientation when their view of the sky was modified using a polarizing filter.

The wall blocking the landscape view beyond the nest was the only treatment to result in random initial directions, greater numbers of search loops and returns to the drop site, and increased time to reach the nest, supporting the hypothesis that snapshot memories are used during homeward navigation. Other studies of ants demonstrate that snapshot memories could be used either along with or independently of path integration (Collett et al. 2001; Fukushima and Wehner 2004), but I did not specifically address that issue in the present experiments.

Overall, I conclude that *Pogonomyrmex* harvesters and workers are able to form snapshot memories before or during a foraging excursion, and then compare that information to snapshots they are seeing as they navigate homeward. The present results also demonstrate that ants move shorter distances in the initial movement in the fictive nest direction with increasing levels of displacement, suggesting increasing levels of disorientation. This finding means that ants pick up on incompatibilities in the incoming information, whether these are related to panoramic information, path integration, or both, and react by initiating search loops and returns to the drop

site. The ability to form snapshots and recognize incompatible information is a surprising feat for such small-brained creatures, and it suggests the presence of cognitive adaptations.

These results shed further light on the relative importance of navigational information sources, but there are still questions for future research. What I have found is that panoramic information is crucial to the initial and searching phases of homeward navigation, while the other tested information sources are not. However, every ant eventually returned to the nest, even while the information source being targeted by a treatment was still obscured. This leaves further questions about the final stages of homeward navigation and the ants' ability to find their nest after time spent searching while disoriented. The search loops eventually bring the ant closer to their nest, and they are somehow able to recognize that they are near their goal location. This could be the result of a nearby landmark (Tinbergen 1951) or any of a variety of other information sources, including olfactory information. Focusing on the final stages could form a more complete understanding of the cognitive mechanisms being used by *Pogonomyrmex occidentalis* during homeward navigation. Gould (2004) suggests that navigation-dependent animals do not rely on one information source, but instead have access to multiple that they can use, according to which best suits a given situation. Therefore, it is possible that information sources that were less important during initial and mid-phase homeward navigation begin to play a more important role during the final stages.

My results provide information about homeward navigation in *Pogonomyrmex occidentalis*, as well as the importance of snapshot memories. This study follows up on the findings of Fukushi (2001), and contributes to the overall understanding of the importance of panoramic information. It sheds light on navigational adaptations of *Pogonomyrmex occidentalis* and cognitive mechanisms that enable ants to find their way home during navigation.

Understanding these processes can contribute to the “bottom-up” approach to comparative spatial cognition research; a better understanding of these simple processes can facilitate the understanding of more complex cognitive processes in the more advanced birds and mammals, including humans (Wystrach and Graham 2012).

The importance of snapshot memories in *Pogonomyrmex occidentalis* homeward navigation contributes to a growing collection of research that demonstrates both the simplicity and sophistication of the mental patterns used by invertebrates during navigation (Graham 2010). The ever-growing body of evidence that navigational behavior in vertebrates may be partially explained by similar simple mechanisms highlights the importance of research in insect navigation. While there is no evidence that insects are capable of creating mental maps of their surroundings, their cognitive adaptations demonstrate that even simple, small-brained creatures can solve real-world navigational problems (Graham 2010).

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