

Coordinating Individual Behavior in Collective Processes;
Seed Choice in Harvester Ants (*Pogonomyrmex californicus*)

by

Ioulia Beshpalova

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved January 2020 by the
Graduate Supervisory Committee:

Jennifer Fewell, Chair
Bert Hölldobler
Jürgen Liebig
Noa Pinter-Wollman
Stephen Pratt

ARIZONA STATE UNIVERSITY

May 2020

ProQuest Number:27956294

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent on the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 27956294

Published by ProQuest LLC (2020). Copyright of the Dissertation is held by the Author.

All Rights Reserved.

This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ABSTRACT

Social animals benefit from the aggregation of knowledge and cognitive processing power. Part of this benefit comes from individual heterogeneity, which provides the basis to group-level strategies, such as division of labor and collective intelligence. In turn, the outcomes of collective choices, as well as the needs of the society at large, influence the behavior of individuals within it. My dissertation research addresses how the feedback between individual and group-level behavior affects individuals and promotes collective change. I study this question in the context of seed selection in the seed harvester ant, *Pogonomyrmex californicus*. I use both field and laboratory studies to explore questions relating to individual behavior: how forager decision-making is affected through information available in the nest and at the seed pile; how workers interact with seeds in the nest; and how forager preferences diverge from each other's and the colony's preference. I also explore the integration between individual and colony behavior, specifically: how interactions between the foraging and processing tasks affect colony collection behavior; how individual behavior changes affect colony preference changes and whether colony preference changes can be considered learning behavior.

To answer these questions, I provided colonies with binary choices between seeds of unequal or similar quality, and measured individual, task group, and colony-level behavior. I found that colonies are capable of learning to discriminate between seeds, and learned information lasts at least one month without seed interaction outside of the nest. I also found that colony learning was coordinated by foragers receiving updated information from seeds in the nest to better discriminate and make choices between seed quality during searches for seeds outside of the nest. My results show that seed processing is essential for stimulating collection of novel

seeds, and that foraging and processing are conducted by behaviorally and spatially overlapping but distinct groups of workers. Finally, I found that foragers' preferences are diverse yet flexible, even when colonies are consistent in their preference at the population level. These combined experiments generate a more detailed and complete understanding of the mechanisms behind the flexibility of collective colony choices, how colonies incorporate new information, and how workers individually and collectively make foraging decisions for the colony in a decentralized manner.

PREVIEW

ACKNOWLEDGMENTS

Thank you to Jennifer Fewell for her encouragement, clarity, support, and patient mentorship. Thank you to my committee, Bert Hölldobler, Jürgen Liebig, Noa Pinter-Wollman, and Stephen Pratt, for their help in editing and analysis, helpful suggestions, and conversations.

A special thank-you to Kaitlin Baudier, Daniel Coven, Richard Gerkin, Xiaohui Guo, Jon Harrison, Christina Kwapich, Michael Lin, Colin Lynch, Madeline Ostwald, Nathan E. Smith, and Kate Weiss for the statistics lessons, conversations, edits and comments, and paper suggestions that helped this dissertation come to be.

A big thank you to Victoria Depner, Ian Gabriel, Xiaohui Guo, Corey Hulse, Natalie Newton, Alison Willis, and Kristofor Yap for their gracious and patient help in video analysis and in the field.

I am very grateful for the support and comradery of my academic family – the present and past members of the Social Insect Research Group. A special thank-you to those not previously mentioned - James Waters, Ben Pyenson, Ted Pavlic, John McKay, Jon Jackson, Brian Haney, Kevin Haight, Abigail Finkelstein, Ti Eriksson, Jessie Ebie, Anthony Basile, Rebecca Clark, and Elizabeth Cash.

Thank you to Elena Bespalova and Ivan Bespalov, for teaching me the most important skills used for my academic work: a love of knowledge, nature, science, and arts and crafts. Thank you for moving me from Belarus to the U.S.A, where girls have a better chance of growing up to be myrmecologists.

I'm grateful to the Arizona State University School of Life Sciences, and the National Science Foundation Graduate Research Fellowship Program, for funding this work.

TABLE OF CONTENTS

	Page
LIST OF FIGURES	vi
CHAPTER	
1 INTRODUCTION	1
2 COLONY LEARNING IN A COMPLEX ENVIRONMENT	7
Abstract	7
Introduction	8
Methods	13
Results	19
Discussion	23
3 INDIVIDUALS WITH DIVERSE AND FLEXIBLE BIASES SHAPE COLLECTIVE DECISION-MAKING	37
Abstract.....	37
Introduction	38
Methods	42
Results.....	50
Discussion.....	53
4 PROCESSED SEEDS PRIME NAÏVE HARVESTER ANT FORAGERS TO RECOGNIZE AND EVALUATE NOVEL SEEDS	68
Abstract.....	68
Introduction	69
Methods	73
Results.....	84
Discussion.....	89

CHAPTER	Page
5 CONCLUSION	106
REFERENCES	112
APPENDIX	
A: SUPPLEMENTARY MATERIAL	125
Lipid Analysis	126
Approximating Seed Amounts Given to Colonies.....	127
Supplementary Material for Chapter 3	128
Supplementary Material for Chapter 4.....	131

PREVIEW

LIST OF FIGURES

Figure		Page
1.	Ch. 2 Figure 1: Diagram of Experimental Set-up in the Field	31
2.	Ch. 2 Figure 2: Effect of Experience over Days on Colony Behavior	32
3.	Ch. 2 Figure 3: Effect of Experience over 30 Min on Colony Behavior.....	34
4.	Ch. 2 Figure 4: Relationship between Rate and Bias	36
5.	Ch. 3 Figure 1: Diagram of Experimental Set-up in the Field	60
6.	Ch. 3 Figure 2: Change in Preference of Colonies across Two Days	62
7.	Ch. 3 Figure 3: Change in Preference of Individuals across Two Days	63
8.	Ch. 3 Figure 4: Change in Diversity of Individuals across Two Days.....	64
9.	Ch. 3 Figure 5: Change in Colonies and Individuals over Six Days.....	65
10.	Ch. 3 Figure 6: Forager Behavior When Favorite Seed Disappears.....	67
11.	Ch. 4 Figure 1: Experimental Set-up and Manipulations	97
12.	Ch. 4 Figure 2: Seed Consumption and Collection by Subset Nests.....	99
13.	Ch. 4 Figure 3: Interactions between Workers and Seeds in the Nest.....	101
14.	Ch. 4 Figure 4: Locations of Foragers and Processors	103
15.	Ch. 4 Figure 5: Seed Consumption and Collection by Field Foragers	104
16.	Ch. 4 Figure 6: Seed Consumption Effect on Collection in Field Workers .	105
17.	Ch. 5 Figure 1: Diagram of Colony Information Integration.....	111

CHAPTER 1

INTRODUCTION

Animals in social groups benefit from the aggregation of knowledge and cognitive processing power. They gain advantages in safety, information and resource exchange, and collective efficiency (Brown 1988, Roberts 1996, Galef & Giraldeau 2000, Galef & Laland 2005, Couzin 2008). In highly-integrated social groups, including human societies, individual idiosyncrasies lead to group-level benefits like division of labor and collective intelligence (Beshers & Fewell 2001, Surowiecki 2004, Sumpter 2006, Couzin 2008). Meanwhile, the outcomes of collective choices as well as the needs of the society and the problems that it faces, influence the behavior of individuals within it (Dussutour & Simpson 2009, Kao *et al.* 2014).

Social insect colonies are model organisms for studying the interplay between diverse individual behaviors and collective functioning. Colonies rely on a system of decentralized control, meaning that colony behavior is based on the individual worker's capacity for self-driven decision-making. However, though colony members decide how to behave individually, they do not behave independently. Colonies rely on interactions between individuals and the exchange of social information to organize decision processes across individuals (Fewell 2003). The kinds of collective behaviors that colonies achieve from decentralized group decision making range from convergence on a single behavioral outcome, such as when choosing a new nest site (Seeley 2003, Franks *et al.* 2002, Visscher 2007), to distributing workers appropriately across tasks (Beshers & Fewell 2001), to the coordinated but also differentiated decisions of foragers collecting food from different sources to provide nutrition for the colony (Detrain & Deneubourg 2008).

Social insect colonies are complex adaptive systems, meaning that distributed decision-making also governs collective changes in response to new information (Bonabeau 1998). The integration of information flowing between distributed parts is facilitated by the nest environment, which acts as an information center (Seeley 1985). For example, during selection of a new nest site, both ants and honeybees will individually evaluate a distant potential site on their own, decide its quality, then come to the nest to recruit others, which in turn evaluate it for themselves. After a quorum is reached in favor of a site, the colony chooses that option and moves. Recruitment effort is higher for sites deemed to be better quality, so a better site found late can be chosen over a poorer site found earlier (Seeley 2003, Visscher 2007, Mallon *et al.* 2001). During foraging, ants and bees evaluate resources on their own, then lay a recruitment trail to the nest or return to dance for their site only if it meets their threshold of quality. The number of recruiters and strength of pheromone trails laid by multiple assessors relay these estimates, which wane as resources are depleted (Seeley *et al.* 1991, Seeley & Towne 1992, Detrain & Deneubourg 2008). The combination of individual evaluation and information sharing at the nest thus allows for the flexibility and democracy necessary for the colony to evaluate minute differences between sites, and track changing resources (Seeley & Towne 1992, Sasaki *et al.* 2013).

Beyond updating information about changes in an environment, the colony can also integrate information from past interactions with the environment, leading colonies to learn from previous experiences (Johnson 1991, Franks & Sendova-Franks 2013, Sasaki & Pratt 2018, Herz *et al.* 2008). Collective learning is facilitated through individuals that integrate new information from past collective choice, and change their behavior appropriately. For example, leafcutter ants integrate information about the effect of their past leaf choices on their fungus gardens, and

can learn to reject poor-quality leaves (Herz *et al.* 2008, Saverschek *et al.* 2010, Saverschek & Roces 2011). Similarly, nest scouting ants learn from past nest choices which heuristics are most likely to determine nest quality, and focus on these factors when choosing future nests (Sasaki & Pratt 2013). Learning can also happen through changes in the interactions between individuals that perform different tasks (Franks & Sendova-Franks 2013).

In this dissertation, I explore how individual decision-making is affected through information provided in the nest, and how this feeds into behavior and learning at the colony level. To do this, I look at both individual-level decision-making, information integration, and collective changes in colonies of harvester ants as they make choices about seed collection. Seed foraging by the “bearded” harvester ants of the genus *Pogonomyrmex* provides a rich context in which to understand the integration of individual decision making into complex collective decision processes. Colonies choose from seed species that are nutritionally, chemically and morphologically variable. Seeds also represent a resource that is heterogeneously distributed and constantly in flux (Brown *et al.* 1979). Many species of seed are available in the environment at any one time, yet colonies show clear preferences for certain species and can be quite thorough in their exploitation of favorites and rejection of other seeds (MacMahon *et al.* 2000). Colony preferences are influenced by nutritional content, toxin presence, size, and novelty of seeds (Gordon 1980, Rissing 1981, Fewell & Harrison 1991, Crist & MacMahon 1992, Knoch *et al.* 1993, Pirk & Lopez de Casenave 2010). Colonies also have been found to adjust relative seed preferences as they gather new information, and to learn to better recognize and handle seeds over time (Rissing 1981, Johnson 1991, Fewell & Harrison 1991). Whether colony changes in preference are maintained over time,

suggesting learning behavior, or whether they simply track changes in nutritional state of the colony has yet to be determined.

Foraging in harvester ant colonies is driven by decentralized decision-making. Foragers, who typically select only one seed at a time to carry to the nest, use individual assessments of quality when they come in contact with a seed. After evaluating a seed, they either choose to bring it to the nest, or continue to search the environment. Though colonies tend to show similar preferences for seed types (Pirk & Lopez de Casenave 2011), individuals exhibit diverse preferences that do not necessarily match colony preferences. Some species' foragers have been found to specialize to one seed type (Nickle & Neal 1972, Rissing 1981), while other species vary in which seed is preferred and their level of bias (Crist & MacMahon 1991, Fewell & Harrison 1991). Individuals can also change their preference, either randomly, or in a similar direction to others, facilitating changes in colony preference (Rissing 1981, Fewell & Harrison 1991). How individual preferences change over a longer time-span of information integration has only been characterized for one colony in one species; in this case, individuals became more specialized over time (*P. rugosus*, Rissing 1981). More work, in other species, is needed to understand if individual specialization is the typical outcome when colonies forage on the same seeds for an extended time period. Furthermore, when and where foragers gain information is still unclear. Information may be gained during foraging, at the seed pile, as well as inside the nest (Johnson 1991, Fewell & Harrison 1991, Weser 2005). Seed shells may preclude individuals from gaining all the necessary nutritional information while they are foraging, requiring interactions with seeds in the nest after the seed is husked (Lundgren 2009). Though we know how foragers interact with seeds outside of the nest, we are not clear on how they behave around seeds in the nest; do they interact with cached seeds, or help with seed processing?

The species I used in this study is *Pogonomyrmex californicus*, a desert harvester ant found throughout the Southwestern U.S. and Northern Mexico. Colonies are composed of 2000-4500 workers (Erickson 1972, Johnson 2000), and have an average foraging range of 1.5-3 meters from the colony (De Vita 1979). Colonies mostly subsist on seeds, though they also collect plant parts, and arthropods (MacMahon *et al.* 2000). This species does not use trunk trails as many of its oft-studied congeners, but instead employs a mixed strategy of diffuse foraging for scattered seed, where foragers leave the nest and look for seed alone, with occasional recruitment to dense seed patches (Hölldobler 1976, Traniello 1989, Johnson 2000).

In this study, I investigated the decision-making process of foragers and colonies in *P. californicus*, to **1)** assess how colonies integrate information about seeds (during foraging from the pile, or after seeds had been collected into the nest), and whether preferences are maintained long-term (**Chapter 2**). Next, I characterized **2)** forager preference diversity, flexibility, and how information is integrated at the individual level to affect colony-level changes (**Chapter 3**). Finally, I explored **3)** how seeds in the nest inform forager choices, by examining in-nest seed processing and its effect on collection behavior (**Chapter 4**). To address the questions presented above, I manipulated *P. californicus* colonies in a series of field and laboratory studies in which foraging choices and associated behaviors of individually marked workers and colonies were monitored over days. Three seed species were offered in binary choices between seed with similar or very different quality and caloric value. In field experiments, I offered colonies these binary seed choices to understand the development of seed preference in foragers and by colonies. I also tested forager flexibility by forcing foragers to switch between seed species. In laboratory experiments, I manipulated the availability of seeds in

observation nests and followed interactions between foragers and seeds, assessing how seed processing affected information integration. These combined experiments generate a more detailed and complete understanding of the mechanisms behind the coordination of individual choices, as workers individually and collectively make foraging decisions for the colony.

PREVIEW

CHAPTER 2

Colony learning in a complex environment: harvester ant colonies (*Pogonomyrmex californicus*) become more selective as they process information about seeds

ABSTRACT

Groups often make decisions as a collective, and in some cases may benefit from learning from previous choices. When groups learn, both individual cognitive processes and inter-individual interactions shape learning and memory. In social insects, inter-individual differences interact to create a colony division of labor, which can affect learning dynamics. In this study, we first explored whether field colonies of harvester ants learn to refine their seed preferences over multiple days of choosing between two types of novel seeds, and whether preferences are remembered. We found that colonies did increase their preference for higher-quality seeds across collection days in a manner consistent with learning, and most colonies retained learned preferences over a four-week span during which the seeds were absent. Preference changes due to familiarity with seeds, or improvement to nutritional diversity were ruled out by offering colonies different relative amounts of the seed types. Effects of satiety were ruled out because colonies generally did not decrease their collection rate as their preferences changed. Next, we further asked whether colony division of labor, specifically the foraging tasks' focus on seed collection and recruitment, affected learning. We found that, over 30 minutes of collection, colonies increased collection rate, but not preference for high quality seeds. Furthermore, colonies that were faster collectors were the least accurate at selecting the higher quality seeds. Together, the results suggest that within-nest information exchange shapes learning between collection days, while colonies

prioritize fast seed collection rather than seed processing and learning in the short term during active collection. Further, colonies retain information about seed quality across time spans from successive days to a month past last seed encounter. Despite no change in collection rate as colonies learned over days, colonies with a faster collection rate were less accurate during collection, suggesting that speed/accuracy tradeoffs may affect colony choices independent of learning. Most colonies retained learned preferences even when seeds were absent in their environment. Because active foragers tend to die at a regular rate, this group memory may last despite changes in forager group membership.

INTRODUCTION

Groups often make decisions as a collective, and can benefit from learning from previous choices (Franks & Sendova-Franks 2013, Kao *et al.* 2014, Biro *et al.* 2016, Sasaki & Pratt 2018). Group-level learning is governed by a mix of individual cognitive processes as well as social interactions (Fewell 2003, Kao *et al.* 2014). Social factors can affect the timescales over which group learning occurs; the speed of information flow, shaped by inter-individual differences in behavior and amplified through a shared environment, determines how quickly new information travels through groups (Waters & Fewell 2012, Pinter-Wollman *et al.* 2013, Franks & Sendova-Franks 2013, Kao *et al.* 2014, Sasaki & Pratt 2018). Furthermore, group memory can outlast the capacity of individual memory through direct or indirect information transfer that act as individual reminders, or act to pass information on through changes in membership (Johnson 1991, Weser 2005, Thornton & Clutton-Brock 2011, Biro *et al.* 2016, Sasaki & Biro 2017).

Because of the tight integration between individual and group-level goals, social insect colonies make for a useful system within which to explore group

learning. Colonies make decisions around a variety of objectives, and in some cases, learning has been found to affect decision-making. Learning behavior has been identified in situations where the group is tasked with choosing one option out of several, specifically when choosing a new nest site. *Temnothorax* ant colonies use learned attributes when choosing new nests, and learning can help expedite decision-making and relocation (Sasaki & Pratt 2018). Relocation speed is improved through streamlining interactions between ants performing different tasks (Franks & Sendova-Franks 2013). Learning is also critical in dynamic situations like foraging, where several options can be selected in parallel, and decisions are made continuously. During foraging, learning can increase the rate of acquisition of high-quality foods (Johnson 1991, Weser 2005). Learning can help colonies discriminate between food sources, for example, leafcutter ant colonies learn to reject food sources that negatively affect their edible fungus gardens (Herz *et al.* 2008, Saverschek *et al.* 2010). As colonies continuously respond to a changing resource environment, learning about the value of new food sources may take time, but allows colonies to make appropriate decisions as individuals gather, process, and exchange information about the environment (Detrain & Deneubourg 2008, Behmer 2009).

In this study we ask whether *Pogonomyrmex californicus* harvester ant colonies learn to distinguish between novel seeds, and what factors affect the learning process. This species is part of a genus of ants that interact with a seasonally changing seed landscape, with little known about the role learning plays in seed choice. Harvester ants collect a variety of plant parts and arthropods, but subsist mainly on seeds (MacMahon *et al.* 2000). The seeds available in their environment differ in nutritional content, and in physical and chemical defenses (Janzen 1969, Brown *et al.* 1979, Knoch *et al.* 1993, Lundgren 2009). Harvester ant

colonies are selective in their seed choices, strongly preferring some seed types over others and often ignoring abundant seed options (MacMahon *et al.* 2000). Colonies may further prefer seeds that improve the nutritional diversity of their diets (Fewell & Harrison 1991). Learning is known to improve seed recognition and collection speed in *P. rugosus* and *Veromessor pergandei* (Johnson 1991, Johnson *et al.* 1994, Weser 2005). However, learning to prefer one seed over another should further involve changes in preference that indicate improvement to colony assessment of seed quality. Learning to distinguish relative seed value would be useful during seed selection, because it would allow the workforce to focus on finding and exploiting the most profitable seed patches quickly.

Changes in preference is a difficult metric to judge learning by, as a food's value may depend on internal states (Houston & McNamara 1999). Specifically, evaluating whether an individual or a group learns about food can be confounded by level of satiation or nutritional needs. Hunger decreases with foraging, and has an effect on how selective animals are (Perry 1987, Hileman *et al.* 1994, Seeley 1997), and on what foods they select (Xuwang *et al.* 2011). Such response to hunger occurs at the group level as well. Satiated *P. californicus* colonies have slower collection rates than food deprived colonies, lowered selectivity for higher-calorie seeds, and a smaller niche breadth (Holder Bailey & Polis 1987). A response to immediate internal states, such as hunger or nutritional deficiencies, should be ruled out in order to support that a behavior is a result of learning; colonies should remember their learned preference for durations that are longer than the time scale of immediate changes in hunger or satiety. Learning would also be more likely to result in a colony "memory". That is, learned preferences could persist for periods that exceed the encounter rates with a particular food type in the environment.

In social insect colonies, including harvester ant colonies, inter-individual behavioral differences create a division of labor wherein individuals focus on a subset of discrete tasks (Beshers & Fewell 2001). The specific duties of the foraging task, as well as possible differences in information available about a seed type upon collection compared to after processing may impact learning dynamics. Ants participating in foraging are focused on seed collection, and interactions that regulate recruitment to the collection effort (Gordon *et al.* 2008, Pinter-Wollman *et al.* 2013, Tschinkel & Hanley 2017). Foragers typically collect seeds whole, and the edible portion is removed from the chaff inside the nest (Whitford 1978). Foragers make categorical decisions about whether or not to collect a seed as they are encountered in the environment, and in some cases, taste cues on the seed hull may provide immediate information about seed quality (Gordon 1980). If cues on the hull provide reliable information about seed quality, an individual's past experiences may inform their decisions across successive trips. However, learning to discriminate based on quality may be impeded by seeds' natural protective measures; a seed's hull may be an impediment to feeding and a barrier to nutritional information (Janzen 1969, Lundgren 2009). Potential toxins in the seed may require time to act, causing delayed learning (Rissing 1981, Knoch *et al.* 1993). If the seed's protective measures necessitate seed processing, the forager's focus on collection and recruitment may not leave them much time to gain an accurate understanding of seed quality as they collect, which may delay colony preference learning. The possibility of delayed preference learning is supported by studies which have found large increases in seed recognition between the first and second day a novel seed is offered, suggesting that extended time enhanced colony seed recognition (Johnson 1991, Johnson *et al.* 1994, Weser 2005).

Finally, colonies may also vary in speed/accuracy tradeoffs, independent of learning ability. For example, colonies of *Temnothorax* house-hunting ants modulate speed and accuracy based on relative importance during nest site choice (Franks *et al.* 2003). If harvester ant colonies vary in the accuracy with which they discriminate between seeds independently of their collection rate, this would suggest some colonies may be better learners than others. However, if colonies do not tend to change collection speed when they learn, yet high accuracy is correlated with a low collection rate, it is more likely that differences in accuracy between colonies are due to speed-accuracy tradeoffs rather than differences in learning ability.

To evaluate collective learning during seed foraging in harvester ants, we presented colonies of *P. californicus* with two novel seed types that differed in quality. These seeds had hulls that made information about seed quality potentially difficult to access immediately upon collection. We assessed colony learning across days of collection by following changes in preference for the seed types, and further tested whether colonies maintained a long-term memory for their preferences over a span of four weeks. To rule out effects of satiety or nutritional deficiency on preference that would confound the presence of learning, we manipulated the relative proportions of the two seed types available for collection, and looked for changes in collection rate over time. Next, we assessed whether learning happened during collection, and asked if colony differences in accuracy were more likely due to differences in learning ability, or speed-accuracy tradeoffs.

METHODS

Study site and seeds used

We studied colony learning of novel seeds in a population of *P. californicus* at the Coon Bluff Campground in Tonto National Forest (Mesa, AZ; 33°32'N, 111°38'W). Experiments took place between the months of July and October, 2014. Selected colonies were at least 50 m apart to ensure that experiments were performed on separate colonies, rather than on large colonies with multiple entrances. To control for the ants' experience with seeds, we provided seeds that do not occur in the ants' natural environment. To determine if preferences related to nutritional value of seeds, we used seed types that differed from one another in nutritional value. We presented colonies with niger (*Guizotia abyssinica*) and annual ryegrass (*Lolium multiflorum*) seeds. These two seed types have a similar wet mass (niger: 3.4 mg, ryegrass: 4.3 mg, average weight of 100 seeds) but they vary in caloric value, with niger having a higher lipid content and thus higher energy value (mean \pm SE: niger: 40.8% \pm 0.3% lipid or 1.39 mg/seed, ryegrass: 3.5% \pm 0.2% lipid, or 0.56 mg/seed. For lipid analysis, see Supplementary Material). Based on previous studies in which harvester ants rejected ryegrass (Rissing 1981), and given the caloric value of the two seed types, our *a priori* expectation was that niger would be a more preferred resource than ryegrass (Gordon 1980, Fewell & Harrison 1991, Crist & MacMahon 1992). Seeds of both species have a natural hull that needs to be removed to access the seed contents. This sets up the opportunity for time delayed learning, because the hull provides a barrier to evaluating seed contents upon collection. Seeds are hulled inside the nest, likely by workers other than foragers (Oettler & Johnson 2009).

General protocol

To test how experience influenced colony seed preference, we exposed each colony to the two seeds over a four-day period. We first familiarized each colony with approximately 400 seeds of each type for one day (Day 1) to ensure that colonies would recognize and collect the novel seeds (Johnson 1991), and to allow them to form an initial preference. The next day (Day 2), we assessed colony baseline seed preference and collection speed in a choice test, in which we presented colonies with equal proportions of niger and ryegrass *ad libitum* in a single pile and counted the number of seeds of each type removed over 30 minutes (detailed below). We next manipulated the colony experience with seed abundance and nutritional diversity by presenting colonies with niger and ryegrass seeds in different proportions immediately after the baseline test (Day 2), and on the next day (Day 3, detailed below). On the fourth day (Day 4), we assessed changes in collection behavior by conducting another “post-manipulation” choice test using equal proportions of *ad libitum* seeds. To test for long term memory, we assayed the seed preference of a subset of colonies 27-28 days (~ 1 month) after the post-manipulation test, again using equal proportions and *ad libitum* sampling, in a ‘memory test’ (Fig.1).

Fifteen colonies were tested in July. These colonies had previously been used during pilot studies in which they were exposed to niger and ryegrass, one month or more prior to the experiments described here. These are henceforth referred to as ‘summer colonies’. Eighteen additional colonies were tested in September and had never experienced the ryegrass or niger seeds previously. Fourteen of these colonies were tested again about four weeks later for the existence of long term memory. The 18 colonies first tested in September are henceforth referred to as ‘fall colonies’.

To test whether greater exposure to one seed type affected colony preference or collection rate, colonies were presented with different proportions of seeds on

Days 2 and 3. Colonies were given either a 3:1 ratio of ryegrass to niger ("More Ryegrass"), a 1:3 ratio of ryegrass to niger ("More Niger"), or equal amounts of both seeds ("Equal"). Colonies were given approximately 1,200 seeds per day over the two treatment days (for seed number approximation procedure, see Supplementary Material). We tested five colonies per treatment in the summer and an additional six colonies per treatment in the fall. Six of the 'More Niger', five 'More Ryegrass', and three 'Equal' treatment colonies were later used for the memory test. The other four colonies had either entered hibernation or relocated and could not be found when conducting the memory test.

We used the counts of seeds collected in choice tests to assess changes in preference, bias, and collection rate both during and across choice tests. Preference was measured as the ratio of ryegrass to niger (R:N) collected, while bias was measured as the ratio of the less preferred seed over the more preferred seed regardless of type (LP:MP). Collection rate was calculated as the number of seeds collected per minute (seeds/min). To assess whether colonies exhibited speed/accuracy tradeoffs we regressed seed bias against collection rate during post-manipulation tests. Bias was used for this assessment instead of preference because two colonies collected a higher proportion of ryegrass than niger. Accuracy in speed-accuracy tradeoffs is typically thought of as a difference from random choice, and in our case, random choice would be a 1:1 ratio of niger to ryegrass.

To allow colonies to find seeds and learn to handle them, seeds were presented in a single well-mixed pile located approximately 1m from the colony's nest entrance in the direction that most ants were foraging. To maintain a consistent mix of the two seeds while still providing *ad libitum* sampling, seed piles were monitored during choice tests, and piles were removed and replaced with fresh mixes of niger and ryegrass at regular intervals throughout the 30 minutes.

Seeds were placed in the same location every day, and were mixed immediately before being placed to mitigate seed odor cross-contamination. Tests were conducted during peak foraging activity in the mornings (7:00-11:00). Because this limited the number of colonies that could be tracked each day, only one replicate of each treatment was conducted per day. Of the seeds provided in differing proportions on Days 2 and 3, colonies typically collected all the provided seeds unless the colony was specifically rejecting one of the two seed types. Seed rejection happened rarely, and did not occur until the second presentation of the treatment pile (Day 3). Thus, all colonies experienced both seed types.

Colonies in this population occasionally relocated nests or opened new nest entrances. If a nest entrance moved overnight before a choice test, the seed pile was repositioned as needed. Fourteen of the 33 colonies in this study used a different entrance location during their baseline or post-manipulation test (compared to starting location). Four of these colonies were in the process of relocating to a new nest during the choice test. Eleven of 14 colonies moved their nest entrance between the post-manipulation test and the long-term memory test. We continued to use colonies only if the new nest entrance was within ~ 1 m of the old one. Because colonies still collected seeds while relocating, and still exhibited preferences for a particular seed type, they were included in the analysis. Seed preference and collection rates during nest relocation were within the range of values observed for colonies not relocating.

Video analysis procedure for seed preference, bias, and collection rate

We used 30-minute video recordings of forager behavior at the seed pile during choice tests to determine colony seed preference and collection rate on a particular day. The type of each seed collected, at the moment it was collected, was