

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

by

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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.

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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.

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

manually coded using the software CowLog (Version 3.0.2; Hänninen & Pastell 2009), which allows logging behaviors using keystrokes tied to video-based time-stamps. For most colonies, the 30-minute recordings started when the first forager arrived at the pile. For the colonies with very slow seed collection in the first 10 minutes (< 10 seeds collected), we discarded the first 10 minutes of the video and added 10 minutes to the recording. This ensured that enough collection events were recorded to accurately estimate seed preference. A visual check for whether both seeds types were within one body length of each forager arriving at the pile was conducted during video analysis to ensure that seed choices were only analyzed if foragers had an equal opportunity to evaluate both seed types. If this rule was not met, collection for that portion of the video was not coded. This procedure supplemented our replacement of the entire seed pile during collection tests whenever seed proportions became unequal. For collection rate analysis, we excluded periods when seeds were not available due to the pile being removed and replaced.

Data analysis

Data were analyzed using linear mixed-effects models (LMM). Preference (R:N ratio during choice test) or collection rate (seeds/min collected during choice test) were response variables. Choice test day (factor = Test Day: Day 2 baseline vs. Day 4 post-manipulation vs. Day 31-32 memory test) and seed manipulation (factor = Manipulation: More Niger, More Ryegrass, or Equal) were fixed effects. We included a random intercept for each colony, as there was a large amount of inter-colony variation in preference. An interaction term between seed manipulation and the choice test day was used to determine how exposure to varying seed proportions affected collection behavior. If there was no interaction effect, we removed it to

examine the effect of choice test day alone. To mitigate unequal variance between choice test days, ratio data were squared when comparing the behavior of summer colonies, and rate data were log transformed when comparing fall colonies.

The LMM's capture the general effect of the manipulations and experience with the seeds on seed collection, but we were also interested in the differences in individual colony responses. To characterize the diversity of responses to experience, we performed separate χ^2 tests on each colony that evaluated whether the proportion of seeds they collected changed between preference tests.

To evaluate changes in collection behavior during choice tests, we used a Paired T-test to determine whether colony preference (R:N) or collection rate (seed/min) changed between the first and last 10 minutes of the baseline and the post-manipulation choice tests. Colonies collecting fewer than 10 seeds during a 10-minute segment were excluded. When testing for speed-accuracy tradeoffs, colony bias and collection rate data were non-normally distributed, so a Spearman's correlation coefficient was calculated for the correlation between the level of bias (LP:MP) and the collection rate (seeds/min) exhibited during the Day 4 post-manipulation tests. A separate regression was conducted for summer and fall colonies.

All statistical tests, were conducted in R (R Core Team 2013). We used the *'lme4'* package (Bates *et al.* 2015) for LMMs, the *'car'* package (Fox & Weisberg 2011) for type-II analysis-of-variance, and the *'multcomp'* package (Hothorn *et al.* 2008) for post-hoc Tukey HSD tests. Assumptions of normality of the residuals or of data distributions were evaluated visually (*'car'* package), and with the Shapiro-Wilk test. Homoscedasticity of residuals was evaluated visually and with the Levene's test (*'car'* package).

RESULTS

Influence of experience and learning on collection behavior across days

In the summer trials, a total of 15 colonies were tested with equal proportions of niger and ryegrass on Day 2, followed by exposure to: equal proportions; 3:1 ryegrass:niger; or 1:3 ryegrass:niger during the manipulation phase of the experiment (Days 2 and 3), and then tested again with equal proportions of the two seed types on Day 4. Baseline (Day 2) and post-manipulation (Day 4) collections were compared for changes in preference (measured as R:N) and collection rate (seeds/min). We found that colonies showed a general increase in preference for niger over ryegrass with experience. Colony preference for ryegrass dropped from a mean of 0.77 (SE \pm 0.10) R:N on Day 2 to a mean of 0.57 (SE \pm 0.09) R:N on Day 4; LMM: Test Day $X_{21} = 7.17$, $P = 0.007$; Manipulation $X_{22} = 0.03$, $P = 0.60$; $N = 15$; Fig. 2A). This preference shift occurred independently of relative availability of either niger or ryegrass on Days 2-3, as there was no significant interaction between manipulation and choice test day ($X_{22} = 2.79$, $P = 0.25$, $N = 5$ colonies per manipulation).

Summer colonies had experienced at least one of the seed types several months prior in pilot studies, so the experiment was repeated in Fall for a set of 18 colonies with no previous experience with seeds. As before, colonies were subjected to choice tests using equal proportions of ryegrass and niger on Day 2 and 4, with exposure to either 1:1, 1:3, or 3:1 ryegrass:niger on Days 2 and 3. This set of colonies was additionally tested 27-28 days (Day 31-32) after the post-manipulation test to assess the length of colony memory. As with the summer data, there was a significant effect of choice test day on colony preference, with colonies again showing a proportional decrease in preference for ryegrass with experience (LMM: Test Day

$X_{2,2} = 18.88, P < 0.001$; Manipulation $X_{22} = 0.33, P = 0.85$; N for Day 2, 4, and 31-32 = 18, 18, 14, mean \pm SE Day 2 = 0.76 ± 0.10 R:N; mean \pm SE Day 4 = 0.36 ± 0.10 R:N, Tukey test: $|z| = 4.08, P = < 0.001$; Fig. 2B).

Colonies also maintained a lower preference for ryegrass in memory tests; a similar mean proportion of ryegrass was collected between post-manipulation tests on Day 4 and memory tests on Day 31-32 (Day 31-32 mean \pm SE of 0.40 ± 0.09 R:N, Tukey test: $|z| = 0.50, P = 0.85$; Fig. 2B). Most colonies continued to collect proportionally less ryegrass in memory tests than they did in baseline tests (Tukey test: $|z| = 3.19, P = 0.004$; Fig. 2B). As with summer colonies, there was no significant interaction between the proportion of niger vs. ryegrass given during the exposure manipulations and changes in preference among choice tests (LMM: $X_{24} = 1.98, P = 0.74, N = 6$ colonies per manipulation for baseline and post-manipulation tests, $N = 6, 5, 3$ colonies for the More Niger, More Ryegrass, and Equal ratio manipulations, respectively, for memory tests).

Although preference changes were observed between days of seed exposure, collection rate did not change over this time period. In summer, collection rate changed between baseline and post-manipulation tests from a mean of 40.01 (SE \pm 5.38) to 40.05 (SE \pm 8.16) seeds/min ($N = 14$ colonies; one colony was excluded as it had moved 15 cm closer the pile). This change was not significant (LMM: Test Day $X_{21} < 0.001, P = 0.996$; Manipulation $X_{21} = 0.21, P = 0.898$; Fig. 2C). Similarly, for fall colonies, collection rate changed from 25.96 (SE \pm 3.21) to 26.32 (SE \pm 3.36) seeds/min between baseline and post-manipulation ($N = 18$ colonies). However, mean collection rate dropped during the memory tests to 16.36 (SE \pm 1.36) seeds/min ($N = 14$ colonies). This led to an overall significant effect of experience on collection rate (LMM: Test Day $X_{22} = 11.12, P = 0.004$; Manipulation $X_{22} = 0.18, P = 0.92$; Fig. 2D). Specifically, the change between baseline and post-manipulation

tests was not significant (Tukey test: $|z| = 0.19$, $P = 0.98$; Fig. 2D). But collection rate was significantly slower 27-28 days later, both as compared to baseline tests (Tukey test: $|z| = 3.05$, $P = 0.006$; Fig. 2D) and post-manipulation tests (Tukey test: $|z| = 2.88$, $P = 0.01$; Fig. 2D).

Manipulating the relative proportion of ryegrass and niger on Days 2 and 3 had no discernable effect on collection rate, as there was no significant interaction effect of manipulation and choice test day. This was true both for colonies tested in the summer (LMM: $X_{22} = 1.93$, $P = 0.38$, More Niger & More Ryegrass $N = 5$, Equal $N = 4$; Fig. 2C) and fall (LMM: $X_{24} = 4.45$, $P = 0.35$, $N = 6$ colonies per manipulation for baseline and post-manipulation tests, for memory test $N = 6, 5, 3$ for More Niger, More Ryegrass, and Equal, respectively).

The general trend was to increase niger collection between baseline and post-manipulation tests, however, colonies differed in their individual behavior between choice tests. In summer tests, 10 colonies increased niger preference, one colony decreased niger preference and four kept the same preference between baseline and post-manipulation tests (X_2 test, $\alpha = 0.05$). In fall tests, 15 colonies increased niger preference, one decreased niger preference and two kept the same preference. Similarly, there was variation in the extent to which colonies changed by the fall memory test. Between baseline and memory tests, 11 colonies increased niger preference, two decreased niger preference, and one kept the same preference. Between post-manipulation and memory tests, five colonies kept the same preference, three colonies increased niger preference, and six decreased niger preference (X_2 test, $\alpha = 0.05$).

Changes in foraging rate and preference within choice tests

Colony seed collection rates increased over time within individual 30-minute choice tests, but colonies did not show shifts in preference toward niger within this time frame, despite interacting with, on average (\pm SD) 850 ± 552 seeds over this timeframe. There was a significant difference in average collection rate between the first and last 10 minutes of collection tests, both during baseline (Paired t-test: *Summer* $|t|_{13} = 5.86, P < 0.001$; *Fall* $|t|_{16} = 5.33, P < 0.001$; Fig. 3AB) and post-manipulation tests (Paired t-test: *Summer* $|t|_{13} = 3.78, P = 0.002$; *Fall*: $|t|_{16} = 5.72, P < 0.001$; Fig. 3AB). Summer colonies increased their collection rate from a mean (\pm SE) of 11.94 ± 1.25 to 61.33 ± 8.63 seeds/min during baseline tests, and from 12.16 ± 1.38 to 53.94 ± 11.72 seeds/min during post-manipulation tests ($N = 14$ colonies for both; Fig. 3AB). Fall colonies increased their collection rate from a mean (\pm SE) of 9.66 ± 0.68 to 38.39 ± 5.58 seeds/min during baseline tests, and from 10.74 ± 1.24 to 40.26 ± 5.76 seeds/min during post-manipulation tests ($N = 17$ colonies for both; Fig. 3AB).

While collection rate increased during all choice tests, preference during most choice tests did not change. We did not detect a significant change in average preference during either season's post-manipulation tests (Paired t-test: *Summer* $|t|_{13} = 0.04, P = 0.97$; *Fall* $|t|_{16} = 1.30, P = 0.21$; Fig. 3CD), or during Fall colony's baseline tests (Paired t-test: $|t|_{16} = 1.72, P = 0.10$; Fig. 3D). Summer colony preferences changed from a mean (\pm SE) of 0.38 ± 0.10 to 0.36 ± 0.10 R:N during post-manipulation tests ($N = 14$ colonies). Fall colonies preferences changed from a mean (\pm SE) of 0.84 ± 0.14 to 0.70 ± 0.11 R:N during baseline tests, and from 0.33 ± 0.10 to 0.39 ± 0.11 R:N during post-manipulation tests ($N = 17$ colonies for both). However, in summer, colonies did increase the proportion of ryegrass they collected during baseline tests. This behavior was the opposite of the preference change that

colonies exhibited across testing days. During summer baseline tests, colonies increased from a mean (\pm SE) of 0.56 ± 0.12 to 0.81 ± 0.11 ryegrass per niger collected (Paired t-test: $|t|_{13} = 2.2$, $P = 0.045$, $N = 14$ colonies; Fig. 3A).

Relationship between collection rate and bias (speed/accuracy tradeoff)

Colonies that were more accurate during post-manipulation choice tests tended to be slower in collection rates. The correlation between seed bias ratio (number collected of least preferred (LP) to most preferred (MP) seed) and collection rate during post-manipulation tests was significant for both the colonies tested in summer (Spearman's rho: $r_s = 0.6$, $S = 182$, $P < 0.01$, $N = 15$), and the fall (Spearman's rho: $r_s = 0.7$, $S = 256$, $P = 0.001$, $N = 18$; Fig. 4).

DISCUSSION

Our results show that harvester ant colonies learn to discriminate between novel seeds across successive collection days, and maintain these learned associations for at least one month during which seeds are unavailable for collection. This process involves iterative binary decisions of which seed to choose by foragers at the seed pile, but results in a collective decision that is proportional and fairly consistent across colonies. Thus, colony learning integrates from multiple individual to collective decisions. The process of learning likely does not take place immediately at the seed pile, given that proportional seed selection did not change across the time span of a single (30-minute) exposure to the seed pile. Instead, colonies changed foraging proportion across days, suggesting that learning is shaped by the behavioral interactions within the nest. Specifically, as foragers focus on collection and recruitment, learning does not occur. But across days, as the colony has a

chance to process and distribute newly collected seeds, foragers receive new information that affects choices on subsequent days.

Colony learning was robust to differences in the relative amount of each seed provided, and collection rate did not decrease over days when seeds were continuously provided. This suggests that colony assessment of quality, rather than quantity, nutritional diversity, or caloric satiety, was the principle driver of collection decisions in the context of this experiment. However, it is notable that all colonies collected a significant number of both seed types, even after shifts in preference had occurred. This suggests that maintaining nutritional diversity may still play some role in foraging preferences and colony decisions.

Our findings support a previous study that found a colony of *P. rugosus* lowered its preference for ryegrass compared to two other seeds between the morning and afternoon of the 3rd day of collection, maintaining this lowered preference for at least nine days; meanwhile, ryegrass was brought out of the colony and discarded (Rissing 1981). Similarly, *P. rugosus* and *Veromessor pergandei* colonies have been found to learn to handle seeds more efficiently over days of collection; colonies have very slow collection on the first day a novel seed is offered, with handling time and seed collection rate increasing greatly over the next several days of collection (Johnson 1991). Leafcutter ant colonies have also been shown to exhibit learning behavior and delay in leaf avoidance behavior on the timescale of days (Saverschek *et al.* 2010). Leafcutter ants collect leaves for their fungus garden, and show delayed avoidance (collecting leaves one day then rejecting them the next) of leaves toxic to their symbiotic fungus (Herz *et al.* 2008). Our findings suggest that colony-level learning may be generally present in ants in the context of foraging.

Experience in the nest is more important for learning than collection

We offered colonies two seed types that differed in energy content, and we predicted a preference for the higher calorie seed. However, colonies did not initially display a strong preference based on caloric value; instead they significantly increased their proportional collection of the higher-calorie seed over days. This timeframe suggests that information exchange in the nest is important to foraging, likely as seeds are processed and seed contents exposed (Chapter 4).

During choice tests, collection rate increased, suggesting new foragers were stimulated and perhaps recruited to come and forage at the pile (Gordon *et al.* 2008, Hölldobler 1976). However, preference for niger did not increase within this timeframe, suggesting that: 1) new foragers coming to the pile were not more informed than previous foragers about the differences between the seed types, and 2) current foragers did not re-assess seed quality in the timeframe of a single collection event or over a session of foraging within a day. Similarly, previous studies in *P. rugosus* and *V. pergandei* found that colonies did not reach their full collection efficiency during the first day interacting with novel seeds, instead showing dramatic increases in handling efficiency and seed recognition, and in the number of foragers that came to the pile, by the second day of collection (Johnson 1991).

Our results suggest that foragers gain information when they spend time in the nest, during times when they are not collecting – perhaps overnight. Rissing (1981) found that a colony of *P. rugosus* took several days of collection to reject ryegrass seeds as well. Both Johnson (1991) and Rissing (1981) suggested that forager interactions with seeds in the nest facilitated this change. It is likely that workers other than the foragers that initially collected the seeds are involved in processing (McCook, 1879; Chapter 4). Thus, there is a possibility that interactions between foragers and workers that focus on processing could be important in colony

learning as well. In *Temnothorax*, colony emigration to a new nest site becomes more efficient with experience, due to behavioral changes in the workers who transport items to the new site, and their interactions with the nurse workers who take care of brood. Experienced transporters move more items, more quickly, and nurses give up brood to transporters more quickly with multiple emigrations (Franks & Sendova-Franks 2013).

The ecology of seed learning

A variety of seed-related factors could have contributed to seed learning in our study. Seeds are under ecological pressure to evolve ways to hide from or repulse predators (Janzen 1969, Crawley 1992) – especially in deserts, where plants are less constrained by space, and depend more on seed survival until adequate rain has fallen (Brown & Heske 1990, Crawley 1992). Defenses such as containing toxins, morphology that makes seeds hard to carry, or producing sticky outer layers when wet are known to help seeds repulse, deter, or hide from ants (Carroll & Janzen 1973, Fuller & Hay 1983, Knoch *et al.* 1993, Ready & Vinson 1995, Lundgren 2009).

The seed coat provides an additional barrier, and may mask scents from the endosperm that would inform about seed quality (Borges 2015). Studies of the natural seed preferences of *Solenopsis* fire ants found that some seeds were more readily collected when their seed coats were removed (Ready & Vinson 1995). In our study, it may be that the removal of the seed coat was necessary to inform ants of the nutrients inside niger and ryegrass seeds, and it suggest that over time ants can perform associative learning between the seed contents and the seed coat. Ryegrass as well as the related grass Tall Fescue is also known to become infected with an endophytic fungus (Australian Office of the Gene Technology Regulator 2017). Tall Fescue seeds infected with entophytic fungi deterred seed collection in comparison to

non-infected seeds in *P. rugosus*, though not *P. occidentalis* (Knoch *et al.* 1993). Ryegrass was also rejected by a *P. rugosus* colony (Rissing 1981). Therefore, the learning processes we captured may have included both delayed learning due to the seed coat and availability of toxin information pre-consumption.

Learning and long-term memory for seeds

Colonies also showed long-term memories for their learned seed preferences that lasted at least a month past exposure. Long-term memories could theoretically persist both in individual memories or through social mechanisms, with both likely contributing to colony memory. Studies in nectivorous and insectivorous ants have found that workers form long-term memories for novel odors and can maintain them for the rest of their lives (Josens *et al.* 2009, Huber & Knaden 2018). For *Pogonomyrmex* foragers, there may be a benefit to recognizing seeds long-term as seeds can persist without germinating for a long time in deserts due to infrequent rains (Brown *et al.* 1979, Crawley 1992). Foragers and in-nest workers could also continue interacting with surplus seeds that are stored in granaries. In leafcutter ants, naïve foragers can learn novel leaf odors and their effects on the fungus from interacting with the fungus or trash piles (Weser 2005, Arenas & Roces 2018). In colonies of *P. rugosus*, and *V. pergandei*, fewer foragers recognize a novel seed as time passes without those seeds in the environment, but *P. rugosus* recognize seed for longer if they are given a larger amount of seed to collect, with some colony memories lasting at least 260 days (Johnson *et al.* 1994, Weser 2005).

As harvester ants display age-driven division of labor, foragers are some of the oldest ants, and *P. rugosus* foragers are predicted to live for only about 30-35 days once they start foraging (Gordon & Hölldobler 1987, Oettler & Johnson 2009) – much less than the timespan that colony seed recognition was found to last in this

species (Johnson *et al.* 1994, Weser 2005). Forager lifespans have been estimated for *P. salinus* (up to 20 days, Porter & Jorgensen 1981), *P. barbatus* (up to 30 days, Gordon & Hölldobler 1987), and *P. badius* (at least 48-35 days Kwapich & Tschinkel 2013). Though we do not know how long *P. californicus* workers live once they start foraging, we know *P. californicus* colonies lose foragers over time (De Vita 1979) and likely only some of the original foragers remained during our memory tests. New foragers could have been in-nest workers that had interacted with the seeds, preserving colony memory despite the change in forager group membership (Biro *et al.* 2016). Colonies also exhibited variation in whether they maintained the same preference, or increased or decreased niger preference between the post-manipulation and memory test. This can be interpreted as some colonies continuing to learn while others started to forget. This suggests that stored food and mortality rate could affect the dynamics of long-term colony memory in harvester ants.

Seed preferences were independent of relative quantity or satiety

Colonies did not show signs of becoming satiated (i.e. lowered collection rates) during the time seeds were offered, and so internal state changes due to satiety are not likely to be the cause of the observed preference changes. Previous studies on *P. californicus* found that satiated colonies had a lower foraging rate than food restricted colonies but were slightly more likely to collect lower calorie seeds at further distances from the nest, suggesting that satiated colonies can be less selective (Holder Bailey & Polis 1987). Similarly, a study in *Messor barbarus* harvester ants found that colonies became both slower and less biased as their seed stores filled up (Reyes-Lopez & Fernandez-Haeger 2002). Experience with seeds over four days of collection lead colonies to become more biased toward higher-calorie seeds without an effect on collection rate, suggesting that we did not offer enough

seeds for colonies to become satiated. Furthermore, colonies fed more of the high-calorie seed did not have a lower collection rate or different preference than those fed equal amounts or more of the low-calorie seed. Colonies did lower their collection rate in the four weeks without seeds, perhaps because of lower temperatures, which affect collection rate (Johnson 1991). However, this lowered collection rate did not correspond with an average change in preference. A change in preference would be expected if end-of-season satiety affected seed choices in our study.

The indifference to the relative amount of niger and ryegrass collected also suggests colonies were not selecting seeds based on how they would add to the nutritional diversity of their seed cache. This result differs from previous studies; *P. occidentalis* colonies were found to prefer novel seeds that increased cache nutrient diversity (Fewell & Harrison 1991), and *P. badius* grew to dislike seeds collected in large amounts (Kwapich & Tschinkel 2013). If ryegrass harbors toxic compounds, toxin avoidance could be more important than maintaining nutritional diversity. On the other hand, very few colonies rejected ryegrass outright; despite a clear preference for niger, most colonies collected some ryegrass as well.

Field studies of harvester ants collecting native seeds have found that colonies typically collect a few seed types in large amounts, and many other seed types in small quantities (Pirk & Lopez de Casenave 2011, Briggs & Redak 2016), generally selecting a diverse array of available seed types in from their environment (Tevis 1958, Crist & MacMahon 1992, Detrain & Pasteels 2000, Pirk & Lopez de Casenave 2006, Schmasow & Robertson 2016). Harvester ants have also been shown to maintain a diverse seed intake, even when the relative proportions of seed types in the diet fluctuates (Pirk *et al.* 2009). Our colonies could have perhaps been maintaining diversity by collecting both types of seeds.

Slower colonies were more precise in their collection

Colonies that were least biased tended to collect the fastest, while the most biased colonies were more likely to be slow collectors. Variation in collection behavior among colonies itself is not surprising, as social insect colonies often vary in performance of various collective behaviors (Jandt *et al.* 2014, Marting *et al.* 2018), and harvester ants specifically have been shown to vary in traits like foraging rate (Gordon *et al.* 2013), response to high temperature and humidity (Gordon 2013), and responses to stimuli like novel food, debris, or intruders (Pinter-Wollman *et al.* 2012). Some variation may be due to external factors, and some to genetics. Differences in colony genetic diversity affect the length of time *P. occidentalis* foragers spend foraging (Cole *et al.* 2010). Moreover, inter-colony variation in foraging behavior has been linked to differences in fitness in both honeybees and harvester ants (Wray *et al.* 2011, Gordon 2013). However, the fact that variation in foraging rate was correlated with preference is interesting and merits further study. Colonies could be displaying different behavioral strategies, as individual honeybees, and colonies of house hunting ants have been found to do (Chittka *et al.* 2003, Franks *et al.* 2003, Burns & Dyer 2006).

Conclusion

Harvester ant seed choice is a group learning behavior involving delayed learning and long term memory. The timescale over which colony learning takes place is affected by social processes; division of labor may slow learning, but the shared nest may act to maintain colony memory even as the membership of the foraging force changed (Biro *et al.* 2016). Harvester ants give us novel insights into collective decision-making, and the capabilities of collective intelligence and problem-solving.

FIGURES

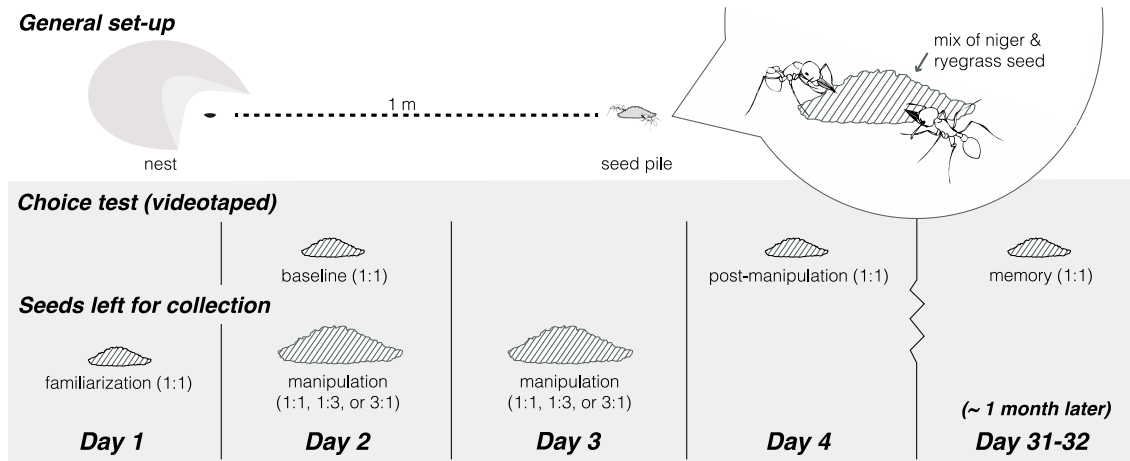


Figure 1. Diagram of experimental set-up in the field. Colonies were given a mix of two seeds (niger and ryegrass) for four days in piles placed approximately 1 m from the nest. A baseline test established their initial preference and collection rate, and a post-manipulation test evaluated changes to collection behavior. A subset of colonies were given a memory test approximately four weeks after the post-manipulation test. During Days 2 and 3, piles contained varying proportions of the two seeds (ratios are in parentheses), to determine how the proportion of seeds collected affected colony learning and preference.

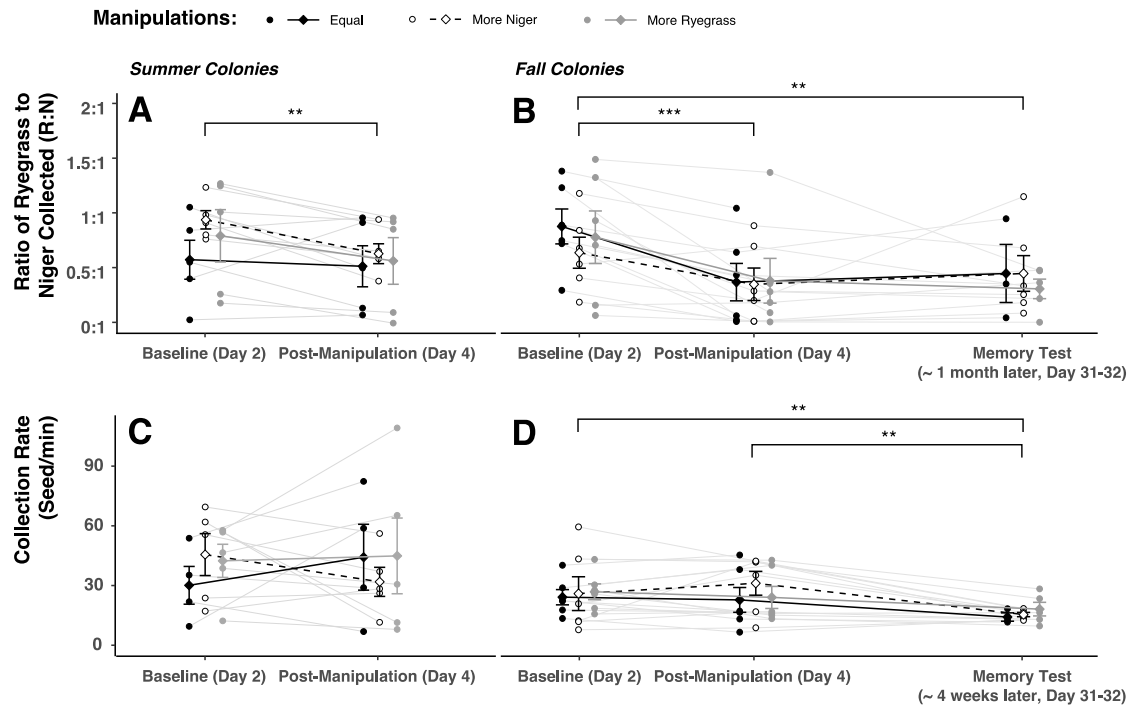


Figure 2. Effect of experience on seed preference of colonies across days. Circles represent individual colonies; diamonds represent manipulation means with bars as SE. Thick lines connect means of each treatment across days, thin lines connect individual colonies across days. Points are displaced along the x axis by manipulation to improve visibility. Colonies tested in the summer had been previously exposed to the two seed types 1-2 months prior, while fall colonies had never experienced the seeds before. (A) Summer colonies changed the ratio of R:N collected between baseline collection at Day 2 to Day 4 after daily exposure, regardless of the relative amounts of seeds given (see: manipulations). (B) Fall colonies similarly changed the ratio of R:N collected with daily exposure between baseline collection at Day 2 to Day 4, and maintained a lowered R:N ratio when tested during memory tests four weeks later. Manipulating the relative proportions of seeds colonies were exposed to in the fall similarly did not affect preference. (C) The collection rate of summer colonies did not change and was not affected by manipulation. (D) Fall colony collection rate did

not change between Day 2 and 4, but was lower one month later during memory tests. Rate was not affected by manipulation. Ratios and rates are based on seeds collected in 30 min while choosing from a pile with both seeds present *ad libitum*. For summer colonies, average (\pm SE) preference and collection rate was 0.77 ± 0.10 and 0.57 ± 0.09 ryegrass for every niger, and 40.01 ± 5.38 and 40.05 ± 8.16 seeds per minute, on Days 2, 4, respectively. For fall, Average (\pm SE) preference and collection rate was 0.76 ± 0.10 , 0.36 ± 0.10 , and 0.40 ± 0.09 ryegrass for every niger, and 25.96 ± 3.21 , 26.32 ± 3.36 , and 16.36 ± 1.36 seeds per minute on Days 2, 4, and 31-32, respectively. Colonies were exposed to two seed types in different proportions on Days 2 and 3: exposure to equal R:N (black fill, solid line); lower R:N (white fill, dashed line); higher R:N (grey fill, solid line). Asterisk represent significant differences in means between days; ** = $P < 0.01$, *** = $P < 0.001$.

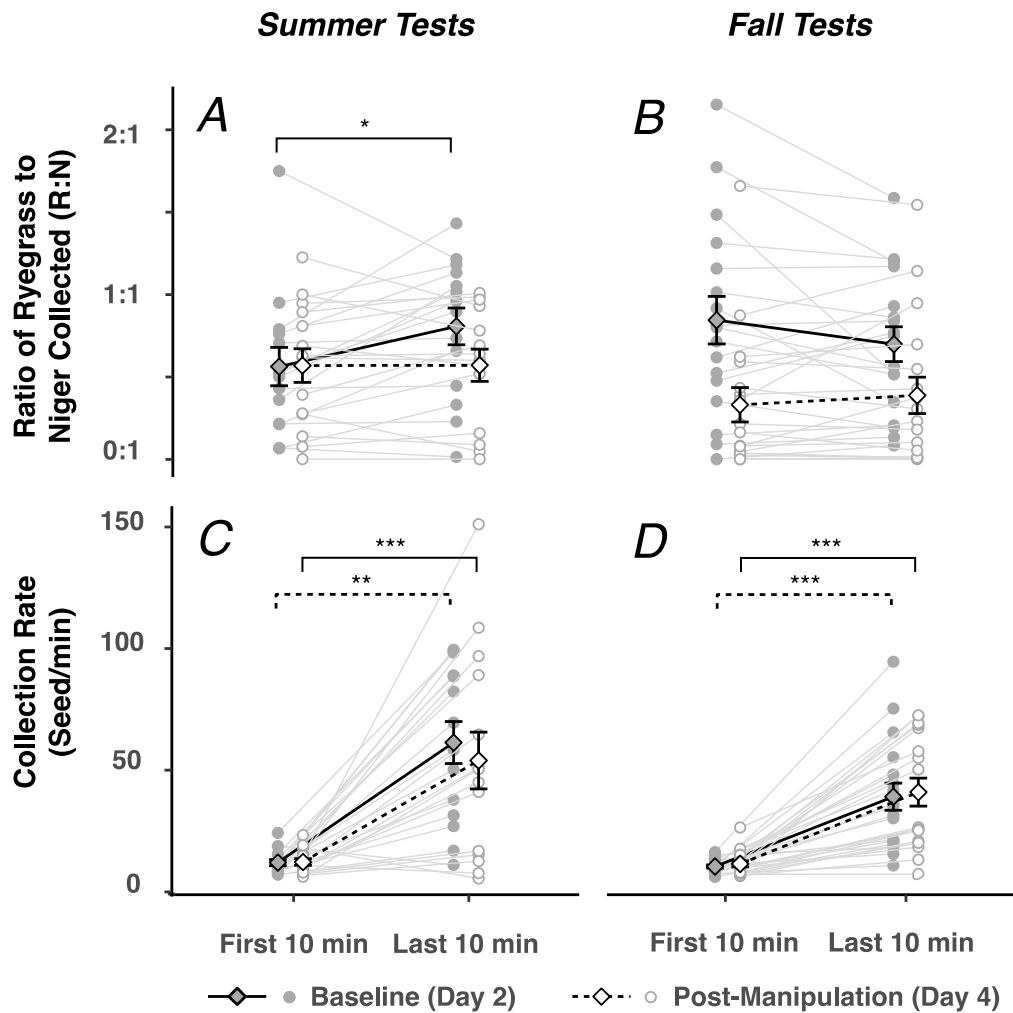


Figure 3. Effect of experience on behavior of colonies during 30 minutes. Circles represents a colony's behavior during 10 minutes, diamonds represent means, and bars are the SE. Thick lines connect means across the first and last 10 minutes of each 30-min choice test, thin lines connect colonies across these time points. Fill and line type indicate whether data is from baseline (grey fill, solid line) or post-manipulation (white fill, dashed line) tests. Points are displaced along the x axis by choice test to improve visibility. (A) Summer colonies significantly increase the average ratio of R:N they collected between the first and last 10 minutes of the baseline choice test, but not the post-manipulation choice test. (B) Fall colonies did

not significantly change their average ratio of R:N over either baseline or post-manipulation choice tests. (C+D) Colonies significantly increased their average seeds collected/min during all choice tests in both seasons. Colonies tested in the summer had been previously exposed to the two seed types 1-2 months prior, while fall colonies had never experienced the seeds before. Asterisk represent significant differences in means; * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

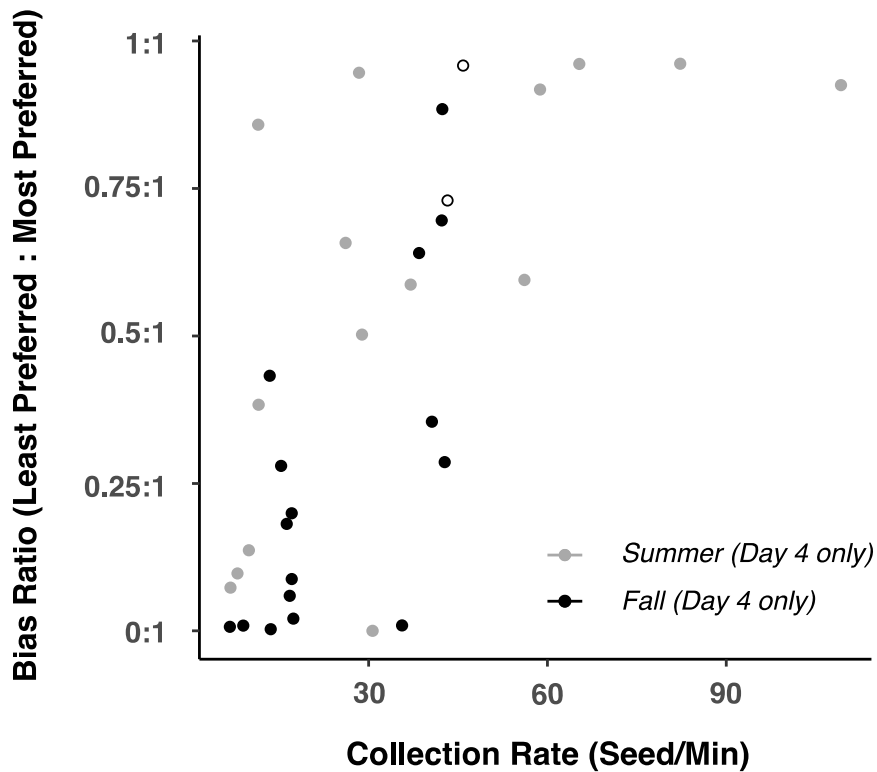


Figure 4. Relationship between rate and bias. Circles represents colony behavior during the 30-minute post-manipulation choice test on Day 4. Fill indicates whether colonies collected a higher proportion of niger (solid circle) or ryegrass (open circle), color indicates whether colonies were from the experiment conducted in summer (grey), or fall (black). Colonies that had a slower collection rate tended to show a stronger bias for niger during post-manipulation choice tests (Day 4 of experiencing seeds).

CHAPTER 3

Individuals with diverse and flexible biases shape collective decision-making in seed harvester ants (*Pogonomyrmex californicus*)

ABSTRACT

Collectives benefit from behavioral diversity, and collective wisdom benefits from a diversity of opinion and inference strategy. Collective wisdom also benefits from error reduction, which can lead individual decisions to become more similar to each other over time, while improving collective estimates. The interaction between individual diversity and choice optimization provides an important context for understanding the dynamics of collective decision-making. In this study, we examined individual seed type preferences of harvester ant foragers, and asked how they change as colonies gain information from repeated days of decision-making during foraging, and how this translates to colony-level change. We presented colonies with two scenarios using paired seed types. In one pairing, one seed was a nutritionally better choice, and in the other pairing, both seeds were more similar in quality. We show that, in both scenarios, colonies chose a consistent ratio of seed types that tracked seed quality differences, while foragers maintained diversity in which seed they preferred. Not all, but most foragers who initially preferred the poor-quality seed could integrate information from past colony choices to collect a higher proportion of better seed types in future choices, changing the proportion of high quality seeds collected by the colony. Foragers collecting from similar quality seeds maintained preference diversity over a week of collection, even changing their preference daily, despite little change in overall colony preference. When collecting from similar-quality seeds, all but the most biased foragers were able to switch to collecting their less-preferred seeds when preferred seeds disappeared, without

suffering a loss of collection rate. This study highlights how a diversity of individual preferences and flexibility can be maintained and integrated into collective learning and relative consistency at the collective level.

INTRODUCTION

Behavioral diversity among individuals is a common feature of collective decision-making, and promoting individual diversity is often in the best interest of the collective (Surowiecki 2004, Sumpter 2006, Oldroyd & Fewell 2007, Sasaki & Pratt 2018). However, in successive rounds of collective decision-making, individuals have the opportunity to optimize personal choice by integrating new information from the outcome past decisions, and reducing individual error (King *et al.* 2012, Sasaki & Pratt 2013, Luo *et al.* 2018).

The interaction between individual diversity and choice optimization provides an important context for understanding the dynamics of collective decision-making. Individual diversity may persist despite new information because of the difficulty or unpredictability of the problem, or limitations on cognitive ability (Surowiecki 2004, Sasaki & Pratt 2018). Physiological differences such as sensitivity to certain stimuli, cognitive processing differences, and even morphological differences can all affect an individual's final choice (Beshers & Fewell 2001, Jandt *et al.* 2014, Chittka *et al.* 2003). If the optimal answer can only be estimated, groups benefit from diverse inference strategies as they can counteract errors inherent to particular strategies (Hong & Page 2004, Krause *et al.* 2011, Fujisaki *et al.* 2018). Finally, diversity may be the optimal approach at the collective level, as it helps groups respond to a wider range of environmental conditions, and can promote resiliency and homeostasis in a changing environment (Oldroyd & Fewell 2007, Cole *et al.* 2010, Cote *et al.* 2011).

In this study, we examine individual behavioral changes in social insect colonies as they integrate new information from repeated interactions with the environment. Specifically, we examine how new information affects individual choices, and how this affects colony learning and the distribution of diverse preferences at the colony-level. Social insect colonies are known to change their behavior as they learn from repeated collective decisions (Franks & Sendova-Franks 2013, Sasaki *et al.* 2013, Chapter 2). Colonies also employ individual heterogeneity to their advantage during collective decision-making (Jandt *et al.* 2014). For example, when choosing a new nest site, individuals rely on a variety of heuristics when evaluating a site, and usually assess quality without comparing among sites (Mallon *et al.* 2001, Seeley 2003, Robinson *et al.* 2009, O'Shea-Wheller *et al.* 2017). In this way, colonies evaluate multiple sites using many independent estimates of quality, without individuals experiencing cognitive overload (Sasaki & Pratt 2018). This method also allows colonies to make fine discriminations between nests, and prevent errors from biased inference strategies (Sasaki & Pratt 2011, 2012).

When reaching a colony-wide consensus is not necessary, such as during foraging, individual behavioral diversity allows colonies to efficiently divide workers among resources and react to resource changes. Individual bees are known to vary in their choice of foraging location and macronutrients, and even focus on different flowers within a patch (Seeley 1986, Fewell & Page 1993, Chittka *et al.* 1999). When choosing between flowers, some bees are more accurate, while others favor speed over accuracy (Chittka *et al.* 2003, Burns & Dyer 2006). Behavioral diversity can help colonies maintain a high rate of food intake as resource availability changes (Seeley 1986, Burns & Dyer 2006).

Here, we focus on changes in individual and collective behavior during seed choice in *Pogonomyrmex californicus* harvester ants. Colonies are selective in their

seed choices, strongly preferring some seed types over other abundant options (MacMahon *et al.* 2000). Harvester ant colonies integrate information from past seed choices to make better future choices, and their foragers show individual variability in seed preferences (Nickle & Neal 1972, Rissing 1981, Crist & MacMahon 1991, Fewell & Harrison 1991, Chapter 2). As colonies collect seeds into the nest, this shared resource provides information about past choices and can inform future ones (Weser 2005, Fewell & Harrison 1991). Foragers have the opportunity to receive updated information on seed quality and/or colony seed preferences between days of foraging (Johnson 1991, Chapter 2), and colonies learn over time to reject poor-quality seeds (*P. rugosus* and *P. californicus*: Rissing 1981, Chapter 2).

There is some indication that colony-level learning is at least partially driven by individuals changing their preference for poor-quality seeds over time (*P. rugosus* Rissing 1981). Foragers of *P. occidentalis* also were found to show less variation when selecting from seeds that differed more in caloric value, suggesting that individuals do attempt to optimize their choices based on nutritional information (Fewell & Harrison 1991). Thus, colony learning in *P. californicus* may be driven by individuals gaining additional information from past colony collection experiences, and may lead to individuals becoming more similar to each other as they come to better discriminate between good and poor quality seeds. Colony change could also be organized through individuals who had collected poor quality seeds abandoning the seed patch. Honeybees abandon poor nectar patches, and bees as well as ants abandon poorer quality nests when choosing a new nest site (Seeley *et al.* 1991, Seeley 2003, Robinson *et al.* 2009). Large-scale studies on how colony change in seed preferences are coordinated at the individual level have yet to be conducted. Indeed, the question of how individual diversity integrates into consistency in group level decisions remains a poorly understood component of collective decision making.

Harvester ant foragers also show individual diversity of preferences when collecting from environments containing a variety of high-quality seed types. In some species, foragers were found to specialize on a single seed type, even when given equal access to different high-quality options (*P. badius* and *P. rugosus*: Nickle & Neal 1972, Rissing 1981). In one colony of *P. rugosus*, foragers became more specialized over multiple days of collection, suggesting that increased experience may promote preference diversity in some species (Rissing 1991). Foragers of *P. occidentalis* also hold a diversity of preferences, and vary in their degree of specialization (Crist & MacMahon 1991, Fewell & Harrison 1991). It is unknown whether *P. californicus* specialize or show preference diversity, and how individuals integrate information from long-term collection. Furthermore, individual preferences may vary in flexibility, from choosing randomly to high selectivity. If a forager spends too long looking for a preferred seed type when other good quality seeds are available, this may unnecessarily slow their foraging, especially as foragers typically return to their nest with one seed at a time. If forager preferences are inflexible, foragers that slow collection when preferred seed disappears may be counteracted by foragers increasing collection when their preferred seed is more available. Foragers may also flexibly adjust their collection to select less-preferred seeds.

To evaluate forager preference, flexibility, and how information is integrated at the individual level to affect colony-level changes in preference and diversity, we presented colonies of *P. californicus* with novel seeds of varying quality over several days. We first asked how individual foragers behave in colonies that are learning to discriminate between two seed types with large differences in caloric value (described in Chapter 2), compared to colonies collecting from two seed types with small differences in caloric quality. For both scenarios, we tested whether an individual's initial preference affected the degree to which they changed preference

(suggesting they incorporated information from earlier collection) or propensity to abandon the seed pile. We also tested whether individual preferences converged on the mean colony preference between days of collection. We further explored whether individual variability in preference in the similar-quality seed scenario disappears with prolonged colony collection. Finally, to assess preference flexibility, we tested how foragers' preferences affected seed selection rate when only preferred, or non-preferred seeds were available.

METHODS

Study site and seeds used

We measured changes in individual and colony behaviors during successive days of collective decision-making in a population of *P. californicus*, sampled at two sites in the Tonto National Forest (Mesa, AZ). One site was Coon Bluff Campground (33°32'N, 111°38'W), the other was 2.3 km away at the end of Usury Pass Rd (33°55'N, 111°62'W). Ants in both areas were located in similar environments; mesquite bosques near the Salt River (AZ). Experiments took place over three years during the months of May (2017), July-August (2016), October (2015, 2016, 2017), up to November 1 (2016). Selected colonies were actively foraging at the time of experiments, and readily collected seeds 1 m away from their nest. Colonies were at least 50 m apart from one another, and had never been used in a previous study in any year. This was to ensure that experiments were performed on separate colonies rather than on large individual colonies with multiple entrances, and that colonies were naïve to the offered seeds.

To control the ants' experience with seeds, we presented colonies with three seed species that are not present in the ants' natural environment: niger (*Guizotia*

abyssinica), annual ryegrass (*Lolium multiflorum*), and sesame (*Sesamum indicum*). The three species have a similar wet mass (niger: 3.4 mg, sesame: 3.1 mg, ryegrass: 4.3 mg, average weight of 100 seeds). Niger and sesame are the most similar to each other in lipid content (mean \pm SD: 41% \pm 0.3% or 1.39 mg/seed, and 50% \pm 0.1% or 1.55 mg/seed lipid content, respectively), and have a much higher lipid content than ryegrass (3% \pm 0.2% SD, or 0.56 mg/seed: for lipid analysis, see Supplementary Material); thus sesame has a slightly higher caloric value than niger, and both have a much higher caloric value than ryegrass (Suttie 1977). Ryegrass seed may also harbor toxic endophytes (Australian Office of the Gene Technology Regulator 2017). We presented colonies with pairs of seeds, either niger and ryegrass, or niger and sesame.

Harvester ants tend to prefer seeds with high caloric value (Fewell & Harrison 1991, Crist & MacMahon 1992, Gordon 1980), and previous studies have shown that colonies given niger and ryegrass shift their collection behavior over days toward a higher collection of niger (up to a mean (\pm SE) of 78% \pm 5% by the fourth day of collection, Chapter 2). Previous work in a related species (*P. rugosus*) found that colonies reject ryegrass after a period of collection (Rissing 1981). For choice tests between niger and sesame, our expectation was that collection of sesame and niger should be approximately equal or slightly biased to sesame if seed caloric content is the most important nutritional feature.

General methods

First, we explored how changes in colony preference were coordinated at the individual level, and whether large differences in caloric value between seeds (niger and ryegrass) led to better individual discrimination between seed value than small differences (niger and sesame), and a narrower distribution of individual bias. For

this comparison, we presented seven colonies with piles of mixed niger and ryegrass, and seven with piles of niger and sesame, and recorded changes in colony and individual behavior over repeated days of exposure. We first familiarized each colony by providing mixed piles with ~200 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. For the next two days (Day 2, Day 3), we assessed colony and individual seed preferences from a “choice test”, in which colonies were presented equal mixes of their assigned seed types *ad libitum*. Choice tests were video-recorded and lasted 30 minutes.

Individual foragers were uniquely marked using oil-based paint markers before recording on Day 2. Foragers were marked as they chose seeds from the pile; 15 foragers were selected while carrying niger, and 15 while carrying ryegrass or sesame, to ensure a diverse mix of personal preferences. Marking lasted ~30-70 minutes. To assure consistency between time points, and to give marked ants time to find the pile, on Day 3, ants were given time to forager freely on their assigned seeds for the same amount of time as marking had taken place the previous day. The Day 3 choice test began immediately after this free foraging period (Fig. 1B).

To explore how individual preference diversity changed over repeated days of experience with seeds, we presented four colonies with an equal mix of niger and sesame over six days, and periodically sampled colony and individual forager preference. We again allowed colonies to familiarize themselves with ~200 seeds of each type for one day (Day 1). Colonies collected all the seeds provided during the familiarization day (Day 1). Thus, all colonies experienced both seed types (Fig. 1A). We then assessed individual and colony preference in choice tests (same as described above) on Days 2,3,5 and 6. Before the Day 2 and Day 5 choice tests, foragers were again marked as they chose seeds from the pile. This time, 10

foragers collecting niger and 10 collecting sesame were marked on each day. Marking took a similar amount of time on Days 2 and 5 (~ 20-50 min depending on the colony). On Days 3 and 6, colonies were again allowed to freely forage on niger and sesame for the same amount of time as marking had taken place on Day 2. Choice tests were not recorded on Day 4, and no seed piles were given on this day (Fig. 1C).

Finally, we assessed forager flexibility in a changing resource environment. Using the four colonies that had been trained on sesame and niger over six days, we performed a “flexibility test” on a subsequent day of testing (Day 7 or 8). For this, colonies were first allowed to freely forage on niger and sesame for the same amount of time as marking had taken place on Day 2. Next, individual preference was assessed in a 30-minute choice test (as described above), while colonies collected *ad libitum* from an equal mix of niger and sesame. Finally, colonies were given either only niger, then only sesame, or *vice versa* for 20 minutes each (2 colonies per treatment order), and individual foraging decisions recorded. The full 70-minute flexibility test was video-recorded (Fig. 1D).

We used the counts of seeds collected during choice tests by marked foragers and the colony as a whole, to assess 1) how colony preference changed over time, and 2) whether foragers converged on the colony preference over time. Preference was measured as the % niger collected out of the total seeds selected. Convergence was measured by taking the absolute value of the difference between an individual’s preference, and the colony preference (% niger collected by individual - % niger collected by the colony). For the first set of experiments, we also assessed how individual preference changed between Days 2 and 3, to determine whether foragers were more likely to change their preference if they had previously preferred the lower-quality seed (ryegrass). To assess whether colony collection was regulated

through patch abandonment, we asked whether the forager preference on Day 2 predicted likelihood of being absent from the patch on Day 3. To assess forager flexibility, we calculated the initial preference of marked foragers, then the number of seeds collected from the all niger pile, and the number collected from the all sesame pile.

Seeds were always 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 two seed types while still providing *ad libitum* sampling, seed piles were monitored during choice tests, and piles were removed and replaced with fresh seed mixes 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 morning and early afternoon (8:00-13:30).

Data analysis

We used video recordings of forager behavior at the seed pile during choice and flexibility tests to determine individual and colony metrics like preference, number of seeds collected, and whether a forager returned to a pile on a subsequent day. The type of each seed collected, as well as the color code of any marked ant that was collecting, was manually coded using CowLog (Version 3.0.2; Hänninen & Pastell 2009), which allows logging behaviors using keystrokes tied to video-based time-stamps. To ensure that colonies were only analyzed if foragers had equal opportunity to evaluate both seeds, a visual check for whether both seeds types were within one body length of foragers coming to the pile was conducted during

video analysis. If this rule was not met, collection was not coded for that portion of the video.

We assessed changes in colony preference by evaluating the change in % niger collected between Day 2 and Day 3. We used a linear mixed-effect model (LMM) that included a random intercept for each colony. A variance-stabilizing transformation was not necessary, as residuals were mostly normally distributed, and residual variance was equal between groups. Change in colony preference over time was not analyzed for the six-day trials, as the sample size was small (four colonies).

To evaluate how colony-level change was coordinated through individual preference changes, we tested whether foragers who chose more lower-quality seeds (ryegrass) were more likely to change preference than those who collected higher quality seeds (niger or sesame). For this, we calculated marked worker's change in preference by Day 3. This was calculated as the absolute value of % niger collected by an individual on Day 3 – Day 2. This metric was regressed on the % niger each worker collected on Day 2. A LMM was used for this analysis that included a random intercept for each colony. Only foragers who collected at least 10 seeds during choice tests were considered. If foragers who preferred poor-quality seeds were most likely to change their preference, we expected a negative slope for the unequal-quality seed scenario, and a slope of 0 in the equal quality scenario. The absolute value was used instead of the raw degree of change (negative and positive values), because the proportions were bounded by 0 and 100%, which meant that ants starting at a low preference for niger had more space to change to positive values, and *vice versa* for those starting with a high preference for niger on Day 2. This created an uninformative negative slope even in a scenario where foragers were changing preferences randomly. A figure showing raw differences is available in the

supplemental material for comparison (SM Fig. 3.1). For the statistical analysis, we used square root transformed values to meet assumptions of equal variance and normality of residuals. The slope and intercept for figures was calculated from untransformed values.

We next assessed whether colony-level change was coordinated through individuals abandoning what they considered to be a poor-quality patch. For this, ants that collected at least 10 seeds on Day 2 were scored for presence or absence at the pile on Day 3. For both the equal and unequal-quality scenario, the preference (% niger collected) on Day 2 of those who returned was compared to the preference on Day 2 of those that didn't, to see if previous day's preference predicted likelihood to return. This was evaluated with an LMM. A random intercept for each colony was included in the model. The proportion niger collected on Day 2 was logit transformed, so that values would meet assumptions of normality.

To evaluate whether individual preferences converged on colony preference, a convergence measure that represented the similarity between an individual's preference and the collective preference was calculated for each day that preferences were assayed. This convergence measure was calculated as the absolute value of the difference between the % niger collected by an individual and % niger collected by the colony. This measure was compared across days, both for the trials comparing pairings of niger and ryegrass, and niger and sesame (Day 2 – Day 3), and for the trials testing colonies on niger and sesame for an extended time period (Day 2,3,5, and 6). The data were analyzed using a LMM that included a random intercept for each colony, and for each ant within each colony. Only foragers who collected at least 10 seeds during choice tests were considered. If the absolute value of the difference were to decrease (move closer to zero) over days, it would mean foragers were becoming more similar to each other with experience. The absolute value of the

convergence measure was square root transformed for the Day 2- 3 comparisons to meet assumptions of equal variance and normality of residuals. Transformations were not necessary for the Day 2, 3, 5, or 6 comparisons. We performed separate χ^2 tests of independence for each individual, to better visualize when individual preference was statistically different from colony preference.

Individual flexibility in a changing resource environment was assessed by evaluating how large the standardized difference was between the number of seeds collected between the niger only and the sesame only trials, given a forager's initial niger preference when both niger and sesame were available. The standardized difference was calculated as number seeds collected when only niger was present minus when only sesame was present, divided by the total number of seeds collected from the sesame only and niger only piles. This standardized measure was used because there was inter-individual variation in collection rate. This relationship was analyzed with a LMM that included a random intercept for each colony. Only foragers that collected at least 8 seeds during the initial preference tests were analyzed. The cutoff was relaxed to 8 seeds collected rather than 10, in order to increase the available sample size.

Statistical Analysis

All statistical tests were conducted in R (R Core Team 2013). The 'lme4' package (Bates *et al.* 2015) was used for the LMM models, 'car' package (Fox & Weisberg 2011) for type-II analysis-of-variance tables and logit transformation. Assumptions of normality of the residuals were evaluated visually ('car' package), and with the Shapiro-Wilk test. Homoscedasticity of residuals was evaluated visually and with the Levene's test ('car' package). Values were not transformed when

deviations from normality were slight, as ANOVA is robust to deviations from normality.

RESULTS

Integration of individual behavior and colony change

A total of seven colonies were presented with a mix of two seeds that differed in quality (niger and ryegrass), and another seven were presented with a mix of two seeds that were more similar in quality (niger and sesame). In the colonies that selected from niger and ryegrass, colony preferences changed to track seed quality. Colonies preferred niger over ryegrass starting on the second day of collection, collecting 59.7% (\pm SE 7.6%) niger from the mix. Colonies further increased their preference for niger by the third day of collection to 75.7% (\pm SE 4.0%. LMM: $X_{21} = 9.46$, $P = 0.002$, $N = 7$ colonies, Fig. 2). In colonies selecting from niger and sesame, colonies preferred sesame to niger on both tested days, and they did not change their preference by the third day (means presented \pm SE: Day 2: 34.85% \pm 3.04% niger collected; Day 3 38.7% \pm 3.5% niger collected. LMM: $X_{21} = 1.57$, $P = 0.21$, $N = 7$ colonies).

The change in preference seen in the colonies given niger and ryegrass emerged from foragers who had preferred ryegrass on Day 2 changing their preference by Day 3. Foragers that had preferred ryegrass more changed their preference more, and foragers with the strongest initial preference for niger tended to change the least, resulting in a negative relationship between initial preference for niger and degree of change (LMM: $X_{21} = 72.01$, $P < 0.001$, $N = 80$ ants from 7 colonies, Fig. 3A). Fifteen foragers exclusively collected niger on Day 3, while 7 exclusively collected niger on Day 4 (out of 80 total ants), suggesting that many still

collected a mix of the two seeds. Interestingly, there were some ryegrass-preferring foragers that changed preference very little, and others who came to prefer ryegrass more. In the colonies given niger and sesame, forager preference changes were not correlated with their preference on the previous day (LMM: $X_{21} = 1.62$, $P = 0.20$, $N = 89$ ants from 7 colonies, Fig. 3B). However, many ants did change preference, some to a large degree.

We explored whether a possible mechanism of colony preference change is through foragers that prefer an unpopular seed integrating this information and abandoning the pile. However, no association was found between an active forager's seed preference on Day 2, and their presence or absence on Day 3 (Niger & Ryegrass: LMM: $X_{21} = 0.05$, $P = 0.82$, $N = 90$ ants that came back, 67 that did not, from 7 colonies; Niger & Sesame: LMM: $X_{21} = 0.04$, $P = 0.85$, $N = 99$ ants that came back, 45 that did not, from 7 colonies, figure in Supplementary Material, SM Fig. 3.2).

Foragers were diverse in their seed preference, and they did not greatly decrease this diversity by the second day of testing. Foragers collecting from ryegrass and niger had preferences ranging between 0-100% niger on Day 2, and 4-100% niger on Day 3. Foragers collecting from niger and sesame were similarly diverse, ranging from 0-100% niger on Day 2 and Day 3. There was no significant change in how different individual preferences were from their colony's preference (preference being the % Niger collected, and difference calculated by subtracting colony preference from individual preference and taking the absolute value) between Day 2 and 4 (Niger & Sesame: LMM: $X_{21} = 0.28$, $P = 0.59$, $N = 89$ ants from 7 colonies; Niger & Ryegrass: LMM: $X_{21} = 2.88$, $P = 0.09$, $N = 80$ ants from 7 colonies, Fig. 4).

Forager diversity in colonies with prolonged decision-making experience

To explore whether preference diversity was maintained after a prolonged information-integration period, a total of four colonies were presented with niger and sesame seeds over a span of six days. Over this period, the four colonies maintained a preference range between 32% and 51% niger collected, and did not change more than 7.5% across days (Fig.5A). The diversity of forager preferences remained high and diversity levels changed little over the same time span. Forager preferences ranged between 0-100% niger preference on Days 2, 3 and 5, and 0-95% niger preference on Day 6. The average difference in preference between individuals and the colony was 21% ($\pm 2\%$ SE) on Day 2, and 24% ($\pm 2\%$ SE) on Day 6; it did not significantly change over this timeframe (LMM: $X_{23} = 5.32$, $P = 0.15$, N on Days 2,3,5 and 6 = 53, 42, 58, and 46 ants, respectively, Fig. 5B). Approximately half of the marked foragers still had a preference significantly different from their colony's by Day 6 of collection (22 out of 46, evaluated with X_2 test, $\alpha=0.05$).

Foragers were found to change preference frequently between days (figure in Supplementary Material, SM Fig.3.3). Of the 12 foragers that were seen on all four days, half ($N = 6$) changed from being less seed biased (% niger collected = 25-75%), to more biased (% niger collected < 15% or > 85%) towards a seed type. All six of these were from the same colony. Only one forager remained highly biased (%N > 95%) throughout, while one switched from bias toward sesame, to bias toward niger (collecting first < 25% niger, then > 75% niger).

Forager flexibility when preferred seed disappears

To examine how flexible foragers were when their preferred seed disappeared, we provided the four colonies tested over six days with a follow-up flexibility test on Day 7 or 8. Initial forager preference was determined from their

relative collection amounts at an equal mix of niger and sesame, then their collection when given a pile of either only niger, then only sesame, or *vice versa*.

Apart from absolute seed specialists (those collecting 100% sesame or niger), foragers' preferences affected their collection rates very little; however, specialists were more strongly affected by the switch. This led to a significant positive relationship between the % niger collected from an equal-mix pile, and whether foragers collected more seeds in the niger only pile (LMM: $X_{21} = 13.49$, $P < 0.001$, $N = 32$, Fig. 6). When specialists were removed from the analysis, the effect of the % niger collected from an equal-mix pile on collection rate between niger-only or sesame-only piles was marginally non-significant (LMM: $X_{21} = 3.75$, $P = 0.053$, $N = 27$).

DISCUSSION

Collective decisions often make use of many imprecise individual estimates (Surowiecki 2004, Sumpter 2006, Sasaki & Pratt 2018). Particularly when decisions are not absolute, they also benefit from individuals who use diverse inference strategies, and those who respond differently to stimuli (Seeley 1986, Chittka *et al.* 2003, Hong & Page 2004, Burns & Dyer 2006, Oldroyd & Fewell 2007, Krause *et al.* 2011, Fujisaki *et al.* 2018). Our results show that harvester ant colonies choosing between seed types maintain a diversity of preferences, and show diversity in their flexibility as the colony gains new information or the environment changes. The group-level changes observed here were organized through a variety of individual-level adjustments, with some individuals changing in the opposite direction of colony preference, yet with the majority coordinating to varying degrees to increase the proportion of high-quality seeds collected by the colony. There are also individual-level adjustments to preference over successive days of seed intake, even when

colony preferences remain relatively stable. Furthermore, most foragers can flexibly switch to collecting a less preferred seed without any cost to their collection rate - only seed specialists slow down when their favorite seed is unavailable. This suggests that foraging ants preserve independent preferences, even as they interface in the shared colony, and despite the ability to recognize all collectively chosen seeds.

Individual flexibility drives colony learning

In this study, colony learning was coordinated through individuals using information from previous collection experiences to adjust their personal preferences, generally in favor of a better-quality seed (niger being higher in caloric value than ryegrass). This finding elucidates our understanding of the mechanisms through which *P. californicus* colonies learn about seed quality, first discussed in Chapter 2. Interestingly, individual preference adjustment did not affect the overall preference diversity of the colonies in this study. It is still possible that extended experience with the seed would lead to convergence on niger and complete rejection of ryegrass, particularly if this species contains toxins that may not show initial effects. As an example, in a study by Rissing (1981) a colony of *P. rugosus* eventually rejected ryegrass completely with extended collection (Rissing 1981).

Previously, we found that collective learning in *P. californicus* is facilitated by within-nest activities, likely through communication of information gained after seed processing (Johnson 1991, Chapters 2, 4). Exactly how this information is gained within field colonies is unclear. Excavations of *P. badius* nests have found that foragers are usually located at the top of the nest, while seeds are stored in chambers below (Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017), suggesting that foragers may not have direct access to stored seeds.

We also do not know if foragers gained information solely from seeds they collected themselves, or additionally from seeds collected by others. Indeed, the diversity in preference, and the fact that some foragers did not improve their seed choices or even changed to prefer lower quality seeds, suggests that all may not have an equal opportunity or interest in comparing among seeds. Honey bees are known to ignore information that is ostensibly available by randomly choosing dances to follow instead of comparing among dances (Seeley & Towne 1992), so perhaps harvester ant foragers do not seek out opportunities to compare seeds in the nest. Different preferences may also partially depend on differences in cognitive ability, speed and accuracy tradeoffs, or sensitivities to stimuli. Bees are known to vary in their ability to learn, their speed-accuracy tradeoffs, and their proclivity to prefer certain macronutrients over others (Fewell & Page 1993, Laloï *et al.* 1999, Chittka *et al.* 2003). Others have suggested individual preference differences in ants can come from differences in age (Therrien 1988), or experience with the environment (Johnson *et al.* 1994). Foragers tend to be older than in-nest workers, but the age and experience of foragers at the test seed piles undoubtedly varied.

We found that some foragers maintained collection of low-quality ryegrass seeds, even as others changed preference to the higher-quality niger seeds. Foragers that prefer or maintain collection of low-quality seeds may be important in seed-poor environments, or when resources become depleted (Seeley 1986). Similarly, it has been suggested that *T. albipennis* individuals with poor taste in future nest sites can be useful to collective decisions in situations where only poor quality options are available, as they prevent collective indecision (Mallon *et al.* 2001). In leaf choice by leafcutter ants, one forager was also found to prefer leaves ranked lowest by its colony and most other colony members (Therrien 1988), suggesting that small numbers of individuals with preferences for poor-quality

options may be a widespread phenomenon. This, as well as the fact that forager preferences did not converge on collective preference, suggests that social influence does not completely control a forager's seed choice. For human groups, social influence can take away from individual independence, causing correlation between individual's estimates without improving the collective estimate (Lorenz *et al.* 2011, Kao *et al.* 2018).

Individual diversity is maintained even with repeated decision-making

We found that foragers retained a diversity of seed preferences over an extended period of time (six days) when collecting from seeds of similar quality (sesame being slightly higher in calories than niger). Individuals spanned the gamut of preferences, from 0-100% niger collection, while colonies showed less variation in their preference. Unlike previous studies in other species, foragers in our study did not all specialize on one of the two seeds (*P. badius* and *P. rugosus*, Nickle & Neal 1972, Rissing 1981). Instead, they showed a range of seed biases from equal preference to specialization, more similar to what has been described for *P. occidentalis* (Crist and MacMahon 1991, Fewell & Harrison 1991). Differences in individual preference for leaf species have also been found in leafcutter ants (Therrien 1988), and preferences for nests and building materials vary among individuals of *Temnothorax albipennis* (Aleksiev *et al.* 2007, O'Shea-Wheller *et al.* 2017).

In our study, some individuals changed their level of bias, and which seed they preferred more, over the six-day collection period. Foragers were also found to change their bias in the seven colonies tested on niger and ryegrass over two days. Similarly, *P. occidentalis* workers have inconsistent preferences for seed types across days (Fewell & Harrison 1991), as do leafcutter ants (Therrien 1988). We found that

changes in preference did not lead to convergence on colony preference. In human groups, receiving accurate information about previous rounds of decision-making tends to reduce the diversity of individual estimates by correcting errors, and better the group's response (King *et al.* 2012, Jayles *et al.* 2017, Luo *et al.* 2018).

Nevertheless, collective responses that maintain a high level of individual error can be as good as those with a low level of individual error (Luo *et al.* 2018). In our study, foragers either did not, or could not decide on a single best response.

This lack of convergence in preference suggests that, even with information potentially available in the colony, foragers form their own preferences about seed quality. Foragers have access to the same information at the seed pile; they experience both seeds during collection, and perhaps in the colony as well. However, foragers may use different heuristics or have access to different kinds of information when judging seed quality. Studies on animals, including social insects, find that if an individual has information which satisfies their goals, they tend to use this personal information rather than seeking to update it (Grüter & Leadbeater 2014). Honeybees often fly to a previously known feeder location, after being activated by following a dancer dancing for a different location (Grüter *et al.* 2008). Rats and guppies also follow conspecifics when food options are unknown, but follow personal experience when they are familiar with one of the options (Laland 2004). Ants that use pheromone trails will often rely on their personal information of a good site rather than follow a trail (Harrison *et al.* 1989, Grüter *et al.* 2011, Cronin 2013).

Furthermore, models of collective decision-making predict that groups composed of members with diverse problem-solving rules or inference strategies can be better at collective problem solving than groups with members using similar approaches to a problem (Hong & Page 2004, Fujisaki *et al.* 2018). Harvester ants

may similarly benefit from preserving the formation of varied independent preferences.

Individuals largely show flexible adjustment to the loss of preferred seed

When foragers were forced to collect from piles of only niger or only sesame, we found that most forager's collection rates were independent of previous preference. This suggests that preferences are labile, and that most foragers can relatively easily switch which seeds they select in response to the current environment. *P. occidentals* is similarly labile in preference, choosing seeds during collection using heuristics based on relative quality (Fewell & Harrison 1991). Additionally, *P. californicus* colonies are known to accept more poor-quality seed when collecting from a seed pile where the high-quality seed runs out (Holder Bailey & Polis 1987).

On the other hand, our foragers were selecting from a patch where seed density was high, and the absence of their favorite seed could quickly be assessed. Personal preferences may have more of an effect on collection rate when seeds are less densely scattered. Also, the foragers who showed complete specialization on a specific seed type when choosing from two seeds, did have trouble collecting the non-preferred seed when it was the only seed offered. Strongly specialized ants may be using other strategies for selection compared to the less-selective ants, such as high accuracy rather than speed (Chittka *et al.* 2003, Burns & Dyer 2006), or they may simply be at the extreme in ability to learn (Therrien 1988, Dukas 2008).

Conclusion

Individuals are often concerned with choosing the best strategy to optimize individual decision-making. However, studies of collective decision-making find that

groups can make good decisions, despite individual inaccuracy, and sometimes benefit from a diversity of behavioral strategies (Surowiecki 2004, Hong & Page 2004, Sumpter 2006, Krause *et al.* 2011) especially when groups are tasked with making choices in variable environments (Seeley 1986, Oldroyd & Fewell 2007, Fujisaki *et al.* 2018). Social insects are a model system for collective decision-making, and a diversity of individual heuristics are consistently found to be the core of precise discrimination between options (Sasaki & Pratt 2018). Our study shows that collective changes do not necessitate convergence of preference, and good collective choices do not necessarily need rely on individual error reduction.

FIGURES

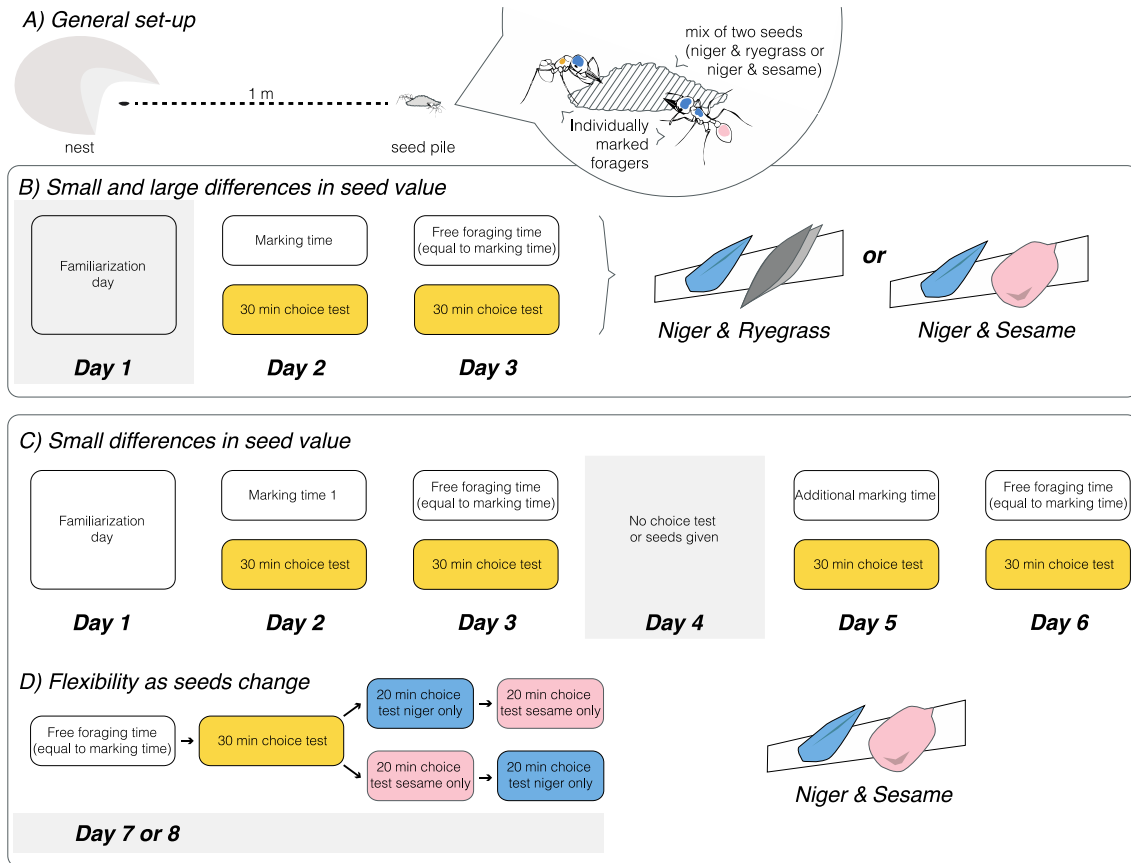


Figure 1: Diagram of experimental set-up in the field. (A) Piles of two seeds, equal in ratio and well-mixed, were placed 1 m away from nests. A selection of foragers were individually marked. (B) Colonies were given seeds niger and ryegrass (large difference in caloric value), or niger and sesame (small difference in caloric value) for three days. After an initial day of familiarization (Day 1), foragers were marked, then given a video-recorded choice test (Day 2). The next day (Day 3), colonies were allowed free foraging time equivalent to the time spent marking, then given a second video-recorded choice test. (C) Four new colonies were given niger and sesame over a span of six days. After a day of familiarization (Day 1), ants were marked and given video-recorded choice tests (Day 2). The next day, after a period of free

foraging time equivalent to the time spent marking, colonies were given a second video-recorded choice test (Day 3). The same was done for Day 4 and 5 as was done for Day 2 and 3. No seeds were given or video recorded on Day 4. (D) The four colonies tested in (C) were given a subsequent day of flexibility tests (Day 7 or 8). Foragers were allowed to freely forage for a time period equivalent to time spent marking on Days 2 and 5. Then, a choice test with equal amounts of niger and sesame was provided. After this, the pile was switched to all niger, then all sesame, or vice versa.

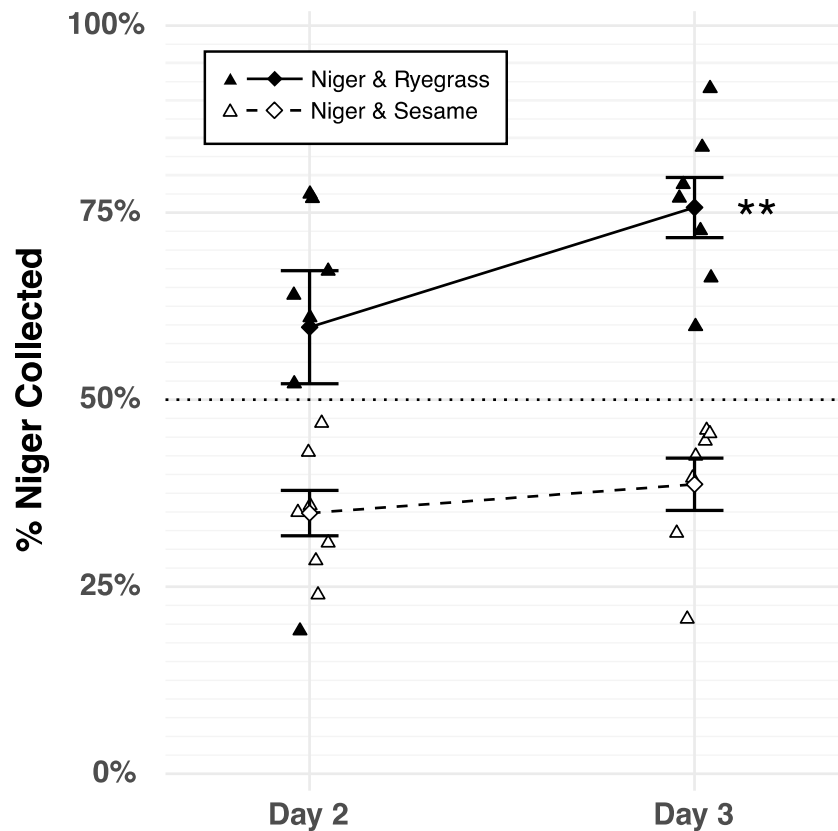


Figure 2: Changes in seed preference of colonies across days. Preference is measured as the proportion of niger collected out of niger and ryegrass, or niger and sesame. Niger and ryegrass were more different in caloric value than niger and sesame. Each triangle represents the preference of the colony for that day. Diamonds represent means and bars represent standard error. Lines connect means across days. Colonies collecting from niger and ryegrass (black triangles, black diamonds, solid line) increased preference for niger by the third day, but colonies collecting from niger and sesame (white diamonds, white triangles, dashed line) did not change their preference. Mean \pm SE for niger and ryegrass, Day 2: 59.69% \pm 7.55%, Day 3: 75.69% \pm 4.02%. For niger and sesame, Day 2: 34.85% \pm 3.04%, Day 3: 38.69% \pm 3.5%. ** = $P < 0.01$.

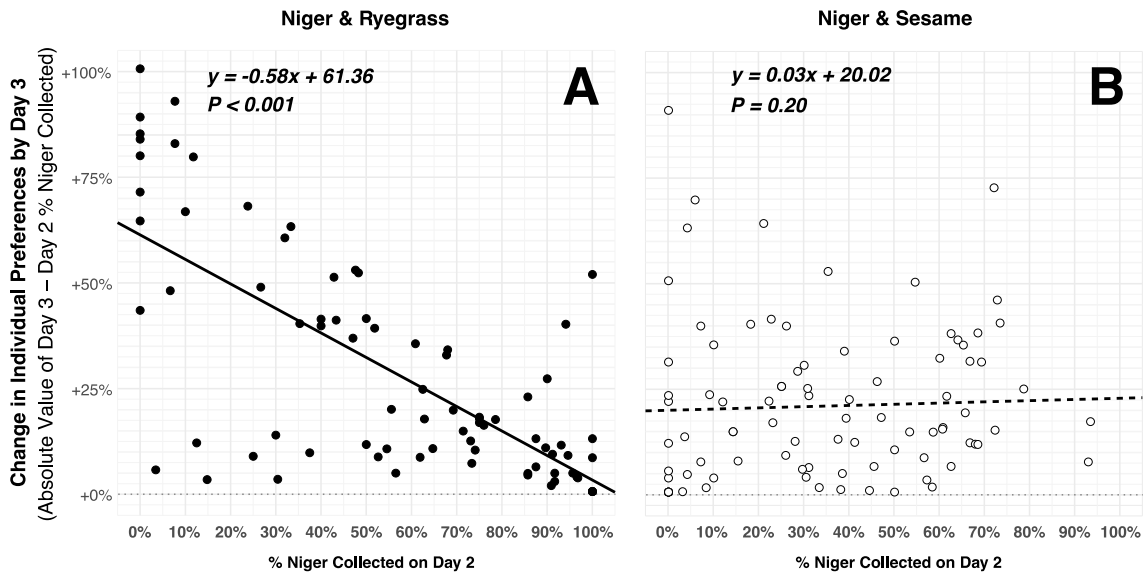


Figure 3: Changes in seed preference of individuals across days. Circles represent a forager's change in preference between Day 2 and Day 3. Horizontal dotted lines mark $y=0$ (no change in preference). (A) Foragers collecting from niger and ryegrass (filled circles) changed more if they had preferred ryegrass on Day 2, rather than niger (relationship represented by solid line). (B) The preference changes of foragers collecting from niger and sesame (open circles) were not affected by their preference on Day 2 (relationship represented by dashed line). Equation for the line is in the top left corner of its respective graph. Only ants that collected at least 10 seeds are represented in this figure. Niger & ryegrass $N = 80$ ants from 7 colonies; Niger & sesame $N = 89$ ants from 7 colonies.

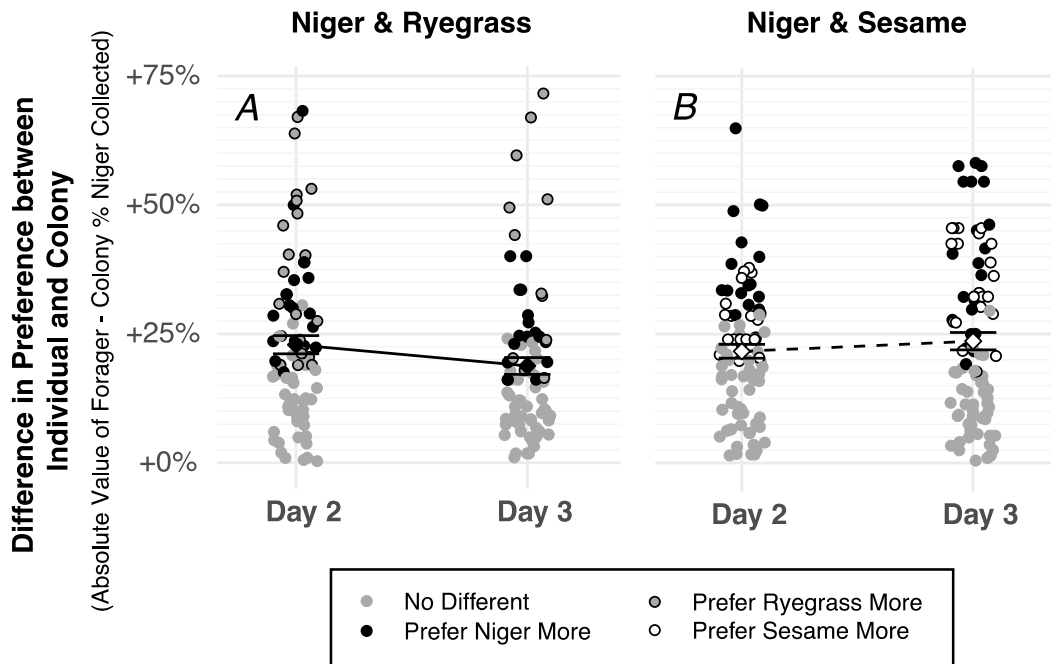


Figure 4: Change in individual deviance from the colony mean. A forager's difference in preference from its colony's, calculated by subtracting colony preference (%N) from individual preference (%N) and taking the absolute value, is represented by circles. Solid grey circles are ants whose preference was not different from their colony's, while black circles are ants that preferred niger more, black and white circles are ants that preferred sesame more, and black and grey circles are ants that preferred ryegrass more than their colony (evaluated with a χ^2 test, $\alpha=0.05$). Diamonds represent mean difference from the colony, and bars represent standard error. Lines connect means across days. (A) Foragers collecting niger and ryegrass maintained a similar deviance from the colony preference on both days, even as the colonies tended to decrease preference for ryegrass (black diamond, solid line). (B) Foragers collecting niger and sesame also maintained a similar deviance from the colony preference on both days (white diamond, dashed line). Only ants that collected at least 10 seeds on both days are represented in this figure. Niger & ryegrass $N = 80$ ants from 7 colonies; Niger & Sesame $N = 89$ ants from 7 colonies).

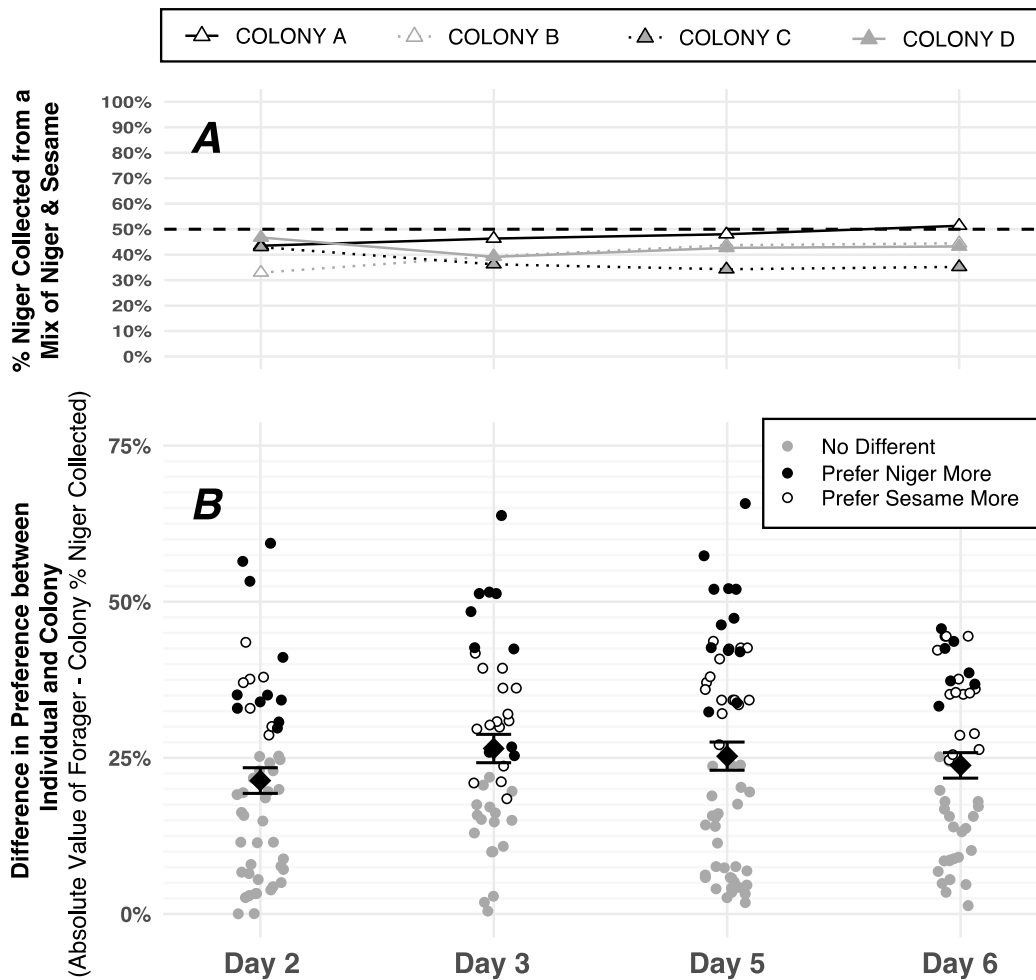


Figure 5: Colony variation and difference in preference between individuals and the colony over an extended period of time. Colonies and foragers were assessed in four choice tests, sampled over six days of collection, as they collected from niger and sesame (small difference in caloric value). (A) Preferences of colonies (Colony A-D) are depicted by triangles, lines connect preferences across days. Colonies maintained a sesame-biased but consistent preference over the time span. (B) Forager preferences calculated by subtracting colony preference (%N) from individual preference (%N) and taking the absolute value, is represented by circles; grey circles are ants that were not significantly different from the colony, white circles are

ants that preferred sesame more, while black circles are ants that preferred niger more than the colony (evaluated with a χ^2 test, $\alpha=0.05$). Diamonds represent mean difference from the colony, and bars represent standard error. Foragers maintained a similar difference in preferences around the colony preference on all tested days. Only foragers that collected at least 10 seeds were used for this analysis. N on days 2, 3, 5 and 6 = 53, 42, 58, and 46 ants, respectively.

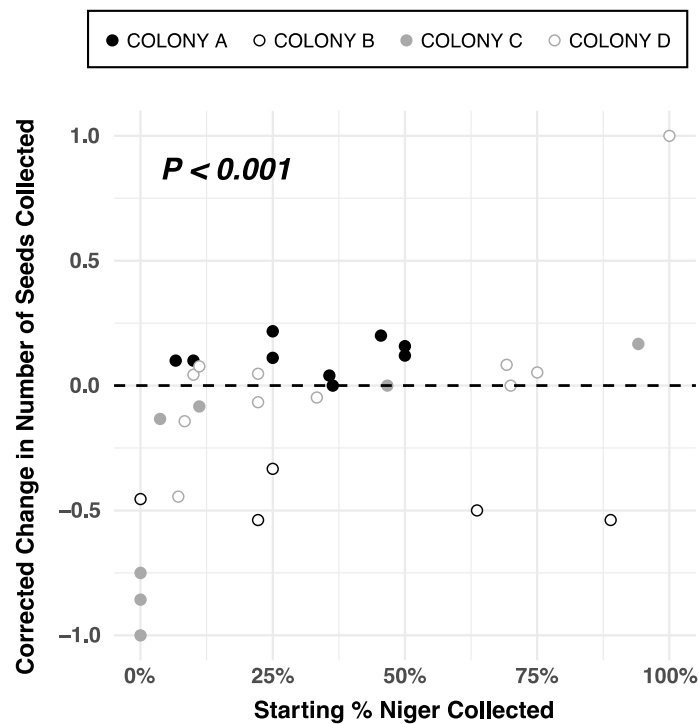


Figure 6: Forager collection behavior when favorite seed disappears. Circles represent the behavior of a single ant, circle shading represents the colony they were a part of. This figure shows the relationship between the preference exhibited by ants during an initial choice test where an equal mix of niger and sesame (small difference in caloric value) was presented, and the corrected difference in the number of seeds they collected when only niger was present, vs. only sesame present (corrected by number of seeds collected in the single-seed piles). Negative values mean forager collected more seeds when only sesame was present, positive values mean forager collected more seeds when only niger was present, and values of -1 or 1 means the forager only collected seeds from the pure sesame environment, or the pure niger environment (respectively). Those ants who specialized to a seed were likely to collect very little when their preferred seed was not present. Only ants that collected at least 8 seeds during the initial test were used for this analysis.

CHAPTER 4

Processed seeds prime naïve harvester ant foragers to recognize and evaluate novel seeds (*Pogonomyrmex californicus*)

ABSTRACT

The shared space of a nest or roost is an important locus for the consolidation of information. This can be particularly useful in the context of social foraging, when group members individually gather information about the availability and quality of resources. However, the transfer of information from novel food finder to naïve forager is not necessarily a single-step process. Especially for large and highly cooperative societies, such as in the social Hymenoptera, information exchange about food can involve different groups of workers and take place in various locations in the nest. These intermediary steps set up the potential for mechanisms to regulate information about resource availability relative to colony needs. In this study we explore how foragers of the harvester ant *Pogonomyrmex californicus* gain information about novel seeds from the nest, and what factors affect this information transfer. Using groups comprised of foragers alone, or groups with foragers and other within-nest workers, we manipulated the presence of seeds and larvae in nests in the laboratory, and tracked interactions with the seeds, as well as the forager's interest in collecting the same seed types in a foraging arena. We used different seed types that were novel to the worker's source colonies, and for which colony preference in the field had previously been established (Chapter 2, 3). We found that foragers are more likely to collect novel seeds of high quality if their group has consumed them. Groups consumed and collected fewer low quality seeds, although almost all groups husked at least one. This suggests that the husking of a novel seed releases information to foragers. This information influences them to collect seeds,

and discriminate between seeds of different quality. We also found that foragers can husk seeds on their own; however, in groups comprised of different tasks, foragers spend more time searching outside the nest than do the workers husking seeds. This sets up an efficient division of labor between food collection and food processing, but could affect the time it takes for a forager to update information about seed quality or colony preference.

INTRODUCTION

For group-living animals, the nest, burrow, or roost can be an important location for gathering information (Seeley 1985, Brown 1988, Zentall & Galef 1988, Bijleveld *et al.* 2010). In particular, information gained from group members about food items or foraging locations can cut search time and help animals focus their foraging effort (Galef 1988, Lefebvre & Palameta 1988, Rapaport 2006, and refs. therein). Information about novel food quality, handling techniques, and availability can be accessed through shared food, surplus food stored in the nest, or through simply observing which food is chosen by others (Mason 1988, Thornton & Clutton-Brock 2011, Dornhaus & Chittka 2005). In this way, the nest, burrow, or roost can act as an information center where knowledge about potential forage items is consolidated and can be used to optimize decision-making (Galef 1988, Ratcliffe & Hofstede 2006).

In this study, we focus on information use in the nest by foragers of seed harvesting ant colonies - specifically, the information foragers gain from food in the nest, and how behavioral and spatial factors affect this information transfer. Social Hymenoptera of all kinds (ants, bees, wasps) have been found to use information gained in the nest to locate or identify novel foods, and nests offer a variety of sources of information for their foraging workers (Seeley 1985, Jandt & Jeanne 2005,

Farina *et al.* 2005, Provecho & Josens 2009, McCabe & Farina 2009, Reichle *et al.* 2010). Foragers can gain information about novel foods directly from others, most commonly through liquid food regurgitated by other foragers (Von Frisch & Chadwick 1967, Farina *et al.* 2005, Provecho & Josens 2009). In addition, nests can contain surplus food stores that potentially act to spread information more widely. For instance, bumblebee foragers have been found to sample honeypots and learn novel odors that inform foraging choices (Dornhaus & Chittka 2005, McAulay *et al.* 2015). The byproducts of food processing can influence forager choices as well, notably, leafcutter ants who forage for substrate to feed their cultivated fungus gardens use leaf odors on healthy or dead fungus to inform their foraging choices (Weser 2005, Herz *et al.* 2008, Saverschek *et al.* 2010, Arenas & Roces 2017, 2018).

Division of labor, as well as features of the nest structure create a network of information flow where tasks can function as information sources (Fewell 2003, Weser 2005). Foragers of a social hymenopteran colony are often specialized to the task of recognizing and collecting food outside of the nest, while the food they bring into the nest is distributed, processed, and stored by other workers (Anderson & Ratnieks 2000). While in some cases, information can flow directly from foragers with food information to naïve foragers seeking such information, such as in the case of honeybee foragers providing nectar to recruits (Von Frisch & Chadwick 1967), the information transfer is often not direct. For example, leaf quality only becomes apparent to leafcutter ant foragers after novel leaves are fed to the fungus by other workers (Herz *et al.* 2008, Saverschek *et al.* 2010). Thus, it is necessary to sort out the practical use of within-nest information sources from their potential uses by following the network of food transfer among and between task groups.

It has been suggested that seed harvester ant colonies can use information from cached seeds to inform their collection decisions (Fewell & Harrison 1991,

Johnson 1991, Weser 2005). The importance of within-nest seed interactions is implied in studies of handling efficiency changes in *P. rugosus*; improvements in collection efficiency of novel seeds dramatically increases between the first and second day of colony experience with the seeds (Johnson 1991). Within-nest interactions are also implicated in the changes seen in forager seed preferences across days, after the accumulation of colony experience (Rissing 1981, Fewell & Harrison 1991, Chapter 2, 3). In previous work, we found that foragers often do not immediately differentiate seeds of varying quality during initial collection, and that colonies learn about seed quality across successive days of collection. This suggests that information exchange within the nest, possibly after seeds are processed, may be a critical link in information flow and colony decision making around resources. However, we know very little about the specifics of how interactions inside the nest, either with other task groups, such as food processors or nurses feeding brood, or with seeds as information centers, affect forager behavior.

Information about seed quality can follow a circuitous route on its way to foragers. Though seeds have odor cues that foragers can use for information (Gordon 1978, Gordon 1980, Greene *et al.* 2013), and foragers can easily discriminate between different seed types outside of the nest, they seem not to update comparative information about the nutritional quality of different seeds while they are foraging on them (Chapter 2). This may be due to some seeds having shells that effectively mask nutritional information (Lundgren 2009), making it necessary for seeds to be processed (i.e., husked) inside the nest for this information to be exposed and evaluated. It may also be related to time costs of identifying and selecting seeds with limited immediate information.

Additionally, some have suggested that in seed-eating ants there is a division of labor between adult workers who husk and prepare seeds, and larvae who

responsible for digesting seeds and then feeding workers via exudates (Went *et al.* 1972, Davison 1982, Hölldobler & Wilson 1990). Larvae drive forager food choices in other genera (Dussutour & Simpson 2009). As such, information about a seed's nutritional value or toxicity may necessitate larval seed processing. However, foragers can feed on some part of the seeds themselves as well (Moggridge 1873, McCook 1879, Wheeler 1902). A further complication is that foragers may not have the opportunity to spend much time interacting with seeds after collecting them. Studies of the spatial distribution of *P. badius* workers in the nest found foragers to remain almost exclusively toward the top of the nest, above the main seed storage chambers, while seeds are moved deeper into the colony by intermediary transfer workers (Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017), setting up a spatial environment in which different task groups obtain different information about seed availability (foragers) and quality (seed processors and consumers).

To gain a better understanding of how seeds in the nest inform forager choices, we examined within-nest seed processing and its effect on collection behavior by the harvester ant, *P. californicus*. We asked whether seed collection and seed processing tasks were partitioned through division of labor and whether they were spatially separated. Specifically, we asked whether husking and the consumption of seed nutrients was essential for stimulating collection, and whether larval presence is essential for information about seed nutrition to flow back to foragers. We also explored how the relative proportion of novel seeds that were husked and consumed affected the proportion collected. To this end, we observed both groups of workers from laboratory-reared colonies, and worker groups brought into the laboratory from the field, as they interacted with novel seeds both in and outside of observation nests. The novel seeds we used were niger, ryegrass, and sesame; colony and forager-level preferences for these seeds have already been

established in previous studies (Chapter 2, 3). Colonies prefer collecting niger seeds to ryegrass seeds, and both colonies and individuals differentiate the quality of these seeds better with more days of collection. Sesame and niger are collected in similar amounts and colony preference for one over the other does not change much over days (Chapter 3). Through this work, we evaluate the effectiveness of the nest as an information center for foragers to gain information about seeds before they encounter them while foraging.

METHODS

General methods

We conducted a series of experiments to assess how within-nest information sources influence foraging decisions. The first experiment was conducted on subsets of workers taken out of laboratory-reared source colonies, as this allowed us to have colony members of all types present in the nest. We assessed two foci that may serve as information centers for foraging: larval seed consumption and worker seed processing. We manipulated the presence/absence of larvae and proportions of seeds varying in quality and tested the effect on collection. We asked: a) how within-nest seed interaction frequency affected foraging decisions, and b) whether there was a division of work between processors and foragers.

In the second experiment, we collected foraging workers from field colonies and established them in laboratory nests, to ask whether foragers are able to process seeds and use information from the nest to effectively choose between seeds of high and low quality. For these two experiments, we offered colonies a choice of niger (*Guizotia abyssinica*), and annual ryegrass (*Lolium multiflorum*), both of which were novel to the groups being tested. The two species have a similar wet mass, but

niger has a much higher lipid and caloric content (see Chapter 3, and Supplementary Material). In field trials, colonies showed a clear preference for niger over ryegrass, but that preference was learned over several days during which time colonies interacted with both seed types in the nest (Chapter 2).

Finally, in a third experiment, foragers and in-nest workers collected from field nests were used to evaluate whether foragers preferred seeds that they encountered more often in the nest. The use of foragers from field nests allowed us to assess behavior in individuals that have engaged in foraging under more natural conditions. Field collections also allowed for larger sample sizes for foraging assessments. Thus, the lab nest and forager experiments provide complementary assessments of information exchange in the context of foraging.

Field-captured foragers were collected as they were bringing back seeds from the nest, and in-nest workers were collected by shallowly excavating the nest. The workers close to the surface were likely either inactive foragers or in-nest workers that we believed may help with seed processing. Workers were again placed into laboratory colonies for assessment, and were offered seeds of niger and sesame (*Sesamum indicum*). These species have a similar wet mass, with sesame having a slightly higher lipid content. Although sesame has a slightly higher caloric value than niger, both have a much higher caloric value than ryegrass (Suttie 1977). Ryegrass seed may also harbor toxic endophytes (Australian Office of the Gene Technology Regulator 2017).

Experiment 1: Forager and processor interactions with seeds in lab-reared colonies

Prior to experiments, we first established a series of three subset nests from each of three laboratory-reared source colonies ($N= 9$ subset nests). Each of the

source colonies was started from a single newly mated *P. californicus* queen, collected in 2015 from a population in the Tonto National Forest (Mesa, AZ). These colonies contained ~1,000 – 1,600 workers when experiments began in December-January 2017-2018. Each source colony was divided into worker groups of approximately similar size, and with similar proportions of tasks groups from the source colony (foragers, within-nest workers, and brood care workers). Because task participation in ants tends to be location-specific, we ensured that all tasks were represented in the worker groups by taking equal numbers of workers from each part of the source colony's nest for each subset of workers. Two source colonies yielded ~500 worker for each subset, and the third yielded ~300 workers. Worker subsets were queenless; the queen was kept in the parent colony. Worker subsets were placed in observation nests consisting of two Plexiglas chambers attached to a small open "Foraging Arena" (26 x 11 x 8 cm) with a base covered in sand. One of the chambers contained moist plaster ("Moist Plaster Chamber"), the other was empty and dry ("Seed Chamber"). These observation nests containing subsets of workers from a parent colony are henceforth referred to as "nests".

To test how larval presence and seed processing affected forager seed choice, we designed three treatments that manipulated the following parameters: (1) nest with larvae and seeds in the seed chamber; (2) nest without larvae but with seeds in the seed chamber; (3) nest with larvae but no seeds in the seed chamber (Fig. 1A). Nests with seeds placed into the seed chamber received both niger and ryegrass seeds. First, a baseline collection test assessed initial collection level of the novel seeds. Immediately after this initial test, those in treatments that were to receive seeds were given seeds (described below). Because of our small sample size, and because our main interest in including or excluding larvae was to determine if their presence was necessary for seed consumption, we did not treat these groups as

separate treatment groups, but simply observed whether seeds were consumed when no larvae were present (see statistical model details below).

All nests were provided with seeds in the foraging arena once a day in the morning for five days, and their collection was monitored. Collection tests consisted of placing a dish of approximately 200 seeds each of niger and ryegrass at the far end of the foraging arena for 30 minutes (how seed numbers were approximated is provided in Supplementary Material). Immediately after a test, we removed the dishes, and any seeds dropped in the foraging arena. We also counted and removed all seeds moved from the dish into the nest before they could be processed. By removing the collected seeds, we could continuously control whether nests had seeds available to process; nests in the treatment groups allowed seeds were then given seeds added directly into the seed chamber.

The first collection test (Day 1) was used to establish baseline collection levels for the two seed types. Immediately after, we placed seeds of niger and ryegrass into the seed chamber of those nests chosen to receive them. We gave 15 seeds of each type to the nests containing ~300 workers, and 25 to nests containing ~500 workers (full timeline in Fig. 1B). On Days 2-5 before collection tests began, we removed any remaining whole seeds from the nest so as not to confuse them with collected seeds, and replaced them back after all seeds from the collection tests were removed.

The data collected for this experiment included: a) the number of seeds given to process that were missing (i.e. consumed) by each nest between collection tests, and b) the daily number and type of seeds collected by nests during collection tests. To determine whether nests differentially consumed seeds based on quality, we evaluated what proportion of the total niger and ryegrass given was consumed (consumed/given) with a Paired T-test (values were logit transformed, paired by

subset nest, i.e. the difference between proportion niger and proportion ryegrass consumed within each nest).

We also evaluated how seed consumption, Day, and Colony affected the number of seeds collected, with a Negative Binomial Model (GLM-NB). For each nest, days of the experiment were coded as either "before consumption" or "after consumption", depending on when colonies first began to process seeds within the nest. All nests were coded as "before consumption" on Day 1, because the initial collection test was conducted before any novel seeds were placed in any nest. All nests that were never given novel seeds to process in the nest were coded as "before consumption" for all five days. Of the nests in the "seeds present" treatments, all but one consumed seeds prior to the next day's test. These were marked as "after consumption" for Days 2-5. One nest given seeds began to process seeds after Day 2. This was coded as "after consumption" on Days 3-4, and "before consumption" on Day 2. We coded Day numerically (1-5), not categorically, because we assumed each day's effect would be summative. We included Colony as a random effect to account for differences in collection rate. The interdependence between days was captured by coding Day numerically.

In those nests provided with seeds to process, we determined how foragers and other workers interacted with the seeds provided by tracking their within-nest behaviors via scan sampling. To track individuals over time, workers were uniquely marked using oil-based paint markers in the days before the experiments began, with 31-37% of workers marked in each nest. Because we wanted to connect novel seed interactions to collection behavior, we focused our observations on the time span before the foragers first brought novel seeds into the nest. For most nests, this was over the 24 hours after seeds were first placed; a timespan covering Days 1-2.

One nest did not collect until Day 3, and other did not collect until Day 4; the timespan covered for them is Days 2-3, and Days 3-4 respectively.

Scan samples were conducted at 20-minute intervals between the hours of 6 or 7 AM to 8 or 9 PM. Nests were not observed overnight, or during the time it took to conduct a collection test and remove seeds (30 min - 1 hour). This yielded 29 scans after seeds were first put in the nest until nightfall, and 11 scans the morning before the second day's collection test. For those nests where seed collection did not occur until Day 3 or Day 4, additional scans were collected between the Day 2, Day 3, and Day 4 collection tests to verify whether seeds were being processed (39 scans per day). Scan samples consisted of taking a video scan of each sub-nest in series, with intermittent high-resolution photos. Collection tests were also video-recorded, to identify which individuals foraged.

To evaluate whether frequency of seed encounter is related to frequency of collection, we evaluated the scan samples and the collection test video and extracted the following data: a) the identity of the ants that were biting or carrying a seed or husk; the type of seed or husk; whether the seed was open (husked) or closed (still had an intact shell). We also identified b) the location of the interaction (Foraging Arena, Seed Chamber, or Moist Plaster Chamber). The data described above were only extracted for the time period before a subset first collected seeds into the nest. We also gathered data on the identity of the ants collecting seeds, and the number each collected, during the collection test in which a nest first collected novel seeds. Using a Spearman's correlation test, we analyzed the relationship between number of seeds collected by each individual forager, and number of scans in which that forager was seen interacting with seeds (biting or carrying). We also evaluated whether most within-nest processing took place in the seed chamber or plaster chamber, by calculating the proportions of scans in which they processed in the

plaster chamber, and evaluating difference from random using a One-sample T-test with expected mu of 50%.

We next determined whether foragers were spatially segregated from seed processors. To do this, we chose ants that were representative of the foraging or processing task and tracked their locations ($N = 12$ foragers, 14 processors, sampled from 5 subset nests). Representative ants were seen processing frequently (observed biting seeds in 4+ scans) or collecting frequently (collected 4+ seeds on the sub nest's first collection day). To maintain a similar number of observations between nests, we only followed individuals during the 24-hour timespan before the first collection of novel seed, even for subsets who did not collect until Days 3 or 4. We were able to locate focal ants in at least 36 of all 39-40 scans taken during this 24-hour time period. To analyze whether foragers and processors were spatially segregated, a Linear Mixed-effects Model (LMM) was used to test whether high frequency foragers were found in the foraging arena in a higher proportion of scans than the high frequency processors. A random intercept for each source colony included as a random effect.

Experiment 2: Information gathering from seeds in the nest by isolated foragers from the field

We performed a second experiment on groups of foragers collected from field colonies to test whether foragers could gain information from seeds within the nest in the absence of brood or other task groups. The foragers were collected from six field colonies located at Coon Bluff Campground in Tonto National Forest (Mesa, AZ; 33°32'N, 111°38'W) in July 2019. Those individuals carrying food to the nest were identified as foragers. Approximately 300 foragers were collected from each nest, and brought back to the lab, where they were placed in observation nests similar to

those described above. The foragers were allowed to rest for 1-2 days before experiments began. Foragers had a few oats and moist plaster provided water.

We again provided novel seeds inside the observation nest by placing niger and ryegrass in each nest's seed chamber. Fifteen of each type of seed were placed into each nest every morning for three days (Days 1-3), with any uneaten seeds removed and counted the next day, and replaced with a fresh 15 seeds. When removing these seeds, the sand in the foraging arena was sifted to remove any dropped seeds. If fewer than 15 seeds of either type were found, we considered the missing seeds "consumed". We evaluated what proportion of the niger and ryegrass seeds were consumed with a Paired T-test (values were logit transformed, paired by forager group). We also noted if processed but unconsumed seeds were still present at this time.

We gave foragers collection tests as described above, by placing a mixed pile of ~ 200 each of niger and ryegrass into the foraging arena for 30 minutes each day for four days. As before, we removed any seeds taken from the dish into the nest before they could be processed, to control the number of seeds available for processing, and to count them. The foraging arena sand was sifted to remove any dropped seeds. The first collection test (Day 1) was given before any seeds were placed in the nest for processing. For the other collection tests (Day 2-4), the seeds given for processing the previous day were removed before the collection test, and the fresh seeds were placed in the nest after the collection test.

Three out of the six forager groups showed little to no seed collection behavior by the end of Day 4 (collected 0-6 seeds). To see if giving them open seeds prompted them to collect, we gave these three groups a fourth day of seeds in the nest, but cut two of each seed in half to expose the inside. Their final choice test was given the next day (Day 5).

To determine whether subsets consumed seeds based on their quality, we analyzed what proportion of the niger and ryegrass seeds were consumed, using a Paired T-test (values logit transformed, paired by forager group). We also evaluated whether foragers could gain information about seed quality by examining how their seed collection preferences by the end of experiments differed from random, using a One-sample T-test with mu of 50%. We also evaluated how seed consumption ("before consumption", and "after consumption") and Day affected the number of seeds collected, with a GLM-NB. Day was again coded numerically (1-4). Seed consumption was coded for each day as either "before consumption" or "after consumption"; before consumption was the period of time before any seeds of either type were consumed by the nest (i.e., Day 1 for all groups, up to Day 4 for some groups). We coded Day numerically (1-5), not categorically, because we assumed each day's effect would be summative. We did not include source colony in the model, as the interdependence between days was captured by coding Day numerically, and we assumed any variation between subset nests was captured by the random error in the model.

Experiment 3: Testing for an effect of seed processing on preference in workers from the field

We performed a second set of experiments on workers collected from field colonies to assess the relationship between within-nest experience with seeds and collection behavior, this time using two seeds of similar quality: sesame and niger. Field colonies readily collect both seeds, and both are of similar caloric value (Chapter 3). We collected workers from 16 colonies of *P. californicus* colonies found in the Coon Bluff Campground in Tonto National Forest (Mesa, AZ; 33°32'N, 111°38'W), in Sep-Oct 2018, and Oct 2019. Some of the workers we collected were

foragers, identified as ants carrying food to the nest. We also dug into the top 20-30 cm of the nest, to remove upper-nest workers, which are likely to be resting foragers and/or other in-nest workers that could help with processing. In total, 300 workers were collected, with 26-50% confirmed as foragers by their collection behavior. We moved these foragers and within-nest workers to observation nests as described above, and allowed them to settle for 3 days. Again, no brood were present in this experiment, workers had a few oats, and moist plaster provided water.

To test how colony seed consumption relates to seed preference, worker groups were tested for their preference for sesame or niger after experiencing different proportions of these seeds in the nest. As before, seeds were placed into the seed chamber for processing, and 30-minute collection tests were administered each day. However, in this set of experiments, different proportions of sesame and niger seeds were placed in the nest every day. After the initial baseline collection test (Day 1), eight observation nests were given two niger and six sesame (more sesame treatment), and the other eight were given six niger and two sesame (more niger treatment) to process. Immediately after the second day's collection test, all observation nests were given four niger and four sesame each to process (equal treatment). Immediately after the third day's test, colonies were given the opposite treatment to the one given after the first test. The provided seeds were slightly crushed, to promote feeding and processing behavior, and all seeds were consumed by the next day. Seeds meant for processing and consumption were placed into the nest immediately after each day's collection test. As before, any seeds brought by foragers into the nest during the collection test were removed to control seed consumption, the number of each type collected was counted, and the sand was sifted to remove any dropped seeds.

To evaluate whether seed consumption affected preference, the proportion of niger collected was calculated for each test day, and the effect of the proportion given to consume each day, as well as the original proportion given in the nest ("More sesame" or "More Niger" given after Day 1 collection tests) was evaluated with a LMM, with a random intercept for each source colony included as a random effect. At least eight seeds must have been collected by a worker group on a given day to be included as a data point. We also analyzed whether seed consumption affected the number of niger and sesame collected. We analyzed each seed type separately, to see if the effect of processing on collection was different for the two seeds. The change in total number of sesame or niger collected over days, and the effect of treatment was evaluated with a LMM, with a random intercept for each source colony included as a random effect. The interaction between Original treatment and Day was also analyzed for its effect on both proportion and number collected.

Statistical Analysis

All statistical tests were conducted in R (R Core Team 2013). The 'lme4' package (Bates *et al.* 2015) was used for the LMM analysis, MASS package for GLM-NB (Venables & Ripley 2002), 'car' package (Fox & Weisberg 2011) for type-II analysis-of-variance tables, and multcomp package (Torsten *et al.* 2008) for Tukey HSD. Assumptions of normality and homoscedasticity of the residuals were evaluated visually, and with a Shapiro-Wilk test or Levene's test (the 'car' package).

RESULTS

Seed collection and processing by laboratory-raised workers

We first looked at the behavior of worker subset colonies created from three laboratory source colonies to see how seed consumption, and experience with seeds in the Foraging Arena, affected collection ($N= 9$ nests). We found that the number of seeds collected increased over days, and interacted with seed consumption – that is, seeds were more likely to be collected across days generally, but with a larger increase after the colony had consumed seeds (GLM-NB: Day: $LR X_{21} = 13.27$, $P < 0.001$, Consumption: $LR X_{21} = 34.47$, $P < 0.001$, Colony: $LR X_{22} = 7.04$, $P = 0.03$, Interaction between Day and Consumption: $LR X_{21} = 4.24$, $P = 0.04$, $N = 9$ nests, Fig. 2).

The estimate of the effect of consuming the seeds was larger than that of day, suggesting that seed consumption caused collection to increase more than simply interaction with unhusked seeds over time (estimate of β Day: 1.36, β Consumption: 6.36, full table of coefficients provided in Supplementary Material SM Table 4.1). Nests given seeds to process did not collect any seeds before consuming some from the nest. The nests not given seeds to process also collected seeds, but in generally lower amounts than nests given seeds: the “With larvae and seeds”, “No larvae, with seeds, and “With larvae, no seeds” treatment nests from Colony A collected 101, 468, 10; Colony B collected 65, 84, 1; Colony C collected 162, 4, 20 seeds in total over five days (respectively, Fig. 2).

Our findings also suggested that consumption of seeds by larvae is not the only way to gain information about the poor quality or potential toxin presence of ryegrass. Two of the nests that were given seeds but no larvae, collected almost exclusively niger seeds (99%-100%), despite opening at least one of each type of

seed in the nest. Niger seeds were preferred over ryegrass in our previous studies as well (Chapter 2,3). Two with larvae collected almost exclusively niger seeds as well (98%-100%), while one with larvae collected a mix of both. Nests not only favored niger for collection, but they processed more niger as well. The nests given seeds to process consumed more niger than ryegrass (Paired t-test: $t_5 = 3.4$, $P = 0.02$, $N = 6$).

Interaction with seeds in the nest by foragers and other workers

The interactions of marked workers identified as foragers were tracked to establish whether there was a relationship between foraging intensity and interactions with seeds in the nest (i.e. were foragers who collected the most seeds also most drawn to interacting with seeds and shells in the nest). Worker interactions with seeds were observed in the time period before the nest (and thus, the marked foragers) first collected seeds (24-72 hrs), barring 10 hour time spans overnight where colonies were not observed. Only nests that were given seeds to process were tracked, excluding one that collected only four seeds in five days ($N=5$). Twenty five out of 42 marked foragers were seen to interact with seeds in the nest, of these 13 interacted with either open seeds or seed shells. This count only includes within-nest interactions, not interactions in the foraging arena during scans (of which there was only one) or at the pile during collection tests on prior days (foragers sometimes interacted with seeds without collecting them). Foragers interacted with both niger and ryegrass in the nest, but no marked foragers collected ryegrass. There was no relationship between the number of seeds a forager collected and how much of the observed time it spent (i.e. in how many scans it was seen) interacting with seeds in the nest (Spearman's rho: $r_s = -0.03$, $S = 12721$, $P = 0.85$, $N = 42$). An example of the interactions between foragers and seeds, and other within-nest workers and

seeds can be seen in Figure 3, similar visualizations for other nests can be found in Supplementary Material (SM Fig. 4.1). These figures show that the majority of marked workers that interacted with either seeds did not collect any seeds the next day (87-100%). This suggests that foragers are not more drawn to within-nest seed processing tasks than other workers, and in fact the majority of workers who interact with seeds in the nest do not forage for them.

Ants typically took seeds from the seed chamber into the moist plaster chamber. A majority (mean \pm SE: 79.17% \pm 6.23%) of seed processing events occurred in the moist plaster chamber rather than the seed chamber (one ant biting a seed in one scan was counted as one event; One-sample t-test: $\mu = 50\%$, $t_4 = 4.68$, $P < 0.01$, $N=5$). Seeds were most often removed from their shells and consumed in this chamber. Larvae and pupae were located in both chambers. During behavior scans, only once did we observe an ant process a seed in the foraging arena (i.e. bite a closed seed), though seed biting was also observed at the pile during collection tests. Seeds were never husked in the foraging arena.

Spatial distribution of foraging and processing tasks

We asked whether ants identified as high frequency foragers (collected 4+ seeds) were spatially segregated from high frequency processors (seen processing either seed in 4+ scans). To maintain a similar number of observations between nests, we only followed individuals during the 24-hour timespan before the first collection of novel seed, even for subsets who did not collect until Day 3 or 4 (nests were not observed for 10 hours overnight). In this timespan, there were no high frequency foragers who were also high-frequency processors. Though there was some overlap in spatial distribution, we found that high frequency foragers spent more time in the foraging arena than high-frequency processors (LMM: $X_{21} = 12.0$, P

< 0.001, N of High Freq. Foragers = 12 with 1-4 ants from each of 5 nests, High Freq. Processors N = 14 with 1-6 ants from each of 5 nests, Fig. 4.) One nest had no single forager that collected at least four seeds, and the nest as a whole only collected four seeds over 5 days, so it was excluded from analysis.

Field colony forager collection behavior

Foragers were collected from field colonies and set up in laboratory nests, so that seeds in the nest could be manipulated. The foragers were set up in the absence of larvae, to confirm that larvae were not necessary for seed consumption, and to compare the behavior of lab-raised and field-raised foragers. Foragers from all six sampled field colonies were observed over either 4 or 5 days. We found that novel seed consumption in the nest caused an increase in collection, and there was no effect of Day (GLM-NB: Day: $LR X_{21} = 0.42$, $P = 0.52$, Consumption (Before or After): $LR X_{21} = 58.03$, $P < 0.001$, Interaction: $LR X_{21} = 0.19$, $P = 0.66$, $N = 6$ forager groups, Fig. 5). The three colonies that did not collect many seeds over the first four test days had not consumed any niger seeds in the timespan, though two consumed one ryegrass each. However, we saw foragers in these nests interacting with the cut niger seeds soon after they were given to them after day four's choice test. By day five, these three fragments had consumed between 5-15 niger seeds per colony and no ryegrass, and collected only niger (11-33 niger seeds total). Of the total seed consumed over the timespan that forager groups were observed (either four or five days), a higher number of niger was consumed than ryegrass (Paired T-test: $t_5 = 5.26$, $P = 0.003$, $N = 6$). Forager groups also collected a higher proportion of niger than ryegrass by their last day of collection (88-100% niger, One Sample t-test: $\mu = 50\%$, $t_5 = 24.3$, $P < 0.001$, $N = 6$). All groups at least partially husked both niger and ryegrass in the nest.

Field forager and top-of-nest worker collection behavior after processing different proportions of seed

Foragers and upper-nest workers were collected from the field and set up in the laboratory, so that seeds in the nest could be manipulated. Workers were provided varying proportions of niger and sesame (seeds of similar quality) in the nest over four days, to test whether the proportion of seeds processed in the nest affected preference; one half of the groups received more niger first, then equal seeds the next day, and finally received more sesame. The other half received the opposite treatment. Workers were not given any niger or sesame seeds before the first day's choice test, to look at their baseline preference before they had an opportunity to process seeds. We found that the proportion of niger collected changes across days (LMM: Day: $X_{21} = 111.51$, $P < 0.001$, N on Day 1-4 = 10, 16, 16, 16 respectively, Fig. 6A. Five colonies collected four or fewer seeds on the first day of collection, and were excluded from Day 1 preference analysis; the rest collected at least 8 seeds).

Groups preferred sesame more before seeds were given to process in the nest, and preferred niger more after processing seeds; the mean % niger collected on Day 1 was 19.2% (SE \pm 5.3%), while on Day 2, 3, and 4 the mean % niger collected was 50.3% (SE \pm 5.6%), 64.7% (SE \pm 5.5%), and 69.8% (SE \pm 4.3%), respectively (Tukey HSD: Day 1 vs. 2,3,4 $|z| = 6.41, 9.01, 10$, $P < 0.001$. Groups collected the highest proportion of niger on Day 3 and 4 (Tukey HSD: Day 2 vs. 3,4 $|z| = 3.12, 4.21$, $P < 0.01$). All fragments consumed all seeds given in the nest, likely because both were of high quality. This, again, took place without larvae present in the nest. Whether more niger or more sesame was originally processed in the nest did not affect colony preference for niger (LMM: Original Treatment: $X_{21} = 2.73$, $P =$

0.1). There was no significant interaction between day and the original treatment (LMM: Interaction Original Treatment by Day: $X_{23} = 2.54$, $P = 0.47$).

Furthermore, we found that workers collected fewer seeds of both niger and sesame during the baseline collection tests, before they were given novel seeds to process. The effect of test day was significant for sesame and niger (LMM: Sesame: $X_{23} = 31.52$, $P < 0.001$, $N = 16$, Fig. 6B; Niger: $X_{23} = 98.13$, $P < 0.001$, $N = 16$). Specifically, the number of seeds collected on Day 1 was lower than any other day (Tukey HSD: Sesame Day 1 – Day 2, 3, 4 $|z| = 4.07, 4.82, 4.72$, $P < 0.001$; Niger Day 1 – Day 2, 3, 4 $|z| = 4.69, 7.19, 9.43$, $P < 0.001$). Colonies also collected more niger on Day 4 than they did Day 2 ($|z| = 4.74$, $P < 0.001$). Whether more niger or more sesame was originally processed in the nest did not affect the number of seeds collected (LMM: Sesame: $X_{21} = 0.40$, $P = 0.53$; Niger: $X_{21} = 0.22$, $P = 0.64$). There was also no interaction effect between day and the original treatment for either seed type (LMM: Sesame: $X_{23} = 1.8$, $P = 0.60$; Niger: $X_{23} = 2.40$, $P = 0.49$). The results of the preference and number collected analysis suggest that the proportion of seeds processed in the nest did not affect collection, but consumption of seed did. Furthermore, though workers preferred sesame less than niger by the second day of collection, they still collected a larger number of sesame seeds by the second day than the first day.

DISCUSSION

Harvester ant colonies are selective about what seeds they collect, focusing preferentially on certain types based on their caloric value and low toxin levels, while also maintaining diversity in the seed types collected (reviewed in Chapter 2). To this end, the nest can act as an information center, where knowledge is spread to foragers about what seeds are available in the environment and their quality, and to

prime them to make better foraging decisions. This study confirmed that naïve foragers use seeds in the nest to inform their choices outside of the nest, as several previous studies have suggested (Johnson 1991, Weser 2005, Fewell & Harrison 1991). However, we show that the importance of the nest environment for gaining information about novel seeds has perhaps been underestimated. Processing (husking and consuming) novel seeds in the nest was a crucial step to stimulating further collection of high quality novel seeds. This suggests that the quality of some seeds is difficult to evaluate upon first encounter, and may require associative learning between the shell and the nutritional properties. If brought in by a few intrepid foragers, colonies can uncover the seed's true value by processing the seeds in the nest, then disseminating this information widely.

We also clarified the network of food transfer and use within a nest, and how it affected seed information exchange and forager behavior. We found that, although larvae are fed seeds, their absence did not prevent foragers from gaining information that stimulated seed collection or from appropriately differentiating between high calorie and low calorie seeds. Workers also consumed seeds, though what portion was digested is unclear (McCook 1879, Went *et al.* 1972). Studies in other harvester ant species have found that foragers tend to have lower energy reserves than within-nest workers (Porter & Jorgensen 1981, Tschinkel 1998). However, foragers in colonies fed excess food have been found to have higher fat content than unfed colonies, suggesting foragers do feed opportunistically (Kwapich & Tschinkel 2016).

Spatial separation and division of labor between foragers and seed processors was observed, even in the close quarters of our small observation nests. Foragers were seen to help husk seeds on occasion, but husking and foraging was mostly performed by different groups of workers, and workers identified as foragers vs. food processors tended to occupy different areas of the nest. This suggests that foragers

are capable of tasting seed contents as needed to gain information, but in practice, such direct interactions may be moderated by task-related behavior and the nest environment. This may be especially true in the larger nests of field colonies, where foragers have been found to be spatially restricted to the top of the nest, above where seeds are typically cached (Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017). A division of labor between foragers and processors would allow foragers to focus on collecting within a daily foraging cycle, while processors focus on husking. This does come at a potential cost to the speed at which updated information about seed quality can be accessed by foragers. However, collecting poor quality seeds does not necessarily affect the welfare of larvae or other workers in the nest as seed quality control happens inside the nest as well. In-nest workers consumed more high-quality seeds than low-quality seeds, though we placed equal amounts into the nest.

Seeds as a delayed but easily "spreadable" informational cue

The information about seed quality for at least some novel seeds seems to be time-delayed. Slow collection on the first and sometimes second day that colonies encounter a novel seed has been reported for *P. rugosus* and *V. pergandei* harvester ants, with various metrics of seed acceptance and collection efficiency increasing dramatically in subsequent days (Johnson 1991). In previous studies of this system, we found that field colonies increase niger preference between collection days, but remained consistent in their preference during a 30-minute collection period (Chapter 2, 3).

The discovery that collection rose after the husking and consumption of high quality seeds, but not during the time unhusked seeds were in the nest or presented daily in the foraging arena, nor in the nests given no seeds to process, suggests that

this time delay is due to the difficulty of gathering seed quality information from seeds still in their shell. Seed shells are a mechanical barrier to nutritional access for many granivorous animals (Lundgren 2009, Borges 2015), and it is possible there are not enough cues on the shells of some seeds to easily identify a seed's nutritional content before it is husked. Even if there are odors that help differentiate between seeds (Gordon 1980), odor alone may not be enough to stimulate interest in a novel food item.

Bees similarly do not learn from novel odors in the hive, unless the odors are paired with taste stimuli – in fact, odor unpaired to a sugar reward causes a habituation response (Farina *et al.* 2005, Farina *et al.* 2007, Fernández *et al.* 2009). Ants have been shown to learn to pair novel odors with food (Cammaerts 2004, Dupuy *et al.* 2006, Huber & Knaden 2018), suggesting that associative learning is a possible mechanism for pairing cues on the shell with the nutrient content inside. There may also be a general aversion to novel food before it is tasted or observed to be eaten by others, as had been found for mammals (Galef 1988, Thornton & Clutton-Brock 2011). In our study, though we found groups of workers already showed a preference for unhusked sesame seeds before they had a chance to process them, they did increase the number collected further after processing them in the nest – though proportionally, the groups collected more niger than sesame. Collection of sesame before processing could be due to there being more oils on the shell, giving more immediate information about quality. More work could be done to explore to what extent odors on the seed undergo associative learning by harvester ants, and whether novelty delays seed collection.

Seeds in the nest are a source of information that has the potential to spread to more individuals than would be spread through one-to-one interactions like those that take place during trophallaxis (Richardson & Gorochofski 2015). Previous

observations of *Pogonomyrmex* and *Messor* harvester ants, as well as our own in this study, found that seeds were passed from worker to worker, with workers interacting with seeds in short feeding bouts, no one bout enough time to consume a seed entirely (Moggridge 1873, McCook 1879, Eddy 1970). Feeding on seeds may give ants direct access to nutritional quality content. The maxillary palps have receptors that help insects discriminate between foods (De Boer 2006). The cast-off shells, or even oils left over on the body after feeding may additionally promote information transfer. We observed workers chewing and carrying seed shells, and grooming workers that had previously helped open seeds. Many of the ants we saw forage were not seen to interact directly with seeds, so these indirect seed byproducts may be an important additional source of information. Leafcutter ants have been found to use food byproducts for learning; foragers can learn entirely from refuse dumps produced by their colony (Arenas & Roces 2017). Harvester ant foragers may similarly use dumps, or the act of taking trash out of the nest, to familiarize themselves with novel seed odors (Tschinkel & Hanley 2017).

Seed information is segregated by division of labor and space

In harvester ants, the movement of seeds is reliant on interactions between several different task groups that are spatially segregated in the nest. Worker age is highly related to their location in their natural nest, with the youngest, larvae and nurses, being located in the bottom portion and the oldest, foragers and midden workers, being located largely in the top and external to the nest (Porter & Jorgensen 1981, MacKay 1983, Gordon *et al.* 2005, Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017). Seeds must first move down to the larvae, and shells must move back up to the midden workers. In *P. badius* nests, a group separate from foragers or nurses, identified as transfer workers, bring seeds from the upper

levels where they are dropped off by foragers, into the lower levels where the brood, seed storage chambers, and nurses are located (Kwapich & Tschinkel 2013, Tschinkel *et al.* 2015).

We similarly found that the ants that collected the most seeds also spent more time exploring the foraging arena outside of the nest. Those who were processing seeds were located primarily inside the nest, mostly in the furthest and most wet chamber. Harvester ants bring whole seeds into the nest rather than husking them outside the nest (Lavigne 1969, Whitford 1978), and we did not observe seeds being opened in the foraging arena. In the small area of our observation nests, foragers had easy access to the chambers where seeds were being husked, with ample opportunity to interact with seeds, yet there was no relationship between foraging frequency and processing frequency. About 40% of foragers were never seen interacting with seeds, suggesting they do so infrequently enough that this behavior was often not captured. This suggests that foragers spend more time in locations where husked seeds are not present (outside of the nest), and are not behaviorally keen to help with the seed processing task. Given that *P. californicus* colonies can be composed of 2000-4500 workers (Erickson 1972, Johnson 2000, compare to our 300-500 worker groups) with a much larger physical nest area, increased colony size could lead to a more drastic spatial division between foragers and within-nest seed information in field colonies.

Because of this spatial segregation, nest architecture can affect the flow of information within nests (Pinter-Wollman *et al.* 2018, Tschinkel & Hanley 2017). Laboratory nests often do not appropriately capture the full effect of spatial complexity on within-nest interactions (Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017), and in our small laboratory nests, foragers brought in from the field quickly identified niger as the higher quality seed compared to ryegrass, collecting

88-99% niger out of niger and ryegrass by the fourth day after being given seeds to process, and 100% niger after being given opened seeds in the nest. In previous field studies, colonies collected between 50-100% niger out of niger and ryegrass by their fourth day of being given seeds to collect, with faster-collecting colonies collecting a lower proportion of niger. This discrepancy could be explained if information flow in the field was affected by colony size and nest structure. In our small nests, foragers could potentially taste all available seeds or interact with seed byproducts of both types of seed to form direct associational links. In larger field colonies, this may not be as likely an occurrence. The interaction between nest architecture and behavioral segregation could potentially modulate information flow and lead to differences between colonies in their ability to spread information to foragers. This would be an interesting future avenue for research effort.

Information transfer in the nest, amplification, independent assessment, and potential effects of the size of the interaction space

A social insect nest can be thought of as a network, with workers within tasks as nodes, and workers who connect between tasks as links (Fewell 2003, Weser 2005). In this framework, the food information network in the colony can be thought of as having food resources as nodes, with tasks involving food collection and processing as links connecting these resources. The food resources outside of the nest are moved by foragers into the nest, where they are stored for a time. The processing task moves closed seeds from "potential information" to "available information". The processing task is selective in which seeds are turned into available information, as we saw that more niger seeds were consumed than ryegrass seeds by our worker groups. Then, foragers interacting with these seeds or their byproducts, or through behavioral interactions with processors, become informed

and use this to select more seeds from the outside environment. Additionally, a surplus of seeds could be stored and provide a prolonged source of information, as workers age into the foraging task, as suggested by other studies (Johnson 1991, Weser 2005, Tschinkel & Hanley 2017). The nest environment also allows for consolidation of information from the environment, and given the opportunity, foragers do sample multiple available options. We saw this in this study, where providing different proportions of high-quality seeds (niger and sesame) in the nest did not affect forager preferences. This suggests that when seeds are both of high quality, the nest provides the opportunity for direct comparisons, to establish a better understanding of relative quality.

Conclusion

Harvester ant colonies use their nest as an information center (Seeley 1985), both to evaluate the quality of seeds, and expose information that stimulates the collection of novel seeds (Chapter 2, this chapter). Information is transferred between seed collectors, and seed processors, which are overlapping but separate task groups (Fewell 2003). This study opens new potential avenues of exploration into how the spatial structure of an information center, and the structure created through interactions between individuals performing different behaviors, shapes information flow through information centers in other animals (Brown 1988, Zentall & Galef 1988, Bijleveld *et al.* 2010).

FIGURES

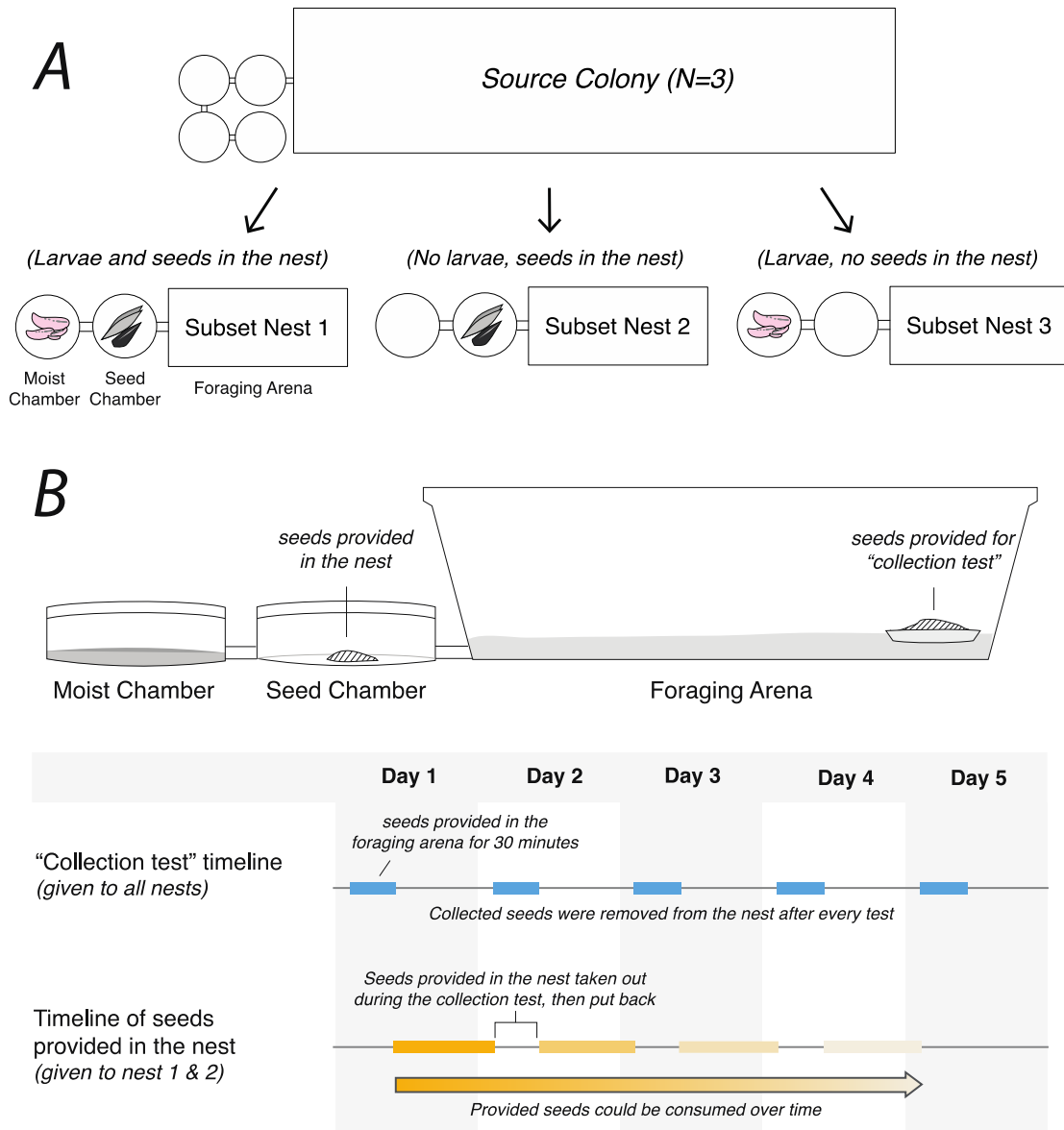


Figure 1: Experimental set-up and manipulations. (A) Workers from each of three lab colonies were divided into three subsets of workers and put into observation nests, each given a different manipulation. Subset 1 was given both larvae and seeds in the nest; Subset 2 was given no larvae, but was given seeds in the nest; Subset 3 was given larvae, but no seeds in the nest. Observation nest had a moist chamber, and a dry "seed chamber", connected to an open foraging arena with base covered in

sand. (B) The experiment was conducted over five days, with collection tests performed every morning. During collection tests, seeds were offered in the foraging arena for 30 minutes, then removed from observation nests immediately after, including any seed collected into the chambers or dropped in the foraging arena. The experiment began with a baseline seed collection test. Following this first test, seeds were placed into the 6 nests that received them for within-nest interaction. These seeds were removed and then placed back before and after each subsequent collection test.

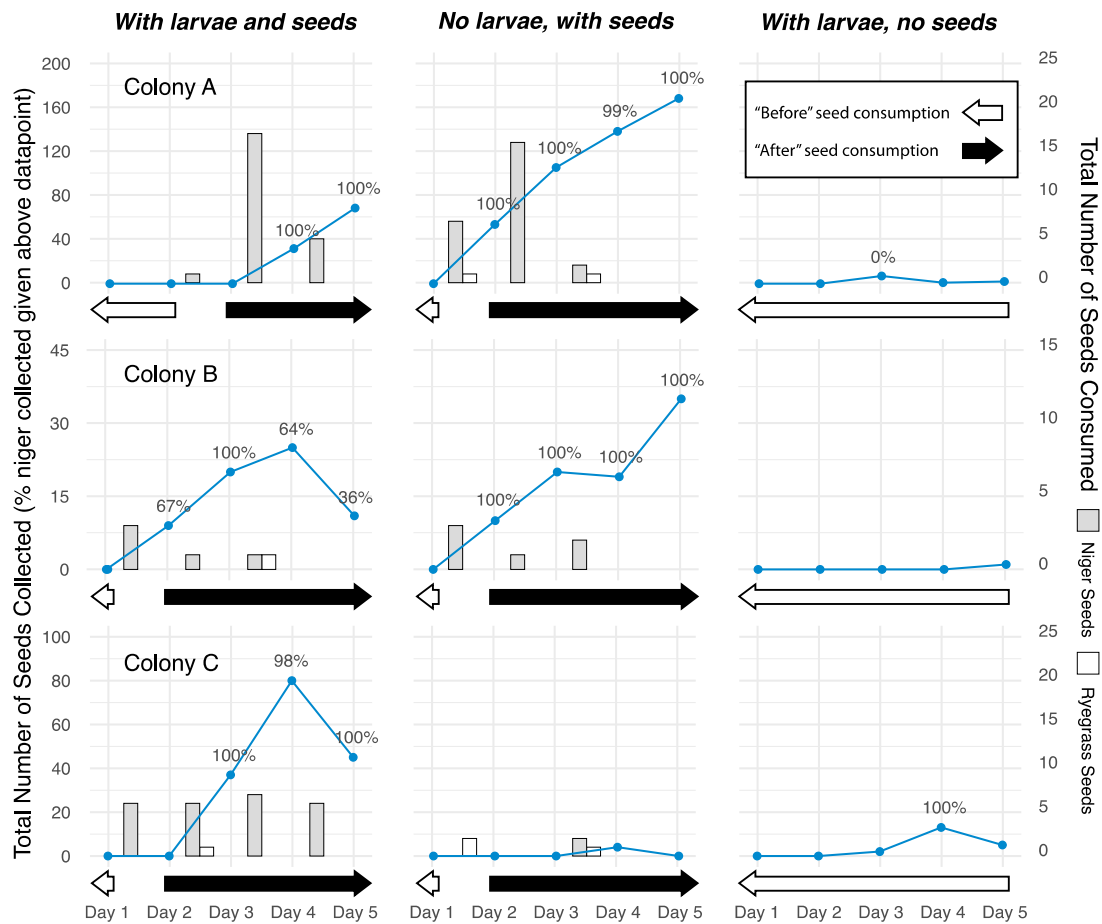


Figure 2: Seed consumption and collection by nests. Nine nests were created from equal numbers of workers selected from three laboratory colonies. Each row represents nests created from one colony. One nest per colony was placed into one of three treatments: (1) given larvae and seeds in the nest to process (left column), (2) no larvae but given seeds to process (middle column) or (3) given larvae but no seeds to process (right column). This figure shows the number of seeds collected (blue dots connected by lines), proportion of niger collected (black text above blue dots, only days where at least 6 seeds were collected are presented), the number of niger consumed (grey bars) and the number of ryegrass consumed (white bars) over five days. More seeds were collected as the days went on, and especially after seed

consumption (Day and Consumption $P < 0.001$, Interaction effect: $P = 0.04$, "before" and "after" consumption time spans shown with black and white arrows). Nests given seeds to process consumed more niger than ryegrass ($P = 0.02$).

Colony A Subset Given Seeds in the Nest, but no Larvae

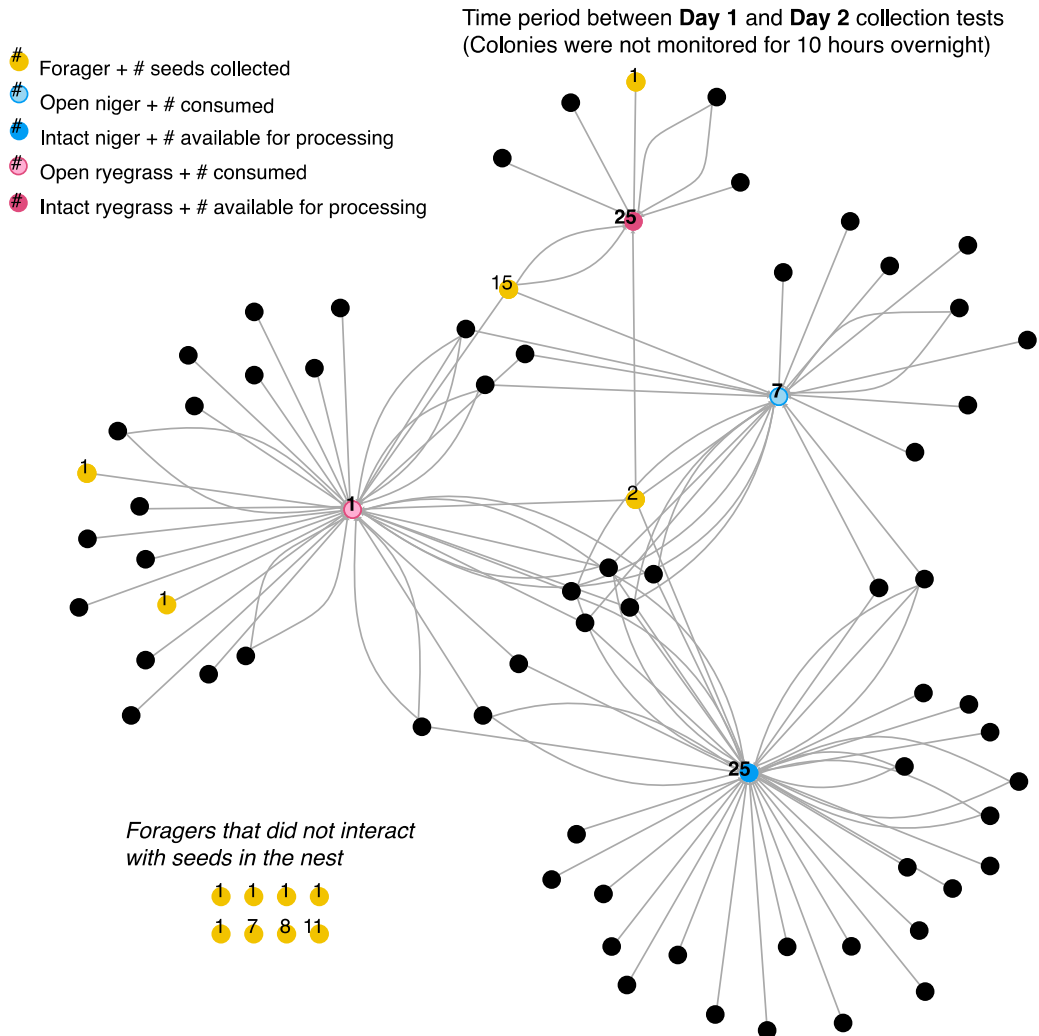


Figure 3: Interactions between workers and seeds in the nest. The interactions (biting and carrying events, lines) between within-nest workers (black dots), ants that foraged for seeds the next day (yellow dots), niger seeds placed in the nest (light blue dots), and ryegrass seeds placed in the nest (pink dots), in one subset of workers. Whether the seed was out of its shell (open circles with light fill) or still in it (filled circles) is shown. The number of seeds available for interaction, and the

number of seeds consumed during this time period are provided within pink and blue circles. Many of the interactions with intact seeds were from foragers biting a seed in the moist chamber, in order to husk it. This data is from the 24-hour time span before the first seeds were collected by the subset nest, barring a span of 10 hours overnight when subset nests were not observed. Only marked foragers that helped during this first seed collection event are shown; unmarked foragers also helped with collection. Workers and foragers in the nest had the opportunity to interact with both opened niger and ryegrass seeds. However, this nest (from source Colony A, given seeds to process but no larvae) collected no ryegrass the next day. Many more workers interacted with seeds than foraged on them. In this nest, 62 out of 68 marked seed-interactors (97%) did not forage for seed the next day.

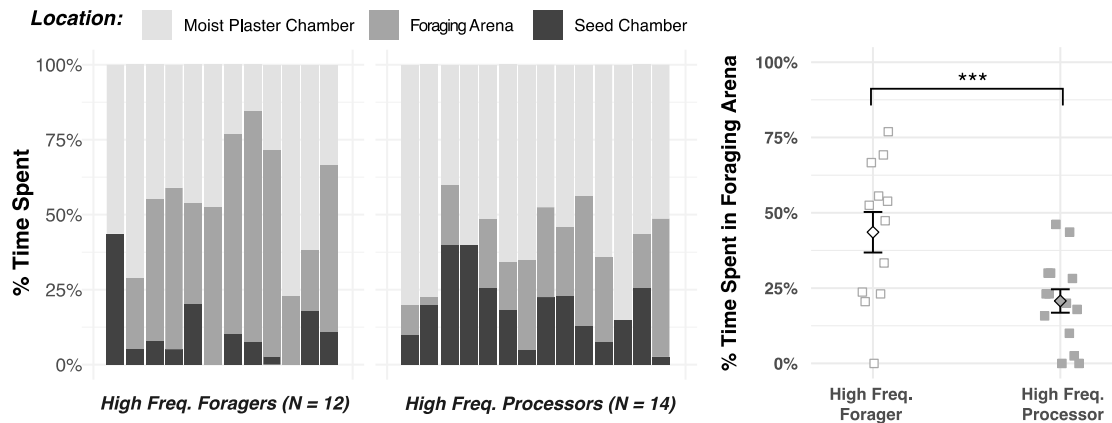


Figure 4: Locations of foragers and processors. The locations of high frequency foragers (workers who collected 4+ seeds) and high frequency processors (workers who bit seeds in 4+ behavior scans) were compared to see whether they were spatially segregated tasks in the nest. Though there was overlap in where these foragers and processors were located, the foragers spent more time overall in the foraging arena, where seed processing did not take place. In the figure to the left, bars represent individuals and the proportion of time they spent in one of three chambers. In the figure to the right, squares represent the proportion of time an individual spent in the foraging arena, diamonds show means, bars show SE, colors represent foragers (white) and processors (grey), *** $P < 0.001$. In-nest seed interactions were monitored within a 24-hour time span before collection occurred, though nests were not monitored overnight.

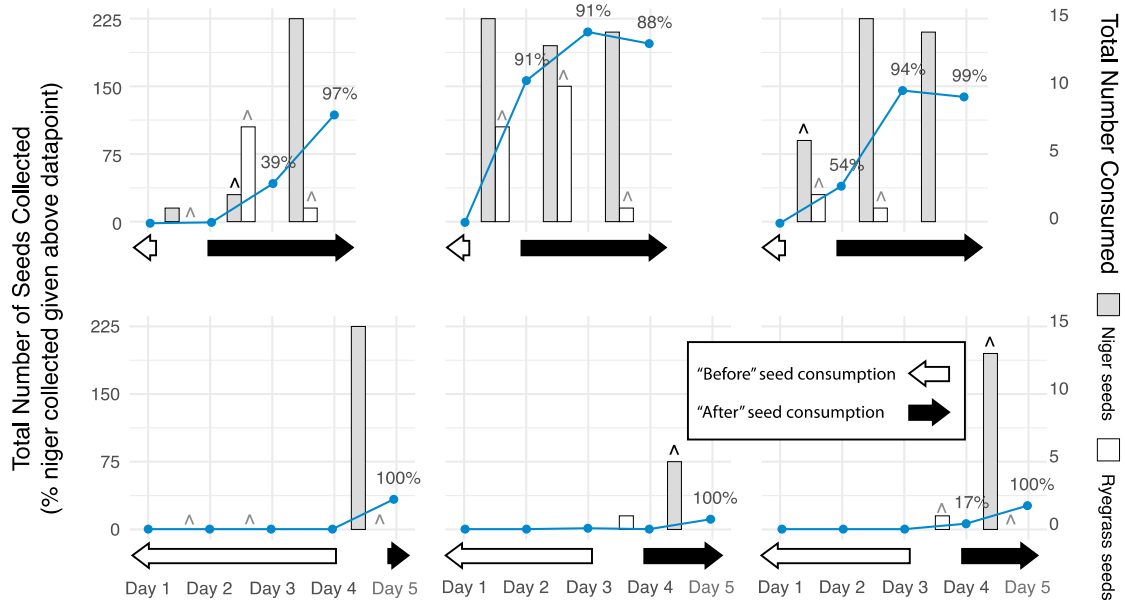


Figure 5: Seed consumption and collection by foragers from the field. Groups of foragers from field colonies were brought into the laboratory and their foraging behavior was examined. Each graph represents the behavior of one colony's foragers, with the foragers who were given opened seeds and another day of collection tests in the bottom row. This figure shows the number of seeds collected (blue dots connected by lines), proportion of niger collected (black text above blue dots, only days where at least 6 seeds were collected are presented), the number of niger consumed (grey bars) and the number of ryegrass consumed (white bars) over five days. ^ symbol indicates some seeds were husked but not consumed (grey: ryegrass, black: niger). Seed consumption was summed over the time period between collection tests (e.g. the time period between Day 1 and Day 2 collection tests). More seeds were collected "after" consumption than "before" ($P < 0.001$, time spans shown with black and white arrows). Foragers consumed more niger than ryegrass ($P < 0.01$), and collected a higher proportion of niger by the last day of collection ($P < 0.001$).

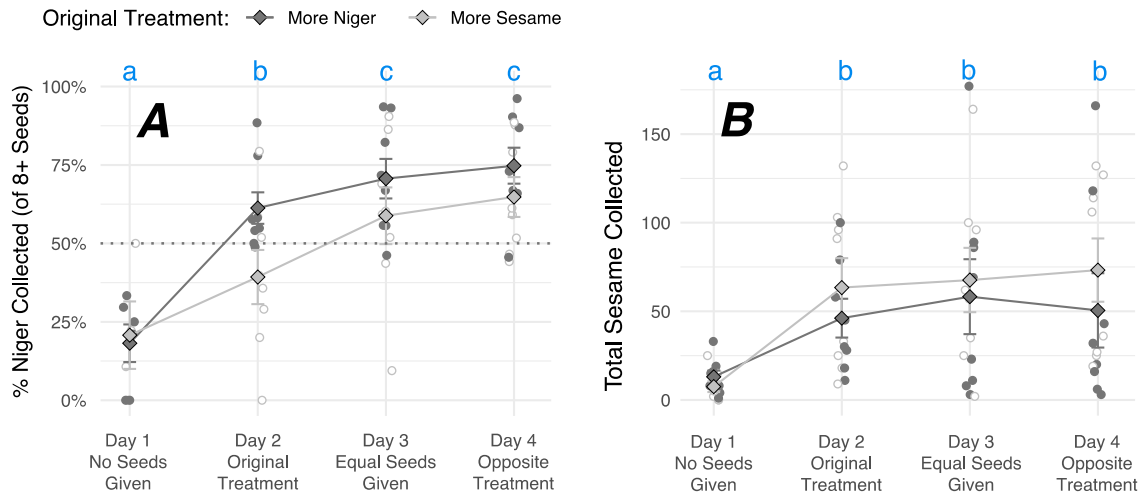


Figure 6. Interaction between seed processing and preference in field workers.

Foragers and upper-nest workers from field colonies were brought into the laboratory and their foraging behavior was examined. Changes in (A) the proportion of niger collected and (B) the number of sesame seeds collected out of niger and sesame are shown. No seeds were given in the nest before Day 1, though seeds were provided in the foraging arena during the collection test on this day. Different proportions of seeds were placed in the nest for processing on subsequent days. Foragers and top-nest workers collected the fewest sesame seeds but showed the highest sesame preference on the first day of collection (Day 1), before seeds were given to the nest for processing ($P < 0.001$). Niger preference also increased between Days 2 and 3 ($P < 0.01$). The number of niger collected similarly increased between Days 1 and 2, and the number of niger collected on Day 4 was higher than Day 2 ($P < 0.001$, graph not shown). There was no significant difference between groups initially given more niger, or more sesame, in their preference or the number of seeds they collected over days, suggesting the proportion of seeds consumed did not influence preference tendencies in this study. Blue letters denote significance differences between days.

CHAPTER 5

CONCLUSION

In this dissertation I investigated how changes in collective decision-making are coordinated through a decentralized system of diverse individual choices. I also explored how individuals and colonies integrate new information over successive rounds of collective decision-making. To do this, I looked at both individual-level decision-making, novel information integration, and collective changes in colonies of *Pogonomyrmex californicus* harvester ants as they made choices about which seeds to collect. My findings suggest that information flow from collection by foragers leads to colony processing by processors, processing feeds into an information exchange within the colony, with new information becoming available for current and perhaps future foragers to evaluate at their discretion. Then, foragers make new, perhaps adjusted choices that feed back into colony collection (Fig. 1). This process is necessary for promoting collection of a novel seed species, and can iterate as the colony learns to collect less of a poor-quality one. Foragers may also gain information and change individual behavior without greatly affecting the preferences of the colony. This loop is the route used by colonies to use past collection experience to fine tune foragers and to foster collective changes, while maintaining decentralized decision-making.

To build this framework, I found through my research that colonies can learn through integrating information about seed quality in the nest through processing the seeds (Chapter 2, Chapter 4). When novel high-quality seeds first appear in the environment, some seeds may be collected, but seed processing is necessary to stimulate a large colony response (Chapter 4, Johnson 1991). Further processing of collected seeds likely provides new information; colonies continue to refine choices after the initial day of collection, likely through in-nest processing (Chapter 2, Rissing

1981). Though in-nest processing slows colony-level learning, it perhaps allows for a more efficient distribution of workers among tasks. Foragers are not the main seed processors of the colony, which allows foragers to focus on searching for and collecting seeds, while processing takes place in parallel (Chapter 4). Furthermore, colonies can maintain learned seed preferences over a time period without seeds in the environment; some even come to more strongly prefer a better quality seed over this time, without needing to interact with the seed in the environment (Chapter 2). This suggests that seed processing in the nest can allow for continued colony improvement, and may perhaps facilitate information transfer to new foragers as they enter the work-force.

These results show that colony learning in harvester ants is distributed among various subgroups of workers, and necessitates communication between different tasks (Fewell 2003). Colony-level learning similarly involves communication between different component parts in leaf choices of leafcutter ants, and in nest choice in *Temnothorax*. In leafcutter ants, foragers learn to associate leaf odor with detrimental effects on the fungus garden, only after the leaves have been integrated into the garden by fungus-tenders, or after they have been placed into the trash (Herz *et al.* 2008, Saverschek *et al.* 2010, Arenas & Roces 2018). In *Temnothorax*, colonies learn to be more efficient through brood care workers transferring brood more quickly to the workers involved in carrying colony members to the new nest (Franks & Sendova-Franks 2013).

An understanding of colony learning in harvester ants would also further benefit from a better understanding of the behavior of processors. Namely; how does individual preference affect which seeds they process, and do they learn to process faster over time? If processing efficiency increases with experience, this may spread information to a larger foraging force more quickly, and speed up learning.

Additionally, careful study is still needed to determine whether foragers perform associative learning between olfactory cues on the seed shell, and nutritional cues from husked seed. It is likely that they do; other ants are able learn nutritional information that aids them in foraging (Josens *et al.* 2009, Huber & Knaden 2018, Saverschek & Roces 2011). We would also gain from a better understanding of how colony memory is maintained: to what degree is colony long-term memory due to workers maintaining a long-term memory, and how might prior experience with seeds in the nest affect the choices of workers as they transition into foraging from other inside-nest tasks (Oettler & Johnson 2009, Tschinkel *et al.* 2015).

Only a subset of workers are foragers, yet they are tasked with providing food for the colony; foragers that have a good understanding of seed quality are crucial to the efficient provision of nutrients. I found that, while individuals sometimes randomly change their preference between days of collection, when the discrepancy in seed quality was high, most changes in preference were in favor of the higher-quality seed (Chapter 3). Foragers integrate information about seed quality in the nest, after seeds have been processed by the colony; some of this information may come from direct interaction with (eating of) husked seeds (Chapter 4). Accounts of harvester ant behavior as far back as the 19th century have described seed-eating by workers; workers were seen to bite seeds to eat fine particles and oils (Moggridge 1873, McCook 1879, Wheeler 1902). However, other ways of transferring information, such as byproducts of seed processing like oils on the body or chaff, or antennation interaction, may also be important for information transfer and merit further research. Leafcutter ants gain information about leaf quality from trash, and harvester ants use antennation to regulate foraging (Arenas & Roces 2018, Pinter-Wollman *et al.* 2013).

Collective decisions in animals, including humans, often make use of many imprecise individual estimates and benefit from individuals who use diverse inference strategies or respond differently to stimuli (Surowiecki 2004, Sumpter 2006, Oldroyd & Fewell 2007, Couzin 2008, Krause *et al.* 2011, Fujisaki *et al.* 2018, Sasaki & Pratt 2018). Though individuals often try to optimize their decision-making, which reduces individual error and makes individual choices more similar to each other, sometimes a single best choice cannot be agreed upon. This is especially true if the best choice can only be estimated, or if individuals are unable to compute all the available information themselves (Surowiecki 2004, Sasaki & Pratt 2018). I found that individuals within colonies of harvester ants also preserve diverse preferences for seeds of similar value, yet that these preferences remain flexible within and between days. Diversity is preserved even with many repeated days of information gathering, and despite the colony maintaining a stable preference within range of other colony preferences in the population (Chapter 3).

The proximate cause of this diversity was beyond the scope of my study, but future studies could explore how cognitive limitations, differences in choice determination heuristics, or experience in the nest promotes inter-individual differences (Mallon *et al.* 2001, Burns & Dyer 2006, Sasaki & Pratt 2012, Grüter & Leadbeater 2014). It would be particularly interesting to characterize the proximate cause of inflexible specialist foragers, and how they affect colony collection efficiency.

Colonies had different likelihoods of specializing to high quality-seeds seed in the field (Chapter 2,3), compared to worker subsets in the laboratory (Chapter 4). Field colonies rarely exclusively selected the highest quality seed out of a mix of high and low quality seeds, however, groups of foragers isolated in our laboratory nests – including foragers collected from field colonies – collected a higher proportion of the

high-quality seeds, and more than half selected only high quality seeds, compared to whole field colonies. This suggests that foragers do try to optimize seed choice when they have enough information, and that the high variation for high and low quality seeds exhibited in Chapters 2 and 3 was perhaps due to inefficient information transfer within the colony. One possible explanation is that the smaller laboratory nests provided foragers more access to information than natural nests do. Natural nests are large with many chambers (Tschinkel 2015). The shape of the nest and the spatial division of workers within it may affect information flow between processed seeds and foragers (Kwapich & Tschinkel 2013, Tschinkel & Hanley 2017, Pinter-Wollman *et al.* 2018). This could be an explanation for why nests showed a tradeoff between speed and accuracy (Chapter 2). Further study on the effect of nest structure on colony learning could provide interesting results. On the other hand, some foragers in laboratory nests did maintain collection of low quality seeds, suggesting that foragers use diverse heuristics to evaluate seeds.

Animal collectives live in a complex and a constantly changing environment. Their effectiveness depends on the individuals within it striving to make good decisions given their individual limitations. Cohesion is facilitated by the movement of information between individuals. My studies add to our understanding of the factors that shape adaptive changes, mainly: diversity, flexibility, and the ability to use information from past collective decisions towards future goals.

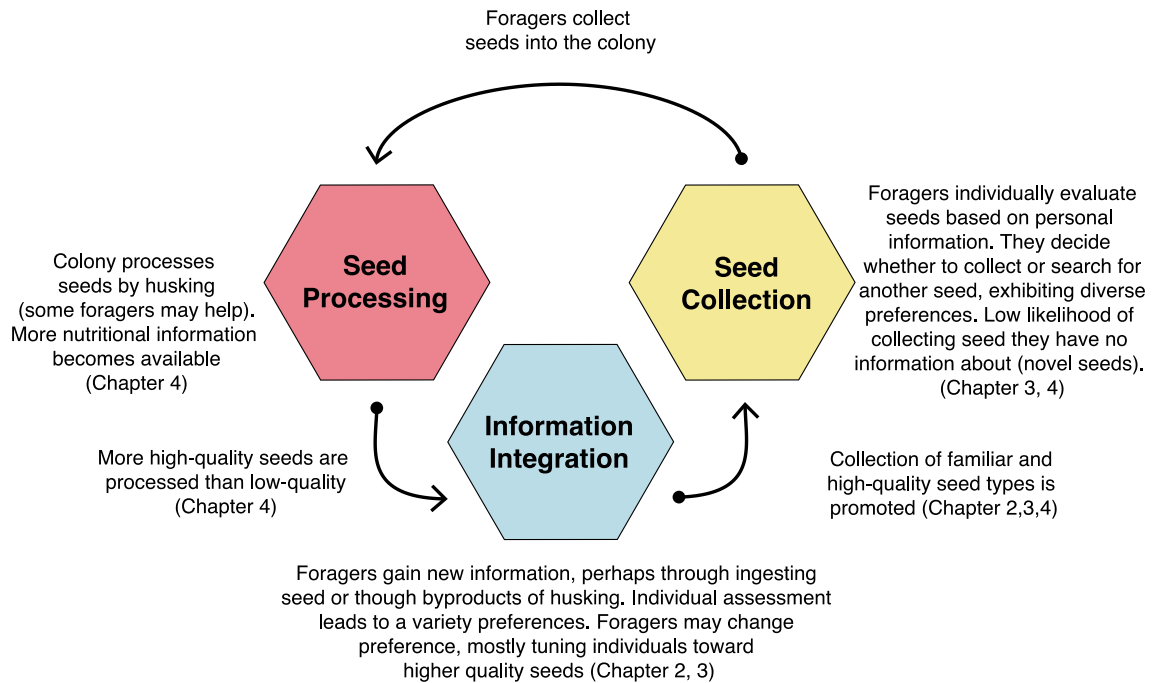


Figure 1. Diagram of colony information integration. This is the proposed process through which collective decisions change over time. Individuals maintain decentralized decision-making and while flexibly responding to new information from the nest. This facilitates colony responses to new seeds in the environment, and colony flexibility in response to information from past choices, to enable colony learning.

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APPENDIX A
SUPPLEMENTARY MATERIAL

LIPID ANALYSIS

We ran chemical assays to test for lipid content of Niger (*Guizotia abyssinica*), annual ryegrass (*Lolium multiflorum*), and Sesame (*Sesamum indicum*) seed. Seeds were dried at 45°C until seed weights no longer changed (at least six days). Dried seeds were ground for 30 s at 30 Hz using a Retsch MM 400 ball mill. Lipid analysis was performed by extraction using diethyl ether, according to methods modified from Williams *et al.* (2011). Ten replicates of 300 mg of each seed type was wrapped in filter paper and weighed. The parcels were fully submerged in diethyl ether, each in their own 10 ml test tube. Diethyl ether was replaced every day for three to four days ($N = 8$ for three days, $N = 2$ for four days). Weight of dried ground seed after lipid extraction was subtracted from the weight before lipid extraction, to find the lipid fraction. Samples extracted for four days had similar weights to those extracted over three days (SM Table 1). The values calculated for niger and sesame are similar to values previously reported in the literature (Shahidi *et al.* 2013, Yermanos *et al.* 1972). Lipid content has not previously been analyzed for *L. multiflorum*.

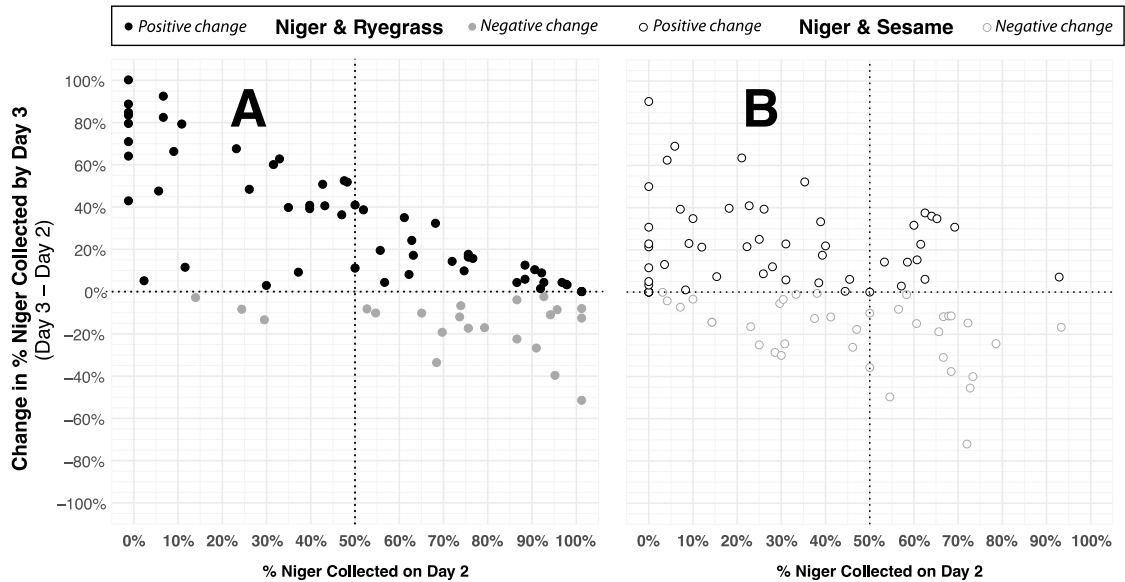
Seed	Three-day extraction (N=8)	Four-day extraction	Pooled (Days 3-4)	Reported values	Reference
Niger	41% ± 0.2%	40.2%, 39.4%	40.8% ± 0.3%	40%	Shahidi <i>et al.</i> 2013
Ryegrass	3.3% ± 0.2%	5%, 3.4%	3.5% ± 0.2%	N/A	N/A
Sesame	50.2% ± 0.2%	50.5%, 50.5%	50.2% ± 0.1%	53.1%	Yermanos <i>et al.</i> 1972

SM Table 1. Lipid content of seeds based on lipid extraction with diethyl ether. Mean lipid content of seeds provided ± standard error. Three days of extraction gave similar results to four days of extraction. Values from our methods are compared to other values reported in the literature.

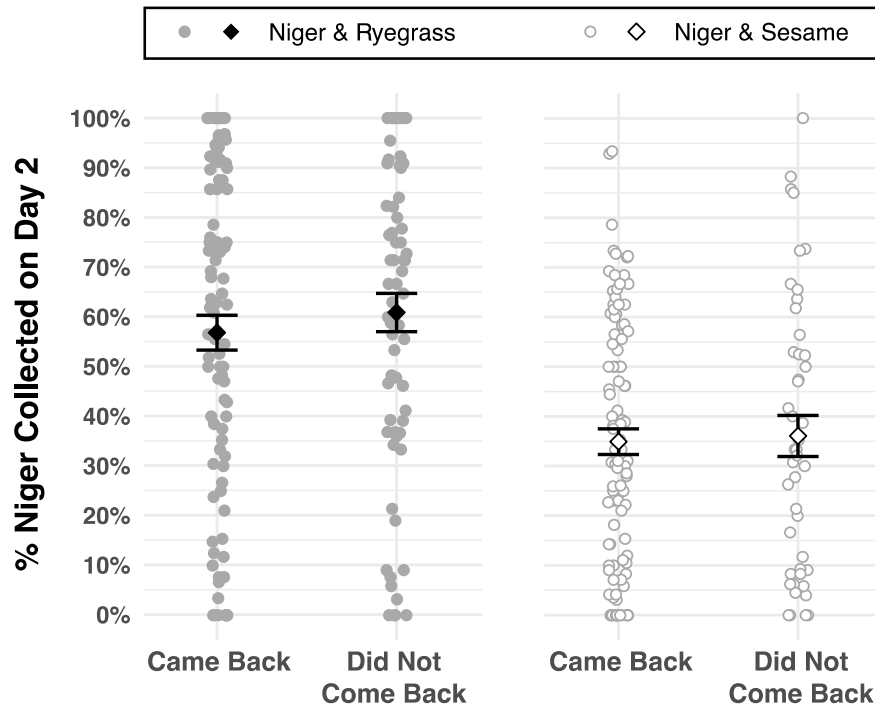
APPROXIMATING SEED AMOUNTS GIVEN TO COLONIES

The number of each seed type that was given to colonies was approximated by volumes marked on a centrifuge tube. Seeds of each type were counted by hand into separate 50 ml centrifuge tubes. A line was drawn to mark the volume taken up by 100 and then 200 seeds. Using these marked tubes, lines denoting 300, 600, and 900 seeds were also marked on the tube, creating tubes with specific volume markings for the different seed types.

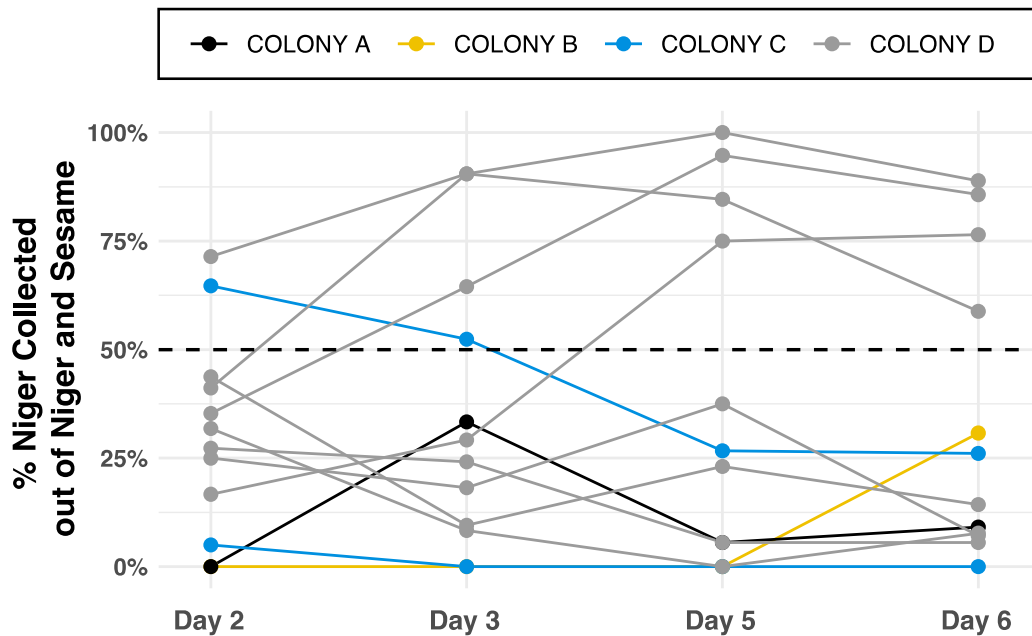
SUPPLEMENTARY MATERIAL FOR CHAPTER 3



SM Figure 3.1: Changes in % niger collected by individuals in relation to their beginning niger preference on Day 2. Circles represent the behavior of foragers. Black circles represent a positive change in niger preference, grey circles represent a negative change in niger preference (i.e. increase in ryegrass or sesame preference). Horizontal dotted lines highlight $y=0$ (no change in preference). Vertical dotted lines denote ants that began with an equal preference for both offered seeds. (A) Foragers collecting from niger and ryegrass (large difference in caloric value, filled circles). (B) Foragers collecting from niger and sesame (smaller difference in caloric value, open circles). (Ryegrass & Niger $N = 80$; Sesame & Niger $N = 89$). Niger & ryegrass $N = 80$ ants from 7 colonies; Niger & sesame $N = 89$ ants from 7 colonies).

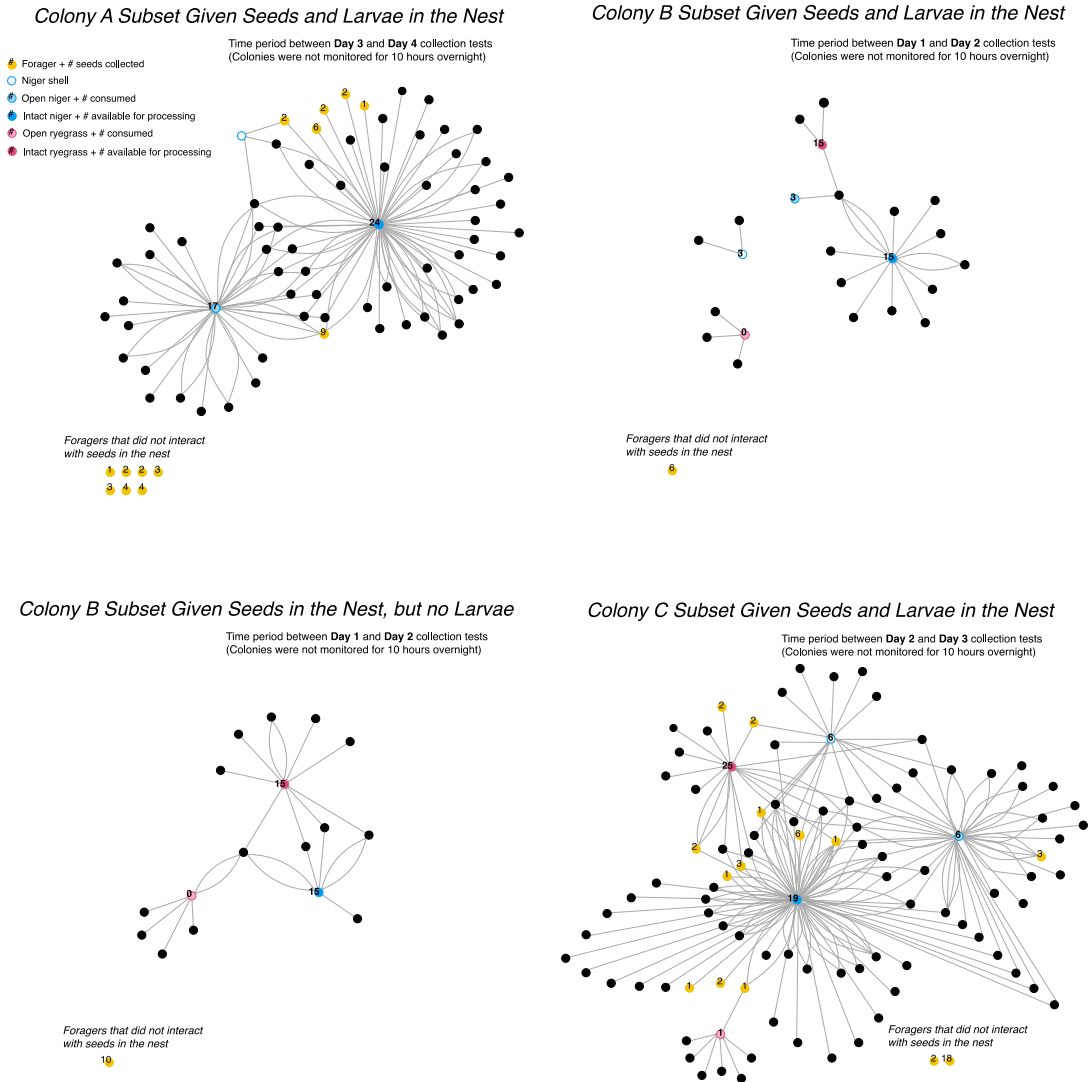


SM Figure 3.2: Relationship between preference and likelihood of abandoning collection. Circles represent the Day 2 preference of individual foragers in relation to whether they abandoned the pile on Day 3. Diamonds represent means and bars represent standard error. There was no relationship between Day 2 preference, and the likelihood of abandoning collection on Day 3, either for ants collecting from niger and ryegrass (large difference in caloric value, grey circles, black diamonds), or from those collecting from niger and sesame (smaller difference in caloric value, white circles, white diamonds). Only ants that collected at least 10 seeds on Day 2 were used for this analysis.



SM Figure 3.3: Individual's preference changes over six days of collection. Circles represent individual preferences of marked foragers that collected at least 10 seeds on all four days that preference was tested (Day 2,3,5 and 6). Lines connect individual preferences across days, and colors denote the colony the forager belonged to. Most foragers changed their preferences. Individuals from Colony D generally became more biased over time.

SUPPLEMENTARY MATERIAL FOR CHAPTER 4



SM Figure 4.1: Interactions between workers and seeds in the nest. The interactions (biting and carrying events, lines) between within-nest workers (black dots), ants that foraged for seeds (yellow dots), niger seeds placed in the nest (light blue dots), and ryegrass seeds placed in the nest (pink dots), in four nests containing subsets of workers from three source colonies (Colony A, B, C). Whether the seed was out of its shell (open circles with light fill) or still in it (filled circles) is shown. Interactions with

niger shells (blue circle no fill) are also shown. Interactions with niger shells likely were due to the fact that they were oily. The number of seeds available for interaction, and the number of seeds consumed during this time period are provided within pink and blue circles. Many of the interactions with in-tact seeds were from foragers biting a seed in the moist chamber, in order to husk it. This data is from the 24-hour time span before the first seeds were collected by a nest, barring a span of 10 hours overnight when nests were not observed. Only marked foragers that helped during this first seed collection event are shown; unmarked foragers also helped with collection.

	ESTIMATE	STD. ERROR	Z VALUE	PR(> Z)
(INTERCEPT)	-3.5426	1.2778	-2.772	0.00557
DAY	1.3617	0.3366	4.045	5.22e-05
CONSUMPTION (AFTER)	6.3638	1.6094	3.954	7.68e-05
COLONY B	-1.5184	0.5852	-2.595	0.00947
COLONY C	-0.8452	0.5718	-1.478	0.13938
DAY:CONSUMPTION (AFTER)	-0.9777	0.4235	-2.308	0.02098

SM Table 4.1: Coefficients of a model fit using a Negative binomial regression analysis testing the effect of Day (1-5) and Seed Consumption (Before or After) on the number of seeds collected. Laboratory source colony was included as a fixed effect. Both Day of experiment and consumption had an effect on the number of seeds collected, but consumption had a stronger effect than day.