Behavioral, Physiological, and Transcriptomic Variation among colonies of the Red Harvester Ant (*Pogonomyrmex barbatus*)

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Abstract:

Social insect colony behavior arises within a specific ecological context from patterns of interactions of nestmates with each other. The neurophysiological basis of behavior in the social insects has primarily been studied in the context of behavioral differences among nestmates, for example between nursing and foraging workers. Many conserved pathways that regulate behavior in other animals, such as the neurohormonal and biogenic amine neurotransmitter signaling pathways, are also involved in generating behavioral variation among social insect nestmates. Less is known from a molecular perspective about how worker neurophysiological variation is associated with colony-level, collective, behaviors. In this thesis, I consider how physiological differences among colonies of the red harvester ant (*Pogonomyrmex barbatus*) are associated with colony behavioral differences, and with the evolution of collective behavior.

Chapter 1 uses transcriptomic profiling of forager brains from *P. barbatus* colonies to explore differences in gene expression between groups of colonies that vary in how they regulate foraging in dry conditions. Forager brains of different colonies significantly varied in brain biogenic amine titers, as well as in the expression of multiple neurophysiological signaling pathways involved in regulating foraging in social and solitary insect species. Pharmacological experiments demonstrated that increases in forager brain dopamine titer resulted in increases in foraging activity, whereas decreases in brain dopamine decreased foraging activity.

Chapter 2 investigates the relationship between colony foraging behavior, colony reproductive success, and forager desiccation physiology. Foragers from colonies that reduced foraging activity in dry conditions lose water and motor coordination more rapidly than foragers from colonies that did not reduce foraging in dry conditions. Manipulative experiments in the field show that hydrated foragers go on significantly more foraging trips than their unhydrated nestmates, and that this effect increases in strength as conditions get drier.

Chapter 3 uses RNA-seq on single forager brains to investigate how variation in gene expression variation within and among colonies is associated with colony traits and with the degree of protein coding sequence constraint over evolutionary time. Hundreds of genes had expression and coexpression patterns correlated with colony traits. Gene

coexpression modules were significantly differentially utilized among colonies, and these modules were enriched in neurophysiologically-relevant functions related to the regulation of biogenic metabolism and signaling. Loci that are more central to coexpression networks tend to be better correlated with colony traits, and are evolving under increased coding sequencing constraint relative to less central loci.

Chapter 4 uses pharmacological experiments on individually-marked foragers to characterize how heterogeneity among nestmates in foraging activity was related to the effect of hydration and dopamine treatment on increasing overall foraging trips. The overall stimulatory effect of hydration and dopamine treatment was not due to a small subset of ants. The relationship between humidity and foraging activity was more variable within a day and between colonies, than between different treatment groups.

Natural selection shapes patterns of behavioral variation among ant colonies via the differential reproductive success of colonies with different phenotypes. Colony behavioral variation arises from a complex nexus of both heritable and non-heritable factors. All molecular factors exert their influence on colony behavior only to the extent that they modulate worker physiology to alter how workers respond to different types of interactions. This thesis begins to characterize the neurophysiological basis of variation among red harvester ant colonies in foraging behavior.

Contributions:

Here I describe my role in each of the Chapters.

Chapter 1:

I performed the field work, collected the samples, dissected and prepared ant samples, performed the statistical analyses, and was the primary author of the manuscript. This Chapter was published in 2018 as:

Friedman DA, Pilko A, Skowronska-Krawczyk D, Krasinska K, Parker JW, Hirsh J, DM Gordon, "The Role of Dopamine in the Collective Regulation of Foraging in Harvester Ants". *iScience* 8, 283–294 (2018).

Chapter 2:

I performed the field work related to the hydration experiments (but not the desiccation experiments). I performed the statistical analyses for the paper and was the primary author of the manuscript. This Chapter was published in 2019 as:

Friedman DA, Greene MJ & DM Gordon, "The physiology of forager hydration and variation among harvester ant (*Pogonomyrmex barbatus*) colonies in collective foraging behavior. *Science Reports* 9, 5126 (2019).

Chapter 3:

I performed the field work, coordination, and preparation of ant samples for RNA-seq. I was a
primary collaborator on the bioinformatic analyses, and was the primary author of the manuscript.

Chapter 4:

I performed the field work, statistical analyses, and primary writing of this manuscript.

Acknowledgments:

Symbolic languages does not adequately express my gratitude to:

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Family; my nestmates.
Stanford University; my nest.
Friends and Colleagues; my ecosystem.
Professor Deborah M Gordon; my primary advisor.
Thesis Committee members and Stanford Faculty; my mentors.
You, the reader of this document; that it & I might be of service to you.
To those not listed, and those who are departed: I feel your presence as well.
To past Spiritual and Intellectual heroes, you keepers of the faith; guiding lights.
And of course to the Ants, who sacrifice their seeds & brains so that we might learn.
Together you've taught me that "*laboratorium est oratorium*".

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Introduction

Diverse ant species thrive in ecosystems from the deepest rainforests to the driest deserts [1,2]. In all ant species, the collective behavior of the colony is the aggregate outcome of nestmate workers, each responding to their local experiences [3–5]. Because the regulation of ant colony behavior is distributed (without central control), colonies can respond to challenges exceeding the sensory, cognitive, and physical capabilities of any single worker [6–8]. Ant colony behavior evolves via changes to how workers respond to the rate and type of interactions that they experience [3,4,9]. Thus the role of a worker ant in colony behavior is akin to the role of a neuron in a brain: the neuron responds only to local cues, yet participates in decentralized cognitive processes occurring over entire brain regions [10–13]. The resilience, flexibility, and tractability of ant colonies make them attractive systems in which to learn about how distributed systems function and evolve [3,14].

The challenge of behavioral ecology in ants is to understand how colony-level outcomes, such as the adaptive allocation of colony labor, arise from the response of workers to their local interactions [4,15]. This requires an integrated understanding of the natural history of the ant species [16–18], the algorithmic processes by which interaction patterns among workers result in colony outcomes [19], and the neurophysiological mechanisms that mediate the worker's response to stimuli [20–23]. Here we focus on the case of desert ants. Desert ants express behavioral and physiological adaptations that allow them to live in harsh climates. Examples of physiological adaptations in desert ant workers include increases to their maximum thermal limit that allow activity during the hottest parts of the day [24–26], and changes to their exoskeleton that result in reduced cuticular water loss rates [27–30] or direct dissipation of thermal energy [31]. Examples of behavioral adaptations in desert ant workers include living in humidified underground nests, or adjusting the times of day that they are active outside of the nest to disfavor especially desiccating periods [29,32,33].

From neuroanatomical and molecular perspectives, the ant brain is similar to the brain of other insects [23,34–38]. The neurophysiological basis of behavior in insects has been most explored in the fruit fly *Drosophila melanogaster* [39–42], and to a lesser extent in the

honey bee *Apis mellifera* [43–46]. Many of the molecules involved in regulating foraging behavior in ants also regulate foraging activity in flies and bees, such as protein kinase-based signal transduction pathways (“*foraging”* gene) [47–49] and peptidergic neurohormones [50–53]. The biogenic amine neurotransmitters, such as dopamine and serotonin, regulate many types of behavior in social insects, including the regulation of foraging activity [21,23,54,55]. In ants and bees, foraging workers tend to have higher brain dopamine-to-serotonin ratios than nursing workers [20,23,37], and experimental increases in dopamine signaling increase foraging activity [56–58].

Natural selection occurs whenever there is heritable variation in a phenotype that is correlated with reproductive success in a population [59]. As the colony is the reproductive unit in ants, the physiological and behavioral adaptations of workers are the outcome of natural selection acting on populations of colonies [18,60,61]. Thus natural selection shapes worker behavior and physiology whenever variation in colony-level phenotype is associated with differences in colony reproductive success [18,19,62]. Natural selection is efficient in populations of ant colonies to the extent that phenotype-fitness correlations are heritable between the generations of colonies [63–67]. It is important to note here that most molecular studies in social insects have focused on the differences in gene expression and metabolism between nestmates that differ in reproductive status (e.g. queen vs. worker), or workers that differ in task performance (e.g. nurse vs. forager) [37,54,68–70]. This characterization of the tissue-specific molecular differences among groups of nestmates has provided insight into the origin and elaborations of the eusocial lifestyle in the social insects [71–74]. However, relatively few studies have characterized natural variation among colonies in gene expression or metabolism, of ants of the same task group [75–77]. These molecular differences among colonies could reflect the worker-level basis of behavioral variation among colonies, and thus a possible source of the phenotypic heterogeneity of ant colonies in natural settings [76,77].

In this thesis I focus on the evolution of behavior and physiology in red harvester ants (*Pogonomyrmex barbatus*), with an emphasis on the physiological basis of variation among colonies in how they regulate foraging in dry conditions. Foraging *P. barbatus* workers leave the nest to acquire seeds for the colony, which provide nutrition and hydration for the colony [78,79]. Colonies of *P. barbatus* vary in how they regulate

collective foraging activity in dry conditions [80,81]. These differences among colonies in foraging activity are stable across years, and associated with differences among colonies in reproductive success [64]. Additionally, daughter colonies of *P. barbatus* resemble their mothers in the days which they reduce foraging activity [64], suggesting that collective behavior differences among colonies are heritable. Thus variation among colonies in how they regulate foraging in dry conditions is an ecologically important trait which may have a molecular component that is being shaped by selection acting on colony behavior. Here I present a series of transcriptomic, pharmacological, and behavioral ecological studies that address the physiological basis of variation among *P. barbatus* colonies in foraging activity.

In Chapter 1, "*The Role of Dopamine in the Collective Regulation of Foraging in Harvester Ants*" [82], we characterize molecular variation in gene expression and biogenic amine metabolism among colonies of *P. barbatus* that differ in collective foraging behavior, and perform pharmacological experiments in natural settings to support the role of dopamine in regulating individual foraging activity. First we used RNA sequencing on *P. barbatus* forager brains to determine how gene expression differences were associated with the behavioral differences between two sets of colonies that did or did not strongly reduce foraging in dry condition. Gene expression and coexpression analysis highlighted differential usage of neurohormonal pathways, as well as biogenic amine signaling and metabolism. Dopamine specifically is known to regulate foraging activity in ants and other insects [20,42,56,58], and multiple metabolic enzymes, receptors, and downstream signaling proteins related to dopamine were differentially expressed between the two groups of colonies. To test the behavioral effect of increased dopamine on foraging activity, we used mass spectrometry to validate a protocol for significantly elevating forager brain dopamine levels. In two years of field experiments with 9 *P. barbatus* colonies, exogenous dopamine treatment significantly increased foraging activity, and administration of a drug that reduces foraging activity (3-iodotyrosine, [83]) reduced foraging activity. Colonies significantly varied in forager brain dopamine-to-serotonin ratio, the first demonstration of variation among ant colonies in neurophysiology in natural settings. This Chapter demonstrates that colonies that differ in behavior also differ

in forager brain gene expression and biogenic amine metabolism, and demonstrates that increases in forager brain dopamine lead to increases in foraging activity.

In Chapter 2, "*The physiology of forager hydration and variation among harvester ant (Pogonomyrmex barbatus) colonies in collective foraging behavior*" [84], we test how colonies vary in forager water loss physiology, and the effect of exogenous hydration on foraging activity. Colonies that reduce foraging in dry condition, and colonies with offspring colonies, were found to lose water at a faster rate, and lose motor coordination more rapidly in desiccating conditions. In a separate set of colonies, exogenous hydration increased foraging activity relative to unhydrated ants the day after administration, and the effect of hydration on increasing foraging activity was stronger as conditions became increasingly dry. This Chapter demonstrates that colonies that differ in behavior and reproductive success also differ in forager water loss physiology, and that manipulations of forager hydration physiology can lead to changes in forager behavior, especially in dry conditions.

In Chapter 3, "*Forager brain gene expression is variable among ant colonies in natural settings and associated with differences in colony-level traits*", we use RNA sequencing on single forager brains to characterize patterns of gene expression variation among nestmates, in a set of colonies that vary in forager brain biogenic amine metabolism and natural behavior. Hundreds of genes show expression patterns significantly correlated with colony behavior and physiology, highlighting biogenic amine signaling metabolic and signaling pathways. Analysis of gene coexpression patterns shows that colonies significantly differ in the use of multiple coexpression modules that are also significantly correlated with colony traits, and functionally enriched in loci related to neurophysiology. Evolutionary genomic analysis shows that loci with expression patterns more central to coexpression modules are better correlated with colony traits, and also are evolving under increased coding sequence constraint. This Chapter demonstrates that transcriptomic differences in single forager brain among colonies are associated with differences in colony traits, enriched in neurophysiological

mechanisms including biogenic amine signaling, and may reflect the genes involved in the evolution of collective behavior over deep time.

In Chapter 4, "*The physiology of forager hydration and variation among harvester ant (Pogonomyrmex barbatus) colonies in collective foraging behavior*", we use pharmacology experiments on individually marked ants to test the effect of exogenous and hydration on forager trip distributions. Hydration and dopamine treatment both have a significant effect on forager trip distribution shape and mean number of trips. Trimmed-means tests demonstrate that hydration significantly increases the mean number of trips each forager makes relative to the unhydrated control, and that dopamine treatment significantly increases the mean number of trips each forager makes relative to the hydration group. This Chapter demonstrates that the effect of hydration and dopamine on increasing *P. barbatus* foraging activity is due to a slight increase in activity from many treated ants, not from a large increase in activity of a few treated foragers.

Chapter 1

The role of dopamine in the regulation of foraging in the red harvester ant

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Abstract

This study investigated how variation in the neurophysiology of individual foragers is linked to ecologically important variation among colonies in foraging behavior. Colonies of the red harvester ant (*Pogonomyrmex barbatus*) vary in the collective regulation of foraging activity in response to dry conditions. RNA sequencing of forager brain tissue was used to characterize differences in gene expression between two sets of colonies that differ in the regulation of foraging. The forager brain transcriptomes of the two sets of colonies differed in the expression of genes involved in biogenic amine metabolism, including phenylalanine hydroxylase and tyramine beta-hydroxylase. Pharmacologically induced increases in the brain dopamine titers of foragers led to a significantly increased number of foraging trips in field colonies. Variation among colonies in the dopamine metabolism of ants may lead to variation in the collective regulation of foraging.

Summary statement: Two groups of harvester ant colonies that differed in foraging behavior showed differences in expression of biogenic amine metabolism genes. In field experiments, raised brain dopamine titers increased foraging activity.

Key words: Dopamine, Foraging, *Pogonomyrmex*, Collective behavior, Ants, Social insects.

Introduction

Decentralized biological systems from brains to insect colonies are regulated by distributed control. Group outcomes arise through the collective response of individual agents to their local stimuli. In social insects, colony outcomes are regulated through interactions among workers [3,6,85,86]. For example, in leaf-cutting ants, foraging behavior is mediated by the rate of head-on collisions between incoming and outgoing foragers [87], and in the desert ant *Cataglyphis,* foragers convey information about food availability through patterns of tactile interactions [5]. Colonies of social insects vary in collective behavioral traits. For example, colonies of the harvester ant *Pogonomyrmex occidentalis* vary in the duration of the daily foraging activity period [88], apparently due to differences among colonies in the thermal sensitivity of foragers [89]. Similarly, honey bee colonies vary in foraging behavior [63], perhaps due to differences in forager sensitivity to nutritional cues [90–92]. The outstanding question about variation among colonies in collective behavior is how it arises from individual differences in physiology and response to environmental factors [75–77].

Here we investigate the neurophysiological basis of variation among colonies in the regulation of foraging behavior in the red harvester ant, *Pogonomyrmex barbatus.* Colonies of *P. barbatus* forage in the desert for seeds which provide both food and water [4]. Foragers lose water while out in the desert sun, and water loss increases with temperature and low humidity [27,28]. To manage the tradeoff between food accumulation and water loss, colonies adjust foraging activity to changes in ambient conditions, especially changes in humidity [64,93,94]. Colonies of *P. barbatus* regulate foraging collectively, through interactions among foragers in the entrance chamber of their nest. An outgoing forager is stimulated to leave the nest by its rate of olfactory interactions

with incoming seed-carrying foragers [95–98]. Since a forager continues to search until it finds a seed, the rate of forager return is related to food availability [80].

Colonies of *P. barbatus* vary in the regulation of foraging activity, as measured by the proportion of days that they are active outside the nest [80], baseline foraging rate [81,94], response to reduction in the rate of returning foragers [81], and how likely they are to reduce foraging activity in dry conditions [64]. Behavioral differences among colonies of *P. barbatus* persist from year to year [64,80,81], though conditions differ between years [94]. Since workers live only a year [99], persistent differences in colony behavior suggest that successive cohorts of workers inherit similar responses to environmental conditions from their mother, the colony's single queen. Additionally, daughter colonies resemble parent colonies in response to hot, dry conditions, similarly indicating some heritable factor [64]. Colony lifetime reproductive success is associated with the regulation of foraging in poor conditions [64,100]. Colonies of *P. barbatus* store seeds in the nest [101], and reduced foraging activity does not affect colony survival [64].

Differences among social insect colonies in the regulation of foraging activity may be related to differences in the physiology of individual foragers. In honey bees and several ant species, there are transcriptomic differences between queens and workers, as well as between foragers and workers performing other tasks [36,37,68,102]. For example, in *P. barbatus* there is brain-specific differential expression of the *foraging* gene within colonies, between foragers and workers performing other tasks [48,103]. However, there have been very few high-throughput transcriptomic studies that compare ants of similar physiological state across colonies (e.g. in founding *Pogonomyrmex californicus* queens, [104]), and no transcriptomic comparisons of foragers from different colonies in any ant species.

Several biogenic amine compounds have been implicated in foraging behavior in the social Hymenoptera, including dopamine and octopamine. In many ant and bee species, foragers have increased brain dopamine titers relative to workers performing other tasks [23,105–107]. Pharmacological manipulation of dopaminergic signaling in ants and bees indicates that increases in dopamine signaling lead to increases in foraging activity [56,58,108–110]. In many animals, dopamine signaling plays a conserved role in the regulation of foraging activity [22,111–114], and increases in dopamine titers and signaling are

consistently associated with overvaluation of risky options in vertebrate and invertebrate species [23,57,108,115]. Octopamine, another biogenic amine neurotransmitter, has also been implicated in the regulation of foraging activity in social insects. In honey bees, brain octopamine titers are higher in foragers than nurses [105,116] and treatment with octopamine accelerates the behavioral transition to foraging work [117]. In ants, octopamine plays a role in aggression and nestmate recognition [23,118,119]. However, octopamine has not consistently been associated with differences among nestmates in individual foraging activity [54,106,120].

Here we examine the neurophysiological differences among foragers that might give rise to variation among colonies in the regulation of foraging. We compared colonies of *P. barbatus* to investigate how brain gene expression differences in the metabolism of biogenic amines are associated with behavioral variation among colonies in foraging behavior. We compared foragers from two sets of colonies: 1) colonies that strongly reduce foraging in dry conditions and 2) colonies that do not strongly reduce foraging in dry conditions. Our transcriptomic analysis showed that foragers from the two sets of colonies differed in brain gene expression of transcripts related to biogenic amine signaling and metabolism, and that the list of transcripts upregulated in colonies that strongly reduced foraging activity was enriched in the GO term "dopamine metabolic process". We then developed a non-invasive method to pharmacologically manipulate the brain dopamine content of foragers, and performed experiments in the field to measure foraging activity in foragers with increased brain dopamine titers to determine whether this increases individual foraging activity.

Materials and Methods

Transcriptomic Methods

Foragers of *Pogonomyrmex barbatus* were collected into liquid nitrogen between 06:00-08:00 on 8/20/2014, from colonies at a long-term field site near Rodeo NM, at which all colonies have been identified and censused since 1985 [100]. Foragers were collected as soon as they left the nest entrance, not carrying anything, and moved off the nest mound onto a foraging trail or fan. Foragers were collected from 6 mature colonies in which foraging behavior had been monitored in previous work. Three of the colonies

tended to show strongly reduced foraging on dry days relative to humid days, in counts of foraging activity made in in 2011 and 2012 [64], while 3 colonies did not strongly reduce foraging activity on dry days. There were similar differences between some of the colonies in each group in foraging activity measured in 2009 [81]. The three colonies that strongly reduced foraging on dry days all had offspring colonies; in the other group of three colonies, none had offspring colonies [100]. Other work indicates an association between reproductive success and the tendency to reduce foraging on dry days [64], and shows that colonies are consistent from year to year in foraging activity [80]. No ethical precautions were required for this study.

Samples were shipped from the field site to the laboratory in liquid nitrogen and stored at -80C. Whole brains were cleanly dissected away from muscular, glandular, and connective tissue in cold RNAlater buffer (Thermo Fisher Scientific, Fremont, CA, USA). Dissected brains were frozen at -80C until RNA extraction. Total RNA was extracted from dissected brains using a Direct-zol RNA extraction kit (Zymo Research, Irvine, CA, USA). RNA concentration was assessed using Qubit 2.0 RNA HS reagents (Thermo Fisher Scientific, Fremont, CA, USA) and purity using a NanoDrop (ND 2000, Thermo Fisher Scientific, Fremont, CA, USA). Total RNA was assessed for quality using a BioAnalyzer tapestation (Agilent Technologies, Santa Clara, CA, USA), and samples with RNA Integrity Number (RIN) > 8.0 were used to make RNA libraries. 3 libraries were made for each of 6 colonies. Each library consisted of poly-AAA+ mRNA extracted from the pooled dissected brains of 3 foragers. Libraries were generated using Illumina's TruSeq Stranded mRNA Sample Prep Kit (Illumina Inc., San Diego, CA, USA) using 200 ng of total RNA. Libraries were multiplexed and sequenced with 75 basepair paired-end reads (PE75) on an Illumina HiSeq2500 Rapid Run (at the UCSD IGM Genomics Core).

RNA-seq reads were demultiplexed and FASTQ files generated using CASAVA v1.8.2. Read quality was analyzed with FastQC [121]. The *P. barbatus* reference transcriptome was downloaded from NCBI [122] (accessed: 4/5/2016, updated assembly Pbar_UMD_V03). For the kallisto/sleuth differential gene expression analysis pipeline, the reference transcriptome was indexed by kallisto v.0.42.5 [123]. RNA-seq reads were pseudoaligned to the indexed reference transcriptome with kallisto. The k-mer bias correction option was

implemented and 100 bootstrapped transcriptomes were generated for each library to estimate the variation of expression for each transcript. The kallisto output was analyzed using the sleuth v0.28.0 package [124] in R v.3.3.0 [125]. Across the 18 libraries from 6 colonies, there were a total of ~355 million 75 basepair paired-end RNA-seq reads. All workers sampled in this study are part of the same interbreeding J1/J2 population of *P. barbatus* at a long-term study site [100] and no colonies or libraries displayed a mapping bias to the reference transcriptome used.

The reference transcriptome was annotated with GO terms using Blast2GO 3 [126]. GO terms were determined by querying translated protein sequences against the NCBI database of Arthropod proteins using blastx. An E-value cutoff of 10^-4 was used to call significant blastx homology. A maximum of 15 Arthropod hits were pulled from the NCBI results and saved in XML format. GO terms for *P. barbatus* transcripts were inferred from orthology to the BLAST hits, as assessed in 6/2015. Additionally, InterProScan [127] was used to query each transcript's predicted protein translation against protein databases (Profile HMM models: CATH-Gene3D, Superfamily, PIRSF, TIGRFAMs, Panther, Pfam, and Smart. Profile models: HAMAP, Prosite, ProDom. Pattern models: PRINTS, Prosite). InterProScan protein domain-level GO terms were merged with the GO terms inferred by blastx homology. Annotation augmentation (ANNEX) was performed in Blast2GO. Finally the annotations were trimmed using a true-by-path validation rule, in which redundant general GO terms are replaced by more-specific GO terms for each transcript (e.g. "ion homeostasis" is implied in the term "calcium homeostasis"). Lists of transcripts identified via differential expression or co-expression modules were tested for GO term enrichment using Fisher's Exact Test in Blast2GO. Multiple test correction was implemented according to the False Discovery Rate [128] and results were filtered at an FDR < 0.1. The resulting GO enrichments were semantically reduced to their most specific child terms using Blast2GO.

To generate the co-expression network, transcript-level expression levels for each library were loaded into Cytoscape v3.4.0 [129]. Transcripts that had less than 1 tpm of expression in any colony were excluded from this analysis. We used the ExpressionCorrelation plug-in [130] in Cytoscape to generate a transcriptome-wide co-expression network, using gene expression data from all 6 colonies considered together. The final co-expression graph

included 1,933 nodes representing specific transcripts, and 6,885 edges representing a strong pairwise correlation between the two connected nodes.

Dopamine Oral Administration Methods

All solutions were administered to ants as follows: an ant was collected with an aspirator and placed in a 50 mL tube. The 50 mL tube was immersed in ice until the ant stopped moving. The ant was tapped out onto a paper towel, and gently grasped by a rear leg. A small dab of oil-based paint (Uniball Uni-Paint PX-20) was placed on the back of the ant's head using a small toothpick, using a unique color for each of the two treatment groups. To feed the solution to the ant, 0.2 μL of aqueous solution was placed on the mandibles of the anesthetized ant. The droplet is captured between the mandibles via surface tension. The contents of the solution used in each experiment are described below. After administering a solution to an ant, the ant was placed on its lateral side, and it eventually began to move around.

Dopamine Brain Quantification Methods

To assess the effect of orally ingested dopamine on individual ant brain dopamine titers, we used mass spectroscopy to quantify the brain biogenic amine levels of single ants after feeding them either water or water with dissolved dopamine at a concentration of either 3 mg/mL or 30 mg/mL (Sigma-Aldrich, St. Louis, MO, USA). Water was chosen as a solvent to isolate the effect of exogenous dopamine on brain dopamine titers. At 1 and 3 days after treatment, ants were frozen in liquid nitrogen at 10 a.m. and brains were dissected out in cold phosphate-buffed saline (PBS) (Electron Microscopy Sciences, Hatfield, PA, USA). Brain dopamine titers of single *P. barbatus* workers were quantified via mass spectroscopy with an internal radiolabeled dopamine standard (Cambridge Isotope Laboratory, Tewksbury, MA, USA). Full dopamine quantification methods are provided in the Appendix.

Field Behavioral Assays

Experiments were performed with ants from 10 colonies between 7/18/2016 and 8/3/2016. The colonies were near but not on the long-term study site [100]. Ants were

collected 1-2 meters from the nest entrance, identified as foragers because they were not carrying anything, and walked in a straight line off the nest mound onto a foraging trail or fan [131]. The ants were brought back to the laboratory at the Southwest Research Station and randomly sorted into two treatment groups. Each group was administered either 0 mg/mL or 3 mg/mL dopamine in 1x PBS. For our behavioral assays we used PBS as the vehicle because PBS would mask any possible taste effects of dopamine, and also the two solutions would not differ drastically in osmolarity, which might affect salt balance and thus foraging activity in the desert. Dopamine solutions were made immediately before administration. There were 100 ants per treatment per colony per day. Ants were returned to their nest later the same day. Foragers of *P. barbatus* tend to be the oldest ants in the colony, and workers marked while foraging do not later switch to perform other tasks [132].

Observations began early the following day before foraging began. Counts of foraging trips by marked ants began when the first marked ant was observed to leave the nest. For colonies with a single foraging trail, a foraging trip was recorded when a marked ant crossed a line ~2 meters from the nest entrance on the trail. For colonies with more than one foraging trail, a foraging trip was recorded when a marked ant was observed leaving the nest entrance, carrying nothing, and walking in a straight line off of the nest mound [131]. Two colonies were observed each morning in alternating observation periods of 15-20 minutes. During each 15-20 minute observation period, we continually recorded the number of foraging trips made by marked ants, recording counts in 30-second intervals. Foraging counts ended when the colony had stopped foraging for the morning and no ants had left the nest for 3 minutes. The overall number of foraging trips taken by marked ants per colony ranged from 126 to 588.

For each colony we calculated the increase in foraging trips made by dopamine-treated ants as ratio of the total number of observed foraging trips made by dopamine-treated ants divided by the total number of observed foraging trips made by control-treated foragers. This design minimizes the effects of day, as all comparisons are being made between two groups of foragers within the same colony on the same day.

Results

Transcriptomics.

We found significant gene expression differences in the brains of foragers of two sets of colonies of *P. barbatus* that differed in how strongly they reduce foraging activity in dry conditions. Using the kallisto/sleuth RNA-seq analysis pipeline [123] to align reads to the *P. barbatus* reference transcriptome [122], we detected 273 transcripts out of 20,387 transcripts in the reference transcriptome to be significantly differentially expressed in whole forager brains between the two sets of colonies (q-value < 0.01 using FDR correction, 6 colonies, 3 libraries per colony of 3 forager brains each, average of 20,723,464 mapped reads per colony). Of these 273 significantly differentially expressed transcripts, 113 transcripts were upregulated in colonies that do not strongly reduce foraging on dry days, and 160 transcripts were upregulated in colonies that strongly reduce foraging on dry days. Across the whole transcriptome, per-transcript mean expression levels were very similar in the two groups of colonies ($r^2 > 0.99$). A linear Principal Component Analysis (PCA) was used to visualize the expression data of all transcripts in sleuth [123], and colony transcriptomes did not cluster together by behavioral type.

Brain tissue of *P. barbatus* foragers from colonies that strongly reduced foraging on dry days displayed higher expression of transcripts homologous to the metabolic enzymes phenylalanine hydroxylase (3.17-fold change, XM_011648879.1, q-value = 0.0049) and tyramine beta-hydroxylase (1.55-fold change, XM_011649732.1, q-value = 0.00011, 31.6 vs. 20.4 tpm, other transcript from same locus XM_011649733.1 upregulated 1.44-fold in low-foragers, 47.3 vs. 32.9 tpm, q-value = 1.60E-07). Several specific transcripts from other pathways known to play a role in the regulation of insect foraging behavior were upregulated in the brains of foragers from colonies that strongly reduced foraging in dry conditions. Brain tissue of foragers from colonies that strongly reduced foraging activity on dry days had significantly higher expression of the FMRFamide receptor (1.69-fold change, XM_011639920.1, q-value = 0.0036), an allatostatin peptide hormone (1.2-fold change, XM_011640492.1, q-value = 1.77e-05) and the hypertrehalosaemic prohormone (1.82-fold change, XM_011643332.1, q-value 1.60e-07), three genes that are important in insect neurohormonal signaling and the regulation of foraging in solitary insects [133–135]. Foragers from colonies that strongly reduced foraging on dry days had significantly higher expression of an inositol

monophosphatase (3.1-fold change, XM_011632239.1, 5.38E-05), a phosphoinositide phospholipase (1.33-fold change, XM_011632265.1, q-value = 0.0021), and the GSK3β interaction protein (1.54- fold change, XM_011646061.1, q-value 0.0002). These three genes are involved in the inositol-phosphate signaling pathway [136], which is implicated in the transcriptomic changes between nurse and forager honey bees [137].

To assess functional enrichment of differentially expressed genes, a Gene Ontology (GO) annotation was generated with Blast2GO [126,138] to describe the *P. barbatus* reference transcriptome (Appendix). Relative to all annotated transcripts in the reference transcriptome, the list of 273 differentially-expressed transcripts was not significantly enriched or depleted for any GO terms (Fisher's Exact Test, FDR < 0.1 cutoff). We then considered GO term enrichments for the lists of transcripts upregulated in each group of colonies, relative to the other. The list of 160 transcripts upregulated in colonies that reduced foraging in dry conditions was significantly enriched in the GO term "hormone activity" (p = 1.9e-6, FDR = 9.5e-3). There were several other GO terms enrichments that had significant single-test enrichment p-values in this list of 160 transcripts, but not at a significant FDR (e.g. "dopamine metabolic process" p = 6.98e-4, FDR = 0.34; "neuropeptide signaling pathway", p = 5.57e-4, FDR = 0.34). The list of 113 transcripts upregulated in foragers from colonies that did not strongly reduce foraging on dry days was not statistically enriched in any GO terms (FDR < 0.1).

Topological co-expression analysis

To further examine the functional relationships among brain-expressed transcripts in *P. barbatus* foragers, we performed a transcriptomic co-expression network analysis using Cytoscape [129]. Gene co-expression networks represent transcripts as "nodes" and highly correlated expression levels between two transcripts across samples as connecting "edges". Gene co-expression analyses are used to understand how complex phenotypes are regulated through coordinated changes in the expression of modules of genes [139], and have recently been used in transcriptomic studies of various ant species [69,140]. We found transcript-transcript correlations among all transcripts with expression level above 1 transcript per million (tpm) in all colonies [141], using a correlation cutoff ($r^2 > 0.93$) that generated distinct modules of transcripts. The final co-expression network consisted of