Title: Queen personality type predicts nest guarding behavior, colony size, and the subsequent collective aggressiveness of the colony

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25 **ABSTRACT**

26 The founding individuals of a new group or lineage are uniquely poised to have long-lasting
27 effects on their descendants. Here we explore how the behavioural traits of new
28 foundresses influence their task allocation strategies and the downstream collective
29 behaviour of their colonies. We collected individual paper wasp (*Polistes metricus*) queens
30 from the wild, and measured their boldness, aggressiveness, exploration, activity, and time
31 budgets in the laboratory. We then provided queens with food and nest materials and
32 waited for their workers to eclose. We subsequently tested for links between queen
33 behavioural traits (e.g., boldness, aggressiveness) and the collective aggressiveness of their
34 colonies, as estimated by the number of workers that responded to and stung a model
35 predator. We found that bolder queens (i.e., queens that were less likely to leave their nests
36 after repeated exposure to an agonistic stimulus) spent more time nest guarding, reared
37 more workers, and produced groups that were less responsive to a model predator. Thus,
38 there appears to be parent-offspring, or queen-worker, resemblance in the degree to which
39 individuals remain on their nests in different social contexts (solitary queen vs. queen + her
40 worker group). To our knowledge this represents the first study to explicitly test for links
41 between individual variation in foundress behavior, worker production, and inter-colony
42 collective behaviour; the results highlight the possibility that cross-contextual and trans-
43 generational behavioural links could be a reasonably common and potentially important
44 phenomenon in complex societies.

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INTRODUCTION

The founders of a new lineage or group, for better or for worse, often have large influences on the subsequent performance of their descendants. Perhaps the easiest way founders influence their descendants is through direct genetic transmission, though other mechanisms may include learning, chemical manipulation, or through inheriting environments altered by the founders. Social animals in particular provide us with an intriguing system to study how the traits of founders influence the downstream collective traits of their future colonies. This is because many social species (e.g. some ants and wasps) often initiate a new colony as a single founding queen (haplometrosis) rather than founding colonies in groups (pleometrosis) or through colony fission such as in honeybees. In instances where founding events are a cyclical component of a species' life history, we might predict novel adaptations to evolve in response to selection imposed during the founding stage. Additionally, given the vastly different ecologies experienced in the founding (solitary) vs. worker (social) phases of haplometrotic colonies, we might expect different or even opposing selection pressures to act upon these distinct stages.

In some social spiders, for instance, colonies often arise from the long distance dispersal ('ballooning') of one or a few mated females into new habitats (Schneider, Roos, Lubin, & Henschel, 2001; Wickler & Seibt, 1986). The resulting colonies are often highly inbred (Duncan, Riechert, Fitzpatrick, & Fordyce, 2010; Henschel, Lubin, & Schneider, 1995). In one species of spider (*Anelosimus studiosus*), the behavioural phenotypes of colony founders predict the downstream collective behaviour and success of their descendent colonies, even generations later (J. N. Pruitt, 2012, 2013). Studies on other social systems have highlighted the fact that inter-colony differences in collective

behaviour (aka 'colony-level personality') can be important for predicting colony success, and several of these studies have proposed that the traits of founders could be an important determinant of such inter-colony differences, reviewed in Modlmeier et al. (2014) and Bengston & Jandt (2014). For a sense of generality, associations between 'colony-level personality' and group success have been noted in many social organisms, including ants (Chapman, Thain, Coughlin, & Hughes, 2011; Holway & Case, 2001; Lichtenstein, Pruitt, & Modlmeier, 2015; Modlmeier, Keiser, Shearer, & Pruitt, 2014), bees (Raine, Ings, Ramos-Rodriguez, & Chittka, 2006; Wray, Mattila, & Seeley, 2011), wasps (Bruschini, Cervo, & Turillazzi, 2005; Karsai & Wenzel, 1998), termites (Mizumoto & Matsuura, 2013), spiders (Keiser & Pruitt, 2014; Jonathan N. Pruitt, Grinsted, & Settepani, 2013), birds (Lucy M. Aplin, Farine, Mann, & Sheldon, 2014; L. M. Aplin et al., 2013), fish (C. Brown & Irving, 2014; Dyer, Croft, Morrell, & Krause, 2009) and even caterpillars (Dussutour, Nicolis, Despland, & Simpson, 2008).

The importance of colony level personality observed in many systems raises the question: *to what extent do founder traits help determine such inter-colony differences?* Testing for links between founder traits and subsequent group traits is important (i) because only rarely can we actually identify the mechanisms driving inter-group differences in collective behaviour, despite the fact that group traits are often important for determining individual fitness and group success, (ii) there exists the possibility for cross-contextual performance trade-offs when or if the traits that beget success in founders yield suboptimal collective behaviour in groups, and (iii) transgenerational and cross-contextual links in behaviour potentially can imply a genetic component to the behaviour being examined, although there are other plausible explanations as well.

Social insects have served as the choice model for studies on colony-level personality, and it stands to reason that the traits of a colony founder (i.e., queens) could play a large role in determining colony phenotypes. Unfortunately, identifying links between founder behaviour and group behaviour in many social insect species can be impractical or daunting. This is because foundresses (and their colonies) of many species are frequently encased in nests surrounded by sediment or various biomaterials (Theraulaz, Bonabeau, & Deneubourg, 1998), often preventing direct observation of both queen and worker behaviour. Additionally, in most social insects, the queens and workers behave in strikingly different ways, thus preventing direct comparisons in their behaviour (M. J. F. Brown, 1999; M. J. F. Brown & Bonhoeffer, 2003) and some species lack a distinct founding phase (Kronauer, Schoning, Pedersen, Boomsma, & Gadau, 2004; Seeley, 1985). Paper wasps (Vespidae: Polistinae) are an excellent system for investigating the consequences of queen behaviour on colony behaviour, because these animals circumvent many of these difficulties. We selected *Polistes metricus* for this study due to the tendency for its queens to found nests solitarily (Bohm & Stockhammer, 1977), rather than in groups, which is common in many paper wasp species (E. A. Tibbetts & Reeve, 2003; West, 1967).

Polistes metricus is native to North America and is distributed throughout most of the central and eastern United States and parts of southeastern Canada. In the early spring, single queens emerge from winter dormancy to found new nests. They nest naturally in trees and shrubs, but are more commonly found nesting in the eaves of houses and other manmade wooden structures (Isely, 1922). Their nests are small and open-faced, making observations in both the field and laboratory easy. Before workers emerge, the solitary

foundress performs a wide variety of tasks necessary for colony success: egg laying, nest building, foraging for food, water, and nesting material, nest defense, and brood care. Once workers begin to emerge in mid-spring, queens increase their egg laying and allow their workers to perform all other tasks. Toward the end of the summer, males begin to emerge from colonies, followed by unmated potential queens (gynes). Males travel from colony to colony to mate with gynes, and these gynes will then overwinter and emerge the following spring as a new generation of queens, starting the cycle anew (Bohm & Stockhammer, 1977). A previous study found that queens varied greatly in their levels of aggressiveness, boldness, exploration and activity, and that aggressiveness and boldness formed a 'behavioural syndrome' (*sensu* Sih et al. (2004)) in this species (Wright et al., 2016). Thus, *P. metricus* is well suited to examine the long-term consequences of queen behaviour on the collective behaviour and success of their future colonies.

In the present study, we collected 30 newly founded *P. metricus* queens with their nests from the field and transported them back to the laboratory where they were placed individually into 30x30cm nest boxes. We recorded queen head width and nest size (# of cells) and, prior to worker emergence, we assayed the behavioural tendencies of each solitary queen along five personality axes: aggressiveness, boldness, exploration, activity, and time budgets (all defined below) for a variety of tasks. We also recorded the number of larvae queens rejected over time, as well as the number of workers each colony produced. Once workers emerged, we tested each colony for colony-level aggressiveness toward a visual stimulus only, followed by a visual + vibrational stimulus based loosely on methods used in Bruschini et al (2005).

We used these data to ask the following questions: Does queen personality predict (1) pre-worker colony growth, (2) task time budgets, (3) queen larva rejection, (4) the total number of workers produced, and (5) the downstream collective aggressiveness of entire colonies? If queen traits are strong drivers of colony phenotypes, then we would predict tight associations between queen personality and several of these outcomes.

METHODS

P. metricus queens were collected with their intact nests in the early spring of 2015 from the University of Pittsburgh's Pymatuning Laboratory of Ecology (PLE). The queens ($N = 32$) were then taken into PLE lab space where we measured head width and wing length, both of which are highly correlated with body size and mass (Eickwort 1969). We then recorded the current state of the nests, which included the number of cells and the number of both eggs and early stage larvae. The queens were then placed inside their own 30×30×30 cm metal mesocosm with clear plastic and chiffon siding (BIOQUIP). The nests were glued to cardboard in the top-center of the mesocosm, and the queens also used this cardboard as nesting material. The wasps were fed an *ad libitum* diet of wax worms (*Galleria mellonella*), maggots, and sugar cubes and their water dishes were kept clean and full. The wasps were kept on a 16:8 h light:dark schedule using two 500W incandescent lights, and the temperature was maintained at approximately 25 °C while in the lab.

All behavioural assays were performed between 10:00am and 4:00pm within two weeks of collection to ensure all nests were in similar stages of development. Colonies were monitored closely for the appearance of cell caps as well as worker emergence. Cell and worker counts were recorded every week. Additionally, we recorded every instance of

larvae rejection. Larvae rejection was evidenced by the presence of wasp larvae on the mesocosm floor during our routine weekly cell counts. Multiple behavioural assays were not performed on the same day to avoid any potential influences that one assay may have on subsequent behavioural assays. Behavioural assays were conducted in the order presented here.

Individual boldness and aggressiveness

Boldness is defined as an individual's propensity to engage in risky behaviour (Sloan Wilson, Clark, Coleman, & Dearstyne, 1994). Our boldness assays were performed while queens were perched on their nests. These assays consisted of prodding the wasps anteriorly to the clypeus with a small plastic brush up to 50 times in three-second intervals. This assay was designed to imitate a generalized aversive stimulus, such as an encounter with a foreign rival or a novel predator such as a bird. The number of prods it took (up to 50) for a queen to leave their nest was our measure of boldness. Wasps that did not leave and held their ground after frequent prods were considered bolder, as standing one's ground in the face of consistent threat potentially risks both injury and death. Wasps that readily abandoned their nests after only a few prods were considered shyer. This assay was performed four times for each queen, and the final measure of boldness was the average value across these four trials. Aggressiveness was measured as the proportion of the first ten prods the queens acted aggressively towards during boldness assays. Aggressive actions included biting and stinging the brush. The final aggressiveness score was the average proportion of prods that elicited an aggressive response across all four trials.

Exploration and activity

We measured individual queen exploration and activity levels by placing individuals into a small plastic cylinder that was then slid over the opening of a 29L×16W×4H cm exploration arena. This arena consists of ten compartments that the queen could enter freely without hindrance. Once the queens entered the opening located at the center of the exploration arena, we started a timer and recorded the number of novel chambers the queens explored over ten minutes (exploration), as well as the proportion of this time the queens remained active (activity level). We operationally define activity as walking. Standing in place grooming, for instance, was not considered being active. This assay was performed four times for each queen. Final exploration and activity scores were the average across these four trials.

Time budgets

To determine whether personality type correlated with the time queens allocated to various tasks, we performed scan observations of queen activity every hour for nine hours over four consecutive days. During a scan, we recorded whether the queen was on or off her nest. If the queen was on her nest, we observed whether she was nest building, laying eggs, feeding young, or simply standing guard. If she was spotted off her nest, we noted whether she was flying, consuming food, sugar or water, gathering nest-building materials, walking around the container, or standing motionless. This gives us a sense of the ways in which queens partition their time across various tasks, which may have a basis in individual personality and could be informative for any observed differences in colony success.

Colony-level aggressiveness

Colony-level aggressiveness was assayed using a modified procedure of Bruschini *et al.* (2005). Colonies were tested individually. A metronome was placed 10cm from the front of the wasp box on the side containing a transparent observation window. The metronome was then set at 160bpm. A black paper disk (radius = 3.5cm) was placed atop the arm of the metronome. This device, when set into motion, provides a novel visual stimulus to the wasps and causes them to respond aggressively. We recorded each colony using a Canon VIXIA HF R600 high definition (1080p) camcorder. The metronome was turned on and we recorded the number of wasps that attempted to attack the visual stimulus, measured by recording the number of wasps located on the front panel of the wasp box every ten seconds for one minute following the stimulus (six observations) on video playback. After the one-minute visual stimulus, we subjected the wasp boxes to an additional one-minute vibratory stimulus in concert with the visual stimulus and again recorded the number of wasps on the front of the box every ten seconds for one minute on video playback. The vibrations were provided using a handheld vibrator strapped to a piece of flat cardboard, which was placed on top of the wasps' nest box. The vibrator was set to a "pulse" setting that provided vibrations at short intervals (about 3/s) as opposed to a continuous vibration. We reasoned that the pulse setting provided a more realistic sensation of being disturbed by a predator than would a constant vibration. For clarity, the assay lasted a total of two minutes—the first minute consisted of a visual stimulus only, and the second minute constituted a visual + vibratory stimulus. All colonies were tested once, as we wanted to test their response to a completely novel stimulus, and thus minimize effects of habituation.

229 Lastly, we made sure that workers were at least two weeks old before performing this
230 assay, as workers tend to perform limited tasks immediately after eclosion.

232 *Statistical methods*

233 All statistics were performed using generalized linear mixed models (GLMMs).
234 Predictor variables (fixed effects) included average queen aggressiveness, boldness, and
235 activity. Activity and exploration were highly correlated, and so we removed exploration
236 from our models to both increase our statistical power and reduce overparameterisation.
237 Response variables included cell # increase, # of workers produced over the course of the
238 season, # of rejected larvae over the course of the season, time budgets (time spent on/off
239 nest, and time spent nest guarding), and colony level aggressiveness. Colony-level
240 aggressiveness also included the number of workers as a covariate. The response variables
241 cell # increase, proportion of time on/off nests, and nest guarding used a normal
242 distribution and identity link function, while # of workers, # of rejected larvae, and colony
243 level aggressiveness used a Poisson distribution with log link function. Queen head width
244 and starting nest size (which equaled the # of eggs in the nests) were included as random
245 effects in all models. All stats were performed using JMP version 12 (SAS Institute, Cary
246 NC).

247 **RESULTS**

248 No queen personality traits were able to predict colony growth, the number of
249 larvae rejected, or colony aggressiveness to a visual stimulus only (Table 1). However,
250 queen boldness was positively associated with the proportion of time queens spent on
251 their nests ($p = 0.01$)(Figure 1), and boldness was also associated with the amount of time

that females devoted to stationary nest guarding ($p = 0.018$)(Figure 2). Additionally, bolder queens tended to produce more workers on average than shy queens ($p = 0.027$. For colony-level aggressiveness, in response to a combination of visual and vibrational stimuli, shy queens tended to give rise to colonies that attacked more readily ($p = 0.022$)(Figures 3 & 4 respectively). For a full list of all significant and non-significant p -values, please refer to Table 1. Additionally, for a breakdown of *P. metricus* time budget results, see Table 2.

DISCUSSION

This study is the first to demonstrate that queen traits can help determine the downstream collective behaviours of their future colonies. Specifically, we found that bold queens, or those more likely to stand their ground after repeated antagonistic encounters, also spent more time on their nests standing guard generally, produced more workers, and gave rise to colonies where workers were also more likely to remain on their nest during a simulated predator attack. So larger colonies, though harboring more workers capable of mounting an impressive attack, don't necessarily do so; this depends on how bold their queen is. This is potentially an impactful result, as it shows that one can predict how a queen's workers will react toward predators simply by knowing how the queen reacted in similar situations *many weeks before workers even emerge*. It is not difficult to imagine that the tendency to stand one's ground during stressful encounters could have large fitness consequences, both for solitary queens and their resulting colonies. Moreover, we reason that the relative costs versus benefits of remaining near the nest during such encounters could change considerably depending on whether an individual is the sole individual occupant of the nest or whether the individual is part of a sizable mobbing force. Finally,

we found, more surprisingly, that less exploratory queens also tended to give rise to colonies that were more responsive to antagonistic predator encounters.

The common thread running through our findings appears to be a measure of the tendency of a queen or worker to leave their nest. For queens, leaving the nest means retreating in the face of danger; for workers, however, leaving the nest means aggressively attacking an intruder. During our staged agonistic encounters, we regularly observed wasps stinging at the observation window. Flight in this particular context was obviously not of the fearful sort. Thus, what we have originally described as queen boldness and colony level aggressiveness might be more appropriately described as a measure of the tendency or willingness to leave the nest. This interpretation distinguishes this behaviour from boldness *per se*, which is a general tendency to engage in risky behaviour (Sloan Wilson et al., 1994). A queen may be considered to be bold by remaining on the nest during an attack, but it is unclear whether entire colonies that refrain from leaving the nest to attack may also be considered bold, as this may in fact be less risky than attacking. It is not difficult to devise a rationale for either interpretation. While tempting, there are similar problems with describing our findings on the proactive-reactive axis of behaviour (Koolhaas et al., 1999) for both queens and colonies alike. Can queens that are more likely to retreat and colonies that are more likely to attack *both* be considered either proactive or reactive? This is not clear. Because of these problems, and for simplicity, we have chosen to describe this *tendency to leave the nest* as a measure of queen and colony *obstinacy*, where more *obstinate* queens and colonies are characterized by less willingness to leave the nest, regardless of context.

There may be benefits and disadvantages associated with queen and colony obstinacy, and these relative costs and benefits plausibly change depending on the stage of a colony's lifecycle (i.e., pre- vs. post-worker emergence colonies). In pre-emergence nests (i.e., nests with no workers), *P. metricus* queens, as well as many other *Polistes* species, are subject to intra- and interspecific usurpers (Cervo, 2006; Field, 1992; Klahn, 1988). Soon before worker emergence, nest-less queens often attempt to usurp the nests of other, usually solitary, queens (Gamboa, 1978). These encounters are aggressive in nature, and involve grappling, biting, and stinging until one of the queens relent or is killed in the confrontation (Cervo, 2006; Field, 1992). Threat from usurpers can be nearly constant, with encounters happening every two hours in some species (Gamboa et al., 2004). Queens more commonly prevail over usurpers, but are sometimes driven away or are forced into a subordinate role when the usurper is larger or possesses higher fighting ability (Elizabeth A. Tibbetts & Shorter, 2009). Our findings may be important for determining the outcomes of such contests, that is, if more obstinate queens are more difficult to oust during usurpation attempts. Even against larger usurpers, a willingness to stand and fight, coupled with the fact that queens may value their nests more than usurpers (Elizabeth A. Tibbetts & Shorter, 2009), could give queens an edge over usurpers, especially in areas of high wasp density where such contests are frequent (Gamboa, 1978). However, while obstinacy may be beneficial for thwarting usurpers during the solitary, pre-emergence phase, it may be detrimental to both pre- and post-emergence colonies during more one-sided antagonistic encounters, such as those involving vertebrate predators like mice, rats, and birds. It is difficult to imagine in such situations how obstinacy would be preferred in solitary queens, who would likely die in such encounters, or in workers where aggressively attacking the

intruder would seem more effective. Although, it is perhaps plausible that greater obstinacy could help conceal the precise location of the colony and its members, which could afford some benefit.

Another potential benefit of queen and colony obstinacy may be elevated vigilance against nest parasites. *Polistes metricus*, as well as other *Polistes* species, are afflicted by a taxonomically diverse array of colony parasites (A. C. Hodges, Hodges, & Espelie, 2003; Starr, 1976). For example, the most common nest parasite of *P. metricus* is the pyralid moth *Chalcoela ipitalis*. This moth has been estimated to infect 60% or more of *P. metricus* nests in some areas (Nelson, 1968). Other nest parasites include the moth *C. pegasalis*, Ichneumonid wasps *Pachysomoides fulvus* and *P. stupidus*, and the Eulophid wasp *Elasmus polistis* to name only a few (Amanda Coleen Hodges, 2002; A. C. Hodges et al., 2003; Nelson, 1968; Starr, 1976). These parasites generally lay their eggs in the cells of late-stage *P. metricus* larvae before they cap their cells in preparation to pupate. Once capped, the parasites' eggs hatch and consume the *P. metricus* larvae in the safety of the capped cell to later emerge as adults. Given that obstinate queens were also observed spending a larger portion of their time engaged in nest guarding, it may be that these queens are less susceptible to intrusion by nest parasites. Thus, this obstinate tactic might be beneficial if the negative effects of heterospecific parasites and conspecific usurpers outweigh depredation by vertebrates. This explanation does assume that although variation in queen boldness and colony aggressiveness exists, individual *P. metricus* queens and colonies are relatively inflexible during antagonistic encounters. Additionally, more time spent on the nest means less time spent foraging, and thus the benefits of queen and colony obstinacy may fluctuate depending on the degree of parasite pressure, resource availabilities, and

343 predation risk. Field studies will be needed to flesh out these possibilities.

344 Our discovery of a link between queen behavior, colony size, and downstream
345 colony collective behaviour has potentially far-reaching implications for studies of complex
346 sociality in general. While most studies attempt to explain colony-level behaviour by
347 observing colony environment (Pinter-Wollman, 2015; Pinter-Wollman & Brown, 2015;
348 Pinter-Wollman, Gordon, & Holmes, 2012) or by elucidating local behavioural rules that
349 facilitate effective self-organization (Couzin & Krause, 2003; Katz, Tunstrom, Ioannou,
350 Huepe, & Couzin, 2011), our study demonstrates—through an even more reductionist
351 view—that colony-level behaviour can in some instances be shaped by the behavioural
352 traits of the queen or founding individual *many weeks before workers emerge*. A few weeks
353 might sound superficially trivial, but for annual species like *P. metricus*, this time frame
354 represents a significant fraction of the total colony lifecycle (Bohm & Stockhammer, 1977).
355 We currently do not know the mechanism or mechanisms by which queens shape the
356 behaviour of their colonies. However, likely explanations include direct genetic
357 transmission of behavioural traits from queens to workers, worker learning through
358 queen-worker social interactions, or chemical or behavioural manipulation by the queen.
359 Future studies are needed to discern the nature of queen and colony obstinacy, such as
360 whether obstinacy is related to more mechanistically explicit behavioural metrics like fixed
361 response threshold responses (Beshers, 2001; Jeanson, 2013). Lastly, we encourage other
362 researchers to probe for similar links between queen traits and colony-level behaviour in
363 their own systems, as we see little reason that our findings should be unique to *Polistes*
364 wasps.

ACKNOWLEDGMENTS

We would like to thank the National Science Foundation (NSF) and the Pape family for providing funding for this research project (CMW) as well as the Pymatuning Laboratory of Ecology at the University of Pittsburgh for providing lab space.

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TABLE HEADINGS AND FIGURE LEGENDS

Figure 1: The proportion of time queens spent on their nest as predicted by average queen aggressiveness and boldness. More aggressive bold queens spent significantly more time on their nests than less aggressive and shy queens.

Figure 2: The proportion of time queens spent on their nests actively guarding their nests as predicted by average queen boldness. Bolder queens spent more time guarding their nests than shy queens.

Figure 3: The relationship between average queen boldness and colony level aggressiveness. Here we see that bolder (or more obstinate) queens give rise to less aggressive (or more obstinate) colonies.

550 **Table 1:** The significant and non-significant p-values on the effects of queen
551 aggressiveness, boldness, exploration, and activity on numerous response variables. Table
552 contains the number of individuals sampled (N), degrees of freedom (DF), Chi-square test
553 statistic (χ^2), and the significance value (**Prob** > χ^2). Values in **bold** and with an asterisk (*)
554 are significant.

	<i>N</i>	<i>DF</i>	χ^2	Prob > χ^2
<u>Cell # increase</u>				
Boldness	30	1	1.28	0.25
Aggressiveness	30	1	0.197	0.65
Activity	30	1	1.76	0.18
Whole model	30	3	2.86	0.41
<u>Total # of workers</u>				
Boldness	30	1	4.85	0.027*
Aggressiveness	30	1	0.239	0.62
Activity	30	1	0.0848	0.77
Whole model	30	3	5.08	0.016*
<u># of rejected larvae</u>				
Boldness	30	1	0.721	0.39
Aggressiveness	30	1	0.828	0.36
Activity	30	1	0.0114	0.91
Whole model	30	3	5.88	0.21
<u>Time on nest</u>				
Boldness	29	1	6.64	0.01*
Aggressiveness	29	1	0.721	0.39
Activity	29	1	0.000406	0.98
Whole model	29	3	10.5	0.014*
<u>Nest guarding</u>				
Boldness	29	1	5.54	0.018*
Aggressiveness	29	1	0.365	0.54
Activity	29	1	0.0762	0.78
Whole model	29	3	8.37	0.038*
<u>Colony attack (visual)</u>				
Boldness	19	1	0.57	0.29

Aggressiveness	19	1	1.16	0.48
Activity	19	1	0.51	0.54
Whole model	19	3	0.36	0.27
<u>Colony attack (vis. + vib.)</u>				
Boldness	19	1	5.21	0.022*
Aggressiveness	19	1	0.673	0.41
Activity	19	1	0.498	0.48
# of workers (covariate)	19	1	19.4	< 0.0001*
Whole model	19	4	42.5	< 0.0001*

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Table 2:

This table provides the results of scan sampling on queen activity budgets. The first column lists the various activities queens were seen performing. The second column shows the average total amount of time queens were observed performing various tasks, and the third column shows the average relative time queens performed various tasks while ON or OFF their nests. For instance, of the time queens spent on their nests, 93% of that time was spent nest guarding, and of the time queens spent off their nests, 31% of that time was spent feeding on sugar.

	Average total proportion	Average relative proportion
Time ON nest	0.85	-
Guarding	0.79	0.93
Nest building	0.013	0.015
Feeding brood	0.047	0.055
Time OFF nest	0.15	-
Flying	0.012	0.08
Feeding (sugar)	0.047	0.31
Feeding (protein)	0.007	0.047
Drinking	0.002	0.013
Gathering wood	0.016	0.11
Exploring	0.045	0.3
Sitting	0.02	0.13

567 **Figure 1:**

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570 **Figure 2:**

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