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Yarira Ortiz-Alvarado[1*], Bert Rivera-Marchand[2]

[1]University of Puerto Rico, Puerto Rico, [2]InterAmerican University of Puerto Rico, Puerto Rico

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Worker queens? Behavioral flexibility of queens in the little fire ant *Wasmannia auropunctata*

Yarira Ortiz-Alvarado[1*] and Bert Rivera-Marchand[2]

[1]University of Puerto Rico, Department of Biology, Rio Piedras, Puerto Rico

[2]Inter American University, Department of Natural Sciences and Mathematics, Bayamon, Puerto Rico

***Correspondence:**

Yarira Ortiz-Alvarado, PhD.

y.ortizal@gmail.com

urn:lsid:zoobank.org:act:8A719B0D-0E2C-4661-AA0F-A05367A1D837

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Abstract

Many species of social Hymenoptera demonstrate behavioral flexibility, where older workers that typically forage can revert to younger worker tasks, such as nursing, when these are absent. This flexibility is typical of the sterile worker class, yet rare in queens. In the little fire ant (*Wasmannia auropunctata*), queens have been reported to perform only egg laying. We examined behavior of queens of *W. auropunctata* after demographic manipulation. When half of the workers were removed from the colony, queens were observed caring for eggs, larvae and pupae as well as eating outside of the nest, like forager workers. We examined the relationship between these atypical queen behaviors and their juvenile hormone binding protein (JHbp) and vitellogenin (Vg) expression via QRT-PCR method. *JHbp* and *Vg* expression decreased when queens were performing worker tasks, resembling the expected expression pattern of typical sterile workers. Flexibility in queen behaviors in the little fire ant may be an important adaptation to changing environments. As a significant invasive species, such adaptation may increase the probability of colony survival during propagation. Our results not only present new insights in behavioral flexibility in social insects, but also increases our understanding of the success of this significant invasive species.

1. Introduction

Eusocial insects are characterized by having reproductive division of labor (Wilson 1971). Within the colony one or more individuals carry out egg laying while sterile workers perform nest related tasks including queen and brood care (i.e. nursing), defense and foraging (Gordon 1996). Division of labor in workers may be associated with age or morphological differences (Hölldobler & Wilson 1990). Nevertheless, there are species where workers show behavioral flexibility, performing tasks that are not typical of their age or morphology. For example, in honey bees (*Apis mellifera*), nurses are known to forage precociously when foragers are absent, while forgers may revert to nursing according to the needs of the colony (Robinson 1992). Similar behaviors has been observed in the eusocial wasp *Polybia occidentalis* (O'Donnell

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2001). Workers of the ant *Pheidole dentata* are found to increase their behavioral repertory as they age, where older workers perform tasks typical to younger individuals, yet the young workers are not proficient in older worker tasks (Calabi & Traniello 1989; Seid & Traniello 2006; Mertil & Traniello 2009). Although behavioral flexibility in workers is an important adaptation that increases the chances of colony survival when the worker population decreases, it has not been reported in queens (Rüppell et al. 2002).

Queens produce eggs throughout most of their life. In species where the colony goes through a founding stage or independent colony foundation, queens perform worker type tasks until workers are reared. For example, in the red imported fire ant, *Solenopsis invicta*, the founding queen starts with nest construction, lays and tends the first batch of eggs until these emerge as adult workers, which take over worker duties. From this point on queens are known to only lay eggs (Tschinkel 2006). Contrastingly, in species that reproduce through colony budding or dependent colony foundation, where one or more queens depart from the main colony with a group of workers, the queens do not experience a founding stage (Keller 1991; Peeters & Ito 2001). Since these queens keep a group of workers at all times it is not expected that they perform worker-like tasks.

We examined queen behavior in *Wasmannia auropunctata*, the little fire ant, a native of South America and an aggressive invasive species on all other continents except Antarctica (Wetterer & Porter 2003; Le Breton et al. 2003; Mikheyev et al. 2008). Its colonies are composed of 200-500 monomorphic workers and one to twelve larger queens, and reproduce by colony fission (Wetterer & Porter 2003; Foucaud et al. 2006; Mikheyev et al. 2009). Older workers, which typically carry out foraging duties, demonstrate behavioral flexibility by performing nursing duties when young workers (i.e. nurses) are absent (Rivera-Marchand & Fernández-Casas unpublished). The first objective of this study was to determine if queens of *W. auropunctata* demonstrate behavioral flexibility, performing worker tasks when necessary. Since colonies of the little fire ant do not experience a solitary founding stage (Wetterer & Porter 2003; Foucaud et al. 2006; Mikheyev et al. 2009), queens typically have no need to perform worker tasks. We did not expect queens to perform worker duties until we had observed queens manipulating eggs. Based on these preliminary observations we expected that in the absence of workers, queens of *W. auropunctata* should have the behavioral flexibility to perform worker tasks.

Reproductive division of labor in eusocial Hymenoptera (ants, bees and wasps), is under endocrine control (e.g. JH and Vg; Bloch et al. 2002; Amsalem et al. 2014). Juvenile hormone (JH) and vitellogenin (Vg) have important roles in regulating insect physiology (Dolezal et al. 2009; Dolezal et al. 2012) such as development, reproduction, and behavior (Robinson & Vargo 1997; Dong et al. 2009; Azevedo et al. 2016). JH, considered a master hormone, has been found to control behavioral development in honey bees (Robinson & Vargo 1997; Sullivan et al. 2000). It influences physiology in queens and guarding behavior in workers of primitive eusocial wasp *Polistes canadensis* (Giray et al. 2004). JH also affects queen maturation and reproduction in the invasive ant *S. invicta* where, high levels of JH induces alates to begin oogenesis (Vargo & Laurel 1994; Brent & Vargo 2003; Lu et al. 2009). Vg is a yolk precursor protein; its production is typically used to produce egg yolk by oviparous animals (Amdam et al. 2003), but it may also

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affect behavior (Nelson et al. 2007). In honey bees Vg has a role in the reproductive division of labor where concentrations are correlated with the hierarchy of the hive and reproductive division of labor (Corona et al. 2007; Nelson et al. 2007). Similar trends of Vg expression are seen in ants of *S. inivicta* (Lewis et al. 2001; Lu et al. 2009) and *Pogonomyrmex* spp. (Corona et al. 2013; Libbrecht et al. 2013). Therefore, the second objective of this study was to measure gene expression of JH and Vg in relation to the tasks performed by queens. We expected egg laying queens to have higher levels of JH and Vg than worker-like queens.

2. Materials and Methods

2.1 Samples

Nests (N=20) of the little fire ant *W. auropunctata* were collected from dry twigs and leaf litter in the northern region of the Caribbean island of Puerto Rico. They were housed in artificial nests which consisted of plastic boxes (25cm x 13cm x 7.5cm) coated with Fluon (Northern Products Inc., Alsip, IL.) on the sides. The boxes' lids were perforated with a pin to allow air exchange. The nesting area within the nest box consisted of a $3cm^2$ piece of thin (less than 1cm thick) wood elevated 0.5 cm by a strip of clay placed along the edges of the wood. Each nest was kept at 25°C, a relative humidity (RH) between 80% and 85%, and 12 hour light cycles. Nests were fed daily with 0.5 g of feeding mixture containing agar, eggs, honey and vitamin supplements (Hölldobler & Wilson 1994) placed in a feeding arena within the box at approximately 10 cm from the nesting area. Nests used in the study had multiple queens, eggs, larvae and pupae. We marked 52 queens on the thorax or abdomen using unique color combinations of nail polish. Queens are easily distinguishable from workers by being three to four times larger (workers 1.2-1.5 mm long, queens 4.5-5 mm; Wetterer & Porter 2003).

2.2 Behavioral flexibility of queens assays

Six nests (N=6) with a total of 19 queens were used for behavioral flexibility assays. Colonies were kept in the artificial nests three days prior to the start of experiment and fed daily. During the experiment observation period, queens were observed for 10 minutes daily for a total of nineteen days; food was removed after each observation period. The 10 minutes observation period was determined after observing that the proportion of behaviors does not vary significantly in 10, 15, 20 and 30 minute intervals. Observations were made for different tasks, including egg laying (a typical behavior), nursing (i.e. manipulating brood) and foraging (i.e. queens seen feeding in the designated arena), the latter two being non-typical behaviors. During the 10 minutes observational period, we tabulated by number of events, i.e., number of eggs laid, number of times brood was manipulated, and number of times queens walked to the feeding arena and was seen feeding. Control observation periods were performed for a period of five days. After the first five days of observations, worker population per nest was estimated via nest pictures. We then randomly culled approximately 50% of workers from the nest to simulate natural events in the wild. Preliminary observations (Rivera-Marchand & Fernández-Casas unpublished) indicated that the worker caste is equally divided between nurses and foragers. Daily 10 minutes observations continued for fourteen days (Table 1). The remaining nests were used to measure *JHbp* and *Vg* gene expression.

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2.3 Bioinformatic analysis and primer design

Primers were designed for gene sequences related to JH and Vg expression. Since JH is a terpenoid, its gene expression levels were determined indirectly by measuring Juvenile hormone binding protein (JHbp), an associated protein. JHbp is directly correlated with the onset of JH production in the hemolymph (Kramer et al. 1976; Shemshedini & Wilson 1990) because it prevents the absorption and enzymatic hydrolysis of JH, thereby maintaining a steady reservoir of the hormone in the hemolymph. As a consequence, free JH is virtually absent (Roe &Venkatesh 1990; Tan 2007). Also, JHbp/JH interaction is specific and of high affinity ($KD=10^{-9}M$), more than 99% of JH is bound to JHbp (De Kort & Granger 1996; Tan 2007). Other studies have further suggested this direct involvement as well (Prestwich et al. 1996; Hagai et al. 2007). Sequences for JHbp and Vg of *W. auropunctata* were obtained from NCBI Gene Bank. Vg sequences (XM_011697672.1, XM_011697673.1) were aligned using MAFFT (Multiple sequence alignment tool: Katoh et al. 2009). Primers (Table 2) were designed using primer3 from NCBI (Ye et al. 2012) with the obtained consensus sequence for Vg and the JHbp sequence (XM_011708554). Actin and GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) were used as housekeeping genes (Wong & Medrano 2005; Scharlaken et al. 2008).

2.4 RNA extraction, cDNA and qPCR of *JHbp* and *Vg.*

Fourteen nests (N=14), different from the ones used in the first behavioral assay, were used with a total of 33 queens. Nests were randomly assigned to control or experimental groups (nest with workers removed), behavioral assays were repeated. Seven days after worker removal, queens were collected by tasks; from control nests n=15 and from experimental nests n=18 and placed in a microtube with 20 µL of RNAlater reagent (Qiagen Valencia, CA) stored at -80°C for later RNA extraction. Afterwards, samples were placed in a sterilized microtube and mechanically homogenized. RNA extraction was performed using the RNeasy Mini Kit (Qiagen). Extracted RNA was quantified for each sample in µg/µL units using a Nanophotometer (Implen, Westlake Village, CA). RNA was normalized to a concentration (10 µg/µL) in a final volume of 20 µL and treated with DNase 1, following BioLabs (Ipswich, MA) protocol to remove any DNA contamination. cDNA was synthesized from the normalized RNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad Hercules, CA.) following the manufacturer's protocol with 10µL of RNA as a template. cDNA synthesis was verified in an electrophoresis 1% ETBR-gel.

qPCR was performed using the MJ Mini-Opticon Real-Time PCR (Bio-Rad) following the standard protocol of forty cycles; denature at 95°C for 10s, annealing at 56°C for 30s and elongation at 72°C for 15s x40, with post-amplification melt curve analysis. As a standard for quantification purposes, actin and GAPDH were used as reference genes (Wong & Medrano 2005; Scharlaken et al. 2008). Primer efficiency was calculated using the standard curve analysis method where 1 µl of each cDNA sample were pooled and serial diluted in five points at 1:10. Reactions were prepared with 2 µL of first strand cDNA as a template in a master mix of 1 µL of forward and reverse primers per gene at [10 nM] and 5 µL of iTaq Universal SYBR Green Supermix (BioRad) in a final volume of 10 µL. Relative gene expression was calculated using the geometric mean analysis method (Vandesompele et al. 2002), using the following equation:

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$$Relative\ gene\ expression = \frac{(E_{GOI})^{\Delta CtGOI}}{GeoMean}[(E_{REF})^{\Delta CtREF}],$$

E= primer efficiency, GOI= gene of interest, GeoMean= geometric mean and REF= reference gene. ΔCt was calculated using the average Ct values of the control group for each gene (calibrator Ct). The relative expression values presented are relative to the control group.

2.5 Statistical analysis

Behavior Analysis. For each task, relative probability was calculated by the number of queens performing a task with the total number of queens. Differences in relative probability between before and after worker removal were calculated with a Wilcoxon signed-rank test. To compare frequency of queen behaviors, frequency of typical and non-typical tasks were calculated by counting the number of events by queens before and after manipulation. Frequencies were compared using a Friedman test and Dunn's test as a Post-Hoc method.

Gene Expression. In order to compare relative expression between control and experimental samples, a Wilcoxon signed-rank test was used to measure differences in expression of *JHbp* and *Vg*. Here experimental samples were considered queens performing both nursing and/or foraging. To verify relative gene expression of *JHbp* and *Vg* differences among all tasks (egg laying, nursing and foraging), a Kruskal-Wallis rank sum test was used with Dunn's test as a Post-Hoc method.

Data was analyzed using the statistical program R (R Core Team 2014) v. 3.5.2 (2018-12-20) and the package agricolae (Statistical Procedures for Agricultural Research) v. 1.3-1. Graphs were done in Graph Pad Prism 6.0, (GraphPad software, La Jolla California USA).

3. Results

3.1 Behavior Analysis

In the first three days of observations queens laid eggs while workers performed typical tasks. After worker removal queens performed worker tasks for nine consecutive days, which coincided with pupae emergence. During the period after worker removal in which queens behaved as workers, egg laying decreased significantly (Figure 1A: W = 62.5, p-value = 0.01) while nursing and foraging behaviors increased significantly (Figure 1B: W = 2.5, p-value = 0.002; Figure 1C: W = 15, p-value = 0.04). Of note, during the behavior observation period, some of the queens in the nests remained idle.

Throughout the experiment when queens performed non-typical tasks, egg laying decreased until new workers emerged (Figure 2). After new workers emerged, queens returned to egg laying and stopped performing worker tasks. Post hoc comparisons indicate that egg laying frequency from day four through ten are significantly lower (p-value <0.05) than egg laying before worker removal. As egg laying decreased, the frequency of nursing behavior by queens increased significantly (p-value <0.05) from the fourth through tenth day of observations. While performing nursing, queens were seen manipulating eggs, larvae and pupae. Queens (N=6) were also seen foraging during days six through nine. Only on day seven was foraging frequency significantly higher than the rest of the experiment. On day eleven the queens returned to egg

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laying and by day fourteen queen behaviors resembled the behaviors during control period (p-value >0.05), thus showing that the nests endured the experimental period and returned to a typical behavioral pattern.

3.2 Gene Expression

There's a significant difference between the control and the experimental samples for *JHbp* and *Vg* expression. Queens from experimental nests performing worker related tasks have a lower relative gene expression of *JHbp* and *Vg* related to control nest queens (Figure 3A: W = 270, p-value = <0.001; Figure 3C: W = 270, p-value = <0.001). When relative gene expression of *JHbp* and *Vg* were compared between tasks, it shows a difference between egg laying compared to nursing and foraging for both *JHbp* and *Vg*; however, there is no difference in relative gene expression of *JHbp* and *Vg* in queens performing nursing or foraging tasks (Figure 3B:, Kruskal-Wallis chi-squared = 24.21, df = 2, p-value = <0.001; Figure 3D; Kruskal-Wallis chi-squared = 25.19, df = 2, p-value = <0.001).

4. Discussion

Although behavioral flexibility is known to be a trait of workers in many species of social Hymenoptera (Gordon 1991; Robinson 1992; Giray et al. 2004; Seid & Traniello 2006), queens of the little fire ant also demonstrate behavioral flexibility. When worker population decreases they perform worker tasks, principally nursing and, with less frequency, foraging (Figure 2). Although both behaviors were not initially expected, foraging in particular was a surprising outcome. Both egg laying and nursing are tasks done within the nest, so we hypothesize that the reason queens shift primarily to nursing is that it is less risky. Queens are risk aversive, and tend to perform nursing rather than foraging because the former is a less risky worker task. To our knowledge, this is the first time queens have been observed demonstrating behavioral flexibility after the founding stage. Given that *W. auropunctata* queens never experience a solitary founding stage (Foucaud et al. 2006; Mikheyev et al. 2009) and thus under typical conditions never perform worker-like duties, our results are even more remarkable.

During the experiment, there was an apparent transition phase, where queens gradually decreased their egg laying while increasing worker tasks. It is likely that queens may sense the shortage of workers due to a decrease in contact with workers. Studies have shown that ants typically communicate by cuticle hydrocarbons which are perceived by the olfactory organs (Vander Meer et al. 1989; Saïd et al. 2005; Ichinose & Lenoir 2009; Bos et al. 2010), hence a decrease in the amount of cuticle hydrocarbons perceived might be an indicator to queens that the worker population has decreased. Maximum worker behavior frequencies were observed on the eighth day of the experiment, coinciding with minimum egg laying frequencies. We observed individual queens performing both nursing and foraging tasks. After the ninth day an increase in egg laying and a decrease in nursing were observed. From day six to nine, we observed queens eating at the feeding arena. Queens seem to decrease investments in reproduction in order to invest in brood care. As adult workers emerge and take over brood care tasks, queens begin investing in egg laying again. Since energy expenditures due to reproduction tend to be high in social insects (Oster & Wilson 1978), queens of the little fire ant may not be able to invest in both egg laying

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and brood care. It is possible that these queens are diverting energy typically used for egg production to carry other nest duties. In our study queens performing worker tasks were not observed laying eggs. Moreover, studies on the reproductive biology of *W. auropunctata* have shown that all queens within nests produce viable eggs (de Ulloa 2003). Therefore, we worked under the assumption that queens in the experiment were inseminated.

The changes in behaviors of queens were associated with changes in the expression of *JHbp* gene and *Vg*. When queens are performing typical tasks, the levels of *JHbp* and *Vg* tend to be higher, than when a queen displays worker behaviors. This suggests regulation by molecular and physiological mechanisms on behaviors, such as regulation by hormones/protein through gene expression. In honey bees, queens tend to have low levels of JH (Fahrbach et al. 1985; Robinson et al. 1991), the opposite is seen in the little fire ant queens, where queens performing egg laying had higher gene expression of *JHbp* gene than those performing worker related tasks (Figure 3A). When studying Vg, this protein has been found to have an effect on reproductive division of labor on honey bees, where queens have higher levels of Vg which decrease in nurses and foragers (Nelson et al. 2007; Page & Amdam 2007). This is not an exception in other insects such as *S. invicta* and *P. canadensis* (Brent & Vargo 2003; Sumner et al. 2006). The same pattern has been found in *W. auropunctata* queens, where queens have higher expression levels of *Vg* when laying eggs compared to the expression levels of *Vg* in the performance of worker related tasks (Figure 3B). Furthermore, studies have shown precocious foraging in workers induced by downregulation Vg using RNAi (Nelson et al. 2007; Antonio et al. 2008), suggesting changes in behavior are mediated by Vg.

The relationship between JH and Vg has been studied before (Robinson & Vargo 1997; Lewis et al. 2001; Barchuk et al. 2002; Brent & Vargo 2003). In most insects, there is a positive relationship between JH and Vg; increasing levels of JH cause an increase in Vg synthesis (Barchuk et al. 2002; Toth & Robinson 2007). In *S. invicta* queens, high levels of JH and Vg are correlated. JH has been found to be an important promoter of vitellogenic oogenesis (Brent & Vargo 2003; Lu et al. 2009) therefore, a decrease of JH seems to interrupt egg production. Our results show a similar relation with JH (*JHbp*) and Vg as queens' reduction in Vg expression seems to correspond to a reduction in JHbp gene expression.

Behavioral flexibility in social insects ensures survival, particularly where environmental changes may affect colony demography. The adaptive value of behavioral flexibility in workers has been evidenced in honey bees (Robinson 1992; Scheiner et al. 2004), different *Pheidole* species (Seid & Traniello 2006; Mertil & Traniello 2009) and the harvester ant (*Pogonomyrmex barbatus*; Gordon 1991 & 2002), where increased needs for one task due to environmental changes may lead to a behavioral response. Flexibility in queen behaviors in the little fire ant may be an important adaptation to changing environments. As a native to the tropics, this ant may face frequent disturbances that may deplete worker population. Moreover, the colonies of this ant tend to move their nests often (Wetterer & Porter 2003). During the process of moving, workers such as foragers may be left behind as the nest is moved. The probability of colony survival may increase with queens performing worker duties as an adaptation for the population reduction that might occur during propagation. Evidence of this increased probability of survival

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can be seen in the final phase of the experiment where queens returned to their typical behaviors as the worker population increased. The results of this study not only present new insights in behavioral flexibility in social insects, but also increases our understanding of the success of this important invasive species. The little fire ant, is an important invasive species and agricultural pest that has colonized many areas around the world. Various ecological and reproductive adaptations are associated to its success and the unique queen behavior of this study adds to our knowledge of the suite of adaptations allowing this ant to be a successful invader.

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6. Author Contributions Statement

The experiments presented in this article were conceived and designed by YOA and BRM. All of the experiments were performed in Puerto Rico by YOA. Data analysis on behavior and gene expression was performed by YOA under the supervision of BR. The writing of this manuscript, preparation of the figures and editing was performed by YOA and BRM.

7. Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8. Data Availability

The original contributions presented in this study are publicly available. This data can be found here: https://doi.org/10.5061/dryad.j6q573nb2

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Table 1. Observation table. Number of total observations during behavioral assays and extrapolated to 12 hrs periods. Behaviors observed and tabulated were the number of events counted during the observation period.

Table 1: Behavior flexibility assay observations	
Behaviors Observed	# Extrapolated Observations
Egg Laying	2,204
Nursing	1,064
Foraging	54
*Observations 10 min twice a day and extrapolated to 12hr periods for 19 days	

Table 2. Primer table. List of target genes including housekeeping genes primers, their accession number in NCBI and amplification length. Actin and GAPDH primer sequences were taken from Scharlaken et al. 2008.

Gene	**Acc. Number**	**Strand**	**Primer sequence**	**Amplification (bp)**
JHbp	XM_011708554	FW	TGTTGGTGCCCATCGCTAAT	140
		RV	GTCCAGCTTTATCGTCAACTTCG	
Vg	XM_011697672.1, XM_011697673.1	FW	GCCACAACTGATCACAGCCA	218
		RV	GGACCGTCCGGTAATGTAGT	
		RV	GAGTTTGCTGCTGTGTTC	
Actin	AB023025	FW	TGCCAACACTGTCCTTTCTG	155
		RV	AGAATTGACCCACCAATCCA	
GAPDH	XM_393605	FW	GATGCACCCATGTTTGTTTG	203
		RV	TTTGCAGAAGGTGCATCAAC	

Figure 1. Relative probability of tasks in egg laying behavior **(A)**, nursing behavior **(B)** and foraging behavior **(C)**. **(A).** Egg Laying Behavior. Queen egg laying behaviors before and after workers were removed, task decreased significantly, W = 62.5, p-value = 0.01, $Mean_{typical}$=0.75, $SE_{typical}$=0.07, $Mean_{WR}$=0.36, SE_{WR}=0.08. **(B).** Nursing Behavior. Queen nursing behaviors before and after workers were removed, task increased significantly, W = 2.5, p-value = 0.002, $Mean_{typical}$=0.00, $SE_{typical}$=0.00, $Mean_{WR}$=0.55, SE_{WR}=0.05. **(C).** Foraging Behavior. Queen foraging behaviors before and after workers were removed, task increased significantly, W = 15, p-value = 0.04, $Mean_{typical}$=0.00, $SE_{typical}$=0.00, $Mean_{WR}$=0.07, SE_{WR}=0.02. n=19 queens. Behaviors observed tabulated as number of events occurred during the observation period.

Figure 2. Daily behavior rate. Task frequency of Queens (n=19) during the experiment. The shaded area represents tasks during the control period (five days). Workers were removed on day five after final control observations (C5). Egg laying behavior decreased significantly at day

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four; Chi-square = 118.524, df = 18, p-value = <0.001, just as nursing behavior increased significantly; Chi-square = 14.58, df = 18, p-value = <0.001, foraging increased significantly, at day seven; Chi-square = 37.71, df = 18, p-value = <0.001. On day twelve the population began to grow as workers emerged. At day thirteen queens increased egg laying and decreased nursing, completing a cycle. First three days show no significant difference with the last three days; Dunn p-value = >0.05. Behaviors were observed tabulated as number of events occurred during the observation period.

Figure 3. Relative gene expression of JHbp **(A&B)** and Vg **(C&D)** in control and experimental nests. Results show differences in gene expression of JHbp and Vg between control or egg laying queens and experimental or nursing and foraging queens. Expression was measured in relative expression to endogenous genes Actin and GAPDH by geometric mean analysis method. n=33 queens; control queens n=15, experimental queens n=18. **(A).** JHbp expression decreases from control to experimental queens, W = 270, p-value = <0.001, $Mean_{control}$=1.30, SE_{exp}=0.26. **(B).** Comparing JHbp expression by tasks shows difference between egg laying compared to nursing and foraging, there's no difference in expression between nursing or foraging queens Kruskal-Wallis chi-squared = 24.21, df = 2, p-value = <0.001, $Mean_{egg\ laying}$=1.30, $SE_{egg\ laying}$=0.26, $Mean_{nursing}$=0.20, $SE_{nursing}$ =0.002, $Mean_{foraging}$=0.10, $SE_{egg\ laying}$=0.003. **(C&D)**. Similar pattern as JHbp is seen with Vg expression, decreases in experimental queens (W = 270, p-value = <0.001; Kruskal-Wallis chi-squared = 25.19, df = 2, p-value = <0.001, $Mean_{control}$=1.22, SE_{exp}=0.22, $Mean_{egg\ laying}$=1.22, $SE_{egg\ laying}$=0.22, $Mean_{nursing}$=0.10, $SE_{nursing}$ =0.02, $Mean_{foraging}$=0.05, $SE_{egg\ laying}$=0.01; **C&D** respectively.

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Figure 01.JPEG

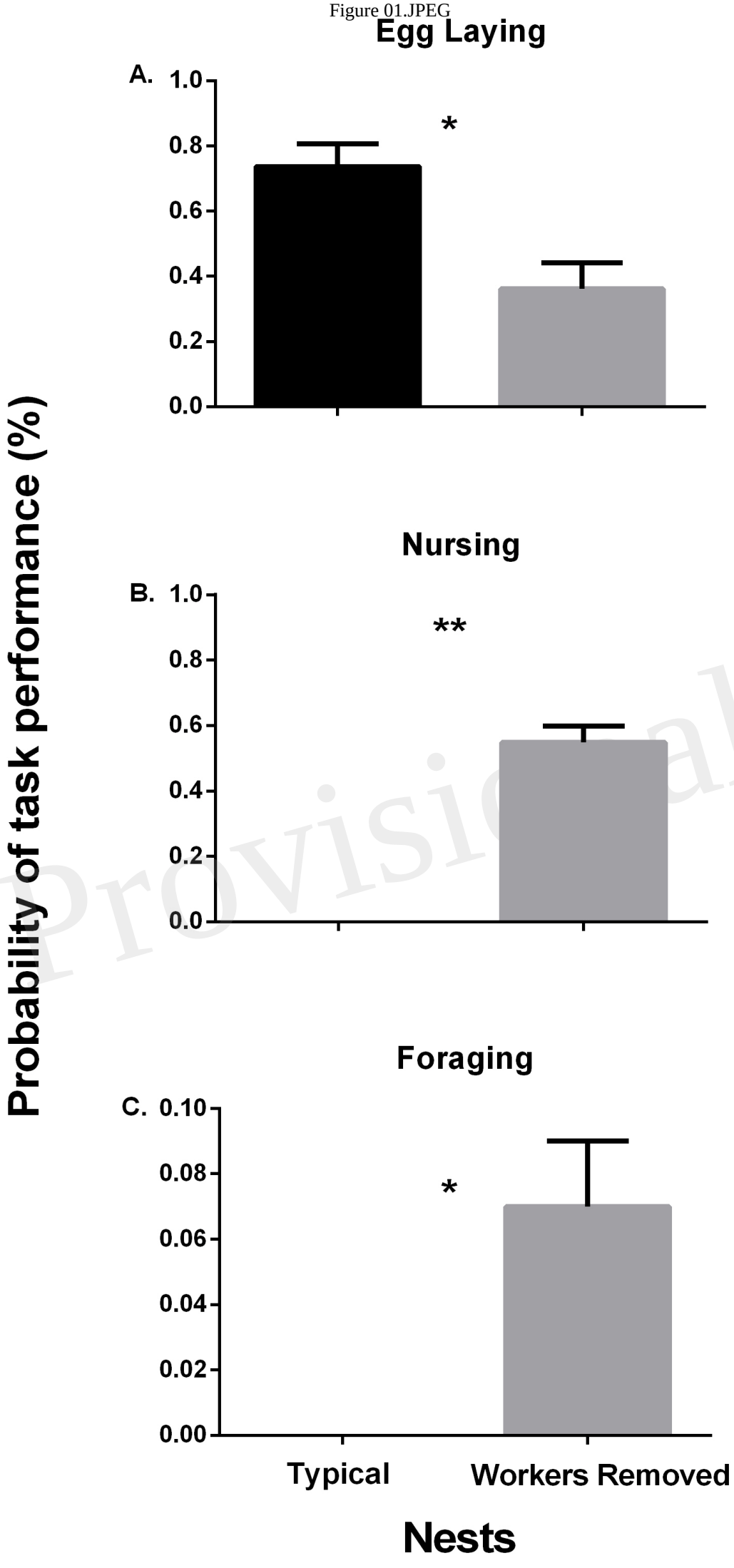

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Figure 02.JPEG

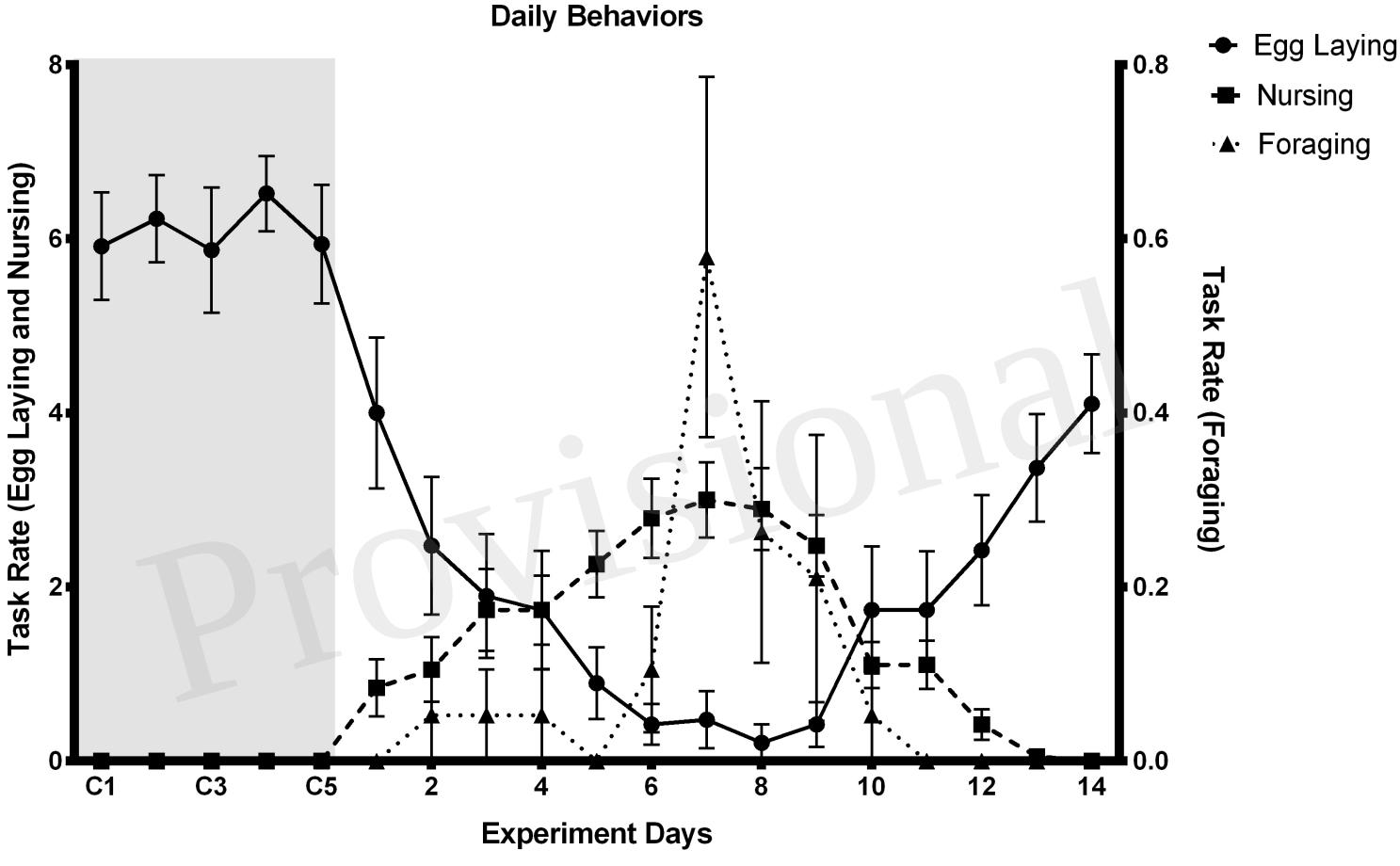

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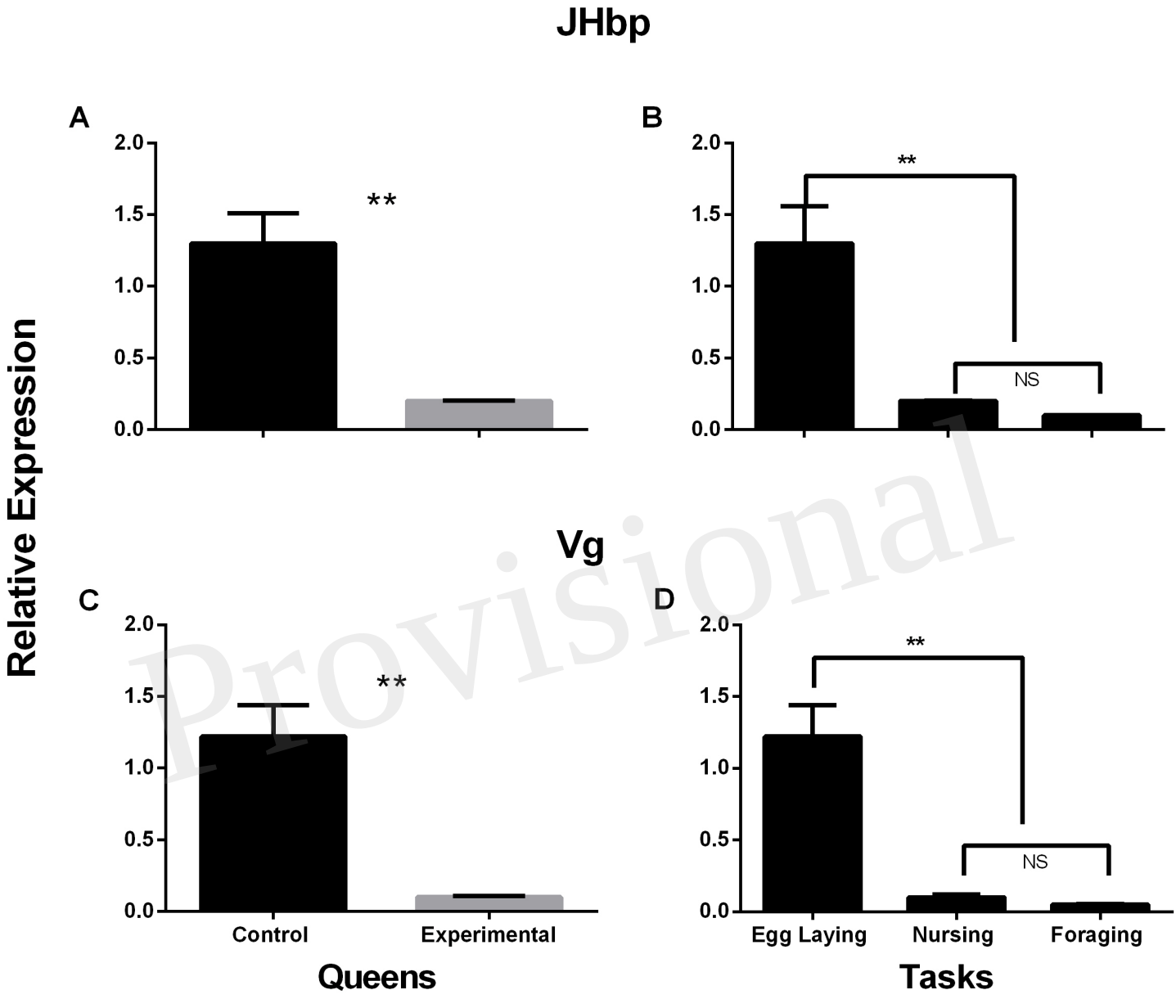
Figure 03.JPEG



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