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Vitellogenin plays a role in regulating honey bee swarming

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Swarming, or colony reproduction, in honey bees (*Apis mellifera*) is an indicator of colony-level fitness. The drivers of swarming remain elusive at both the colony and individual bee level. Floral abundance, rapid colony growth, and congestion are colony correlates and partial triggers of swarming but are not singularly causal. The nutritional and physiological state of individual bees within colonies preparing to swarm has been understudied. We hypothesized that vitellogenin (Vg), a phospholipoglycoprotein that influences the honey bee age-based division of labor in individual bees, might also mediate the cascade of physiological and behavioral processes that lead to reproductive swarming. Over two years, we compared *vitellogenin*(*Vg*) gene expression levels in age-marked worker bees sampled at various intervals before a swarm (pre-swarming colonies) to samples of same-aged bees collected from non-swarming colonies at the same time intervals. *Vg* levels were significantly higher in 10- and 14-day old bees from pre-swarming colonies three days prior and within 24 h of swarm issuance. *Vg* levels normally decrease in 10-14d old bees that are transitioning to the forager behavioral state. We provide a hypothesis for how *Vg* levels in individual bees might influence the colony-level regulatory processes that lead to swarming. This work may show for the first time, the link between a highly conserved protein associated with individual reproduction across oviparous animal taxa and its function as a mechanism of social reproduction in honeybees colonies.

Keywords Vitellogenin, Honey bee, Swarming, Behavioral maturation, Colony reproduction

Vg, a phospholipoglycoprotein synthesized and stored in the honey bee fat body, is an ancient reproduction-associated protein that provides nutrients to eggs in most oviparous animals. Honey bee queens, who produce hundreds of eggs each day, have high levels of *Vg* gene expression. *Vg* levels are also high in the functionally sterile workers, during the period in which they engage in "nursing" behavior. In the social context of a honey bee colony, the Vg produced by workers is still used to provide nutrients to developing young. Instead of provisioning eggs, worker produced Vg is secreted into the hemolymph and transported to the hypopharyngeal glands in the bees' heads where it is converted into brood food or royal jelly for larval nutrition[1].

In eusocial honey bees, Vg has evolved other critical functions as well. Vg is the primary storage protein in honey bees, *Vg* gene expression levels in individual worker bees are dynamic and age-dependent. Nurse bees, responsible for feeding larvae, typically begin feeding when they are approximately 3 days old and after 12 days move to the outer periphery of the brood nest for food storage tasks, although the exact temporal division of labor can vary depending on colony conditions[2]. *Vg* levels are highest in nurse bees and begin to decline significantly as they transition to tasks peripheral to the brood nest[3]. As workers age further, the levels of juvenile hormone (JH), an endocrine factor, increase concomitantly with a decrease in levels of *Vg*, which leads bees into the forager behavioral state[1]. Early life *Vg* expression is also associated with a forager's propensity to collect pollen (protein/lipids) or nectar (carbohydrates)[4,5]. How these associations within individual workers interact with colony-level nutrient stores and needs is still unclear.

Seasonality and environmental conditions, such as pollen availability, also influence Vg dynamics. Its production is highly influenced by the availability of nectar and pollen from flowering landscapes to meet the nutritional needs of colonies[6]. For instance, in the active spring and summer months, workers from resource-stressed colonies display precocious foraging[7]. In the late summer and early autumn, the colony produces winter or diutinus bees that accumulate lipid and vitellogenin stores even as field resources decline[8]. These internal resource stores along with the stored honey, enable the individual workers and colony as a whole to survive the winter months during which no new resources can be collected[3,9].

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Vg's known roles in egg/brood provisioning, regulating behavioral maturation and nutrient storage led us to hypothesize that it may be co-opted into the context of swarming, the social reproductive mechanism of a eusocial honey bee colony. Swarming, a seasonal reproductive process, involves about one-half to two-thirds of the colony leaving with the old queen to establish a new nest, leaving a new queen to inherit the parent colony[10,11]. The bees that leave the colony undergo a short bivouacking stage while scouts locate a new nest site. During this time along with an establishment phase in the new nest, the colony has no access to external nutrient stores[11]. These conditions could demand a buildup of internal energy stores like that seen in winter bees. Known correlates of swarming include seasonal resource abundance, increased colony size, brood nest congestion, and reduced transmission of queen pheromones[12]. However, these colony-level factors alone are not sufficient to induce swarming and do not fully explain the physiological and demographic thresholds required for swarming[12–15].

Given Vg's role in regulating nutritional physiology and worker bee maturation, we hypothesized that this important regulatory protein may be an indicator of the larger processes and signals during swarming preparation. Pre-swarming *A. mellifera* colonies exhibit delayed increases in JH levels[16], suggesting that *Vg* levels may remain elevated during this period[1]. We predicted that nurse-age bees (7–14 days old) in pre-swarming colonies would maintain higher *Vg* levels compared to bees in non-swarming colonies, facilitating a delayed maturation in this age range of bees in colonies preparing to swarm. Our findings draw a new connection between individual age maturation and colony-level reproduction, providing novel insights into the physiological processes underlying swarming.

Materials and methods

Bees

In the summers of 2021 and 2022, *Apis mellifera* colonies ($n = 12$ per year) were established at the University of Minnesota from "packages" (10,000 bees with a mated queen in a screened box) purchased from Olivarez Honey Co., California. Each package was hived in a single Langstroth-style deep box containing 10 frames of foundation (no drawn comb) and fed sugar syrup as needed to promote the construction of wax cells and colony growth. Colonies were maintained in the one box to encourage crowding and potential swarming. Colonies were hived on May 7, 2021, and April 30, 2022.

Starting June 1, 2021, and June 2, 2022, a frame of pre-eclosion pupae was removed weekly (2021) or twice weekly (2022) from each colony, caged, and incubated overnight at 33 °C and 50% RH. The next day, newly emerged worker bees (150–200 bees per session) were paint-marked with Testor's® enamel paint using a unique color for each date and then were returned to their respective colonies. Marked bees were later collected when they were 7 and 14 days old (2021), and 7, 10, and 14 days old (2022), corresponding to known physiological transitions in *Vg* levels[16]. Collections were made from June 8 to July 6, 2021, and from June 9 to July 11, 2022, with 20–30 bees collected per age group per colony on each date. Samples were stored at −80 °C.

Colony conditions were monitored weekly to assess indicators of swarming both years, and in 2022, included estimates of colony strength (frames of brood and bees)[17]. Colony strength was estimated by counting the number of frames completely covered with bees as well as the number of frames with brood (eggs, larvae and/or pupae). *Vg* expression in bees of known ages was analyzed from pre-swarming and non-swarming colonies each year. We analyzed samples of bees from pre-swarming colonies, collected within 24 h prior to the swarm leaving the colony. Samples of bees collected on the same dates from non-swarming colonies were chosen based on having a similar adult bee population and amount of brood to the pre-swarming colony.

Quantification of vitellogenin mRNA

Samples collected in 2021 and 2022 were analyzed at the USDA-ARS Bee Research Lab in Baton Rouge, LA, using real-time quantitative PCR. RNA was extracted from individual bee abdomens (12 per colony) using the Maxwell RSC 48 SimplyRNA Tissue Kit (Promega). Abdomens were homogenized in 200 µL of SimplyRNA homogenization solution, and debris was removed via centrifugation. RNA extractions were automated using Maxwell RSC 48 cartridges with DNase treatment. The extraction process followed the "Simply RNA Tissue Protocol" and was completed in 52 min.

cDNA synthesis was performed following the protocol described by[4]. Quantitative real-time PCR was carried out using the Bio-Rad CFX Connect Real-Time System, targeting the vitellogenin (*Vg*) gene and two reference genes, β-actin and *NDUFA8* (see Table 1 for primer details). Each sample was run in triplicate. PCR reactions were performed with a total volume of 10 µL, using SYBR/FAM dye and an annealing temperature of 59 °C. Thermal cycling conditions were as follows: For *Vg*: 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 57.5 °C for 10 s, and 72 °C for 10 s. For β-actin: 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 52.5 °C for 10

Gene name	Primer direction	Primer sequence
Vitellogenin[4]	FW	GTTGGAGAGCAACATGCAGA
	RE	TCGATCCATTCCTTGATGGT
Actin[4]	FW	TGCCAACACTGTCCTTTCTG
	RW	AGAATTGACCCACCAATCCA
NDUFA8[19]	FW	GCACGATTCACCAAGACCAA
	RW	GGTGGAGCTACAGGCTCAGG

Table 1. Primer sequences used for quantitative reverse transcription polymerase chain reaction (qRT-PCR).

s, and 72 °C for 10 s. For *NDUFA8*: 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 52.5 °C for 10 s, and 72 °C for 10 s. Relative gene expression was calculated using the ΔΔCt method[18].

Statistical analyses

R (Version 2024.09 + 375) was used for comparison of *Vg* levels in bees from pre-swarming and non-swarming colonies on given dates. *Vg* expression was calculated with the ΔCT method using the mean CT values of the two references genes for standardization within each sample. Gene expression data were then log 2-transformed to approximate normality. A linear mixed-effects model (LMM) was employed to evaluate the effects of colony type (pre-swarming or non-swarming) and bee age (7, 10, 14 days), and their interaction as fixed effects, on *Vg* levels. Colony was included as a random effect to account for variability among colonies.

The model was fitted using the lmer() function from the lme4 package. Statistical significance was determined using Type II Wald chi-square tests (Anova() function, car package). Pairwise comparisons and estimated marginal means (EMMs) were computed with the emmeans package, with significance determined at $\alpha = 0.05$.

To compare the log-transformed *Vg* levels in bees during the temporal build-up to swarming, non-parametric Jonckheere-Terpstra (JT) tests (R Version 2024.09 + 375) were used to detect monotonic trends across time points (14, 7, 3, and 0 days before swarming). JT tests were performed using the JonckheereTerpstraTest() function from the DescTools package in R. Pairwise comparisons between time points were carried out using Conover's test (conover.test() function, conover.test package).

Results

Pre-swarm: within 24-hours of swarm

In 2021, six of the 12 colonies swarmed. We analyzed *Vg* levels in bees from three of the six pre-swarming and three non-swarming colonies. Three swarming colonies were excluded from analysis because they swarmed several days between sampling bouts, or we could not confirm the exact date the swarm issued.

In 2022 four of the 12 colonies swarmed and we compared *Vg* levels in bees from the four pre-swarming and four non-swarming colonies. We were able to analyze samples from all four because the samples were collected and frozen within 24 h of the known swarm issuance. Estimates of colony strength of the four pairs, taken within one week of the swarm issuance, revealed no significant differences between them: Colonies that swarmed had 7.88 ± 0.65 frames of brood and those that did not swarm had 8.50 ± 0.50 ($t(6) = -1.53$, $p = 0.176$); colonies that swarmed had 8.50 ± 0.50 frames of bees vs. 8.12 ± 0.68 in colonies that did not swarm ($t(6) = 0.88$, $p = 0.41$).

In 2021, we analyzed 7 and 14 day old stored bees collected within 24 h prior to the swarm and compared them to 7 and 14 day old bees from non-swarming colonies. In 2022, we analyzed 7, 10, and 14 day old stored bees collected within 24 h prior to the swarm. Analysis of year as a factor showed no interaction between year and colony type: swarm vs. no swarm ($F(1) = 388$, $p = 0.203$); therefore, data from both years were combined.

Vg levels were significantly influenced by the interaction between colony type and age group ($F = 11.77$, $df = 2$, $p = 0.0028$). Post-hoc pairwise comparisons showed that *Vg* levels were not significantly different between 7-day old bees ($p = 0.9261$) from pre-swarming vs. non-swarming colonies, however the levels were significantly higher in 10- and 14-day-old bees ($p = 0.0033$ and $p = 0.0193$ respectively), from pre-swarming colonies vs. non-swarming colonies (Fig. 1).

Among colonies that swarmed, *Vg* levels did not increase with increasing age of bees; there was no difference among 7-, 10- and 14-day old bees ($p = 0.266$). However, *Vg* levels in same age bees from non-swarming colonies significantly decreased in bees older than 7 days ($p = 5.58e\text{-}08$).

Temporal build-up to swarm

To investigate temporal changes in *Vg* levels in both pre-swarming and non-swarming colonies, we compared three age groups of worker bees (7-, 10-, and 14-day old) across three time points prior to swarming (14, 7, 3, and 0 days before swarm) from the same colonies. We were not able to analyze all the paired colonies at every time point from pre-swarming and non-swarming colonies. Some of the colonies swarmed early in the experiment and did not have stored, marked bees from 14, 7, or 3 days prior, and in some instances, we did not have enough sampled bees left for a particular age and date. When possible, we analyzed 12 bees from each selected date (Table 2).

7-day-old bees

For pre-swarming colonies the Jonckheere-Terpstra (JT) test revealed the following: *Vg* expression remained relatively unchanged throughout the experiment ($JT = 2902$, $p = 0.072$). For non-swarming colonies, the JT test showed a significant increase in *Vg* levels ($JT = 5064$, $p < 0.001$), with pairwise comparisons revealing significant differences between all time points ($p < 0.05$) (Fig. 2).

10-day-old bees

In pre-swarming colonies, the JT test showed a significant increase in *Vg* levels ($JT = 3711$, $p = 3.608e\text{-}13$). Pairwise comparisons revealed significant differences among all time points ($p = 0$). In non-swarming colonies, the JT test did not detect a significant difference ($JT = 1557$, $p = 0.8254$).

14-day-old bees

For Pre-swarming colonies, the JT test indicated a significant increasing trend in *Vg* levels over time ($JT = 5051$, $p = 1.449e\text{-}08$). Pairwise comparisons revealed significant differences among all time points ($p = 0$). In non-swarming colonies, no significant difference was detected ($JT = 2886$, $p = 0.744$ (Fig. 2).

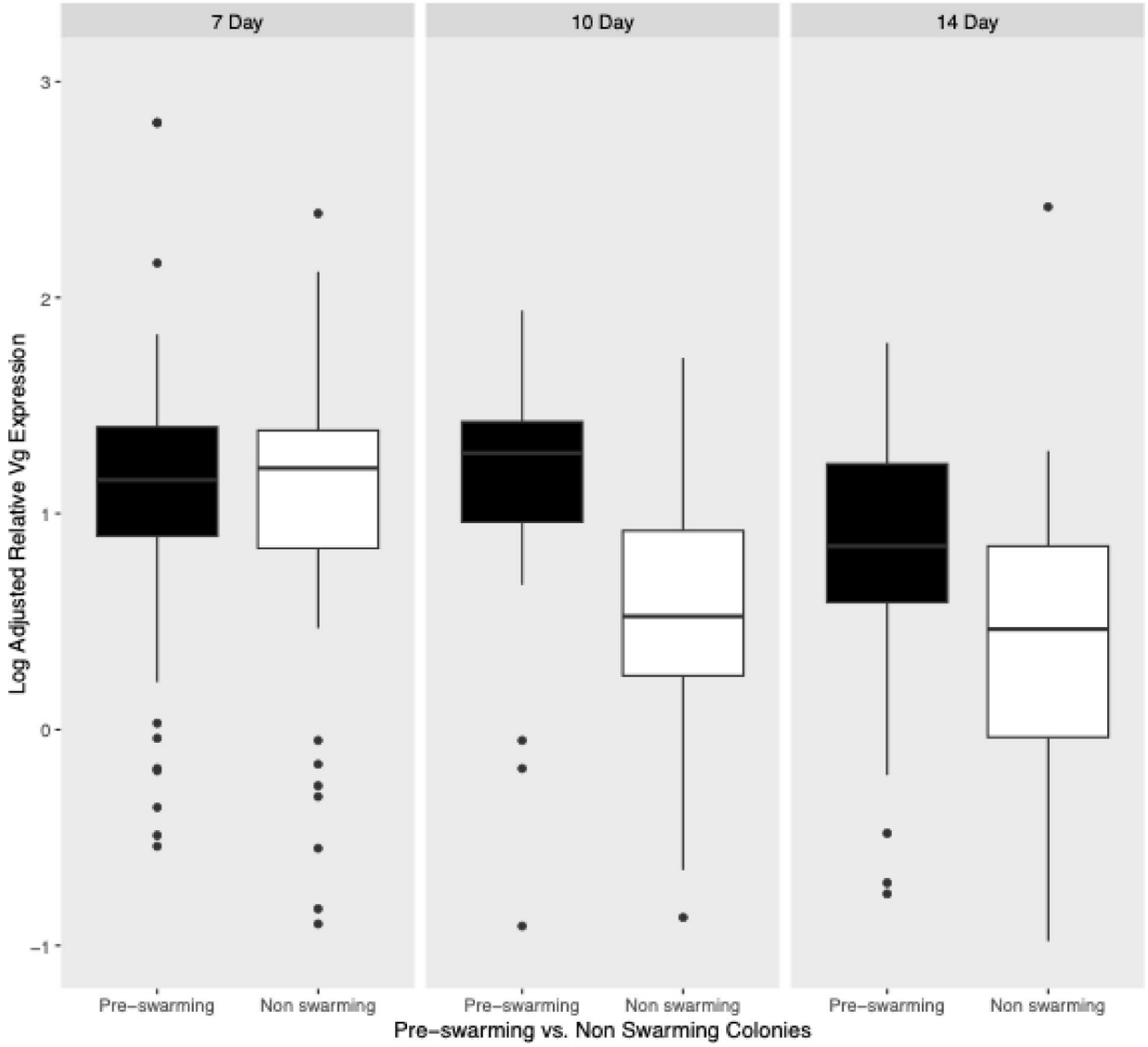

Fig. 1. Log-transformed relative v*itellogenin* (*Vg*) gene expression in honey bee workers from pre-swarming and non-swarming colonies across three age groups: 7, 10, and 14 days from 2021 and 2022 combined. Error bars represent 95% confidence intervals. Different letters indicate significant differences among means ($\alpha = 0.05$) both among colony types (pre-swarm vs. non-swarm) and across age groups. Significant effects of colony type ($F = 4.303$, $df = 1$, $p = 0.038$) and age group ($F = 23.374$, $df = 2$, $p < 0.001$), as well as a significant interaction between colony type and age group ($F = 11.774$, $df = 2$, $p = 0.0028$) were observed. *Vg* levels remained the same among bees from pre-swarming colonies at all ages but decreased significantly in 10- and 14-day old bees from non-swarming colonies. A comparison of bees from pre-swarming vs. non-swarming colonies within each age group, showed no significant differences in *Vg* levels in 7-day old bees ($p = 0.9261$) but significantly higher levels in both 10-day ($p = 0.0033$) and 14 day old bees ($p = 0.0193$), from pre-swarming and non-swarming colonies.

Discussion

Vg is a major driver of individual honey bee longevity and a strong indicator of colony health and nutritional status. High levels of *Vg* expression in adult bees, coinciding with high pollen intake, are a signal of a healthy physiological state of the colony[3,20–23]. Studies have shown that Vg provides resistance to oxidative stress and that the individual life expectancy of honey bees is correlated with levels of *Vg*[24,25]. Our findings highlight yet another way Vg influences honey bee individual and colony dynamics, as it supports colony level preparations for reproductive swarming.

We found higher levels of *Vg* specifically in middle-aged bees (10–14 days old), compared with same age cohort of bees from non-swarming colonies, indicating they are physiologically delayed in maturation, maintaining nurse-like characteristics. The increased levels of *Vg* in 10-14-day old bees from pre-swarming colonies were observed 3 days prior to swarming. Younger, 7-day old bees had relatively high *Vg* levels in both pre-swarming and non-swarming colonies, which is typical of younger nurse bees that consume pollen and

Date of Swarm	Colony #	< 24 h before swarm	3 d before swarm	7 d before swarm	14 d before swarm
		Age of marked bees and sample size analyzed			
2021 June 8	Swarm 269	7 d $n=6$ 14 d $n=0$			
	Non-swarm 220	7 d $n=9$ 14 d $n=12$		7 d $n=9$ 14 d $n=12$	14 d $n=6$
2021 June 29	Swarm 103	7 d $n=12$ 14 d $n=12$		7 d $n=8$ 14 d $n=6$	14 d $n=12$
	Non-swarm 142	7 d $n=10$ 14 d $n=12$		7 d $n=11$ 14 d $n=12$	14 d $n=11$
2021 30 June	Swarm 275	7 d $n=12$ 14 d $n=12$		7 d $n=12$ 14 d $n=5$	14 d $n=9$
	Non-swarm 254	7 d $n=9$ 14 d $n=12$		7 d $n=12$ 14 d $n=12$	
20 June 2022	Swarm 13	7 d $n=6$ 10 d $n=10$ 14 d $n=10$	7 d $n=12$ 10 d $n=12$ 14 d $n=12$		
	Non-swarm 18	7 d $n=9$ 10 d $n=12$ 14 d $n=11$	7 d $n=9$ 10 d $n=10$ 14 d $n=5$		
23 June 2022	Swarm 17	7 d $n=10$ 10 d $n=9$ 14 d $n=12$	14 d $n=12$	14 d $n=12$	10 d $n=12$ 14 d $n=12$
	Non-swarm 19	7 d $n=12$ 10 d $n=11$ 14 d $n=11$	14 d $n=12$		10 d $n=12$ 14 d $n=9$
27 June 2022	Swarm 20	7 d $n=12$ 10 d $n=12$ 14 d $n=12$	7 d $n=11$ 10 d $n=12$ 14 d $n=12$	14 d $n=12$	10 d $n=12$
	Non-swarm 21	7 d $n=11$ 10 d $n=11$ 14 d $n=12$	7 d $n=12$ 10 d $n=12$ 14 d $n=5$	14 d $n=9$	
27 June 2022	Swarm 23	7 d $n=12$ 10 d $n=12$ 14 d $n=11$	7 d $n=12$ 10 d $n=12$ 14 d $n=9$		10 d $n=11$
	Non-swarm 22	7 d $n=12$ 10 d $n=12$ 14 d $n=12$	7 d $n=12$ 10 d $n=12$ 14 d $n=12$	14 d $n=12$	10 d $n=11$

Table 2. Dates of colonies swarming in 2021 ($n=3$ colonies) and in 2022 ($n=4$ colonies), and timing of collection of age marked bees from colonies relative to when the swarm issued. *Vg* levels in age-marked bees from colonies that swarmed were compared to same-aged bees from colonies that did not swarm on the same date. Approximately 9,450 bees were sampled from 24 colonies over the course of 2 years. *Vg* expression was analyzed from approximately 1,104 bees from 15 colonies.

stimulates their hypopharyngeal glands to secrete brood food for the larvae. The high *Vg* levels in bees from pre-swarming colonies persisted as bees aged, but dropped in non-swarming colonies, as they do during the normal transition from the nurse to the forager behavioral state. Our results may provide a missing link in the cascade of physiological and environmental factors that lead to reproductive swarming in honey bees.

We hypothesize that Vg may modulate the swarming process as follows. The seasonal reproductive process begins in late spring and early summer, when the rapid increase of floral resource abundance leads to foragers collecting and storing high amounts of pollen, young nurse bees consume the protein and lipid-rich pollen and convert it into Vg which is synthesized and stored in the fat body and secreted into the hemolymph. The Vg is then transported to the hypopharyngeal glands, where it is converted into brood food or royal jelly to feed larvae, supporting rapid population growth. The enhanced food provisioning for the larvae leads to a rapid increase in the number of young workers[12,26]. As the colony grows, spatial congestion can develop within the hive, leading to a reduction in the effective spread of queen mandibular pheromone (QMP)[26]. This reduced pheromone distribution is a well-documented factor that stimulates queen rearing, a critical step in the swarming process[13]. Weeks before a colony swarms, they begin mass queen rearing by creating multiple wax cups for queen larvae on the bottom of combs and provisioning these cups with ample royal jelly for the developing larvae[27].

Simultaneously, as the buildup of young, Vg-rich workers occurs, foragers—characteristically low in Vg—begin to curtail their external activities in the period just prior to swarming[11]. This reduction in foraging activities leads to an excess of foragers within the hive. This seems to confirm the double repressor hypothesis of an external repressor signal coming from foragers[1] that was later shown to be ethyl oleate, which delays the transition to foraging behavior via trophalaxis[28].The middle aged worker bees in our study had a delayed ontological progression starting three days before swarming, which would coincide with higher numbers of foragers present inside the hive.

At the same time, the buildup of nurse bees consuming high levels of pollen and subsequently maintaining elevated levels of *Vg* provides an internal repressor that inhibits the age-related transition to foraging. This

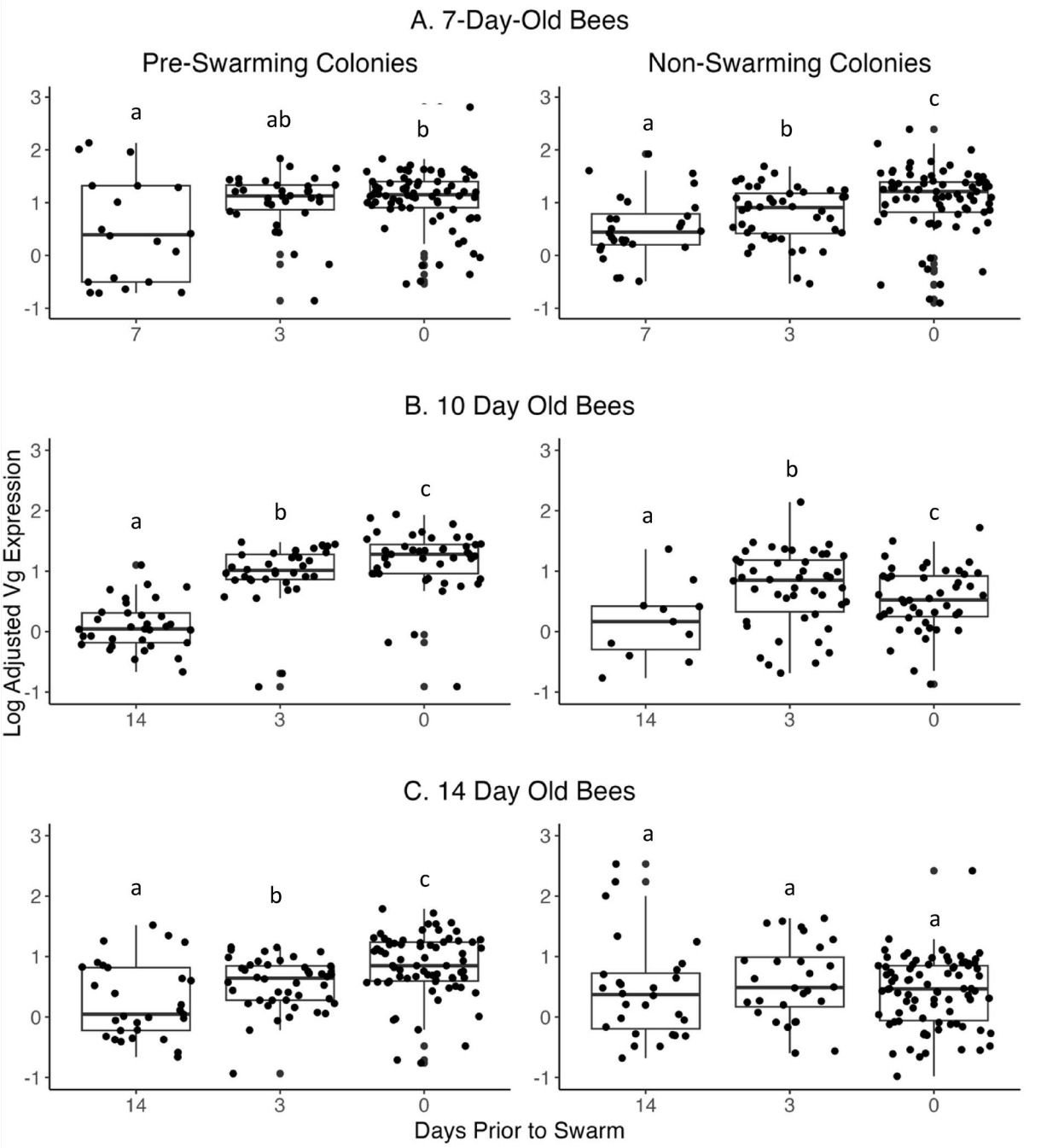

Fig. 2. *Vg* gene expression in 7-, 10-, and 14-day-old worker bees from pre-swarming and non-swarming colonies across three time points leading up to swarming. Boxplots show the distribution of log-transformed *Vg* levels at 7, 3, and 0 days (for 7-day-old bees) or 14, 3, and 0 days (for 10- and 14-day-old bees) prior to swarm issuance. Different letters indicate significant differences ($\alpha = 0.05$) among days within each colony type; no comparisons were made between pre-swarming and non-swarming colonies. Pre-swarming colonies generally showed increasing *Vg* levels toward the swarm event, whereas non-swarming colonies exhibited lower and more variable patterns.

repressor operates through a negative feedback loop between Vg and JH, where high *Vg* levels suppress the rise of JH titers. The resulting delay in the transition from nurse to forager ensures that the colony retains a robust population of physiologically younger bees. These patterns align with the delayed endocrine transitions previously reported in honey bees, where a postponed rise in juvenile hormone (JH) titers in older workers was observed[16]. These bees are essential for feeding the queen and the brood both in the new swarm and within the parent colony. This physiological delay enhances the colony's survival potential during the critical phases throughout swarming preparation until the successful establishment of the new colony. Colonies that are

not preparing to swarm do not exhibit these physiological delays, further supporting the conclusion that Vg dynamics are tightly linked to swarming-specific processes occurring in the days leading up to swarming.

High levels of *Vg* in workers confer significant advantages to colony reproductive fitness, particularly during the swarming phase[29]. Beyond its role in provisioning food for the parent colony's increased brood and queen cells, Vg stored in the fat body of workers contributes to stress resilience and cellular immunity, which are critical for workers transitioning to the new nest site. Additionally, Vg serves a metabolic function, acting as a nutrient reserve that can be broken down to support energy demands during food scarcity or disruption[1]. During swarming there is a significant food disruption during the bivouac period when bees are scouting for a new nest site and then must build up that nest site and get new foraging stores. These combined functions of Vg highlight its central role in preparing colonies for the challenges of swarming and establishing a new colony.

Our study provides valuable insights into the potential role of Vg as an upstream regulator and physiological marker of the swarming preparation process. We quantified the mRNA expression levels of *Vg* as a proxy for protein function. Previous research has demonstrated that *Vg* mRNA levels are often correlated with Vg protein titers[1]. We recognize that gene expression alone may not fully capture the biochemical pathways involved in swarming preparation. Future studies should measure Vg protein levels directly, juvenile hormone titers, and pollen intake. Additional studies that alter *Vg* levels or nutrition could further test the causal relationship between *Vg* expression and swarming behavior. It would also be interesting to study how *Vg* levels might influence flight endurance during swarming as an energetic reserve.

Finally, it would be valuable to establish a gene expression profile and regulatory network specific to swarming bees. Comparing the swarm-specific profile to known networks and profiles, such as those of foragers versus nurses or winter versus summer bees, could reveal unique regulatory mechanisms underpinning swarming behavior. This approach might also identify key genes and pathways that differentiate swarming bees from their non-swarming counterparts.

Numerous environmental and social cues initiate a cascade of physiological and behavioral changes that prepare the colony for swarming. We found that *Vg* levels may be a key indicator of these processes as measured by its gene expression in middle aged workers. Our findings highlight the importance of Vg as an indicator of the colony's physiological state and a modulator of the individual worker state during preparation for swarming. While swarming is ultimately triggered by environmental conditions and colony-level factors, Vg may play an essential role in mediating the internal processes that support colony growth and swarming preparation. The delayed physiological shift in workers ensures the colony retains a robust population of young bees, which are vital for colony fitness during reproductive swarming both for those that swarm and those that are left behind. This study offers a first step in identifying Vg as a molecular signal that modulates the capacity for colony level reproduction. Future studies should focus on the biochemical and biomechanical pathways to further elucidate the novel ways that Vg has been exploited by honey bees.

Data availability

All data supporting the findings of this study have been deposited in Dryad and are available to reviewers via this private link: https://datadryad.org/share/Ov0hm8hqjSWEgNP8XiHiDT0etH_RjWaQW3v54SUd0iA. The dataset DOI is 10.5061/dryad.tb2rbp0dp and will become active upon publication. Please note that the DOI will not resolve to a dataset until publication. To access the data during review, please use the private peer-review link provided above. The point of contact if someone wants to request the data from this study is Katrina Klett, corresponding author.

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Author contributions

K.K. and M.S. conceptualized the study and performed the investigation. All authors contributed to methodology and analysis, and also to manuscript review and editing. K.K. did data curation, prepared figures, and wrote the main manuscript. K.I. and M.S.F provided resources for all lab work. M.S. supervised the project and acquired funding.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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