OP-071 [Bee Biology]
Vitellogenin as a Physiological Marker of Swarming in Honey Bees (Apis mellifera)

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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 to beekeepers and researchers. While floral abundance, rapid colony growth, and nest congestion are commonly associated with swarming, they are not direct causal factors. The nutritional and physiological state of individual bees within colonies preparing to swarm is understudied. We hypothesized that levels of vitellogenin (Vg), a phospholipoglycoprotein that influences the age-based division of labor in individual bees, might also mediate the cascade of physiological and behavioral processes that lead to reproductive swarming. This study aimed to determine whether Vg levels in worker bees are associated with colony swarming behavior. Over two years, we compared Vg levels in age-marked worker bees sampled at various time intervals (14, 7, 3, and 0d) before a swarm was issued (pre-swarming colonies) to samples of same-aged bees collected from non-swarming colonies at the same intervals. Samples of known-aged bees were stored in a -80°C freezer for later analysis of relative Vg levels using quantitative real-time PCR. Vg levels were significantly higher in 10 and 14 day old bees from pre-swarming colonies three days prior and within 24 hours of swarm issuance compared to same aged bees from non-swarming colonies. Vg levels normally decrease in 10-14 day old bees that are transitioning to the forager behavioral state. This finding suggests that Vg may play a role in regulating the physiological and behavioral shifts associated with colony preparations for swarming. We hypothesize that Vg modulates the swarming process by delaying the physiological transition of middle-aged worker bees, ensuring a robust population of young, nutritionally enriched bees that support both the departing swarm and the parent colony. These novel findings provide further clues to long-standing mystery about the factors that trigger swarming behavior in honey bees.

Vitellogenin as a Physiological Marker of Swarming in Honey Bees

Vitellogenin plays a role in workers preparing to swarm

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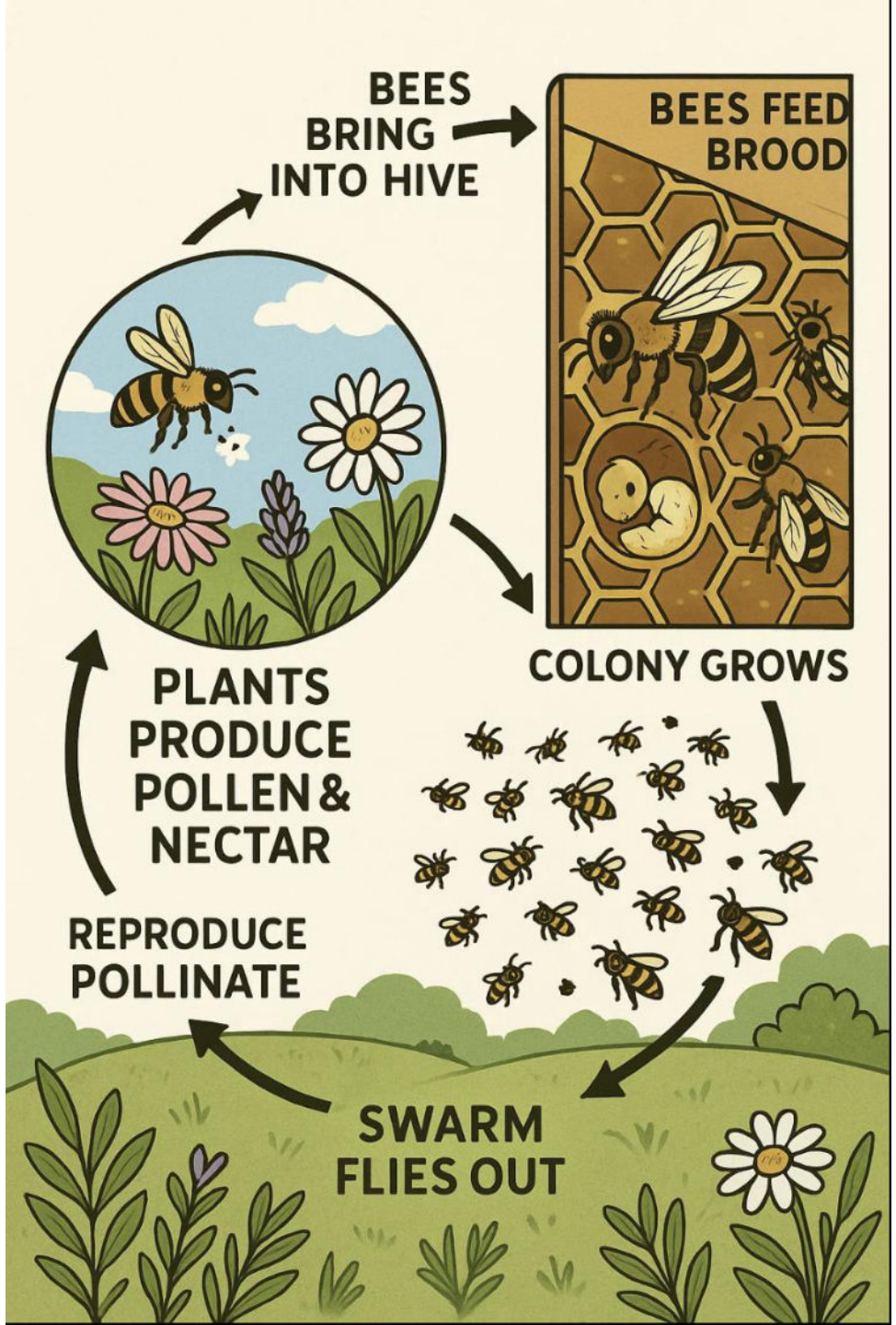
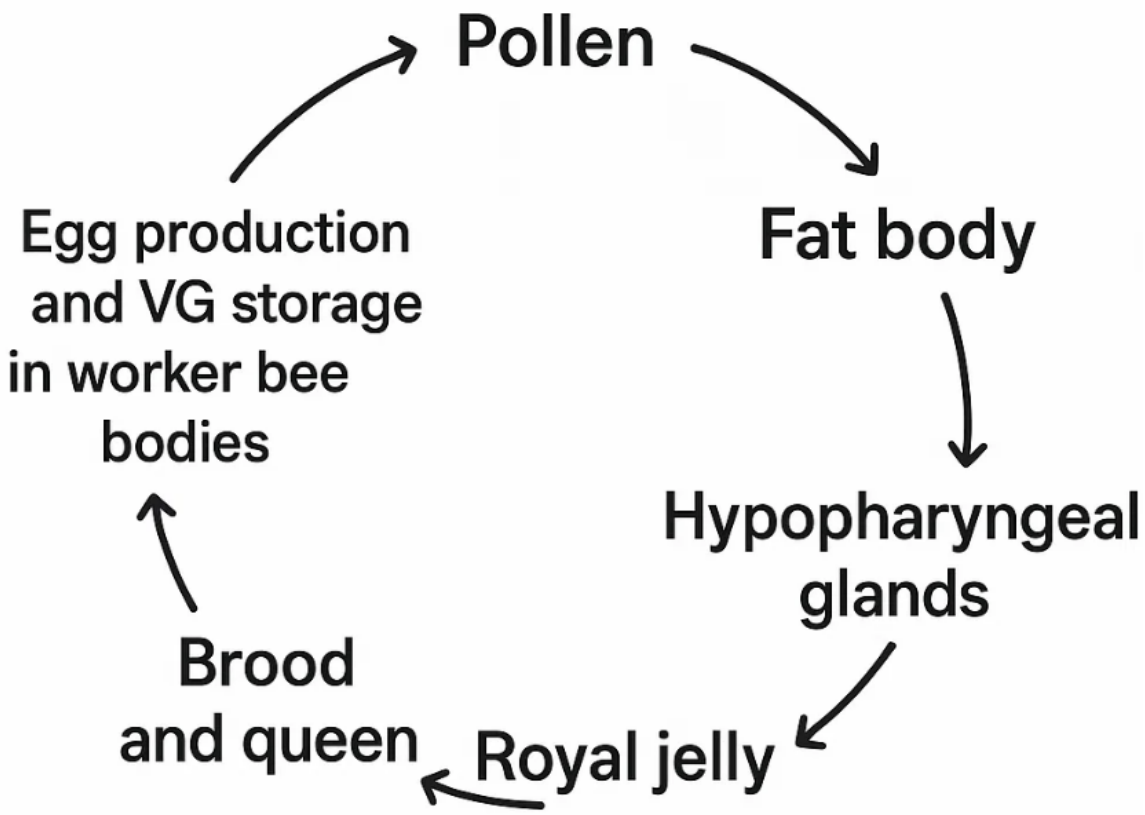
Swarming in Honey Bees

- An indicator of colony level fitness
- No single causal factor (Winston et al., 1991; Grozinger et al., 2013; Rittschof & Seeley, 2008; Rangel & Seeley, 2008)
- We hypothesize that pollen flows may be a critical signal

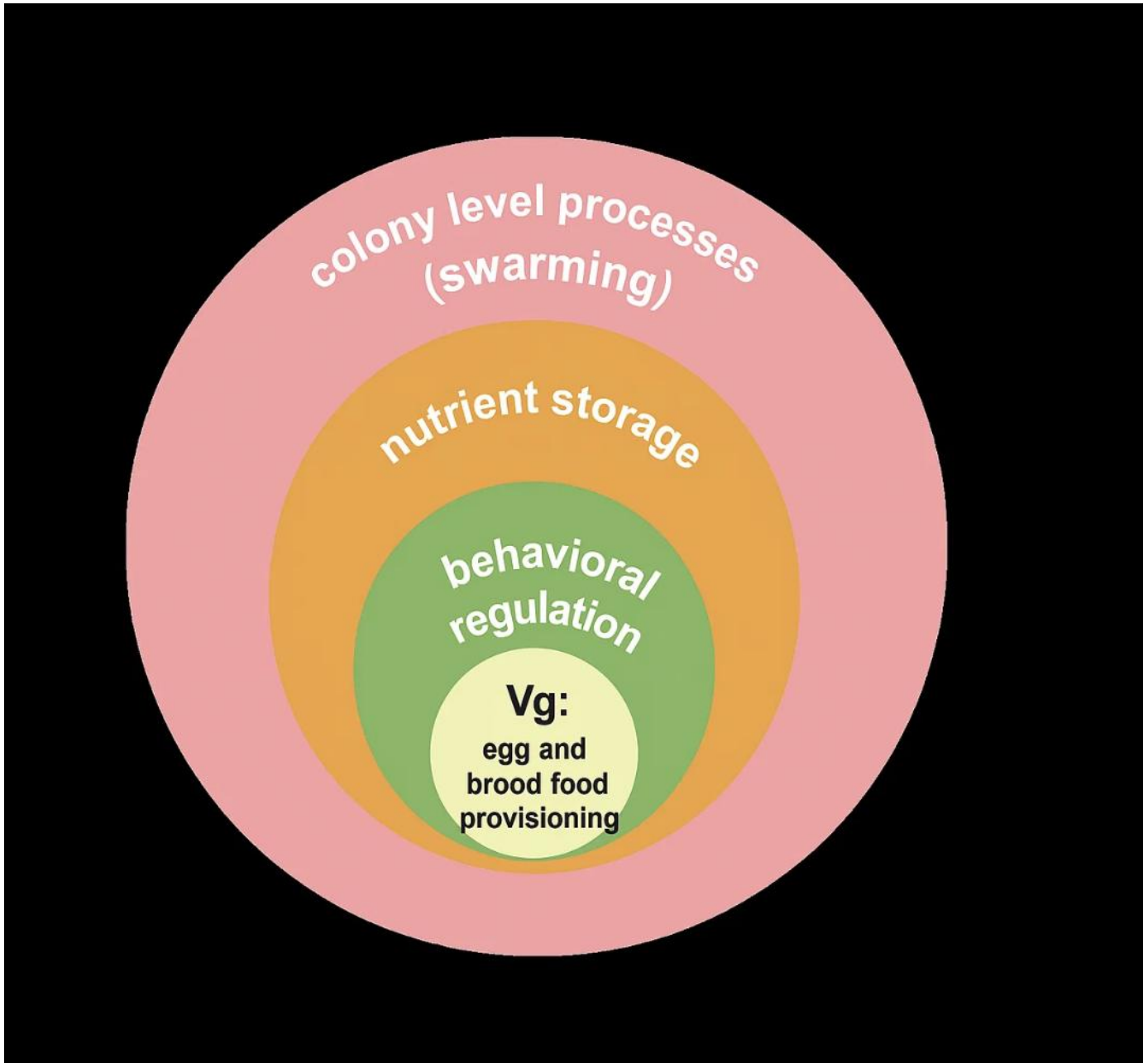


The Pollen to Vitellogenin Flow



Vitellogenin levels are dynamic and age dependent

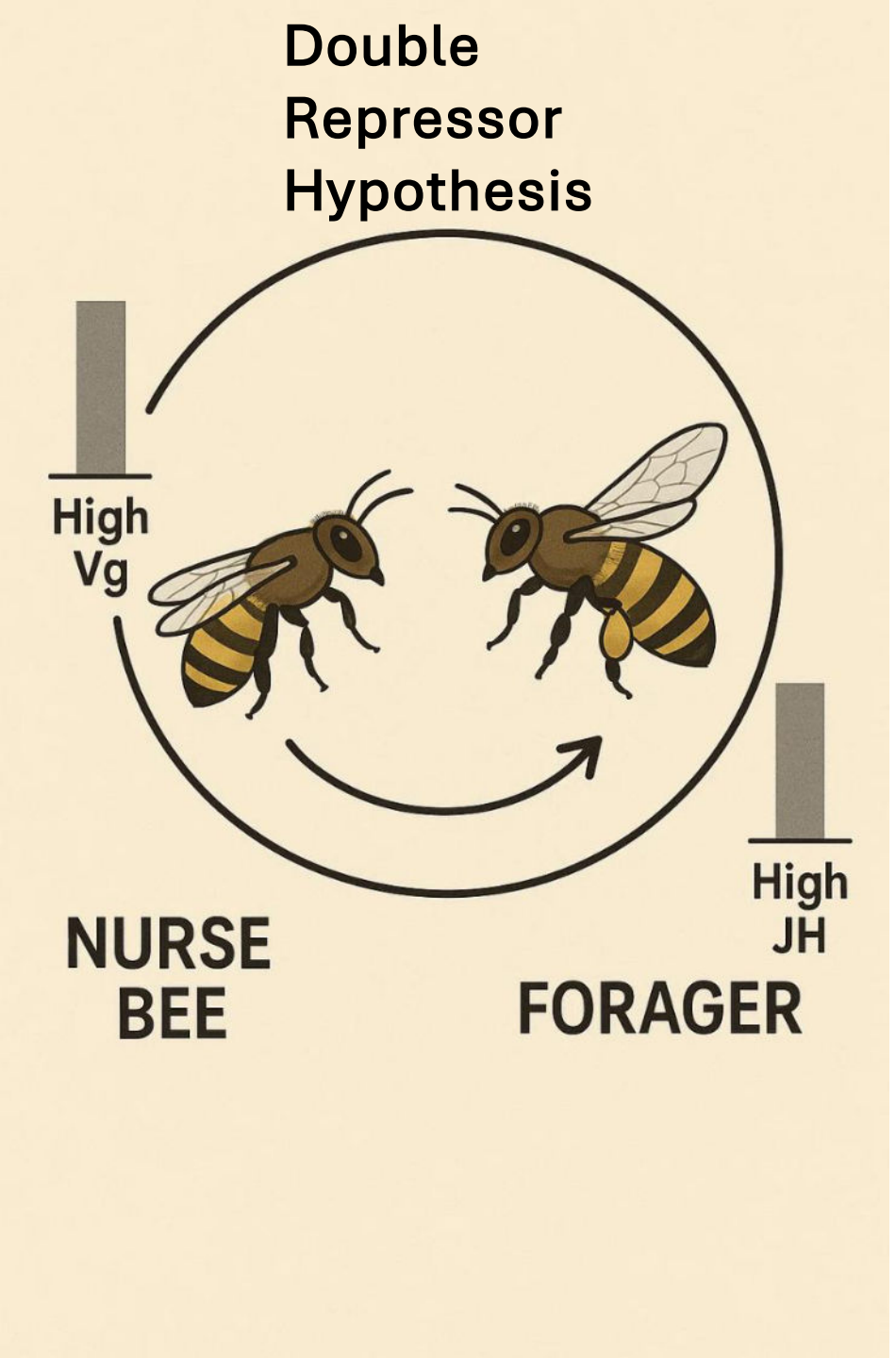
(Mattila & Otis, 2007;
Schulz et al., 1988;
Amdam & Omholt, 2002;
Amdam et al., 2003)

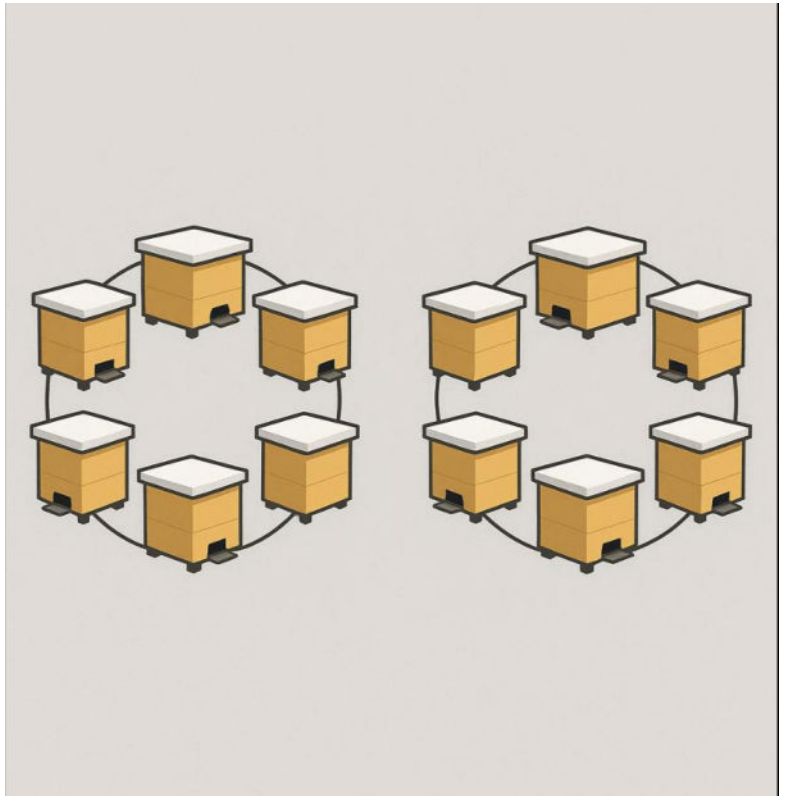


We hypothesized that Vg levels might increase in bees prior to swarming

JH levels have a delayed onset in colonies preparing to swarm

Zeng et al., 2005





Experimental Design

- Started as pkgs
- Maintained in single deep Langstroth box
- marked bees weekly for 4 weeks

- 12 colonies/ year for 2 years
- marked bees and sampled when 7, 10, 14 days of age
- froze samples at -80

Experimental Design

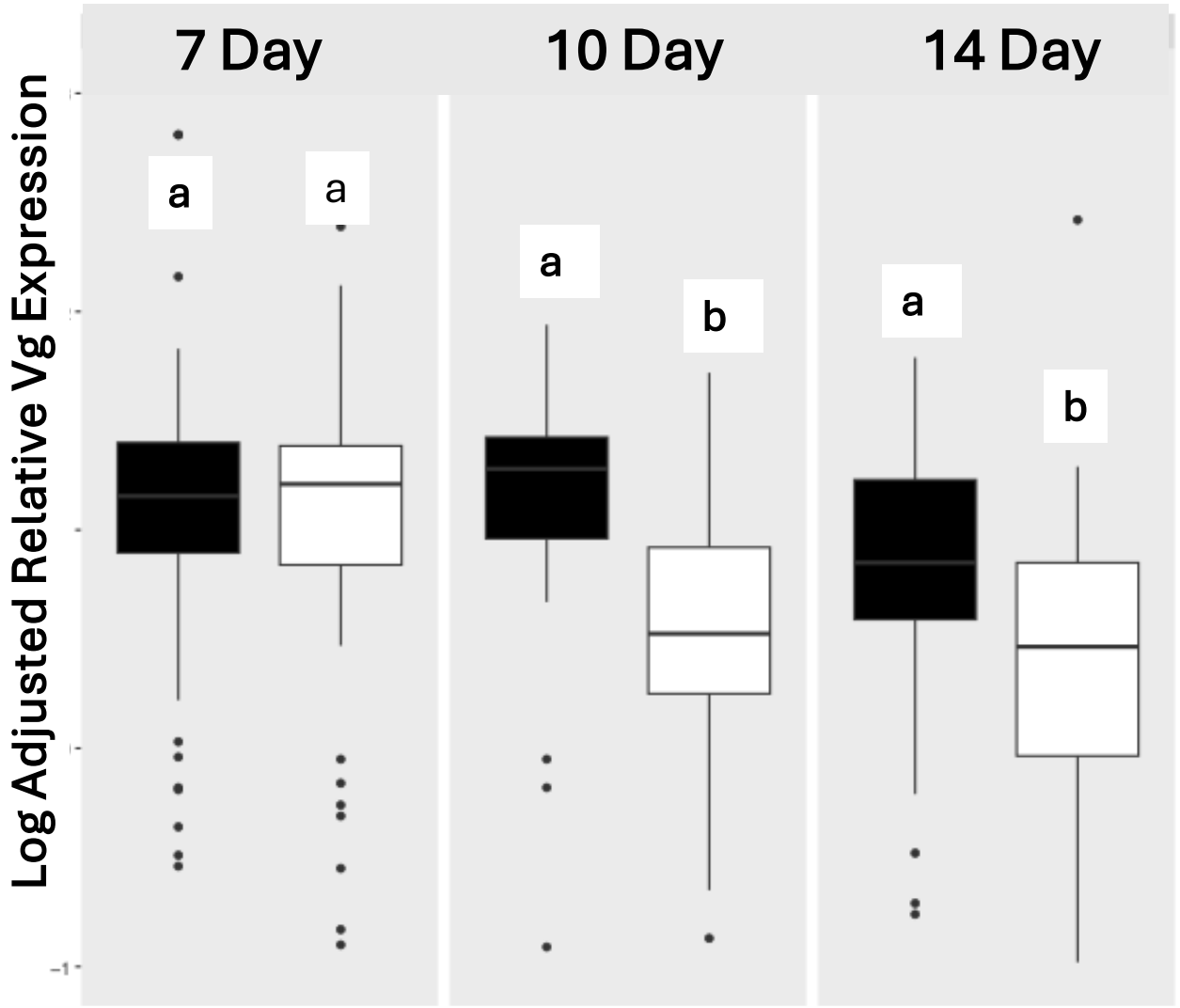
- when colony swarmed, paired with non-swarming colony of similar strength

- 7 paired colonies analyzed for *Vg* expression by qPCR at point of swarming, and 3d, 7d, and 14 d prior to swarm

Vg gene expression in honey bee workers from pre-swarming and non-swarming colonies across three age groups

= Pre-swarming

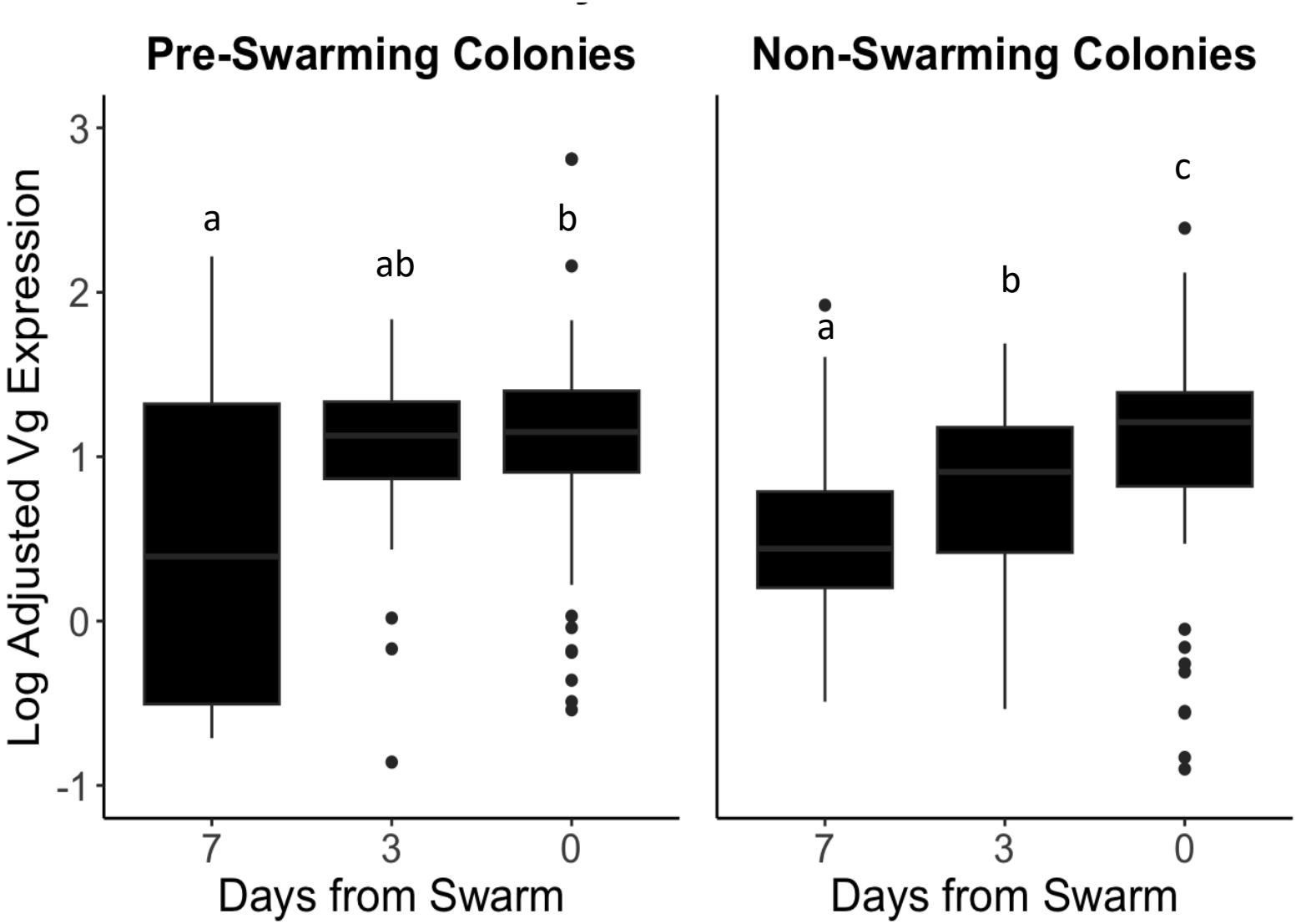
= Non-swarming



One way ANOVA:

- **colony type** (F = 4.303, df = 1, p = 0.038)
- **age group** (F = 23.374, df = 2, p < 0.001
- **interaction** (F = 11.774, df = 2, p = 0.0028)

Temporal Build-Up of Vitellogenin
7d old bees

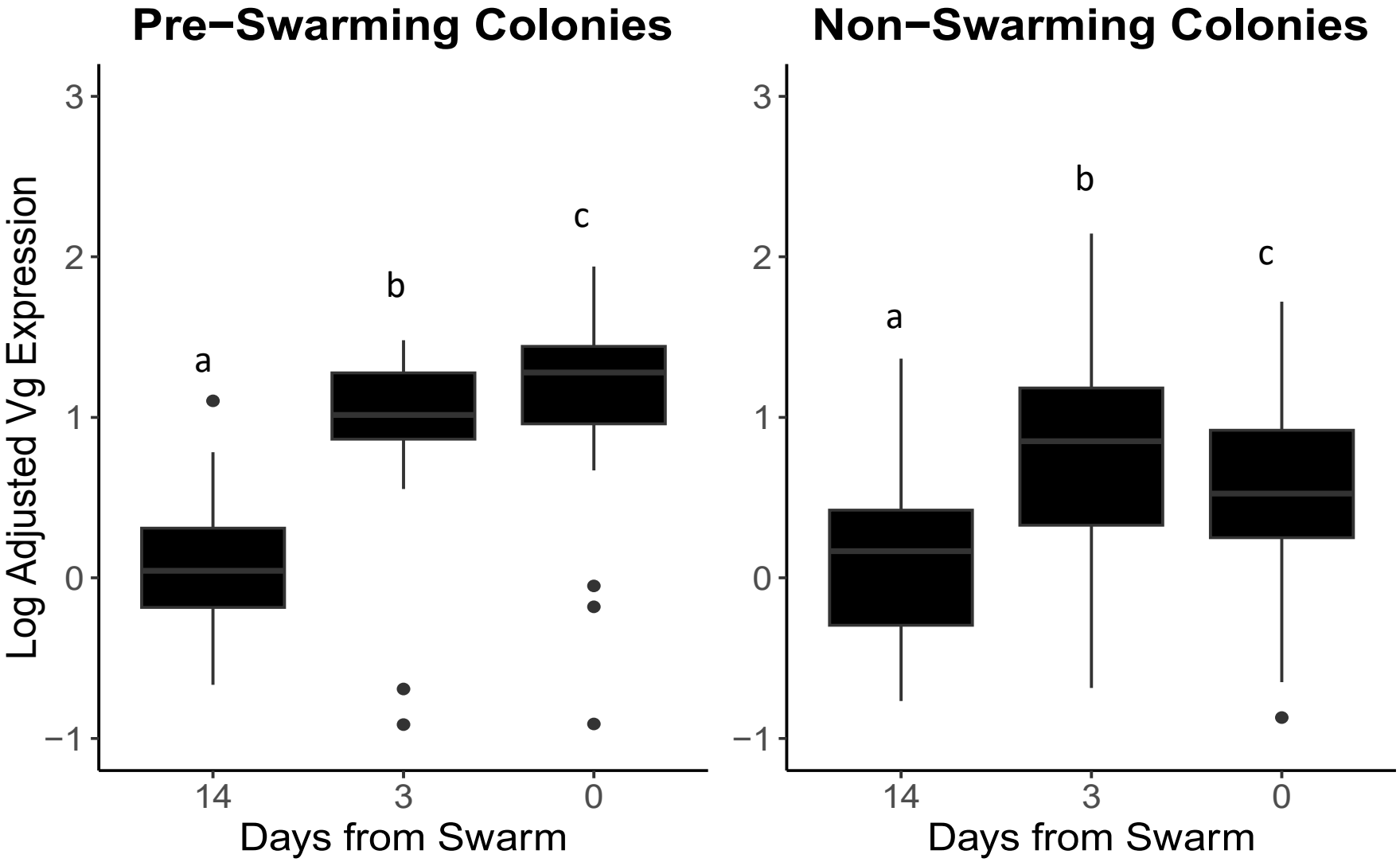


Jonckheere-Terpstra (JT) tests

Pre-swarming
JT=2902, p=0.072

Non-swarming
JT=5064, p<0.001

Temporal Build-Up of Vitellogenin
10d old bees

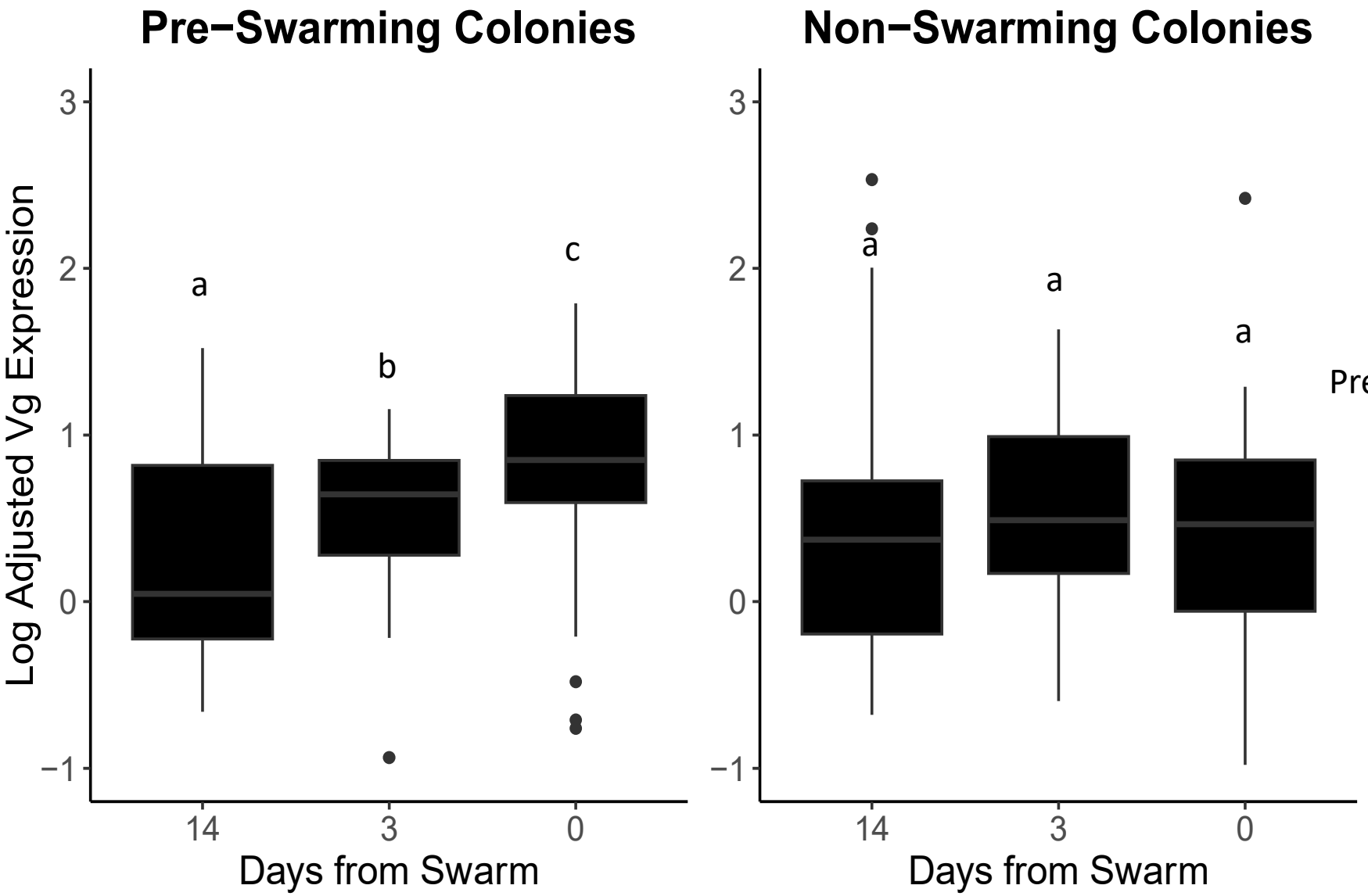


Jonckheere-Terpstra (JT) tests

Pre-swarming JT=3711, p=3.608e-13

Non- swarming JT=1557, p=0.8254

Temporal Build-Up of Vitellogenin
14d old bees



Jonckheere-Terpstra (JT) tests

Pre-swarming JT=5051, p=1.449e-08

Non-swarming JT=2886, p=0.744

Vg also functions as a mechanism for social reproduction in honey bees

Vg delays physiological maturation in pre-swarming worker bees

Vg has been co-opted in this process much like the diutinus bees

Vg levels remain high
(likely because of high pollen intake, and external repressor from foragers inside nest right before take off)

↓

Vg delays physiological aging

↓

builds capacity for the colony to swarm

ensures higher colony level fitness

Future work ideas

- Gene expression alone may not fully capture the biochemical pathways involved in swarming preparation
- Vg in swarming & migrating *Apis cerana*
- Alter Vg levels or nutrition to test the relationship between *Vg* expression and swarming
- Establish a gene expression profile and regulatory network specific to swarming bees