

Queen brood care behavior is plastic and socially regulated in an annually eusocial insect

Blanca R. Peto

`blanca.peto@email.ucr.edu`

University of California, Riverside

Meghan E. Moore

University of California, Riverside

Claudinéia P. Costa

University of California, Riverside

Ysabel M. Giraldo

University of California, Riverside

S. Hollis Woodard

University of California, Riverside

Research Article

Keywords:

Posted Date: September 23rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11013693/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The ability of organisms to shift behaviors in response to their environment, known as behavioral plasticity, is a key facet of survival in many environmental contexts. Organisms living in social groups additionally exhibit behavioral plasticity with changes to the social environment. Within primitively eusocial insect systems, there exists an inherent queen behavioral shift away from brood care as nests move through solitary to social stages. However, whether the onset of sociality within the nest marks a fixed transition or whether queen behaviors remain responsive to the social environment remains unknown. In this study, we examined plasticity in bumble bee queen brood care in response to total worker loss, specifically exploring two key behaviors: feeding and incubation. We further asked whether queens become less plastic across colony developmental stages by creating early, middle, later-stage, and ultimately mature colonies. We found that across all nest developmental stages explored, queens retained their ability to perform brood care. Further, we found that queens regulate feeding and incubation behaviors differently, displaying a greater plasticity in incubation than in feeding following changes in social conditions. Broadly, this work highlights how life history transitions do not necessarily mark the end of an organism's behavioral plasticity.

Significance Statement

Behavioral plasticity allows organisms to adjust their behavior as environmental conditions change, but whether this flexibility persists across major life-history transitions is less clear. In bumble bees, queens shift away from brood care as workers emerge and the colony becomes social with the queen specializing in reproduction. We tested whether queens retain the ability to adjust brood care after this transition by experimentally removing workers across multiple stages of colony development. These findings demonstrate that the transition to social living does not reduce a queen's capacity to respond to changes in social environment. More broadly, our results reveal that reproductive specialization does not entail the loss of previously critical behaviors and that behavioral plasticity can persist beyond the life-history stage in which it is most strongly expressed.

INTRODUCTION

Phenotypic plasticity is the ability of a single genotype to produce different phenotypes, including in response to changing environmental conditions (Sommer 2020; Schlichting and Pigliucci 1998; Houston and McNamara 1992). Parental care, which is highly diverse across the Animal Kingdom, is broadly defined as traits that enhance the fitness of the parent's offspring. Examples of parental care include egg provisioning, brood attendance, and energetically intensive tasks such as feeding or incubation (Meunier and Kramer 2022). Plasticity can be particularly important in the context of parental care, where environmentally responsive shifts in brood care and reproductive investment can be critical to enhancing fitness (Westrick et al. 2023; West-Eberhard 2003; Stein and Bell 2019). Across many taxa, plasticity in parental care behavior is closely tied to life-history transitions, across which parents undergo stereotypical changes in behavior and physiology. These changes can be in response to factors such as

environmental cues, offspring development, and the physical condition of the parents. These shifts in parental care behavior allow parents to integrate the costs of care with other fitness-related demands, including self-maintenance (feeding and maintaining optimal body status), future reproduction, and survival (Wong and Kölliker 2012; Kölliker and Vancassel 2007) .

In eusocial insect systems, brood care is shaped not only by environmental cues, but is also heavily influenced by social conditions, including the presence, number, and identity of group members, and their interactions (Woodard et al. 2013; Sarro et al. 2021; Johnson 2003; Hugar et al. 1994; Robinson 1992; Fisher et al. 2022; Hölldobler and Wilson 1990). These social conditions can be especially dynamic during different stages in colony development, as both the number and roles of social group members change over time. Across all eusocial insect systems, brood care is provided by siblings, and in some species, there is also a stage that precedes the onset of sibling care wherein a solitary founding queen provides parental care. Some of the most extreme examples of brood care plasticity occur in queens with this type of solitary colony founding. In these systems, queens initially function as both the sole caregiver and the primary reproductive, performing all tasks required for early colony establishment (Cronin et al. 2013). After daughter workers emerge, the social environment of the nest changes dramatically, as workers assume many of the tasks previously performed by the queen and the queen transitions toward a primarily reproductive role (Woodard et al. 2013; Sarro et al. 2021; Cronin et al. 2013; Oster and Wilson 1978; Rubenstein and Abbot 2017).

Most bumble bees (genus *Bombus*, family Apidae) follow this stereotypical solitary-founding colony life cycle and provide an excellent model for examining how queen brood care changes across life-history transitions (Woodard et al. 2013; Sarro et al. 2021; Fisher et al. 2022; Shpigler et al. 2013; Yadav et al. 2016; Peto et al. 2025). In bumble bees, the transition from queen-mediated care to worker-mediated care can occur rapidly, with queen brood care declining markedly within days of worker emergence (*B. terrestris* (Woodard et al. 2013) and in response to only a few workers in the nest (in *B. impatiens* (Sarro et al. 2021; Gustilo et al. 2025)). Queens also increase egg-laying when workers are added prematurely (Woodard et al. 2013), but cannot sustain elevated egg-laying when workers are continuously removed during colony development (Peto et al. 2025) These studies together suggest that queen egg-laying and brood care behavior are both tightly regulated by workers in the nest, at least during the earliest stage of nest initiation and development. This is hypothesized to allow queens to mediate a greater investment in reproduction, rather than brood care, right at the time when helpers first emerge in the nest. Little is known, however, about whether queens remain responsive to the presence of workers during later stages of colony development, when workers greatly outnumber the queen and queen brood care is expected to be greatly reduced (Shpigler et al. 2013; Heinrich 1979; Michener 1974; Sladen 1912; Plath 1934). Thus, whether the queen's transition away from brood care represents a fixed life-history shift or remains flexible and socially contingent is still unclear.

Here, we asked if bumble bee queen brood care behaviors remain plastic as colonies mature, and more specifically, if queen expression of brood care behavior is mediated by workers within the later-staged nests. We first quantified queen brood-care behavior, including both feeding and incubation, in nests at

three distinct stages of development of the bumble bee *Bombus impatiens*. This was done to establish a baseline trajectory of queen behavioral change across colony development. We then experimentally removed workers across colonies at these different developmental stages, in a subset of nests, to test whether queens adjust their brood care behavior and egg laying in response to such abrupt changes in the social environment. We hypothesized that queen behavioral plasticity declines as colonies mature. This is predicated on the idea that behavioral plasticity is costly (DeWitt et al. 1998; Snell-Rood 2013), and as queens become specialized on reproduction, it is not beneficial for them to maintain the costs of behavioral plasticity. Specifically, we predicted that queens in early-stage colonies would be more likely to increase brood care following worker removal, whereas queens in later and more mature colonies would be less likely to do so. Our study sheds light on the dynamics of queen brood care across multiple stages of the colony life cycle in a social insect.

METHODS

Bumble bee rearing and colony establishment:

Nine mature *Bombus impatiens* colonies were obtained from Koppert Biological Systems (Howell, MI, USA) and maintained within their commercial boxes at the University of California, Riverside Entomology Building. Colonies were stored at 27°C and 60–70% relative humidity under constant darkness or dim red light, which bumble bees cannot perceive. Individual adult callow queens (< 24 hours old), identified by their silvery appearance and inability to fly, were removed from natal colonies. Queens were placed individually in plastic containers (Hi-Plas plastic containers, 7.5 cm diameter) and provided ad libitum mixed-source honey bee–collected pollen (Koppert Biological Systems) and artificial nectar prepared following the recipe in Boyle et al. 2018.

At three days of adult age, queens were placed daily into mating cages (BugDorm-6E620 insect rearing cages; 60 × 60 × 120 cm) between 7 and 9 hours per day under ambient light conditions and allowed the opportunity to mate until age ten days. Queens remained in mating cages until the first time copulation was observed, and they were not returned to mating cages after mating occurred (Peto et al. 2025; Treanore et al. 2021; Watrous et al. 2019). This ensured that queens were singly mated. Adult males were obtained from three natal colonies and were paired with queens from different colonies to avoid within-colony mating. While in the mating cage, both queens and males were provided pollen and artificial nectar ad libitum (as described above).

Following mating, queens were housed individually in the same Hi-Plas containers (see above) with pollen and artificial nectar in an Invictus Drosophila incubator maintained at 27°C, constant darkness, and 60% relative humidity. At adult ages 12 and 13 days, queens were treated with CO₂ gas for 30 minutes over two days to induce them to bypass diapause (Röseler and van Honk 1990; Amsalem and Grozinger 2017). Following the second CO₂ treatment, queens were placed into plastic nest cages (Biobest Group USA, Inc.; 17 cm × 17 cm × 9.5 cm) containing pollen, artificial nectar, and a honey bee wax-covered pollen ball. Wax was provided as readily accessible wax for queens to utilize in nest

building. Nest cages were then transferred to the University of California, Riverside Insectary and Quarantine Facility and maintained under similar temperature and humidity conditions, with constant darkness except for brief light exposure during feeding or nest inspections. Pollen was replenished every 5–7 days, and artificial nectar was replaced every one to two weeks to prevent spoilage. Nests were inspected daily for new worker emergence (< 24 hours old; identified by silvery appearance), and the dates of adult worker emergence were noted. Once the first worker emerged, nests were transferred to a secondary room within the Insectary and Quarantine Facility with similar environmental conditions as described above (27°C, 60% relative humidity).

After successful mating and colony initiation, nests ($n = 50$) were evenly distributed first among three colony developmental stages, then further into treatment (with worker removal, W-) and control (without removing workers, W) colonies, with the following numbers of nests in each treatment group: early control (E/W; $n = 7$), early treatment (E/W-; $n = 9$), middle control (M/W; $n = 8$), middle treatment (M/W-; $n = 8$), later control (L/W; $n = 9$), and later treatment (L/W-; $n = 9$). To control for non-independence amongst queens, colony source was noted and later used in analyses (see below). These developmental stages and treatment (or sham) groups are further described below. No queens died during the experiment; thus, these queen/nest sample sizes were used for all analyses.

Nests were maintained until they reached worker numbers of approximately 5, 15, or 50 individuals, which we refer to as “early (E),” “middle (M),” or “later (L)” colonies, respectively. These colony developmental stages were chosen to represent different stages of early nest development, across which queens progressively reduce their brood care behavior and increase their egg laying (Woodard et al. 2013; Shpigler et al. 2013). By later stages of colony development (~ 30 days post-first worker emergence, when there may be ~ 50–70 workers in the nest (Peto et al. 2025; Rotheray et al. 2017), queen-provided brood care is typically minimal (< 5% (Shpigler et al. 2013; Leza et al. 2018)). As such, our largest worker group (~ 50 workers) represents a developmental stage in which queen brood care is expected to be rare or nearly absent. All nests in the study produced worker offspring, indicating successful mating and diploid female production.

Worker removal manipulation

Once nests reached a stage of development, according to their assigned group (see above), they were subsequently further divided into treatment and control (sham) groups. Nests assigned to the treatment group experienced total worker removal after the initial 24-hour pre-manipulation recording period (video details described below), whereas sham nests were disturbed but had no workers removed. Workers were manually removed from treatment nests using long forceps while avoiding disturbing the queen and brood. Removing workers from the nest (termed “sociotomy”) is an established, powerful tool for studying social regulation and other social effects by examining responses in remaining individuals and nesting conditions (Gardner et al. 2007; Kolmes and Winston 1988; Backen et al. 2000). After worker removal, nests were monitored for an additional five minutes to allow for the detection and removal of any additional workers that were further hidden within the nest structure. In the control nests, each

worker within the nest was briefly (2–3 seconds) handled with forceps to mimic the disturbance associated with worker removal and was returned to the nest. All treatment and control nests (sham group) were undisturbed after this period.

Video collection, selection, and scoring:

All nests, and thus all six groups, were subjected to a 48-hour recording session, with the first 24-hours used to capture baseline levels of brood care behaviors, and the second 24-hours used to examine brood care behaviors during the post-manipulation (treatment/sham) period.

Infrared security cameras (VIGICA Peashooter QD520) were positioned above each nest to continuously record queen brood care behaviors, following previously described methods (Sarro Gustilo et al. 2023; Fisher et al. 2022). Each colony was recorded for 48 hours, consisting of a 24-hour pre-manipulation phase and a 24-hour post-manipulation phase. The pre-manipulation phase captured baseline queen behaviors prior to either worker removal or sham manipulation, while the post-manipulation phase captured queen behaviors after treatment, either worker removal (W-) or worker presence/sham control (W). Hereon, the full 48-hour recording session (pre/post-manipulation) is called the “entire experimental phase.” These recordings allowed us to compare individual queen behavior before and after worker removal and group-level differences in queen behavior both between and within given colony developmental stages (see Supplementary Fig. 1 for experimental flowchart). Recordings and manipulations were conducted at approximately 10:00 AM for each colony, such that pre- and post-manipulation periods corresponded to equivalent times of day across colonies.

Of the full 48-hour experimental phase, a total of ten hours of video were scored for brood care behaviors. Hours are reported relative to the start of each recording, with hour 0 representing the first hour of recording (0-1h) and hour 23 representing the final hour of a 24-hour recording period (23–24 h). For each nest, two hours were analyzed before treatment, and eight hours were scored after treatment or sham manipulation (Supplementary Fig. 2). The first and final hours of each 24-hour recording period were excluded to account for potential lighting or behavioral disturbances, as well as occasional inconsistencies in recording duration when cameras were reset or new queens were placed under observation. From the remaining 22-hour interval (hours 1–23), pre-manipulation behavior was scored for two one-hour windows: hours 4–5 and 19–20. All nests during the pre-manipulation phase were unaltered, regardless of which treatment group they were assigned to, and thus we would expect two hours to be sufficient to capture behaviors pre-manipulation. A shorter period of observation (two hours) was deemed sufficient to capture individual queen behaviors during this period relative to the post-treatment period. Post-treatment behavioral observations were scored more extensively as this period was of particular interest for our study, and were conducted during eight one-hour sampling windows spaced every three hours, allowing us to capture cumulative brood care behavior across the day while accounting for natural temporal variation and potential circadian effects in queen behavior (Sarro Gustilo et al. 2023; Fisher et al. 2022). Analyses comparing pre- and post-treatment behavior were restricted to two matched time points, using the same two sampling windows in both periods 4–5 and 19–20.

Specifically, these time points were selected to capture behavior relatively close to the initial manipulation period and later in the recording period, after queens had more time to adjust to worker removal. This matched timepoint approach allowed us to compare queen behavior before and after treatment while controlling for potential effects by time of day.

In total, over 500 hours of video were watched across all nests (10 hours per nest over 50 nests). All behavioral observations were performed by a single observer. It was not possible to fully blind behavioral classification because treatment (presence or absence of workers) and colony size-related features (e.g., number of workers present and physical colony size) were visible in the videos. However, the observer was unaware of colony identity during behavioral classification.

In our study, queen brood care behaviors were limited to incubation and brood feeding, the two behaviors in which queens interact most directly with brood, and are defined as follows. For incubation: queen bee is stationary and perched on brood wax or cocoon in a flattened position with the thorax and abdomen contacting the brood, and the abdomen is often pumping (Michener 1974; Sladen 1912; Heinrich 1972), see Supplement Video 1). To ensure accurate scoring, incubation was recorded only when the queen's main body (thorax and abdomen, excluding head and legs) was positioned directly on the brood. For feeding: stereotypical behavior in which the queen pierces the larval wax envelope, inserts her mouthparts through the opening, and regurgitates food, accompanied by an abdominal contraction (Woodard et al. 2013; Fisher et al. 2022; Sladen 1912; Sarro Gustilo 2023; Costa et al. 2021), see Supplement Video 2).

In addition to our primary experimental colonies described above, we also examined queen brood care behaviors within a set of nine mature colonies (five treatments and four controls; pre-gyne emergence; average = 456.56 ± 32.62 workers). This was done to examine brood-care behaviors at a point beyond which queens would be expected to provide any brood care in the nest. Colonies were obtained from Koppert Biological Systems, maintained in the same room in UCR's Insectary and Quarantine, and provided pollen and artificial nectar ad libitum as described above. Nests were acclimated to laboratory conditions for three weeks before observation. Due to the size and structural complexity of these colonies, complete worker removal was not feasible, and approximately 20–40 workers remained in the W- nests following manipulation. Infrared cameras (described above) were also placed above these mature colonies; however, due to the substantially later nest size and colony complexity, cameras were unable to capture the entire nest simultaneously. Consequently, these colonies were not included in statistical analyses, and behavioral data were instead used qualitatively (yes/no was a given behavior observed in the nest). We also only observed incubation in these fully mature colonies, as feeding was more difficult to observe in these videos. For these observations, videos were monitored until the first occurrence of incubation was detected, after which the remaining footage was not analyzed.

Reproductive behavior and colony development:

After the 48-hour recording period, all nests were frozen and stored at -80 C until dissections were performed. During dissections, the number of eggs present in each nest was recorded as a metric of queen reproductive output. Eggs present in the nests at the end of the experiment were assumed to reflect queen egg-laying (rather than eggs laid by workers), given that queens lay the overwhelming majority of eggs in bumble bee nests (Alaux et al. 2004; Alaux et al. 2006; Amsalem et al. 2017; Bloch 1999; Padilla et al. 2016).

In addition to egg counts, other, older brood were visually inspected and classified into developmental categories following Rozen et al. (2018) and Peto et al. (2025): (i) *eggs*; (ii) *younger larvae* corresponding to first and second instars housed in communal chambers; (iii) *older larvae* corresponding to third instar and most fourth instar larvae that begin spinning cocoons; and (iv) *late-stage brood*, which includes post-defecation fourth instar larvae, prepupae, and pupae. These same classifications were used when determining which brood stages were fed or incubated during behavioral observations and for subsequent analyses.

Statistical analyses:

All statistical analyses were conducted in R version 4.3.1 (R Core Team 2023), and statistical significance was assessed at $\alpha = 0.05$. Data distributions were evaluated using Shapiro–Wilk tests, and most response variables deviated from normality. Accordingly, we employed generalized linear mixed models (GLMMs) to examine how social conditions influenced queen brood care behavior. GLMMs were fitted using the *glmmTMB* package (Brooks et al. 2017). Specifically, Gaussian models were used for approximately normally distributed responses (e.g., incubation frequency in some subsets). Gamma distributions were used for non-normal, skewed responses (e.g., incubation duration and total incubation effort), and negative binomial models (*nbinom2*) were used for overdispersed count data (e.g., feeding frequency, final egg counts, non-egg brood counts). In cases where response variables were proportional (e.g., standardized incubation), alternative distributions (e.g., ordered beta) were applied as appropriate. Across all models, natal colony was included as a random effect to account for queens originating from the same natal colony. When relevant, queen-level repeated measures (e.g., pre- vs. post-manipulation) were included as fixed effects. Fixed effects included treatment (worker presence versus removal), colony size (early, middle, and later), and their interactions, depending on the specific hypothesis being tested. See supplementary materials for more detailed definitions.

For each response variable, we constructed a set of candidate models representing all biologically relevant additive and interactive combinations of fixed effects, while holding the random-effects structure constant. Model selection was performed using Akaike's Information Criterion (AIC) via the *model.sel* function in the MuMIn package (Bartoń et al. 2024). The model with the lowest AIC value was considered the best-supported model; however, when competing models differed by less than $\Delta\text{AIC} = 2$, biological interpretability was also considered in model selection (see supplementary information for more details). Following model selection, we evaluated overall model effects using Type II analysis of

variance (ANOVA) from the *car* package. Post hoc pairwise comparisons were conducted using estimated marginal means (emmeans) with Tukey-adjusted contrasts.

To examine relationships between correlated responses, such as total incubation effort and egg production, we accounted for collinearity by using residuals derived from regressions of incubation effort against treatment (following Graham, 2003). This approach allowed us to isolate variation in incubation behavior independent of treatment effects.

To establish a baseline understanding of changes in queen behavior across colony stages in unmanipulated queens, we analyzed the post-manipulation phase in control queens (post-W) only. We used the post-manipulation phase to maximize observational periods across time. Specifically, we tested whether incubation frequency, average individual incubation duration, cumulative incubation duration, and total feeding events varied across colony developmental stages (early, middle, or late). Feeding behavior was analyzed only as a count because each feeding event consisted of a single, quick regurgitation event directed toward a larva or group of larvae. We used Gaussian models for incubation frequency, Gamma models for incubation duration variables, and a negative binomial model for feeding event counts. Candidate models were compared using AIC, and estimated marginal means were used for post-hoc pairwise contrasts.

All data processing and visualization were conducted in R using packages including *tidyverse* and *ggplot2*. Additional details regarding candidate model structures and full model outputs are provided in the Supplementary Materials. For definitions of how analyses were conducted, see the “definitions and description of data used in each analysis” section in the supplement.

RESULTS

Queen brood care does not decline across the unmanipulated colony stages tested

First, we asked if brood care behaviors (incubation and feeding) differed across colony developmental stages in our control queens only, to establish a baseline for the amount of brood care queens provide at different points in colony development. For incubation, we examined three facets: cumulative incubation duration (measured as total minutes across the post-manipulation phase), frequency of incubation events per hour (measured as total events divided by experimental phase hours), and average event duration (measured as the average duration of individual incubation events). In general, across all groups, queens spent between 22–24% (on average) of each hour incubating. We did not detect any differences in any of aspects of queen incubation behavior (cumulative incubation duration: $\chi^2 = 1.2895$, $df = 2$, $p = 0.5248$; incubation frequency per hour: $\chi^2 = 1.0854$, $df = 2$, $p = 0.5812$; average incubation event duration: $\chi^2 = 0.0448$, $df = 1$, $p = 0.8324$; Supplementary Fig. 3–5) across the unmanipulated treatment groups. For more details regarding analyses, refer to the supplement.

Additionally, we did not detect any differences in the total number of queen feeding events across the colony developmental stages in our unmanipulated queens (overall model: $\chi^2 = 0.102$, $df = 2$, $p = 0.9503$). Total feeding events across the matched timeframes for the control queens were generally rare (average number of feeding events observed per group $\pm$ s.e.: E: 1.86 ± 0.553 ; M: 1.62 ± 0.498 ; L: 1.44 ± 0.475).

Queen incubation, but not feeding, increases when workers are removed from the nest

Cumulative duration of brood incubation provided by queens was associated with both treatment and experimental phase, in all colony developmental stages (Overall model: E: $\chi^2 = 6.4357$, $df = 1$, $p < 0.05$; M: $\chi^2 = 18.0507$, $df = 1$, $p < 0.001$; L: $\chi^2 = 13.0811$, $df = 1$, $p < 0.005$). Cumulative incubation duration was higher in treatment groups post manipulation compared to pre-manipulation (Pre/W) and was higher than controls, regardless of experimental phase, across all stages of colony development tested (post-hoc tests: early: Post/E/W – vs. Pre/E/W–: $T = -4.860$, $p < 0.0001$; middle: Post/M/W – vs. Pre/M/W–: $T = -5.058$, $p < 0.001$; later: Post/L/W – vs. Pre/L/W–: $T = -5.101$, $p < 0.001$ (Fig. 1). All other comparisons did not differ significantly in all colony developmental stages (all comparisons $p > 0.05$).

Incubation frequency was also associated with treatment in all colony developmental stages, with higher incubation frequency following worker removal (overall model: E: $\chi^2 = 38.007$, $df = 1$, $p < 0.001$; M: $\chi^2 = 17.5708$, $df = 1$, $p < 0.001$; L: $\chi^2 = 6.6729$, $df = 1$, $p < 0.05$; Supplementary Fig. 6). Post hoc comparisons for the middle and later colony developmental stages only are reported in Supplementary Table 1. The early colony developmental stage is excluded from this table as incubation frequency did not differ between experimental phases.

Finally, the average duration of an individual incubation event was only associated with worker removal treatment and experimental phase in our early nests (E: $\chi^2 = 5.1664$, $df = 1$, $p < 0.05$, M: $\chi^2 = 0.3397$, $df = 1$, $p = 0.5600$; L: $\chi^2 = 1.4923$, $df = 1$, $p = 0.2219$, Supplementary Fig. 7). The effect in our early-stage colonies was driven by shorter durations in our pre-manipulation treatment group (Pre/W-) compared with the post-manipulation treatment (Post/W-) and pre-manipulation control (Pre/W, $Z = -2.635$, $p = 0.0418$), with no significant additional pairwise comparisons.

Finally, in our mature colonies ($n = 9$), queens were observed incubating brood in all nests, regardless of whether workers were present or removed.

The change in the proportion of time queens allocated to incubation, calculated as post-manipulation minus pre-manipulation incubation, differed significantly by treatment ($\chi^2 = 46.495$, $df = 1$, $p < 0.001$). Worker-removal queens (W-) increased incubation allocation after worker removal, whereas control queens (W) showed little to no change from pre-to post manipulation ($p < 0.01$; Supplementary Fig. 8). Across colony developmental stages, there were no significant differences in incubation change within each of the treatment group (Supplementary Fig. 8).

Queen brood feeding behavior was not impacted by worker removal (overall model: E: $\chi^2 = 1.5209$, $df = 1$, $p = 0.2$; M: $\chi^2 = 2.6338$, $df = 1$, $p = 0.05$; L: $\chi^2 = 0.8157$, $df = 1$, $p = 0.4$). Queens were observed feeding brood across all colony developmental stages and experimental phases, but brood feeding was relatively infrequently observed (average number of feeding events observed per stage and treatment: E/W: 2.07 ± 0.57 ; E/W-: 1.44 ± 0.34 ; M/W: 1.81 ± 0.31 ; M/W-: 1.00 ± 0.32 ; L/W: 1.17 ± 0.28 ; L/W-: 0.83 ± 0.26).

Queens incubate late-stage brood most often, but feed only the youngest brood:

Queens incubated late-stage brood the most (84% of events) of any brood type ($n = 2994$ incubation events were on late-stage brood, out of $n = 3527$ total events observed).

We then asked whether queen incubation was allocated differently by brood type after workers were removed. To account for variation among queens in total time spent incubating, we calculated the relative proportion of incubation time allocated to each brood type (hereby standardized incubation duration; see supplement for more details). Standardized cumulative incubation duration was only significantly associated with brood type in the early and later colony developmental stages (E: $\chi^2 = 26.99$, $df = 3$, $p < 0.001$; M: $\chi^2 = 7.08$, $df = 3$, $p = 0.069$; L: $\chi^2 = 9.15$, $df = 3$, $p < 0.05$; Fig. 2). In contrast, worker removal treatment alone was not significantly associated with standardized cumulative incubation duration in any colony developmental stage (E: $\chi^2 = 1.44$, $df = 1$, $p = 0.229$; M: $\chi^2 = 0.80$, $df = 1$, $p = 0.372$; L: $\chi^2 = 1.81$, $df = 1$, $p = 0.179$). Supplementary Table 2 shows the results of emmeans pairwise comparisons of colony developmental stages and treatment groups.

Unlike what was observed for incubation, feeding behavior was overwhelmingly directed towards young larvae. Of the total feeding events observed across all nests, 99.7% (309 of 310 events) involved feeding young larvae, with only one event involving feeding older larvae.

Tradeoff between incubation and egg-laying in later-staged nests:

Total brood increased with colony developmental stage ($\chi^2 = 237.9$, $df = 2$, $p < 0.0001$), with treatment not included in the best-fit model. Total brood generally increased with colony developmental stage, with early stage containing the fewest brood items and late stage containing the most (E vs. M: $Z = -7.251$, $p < 0.0001$; E vs. L: $Z = -14.955$, $p < 0.0001$; M vs. L: $Z = -8.665$, $p < 0.0001$). On average, early-stage colonies contained 71 ± 5.65 brood items, middle-stage colonies contained 161 ± 12 brood items, and later-stage colonies contained 340 ± 17.8 brood items.

We then asked whether the queen's reproduction was influenced by treatment (W/W-). Worker removal had a significant negative impact on the number of eggs in the nest at the end of the experiment, only in the later-stage colonies (GLMM: $\chi^2 = 7.71$, $df = 1$, $p = 0.005$; L/W = 101.9 ± 14.3 eggs, L/W- = 72.5 ± 24.9 eggs (early- and middle-stage colonies (E: $\chi^2 = 0.128$, $df = 1$, $p = 0.721$; M: $\chi^2 = 0.0246$, $df = 1$, $p = 0.875$, see Fig. 3)). Worker removal did not impact the amount of non-egg brood types in the nests (E: $\chi^2 =$

0.056, $df = 1$, $p = 0.812$; M: $\chi^2 = 0.4604$, $df = 1$, $p = 0.497$; L: $\chi^2 = 0.0196$, $df = 1$, $p = 0.889$). There was no relationship between the total amount of time a queen spent incubating and the number of eggs in her nest at the end of the experiment ($p = 0.9441$; see Supplementary Fig. 9).

Discussion

Social conditions can be manipulated to investigate plasticity in brood care behavior, including within the queen caste of eusocial systems where queens found colonies solitarily (Woodard et al. 2013; Robinson 1992; Sarro Gustilo et al. 2023). In bumble bees, solitary founding queens naturally reduce their brood care behavior at the earliest stages of colony development as they transition from solitary to social living. This reduction can be artificially induced by manipulating social conditions within the nest, such as prematurely adding an early cohort of workers (Woodard et al. 2013; Robinson 1992; Sarro Gustilo et al. 2023). This response demonstrates that workers regulate queen bumble bee behavior. However, the permanence and the degree to which workers regulate queen behavior, beyond the incipient stages of nest development (Heinrich 1979; Michener 1974; Sladen 1912; Plath 1934), are not currently known. Here, we used a sociotomy experiment in the bumble bee *B. impatiens*, removing all workers from the nest, to shed new light on the social regulation of queen bumble bee brood care behavior.

We show that queens can increase their expression of brood-care behavior, specifically incubation, when artificially induced to return to conditions in which no other individuals are present to provide care in the nest. Queens in our study increased the proportion of time allocated to incubation after worker removal, and this effect was similar across all colony developmental stages. This demonstrates that queens remain equally plastic in their responses to social conditions as they age and as nests grow. This is contrary to our original hypothesis: that queen behavioral plasticity might also decline as their behavioral repertoire narrows. As behavioral plasticity is thought to be costly (DeWitt et al. 1998; Snell-Rood 2013), this suggests that for bumble bee queens, the benefits of maintaining plasticity in brood care behavior might outweigh these costs. Whether this is true specifically in the context of brood care or also holds for other types of behavior is not currently known. A study by Richardson et al. (2026) found that bumble bee queens in the fully reproductive stage retain relatively high cognitive abilities. Together with our findings, this suggests that bumble bee queens maintain surprising cognitive abilities and behavioral plasticity even as they age, rather than exhibiting declining cognition as their behavioral repertoire narrows across colony development.

Whereas queen incubation was highly responsive to social conditions, queen brood feeding was not. This is consistent with previous work demonstrating that these two behaviors are differently regulated in bumble bee queens, with incubation being the more labile of the two behaviors (Sarro Gustilo 2023). Incubation and feeding are both critical for brood development and thus overall nesting success (Heinrich 1972; Hasselrot 1960; Grad and Gradišek 2018; Vogt 1986; Pendrel and Plowright 1981; Pereboom et al. 2003). Of the two behaviors, incubation is likely more energetically demanding and time-intensive, and it is required for all larval instars and pupae (Heinrich 1979; Heinrich 1972; Vogt 1986; Goulson 2003). Even small decreases in brood temperature can substantially reduce brood

developmental rate, survival, and colony growth (Stewart et al. 2021), and restoring heat to cooled brood is more energetically expensive than maintaining stable temperatures (Tulp and Schekkerman 2006). In contrast, larval feeding is brief (i.e., a few seconds) and occurs far less frequently (Sladen 1912; Sarro Gustilo et al. 2023; Free and Butler 1959). Compared to the consistent requirement of incubation, feeding is periodically elicited by a larval hunger signal (de Boer and Duchateau 2006; Smeets and Duchateau 2001). Thus, incubation and brood feeding may be uniquely regulated because they are fundamentally different behaviors, involve different physiological and behavioral processes, and are differently demanded by developing brood. Here, we focused solely on feeding and incubation behaviors, as queens interact most directly with developing brood in performing these behaviors. Other behaviors that queens were restricted from expressing in our study, such as foraging and nest guarding, are also critical brood care behaviors. If allowed to express these additional behaviors, queens may allocate their time differently, or respond differently to the sociotomy treatment we subjected them to here.

Queens in our study never ceased providing brood care. In our main experiment, queens continued to incubate across all colony stages, and queens were also observed incubating in our examination of a small subset of fully mature nests with over 400 workers. We also observed queens feeding larvae across all colony stages in our main experiment. To our knowledge, this is the first demonstration that bumble bee queens continue to provide brood care at more mature colony stages. We posit that bumble bee queens may never fully relinquish brood care. Interestingly, however, levels of both queen incubation and brood feeding remained consistent across all colony stages. This is even though older nests have more brood, and thus incubation and larval nutritional demands increase as nests grow. Under natural conditions, workers may provide all the additional brood care needed at these later stages. Thus, the queen's proportional contribution to total brood care may decrease, even if her actual level of care remains unchanged, because the overall amount of care increases with the presence of workers. Queens may not increase their incubation when more brood are present because they have an upper limit on the amount of brood care that is constrained for reasons that are currently unknown. Why queens continue to incubate, even when there are hundreds of incubating workers in the nest, is also unclear.

We also saw that queen incubation and brood feeding behavior were directed to different brood types. In our later colony developmental stage, queens performed more incubation toward late-stage brood (late-stage larvae, prepupae, and pupae). This finding with queens aligns with previous reports that bumble bee workers preferentially interact with pupae over larvae (Starkey et al 2019). In contrast, queens in our study directed feeding almost exclusively (99% of events) toward younger larvae. This pattern occurred despite previous work demonstrating that older larvae require more feeding than younger instars (Ribeiro 1994; Ribeiro et al. 1999; Pereboom et al 2003). Perhaps queen feeding behavior may not be related to larval energetic demands, but instead is allocated selectively rather than applied uniformly across all brood. Indeed, the queens' efforts toward specific developmental stages could be adaptive. Queens have a stronger influence, mediated through feeding, on the development of younger larvae (Franco et al. 2023; Cholé et al 2019). Concurrently, incubation of older brood may reduce the time until worker eclosion, at which point queens will have other brood-care-providing individuals in the nest, which may allow queens to more fully specialize on egg laying (Peto et al. 2025). Thus, by directing incubation and

feeding to specific brood stages, queens may influence brood developmental outcomes and, by extension, colony function.

Across animal systems, parental care and reproduction are governed by physiological and temporal trade-offs, in which increased investment in one function can come at the expense of another (Monaghan et al 1998; Visser and Lessells 2001; O'Rourke and Renn 2015). In solitary-founding eusocial systems, these trade-offs are additionally mediated by the emergence of helpers, which allow the primary reproductive individual (the queen) to shift investment away from care and toward reproduction. Within early bumble bee nests, experimental worker removal reduces queen egg laying, whereas the premature addition of workers accelerates egg laying (Peto et al. 2025; Woodard et al. 2013; Sarro et al. 2021). Consistent with this, we detected a significant decline in queen egg laying in our largest colonies approximately twenty-four hours following worker removal. These results suggest that queens can rapidly increase brood care following worker removal, but there may be reproductive costs in doing so. This is consistent with previous findings showing worker loss can influence queen egg laying and that the trade-off between brood care and reproduction can occur within hours of worker removal. Our study examined a much shorter timescale for detecting the effects of worker removal than has been reported in previous studies, where effects were observed over days (Shpigler et al 2013) to weeks (Sarro et al. 2021; Fisher et al. 2022; Sarro Gustilo et al. 2023). Across a longer timeframe than we examined, queens may ultimately become more responsive to the increasing demands of brood, or, alternatively, they may express less brood care as these demands overwhelm their capacity. Studies that extend behavioral analyses for > 24 hours are needed to explore these outcomes.

CONCLUSION

During the onset of nest development in solitary-founding eusocial insect systems, the queen's shift from a primarily caretaking to a reproductive role can be interpreted as a developmental reallocation to maximize egg laying. Our findings show that this transition is not complete, as *B. impatiens* queens continue to provide for brood far into colony development. Moreover, the transition is reversible, and queens can increase their maternal care when social conditions demand it. This persistence and flexibility in queen brood care behavior indicate that reproductive specialization does not necessarily entail a permanent loss of caregiving capacity. Reversibility in queen brood care behavior may be particularly important in unpredictable environments, where offspring survival can be compromised by sudden environmental changes. The ability of bumble bee queens to re-engage in parental care under certain conditions highlights the potential advantages of behavioral flexibility in primitively eusocial systems

As a primitively eusocial model system, bumble bees offer a unique opportunity to study the regulation of queen brood care behavior and reproduction in ways that are not possible in many highly eusocial systems. For example, in swarm-founding systems, new queens never pass through a solitary nest-founding stage (Seeley and Buhrman 1999; Winston 1987), and therefore never directly meet the caregiving demands that exist during early colony development in solitary founding species. The

plasticity documented here may reflect behavioral flexibility retained and possibly maintained by selection from ancestral repertoires, persisting for up to 70–80 million years since the bumble bee lineage is estimated to have first evolved sociality (Cardinal and Danforth 2011). Rather than representing a vestigial and non-functional trait, this plasticity may serve an adaptive role by enabling queens to adjust their behavior in response to changing social conditions, ultimately sustaining colony growth and reproductive success under variable environments.

Declarations

ACKNOWLEDGEMENTS:

We thank the members of the Woodard Lab for their invaluable feedback and review of the manuscript. Special thanks to Liam Grooms, who assisted with bee rearing and preliminary data collection. Finally, we are grateful to Koppert Biological Systems, Inc., USA, for generously supplying the bumble bee colonies used in our experiments. Trenton Jung created the artwork in Figure 1.

FUNDING:

This work was supported by the National Science Foundation (Award no. 2046158) to S.H.W.

CONTRIBUTIONS:

BRP and MEM contributed equally to this manuscript; led the experimental design, carried out the experiments, and led authorship of the manuscript. CPC co-designed the experiments and provided statistical analysis. YMG co-designed the experiments. SHW co-designed the experiments. All co-authors contributed to writing the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL:

All applicable institutional and natural guidelines for the care and use of insects were followed. No permits were required for the described study as *Bombus impatiens* is not a protected species.

COMPETING INTERESTS:

The authors declare no competing interests.

References

1. Alaux C, Jaisson P, Hefetz A (2004) Queen influence on worker reproduction in bumblebees (*Bombus terrestris*) colonies. *Insectes Sociaux* 51:287–293. <https://doi.org/10.1007/s00040-004-0741-5>
2. Alaux C, Jaisson P, Hefetz A (2006) Regulation of worker reproduction in bumblebees (*Bombus terrestris*): Workers eavesdrop on a queen signal. *Behav Ecol Sociobiol* 60:439–446.

<https://doi.org/10.1007/s00265-006-0184-2>

3. Amsalem E, Grozinger CM (2017) Evaluating the molecular, physiological and behavioral impacts of CO₂ narcosis in bumble bees (*Bombus impatiens*). *J Insect Physiol* 101:57–65.
<https://doi.org/10.1016/j.jinsphys.2017.06.014>
4. Amsalem E, Padilla M, Schreiber PM, Altman NS, Hefetz A (2017) Do bumble bee, *Bombus impatiens*, queens signal their reproductive and mating status to their workers? *J Chem Ecol* 43:563–572.
<https://doi.org/10.1007/s10886-017-0858-4>
5. Backen SJ, Sendova-Franks AB, Franks NR (2000) Testing the limits of social resilience in ant colonies. *Behav Ecol Sociobiol* 48:125–131. <https://doi.org/10.1007/s002650000219>
6. Bartoń K (2024) *MuMIn: Multi-model inference* (R package version 1.48.4). CRAN. <https://CRAN.R-project.org/package=MuMIn>
7. Bloch G (1999) Regulation of queen-worker conflict in bumble-bee (*Bombus terrestris*) colonies. *Proceedings of the Royal Society B: Biological Sciences*, 266(1437), 2465–2469.
<https://doi.org/10.1098/rspb.1999.0947>
8. de Boer SPA, Duchateau MJHM (2006) A larval hunger signal in the bumblebee *Bombus terrestris*. *Insectes Sociaux* 53:369–373. <https://doi.org/10.1007/s00040-006-0883-8>
9. Boyle NK, Tripodi AD, Machtley SA, Strange JP, Pitts-Singer TL, Hagler JR (2018) A nonlethal method to examine non-*Apis* bees for mark-capture research. *J Insect Sci* 18(3). Article 10.
<https://doi.org/10.1093/jisesa/iey043>
10. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM (2017) GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9(2):378–400. <https://doi.org/10.32614/RJ-2017-066>
11. Cardinal S, Danforth BN (2011) The antiquity and evolutionary history of social behavior in bees. *PLoS ONE* 6(6) Article e21086. <https://doi.org/10.1371/journal.pone.0021086>
12. Cholé H, Woodard SH, Bloch G (2019) Body size variation in bees: Regulation, mechanisms, and relationship to social organization. *Curr Opin Insect Sci* 35:77–87.
<https://doi.org/10.1016/j.cois.2019.07.006>
13. Costa CP, Fisher K, Guillén BM, Yamanaka N, Bloch G, Woodard SH (2021) Care-giver identity impacts offspring development and performance in an annually social bumble bee. *BMC Ecol Evol* 21., Article 20. <https://doi.org/10.1186/s12862-021-01756-2>
14. Cronin A, Molet M, Doums C, Monnin T, Peeters C (2013) Recurrent evolution of dependent colony foundation across eusocial insects. *Ann Rev Entomol* 58:37–55. <https://doi.org/10.1146/annurev-ento-120811-153643>
15. DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 13(2):77–81. [https://doi.org/10.1016/S0169-5347\(97\)01274-3](https://doi.org/10.1016/S0169-5347(97)01274-3)
16. Fisher K, Sarro E, Miranda CK, Guillen BM, Woodard SH (2022) Worker task organization in incipient bumble bee nests. *Anim Behav* 185:143–161. <https://doi.org/10.1016/j.anbehav.2021.12.005>

17. Franco M, Fassler R, Goldberg TS, Chol   H, Herz Y, Woodard SH, Reichmann D, Bloch G (2023) Substances in the mandibular glands mediate queen effects on larval development and colony organization in an annual bumble bee. *Proc Natl Acad Sci USA* 120(45) Article e2302071120. <https://doi.org/10.1073/pnas.2302071120>
18. Free JB, Butler CG (1959) *Bumblebees*. Collins
19. Gardner KE, Foster RL, O'Donnell S (2007) Experimental analysis of worker division of labor in bumblebee nest thermoregulation (*Bombus huntii*, Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology*, 61(5), 783–792. <https://doi.org/10.1007/s00265-006-0309-7>
20. Goulson D (2003) *Bumblebees: Their behaviour and ecology*. Oxford University Press
21. Grad J, Gradi  ek A (2018) Bumblebee brood temperature and colony development: A field study. *Acta Entomologica Slov* 26:219–232
22. Graham MH (2003) Confronting multicollinearity in ecological multiple regression. *Ecology* 84(11):2809–2815. <https://doi.org/10.1890/02-3114>
23. Gustilo ES, Grover WH, Woodard SH (2025) Wild foundress queen bumble bees make numerous, short foraging trips and exhibit frequent nest failure: Insights from trap-nesting and RFID tracking. *Ecol Evol* 15:e71016. <https://doi.org/10.1002/ece3.71016>
24. Hasselrot TB (1960) Studies on Swedish bumblebees (*Genus Bombus* Latr.), their domestication and biology. *Opuscula Entomologica Supplement* 17:1–192
25. Heinrich B (1972) Physiology of brood incubation in the bumblebee queen, *Bombus vosnesenskii*. *Nature* 239:223–225. <https://doi.org/10.1038/239223a0>
26. Heinrich B (1979) *Bumblebee economics*. Harvard University Press
27. H  lldobler B, Wilson EO (1990) *The ants*. Harvard University Press
28. Houston AI, McNamara JM (1992) Phenotypic plasticity as a state-dependent life-history decision. *Evol Ecol* 6:243–253. <https://doi.org/10.1007/BF02214164>
29. Huang ZY, Robinson GE, Borst DW (1994) Physiological correlates of division of labor among similarly aged honey bees. *J Comp Physiol A* 174:731–739. <https://doi.org/10.1007/BF00192722>
30. Johnson BR (2003) Organization of work in the honeybee: A compromise between division of labour and behavioural flexibility. *Proceedings of the Royal Society B: Biological Sciences*, 270(1511), 147–152. <https://doi.org/10.1098/rspb.2002.2207>
31. K  lliker M, Vancassel M (2007) Maternal attendance and the maintenance of family groups in common earwigs (*Forficula auricularia*): A field experiment. *Ecol Entomol* 32:24–27. <https://doi.org/10.1111/j.1365-2311.2006.00831.x>
32. Kolmes SA, Winston ML (1988) Division of labor among worker honey bees in demographically manipulated colonies. *Insectes Sociaux* 35:262–270. <https://doi.org/10.1007/BF02224059>
33. Leza M, Watrous KM, Bratu J, Woodard SH (2018) Effects of neonicotinoid insecticide exposure and monofloral diet on nest-founding bumblebee queens. *Proceedings of the Royal Society B: Biological Sciences*, 285(1880), Article 20180761. <https://doi.org/10.1098/rspb.2018.0761>

34. Meunier J, Körner M, Kramer J (2022) Parental care. In: Omkar, Mishra G (eds) Reproductive strategies in insects, 1st edn. CRC
35. Michener CD (1974) The social behavior of the bees: A comparative study. Belknap Press of Harvard University
36. Monaghan P, Nager RG, Houston DC (1998) The price of eggs: Increased investment in egg production reduces the offspring-rearing capacity of parents. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 731–735. <https://doi.org/10.1098/rspb.1998.0495>
37. O'Rourke CF, Renn SCP (2015) Integrating adaptive trade-offs between parental care and feeding regulation. *Curr Opin Behav Sci* 6:160–167. <https://doi.org/10.1016/j.cobeha.2015.11.010>
38. Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press
39. Padilla M, Amsalem E, Altman N, Hefetz A, Grozinger CM (2016) Chemical communication is not sufficient to explain reproductive inhibition in the bumblebee *Bombus impatiens*. *Royal Soc Open Sci* 3(10):160576. <https://doi.org/10.1098/rsos.160576>
40. Pendrel BA, Plowright RC (1981) Larval feeding by adult bumble bee workers (Hymenoptera: Apidae). *Behav Ecol Sociobiol* 8:71–76. <https://doi.org/10.1007/BF00300817>
41. Pereboom JJM, Velthuis HHW, Duchateau MJ (2003) The organisation of larval feeding in bumble bees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insectes Sociaux* 50:127–133. <https://doi.org/10.1007/s00040-003-0639-7>
42. Peto BR, Costa CP, Moore ME, Woodard SH (2025) Social control of egg-laying in independently nest-founding bumble bee queens. *BMC Ecol Evol* 25:30. <https://doi.org/10.1186/s12862-025-02364-0>
43. Plath OE (1934) Bumblebees and their ways. Macmillan Company
44. R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing
45. Ribeiro MF (1994) Growth in bumble bee larvae: Relation between development time, mass, and amount of pollen ingested. *Can J Zool* 72(11):1978–1985. <https://doi.org/10.1139/z94-270>
46. Ribeiro MF, Velthuis HHW, Duchateau MJ, van der Tweel I (1999) Feeding frequency and caste differentiation in *Bombus terrestris* larvae. *Insectes Sociaux* 46:306–314. <https://doi.org/10.1007/s000400050150>
47. Richardson LI, Jha S, Jernigan CM, Muth F (2026) Bumblebee queens differ in brain morphology but not learning performance across life stages. *J Exp Biol* 229(9) Article jeb252295. <https://doi.org/10.1242/jeb.252295>
48. Robinson GE (1992) Regulation of division of labour in insect societies. *Ann Rev Entomol* 37:637–665. <https://doi.org/10.1146/annurev.en.37.010192.003225>
49. Röseler PF, van Honk CGJ (1990) Castes and reproduction in bumblebees. In: Engels W (ed) Social insects. Springer, pp 147–166

50. Rotheray E, Osborne J, Goulson D (2017) Quantifying the food requirements and effects of food stress on bumble bee colony development. *J Apic Res* 56:1–12.
<https://doi.org/10.1080/00218839.2017.1307712>
51. Rozen JG Jr., Smith CS, Kocher S, Wyman ES (2018) Developmental biology among corbiculate bees: *Bombus impatiens*, including observations on its egg eclosion. *Am Mus Novit* 3912:1–26.
<https://doi.org/10.1206/3912.1>
52. Rubenstein DR, Abbot P (eds) (2017) *Comparative social evolution*. Cambridge University Press
53. Sarro E, Sun P, Mauck K, Chen Y, Woodard SH (2021) An organizing feature of bumble bee life history: Worker emergence promotes queen reproduction and survival in young nests. *Conserv Physiol* 9:coab047. <https://doi.org/10.1093/conphys/coab047>
54. Sarro Gustilo E, Fisher K, Woodard SH (2023) Parental care behaviour in bumble bee queens is tightly regulated by the number of helpers in incipient nests. *Anim Behav* 203:241–250.
<https://doi.org/10.1016/j.anbehav.2023.07.009>
55. Schlichting C, Pigliucci M (1998) Phenotypic evolution: A reaction norm perspective. Sinauer
56. Seeley T, Buhrman S (1999) Group decision making in swarms of honey bees. *Behav Ecol Sociobiol* 45:19–31. <https://doi.org/10.1007/s002650050536>
57. Shpigler H, Tamarkin M, Gruber Y, Bloch G (2013) Social influences on body size and developmental time in the bumblebee *Bombus terrestris*. *Behav Ecol Sociobiol* 67:1601–1612.
<https://doi.org/10.1007/s00265-013-1571-0>
58. Sladen FWL (1912) The humble-bee: Its life-history and how to domesticate it, with descriptions of all the British species of *Bombus* and *Psithyrus*. Macmillan
59. Smeets P, Duchateau MJ (2001) Feeding behaviour in the bumble bee *Bombus terrestris*. *Belg J Zool*, 131
60. Snell-Rood EC (2013) An overview of the evolutionary causes and consequences of behavioural plasticity. *Anim Behav* 85(5):1004–1011. <https://doi.org/10.1016/j.anbehav.2012.12.031>
61. Sommer RJ (2020) Phenotypic plasticity: From theory and genetics to current and future challenges. *Genetics* 215(1):1–13. <https://doi.org/10.1534/genetics.120.303163>
62. Starkey J, Derstine N, Amsalem E (2019) Do bumble bees produce brood pheromones? *J Chem Ecol* 45:725–734. <https://doi.org/10.1007/s10886-019-01101-4>
63. Stein LR, Bell AM (2019) The role of variation and plasticity in parental care during the adaptive radiation of three-spine sticklebacks. *Evolution* 73(5):1037–1044.
<https://doi.org/10.1111/evo.13711>
64. Stewart T, Bolton-Patel N, Cresswell JE (2021) Eating versus heating: A study of the allocation of workers between foraging and nest incubation in bumble bees. *Ecol Entomol* 46:844–855.
<https://doi.org/10.1111/een.13021>
65. Treanore E, Barie K, Derstine N, Gadebusch K, Orlova M, Porter M, Strange JP (2021) Optimizing laboratory rearing of a key pollinator, *Bombus impatiens*. *Insects* 12(8):673.

<https://doi.org/10.3390/insects12080673>

66. Tulp I, Schekkerman H (2006) Time allocation between feeding and incubation in uniparental arctic-breeding shorebirds: Energy reserves provide leeway in a tight schedule. *J Avian Biol* 37:207–218. <https://doi.org/10.1111/j.2006.0908-8857.03519.x>
67. Visser ME, Lessells CM (2001) The costs of egg production and incubation in great tits (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, 268(1473), 1271–1277. <https://doi.org/10.1098/rspb.2001.1661>
68. Vogt D (1986) Thermoregulation in bumblebee colonies. I. Thermoregulatory versus brood-maintenance behaviors during acute changes in ambient temperature. *Physiological Zool* 59:55–59
69. Watrous KM, Duennes MA, Woodard SH (2019) Pollen diet composition impacts early nesting success in queen bumble bees *Bombus impatiens* Cresson (Hymenoptera: Apidae). *Environ Entomol* 48(3):711–717. <https://doi.org/10.1093/ee/nvz043>
70. West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press
71. Westrick SE, Moss JB, Fischer EK (2023) Who cares? An integrative approach to understanding the evolution of behavioural plasticity in parental care. *Anim Behav* 200:225–236. <https://doi.org/10.1016/j.anbehav.2022.10.005>
72. Winston ML (1987) The biology of the honey bee. Harvard University Press
73. Wong JWY, Kölliker M (2012) The effect of female condition on maternal care in the European earwig. *Ethology* 118:450–459. <https://doi.org/10.1111/j.1439-0310.2012.02030.x>
74. Woodard SH, Bloch G, Band MR, Robinson GE (2013) Social regulation of maternal traits in nest-founding bumble bee (*Bombus terrestris*) queens. *J Exp Biol* 216(18):3474–3482. <https://doi.org/10.1242/jeb.087403>
75. Yadav S, Yadav S, Sharma D, Sangwan N (2016) Bumblebees, life cycle and their role in pollination: A review. *Int J Enhanced Res Sci Technol Eng*, 5(9)

Figures

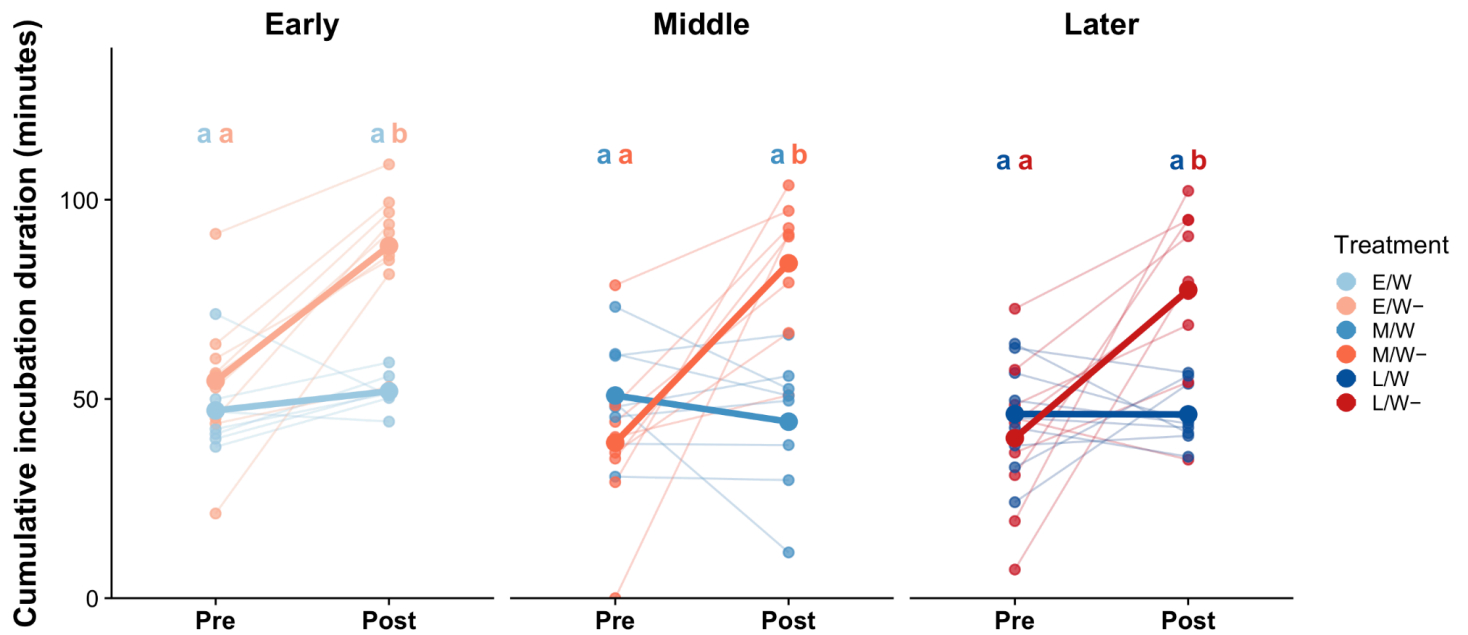


Figure 1

Paired line plot of cumulative incubation duration across the entire experimental phase. Cumulative incubation duration is shown in minutes for individual queens during pre- and post-manipulation experimental phases. Panels represent early (E)-, middle (M)-, and later (L)-stage colonies. Treatment labels combine colony stage and worker condition: E/W, M/W/ L/W indicate control colonies, whereas E/W-, M/W-, and LW- indicate worker-removal colonies for early, middle, and later stages, respectively. Data are shown for individual queens (individual points connected by thinner lines) with group means shown with thicker lines. Letters indicate post hoc groupings, via emmeans, where groups that do not share a letter are significantly different from one another.

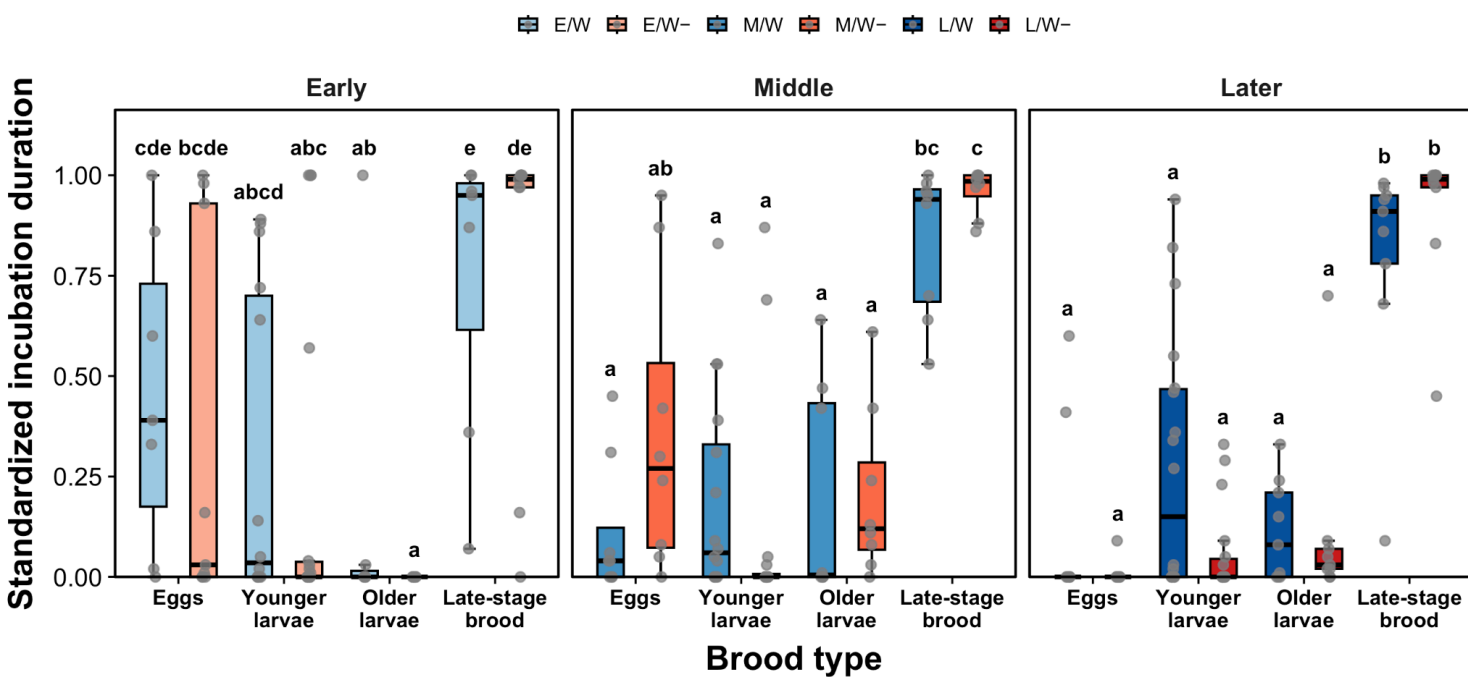


Figure 2

Box plot of standardized incubation duration across brood types for different colony developmental stages. The box plot shows the early (E), middle (M), and later (L)-stage colonies (see tables 2a-c for more information). Treatment labels combine colony stage and worker condition: E/W, M/W/ L/W indicate control colonies, whereas E/W-, M/W-, and LW- indicate worker-removal colonies for early, middle, and later stages, respectively. Grey dots represent individual queens. Letters above groups indicate statistically significant differences; groups sharing the same letter do not differ significantly (Tukey-adjusted $p < 0.05$).

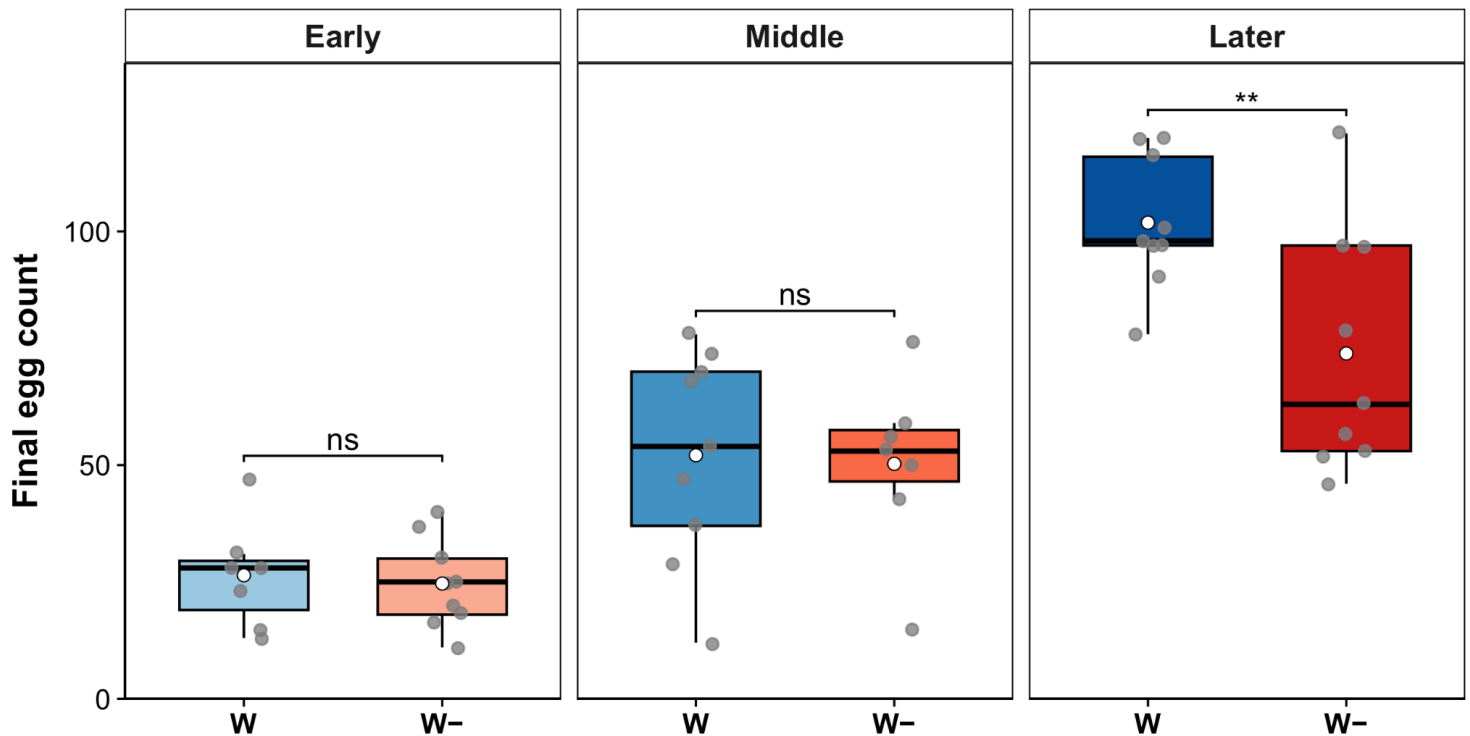


Figure 3

Final egg counts across colony developmental stages and treatments. Box plot shows final egg counts for early (E)-, middle (M)-, and late (L)-stage colonies under control (W) and worker removal (W-) treatments. Grey dots represent individual queens. Brackets indicate pairwise comparisons between treatments within each colony developmental stage based on Tukey-adjusted post hoc contrasts. Significance levels are denoted as ns (not significant), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Queenbroodcaresupplementaryvideos.zip](#)
- [Queenbroodcarebehaviorisplasticandsociallyregulatedinanannuallyeusocialinsectsupplement.docx](#)