nature portfolio

Peer Review File

Bumble bee workers adopt new roles and reshape social networks after queen loss

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript deals with an extremely interesting topic – network properties of the social structure of bumble bee colonies with and without a queen. The authors use a novel methodology to track bumble bee behavior that allows them to collect very large amounts of data on interactions between individuals. This definitely opens a new perspective from which social organizations of insect colonies can be examined. The paper is well written and overall sound but a few details are missing that would strengthen the authors' conclusions and clarify ambiguous points. Please see specific comments below.
1. Introduction
The introduction is well-written and provides a good background for the research question, however, it is unfortunately omitting one important piece of information. The competition point in the colony life cycle is does not just coincide with gyne production by the queen. It results from workers actively monitoring the state of the queen and the brood and starting competition when they acquire information of brood being committed to the gyne pathway (Alaux et al., 2004 and 2006). The queen apparently holds the power to inhibit gyne development and commit larvae to worker pathway (Chole et al., 2022, Franco et al., 2023) and the workers monitor her state to pick up the moment when she ceases to use this power. The main point here is that information flow from the queen to the workers, actively sought by the workers, is instrumental in the regulation of social structure in the colony in queenright conditions, and in queenless conditions information flow changes its direction because the main source of its importance, the queen who inhibits gyne-like development, is absent.
Results
2. Page 5 – top left paragraph: "This small but highly interactive population of bees contained 37 individuals across all five queenless partitions (two to twelve bees per partition). These individuals were slightly, but significantly, larger than non-outlier nestmates (Figure S13, $\chi 2 = -2.50, p = 1.25 * 10-2$). " The figure indicated here doesn't compare the hub bees to their non-hub QL nestmates, it compares QR and QL bees. Also, it would be useful to indicate the actual mean sizes in mm.
3. Page 5 - left column: the authors claim in this part that the hub workers display queenlike behavioral phenotypes by showing differences between hub and non-hub workers. However, there is no comparison between hub workers and queens themselves. Such a comparison is essential to gauge the similarities and the differences between the roles of a queen and of hub workers in the network and to understand how queenlike these workers actually are. Also, while the authors show that the hub bees are more interactive there is no data on who initiates these interactions. For queens the authors showed the percentage of interactions initiated by the queen herself versus those initiated by workers approaching the queen. It would be useful to show this metric for hub workers too. Also, the figure illustrating behavioral differences between hub and non-hub bees would be more useful in the main body of the article rather than in supplementary materials. Another important piece of information that is missing is whether the hub bees were more likely to interact with other hub bees or with non-hub bees. In general, it would be useful to have a figure or a metric showing how interactions of each hub bee were partitioned between hub and non-hub individuals.
4. Page 5 – top right paragraph: "This coincides with a significant association between interactivity and ovary index in queenright colonies (Figure 4c, cor.test: n=100, r=0.45, p=1.81 $*$ 10−8) which is not seen in queenless colonies (Figure 4c, cor.test: n=114, r=0.02, p=0.81)." : probably typo, the figure and further text indicate that it is queenless, not queenright, workers that have correlation between ovaries and interactions.
5. Page 6 – top left paragraph: "Queenless networks were also more efficient (Figure 5c: $\chi 2 = 39.6, p = 6.77 * 10-271$), suggesting that the larger total number of interactions in queenless colonies (Figure 2a: $\chi 2 = 46.4, p = \sim 0$) may facilitate a more rapid spread of information. Finally, queenless colonies were less disassortative than their queenright counterparts (Figure 5d: $\chi 2 = 11.0, p = 1.22 * 10-27$), meaning the tendency of dissimilar individuals to interact was weaker among queenless nestmates, reflecting a shift toward a more neutral pattern of interactions among workers (i.e., neither an

assortative nor disassortative network).” It’s not entirely clear what is meant by “efficiency” (probably the number of interactions per minute but it’s not spelled out). Also, when the authors talk about association between dissimilar individuals it’s not clear in what ways are these individuals dissimilar. That should be stated explicitly.
Discussion
6. Page 7 – top left paragraph: “For example, workers could be using standard interactions as a way to monitor their reproductive dominance status (Alaux, Jaisson, and Hefetz, 2006) or to advertise their sterility, potentially reducing within-colony conflict (Amsalem, Twele, et al., 2009).” Advertising sterility makes sense mainly to avoid aggression, so, if the workers advertise sterility to the queen, they would also advertise it to dominant workers in the QL partition. To determine if this is the case it would be useful to report how likely the dominant workers are to initiate interactions.
Methods
7. My main concern about the SLEAP methodology and the filtering of data is that it’s not clear how the model differentiated between interactions of different valence. Bumble bees display a wide range of aggressive behaviors that differ in their social significance from neutral ones such as antennation or affiliative ones such as huddling (full body contact most often performed by young bees) (Amsalem and Hefetz, 2010 and 2011). Interactions of different valence obviously carry different meaning for the network structure of the colony but may still manifest as head-to-head or head-to-body contact perceived by the SLEAP model. I wonder if there is any way to differentiate aggressive interactions from neutral or affiliative ones using this tool. Also, one of the common aggressive displays in bumble bees is darting – a rapid lunge of one bee towards another stopping short of collision. I wonder if filtering out of interactions by excessive speed might have inadvertently removed the data on darting. To clarify this matter it would be useful to report at what average speed the workers were moving and what speed limit triggered exclusion of an observation from the analysis.

Reviewer #2

(Remarks to the Author)
The authors compare the social networks of queenright and queenless bumblebee colonies using animal tracking data to infer interaction network changes after queen loss. The strength of the study is the detailed tracking of individuals which yields a large data set of individual interactions, and I believe that the quantification of these interactions is novel. The key finding of the study is that the loss of a queen alters network structure, where individuals, who enlarge their ovaries to become reproductive, take on central roles in the network, leading to an increase in behavioral variation between workers. While I can appreciate that a quantification of the network changes after queen loss is important, I also do not find these results too surprising since it is almost by necessity the case that behavioral variation between workers increases when some workers, but not others, change their reproductive physiology.

In their statistical analysis, the authors use linear mixed models to estimate means of responses given treatment and permutation tests to compare the variance between treatments. The permutation tests show that the variance differs by treatment, which leads the authors to conclude that behavioral variation increases with queen loss. However, I believe that there are some problems with these analyses. If the variance differs between treatments, then the variance should be modelled in the linear mixed models. While the estimated means from the linear mixed models will not be affected by between-treatment variation in variance, the estimates of uncertainty will be, and this can bias p-values. If the authors model the variance in the linear mixed models, it is unnecessary to do the permutation tests, but instead the estimated variance from the linear mixed models can simply be compared for queenright vs. queenless colonies. The authors use the lme4 package for their statistical analysis, which cannot model variance. I would recommend the authors to use the glmmTMB package, which can model variance by treatment (with distribution family set to gaussian, this is a lmm). Subsequently, the authors could use the emmeans package to compare the variance estimates for queenright vs. queenless colonies.

In the analyses corresponding to Fig. 4C, the authors use two correlation tests to test the association between interactivity and ovary index. The authors find that this association is significant in queenless colonies but not in queenright colonies (although the text states the reverse; it seems that “queenright” and “queenless” are confused at multiple spots in the corresponding text paragraph). However, the presence of a correlation in one case and its absence in the other does not necessarily imply that there is a significant difference in the slope of interactivity vs. ovary index in queenright vs. queenless colonies. It is therefore advisable to fit the linear mixed model with an interaction term (interactivity ~ queenless/queenright * ovary index + (1|random effects)). This comes at the additional benefit that the two correlation tests are not necessary, but just one linear mixed model.

Fig. 5a says “Queenless influencer” and “Queenless Non-Influencer Worker”. Is this supposed to mean hub bee and non-hub bee? Also see Fig. S3 and caption of Fig. S16 for additional occurrences of “Influencer”.

Reviewer #3

(Remarks to the Author)
The manuscript "Bumble bee workers adopt novel behavioral roles and reshape their social networks in the absence of a queen" demonstrates how reproductive status shapes interactions among nestmates, showing that non-reproductive workers are more likely to initiate contact with individuals of higher reproductive status (queens or workers with well-developed ovaries). This pattern leads to reproductive individuals functioning as “hub bees” with the strongest connections in the interaction network. The authors employ a split-colony design (size-matched workers, half maintained with the queen and half without) combined with automated tracking, allowing them to quantify not only spatial organization over time but also the directionality of interactions within the group. After four days in the absence of the queen, queenless workers self-

organize according to reproductive status.

Overall, the introduction provides a clear rationale for the study, the experimental design is well suited to address the research aims, the results are thorough and appropriately analysed, and the conclusions are generally well supported by the data. I have only a few minor comments that the authors may wish to consider to improve clarity.

1. More information is needed in the methods section.
- In the first sentence of the Methods, it's not completely clear that the study was conducted on bees from 5 colonies that were recorded consistently over 4 days (for the 96 hours of video). These pieces of info can be deduced from the results, but it would be easy to add it here.
- Page 9: "5g of brood from the source colony." Where was the brood placed? Was spatial arrangement relative to brood included? What stage of development was the brood in? Were honey pots also added?

2. What is the biological relevance of studying spatial interactions among members of a bumble bee colony in 2-dimensional space, without access to a separate foraging arena and only a minimal amount of brood provided.
- Page 9: "Four cotton wicks soaked in nectar substitute (equal parts pure sugar water and inverted sugar water with added feeding stimulant and amino acid supplementation) were placed in one corner of the arena to allow ad libitum feeding, and 5g of ground honey bee pollen mixed with nectar substitute at a ratio of 10:1 (pollen:nectar substitute) was added for protein nutrition and a more naturalistic environment." Provisioning food directly inside the space where bumble bees are living is not really "natural" - though it is essential for them to survive.
- What was their motivation for maintaining an interaction network? Without brood to feed, temperature to maintain, or food to collect, can the results collected from this study be applicable to bees living in 3-D space? Often after the queen is gone, workers begin to do strange things in the colony. If they weren't allowed to leave the arena for 4 days, what is the certainty they were not behaving abnormally under such uniform conditions?
- Were the locations of the brood, food, or areas of defecation (presumably in the corners of the box) considered in the analyses? These factors may influence spatial arrangement.

3. Some of the conclusions that are provided are a bit vague or do not seem relevant for this study.
- Page 8: "Future work combining detailed behavioral tracking with molecular and neurobiological approaches will be crucial to uncover how these social traits develop and function, and when and how they influence both individual and collective outcomes in nature." Because this phenomenon regularly occurs at the end of the colony cycle (the queen dies and workers lay unfertilized eggs), it's not clear why this future work is necessary. Why is it crucial to uncover the mechanisms that influence social traits during the decline phase of the colony cycle?
-Page 8: "However, increased interactivity and connectivity may also carry a cost: higher interaction rates and the presence of multiple reproductive individuals could accrue higher energetic costs to the colony (Waters, 2014)." It's not completely clear what the relevance is here - how could multiple reproductive individuals accrue higher energetic costs to the colony based on what is shown in this ms about networks and interactions?
- Consider highlighting how "normal" it is for B impatiens to lose a queen and go through a queenless phase in their colony cycle in the conclusions. This study is particularly interesting because the data show that the structure of the interaction network remains strong even after the queen is gone. Instead of a colony-breakdown, queenless colonies can still maintain interaction networks.

Minor Comments:
Page 2: "we found head-to-head interactions were significantly enriched relative to head-to-body interactions..." Does this mean that there were more head-to-head interactions? The use of the term "enriched" seems to be the wrong term.

Page 2: "We detected a slight circadian effect in the frequency of head-to-head interactions" It's not clear what the circadian effect was. Fig 2 does not show a repeating pattern across the 4 days of testing, and Fig S6 & S7 separate the interactions between day and night, respectively.

Page 2: "In total, we quantified over 80 million undirected pairwise interactions across nearly 65 million frames. Of these, over 25 million were directed interactions initiated by one of the two bees (see Methods)." Of the undirected interactions, 25 million were directed? It's not clear what is being described here.

Page 2: "B. impatiens females exhibit an enrichment of head-to-head interactions in our data (Figure S6) and in previously published datasets (Wang et al., 2022; Wolf et al., 2023), have been historically used to analyze colony behavior" Check the sentence structure, so that B. impatiens females exhibited an ... or B. impatiens females that exhibit an...

Page 3: "Despite the queen interacting and moving more frequently than the workers, she explored relatively less space in the colony" This result has also been shown in Jandt & Dornhaus 2009, Animal Behaviour

Page 5: "Importantly, this lack of behavioral variation in queenright settings is not the result of a lack of physiological variation – there already variation in ovary size between workers in these queenless colonies (Figure 4c)." There seems to be a grammatical error in the sentence. It's also not clear what it being compared in the second part of the sentence. Lack of variation in queenright settings is not because they lacked physiological variation because ovary size variation was found in queenless colonies?

Page 6, Figure 4: The dashed reference lines make the small dots in Fig 4b almost impossible to see. Consider removing the reference lines and/or making the dots bigger and darker coloured. The lines in the other panels are fine, but also seem unnecessary.

Page 6: "Queenless networks were also more efficient..." Consider adding more context as to what they are more efficiently doing.

Page 6: "Queens were largely spatially constrained to the brood area..." Based on what was presented in the results, there were no analyses that included distance to the brood clump. Where does this conclusions come from?

Page 7: "A strong association between behavioral variation and reproductive physiology emerged in queenless conditions that was not observed in the presence of a queen." Technically, wouldn't the queen be a hub bee? The fact that ovarian development doesn't really start until the queen is removed suggests that reproductive physiology is a strong predictor of social organisation across contexts.

Page 7, Figure 5a: What is an "influencer" - should this say "hub bee"?

Page 7: "Queens are substantially larger and express a distinct cuticular chemistry that reflects reproductive status..." Consider adding "Bombus" before "Queens" as Apis was described in the previous sentence.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors addressed my own and other reviewers' comments with great attention and in my opinion the article is ready for publication. There are still a few spots in the paper where "influencer" appears instead of "hub bee" which probably requires some minor editing but otherwise the paper is ready to go.

Reviewer #2

(Remarks to the Author)
I would like to thank the authors for revising the manuscript and taking my suggestions into account. I still have two concerns which have not been fully resolved in the previous revision.

(1) I have previously suggested to model treatment differences in variance with a linear model because the presence of treatment differences in variance can lead to inaccurate estimates of uncertainty and therefore bias p-values. The authors have now used a linear gaussian model to model treatment-specific variance, which I appreciate. However, the authors are still using the p-values from the previous linear model, which does not account for treatment-specific variance, to compare treatment means. The comparisons of treatment-specific means and variances should occur in the same model to avoid biasing p-values, even if this means that some of the previously significant effects will “disappear”. That means there should be just one model that models both the means and the variances.
(2) The authors now use an interaction model for the results corresponding to Figure 5C, which again I appreciate. I am however confused that the model predictions shown in Figure 5C do not seem to have changed at all (the figures in the old and new version of the manuscript seem identical). This makes me wonder what is shown in the figure. The figure should optimally show the mean and 95% confidence interval of the mean that comes directly from the interaction model, which should also be explained in the figure caption. I would encourage the authors to show the model predictions from the interaction model (and not a separate fit from geom_smooth or something alike), if that is not yet done, since this allows the reader to also evaluate how well the model fits the data.

Reviewer #3

(Remarks to the Author)
The authors have addressed all reviewer comments satisfactorily. I have no further comments on the manuscript.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I would like to thank the authors for addressing my concerns and congratulate them to their manuscript.

Reviewer #3

(Remarks to the Author)

My concerns were addressed in the previous version, and I have no further concerns to check.

Response to Reviewers

Bumble bee workers adopt novel behavioral roles and reshape their social networks in the absence of a queen

March 27, 2026

We thank the reviewers for their thoughtful and constructive feedback. Below, we address each comment point-by-point. Reviewer comments are shown in shaded boxes, our responses in plain text, and modified manuscript text is highlighted in red. The revised manuscript, with major updated sections highlighted in red, can be found following this response document. All line references to the revised manuscript are clickable hyperlinks.

Summary of major revisions:

(1) Added `glmmTMB` dispersion sub-models to directly estimate treatment-specific variance, replacing separate permutation tests; fixed-effect comparisons continue to use `lme4` (Reviewer 2, Comment 1).
(2) Replaced separate correlation tests for the ovary–interactivity relationship with a single interaction model using `emtrends` slope comparisons (Reviewers 1 & 2).
(3) Expanded the Introduction with the role of queen influence during social transitions and worker monitoring of queen/brood state (Reviewer 1, Comment 1).
(4) Renamed all instances of “influencer” to “hub bee” throughout the manuscript and codebase (Reviewers 2 & 3).
(5) Added new analyses of hub bee interaction initiation rates (Reviewer 1, Comments 3 & 6).
(6) Clarified definitions of network efficiency and degree assortativity (Reviewer 1, Comment 5).
(7) Added arena design caveats to Methods and framing of queen loss as a natural part of the *B. impatiens* colony cycle in the Conclusions (Reviewer 3, Comments 2 & 3).
(8) Text corrections and additional citations throughout.

Reviewer #1

Expertise: behavioral ecology, social insects

Comment 1 (Introduction): The introduction is well-written and provides a good background for the research question, however, it is unfortunately omitting one important piece of information. The competition point in the colony life cycle does not just coincide with gyne production by the queen. It results from workers actively monitoring the state of the queen and the brood and starting competition when they acquire information of brood being committed to the gyne pathway (Alaux et al., 2004 and 2006). The queen apparently holds the power to inhibit gyne development and commit larvae to worker pathway (Chole et al., 2022, Franco et al., 2023) and the workers monitor her state to pick up the moment when she ceases to use this power. The main point here is that information flow from the queen to the workers, actively sought by the workers, is instrumental in the regulation of social structure in the colony in queenright conditions, and in queenless conditions information flow changes its direction because the main source of its importance, the queen who inhibits gyne-like development, is absent.

We thank the reviewer for this important point and see that our manuscript would benefit from additional detail. We have added a passage to the Introduction that better describes the role of queen influence during social transitions during the colony life cycle, including the role of worker monitoring of queen and brood state. We now explicitly discuss how workers actively assess whether brood have been committed to the gyne developmental pathway (Alaux, Jaisson, and Hefetz, 2004) and how the queen's capacity to inhibit gyne development (Cholé et al., 2022; Franco et al., 2023) shapes the information flow that regulates social structure. We agree that this context strengthens the motivation for studying network dynamics under queen loss, as the removal of the queen eliminates the primary source of this regulatory information.

In *Bombus terrestris*, workers actively monitor queen behavior and brood status and initiate competition when gyne-destined larvae—which develop into the next generation of reproductives—are detected (Alaux, Jaisson, and Hefetz, 2004; Alaux, Jaisson, and Hefetz, 2006). The queen can inhibit gyne development and commit larvae to the worker-destined developmental pathway (Cholé et al., 2022; Franco et al., 2023; Amsalem, Grozinger, et al., 2015), and workers monitor her state to detect when she ceases to exert this influence. Thus, information flow from the queen to the workers is instrumental in the regulation of social structure under queenright conditions.

→ *See revised manuscript, line 99.*

Comment 2 (Results): "This small but highly interactive population of bees contained 37 individuals across all five queenless partitions (two to twelve bees per partition). These individuals were slightly, but significantly, larger than non-outlier nestmates (Figure S13, $\chi^2 = -2.50, p = 1.25 \times 10^{-2}$)." The figure indicated here doesn't compare the hub bees to their non-hub QL nestmates, it compares QR and QL bees. Also, it would be useful to indicate the actual mean sizes in mm.

We thank the reviewer for this observation. We have added the mean body sizes (marginal cell length) directly to the manuscript text. Hub bees had a mean marginal cell length of 2.684 ± 0.244 mm (N=37), compared to 2.642 ± 0.258 mm for non-hub queenless workers (N=217) and 2.616 ± 0.277 mm for queenright workers (N=247). While statistically significant, the difference between hub and non-hub queenless bees is small (0.042 mm), consistent with our description of hub bees being "slightly, but significantly, larger."

hub worker mean marginal cell length $= 2.684 \pm 0.244$ mm, non-hub queenless worker mean $= 2.642 \pm 0.258$ mm

→ *See revised manuscript, line 315.*

> **Comment 3 (Results):** The authors claim that hub workers display queenlike behavioral phenotypes by showing differences between hub and non-hub workers. However, there is no comparison between hub workers and queens themselves. Such a comparison is essential to gauge the similarities and differences between the roles of a queen and of hub workers in the network. Also, while the authors show that hub bees are more interactive, there is no data on who initiates these interactions. For queens the authors showed the percentage of interactions initiated by the queen herself versus those initiated by workers approaching the queen. It would be useful to show this metric for hub workers too. Also, the figure illustrating behavioral differences between hub and non-hub bees would be more useful in the main body of the article rather than in supplementary materials. Another important piece of information that is missing is whether hub bees were more likely to interact with other hub bees or with non-hub bees.

We address each sub-point in turn:

(a) The manuscript already presents queen vs. all-worker comparisons (3) and hub vs. non-hub comparisons in supplementary figures (Figure S14). While a direct three-way comparison (queen vs. hub vs. non-hub) in a single figure would be informative, the existing figures together demonstrate that hub bees approach queen-like levels of interactivity and centrality but do not fully match the queen in betweenness centrality (see Discussion, "We observed a larger betweenness centrality observed in queens compared to hub bees").

(b) We analyzed the proportion of directed interactions initiated by hub workers versus non-hub QL workers. Hub workers initiated 48.4% of their directed interactions (SD = 0.138), compared to 49.7% for non-hub workers (SD = 0.150). A linear mixed model with colony as a random effect showed a negligible effect of hub status on initiation rate (estimate = -0.014, Cohen's d = -0.085). By contrast, queens initiated only 30.9% of their interactions. Thus, unlike queens—who are predominantly approached by workers—hub workers show no meaningful tendency to either initiate or receive interactions disproportionately. See also our response to Comment 6 for further discussion. $\rightarrow$ *See revised manuscript, line 325.*

(c) We agree that the hub/non-hub behavioral comparison figure is informative for the main text. We have referenced the supplementary figure (Figure S14) more prominently in the Results section.

(d) The manuscript already reports that there is "no evidence for increased assortativity between hub bees" (6), indicating consistent mixing regardless of behavioral profile. This addresses the question of whether hub bees preferentially interact with each other.

Additionally, during revision we identified and corrected a minor discrepancy in the hub bee interactivity statistic. The comparison is between hub bees and non-hub queenless workers; the updated test statistic is $\chi^2 = 42.1$ (previously reported as 47.2). All other statistics in this section were verified and remain unchanged.

Hub bees were more interactive than non-hub queenless workers [...] ($\chi^2 = 42.1, p \approx 0$)
$\rightarrow$ *See revised manuscript, line 325.*

> **Comment 4 (Results):** "This coincides with a significant association between interactivity and ovary index in queenright colonies (Figure 4c, cor.test: n=100, r=0.45, $p = 1.81 \times 10^{-8}$) which is not seen in queenless colonies...": probably typo, the figure and further text indicate that it is queenless, not queenright, workers that have correlation between ovaries and interactions.

Thank you for catching this — the labels were swapped. We have corrected the text so that the significant association is attributed to queenless colonies, not queenright. In the revised manuscript, we have also replaced the two separate correlation tests with a single interaction model (see Reviewer #2, Comment 2), which renders this label swap moot as the new text describes the interaction term directly.

This coincides with a significant interaction between condition and ovary index on interactivity (Degree $\sim$ QR $\times$ ovary_idx $+$ (1|Trial), glmmTMB: $n = 264$, interaction $\beta = 10541$, $z = 6.42$, $p = 1.37 \times 10^{-10}$)

[...] Specifically, queenless workers showed a significant positive relationship between interactivity and ovary index (emtrends slope $= 8843$, SE $= 1072$), while queenright workers did not (slope $= -1698$, SE $= 1281$; pairwise slope comparison: $t = -6.42$, $p < 0.0001$).
$\rightarrow$ *See revised manuscript, line 369.*

Comment 5 (Results): It's not entirely clear what is meant by "efficiency" (probably the number of interactions per minute but it's not spelled out). Also, when the authors talk about association between dissimilar individuals, it's not clear in what ways these individuals are dissimilar. That should be stated explicitly.

We agree that these terms needed clarification. "Efficiency" refers to global network efficiency (Latora and Marchiori, 2001): the average inverse shortest path length between all pairs of nodes, which serves as a proxy for the potential speed and ease of information transfer through the network. "Dissimilar" refers to degree-based assortativity — i.e., whether individuals with different numbers of connections (degree) tend to interact. We have revised the manuscript text to define both terms explicitly at their first use.

We next explored global efficiency, a measure of information flow that tracks the shortest path length between all pairs of nodes within a network (Latora and Marchiori, 2001). Queenless networks were more efficient, [...] meaning the tendency of individuals with dissimilar numbers of connections (i.e., degree) to interact was weaker among queenless nestmates.
$\rightarrow$ *See revised manuscript, line 427.*

Comment 6 (Discussion): "For example, workers could be using standard interactions as a way to monitor their reproductive dominance status (Alaux, Jaisson, and Hefetz, 2006) or to advertise their sterility..." Advertising sterility makes sense mainly to avoid aggression, so, if the workers advertise sterility to the queen, they would also advertise it to dominant workers in the QL partition. To determine if this is the case it would be useful to report how likely the dominant workers are to initiate interactions.

Thank you for this suggestion. We analyzed the proportion of directed interactions initiated by hub workers (i.e., dominant workers in QL colonies) compared to non-hub QL workers. Hub workers initiated 48.4% of their directed interactions (SD = 0.138, n = 3,552 bee-hour observations), while non-hub QL workers initiated 49.7% (SD = 0.150, n = 20,762). Both values are close to 50% (chance level). A linear mixed model with colony as a random effect confirmed that hub status was not a meaningful predictor of initiation rate (estimate = -0.014, t = -4.97; Cohen's d = -0.085, a negligible effect size). Although the large sample size yields statistical significance, the difference of 1.3 percentage points is unlikely to be biologically meaningful.
By contrast, queens initiated only 30.9% of their directed interactions (SD = 0.147, n = 479), consistent with workers approaching queens rather than queens initiating contact.
These results indicate that hub workers do not preferentially initiate interactions the way one might expect if they were actively "advertising" dominance or sterility. Instead, hub workers appear to receive elevated contact from nestmates in a pattern qualitatively similar to—though weaker than—the pattern seen for queens. This is consistent with the interpretation that hub workers' elevated interaction rates reflect increased social attention from nestmates rather than active solicitation by hub workers themselves (see also our response to Comment 3b).

Comment 7 (Methods): My main concern about the SLEAP methodology and the filtering of data is that it's not clear how the model differentiated between interactions of different valence (aggressive vs. neutral vs. affiliative). Bumble bees display a wide range of aggressive behaviors (Amsalem and Hefetz, 2010 and 2011). Also, one of the common aggressive displays in bumble bees is darting — a rapid lunge of one bee towards another stopping short of collision. I wonder if filtering out of interactions by excessive speed might have inadvertently removed the data on darting. To clarify this matter it would be useful to report at what average speed the workers were moving and what speed limit triggered exclusion of an observation from the analysis.

This is an important methodological concern. Our SLEAP-based tracking pipeline detects spatial proximity between body parts (head-to-head, head-to-body) but does not differentiate interaction valence (aggressive vs. neutral vs. affiliative). We acknowledge this limitation.

Regarding filtering thresholds: Our main filter was for tag-level jumps exceeding 100 pixels (≈6.5 mm). When we look at the distribution of movement (see RWR Figure 1 below), we see a set of bimodal peaks, peak 1 representing model noise and peak 2 representing all other movement (including darting). We selected 100 pixels as it is well past the ends of the bell curve, suggesting that we did not filter out darting behaviors. Our other filter at 10-pixels is also past the bell curve (Mean worker velocity was approximately 4.8 pixels/frame (≈0.31 mm/frame)), but we are not concerned that it would eliminate darting as that filter requires a single node on the SLEAP skeleton for an individual to be moving when no other nodes are moving; in darting, we would expect every node to move. This in sum suggests that we only filter out tracking errors through our filtering analysis.

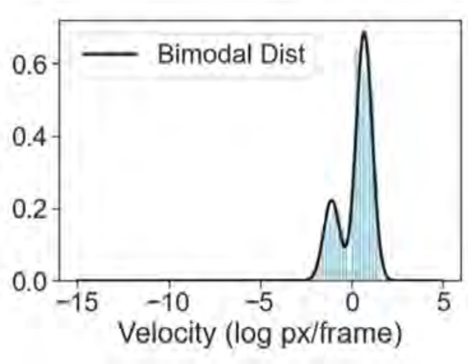

Figure 1. Velocity distribution of a colony on a log2 scale

Importantly, our interaction detection is based on spatial overlap between body parts, not velocity. A darting event that results in close proximity (e.g., antennal contact) would still be captured as an interaction. Only darting events that stop short of the proximity threshold would be missed. We have added millimeter equivalents for all thresholds in the Methods and cited (Amsalem and Hefetz, 2010) for context on bumble bee aggressive behaviors.

Methods – Filtering thresholds (mm equivalents):

[. . .] moved greater than 10 pixels (∼0.65 mm) [. . .] moved greater than 100 pixels (∼6.5 mm) (tag jump).

→ *See revised manuscript, line 746.*

Methods – Velocity and darting:

Across all workers, mean velocity when moving was ∼4.8 pixels/frame (∼0.31 mm/frame), and workers moved an average of ∼56% of the time. The 10-pixel node jump threshold corresponds to approximately 2× the mean velocity, meaning it prevents the capture of tracking artifacts while retaining the range of normal locomotor behaviors, including rapid approaches. We note that our interaction detection is based on spatial overlap of body regions rather than velocity, so brief, high-speed approach events (e.g., darting) that result in antennal contact would still be captured as interactions even if the preceding movement was flagged as a jump (Amsalem and Hefetz, 2010).

→ *See revised manuscript, line 746.*

Reviewer #2

Expertise: social insects, division of labor

Comment 1 (Statistical analysis): In their statistical analysis, the authors use linear mixed models to estimate means of responses given treatment and permutation tests to compare the variance between treatments. If the variance differs between treatments, then the variance should be modelled in the linear mixed models. I would recommend the authors to use the `glmmTMB` package, which can model variance by treatment (with distribution family set to gaussian, this is a LMM). Subsequently, the authors could use the `emmeans` package to compare the variance estimates for queenright vs. queenless colonies.

We thank the reviewer for this excellent suggestion. We have replaced our permutation-based variance tests with `glmmTMB` models using a Gaussian family and a dispersion sub-model (`dispformula` $\sim$ `QR`) that allows residual variance to differ between queenright and queenless conditions. This directly models the heterogeneous variances rather than testing them separately with permutation tests. We use `emmeans` contrasts on the dispersion model to compare treatment-specific variance estimates. Fixed-effect comparisons (e.g., queenright vs. queenless means for network parameters) continue to use `lme4` linear mixed models with Wald chi-squared tests. The Methods section has been updated accordingly.

Linear Mixed Models:
Fixed-effect comparisons (e.g., queenright vs. queenless means) were estimated using linear mixed models fit with the lme4 R package (Bates et al., 2015), with Wald chi-squared tests for significance.

Variance Analysis:
To test whether behavioral variance differed between conditions, we fit separate models using the glmmTMB R package (Brooks et al., 2017) with a Gaussian family and a dispersion sub-model (`dispformula` $\sim$ `QR`), which allows the residual variance to differ between queenright and queenless conditions. We used the emmeans R package (Lenth, 2024) to extract estimated marginal means of the log dispersion for each condition and to perform pairwise contrasts between them.
→ *See revised manuscript, line 806.*

Comment 2 (Fig. 4C analysis): In the analyses corresponding to Fig. 4C, the authors use two correlation tests to test the association between interactivity and ovary index. However, the presence of a correlation in one case and its absence in the other does not necessarily imply a significant difference in slope. It is therefore advisable to fit the linear mixed model with an interaction term (`interactivity` $\sim$ `treatment * ovary_index + (1|random effects)`).

We agree with the reviewer that comparing two separate correlation tests does not formally test whether slopes differ. We have replaced the two `cor.test()` analyses with a single linear mixed model fit using `glmmTMB`: Degree $\sim$ QR $\times$ ovary_idx + (1|Trial). The interaction term directly tests whether the slope of interactivity on ovary index differs between queenright and queenless conditions. The interaction was highly significant ($\beta = 10541$, $z = 6.42$, $p = 1.37 \times 10^{-10}$). We further used `emtrends()` from the `emmeans` package to estimate and compare condition-specific slopes: the queenless slope was 8843 (SE $= 1072$) while the queenright slope was -1698 (SE $= 1281$; pairwise comparison $t = -6.42$, $p < 0.0001$).

Code change: `scripts/manuscript/ovary_interactivity_interaction_model.R` — replaced the two `cor.test` calls with a single interaction model and `emtrends` slope comparison.

This coincides with a significant interaction between condition and ovary index on interactivity (Degree $\sim$ QR $\times$ ovary_idx + (1|Trial), glmmTMB: $n = 264$, interaction $\beta = 10541$, $z = 6.42$, $p = 1.37 \times 10^{-10}$), indicating that the slope of interactivity on ovary index differs between queenright and queenless colonies. Specifically, queenless workers showed a significant positive relationship between interactivity and ovary index (emtrends slope $= 8843$, SE $= 1072$), while queenright workers did not (slope $= -1698$, SE $= 1281$;

pairwise slope comparison: $t = -6.42$, $p < 0.0001$).
→ *See revised manuscript, line 369.*

Comment 3 (Terminology): Fig. 5a says "Queenless influencer" and "Queenless Non-Influencer Worker". Is this supposed to mean hub bee and non-hub bee? Also see Fig. S3 and caption of Fig. S16 for additional occurrences of "Influencer".

Yes, "influencer" should read "hub bee" throughout. We have replaced all instances of "influencer" and "Influencer" with "hub bee" and "Hub Bee" across the manuscript, supplementary materials, figure-generating scripts (R and Python), and data processing code. This includes Figure 5a labels, supplementary figure captions (Figs. S3, S16), and all analysis scripts.

Reviewer #3

Expertise: social insect behaviors

Comment 1 (Methods): More information is needed in the methods section.

- In the first sentence of the Methods, it's not completely clear that the study was conducted on bees from 5 colonies recorded consistently over 4 days (for the 96 hours of video).
- "5g of brood from the source colony." Where was the brood placed? Was spatial arrangement relative to brood included? What stage of development was the brood in? Were honey pots also added?

We have revised the opening sentence of the Methods to make the experimental design explicit: five source colonies, each split into two partitions (one queenright, one queenless), yielding 10 arenas recorded continuously for 96 hours (4 days). Regarding brood, approximately 5 g of brood and pollen were placed in the center of each arena. The brood was at mixed developmental stages (eggs and early-instar larvae). No additional honey pots were provided beyond ad libitum sucrose solution available via feeders.

Our dataset consists of ten 96-hour (4-day) videos from five *Bombus impatiens* source colonies, each split into a queenright and a queenless partition (10 arenas total), recorded continuously under controlled conditions.

→ *See revised manuscript, line 638.*

Comment 2 (Biological relevance): What is the biological relevance of studying spatial interactions among members of a bumble bee colony in 2-dimensional space, without access to a separate foraging arena and only a minimal amount of brood provided? Without brood to feed, temperature to maintain, or food to collect, can the results be applicable to bees living in 3-D space? If they weren't allowed to leave the arena for 4 days, what is the certainty they were not behaving abnormally? Were the locations of the brood, food, or areas of defecation considered in the analyses?

We appreciate the reviewer's concern about the ecological relevance of our 2D arena design. Similar enclosed-arena designs have been widely used to study bumble bee behavior and have demonstrated that key colony-level behaviors—including circadian activity patterns, division of labor, and social interactions – persist under these conditions (Crall, Gravish, Mountcastle, Kocher, et al., 2018; Pandey, Motro, and Bloch, 2020; Wolf et al., 2023). Unlike honey bees, bumble bees do not require outdoor access to take foraging or cleansing flights. We leverage this tractability by tightly controlling the arena environment within and across trials.

We also acknowledge that a lack of foraging opportunities and a full broodnest complement may limit the range of behaviors expressed by workers. However, the critical comparison in our study is between queenright and queenless partitions within the same experimental setup: both conditions experienced identical arena constraints, so any arena-related effects are controlled for in our comparisons.

Regarding spatial covariates, we did not explicitly model the locations of brood, food, or defecation sites in our network analyses. We have added a note in the Methods section acknowledging these design constraints.

We note that our experimental setup used two-dimensional arenas without a separate foraging chamber, which may limit the range of behaviors expressed. However, bumble bee nests are naturally quite flat, consisting of a single layer of brood cells, making a 2D arena a reasonable approximation for within-nest spatial interactions (Goulson, 2010). Similar enclosed-arena designs have been widely used to study bumble bee social behavior and have demonstrated that key colony-level behaviors persist under these conditions (Crall, Gravish, Mountcastle, Kocher, et al., 2018; Pandey, Motro, and Bloch, 2020; Wolf et al., 2023). Although our arenas lacked a foraging chamber and contained only minimal brood (approximately 5 g of brood and pollen; see above), the critical comparison is between queenright and queenless partitions within the same experimental setup, so any arena-related constraints apply equally to both conditions.

→ *See revised manuscript, line 561.*

> **Comment 3 (Conclusions):** Some of the conclusions are a bit vague or do not seem relevant for this study.
> - "Future work combining detailed behavioral tracking with molecular and neurobiological approaches will be crucial. . . " — why is it crucial to uncover mechanisms during the decline phase of the colony cycle?
> - "However, increased interactivity and connectivity may also carry a cost. . . " — how could multiple reproductive individuals accrue higher energetic costs based on what is shown in this ms?
> - Consider highlighting how "normal" it is for *B. impatiens* to lose a queen and go through a queenless phase. The data show that the structure of the interaction network remains strong even after the queen is gone.

We have revised the Conclusions to address each point:

(a) We reframed the call for future mechanistic work around understanding how latent behavioral variation is regulated in the presence of the queen and what mechanisms trigger its expression upon queen loss, rather than framing it narrowly around the decline phase.

(b) We softened the energetic cost claim, noting that increased interaction rates and the presence of multiple reproductive individuals *may* impose additional energetic demands, but that the magnitude of such costs remains to be directly measured.

(c) We added framing that queen loss is a natural event in the annual colony cycle of *B. impatiens*, and that the robust network reorganization we observed — distributed architectures rather than fragmentation — suggests adaptive resilience to this transition.

Our work highlights how social context can modulate the expression of individual traits such that collective dynamics are altered. Here, we compared queenright and queenless partitions of bumble bee colonies to link individual-level behavioral traits to reproductive physiology and colony-level shifts in social organization. Our observations show that, in experimental queenless environments, latent behavioral and physiological variation among workers is unmasked, and that the emergence of these new social phenotypes is associated with a shift in colony-level network organization. In *B. impatiens*, queen loss is a natural part of the annual colony cycle, as colonies transition from a cooperative phase with a single, reproductive queen to a competition phase where multiple workers lay male-destined eggs (Goulson, 2010). Notably, despite this transition, overall network structure remained robust: queenless colonies reorganized into distributed, highly connected architectures rather than fragmenting. Latent behavioral variation among workers could serve as a reservoir of variation that enables rapid transitions between organizational states as conditions change (Blight et al., 2016). A key open question is how this latent variation is regulated in the presence of the queen and what mechanisms trigger its expression upon queen loss. Future work combining detailed behavioral tracking with molecular and neurobiological approaches will be essential to determine how queen-derived signals suppress behavioral and physiological differentiation, and how individual-level variation shapes collective outcomes across the colony cycle.

→ *See revised manuscript, line 600.*

Minor Comments (Reviewer #3)

> "we found head-to-head interactions were significantly enriched relative to head-to-body interactions. . . " Does this mean that there were more head-to-head interactions? The use of the term "enriched" seems to be the wrong term.

We replaced "enriched" with "more frequent" to avoid ambiguity.

[. . .] we found head-to-head interactions were significantly more frequent than head-to-body interactions [. . .]
→ *See revised manuscript, line 188.*

> "We detected a slight circadian effect in the frequency of head-to-head interactions" — It's not clear what the circadian effect was. Fig 2 does not show a repeating pattern across the 4 days.

We replaced "circadian" with "diel" to more accurately describe the day–night difference in interaction rates, which does not imply a full circadian rhythm.

We detected a slight diel effect in the frequency of head-to-head interactions [. . .]
→ *See revised manuscript, line 196.*

> "In total, we quantified over 80 million undirected pairwise interactions. . . Of these, over 25 million were directed interactions initiated by one of the two bees (see Methods)." — Of the undirected interactions, 25 million were directed? It's not clear what is being described here.

We clarified that 80 million total pairwise interaction events were detected, and for 25 million of these, we could assign a clear initiator based on pre-interaction displacement. The remaining interactions had no unambiguous initiator.

[. . .] For over 25 million of these, we could assign a clear initiator based on pre-interaction displacement (see Methods); the remaining interactions had no unambiguous initiator.
→ *See revised manuscript, line 200.*

> "*B. impatiens* females exhibit an enrichment of head-to-head interactions in our data (Figure S6) and in previously published datasets. . . have been historically used to analyze colony behavior." Check the sentence structure.

We split the run-on sentence into two separate statements.

B. impatiens females exhibit an enrichment of head-to-head interactions in our data and in previously published datasets. These interactions have been historically used to analyze colony behavior and are disrupted when bees experience early life social isolation.
→ *See revised manuscript, line 207.*

> "Despite the queen interacting and moving more frequently than the workers, she explored relatively less space in the colony." — This result has also been shown in Jandt & Dornhaus 2009, *Animal Behaviour*.

We added a citation to Jandt & Dornhaus (2009), who also observed restricted queen spatial range in bumble bees.

[. . .] consistent with observations of her primary localization to the brood and prior work in bumble bees (Jandt, Huang, and Dornhaus, 2009).
→ *See revised manuscript, line 237.*

> “Importantly, this lack of behavioral variation in queenright settings is not the result of a lack of physiological variation — there already variation in ovary size between workers in these queenless colonies (Figure 4c).” — Grammatical error. Also not clear what is being compared.

We fixed the grammatical error and swapped the QR/QL labels as noted by Reviewer #1, Comment 4.

This coincides with a significant interaction between condition and ovary index on interactivity [. . .] indicating that the slope of interactivity on ovary index differs between queenright and queenless colonies [. . .] there is already variation in ovary size between workers in these queenless colonies.
→ *See revised manuscript, line 369.*

> Figure 4: The dashed reference lines make the small dots in Fig 4b almost impossible to see. Consider removing the reference lines and/or making the dots bigger and darker coloured.

We have enlarged the dots, darkened the colors, and lightened the dashed reference lines in Figure 4b to improve readability.

> “Queenless networks were also more efficient. . . ” — Consider adding more context as to what they are more efficiently doing.

As noted in our response to Reviewer #1, Comment 5, we have clarified that “efficient” refers to global network efficiency (average inverse shortest path length; (Latora and Marchiori, 2001)), a proxy for how easily information can potentially spread through the network. This definition is now stated explicitly in the manuscript.

> “Queens were largely spatially constrained to the brood area. . . ” — There were no analyses that included distance to the brood clump. Where does this conclusion come from?

We softened this claim and grounded it in published observations rather than implying a direct distance-to-brood analysis.

Queens occupied a relatively restricted spatial range within the colony, consistent with previous observations of queen localization near brood (Cnaani, Schmid-Hempel, and Schmidt, 2002; Jandt, Huang, and Dornhaus, 2009).
→ *See revised manuscript, line 491.*

> “A strong association between behavioral variation and reproductive physiology emerged in queenless conditions that was not observed in the presence of a queen.” — Technically, wouldn’t the queen be a hub bee? The fact that ovarian development doesn’t really start until the queen is removed suggests that reproductive physiology is a strong predictor of social organisation across contexts.

The reviewer raises an excellent point. The queen is indeed the most central node in queenright networks (3), so she is functionally analogous to a “hub bee.” Reproductive physiology predicts network centrality across both queenright contexts (where the queen is the sole reproductive and the most connected individual) and queenless contexts (where workers with the largest ovaries become the most connected). The key distinction is that hub bees *emerge* from among workers only when the queen is absent — in queenright colonies, no worker approaches queen-like centrality. We have added language in the Discussion acknowledging this parallel.

In queenright colonies, the queen herself occupies this central network position, suggesting that reproductive physiology predicts network centrality across both social contexts — but the emergence of hub bees among workers is specific to queenless conditions.
→ *See revised manuscript, line 460.*

> Figure 5a: What is an "influencer" — should this say "hub bee"?

Corrected — see our response to Reviewer #2, Comment 3. All instances of "influencer" have been replaced with "hub bee" throughout, including Figure 5a.

> "Queens are substantially larger and express a distinct cuticular chemistry that reflects reproductive status. . . " — Consider adding "*Bombus*" before "Queens" as *Apis* was described in the previous sentence.

We added "*B. impatiens*" before "queens" to distinguish from *Apis* discussed in the previous sentence.

B. impatiens queens are substantially larger and express a distinct cuticular chemistry that reflects reproductive status. . .
→ *See revised manuscript, line 514.*

Summary of Required Changes

Below is a checklist of all manuscript and code changes referenced above. Each item links to the corresponding label in the revised manuscript.

Manuscript Text Changes (`01_Article_MainText.tex`)

99 Introduction: add competition point mechanism and information flow discussion.
315 Results: add mean hub bee and non-hub body sizes in mm.
325 Results: report hub bee initiation rates; reference supplementary figure more prominently.
369 Results: replace separate cor.tests with interaction model (addresses QR/QL swap).
427 Results: define efficiency and clarify dissimilarity dimension.
746 Methods: add mm equivalents for filtering thresholds; discuss interaction valence and darting.
806 Methods: add glmmTMB dispersion sub-models for variance analysis; retain lme4 for fixed-effect LMMs.
369 Results: replace cor.tests with glmmTMB interaction model and emtrends slopes.
497 Fig 5 caption and all scripts: replace "influencer" with "hub bee."
638 Methods: clarify 5 colonies / 4 days / 10 arenas.
561 Methods: add 2D arena design caveats.
600 Conclusions: add natural queen loss framing; soften energetic cost claim; reframe future work.
460 Discussion: acknowledge queen as functionally analogous hub bee in QR context.

Figure and Code Changes

- `scripts/manuscript/fig_4_new.R`: enlarge dots and increase opacity in ovary index panel (5b).
- `scripts/manuscript/figure_5_hairballs.py`: rename "influencer" → "hub bee."
- `scripts/manuscript/variance_analysis.R`: switch to `glmmTMB` dispersion modeling for variance comparisons.
- `scripts/manuscript/ovary_interactivity_interaction_model.R`: new script replacing separate `cor.test` calls with interaction model.
- `02_Article_Supplementary.tex`: replace "Influencer" with "Hub Bee" in captions.
- 16 additional R/Python scripts: replace "influencer" → "hub bee" in labels, legends, and comments.
- `manuscript-bib.bib`: add Alaux et al. (2004), Chole et al. (2022), Franco et al. (2023), Latora & Marchiori (2001), Amsalem & Hefetz (2010), glmmTMB, emmeans.

End of response to reviewers.

Response to Reviewers — Revision 2

Bumble bee workers adopt novel behavioral roles and reshape their social networks in the absence of a queen

May 19, 2026

We thank both reviewers for their continued careful reading. Reviewer 1 considers the manuscript ready for publication and flagged a few rendered figures with leftover "influencer"/"keystone" labels. Reviewer 2 raised two outstanding statistical concerns, both addressed below. Reviewer comments are shown in shaded boxes, our responses in plain text, and modified manuscript text is highlighted in red.

Summary of changes:

(1) Single `glmmTMB` model with `dispformula` $\sim$ `Treatment` for analyses that report both means and variances, replacing the previous `lme4` + separate-dispersion two-stage setup (R2.1).

(2) Figure 4c lines and 95% CI ribbons now from `predict()` on the interaction model rather than independent `geom_smooth(method="lm")`; caption updated to say so (R2.2).

(3) Regenerated `figures/figure_5.jpg` from the updated source script (legend now "Queenless Hub Bee" / "Non-Hub Bee Worker"), renamed Supplementary Table 1 row "Keystone Assortativity" → "Hub Bee Assortativity", and replotted Figure S16.

Reviewer #1

Expertise: behavioral ecology, social insects

> **Comment 1:** The authors addressed my own and other reviewers' comments with great attention and in my opinion the article is ready for publication. There are still a few spots in the paper where "influencer" appears instead of "hub bee" which probably requires some minor editing but otherwise the paper is ready to go.

Thank you. The leftover instances all came from rendered figures and one supplementary table cell that had not been regenerated from the (already-updated) source. Fixed:

(a) 5 legend now "Queenless Hub Bee" / "Queenless Non-Hub Bee Worker".
(b) Table S1 row renamed to "Hub Bee Assortativity" with matching description.
(c) Figure S16 y-axis now "Hub Bee Assortativity Percentile".

Reviewer #2

Expertise: social insects, division of labor

Comment 1 (Statistical analysis): I have previously suggested to model treatment differences in variance with a linear model because the presence of treatment differences in variance can lead to inaccurate estimates of uncertainty and therefore bias p-values. The authors have now used a linear gaussian model to model treatment-specific variance, which I appreciate. However, the authors are still using the p-values from the previous linear model, which does not account for treatment-specific variance, to compare treatment means. The comparisons of treatment-specific means and variances should occur in the same model to avoid biasing p-values, even if this means that some of the previously significant effects will "disappear". That means there should be just one model that models both the means and the variances.

We agree with the Reviewer's comment and have made changes accordingly. Wherever the manuscript reports both means and variances on the same response, both p-values now come from a single `glmmTMB` fit with `dispformula` ∼ `Treatment`: the Wald χ^2 on the fixed effect for the mean, and pairwise contrasts on the dispersion component via `emmeans` for the variance. This applies to the colony-level network parameters (Figure 5) and to the hub-vs-non-hub comparisons in Figure S14. Other comparisons (queens vs. workers in Figure 2; queens vs. hub bees in the Discussion) report means only and continue to use `lme4`.
As anticipated, several χ^2 values shifted under the unified model, but no qualitative conclusion changed. Updated values appear in the Results text and figure captions, and in the data files reported on GitHub.

Methods — Linear Mixed Models (revised):
Where this study reports both treatment-mean and treatment-variance comparisons on the same response (the colony-level network parameters in Figure 5 and the hub-vs-non-hub comparisons in Figure S14), both come from a single `glmmTMB` (Brooks et al., 2017) Gaussian linear mixed model with random intercept(s) for trial (and hour-of-day where applicable) and a dispersion sub-model `dispformula` ∼ `treatment` that allows the residual variance to differ between conditions. The Wald χ^2 test on the treatment fixed effect provides the mean comparison; pairwise contrasts on the log-dispersion component via `emmeans` (Lenth, 2024) provide the variance comparison. Comparisons that report means only (queens vs. nestmates; queens vs. hub bees) use `lme4` (Bates et al., 2015).
→ *See revised manuscript, line 806.*

Comment 2 (Figure 5C): The authors now use an interaction model for the results corresponding to Figure 5C, which again I appreciate. I am however confused that the model predictions shown in Figure 5C do not seem to have changed at all (the figures in the old and new version of the manuscript seem identical). This makes me wonder what is shown in the figure. The figure should optimally show the mean and 95% confidence interval of the mean that comes directly from the interaction model, which should also be explained in the figure caption. I would encourage the authors to show the model predictions from the interaction model (and not a separate fit from `geom_smooth` or something alike), if that is not yet done, since this allows the reader to also evaluate how well the model fits the data.

The reviewer is correct; the panel is Figure 4c in the current numbering. The interaction model was introduced in the previous revision, but the figure still uses `geom_smooth(method="lm")` per group, independent of that model. For this reason, the figure is unchanged.
Rebuilt: lines and ribbons are now `predict(re.form=NA)` on the same `glmmTMB` interaction model the text reports (`Degree` ∼ `QR_Queen_Condition` ∗ `ovary_idx` + `(1 | Trial)`), with 95% CIs from the fixed-effect covariance. The figure and the reported β, z, p now come from the same fit. The queenright slope stays ≈ -1698 (n.s.); the queenless slope is slightly shrunk vs. the previous OLS line. Qualitative result ($\beta = 10541$, $p = 1.4 \times 10^{-10}$) is unchanged. The Figure 4 caption now states explicitly that lines and ribbons are model predictions with 95% CI.

Code: `scripts/manuscript/fig_4_new.R` (replaces two `geom_smooth` calls with `geom_ribbon` + `geom_-`

`line` from `predict()`).

Figure 4 caption (excerpt):
(c) Standardized interactions per hour relative to ovary index in queenright workers (purple), queenless hub workers (dark yellow), and queenless non-hub workers (yellow). Solid lines and shaded bands show the population-level mean and 95% confidence interval predicted by the `glmmTMB` interaction model (Degree $\sim$ QR_Queen_Condition $\times$ ovary_idx $+$ (1$|$Trial)) reported in the main text; individual points show colony-level mean values per bee.
$\rightarrow$ *See revised Figure 4 caption.*

End of response to reviewers (Revision 2).