Queen loss unmasks cryptic worker influence and decentralizes the bumble bee social network

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Abstract

Bumble bees (*Bombus impatiens*) undergo a radical transformation in social dynamics as a natural part of their colony life cycle. Early in the season, bumble bee societies are eusocial and contain a reproductive queen and functionally sterile workers. As the colony grows, the colony transitions to a competition phase in which reproductive division of labor breaks down and some workers activate their ovaries and lay unfertilized, male-destined eggs. This transition represents a key shift in colony organization, but its underlying social dynamics, including the relationship between individual- and colony-level behavior, are poorly understood. Using multi-animal pose tracking to quantify social interactivity and related movement dynamics, we compared queenright and queenless colonies that capture these distinct cooperative and competitive phases of the colony life cycle. Queenright colonies displayed highly centralized social networks in which the queen received, rather than initiated, the majority of social interactions. In contrast, queenless colonies displayed higher worker interaction rates, and a subset of workers exhibited queen-like social behaviors and higher reproductive potential relative to nestmates. The unmasking of these "influencers" in a queenless, competitive context transformed the colony's social network from a centralized to distributed social organization with higher overall network efficiency. Thus, the bumble bee queen masks a hidden relationship between worker influence and reproductive potential that underlies their latent social flexibility. In the absence of a queen, the colony social structure is transformed, revealing that significant modifications in information transfer can accompany shifts in colony social organization and highlighting a novel layer of resilience in this social system.

social insects | *Bombus impatiens* | social networks | ethology | automated tracking

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Introduction

The influence of individuals within a social network can vary dramatically, and some members can play an outsized role in shaping group behavior and function (Jolles, King, and Killen, 2019; Kralj-Fišer and Schuett, 2014; Cook et al., 2020). Highly influential individuals often serve as central nodes in their networks, mediating information flow and maintaining social stability (Caticha, Calsaverini, and Vicente, 2024; Newman, 2018; Freeman, 1977). This makes their presence particularly consequential for group organization and function (Centola, 2019; McCully and Rose, 2023).

Asymmetries in social influence are particularly pronounced in social insect colonies, where variation in social connectivity often reflects underlying differences in behavior and physiology. In eusocial colonies, one or a few individuals monopolize reproduction, while the majority of group members perform supportive tasks as functionally sterile workers. Reproductive queens suppress worker reproduction through mechanisms that vary with colony social complexity: in smaller colonies, queens often rely on direct physical interactions to maintain dominance, while queens of larger, more elaborate societies (e.g. ants and honey bees) produce chemical signals that inhibit worker reproduction (Van Zweden, 2010; Smith and Liebig, 2017).

The loss of a queen provides a natural opportunity to study how key individuals shape group organization. In the absence of a queen, some of the workers in a colony can activate their ovaries and lay eggs, often leading to competition among nestmates. The colony must respond quickly to minimize such conflict, which can decrease the efficiency of task allocation if supportive tasks are abandoned in favor of individual reproductive opportunities (Mattila, Reeve, and Smith, 2012). Understanding how group members respond, both behaviorally and physiologically, and how these responses interact with collective dynamics, remains a central challenge to our understanding of social systems.

The common eastern bumble bee (*Bombus impatiens*) is an ideal system for exploring the impact of queen loss on social organization and individual behavior and physiology. Bumble bee colony development begins with a cooperative, eusocial phase characterized by a strong reproductive division of labor between a nest-founding queen and her functionally sterile daughter-workers. Physical contact with the queen is required to prevent workers from becoming reproductively active (Padilla et al., 2016). This contact is thought to, in part, mediate the transmission of chemical cues produced by the queen that signal her reproductive status (Orlova, Treanore, and Amsalem, 2020; Orlova and Amsalem, 2021). The later phase of a colony's annual life cycle is characterized by a breakdown of reproductive suppression by the queen, and competition emerges between workers to activate their ovaries and lay haploid, male-destined eggs (Goulson, 2010). Simultaneously, the queen transitions to rearing the next generation of reproductives, termed gynes (Amsalem, Grozinger, et al., 2015). This natural transition, from eusocial cooperation to competition, can be triggered by the artificial removal of the queen or separation of workers from a queenright colony (Cnaani, Wong, and Thomson, 2007). How this transition affects bumble bee social networks, as well as how the expression of individual behavioral and physiological variation among workers shapes network reorganization across contexts, remains poorly understood.

To better understand this process, we compared queenright and queenless *B. impatiens* colonies using a "hybrid" automated tracking tool, NAPS (NAPS is ArUco Plus SLEAP) (Wolf et al., 2023). NAPS combines fiducial marker tracking and pose estimation, enabling high-fidelity tracking of each bee's identity and body part positions in space throughout the duration of an experiment (Pereira et al., 2022; Wolf et al., 2023), thus allowing us to quantify individual interactions between colony members. We leveraged this tool to examine how the presence or absence of the queen shapes colony organization and influences the behavior and physiology of workers.

Results

Automated Monitoring of Bumble Bee Colonies Reveals the Dynamics of Physical Contact Interactions

We previously established a hybrid tracking system, NAPS, to automate the monitoring of bumble bee behaviors within a colony setting (Wolf et al., 2023). NAPS integrates pose estimation performed with SLEAP (Pereira et al., 2022) with tag identification using ArUco (Garrido-Jurado et al., 2014). We developed a SLEAP model using training data from five bumble bee colonies, each divided into queenless and queenright partitions and filmed for 96 hours per cohort (Figure 1). Our SLEAP model yielded accurate representations of workers and queens with minimal error (Figure S1, Figure S2). After matching individual instances to their associated ArUco tag, we generated a dataset of each bee's location. We then filtered our dataset to remove spurious detection events, including bees moving impossibly fast and distances between nodes that were either impossibly larger or small (Figure S3). No filtering step was biased between queenless and queenright colonies (Figure S4). We used antennal presence, calculated as the percentage of frames in which all nodes representing the antennae were visible, as a proxy for each bees' detectability; this value was comparable between queenless and queenright colonies across trials (Figure S5). A single colony (Colony 3, Queenright) had a marginally lower antennal presence compared to other colonies, and we adjusted for this in downstream analyses by normalizing all individual interaction events to antennal presence.

One key advantage to pose estimation is its ability to track and quantify different modalities of physical interactions (Traniello and Kocher, 2024). Knowing that bumble bees rely on their antennae to detect physical and chemical cues (Spaethe et al., 2007), we quantified two distinct types of pairwise antennal contact interactions: head-to-head and head-to-body (Figure 1, Figure S6). We found head-to-head interactions were significantly enriched relative to head-to-body interactions (Figure S6: $t = 61.21, 81.67, 19.39$ for daytime hours (9:00-17:00) in queenright workers, queenless workers, and queens respectively; $p < 2.2 * 10^{-16}$ in all comparisons), despite the bumble bee body being >5x larger than the head. This suggests that head-to-head antennal interactions are enriched in bumble bee colonies as a primary means of physical communication, consistent with results from detailed tracking of two-bee pairings (Wang et al., 2022). We detected a slight circadian effect in the frequency of head-to-head interactions (Figure 2a), so we restricted our analyses to daytime hours. 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).

Queens Play a Central Role in the Queenright Social Network

Focusing our analysis on instances of head-to-head interactions, we tested the hypothesis that the queen regulates the social environment of the colony by maintaining a high interactivity relative to workers. To do this, we generated weighted, undirected social networks between all bees in the colony for each hour of the experiment; weighting was based on the total time spent in each dyadic interaction. Interaction weights were then standardized to antennal presence for each individual, as described above and in Methods. This revealed the queen to be the most central bee in the network, interacting much more frequently than nestmate workers, resulting in a higher degree centrality (Figure 2a-b: $\chi^2 = 26.4, p = 2.09 * 10^{-147}$). This result was also present in unstandardized data (Figure S7a). Relative to interactions between work-

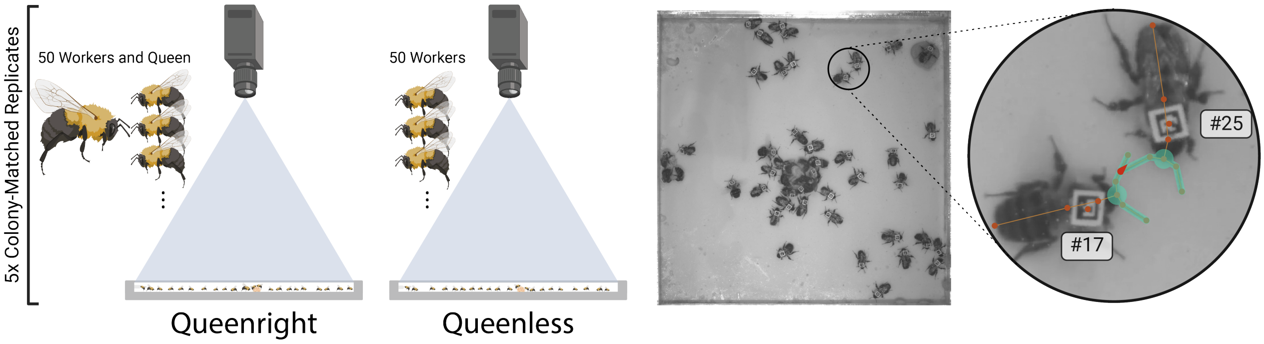

Figure 1. Experimental design and example application of automated behavioral tracking with NAPS. (left) Five colonies of approximately 200 workers and a queen were each split into two partitions: one queenright partition consisting of a queen and 50 (±2) workers and another with 50 (±2) colony-matched workers. The resulting colonies were imaged under infrared light with controlled temperature ($\sim$27 C) and relative humidity ($\sim$30%) for four days. **(right)** Still image from a colony recording: inset shows antennal interaction between adjacent workers, including the localization of nodes and edges (i.e., relevant body parts and their connections, respectively) detected via SLEAP. Unique ArUco identifier is shown in white box next to each bee. Antennal overlap, constituting a head-to-head interaction, is shown in red.

ers, queen-worker interactions were more strongly enriched for head-to-head antennation and longer in duration, suggesting that reproductive status influences physical communication strategies among nestmates (Figure S6, Figure S7b).

Despite the queen interacting and moving more frequently than the workers, she explored relatively less space in the colony (Figure 2c: $\chi^2 = -12.8, p = 2.39 * 10^{-37}$; Figure S7c), consistent with observations of her primary localization to the brood (Cnaani, Schmid-Hempel, and Schmidt, 2002). We further interrogated the dynamics of queen-worker interactivity and found the directionality of these interactions to be imbalanced: queen-worker interactions were initiated by the worker $\sim$70% of the time (Figure 2d: $\chi^2 = -16.9, p = 7.97 * 10^{-63}$). Taken in sum, the queen occupies a smaller home range in which she is visited frequently by workers, resulting in a proportionally high interaction rate.

Individual Variation Unmasked in the Absence of a Queen

In light of the queen's central role in the colony's social environment, we hypothesized that individual workers would vary in their response to her absence. To this end, we analyzed queenless partitions of workers collected from the same source colony as the queenright partition. Associations between body size and aggression have been made in the closely related bumble bee species, *Bombus terrestris*, (Princen et al., 2020), a possible confound we addressed by visually body size-matching each cohort and later using marginal cell length, which serves as a robust proxy for body size, to confirm that our visual assessments were accurate (see Methods). Principal component analysis (PCA) performed on interactive, kinematic, and spatial metrics identified network centrality measures and kinematic variation as the major contributors to the primary axis of variation (i.e., PC1) (Figure 3a, Figure S8). PC1 captured most measures of centrality, including betweenness, closeness, degree, eigenvector centrality, as well as clustering coefficient (Figure S8). Neither of the first two PCs represented variance explained by source colony (Figure S9), suggesting that variance attributed to these PCs was consistently observed across the five colony replicates.

Using permutation tests, we also observed an overall increase in individual variation in the absence of a queen. Queenlessness was associated with increased variation in multiple network parameters, including interactivity and tendency to cluster (standardized interaction count: Figure 3b, $p < 10^{-4}$; clustering coefficient: Figure 3c, $p < 10^{-4}$). Queenless workers were also more varied in percent of time moving (Figure 3d, $p = 2.45 * 10^{-2}$), but not in dispersion (Figure 3e, $p = 1.767 * 10^{-1}$).

To understand the biological basis of the observed variation in network metrics, we investigated differences in reproductive physiology between queenright and queenless workers. Using ovary index (Duchateau, 1989; Cnaani, Schmid-Hempel, and Schmidt, 2002), a measure of ovary activation normalized to body size, we found that worker ovary size was on average larger in queenless partitions (Figure S10, Figure S11), consistent with results from other queen removal experiments (Padilla et al., 2016). This suggests a generalized increase in both physiological and behavioral variation among queenless workers compared to queenright workers.

A Small Number of Queenless Workers Express Queen-Like Traits

We next sought to characterize workers most sensitive to queen removal, hypothesizing a link between their behavioral and reproductive plasticity. Using the embeddings from Figure 3a, which represent individuals clustered according to interactive, kinematic, and

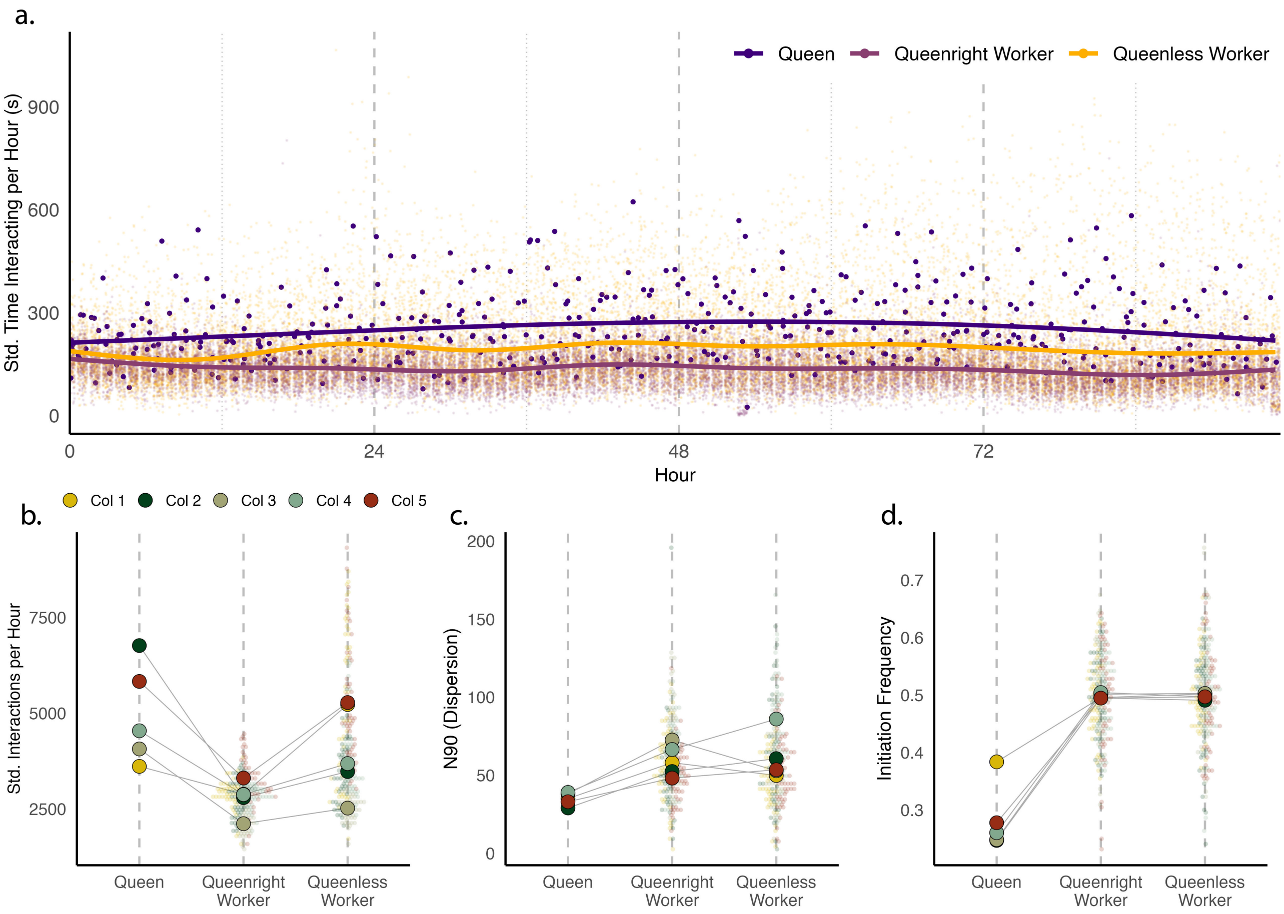

Figure 2. Social interactivity correlates with reproductive state and social condition. (a) Interaction counts (degree centrality) over time aggregated across all colonies within each condition over an hour; counts are standardized to antennal presence for each bee. (b) Standardized interaction counts from (a) averaged for each bee. (c) Dispersion metrics as calculated by N90, the smallest number of 200 x 200 pixel squares that represent 90% of a bee's home range. (d) Proportion of directed interactions in which a given bee is the initiator, as measured by pre-interaction displacement (see Methods). For (b-d), large dots represent the mean for a given colony; for queenright and queenless workers, raw data are shown in the background.

spatial metrics, we identified workers with higher PC1 scores than the highest score for queenright workers in the same PC. We reasoned that these individual bees expressed the most dramatic shifts in behavior in the context of queenlessness relative to the paired queenright partition. This analysis revealed a small but highly interactive population of bees which we term "influencers" due to their higher likelihood of affecting network-level changes in a queenless context. We identified between two and twelve influencers per colony, for a total of 37 influencers across all five queenless partitions. Influencers were slightly but significantly larger than non-influencer nestmates ($\chi^2 = -2.50, p = 1.25 * 10^{-2}$).

Influencers were more interactive than remaining queenright and non-influencer queenless workers, often having a total number of interactions comparable to or greater than their source colony's queen (Figure 4a, $\chi^2 = 47.2, p =\sim 0$). The same pattern could also be observed in the loadings for PC1, though factors other than network centrality contributed to the variance explained by this PC (Figure S8). Similar to the queen, influencers moved more frequently, were higher in other centrality measures, and were significantly less dispersed than remaining workers (movement percent: $\chi^2 = 19.5, p = 1.4 * 10^{-82}$; dispersion Figure 4b: $\chi^2 = -17.0, p = 4.70 * 10^{-64}$; betweenness Figure 4c: $\chi^2 = 33.4, p = 1.17 * 10^{-228}$).

We observed a larger betweenness centrality observed in queens compared to influencers (Figure S12), likely due to betweenness being a measurement of how well an individual connects disparate groups in a network (Farine and Whitehead, 2015). Because queens interact significantly more than workers, they will necessarily be in the most centralized path between disparate individuals. In queenless colonies, which have multiple queen-like influencers, no individual influencer is guaranteed to be on the shortest path.

Because influencers display network behavior profiles similar to their natal colony's queen, we next asked if they also assumed a queen-like reproductive physiology. Indeed, influencers had a larger ovary index than the non-influencer queenless workers,

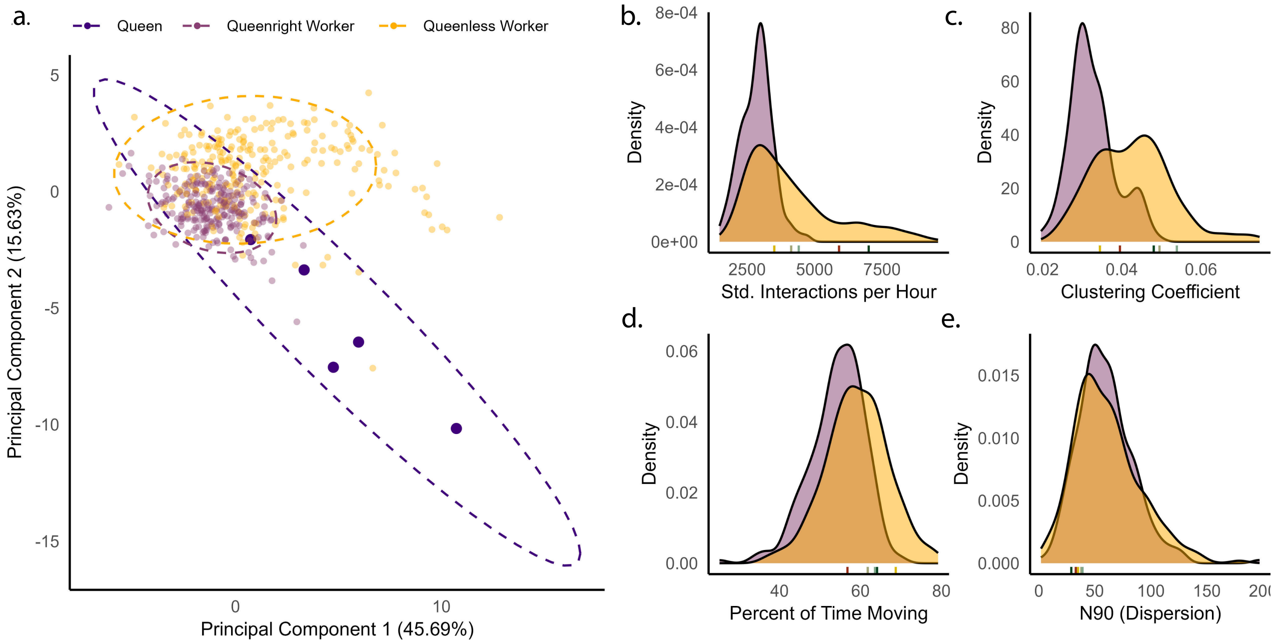

Figure 3. Queenless colonies undergo major shifts in individual- and group-level interaction dynamics. (a) Principal component analysis of interactive, kinematic, and spatial metrics for individual bees in queenright and queenless partitions. Queens and queenright workers are shown in dark and light purple, respectively, and queenless workers are shown in yellow. (b) Density plot of standardized interactions per hour. (c-e) Density plot of (c) clustering coefficient,(d) percent of time moving, and (e) dispersion for queenright and queenless partitions. Queens are indicated in rug plot below each density plot and colored according to source colony. n = 501 workers and five queens from five source colonies.

suggesting that these individuals activate their ovaries most strongly in the absence of the queen (Figure 4d: $\chi^2 = 25.4, p = 2.88 * 10^{-133}$). As a result, worker social network position is highly predictive of reproductive status and *vice versa* in queenless, but not queenright, contexts.

Bumble Bee Social Networks Are Restructured Following Queen Loss

We next asked if the increase in interactivity of influencers was driven by influencer-to-influencer interactions, analogous to dueling tournaments in queenless ponerine ants (Opachaloemphan et al., 2021). To examine this, we calculated assortativity between influencers by testing how enriched influencer-to-influencer interactions were relative to influencer-to-non-influencer interactions. We found no evidence for increased assortativity between influencers (Figure S13), suggesting consistent mixing of queenless workers regardless of reproductive status.

Taken together, our results suggest that queenless influencers express a specific behavioral syndrome in which they are more interactive and more locomotive, yet also more spatially restricted. It is important to note that we cannot disentangle causality between these behavioral phenotypes: bees moving faster will contact one another more frequently, sometimes antennating in the process, but bees may also be moving faster because it allows for an increase in antennation rate.

Finally, we hypothesized that as a result of these changes, the removal of the queen would restructure the collective dynamics of the colony's social network, making it more decentralized and interactive as more workers take on queen-like roles. We found that, while queenright partitions were highly centralized around a single queen, queenless colonies contained multiple highly connected influencers (Figure 5a), and this unmasked variation was consequential for how information transfer occurred in each partition. Transitivity, a measure of "cliquishness" (subgroups formed within a social network) (Table S1), was higher in queenless networks for four of five replicates (Figure 5b: $\chi^2 = 19.7, p = 1.68 * 10^{-80}$). Moreover, queenless networks were 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: $\chi^2 = 46.4, p =\sim 0$) is associated with 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, where we observed a stronger tendency toward a neutral (i.e., neither assortative nor disassortative) network.

Discussion

In social insects, the presence or absence of a queen can profoundly shape group dynamics. We studied how the queen can influence bumble bee colonies by creating queenright and queenless partitions of five *Bombus impatiens* source colonies and tracking over 80 million pairwise interactions among nestmates. Our study revealed multiple ways in which queens can influence

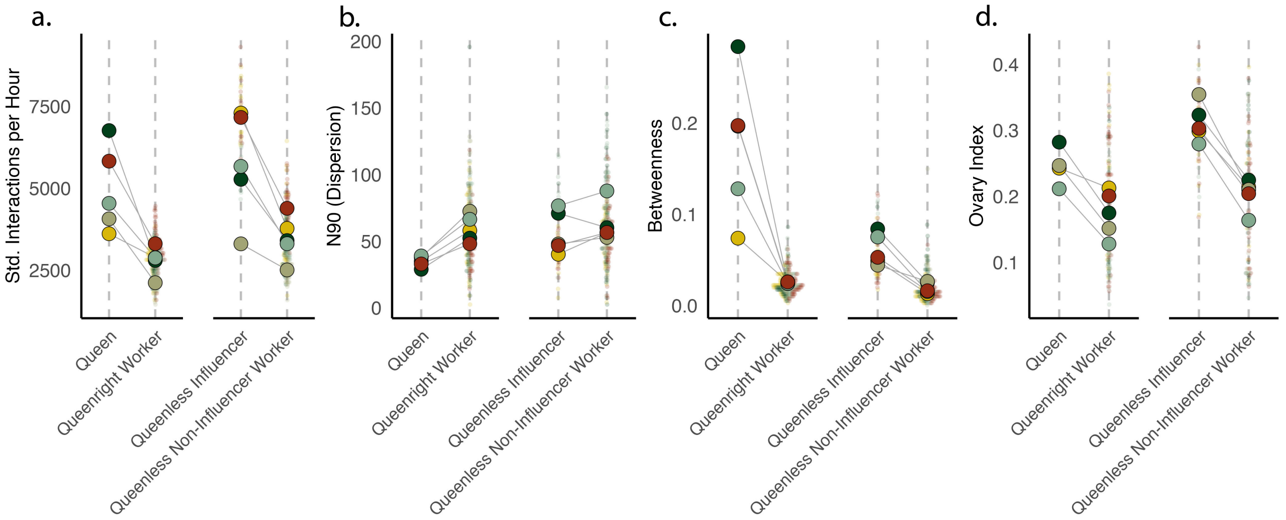

Figure 4. Queen removal disinhibits a small population of workers that adopt queen-like behavioral and physiological traits. Plots showing contrasts between queens and queenright workers and queenless influencers and queenless non-influencer workers. Large dots show colony means and smaller dots show individual measurements for (a) standardized interactions per hour, (b) N90 (dispersion), (c) betweenness centrality, and (d) ovary index.

colony structure and function, from masking latent variation in individual behavior and physiology to centralizing the colony social network.

The Queen Occupies a Central, Yet Passive, Role in the Colony Social Network

Bumble bee colonies are highly interactive, leveraging both physical and chemical communication to coordinate behavior and regulate reproduction (Jandt and Dornhaus, 2011; Padilla et al., 2016; Orlova, Treanore, and Amsalem, 2020). Previous studies have indicated that physical contact between queens and workers is essential for maintaining reproductive division of labor (Padilla et al., 2016); however, the nature of their interactions is largely unknown.

We found that queens occupy a unique position in the colony: they serve as the most central nodes in queenright interaction networks, but these interactions are driven primarily by workers contacting the queen rather than her initiating interactions. This implies that the queen acts as a central hub in the network, but that her role is primarily passive. This pattern differs markedly from both simple eusocial species, which often rely on aggressive enforcement of dominance (Brothers and Michener, 1974; Jandt, Tibbetts, and Toth, 2014), and complex eusocial species like honey bees (*Apis mellifera*), where queens maintain their reproductive monopoly primarily through pheromonal control (Winston, 1987).

While aggression is a commonly used strategy to maintain reproductive dominance within many social insects and vertebrate societies (Holekamp and Strauss, 2016; Tibbetts, Pardo-Sanchez, and Weise, 2022), *B. impatiens* queens rarely displayed overtly aggressive behaviors such as lunging, biting, or stinging. Thus, unlike the closely related species, *B. terrestris* (Pandey, Motro, and Bloch, 2020), *B. impatiens* queens appear to maintain reproductive dominance through mechanisms other than direct aggression. Aggression is unnecessary for social stability if other cues are sufficient to convey dominance status (Tibbetts, Pardo-Sanchez, and Weise, 2022). *B. impatiens* queens are substantially larger and express a distinct cuticular chemistry that reflects reproductive status (Orlova and Amsalem, 2021). Such a signaling system could serve as an honest signal of dominance without the need for aggressive reinforcement. Similar patterns have regularly been observed in honey bees, where queens produce a pheromone with an honest signaling component that reflects their reproductive status (Kocher et al., 2009; Richard, Tarpy, and Grozinger, 2007), and the pheromone bouquet also elicits affiliative licking and grooming behaviors towards the queen (Naumann, 1991; Seely, 1979).

The queen's central yet passive position appears to arise from several factors, including her large body size and more fixed position on the broodnest. As the sole reproductive in a cooperation-phase hive, the queen is likely constrained by the biomechanical costs of egg-laying and thermoregulation of developing workers, exemplifying the reproduction-dispersal tradeoffs observed in other insect societies (Helms and Kaspari, 2015). Because of these constraints, queen centrality is more likely to result from worker-driven strategies. For example, workers in a queenright setting 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).

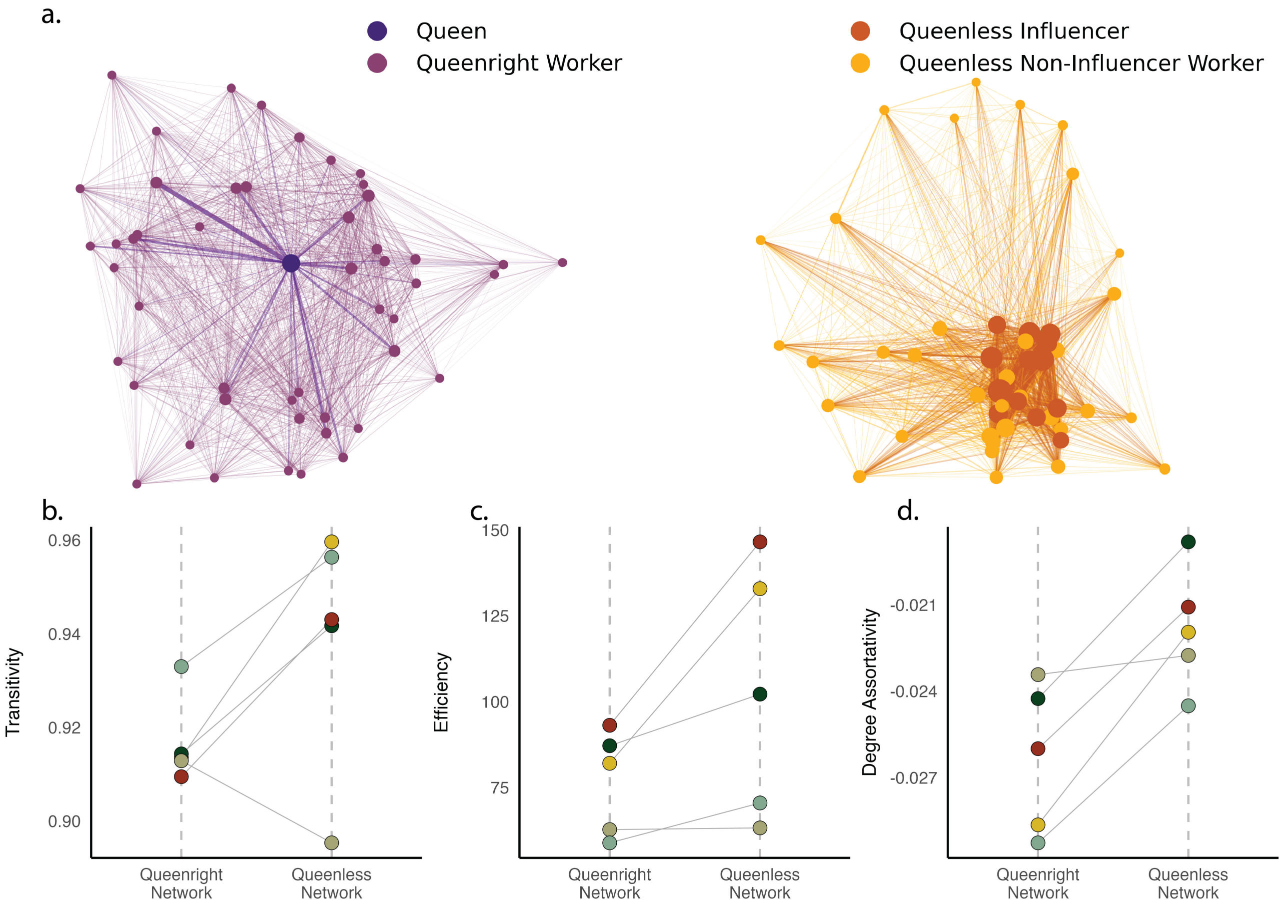

Figure 5. Information flow is more robust in queenless social networks. Network plots of queenright and queenless colony example (Colony 4) and network metrics plotted by colony status. (a) (left) Colony 4 queenright network weighted by pairwise interaction count. (right) Colony 4 queenless network weighted by pairwise interaction count. (b) Network transitivity by queenright/queenless status. (c) Network efficiency by queenright/queenless status. (d) Degree assortativity by queenright/queenless status.

Latent Variation in Worker Behavior and Physiology Is Unmasked in the Queen's Absence

The queen's central position primes her to suppress worker reproduction and mask latent behavioral variation in workers. As expected, we found that worker ovary activation was uncommon in queenright colonies. However, in queenless colonies, a small subset of individuals emerged and expressed high rates of interactivity and overall movement. These bees, which we refer to as "influencers", developed larger ovaries than their nestmates, suggesting the adoption of a queen-like behavioral and physiological profile in the absence of the queen. These individuals may simply have naturally lower thresholds for the expression of queen-like behaviors when the queen is lost (Beshers and Fewell, 2001).

Both queens and reproductively active influencers occupy central network positions, similar to organizational strategies observed in primate social networks (Wooddell, Kaburu, and Dettmer, 2020). While influencers tend to be slightly larger than non-influencer nestmates, size alone does not determine reproductive dominance; influencers were not consistently the largest bees in their respective partitions. Instead, reproductive dominance likely emerges from a constellation of interacting variables that may include factors we did not measure in this study, such as age and experience (Sharma, Gadagkar, and Pinter-Wollman, 2022). Further work is needed to clarify how network behavior interacts with chemical signaling at the colony level, as it is likely that physical contact also disseminates non-volatile chemical cues, as in honey bees (Naumann, 1991).

Highly interactive workers in queenright partitions did not express similar rates of ovary activation, suggesting a link between reproductive physiology and interactivity is unmasked only when the queen is absent. This finding reveals an important complexity in how social and reproductive behaviors are regulated. Bumble bee task allocation cannot be fully explained by a single variable (e.g., size, age, etc.) (Jandt, Huang, and Dornhaus, 2009). This makes the costs and benefits of worker reproduction difficult to assess at the colony level. Indeed, some egg-laying workers in honey bee colonies continue to perform certain tasks, like foraging and brood care (Jones et al., 2020). This suggests that worker reproduction could represent a trade-off between individual- and colony-level fitness rather than being a purely selfish behavior (Korb and Heinze, 2004).

A Subset of Influential Workers Reshape Queenless Colony Dynamics

The emergence of influencer workers in the absence of a queen fundamentally reshapes colony social networks and provides crucial insights into the mechanisms underlying collective organization. Influencers adopting a queen-like phenotype predictably drive a shift in the social structure of the colony by altering the distribution of interaction weights from one to several centralized nodes. As a result, queenless colonies exhibited an increased overall interactivity and higher network efficiency, transitivity, and degree assortativity relative to queenright colonies. In sum, we found that the decentralized structure of queenless social networks was associated with improvements to information-sharing strategies.

Such shifts in network-level dynamics can carry dramatic implications for the physiology of both individual and collective in social insects (Smith, 2018; Bonabeau, Theraulaz, and Deneubourg, 1999; Kay et al., 2024). The transition to a distributed rather than centralized network structure could enhance a colony's ability to fine-tune task allocation (Pradhan, Patra, and Chowdhury, 2021; Fisher et al., 2022), as workers receive more frequent and diverse social signals through the restructured network. Distributed control and frequencies of physical contact play an important role in task allocation in several ant species, including foraging behaviors (Gordon, 2016; Gordon, 1996). 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). Finally, while we did not find influencers to preferentially interact with one another, queenless networks were less disassortative than queenright counterparts. Queen-worker dynamics represent the most asymmetric social relationship in the colony, both in terms of interactivity and reproductive suppression; without a queen, the network may simply trend toward neutrality. Future work will clarify the longer-term impact of the competition phase on preferential worker interactions.

Evolutionary Implications

Bumble bees provide a unique opportunity to study the interplay between individual behavior, social networks, and colony organization because colonies naturally transition from a cooperative phase with a single, reproductive queen to a competition phase where multiple workers lay male-destined eggs (Goulson, 2010). Unlike perennial social insects, such as honey bees or stingless bees, this transition is a natural part of their life cycle. Queen loss leads to the early onset of the naturally occurring competition phase in *B. impatiens*, and the corresponding improvements in network function could facilitate colony survival and fitness in her absence. Similar reasoning underlies the hypothesized evolution of polygynous colonies: multiple queens – or, multiple queen-like workers – may indeed be beneficial under certain ecological constraints, such as low survival or dispersal success rate among reproductive individuals (Nonacs, 1988).

In this study, we find that queenless colonies have a decentralized network structure that results in improved opportunities for information transfer among workers. This raises several intriguing questions about social evolution. For example, if bumble bee societies are more stable and robust in their queenless form, then why is eusocial organization favored during the cooperative phase? Moreover, if the queen suppresses worker ovary activation, then why do workers initiate contact with the queen? These patterns may reflect fundamental trade-offs between individual fitness opportunities and colony-level social organization. For example, premature worker reproduction could reduce colony productivity and fitness, but complete reproductive suppression would eliminate their ability to produce males as queen influence wanes. The rapid emergence of reproductive workers demonstrates how this latent capacity enables colonies to adaptively shift their organization as conditions change.

The ability of social insect colonies to transition between centralized and decentralized social networks highlights an important level of social flexibility that could be a key component of their ecological success. For example, the benefits of a centralized queenright network outweigh its costs during critical periods of colony development (Easton-Calabria et al., 2023), and the ability to shift between organizational states, rather than maintaining either extreme, may help to resolve conflicting selection pressures across the colony life cycle. More work is needed to uncover the potential trade-offs between network robustness and efficiency and understand how network reorganization impacts colony-level energy budgets. In addition, comparative studies across species with different degrees of reproductive division of labor could help to reveal how variation in network organization and reorganization relates to colony resilience and social complexity. Taken together, this work could bring into focus the forces that have shaped collective behavior in social insects.

Conclusions

Our observations of how bumble bee colonies reorganize following queen loss provide insight into the evolution and maintenance of the reproductive division of labor and worker task allocation in social insects. Future work combining detailed behavioral tracking with molecular and neurobiological approaches will be crucial for understanding how these social traits have evolved and how they can shape both individual and collective outcomes in complex social systems.

Methods

Animal Rearing and Tagging

We sequentially performed five biological replicates using healthy *Bombus impatiens* colonies obtained from Koppert Biological Systems (Howell, MI USA); no males nor gynes were observed in any source colony, suggesting each colony arrived in its respective cooperative phase. Each colony was maintained in a warm (28°C), quiet room illuminated by red light (which bees cannot see) to minimize disturbance. Tracking was performed within 10 days of colony arrival to minimize the possibility of stress due to overcrowding, as workers eclose on a daily basis. All experiments were performed between November 11, 2022, and February 7, 2023.

ArUco tags generated from the 5X5_50 set were printed on TerraSlate 5 Mil paper (TerraSlate, Englewood, CO USA), which is waterproof and does not tear, and cut to 4.25 × 4.25 mm using a Silhouette Cameo cutting machine (Silhouette, Lindon, UT USA). On the morning of each tracking experiment, a single queenright colony was moved to a 4°C room to reduce handling stress when the colony was opened and bees removed. Size-matched groups of bees were gently removed with soft forceps and placed in conical vials submerged in wet ice only until no movement was observed. Next, individual bees were removed and a small drop of cyanoacrylate glue (Loctite, Hartford, CT USA) was placed on the dorsal thorax, to which a single ArUco tag was applied using the tip of a pin. Our tagging strategy caused minimal apparent stress as bees actively rewarmed and resumed normal activities within minutes after arena placement. Cyanoacrylate glue does not affect behavior or mortality in bees (Gernat et al., 2018) and previous work only observed an acute increase in grooming following a similar tagging strategy (Crall, Gravish, Mountcastle, and Combes, 2015).

Tagged bees were placed in one of two 27 x 27 cm laser-cut arenas that were designated as "queenless" or "queenright," and the colony's queen was tagged and added to the latter arena (Figure 1). Each partition contained 48-52 workers. Arenas contained silicon matting, on which bees can easily walk, and 5g of brood from the source colony. 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. Temperature was maintained between 27 and 29°C.

Tracking

Arenas were covered with clear acrylic and lit using 7 high-intensity 850nm LED light bars (Smart Vision Lights L300 Linear Light Bar, Norton Shores, MI USA) to allow continuous imaging without disturbance. 5 hours after establishing the partitioned colonies, we imaged the arenas from above using a Basler acA5472-17um (Basler AG, Ahrensburg, Germany) camera recording 3664px × 3664px frames at 20 frames per second (14.5 for Colony 2). Recordings were taken using a modified version of CAMPY, a Python package developed for real-time video compression (Severson, 2021). The resulting videos have a spatial resolution of ∼15.5 pixels/mm, allowing us to capture fine-grained behaviors. Each bumble bee worker varies between approximately 9mm and 14mm in length, so the resulting pixel length of each worker is approximately 139.5px to 217px in the video data (Williams et al., 2014).

After video acquisition, we utilized SLEAP to capture bee pose. We used a 9-node skeleton marking the head (mandible), two thorax points, abdomen, left and right antennal joints, left and right antennae tips, left and right wings, the pretarsus of each leg, and the ArUco tag. We trained two separate models, one for the workers and one for queens to appropriately account for morphological differences. For workers, we trained on 107 frames resulting in a final mean error distance across all nodes of 7.11px (0.46mm) in our validation set. For the queen model, we trained 361 frames and the resulting model has a mean error distance across all nodes of 30.09px (1.94mm). All models are provided in Data Availability.

We utilized NAPS, which integrates SLEAP and ArUco, to ascertain individual bee identities after estimating pose. Using the tracklets identified by SLEAP, a Hungarian matching algorithm was employed to resolve identification ambiguities using the ArUco tags, as described in (Wolf et al., 2023). Post-identification, we filtered data for five anomalies: 'Tag Identity' 'Jumps', 'Between Jumps', 'Skeleton Irregularities', and 'Spatial Irregularities':

(1) We removed all nodes that were mapped with SLEAP to tags not used in the experiment.

(2) Jumps were defined as instances where between two frames a node either (i) moved greater than 10 pixels, when fewer than 80 percent of other nodes moved less than other nodes (node jump) or (ii) when greater than 80 percent of other nodes moved greater than 100 pixels (tag jump).

(3) Between jumps were regions within 10 frames of a tag jump on both directions. In this case we removed

all nodes.
(4) Skeleton irregularities were regions where length of the edge of a skeleton was greater than 5 z-scores above the mean. In this case we removed both connection nodes.
(5) Spatial irregularities were regions where two nodes on the same be were less than 2 pixels away from each other (typically a result of the same node being indicated twice).
The mean filtering of each step of this data is in Supplementary Figure 3.

Touch Detection

We aimed to quantify the modalities of bumble bee contacts, particularly focusing on head-to-head antennation as an indicator of social interaction. To do this, we converted the skeleton into regions of the body by mapping the space a buffer distance B from the skeleton Figure 1 in cyan). This allows us to define physical interactions as any frame with overlapping regions between two bees. Head-head interactions are defined by overlaps between two antennal regions, and Head-body interactions are defined by overlaps between an antennal region and a thorax/abdomen region. To appropriately standardize our data, we calculated the area and perimeter of our regions. The skeleton used to map interactions can be found in Figure S1 and Data Availability.

Network Analysis

Interaction instances were translated into an undirected weighted network. Network data was generated for each hour of our 96-hour recording. Network analyses were conducted using *networkx* version 3.1. We defined weights in two ways: "total Interactions" is the number of unique instances of "bouts" of interaction (lasting at least 2 frames and at least 20 frames away from any other identified interaction between the same two bees). "Interaction Time" is the bouts weighted by their duration such that longer bouts contribute more than shorter bouts.

Using our weighted networks, we calculated both individual-level variables and network level variables as described in (Table S1). These variables were collectively used to create a principal component analysis (Figure S9). We used two different statistical methods to identify the significance of variation in these variables:

Linear Mixed Models. To identify the impact of worker/queen status on given network features (both individual and colony level), we fit linear mixed model using the lme4 R package (Bates et al., 2015) using the following model formula:

$$\begin{aligned}\text{feature} \sim 1 + \text{"Queenright / Queenless Status"} \\ + (1 \mid \text{"Source Colony"}) \\ + (1 \mid \text{"Aligned Hour Of Experiment"})\end{aligned}$$

Queen status is included as a fixed effect, while source colony and experiment hour are included as random effects.

Variance Analysis. To assess the difference in behavioral variance between "Queenright Worker" and "Queenless Worker" conditions, we calculated the variance difference for each experimental trial. Specifically, the variance of worker metric under the "Queenless Worker" condition was subtracted from the variance under the "Queenright Worker" condition within each trial group. The mean of these variance differences across all trials was calculated to obtain the observed mean-variance difference.

To determine the statistical significance of the observed mean-variance difference, a permutation test was conducted. The labels for the "Queenright Worker" and "Queenless Worker" conditions within each trial were randomly shuffled 10,000 times. For each permutation, the mean-variance difference was recalculated, generating a null distribution of mean-variance differences under the assumption of no effect.

The statistical significance of the observed mean-variance difference was then assessed by calculating a p-value. This p-value was defined as the proportion of permuted mean-variance differences that were less than the observed mean-variance difference. This approach allowed us to determine whether the observed variance difference was statistically significant compared to what would be expected by chance alone.

Velocity

To distinguish between actual movement and apparent movement which occurs as a result of noise in the SLEAP model, we created a histogram of the velocities of each individual. We identified two bimodal peaks representing real movement and subpixel movement, the latter of which we considered to be statistical noise. We used a bimodal distribution (in the method of Crall, Gravish, Mountcastle, Kocher, et al., 2018) to calculate a threshold cutoff for movement for each colony, and calculated both the percentage of time each bee is moving and the average velocity of each bee when moving.

Directed Network

Insect networks are commonly based on directed dyadic interactions (Appleby, 1983). To identify directionality within our undirected interactions, we isolated the first frame of each bout of interaction and marked the interaction as "directed" if one of the bees in the interaction traveled at least 65.8 pixels (0.5 bee-lengths) more than the other bee in the second before the interaction. From there, our directed network consists of all the directed interactions, with the initiator being the approaching bee and the receiver being the approached bee. Our undirected network includes both directed and undirected interactions, with no differentiation between initiator and receiver.

Ovary and Body Size Quantification

Ovary dissections were performed at room temperature, and frozen abdomens were allowed to completely thaw in phosphate-buffered saline (PBS) before the tergites T2-T4 were carefully removed. Ovaries were gently lifted out of the abdominal cavity, placed in a new droplet of PBS, and photographed with a Nikon SMZ1270 stereo microscope (Nikon, Tokyo, Japan). The largest oocyte of each ovary was measured in FIJI (Schindelin et al., 2012) and we used the average width across ovaries as a surrogate for reproductive status (Simons and Smith, 2018).

Unlike for honey bees (*Apis mellifera*), bumble bees express size polymorphism that must be accounted for when comparing individuals. Head capsule width, intertegular span, and marginal cell length have all been implemented as proxies for body size (Shpigler et al., 2014; Hagen-Kissling and Dupont, 2013). We measured these structures from $\sim$50 workers from four colonies not included in the tracking experiments to show that all three measurements are strongly correlated with body size (Figure S14, Figure S15). While each structure is therefore similarly informative in estimating size variation, marginal cell length offers three major advantages: 1) it is the most easily measured due to lack of curvature or hair present in the head and thorax, respectively, 2) handling the wings does not risk degradation of the body, and 3) mounting wings under clear tape for imaging generates a permanent tissue archive that is easily stored. Finally, dividing average oocyte width by marginal cell size provided an ovary index, a unitless, normalized measure of ovary activation that can be compared across individual bumble (Shpigler et al., 2014; Cini, Meconcelli, and Cervo, 2013). Because measurements could not be easily taken before or during the experiment, we measured marginal cell size for each queenright and queenless worker for a single colony and found there to be no significant difference in size distribution across partitions (Welch's t-test, t = 1.24, p = 0.219), suggesting our visual size-matching was accurately performed.

Code and Documentation

The analysis code utilized in this study, including scripts for data analysis, visualization, and the generation of network metrics, is publicly available for review and replication purposes. Detailed documentation and source code can be accessed through the following GitHub repositories:

- For the primary analysis code related to this study, please visit: https://github.com/kocherlab/queenright-queenless-analysis.
- For code pertaining to tracking using SLEAP and NAPS, post-processing of tracks, and the computation of network metrics, please refer to: github.com/itraniello/socioQC.

These repositories contain all necessary information for reproducing the tracking, analysis, and network metrics calculations detailed in our study.

Data Availability

The data utilized in this manuscript will all be available through Princeton's DataSpace upon acceptance for publication. Paired with code provided in Code and Documentation, this will provide the code and data required to reproduce this work.

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contributions

DMR, SWW, IMT and SDK conceptualized the project; DMR, SWW, AEW, ESW, MLW, DM, and IMT performed formal analyses; DMR and SWW drafted the initial manuscript. IMT and SDK supervised the project. All authors edited the manuscript and provided critical feedback. All authors approved the publication.

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Supplementary Information

Measure	**Meaning**	**Modality**
Mean Degree	Average number of interactions per node per hour	Network-Level
Efficiency	Mean distance between any two random nodes, using the inverse of weight as our "barrier" to communication	Network-Level
Degree Assortativity	Enrichment (if positive) or depletion (if negative) of interactions between bees with high degrees or low degrees, relative to mean degree	Network-Level
Keystone Assortativity	Enrichment (if positive) or depletion (if negative) of interactions between keystone bees or non-keystone bees, relative to mean degree	Network-Level
Modularity	Proportion of interactions between bees within a module (identified using networkx's greedy modularity community detector) relative to all interactions	Network-Level
Transitivity	Density of complete triads relative to possible triads within a network	Network-Level
Degree Centrality	Sum of all weighted interactions	Individual-Level
Betweenness Centrality	Probability a given bee is on the shortest path between two other individuals, using the inverse of weight as a "barrier" to communication	Individual-Level
Closeness Centrality	Average distance to any other bee, using the inverse of weight as a "barrier" to communication	Individual-Level
Eigenvector Centrality	Measure of a bee's influence in the network, based on both the number of direct connections it has and the importance of the bees it is connected to	Individual-Level
Movement Percentage	Percentage of the hour a bee is moving above the threshold for movement	Individual-Level
Average Velocity	Average pixels per frame a bee is moving when above the threshold for movement	Individual-Level
Home Range (N90)	Smallest number of 200 pixel by 200 pixel frames which represent 90 percent of a bee's time in a given hour	Individual-Level
Presence	Percent of frames a given node or set of nodes is present in	Individual-Level
Clustering Coefficient	Measure of the extent to which a bee's direct connections are also connected to each other, weighted by strength of the connection	Individual-Level

Table S1. Individual and network-level parameters measured in this experiment.

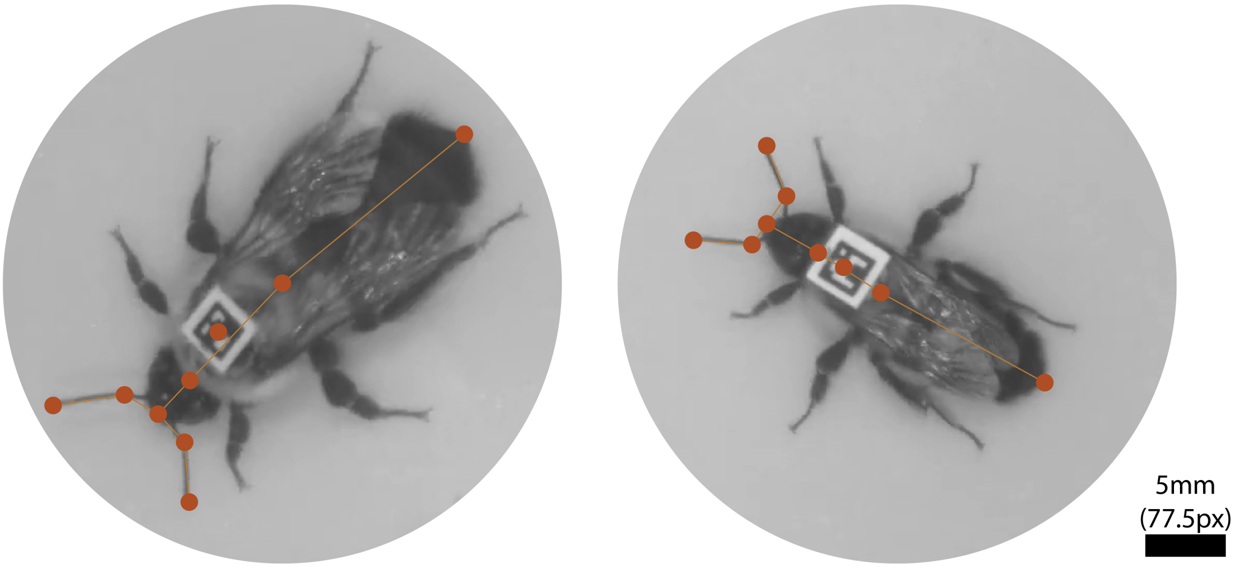


Figure S1. Example SLEAP skeletons on queen and worker showing the nine nodes used for analysis.

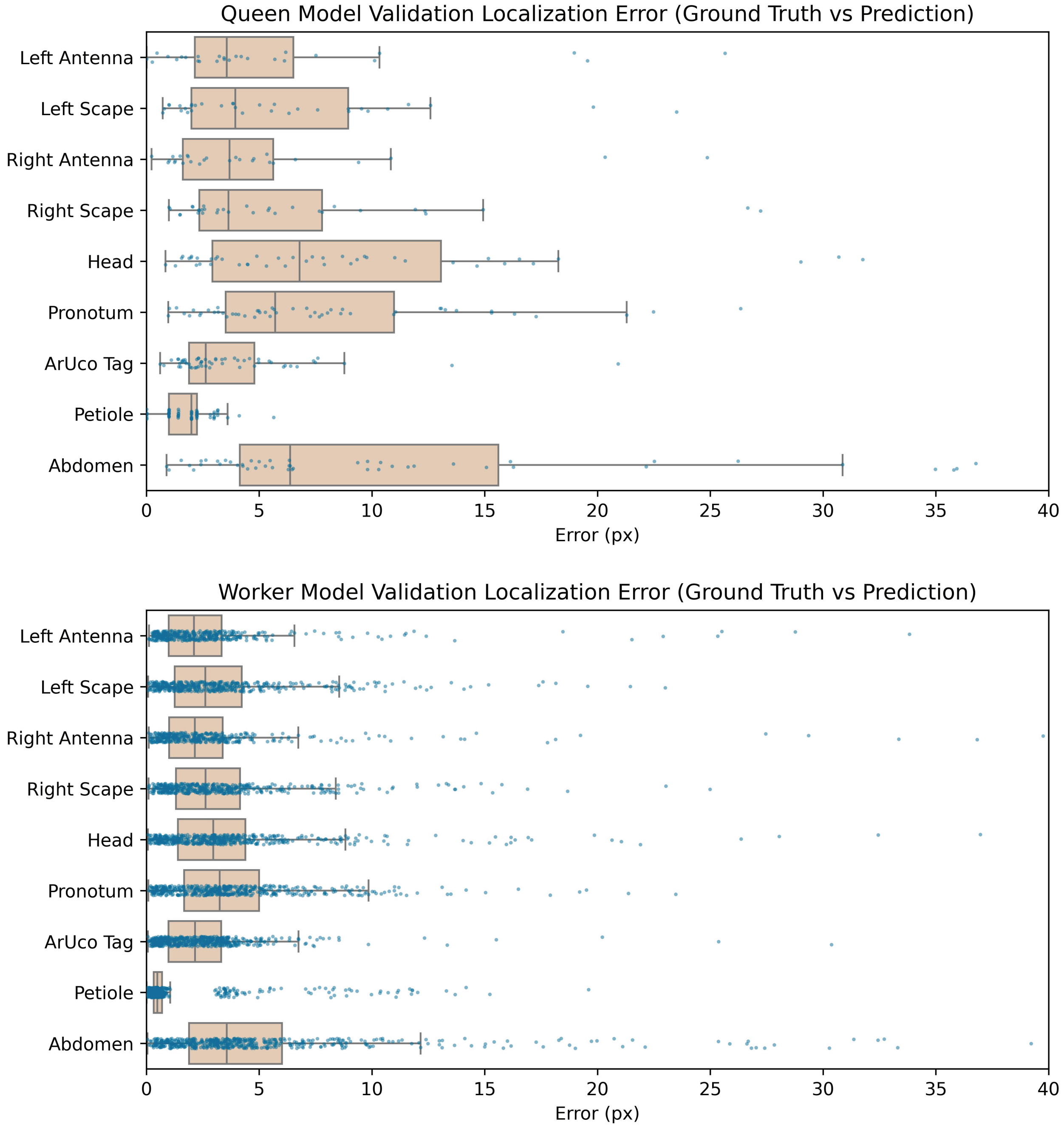
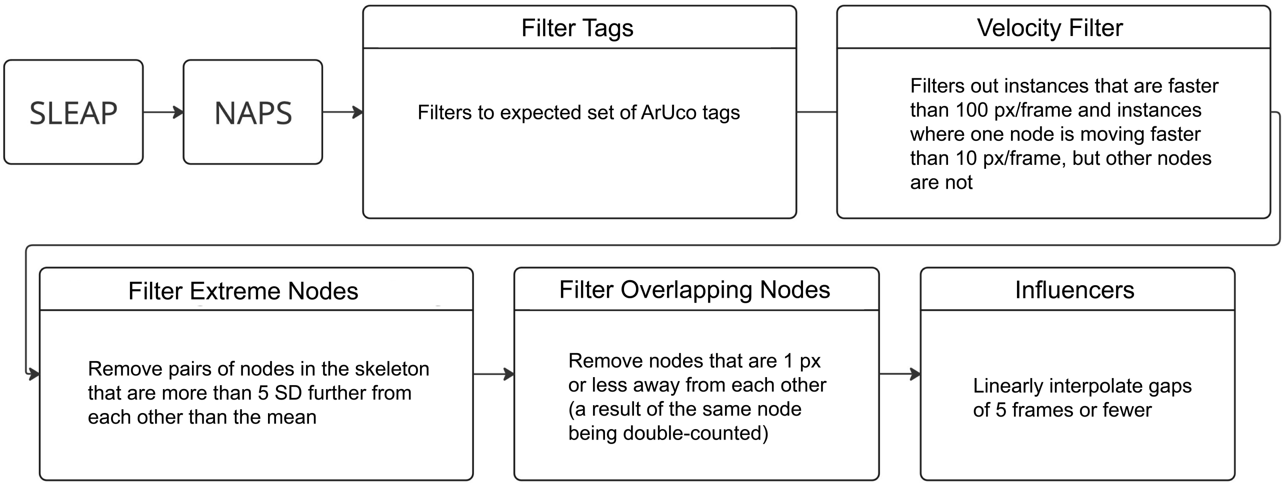


Figure S2. SLEAP model metrics for queen (top) and worker (bottom) models. For this study, px/mm ratio = 15.5, meaning that nearly all localization errors are at the sub-millimeter level. Box and whiskers plots show median, interquartile range (IQR), and upper and lower limits representing 1.5 * IQR above or below the upper and lower quartile, respectively. Each dot represents an instance of detection error in a validation frame.



Filters to expected set of ArUco tags

Filters out instances that are faster than 100 px/frame and instances where one node is moving faster than 10 px/frame, but other nodes are not

Remove pairs of nodes in the skeleton that are more than 5 SD further from each other than the mean

Remove nodes that are 1 px or less away from each other (a result of the same node being double-counted)

Linearly interpolate gaps of 5 frames or fewer

Figure S3. Diagram of filtering steps applied after generating NAPS tracks.

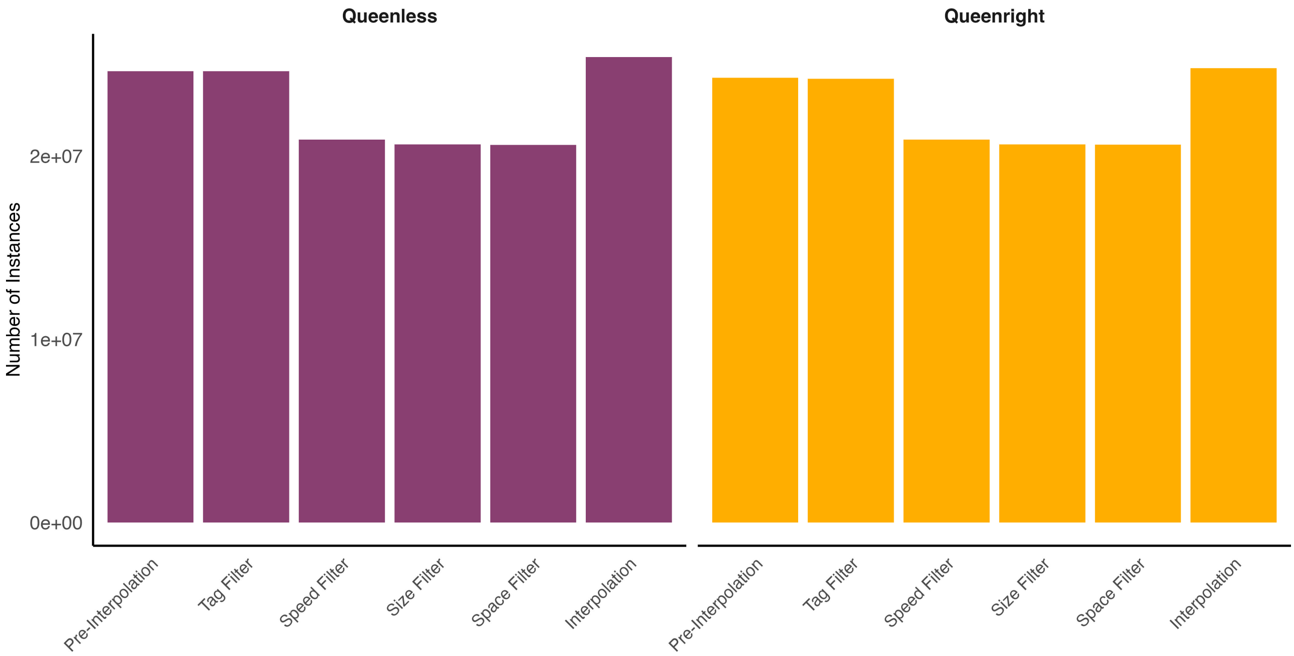


Figure S4. Post-filtering bar plot show total number of instances across all bees across all frames during filtering and interpolation steps.

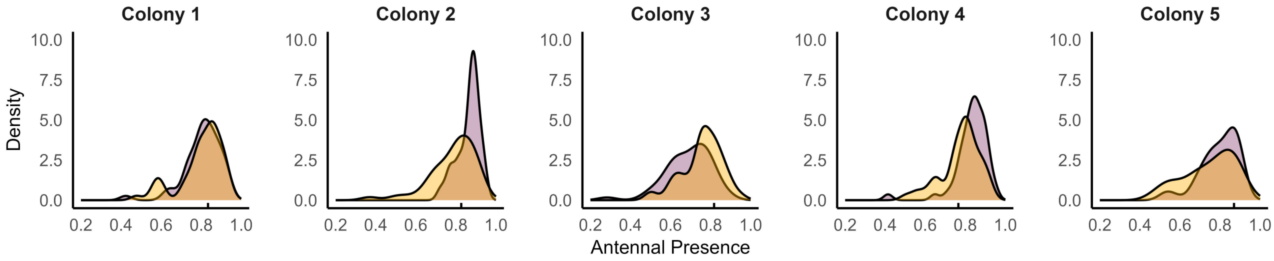


Figure S5. Density plots showing antennal presence per colony.

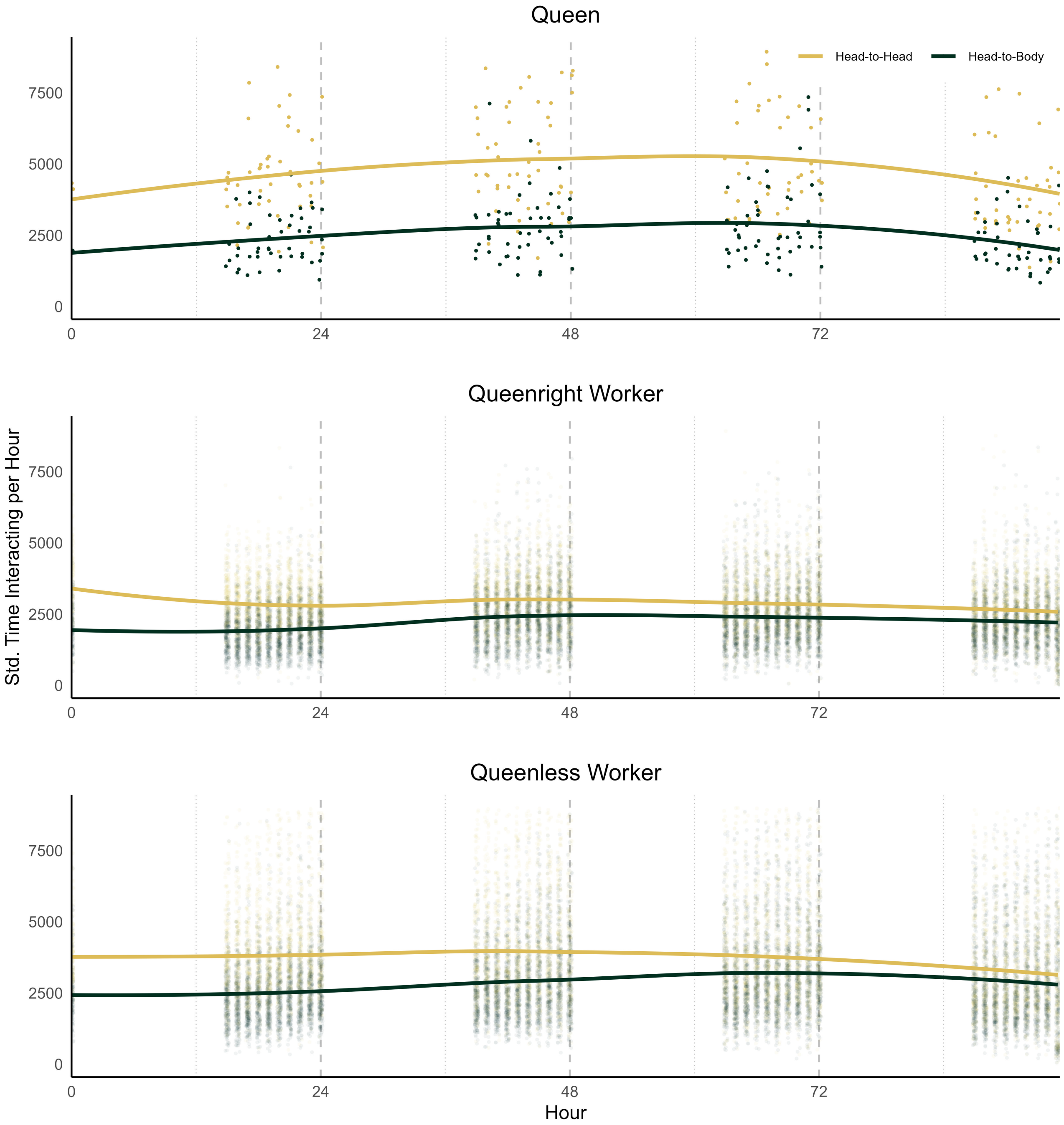


Figure S6. Plots showing standardized interacting time per hour for head-to-head and head-to-body across queens, queenright workers, and queenless workers from 9:00-17:00 each day.

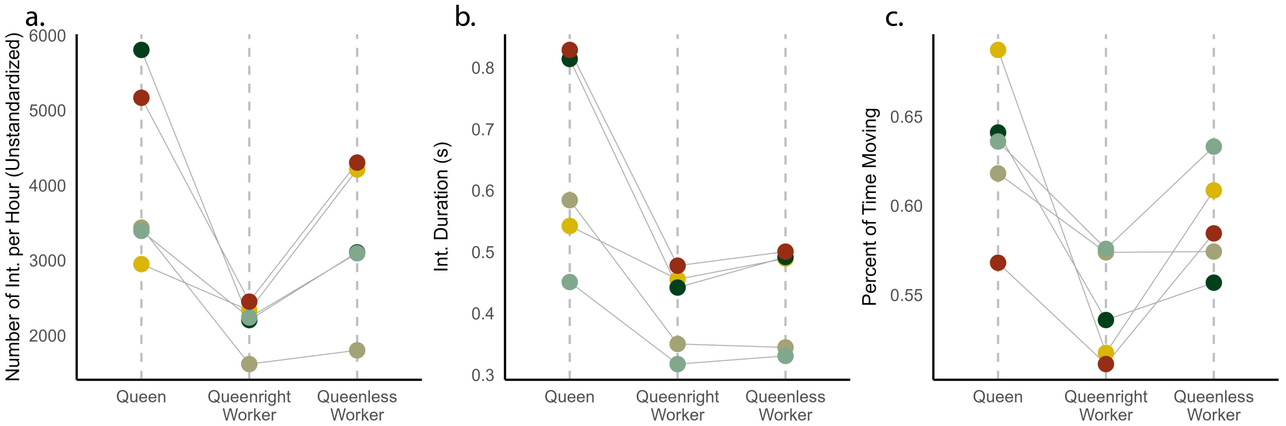


Figure S7. Plots showing status (queen, queenright worker, queenless worker) by individual features: interactions per hour (not standardized to antennal presence), interaction duration, and percent of time moving.

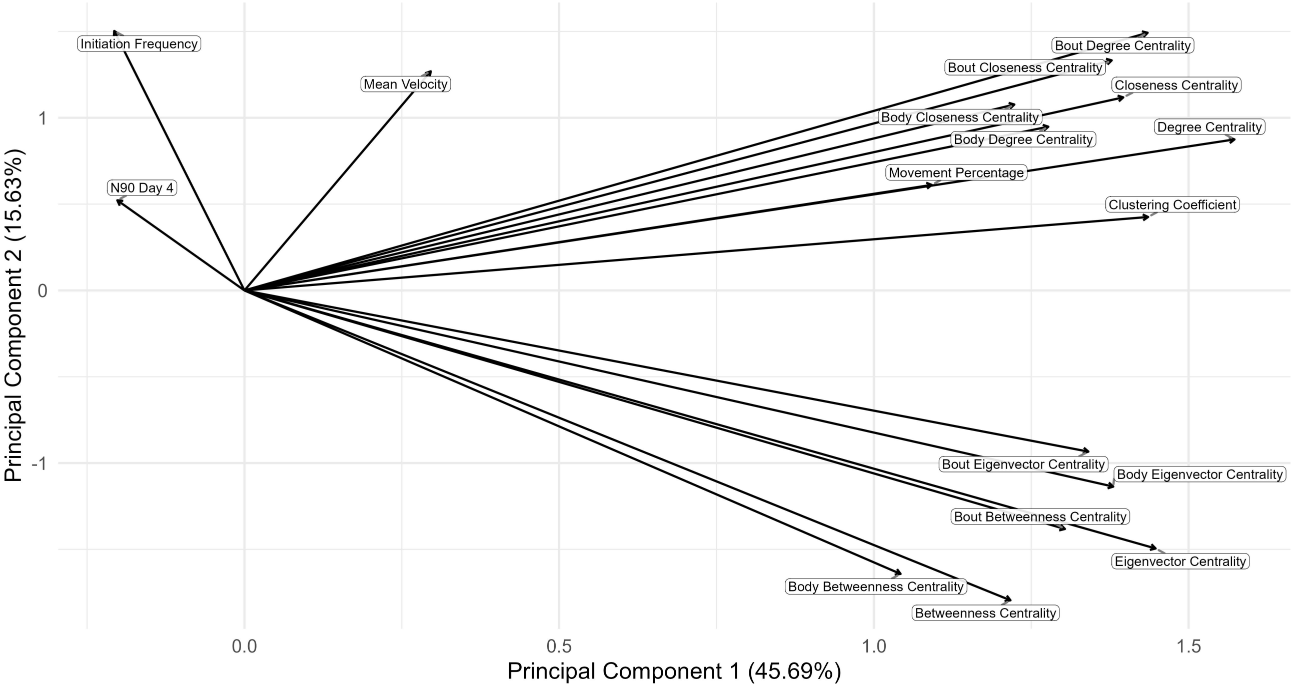


Figure S8. Loading plot showing features used to classify influencer workers.

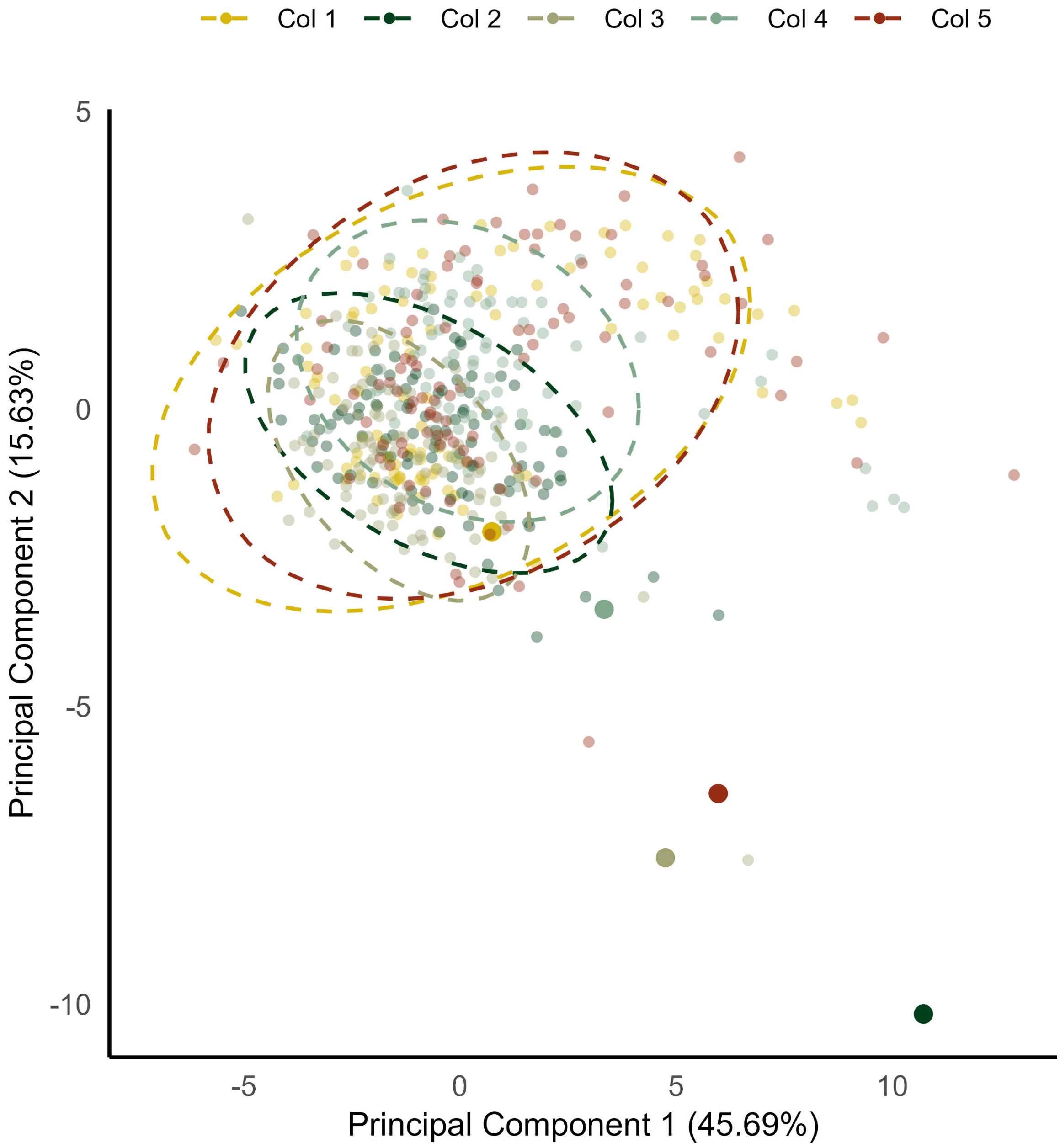


Figure S9. PCA plot from Figure 3a colored by colony with queens enlarged.

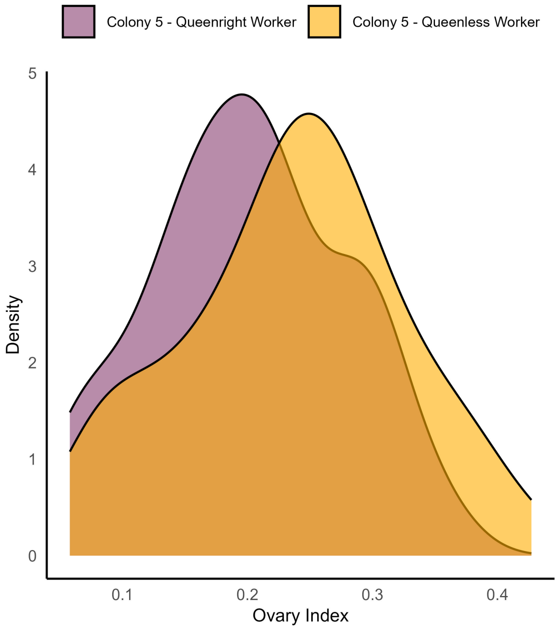


Figure S10. Density plot showing ovary index distributions for Colony 5 grouped by queenright, queenless status.

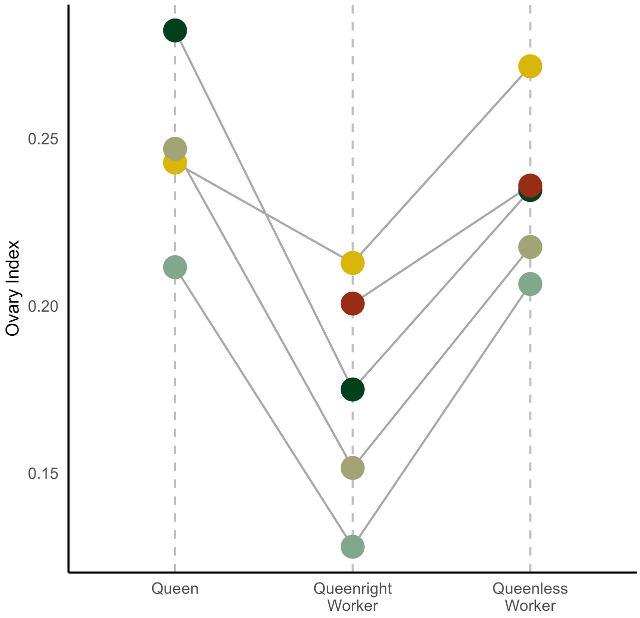


Figure S11. Mean ovary index (width of longest ovariole divided by marginal cell length) by queen, queenright worker, queenless worker status colored by colony.

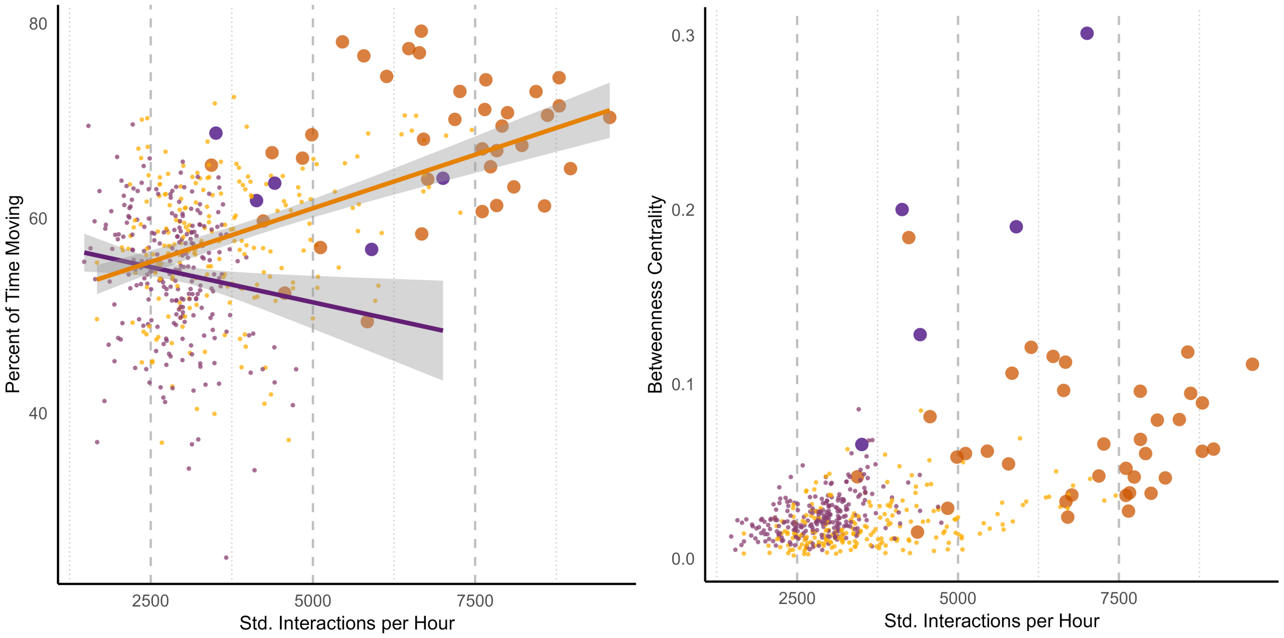


Figure S12. (left) Percent of time moving by standard interactions per hour and (right) betweenness centrality by standard interactions per hour. Purple corresponds to queenright individuals and orange to queenless, enlarged purple dots are queens and enlarged orange dots represent queenless influencers.

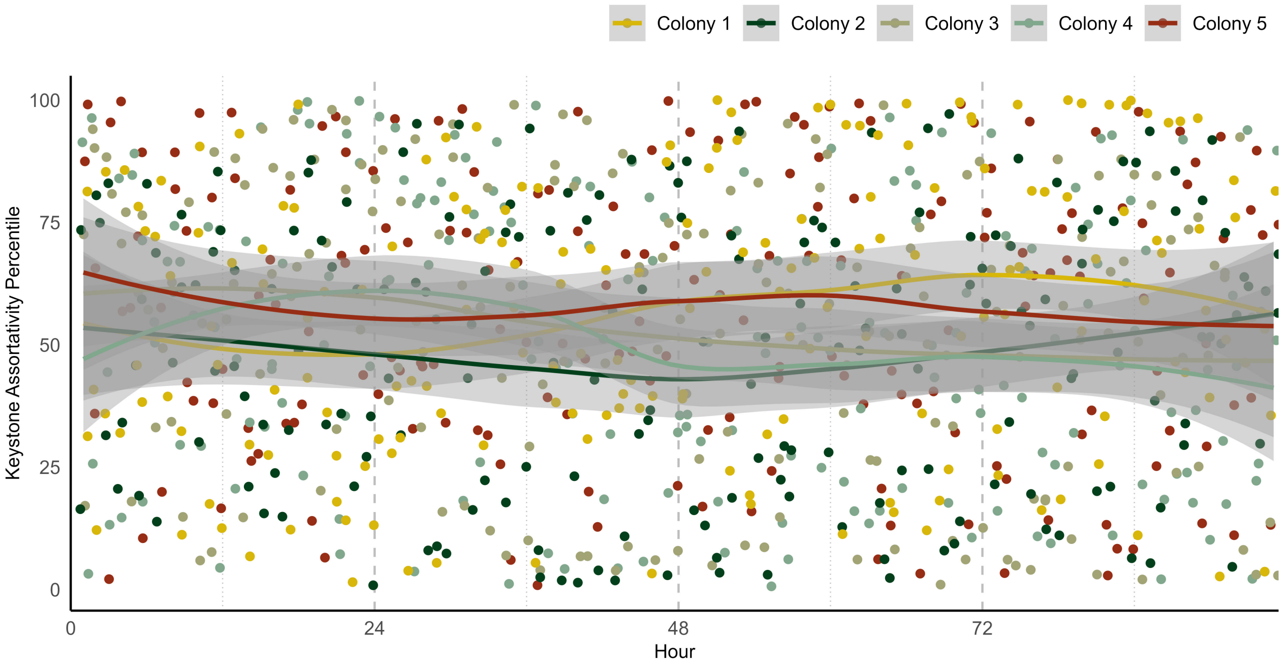


Figure S13. Assortativity between influencers in queenless colonies over time. Colonies are split by color and LOESS fit for each colony is shown.

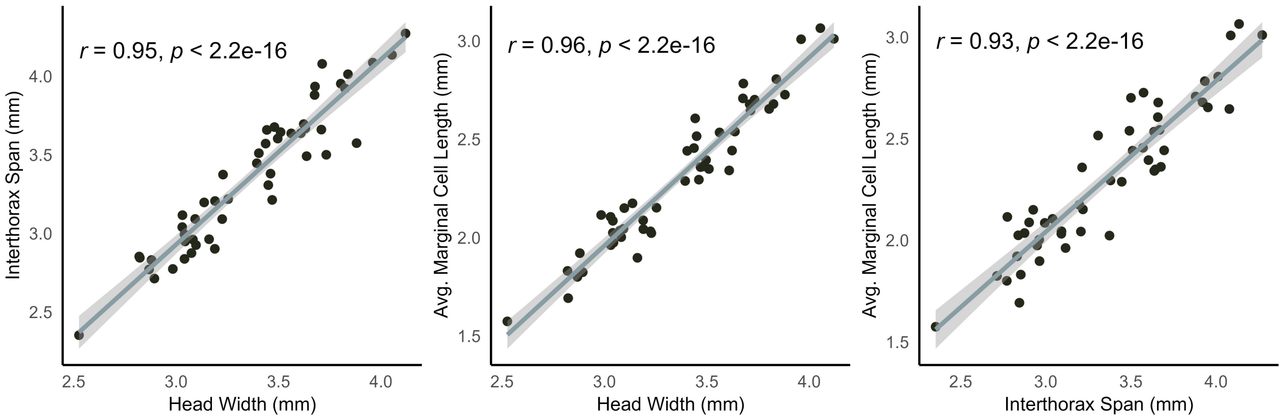


Figure S14. Scatter plots showing relationships between size measurements. (left) Scatter plot showing relationship between interthorax span (mm) and head width (mm). (center) Scatter plot showing relationship between average marginal cell length (mm) and head width (mm). (right) Scatter plot showing relationship between average marginal cell length (mm) and interthorax span (mm).

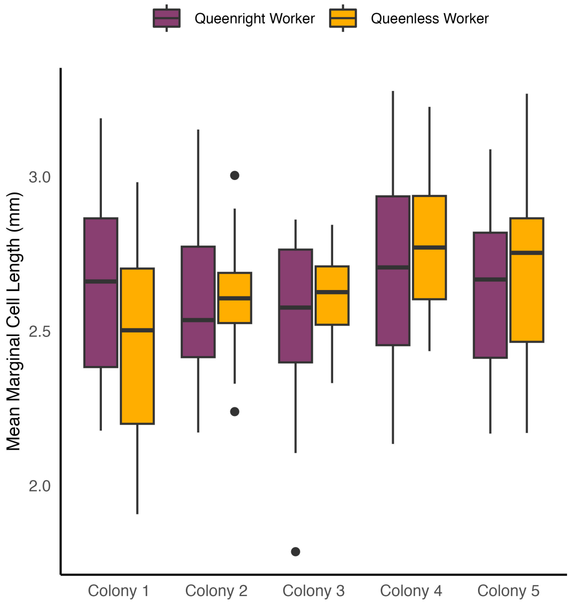


Figure S15. Box plots showing mean marginal cell lengths by queenright (purple)/ queenless (orange) status and source colony.