Physical Interactions Shape Collective Thermoregulatory Behavior in Honey Bees

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

C.E.L: analysis and interpretation of data, revisions of manuscripts of drafts.
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Our study includes researchers across early career stages, including two undergraduate researchers and a graduate researcher. Whenever possible, our research was discussed with local stakeholders to seek feedback on the questions to be tackled and the approach to be considered.

Data Accessibility

Analyses reported in this article can be reproduced using the data provided by Cook and Lambert (2025).

ABSTRACT

Social animals exhibit complex, coordinated behaviors that can drive significant environmental change. Within groups, individuals sense their environment and share information to facilitate collective action. Direct physical contact is a key mechanism of communication in many social species, yet its role in organizing collective behaviors remains underexplored. Here, we investigate how tactile interactions influence thermoregulatory fanning behavior in honey bees (Apis mellifera), a critical collective response to environmental stress. By reducing the ability of bees to engage in physical contact, we demonstrated that direct tactile interactions are necessary to initiate and coordinate fanning. Increasing social density by spatially constraining movement enhanced the likelihood of fanning. Using video tracking, we confirmed that elevated social densities resulted in more frequent direct contacts, identifying tactile interactions - specifically head-to-head contacts - as a driver of fanning behavior. Our findings highlight tactile communication as mechanism for information transfer in an ecologically relevant collective behavior. This work shows how social interactions mediate environmental responses, advancing understanding of the resilience of social systems in a rapidly changing world.

Key Words: Density, Environmental Response, Information Transfer, Physical Interactions, Pollinators, Social Coordination

INTRODUCTION

Social organisms can affect massive change on their environment through coordinated collective behaviors. Locust marches clear croplands as they migrate (Bazazi et al., 2008), termite mounds manage their own internal environment as well as shape savannah ecosystems (Charles et al., 2021; Moe et al., 2017), and mass animal migrations cycle nutrients across the globe (Bauer and Hoye, 2014). Collective behavior emerges from coordination of many individuals, potentially providing resiliency to a changing environment (Gil et al., 2018; Sumpter, 2006). Coordination can occur when individuals sense shifts in local information from others and the environment, and adjust their own behavior accordingly (Couzin, 2009). Information from others can be indirectly perceived, such as when schooling fish simply watching others move in a particular direction and move with them (Sumpter et al., 2008), or it can be direct, such as a honey bee performing a waggle dance to share the location of a food source (Couzin, 2009; Guttal and Couzin, 2010; Von Frisch and Chadwick, 1967). Although there are many opportunities for acquiring information, less is known about how individuals may evaluate then share information to actively coordinate, especially in a changing environment. Understanding how information is shared among individuals provides key insights into how groups and systems adapt and remain resilient when environmental conditions shift.

Social insect societies engage in many mechanisms of communication, enabling them to manage rapidly shifting environments. One major mechanism of communication is direct physical contact. Direct physical contact requires physical touch, often occurring with sensory appendages like antennae, mouthparts, or legs, and can establish and maintain social structures that are the hallmarks of insect societies. Direct physical contact can include aggressive interactions that establish dominance hierarchies and reproductive division of labor, as in many species of *Polistes* wasps (Jandt et al., 2014; Strassmann, 1981; West-Eberhard, 1986). In honey bees, direct physical contact may promote reproductive drone comb building when the colony reaches a critical mass of 4000 workers (Smith et al., 2014). When recruiting nestmates to a new nest site, ants engage in tandem running, which occurs when a recruited ant follows a leader ant by touching the leader's hind legs with its antennae (Franks and Richardson, 2006). Direct physical contact can also coordinate worker division of labor by initiating tasks, such as when inactive foragers begin to forage after an increase in interactions with successful foragers, which also occurs if contact occurs with glass beads that smell like foragers (Greene and Gordon, 2007) and when paper wasps (*Polybia occidentalis*) bite nestmates to stimulate foraging activity after foragers are removed (O'Donnell, 2006). Direct communication is therefore a likely mechanism mediating the coordination of some collective behaviors in response to changing environmental conditions.

Regulating shifts in temperatures via thermoregulation is among the most critical tasks for social insect societies (Jones and Oldroyd, 2006). Honey bees are cavity nesters that rear sedentary offspring (Winston, 1991). As such, adults must work together to manage the temperature inside the colony. Brood must be kept at 35°C; development at higher temperatures may result in impaired learning as adults, physical malformations, or death (Groh et al., 2004; Medina et al., 2020; Tautz et al., 2003). To manage colony temperatures, honey bees perform several thermoregulatory behaviors, such as evaporative cooling via spreading water (Lindauer, 1955), bearding (Hamdan, 2010), heat shielding (Starks and Gilley, 1999) and fanning (Egley and Breed, 2013; Southwick and Moritz, 1987). Fanning occurs throughout the colony as well as at the entrance to circulate air, bringing hot air out and allowing cool air to infiltrate. Fanning is modulated by both thermal and social environment: honey bees are more likely to fan when in groups compared to when as a single individual (Cook and Breed, 2013), and larger groups of adult bees fan earlier to combat rapid rates of temperature change (Cook et al., 2016a; Peters et al., 2019). Individual adult bees are more likely to fan when able to physically touch a larva, but fanning is reduced when separated from it (Cook et al., 2016b). Although the social environment modulates the fanning response, the role that social interactions and information use plays in organizing this important thermoregulatory response is unknown.

Here we create specific social environments to test the hypothesis that tactile cues drive the thermoregulatory fanning response in honey bees. First, we identify whether preventing interactions between individuals reduces the fanning response by physically separating individuals. We then examine whether social density modulates the fanning response by changing the size of the physical space they occupy. To understand the social mechanism driving changes in the fanning response, we tracked individuals in high-and low-density groups. From this analysis, we determine that tactile cues, specifically head-to-head interactions drive the fanning response. Our work highlights the importance of information transfer in collective behavioral responses, which are especially important when organizing to manage a changing environment.

METHODS

Cage Separation Experiment

Husbandry

Ten *Apis mellifera l*. colonies were managed at a bee yard in Boulder, Colorado USA on the University of Colorado's East campus. Colonies were maintained in 10-frame wooden hive bodies with plastic frames. Supplemental feeding of 2:1 sucrose:water solution occurred as needed. Experiments were conducted 1 May 2014 to 1 September 2014.

Identification of Fanners

To identify thermoregulatory fanners, we observed the entrance for bees that were standing still and fanning their wings in place for 10 seconds, with a curved abdomen. Fanners often faced into the colony. The thermoregulatory fanning stance is different from Nasanov fanning (or scenting), which also occurs at the entrance of the colony, but can be distinguished from thermoregulatory fanning as their abdomens are straight, angled upward, and a gland at the tip of the abdomen is often exposed (Free, 1968). Although fanning occurs throughout the colony, we collected fanning from the entrance of the colony due to their accessibility.

To collect bees, we used forceps to grab the legs of the fanners, then placed them into cages. Cages were cylindrical (r=4.25cm, h= 4cm, total area = 226.98cm^3) constructed of 0.25in x 0.25in galvanized steel hardware cloth (McGucken Hardware). Five fanning honey bees were collected into 1 of 3 different cage types: open, divided with 1 layer of hardware cloth, divided with 2 layers of hardware cloth. When the bees were placed into the divided cages, an individual could interact with 2 others, functionally creating groups of 3, so we also tested groups of 3 bees in open cages as a control (Figure 1). The treatment group of 3 bees in open cages was a similar experiment being performed in the same timeframe and has a lower sample size (n=13) compared to our groups of 5 open cages (n=61), single mesh divided cages (n=57), and double mesh divided (n=44). Once bees were collected, they were placed into the heating chamber, described below, for a 25-minute room temperature (average = 25.2°C) acclimation period.

Fanning Assay

To evaluate the fanning response, we heated cages of bees in 1-gallon jars (Uline) on hot plates (Ohaus Guardian 5000). The cages did not touch the glass but were suspended on wooden stands inside the jar. We monitored air temperature with Cole Parmer high accuracy (±0.3°C) digital thermometer. We heated the air temperature inside the jars at 1° C per minute, starting at room temperature. We monitored the cages for fanning behavior, characterized similarly to fanning identification at the hive: flapping wings, curved abdomen, stationary for at least 10 seconds. We recorded the first instance number of fanners and the temperature that they fanned at. Often, several bees would begin fanning with or soon after the first bee; or stop temporarily and resume fanning later in the trial. To account for this variation, we recorded instances of fanning in “bouts." A bout is initiated by an individual fanning for at least 10 seconds (s). To be included in that bout, another individual needed to (1) begin fanning within one minute of initiation, while (2) the first bee was still fanning. If both conditions were not met, a new bout would be logged. Typically, multiple bouts were recorded over the entire trial period. Here we report for each trial the "Initial Bout," which is the first instance of fanning recorded, as well as the "Maximum Number Bout," which was the instance when the greatest number of individuals simultaneously fanned in the trial. Fanning temperature is defined as temperature at the start of the bout. Assays concluded when all activity ceased.

Statistical Analysis

To analyze fanning behavior in the group size and cage separation experiments, we created a four-factor predictor variable which combined the number of bees and the separation of them (i.e. no separation, 5 or 3 bees; 1 layer, 5 bees; 2 layers, 5 bees). To analyze the probability of fanning, we performed a logistic regression with logit transformation using a generalized linear mixed-effect model on probability of fanning. The response variable for this model was a two-column probability of number of fanners and number that did not fan. The temperature of fanning was analyzed as a generalized linear mixed-effect model with a log transformation (with gaussian family and log link), as the temperature response variable was not normally distributed. To analyze the magnitude of effects of the predictor variable, we performed a type II ANOVA Wald Chi square test. Both included the random effect to account for hive effects. For post-hoc pairwise comparisons we performed Tukey tests. All analyses were performed in R ((R Core Team, 2021)version 4.2.2) using RStudio (RStudio Team, 2020)version 2023.06.1+524) packages lme4, car, and emmeans. Graphs were created using ggplot2.

Density Experiment

Colony Husbandry

Ten *Apis mellifera l*. colonies were managed on the roof of Wehr Life Sciences on Marquette University's campus in Milwaukee, Wisconsin, USA. They were maintained in 10-frame Langstroth boxes, and boxes were added as colonies grew through the summer. Frames were plastic (Acorn). Supplemental feeding of 2:1 sucrose:water solution and pollen substitute occurred as needed. Colonies were also treated for mites in August using Apiguard (Mann Lake). All experiments were conducted May 2021-September 2021.

To collect fanners, we identified them using the criteria discussed previously, then used forceps to grab the legs of the bee and placed them into cylindrical cages made of a plywood base, hardware cloth sides, and a plexiglass opening which was a spinning lid to quickly close the cage to prevent escapes. As bees were collected, they were placed directly into two treatment groups: large (r=4.25cm, h= 11.5cm, total area = 652.56cm^3) or small (r=4.25cm, h= 4cm, total area = 226.98cm^3) cages. For each trial, groups of ten bees were collected. Cage collection order was alternated each collection bout. 3-4 collection bouts were typical per day. Collections typically lasted less than 5 minutes, however if they took longer, cages were placed in the shade. Cages were returned to the lab and placed directly into the heating chambers to acclimate for 25 minutes.

Fanning Assay

To evaluate the fanning response, we heated cages of 10 bees placed inside of 1-gallon jars (Uline) on hot plates (Ohaus Guardian 5000). The cages did not touch the glass as they were suspended on wooden stands inside the jar. We monitored air temperature with probes (109, Campbell Scientific) and analog input module (Granite Series Volt 116, Campbell Scientific) using their software Surveyor. We heated the air temperature inside the jars at 1° C per minute, starting at room temperature (average=23.67° C). We monitored the cages for fanning behavior, characterized similarly to fanning identification at the hive: flapping wings, curved abdomen, stationary for 10 seconds. We recorded the number of fanners in the initial and max fanner bout, and the temperature that they fanned at in both bouts.

Statistical Analysis

To analyze the probability of fanning in the two social densities, we performed a logistic regression with logit transformation using a generalized linear mixed-effect model on probability of fanning. The response variable for this model was a two-column probability of number of fanners and number that did not fan. The temperature of fanning was analyzed as a generalized linear mixed-effect model with a log transformation (with a gaussian distribution and log link), as the temperature response variable was not normally distributed. The predictor variable was social density (low or high). To analyze the magnitude of effects of the predictor variables, we performed a type II ANOVA Wald Chi square test. Both included the random effect to account for the different collection hives. For post-hoc pairwise comparisons we performed Tukey tests. All analyses were performed in R ((R Core Team, 2021) version 4.2.2) using Rstudio (RStudio Team, 2020), packages lme4, car, and emmeans. Graphs were created using ggplot2.

Video Assays and Tracking

Recording Conditions

To evaluate whether the behavioral mechanism that drives the fanning response were physical interactions, we quantified number of interactions and interaction duration. To do this, we video recorded fanning assays in high density and low-density social environments. We created a cage as 2-dimentional as possible to enhance video recording and individual tracking quality. These cages were rectangular, as opposed to cylindrical as compared to our previous behavioral assays. Trial cages were constructed with wooden sides, a glass removable top panel, and an aluminum hardware mesh base which was covered with a silicone mat. Trial containers measured 13.5 cm x 14.5 cm x 2.5 cm. Hardware mesh was used to wall off sections of the cage to alter the housing area and encase the temperature probe. The high-density (smaller) housing was 13.5 cm x 7 cm x 2.5 cm; with a floor space of 94.5 cm^2 and total volume of 236.25 cm^3. The low-density (larger) housing was 13.5 cm x 14.5 cm x 2.5 cm but a 3.5 cm x 6.5 cm x 2.5 cm section of was blocked off by the temperature probe for a final floor space of 173 cm^2 and total volume of 432.5 cm^3.

Animal Collection and Preperation

To collect bees, we followed the same protocols as stated above by identifying fanners at the entrance of the colony and using forceps to grab them by their legs. To keep track of individuals during video tracking, we individually marked the bees using different color water-based acrylic paint markers (Montana Brand) as we collected them, before placing them into the two different cage size treatments.

Video Recording

To ensure high quality videos, we created a filming box that was well-lit but reduced reflections. To do this, we created a recording (39cm x 39cm x 57cm dimension) box, painted the inside flat white, and added two circular (14cm diameter) holes for lights on opposite sides. We used two 8.5 watt (60-watt equivalent) daylight bright LED (GE) to light the box. We created a hole at the top of the box for the video camera to cradle in for recording. We used a video camera (Panasonic HC-V800) with a SD card (SanDisk 128mb microSD) to record. Temperature conditions were monitored using a Campbell

Scientific *109 Temperature Probe* wired to a Granite Series *Volt 116* and read and recorded through SURVEYOR version 1.01. Once placed in trial housing, the cages were placed in video recording boxes and allowed to acclimate for 25 minutes. Temperature conditions increased using a hot plate at an average rate of 1°C/min, and all trials were conducted under standardized light conditions. We stored videos locally and backed up on cloud services provided by Marquette University IT through Microsoft Sharepoint.

Animal Tracking

We used a software program called ABCTracker (Rice et al., 2020) to track individual location and interactions. ABCTracker identifies and tracks the body and head of individuals as well as planar coordinates associated with time, which allows for overlap to be identified. A .mp4 video trim was uploaded into ABCTracker and was tracked at a resolution of thirty frames per second. After running the initial process tracking process, tracks were manually corrected. The body of each bee is a rectangle defined to individual dimensions. The head is a square (with sides equal to body width) centered at the “top” of the body; rotated 45 degrees forming a diamond shape. Head is positioned with ½ of the diamond shape extending beyond the body length to incorporate individuals’ antenna. Planar coordinates for the body and head position (x,y) and velocity (pixels/second) per frame and per bee, was extracted and saved as a .csv file.

Tracking Analysis

We compared the interaction dynamics under increasing temperature conditions for groups of ten bees across two cage sizes; high density and low density. Three trials were conducted for each cage size, totaling 6 trials and 60 individual bees.

To ensure comparability across trials, we analyzed the four minutes of activity prior to the start of temperature fanning (disregarding Nasanov fanning). This allows us to account for normal variation in acclimation and initial fanning temperature across groups. An interaction was defined as a period of at least 1 second where the center of the individuals' body or head was within one median bee length (per trail median bee length) of another individual (Guo et al., 2022). Interactions between unique pairs of individuals happening less than 1s apart were combined into a single instance. Interactions were capped at 60s to reduce the effect of individual bees resting next to one another (Wild et al., 2021). Interactions were categorized as being head-to-head (HH), head-to-body (HB), or body-body (BB). As it was possible for multiple interaction types to happen simultaneously, we set an interaction hierarchy which ranked HH first, then HB, then BB. For example, if both a HH and an HB interaction were registered within the frame, it was classified as HH.

We aggregated individual level data, and analyzed the type, number and duration of interactions across the high- and low-density cages. A series of Shapiro-Wilk tests concluded that the data was not normally distributed (Table S1A and S1B), so we used a Wilcoxon Rank-Sum Test to compare results across the two cage sizes. All analyses for this experiment were performed in R version 4.3.1 (R Core Team, 2021) and R studio version (RStudio Team, 2020) 2023.06.0+421.

RESULTS

Physical separation reduces the fanning response

Separating bees significantly reduced the probability of fanning (Figure 2a: Analysis of Deviance: $\chi^2 = 85.38$, $p<0.001$). We found that honey bees were significantly more likely to fan when in open cages; not divided by single ($p<0.001$) or double mesh ($p<0.001$). There was no significant difference in the probability of fanning between single and double mesh cages ($p>0.05$). There was also no significant difference in probability of fanning between groups of 5 bees and groups of 3 freely moving bees in open cages ($p>0.05$).

The temperature at which bees began to fan was predicted by both group size and type of separation (Figure 2b: Analysis of Deviance: $\chi^2 = 15.02$, $p=0.002$). Bees that were freely moving in groups of 5 fanned at an average of 32.7± 1.2°C, which was significantly lower than groups of 3 freely moving bees (mean = 40.5± 2.33°C; $p=0.011$) and single mesh divided groups of 5 (mean = 38.5± 1.5°C, $p=0.014$). Bees in double mesh fanned at an average of 34.1± 1.86°C, which was not significantly different than any of the other groups ($p>0.05$).

Higher Social Density Increases the Fanning Response

When bees are in the high-density social environment, they are significantly more likely to fan, compared to the lower density environment (Figure 3). In the initial bout of fanning, there were significantly more bees fanning in the high social density (average number of fanners ± SD: 3.3 ± 2.95) compared to the low social density (2.1 ± 2.36 fanners on average; Figure 3a; Analysis of Deviance: $\chi^2 = 11.49$, $p>0.001$). Similarly in the maximum fanner bout, on average there were significantly more bees fanning when in high social density (4.1 ± 3.1 fanners in high density on average) compared to low social density (, 3.3 ± 2.63 fanners in low density on average; Figure 3b; Analysis of Deviance: $\chi^2 =4.81$, $p=0.028$). There was no significant difference in the temperature that the fanning bees initiated the fanning response (High density average fanning temperature ± SD: 28.2 ± 5.8, Low density: 29.6 ± 5.36; Figure 4a; Analysis of Deviance: $\chi^2 =0.87$, $p=0.34$) or the temperature that bees were fanning when the highest number of fanners were fanning (High density average fanning temperature ± SD: 30.7 ± 6.3, Low density: 32.8 ± 6.0; Figure 4b; Analysis of Deviance: $\chi^2 =1.58$, $p=0.2$).

Interactions as a social mechanism driving the fanning response

To understand how bees interact leading up to the fanning response, we characterized the types of interactions they were having. Overwhelmingly, bees had more head-to-head (HH) interactions (Figure 5), accounting for 71.7% of interactions in the low-density cages, and 83.0 % of interactions in the high-density cages. Bees in low-density cages engaged in 26.6 % of head-to-body (HB) interactions and 1.6% body-to-body (BB) interactions, compared to 15.3% head-to-body and 1.6% BB interactions in high density cages. Low-density cages engaged in 1712 interactions total and high-density cages engaged in 2808 interactions total (Table S6). The social environment did not significantly impact the type of interactions that the bees were engaging in, as the relative proportions of interaction type remained consistent.

To further explore how the bees interacted with each other, we quantified the number of interactions each individual had. In the high-density environments, individuals were having an average ± SD of 93.6 ± 56.4 interactions in the 4-minute segment before fanning occurred, which was significantly more than the low-density environment where they had an average ± SD of 57.1 ± 20.9 interactions (Table S5; Figure 6a; Wilcoxon test: $p = 0.02$). Honey bees in high-density social environments had significantly more HH interactions than bees in low-density social environments (Figure 6b; Wilcox test: $p < 0.001$). Bees in high density environments also had significantly fewer HB interactions compared to bees interacting in the low-density environments (Figure 6c; Wilcox test: $p = 0.02$). There was no difference in BB interactions in the different social densities (Figure 6d; Wilcox test: $p > 0.05$). Full statistical results in Table S3.

Individuals in low social density had on average longer interactions across all interaction types (Figure 7). Across all interaction durations, low density bees had a higher average interaction time of 5.30 ± 9.99s, compared to 3.14 ± 3.38s for bees in high density social environments (Figure 7a; Wilcoxon test $p=0.01$; low density $n=1712$, high density $n=2808$). Broken down into specific interaction types, in both environments, HH interactions were longest (Figure 7b; low density average 5.95 ± 11.2s, high density average 3.36 ±4.12). In the low-density environment, HB interactions averaged 3.69 ± 5.48, and BB interactions were on average the shortest at an average 2.76 ± 1.42s (Figure c&d). In the high-density environment, HB interactions were on average slightly longer than BB interactions (Figure 7c&d; high density HB average duration 2.07 ± 2.12s, high density BB average duration 2.15 ± 2.30). Full results reported in table S4; S6.

DISCUSSION

Our results support the hypothesis that direct physical contact was necessary for the thermoregulatory fanning response in honey bees. When bees are prevented from interacting with each other with a barrier, they are significantly less likely to fan (Figure 2). This is further supported by our findings that when the same number of bees are in a smaller space, they are significantly more likely to fan compared to when they occupy a larger space (Figure 3). Finally, we confirm that honey bees in higher social density have more interactions compared to bees in lower social density (Figure 5) which correlates with the observed increase in fanning. Together, this work suggests that direct physical contact enables honey bees to coordinate the fanning response.

Physical contact is critical in honey bee colony organization. For example, interactions between older honey bees inhibited the physiological and behavioral development of younger bees. When younger nurse bees were reared in isolation (Huang and Robinson, 1992) or separated by a double or single layer of mesh, nurses foraged earlier (Huang et al., 1998). Interactions between worker bees also impact ovary development, where workers prevented from interacting had smaller ovaries than workers allowed to freely interact (Dor et al., 2005). Here, we hypothesized that physical contact is also important for fanning behavior. We tested this hypothesis by preventing specific interactions from occurring. The single mesh divisions allowed visual cues, perception of vibration, perception of volatile pheromones, but did reduce tactile cues, such as trophallaxis and antennation. The double mesh divisions allowed visual cues, perception of vibration, and perception of volatile pheromones, but eliminated physical contact. We predicted that when honeybees are prevented from touching, they would fan less. Honey bees utilize a myriad of modes to communicate, including tactile (Cao et al., 2007; Gordon, 1989), vibrational (Donahoe et al., 2003), and pheromonal cues (Pankiw and Page, 2003). Our results show that when bees are prevented from fully physically interacting, they are significantly less likely to fan, which indicates that physical contact is necessary to initiate fanning and illustrates the importance tactile information transfer in collective behavior.

We show that density also plays a role in a collective thermoregulatory behavior in honey bees, further supporting our hypothesis. We placed a group of 10 bees into a high-density or low-density social environment by changing the size of the testing arena. We found that groups of 10 worker honey bees were significantly more likely to fan when in a high-density social environment compared to a low density one (Figure 3). Bees in high density were twice as likely to begin to fan than in low density. There was no significant difference in the temperature at which the group began fanning (Figure 4). These results reinforce density-dependent interactions as a mechanism of behavioral shifts that play a role in the division of labor in social insects. For example, inactive red harvester ants (*Pogonomyrmex barbatus*) begin to forage when interactions with patrollers hit a specific threshold of 1 ant every 10 seconds or less, which was confirmed by changing the rate of patroller return by introducing glass beads covered in patroller cuticular hydrocarbons (Greene and Gordon, 2007). Although our results provide evidence that physical contact mediates the fanning response, we cannot rule out that there is a tactile pheromone or other chemical cue that is also transmitted during touch, similar to the cuticular hydrocarbons of foragers in harvester ants. Physical interactions seem to be providing information about the environment, such as food availability or temperature, and honey bee fanning can inform theories about what and how information is transmitted.

Increased social density leads to increased physical interactions, which likely facilitated the increase in the observed fanning response. In our assays, honey bees interacted extensively, and they prioritized head-to-head interactions, regardless of social density (Figure 5). Our findings show that honey bees have more rapid and frequent interactions overall compared to when they are in less dense social environments (Figure 6; Figure 7). Interestingly, in high density environments the increase in number of interactions is not uniform; instead the increased number of interactions seems to be driven by a subset of hyper social individuals. Comparing total interactions, only a third (n=10) of the individuals had inflated interaction numbers that fell outside the range for the low density environment. During head-to-head interactions, eusocial insects engage in antennation and trophallaxis, which are hypothesized to convey important information about environmental conditions (Goyret and Farina, 2003; Goyret and Farina, 2005; Wild et al., 2021). For example, honey bees can learn odor information when rewarded with the touch of a nestmate, similar to when they are rewarded with food (Cholé et al., 2019). Overall, we demonstrate that antennation and trophallaxis that can occur during head-to-head interactions may be key behaviors that regulate the fanning response. The number of those behavioral interactions, and the information they carry, influence whether social insects like honey bees will engage in behaviors, and likely help them coordinate collective behaviors like thermoregulation.

Recent hypotheses identify network information theory as a mechanism of maintaining resilience in a complex systems (Crespi et al., 2021), especially in social animal behavior (Bergman and Beehner, 2023). Complex behaviors like honey bee fanning likely require multiple information inputs, such as temperature of the environment and social environment. Acquired information is potentially synthesized among individuals when they interact to confirm that information, which may help to coordinate subsequent behaviors (Guttal and Couzin, 2010). In previous work, we found that honey bees are more likely to fan when in groups of 10 compared to smaller groups of 3 or when isolated (Cook and Breed, 2013). In these larger groups, honey bees appear to anticipate the rate of temperature change, as they begin fanning earlier when experiencing a fast rate of temperature change (Cook et al., 2016a). As such, individual honey bees may be sensing the environment, but need to communicate with others to properly synthesize the information to initiate a collective response. There is still much work to be done to understand what information may be being communicated or how information is shared.

The performance of collective behaviors relies on effective communication of information between individuals within the group (Sumpter, 2006). Here, we provide further evidence that the collective fanning response is likely dependent on two types of information: thermal information and social information. Direct physical contact provides social context for the fanning response, supported by the reduction in the fanning response when density was reduced. Social information is modified by density in many biological systems, such as cancer metastasis, animal migration, and spread of misinformation (Ballerini et al., 2008; Ioannou and Laskowski, 2023; Jamous et al., 2020; Kameda et al., 2022) and unsurprisingly has an impact on collective behaviors in social insects. In summary, our work here further establishes a tractable system in which to test important hypotheses about how collective animal groups utilize environmental information to respond effectively to a changing environment.

REFERENCES

Ballerini, M., Cabibbo, N., Candelier, R., Cavagna, A., Cisbani, E., Giardina, I., Orlandi, A., Parisi, G., Procaccini, A., Viale, M., et al. (2008). Empirical investigation of starling flocks: a benchmark study in collective animal behaviour. *Animal Behaviour* 76, 201–215.

Bauer, S. and Hoye, B. J. (2014). Migratory Animals Couple Biodiversity and Ecosystem Functioning Worldwide. *Science* 344, 1242552.

Bazazi, S., Buhl, C., Hale, J. J., Anstey, M. L., Sword, G. A., Simpson, S. J. and Couzin, I. D. (2008). Collective Motion and Cannibalism in Locust Migratory Bands. *Current Biology* 18, 735–739.

Bergman, T. J. and Beehner, J. C. (2023). Information Ecology: an integrative framework for studying animal behavior. *Trends in Ecology & Evolution* 38, 1041–1050.

Cao, T. T., Hyland, K. M., Malechuk, a., Lewis, L. a. and Schneider, S. S. (2007). The influence of the vibration signal on worker interactions with the nest and nest mates in established and newly founded colonies of the honey bee, Apis mellifera. *Insectes Sociaux* 54, 144–149.

Charles, G. K., Riginos, C., Veblen, K. E., Kimuyu, D. M. and Young, T. P. (2021). Termite mound cover and abundance respond to herbivore-mediated biotic changes in a Kenyan savanna. *Ecol Evol* 11, 7226–7238.

Cholé, H., Carcaud, J., Mazeau, H., Famié, S., Arnold, G. and Sandoz, J.-C. (2019). Social Contact Acts as Appetitive Reinforcement and Supports Associative Learning in Honeybees. *Current Biology* 29, 1407-1413.e3.

Cook, C. N. and Breed, M. D. (2013). Social context influences the initiation and threshold of thermoregulatory behaviour in honeybees. *Animal Behaviour* 86, 323–329.

Cook, C. N. and Lambert, C. E. (2025). Data From: Physical Interactions Shape Collective Thermoregulatory Behavior in Honey Bees. Behavioral Ecology. 10.6084/m9.figshare.28170452 and 10.6084/m9.figshare.28169405

Cook, C. N., Kaspar, R. E., Flaxman, S. M. and Breed, M. D. (2016a). Rapidly changing environment modulates the thermoregulatory fanning response in honeybee groups. *Animal Behaviour* 115, 237–243.

Cook, C. N., Durzi, S., Scheckel, K. J. and Breed, M. D. (2016b). Larvae influence thermoregulatory fanning behavior in honeybees (Apis mellifera L.). *Insect. Soc.* 63, 271–278.

Couzin, I. D. (2009). Collective cognition in animal groups. *Trends in Cognitive Sciences* 13, 36–43.

Crespi, E., Burnap, R., Chen, J., Das, M., Gassman, N., Rosa, E., Simmons, R., Wada, H., Wang, Z. Q., Xiao, J., et al. (2021). Resolving the Rules of Robustness and Resilience in Biology Across Scales. *Integr Comp Biol* 61, 2163–2179.

Donahoe, K., Lewis, L. a. and Schneider, S. S. (2003). The role of the vibration signal in the house-hunting process of honey bee (Apis mellifera) swarms. *Behavioral Ecology and Sociobiology* 54, 593–600.

Dor, R., Katzav-Gozansky, T. and Hefetz, A. (2005). Dufour’s gland pheromone as a reliable fertility signal among honeybee (Apis mellifera) workers. *Behav Ecol Sociobiol* 58, 270–276.

Egley, R. L. and Breed, M. D. (2013). The Fanner Honey Bee: Behavioral Variability and Environmental Cues in Workers Performing a Specialized Task. *J Insect Behav* 26, 238–245.

Franks, N. R. and Richardson, T. (2006). Teaching in tandem-running ants. *Nature* 439, 153–153.

Free, J. B. (1968). The Conditions Under which Foraging Honeybees Expose their Nasonov Gland. *Journal of Apicultural Research* 7, 139–145.

Gil, M. A., Hein, A. M., Spiegel, O., Baskett, M. L. and Sih, A. (2018). Social Information Links Individual Behavior to Population and Community Dynamics. *Trends in Ecology & Evolution* 33, 535–548.

Gordon, D. M. (1989). Dynamics of task switching in harvester ants. *Animal Behaviour* 38, 194–204.

Goyret, J. and Farina, W. M. (2003). Descriptive study of antennation during trophallactic unloading contacts in honeybees Apis mellifera carnica. *Insect. soc.* 50, 274–276.

Goyret, J. and Farina, W. M. (2005). Non-random nectar unloading interactions between foragers and their receivers in the honeybee hive. *Naturwissenschaften* 92, 440–443.

Greene, M. J. and Gordon, D. M. (2007). Interaction rate informs harvester ant task decisions. *Behavioral Ecology* 18, 451–455.

Groh, C., Tautz, J. and Rossler, W. (2004). Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. *Proceedings of the National Academy of Sciences* 101, 4268–4273.

Guo, X., Lin, M. R., Azizi, A., Saldyt, L. P., Kang, Y., Pavlic, T. P. and Fewell, J. H. (2022). Decoding alarm signal propagation of seed-harvester ants using automated movement tracking and supervised machine learning. *Proc. R. Soc. B.* 289, 20212176.

Guttal, V. and Couzin, I. D. (2010). Social interactions, information use, and the evolution of collective migration. *Proceedings of the National Academy of Sciences* 107, 16172–16177.

Hamdan, K. (2010). The Phenomenon of Bees Bearding. *Bee World* 87, 22–23.

Huang, Z. Y. and Robinson, G. E. (1992). Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proceedings of the National Academy of Sciences* 89, 11726–11729.

Huang, Z.-Y., Plettner, E. and Robinson, G. E. (1998). Effects of social environment and worker mandibular glands on endocrine-mediated behavioral development in honey bees. *J Comp Physiol A* 183, 143–152.

Ioannou, C. C. and Laskowski, K. L. (2023). A multi-scale review of the dynamics of collective behaviour: from rapid responses to ontogeny and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences* 378, 20220059.

Jamous, S., Comba, A., Lowenstein, P. R. and Motsch, S. (2020). Self-organization in brain tumors: How cell morphology and cell density influence glioma pattern formation. *PLoS Comput Biol* 16, e1007611.

Jandt, J. M., Tibbetts, E. A. and Toth, A. L. (2014). Polistes paper wasps: a model genus for the study of social dominance hierarchies. *Insect. Soc.* 61, 11–27.

Jones, J. C. and Oldroyd, B. P. (2006). Nest Thermoregulation in Social Insects. In *Advances in Insect Physiology* (ed. Simpson, S. J.), pp. 153–191. Academic Press.

Kameda, T., Toyokawa, W. and Tindale, R. S. (2022). Information aggregation and collective intelligence beyond the wisdom of crowds. *Nat Rev Psychol* 1, 345–357.

Lindauer, M. (1955). The Water Economy and Temperature Regulation of the Honeybee Colony. *Bee World* 36, 81–92.

Medina, R. G., Paxton, R. J., Hernández-Sotomayor, S. M. T., Pech-Jiménez, C., Medina-Medina, L. A. and Quezada-Euán, J. J. G. (2020). Heat stress during development affects immunocompetence in workers, queens and drones of Africanized honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). *Journal of Thermal Biology* 89, 102541.

Moe, S. R., Eldegard, K., Rannestad, O. T., Okullo, P., Lindtjørn, O., Støen, O. G. and Dale, S. (2017). Strong positive effects of termites on savanna bird abundance and diversity are amplified by large herbivore exclusion. *Ecol Evol* 7, 10079–10088.

O'Donnell, S. (2006). *Polybia* wasp biting interactions recruit foragers following experimental worker removals. *Animal Behaviour* 71, 709–715.

Pankiw, T. and Page, R. E. (2003). Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (Apis mellifera L.). *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* 189, 675–84.

Peters, J. M., Peleg, O. and Mahadevan, L. (2019). Collective ventilation in honeybee nests. *J. R. Soc. Interface.* 16, 20180561.

R Core Team (2021). R: A language and environment for statistical computing.

Rice, L., Tate, S., Farynyk, D., Sun, J., Chism, G., Charbonneau, D., Fasciano, T., Dornhaus, A. and Shin, M. C. (2020). ABCTracker: an easy-to-use, cloud-based application for tracking multiple objects.

RStudio Team (2020). RStudio: Integrated Development for R.

Smith, M. L., Ostwald, M. M., Loftus, J. C. and Seeley, T. D. (2014). A critical number of workers in a honeybee colony triggers investment in reproduction. *Naturwissenschaften* 101, 783–790.

Southwick, E. E. and Moritz, R. F. A. (1987). Social control of air ventilation in colonies of honey bees, *Apis mellifera*. *Journal of Insect Physiology* 33, 623–626.

Starks, P. T. and Gilley, D. C. (1999). Heat Shielding: A Novel Method of Colonial Thermoregulation in Honey Bees. *Naturwissenschaften* 86, 438–440.

Strassmann, J. E. (1981). Wasp Reproduction and Kin Selection: Reproductive Competition and Dominance Hierarchies among Polistes annularis Foundresses. *The Florida Entomologist* 64, 74–88.

Sumpter, D. J. T. (2006). The principles of collective animal behaviour. *Phil. Trans. R. Soc. B* 361, 5–22.

Sumpter, D., Buhl, C., Biro, D. and Couzin, I. (2008). Information transfer in moving animal groups. *Theory Biosci.* 127, 177–186.

Tautz, J., Maier, S., Groh, C., Rossler, W. and Brockmann, A. (2003). Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proceedings of the National Academy of Sciences* 100, 7343–7347.

Von Frisch, K. and Chadwick, L. E. (1967). *The dance language and orientation of bees*. Belknap Press of Harvard University Press Cambridge, MA.

West-Eberhard, M. J. (1986). Dominance Relations in Polistes Canadensis (l.), a Tropical Social Wasp. *Monitore Zoologico Italiano - Italian Journal of Zoology* 20, 263–281.

Wild, B., Dormagen, D. M., Zachariae, A., Smith, M. L., Traynor, K. S., Brockmann, D., Couzin, I. D. and Landgraf, T. (2021). Social networks predict the life and death of honey bees. *Nat Commun* 12, 1110.

Winston, M. L. (1991). *The biology of the honey bee*. harvard university press.

Figure 1: Creating separated cages to reduce interactions. Groups of 5 or 3 bees either moved freely and had full contact, or groups of 5 bees were separated by hardware cloth mesh that reduced or prevented interactions. Bee drawings done by Impact Media Lab.

Figure 2: The fanning response of honey bees in varying group sizes and degrees of separation. A) Honey bees that are able to freely interact in groups of 5 (n=61) or 3 (n=13) are significantly more likely to fan compared to when interactions are restricted by a single layer of mesh (n=57) or a double layer of mesh (n=44). B) Honey bees fan at lower temperatures when they are freely moving in groups of 5, and fan at higher temperature when they are in smaller groups of 3 or divided. Letters above boxes correspond to Tukey post-hoc test at $p<0.05$.

Figure 3: Honey bees are more likely to fan when in high density social environments compared to lower density environments. This includes both the initial fanning bout (a) as well as the fanning bout where the greatest number of bees fan, or max fanner bout (b). Number of small cage trials = 29, number of large cage trials = 32. Dots are the proportion of fanners in a given fanning bout (number of fanners/10). Boxes represent the interquartile range (IQR) and whiskers represent 1.5*IQR. Thick bars are medians and diamonds are means of all the data. Asterisks indicate significance according to logistic regression at $p < 0.05$.

Figure 4: Fanning honey bees fan at the same temperature, regardless of the density of their social environments. a) Honey bees begin to fan at statistically similar temperatures in the initial fanning bout and b) the temperature at which the maximum number of bees are fanning is statistically similar when bees are in high and low density social

environments. N=27 for both groups where fanning was observed. Boxes represent the interquartile range (IQR) and whiskers represent 1.5*IQR. Thick bars are medians and diamonds are means of all the data.

Figure 5: Interacting honey bees prioritized head-to-head interactions over other types of interactions, regardless of social density. Bees interacting in low density social environments, bees had 1228/1712 head-to-head interactions and bees in high density environments had 2332/2808 head-to-head interactions, which comprise about 75% of all interactions in both social environments. This pattern was similar for head-to-body interactions (456/1712) and body-to-body interactions (28/1712) in low density environments and head-to-body interactions (430/2808) and body-to-body interactions (46/2808) in high social density environments (for full data, see Table S6).

Figure 6: Individual bees in high density environments had more head-to-head interactions. When ten bees were in smaller, high-density cages, they had more a) overall interactions and b) head-to-head interactions. There was no difference in the c) head-to-body interactions or the d) body-body interactions. Interactions are characterized as a boundary around the body of the individual bee overlapping. Head-to-head interactions occur when a diamond shaped boundary over the head of the bee overlap with another diamond shaped boundary with another bee. Interactions are ranked: When both occur at the same time, head-tohead interactions are prioritized. Each dot is the raw number of interactions that an individual engages in during the 4 minutes before fanning. Boxes represent 25-75 quartiles, whiskers indicate 95% of the data. Thick bars are medians and diamonds are means of all the data. Asterisks indicate significance according to Wilcox test at $p < 0.05$. Full results described in Table S3; Table S5.

Figure 7: Bees in low density social environments had longer interactions. Results displayed on log10 y-axis scale. When ten bees were in larger, low-density cages they had longer interactions across all categories. Interactions are characterized as a boundary around the body of the individual bee overlapping. Head-tohead interactions occur when a diamond shaped boundary over the head of the bee overlap with another diamond shaped boundary with another bee. Interactions are ranked: When both occur, head-to-head interactions are prioritized. Each dot is the average for each individual in a video. Boxes represent 25-75 quartiles, whiskers indicate 95% of the data. Thick bars are

medians and diamonds are means of all the data. Violin shape represents the distribution of data. Asterisks indicate significance according to Wilcox test at $p < 0.05$. Full results described in Table S4; Table S6.

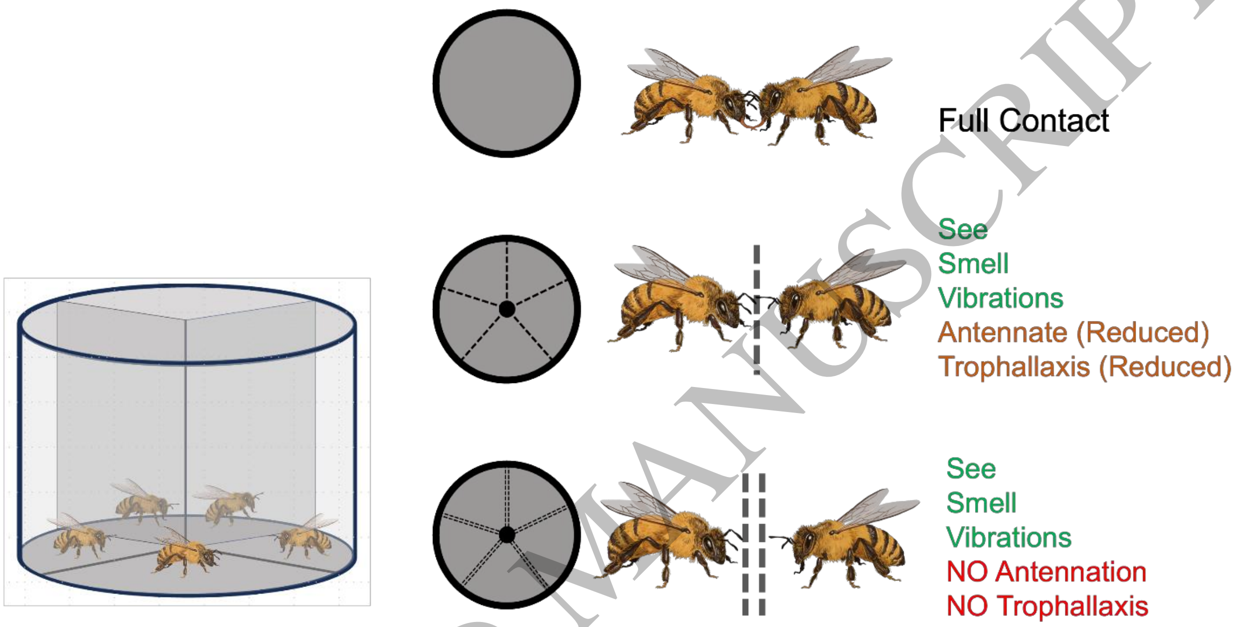

Figure 1
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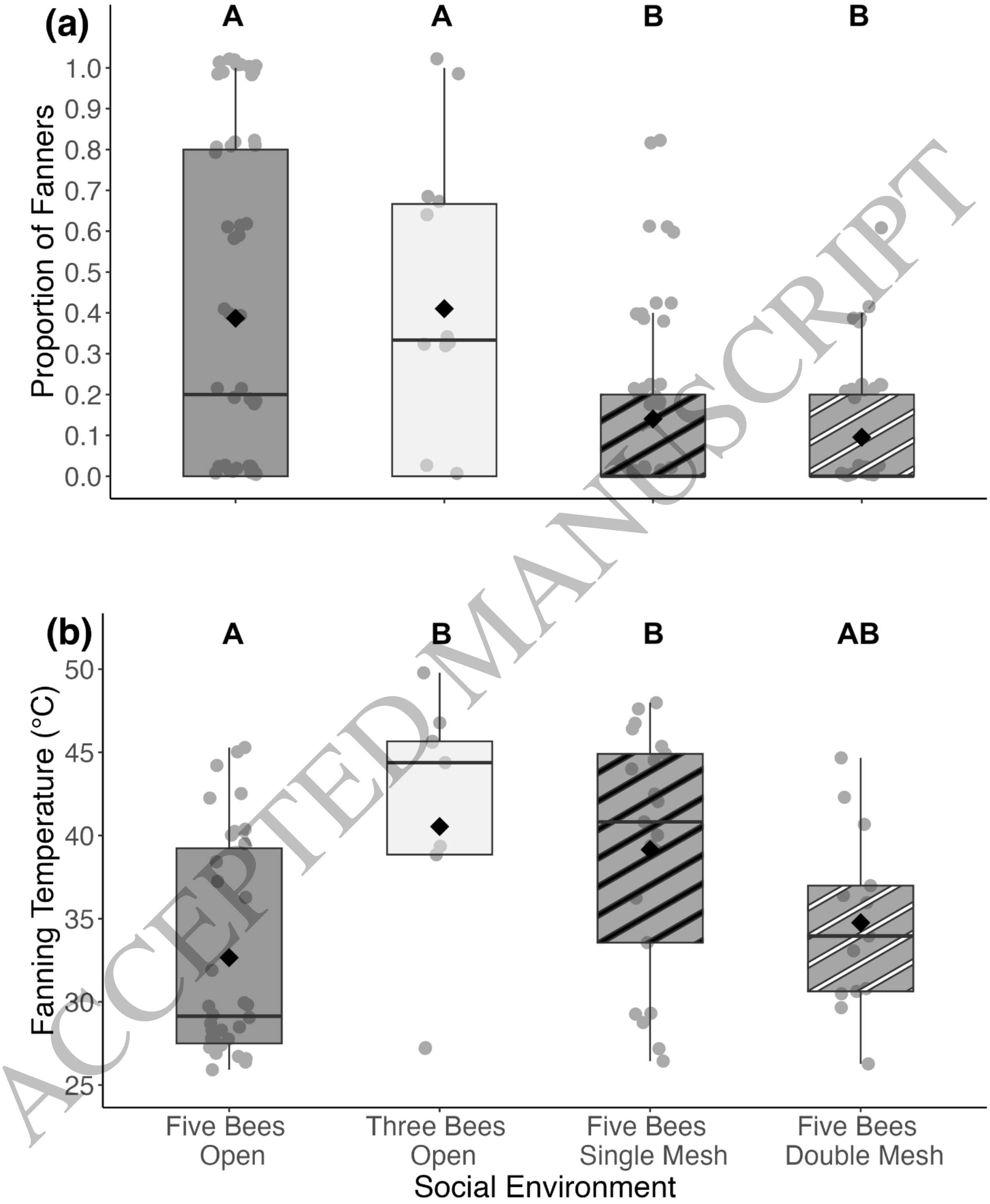

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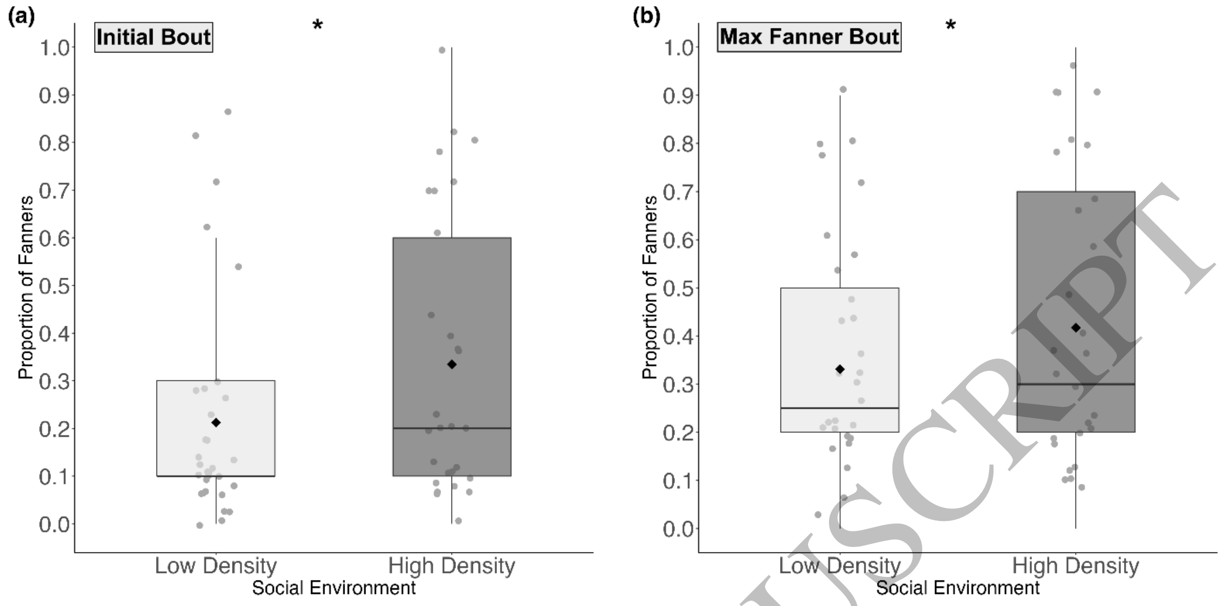

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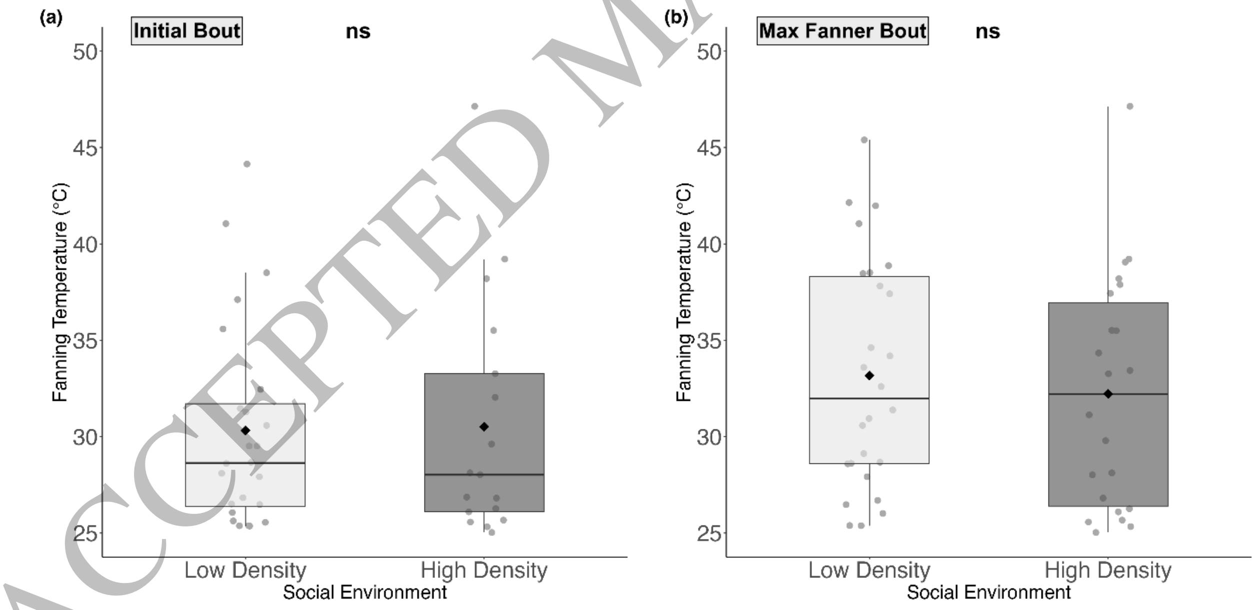

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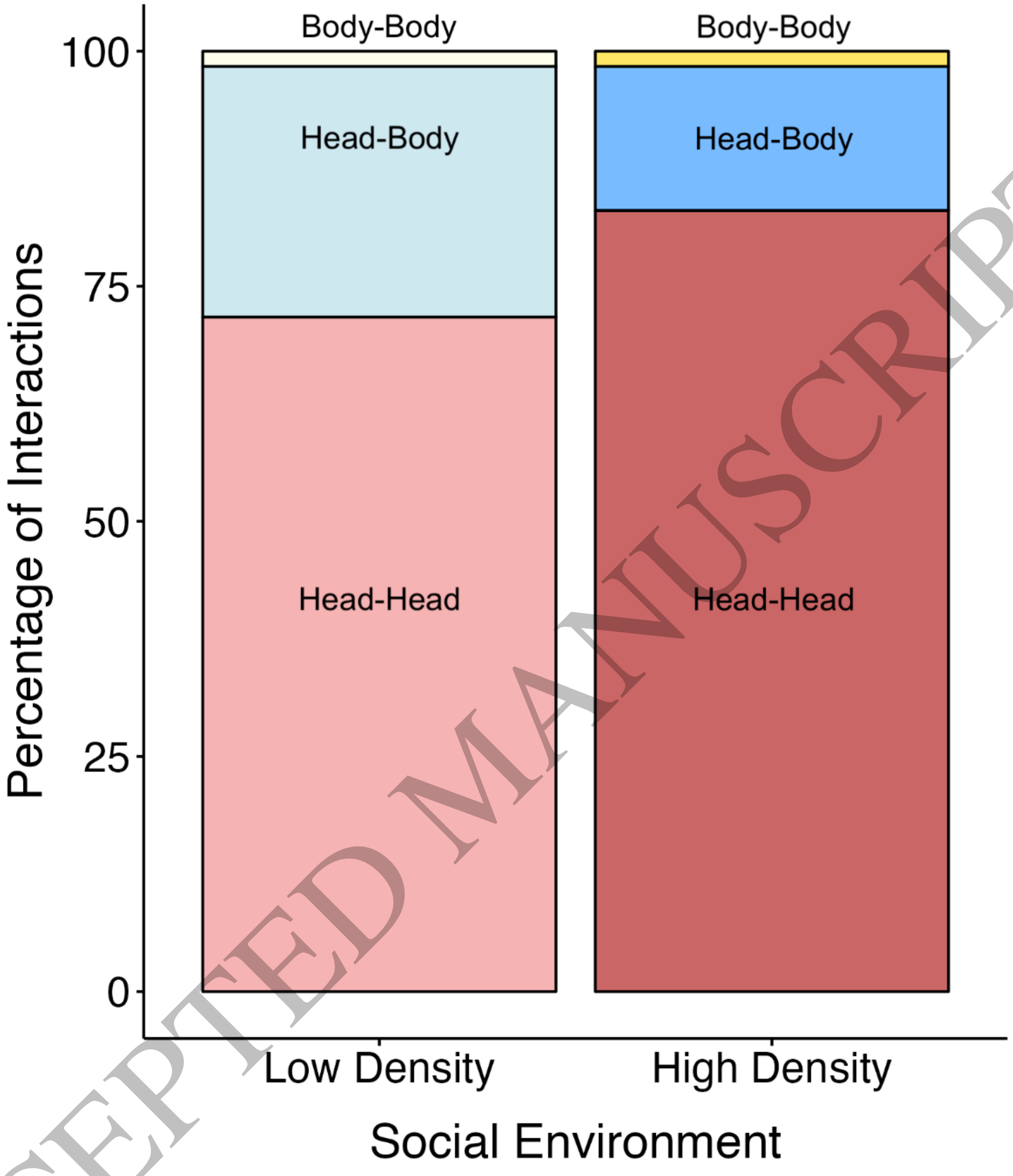

Figure 5
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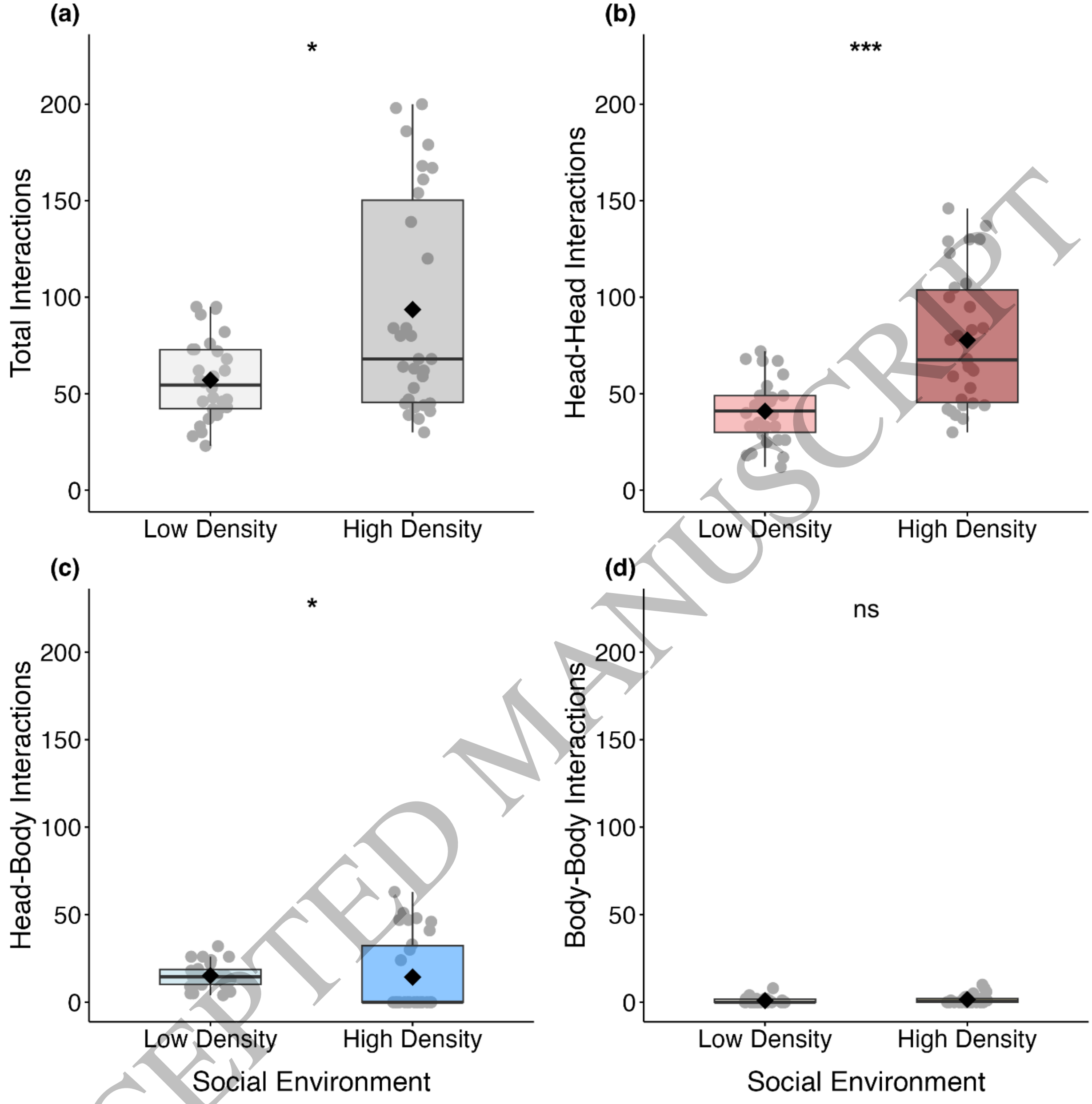

Figure 6
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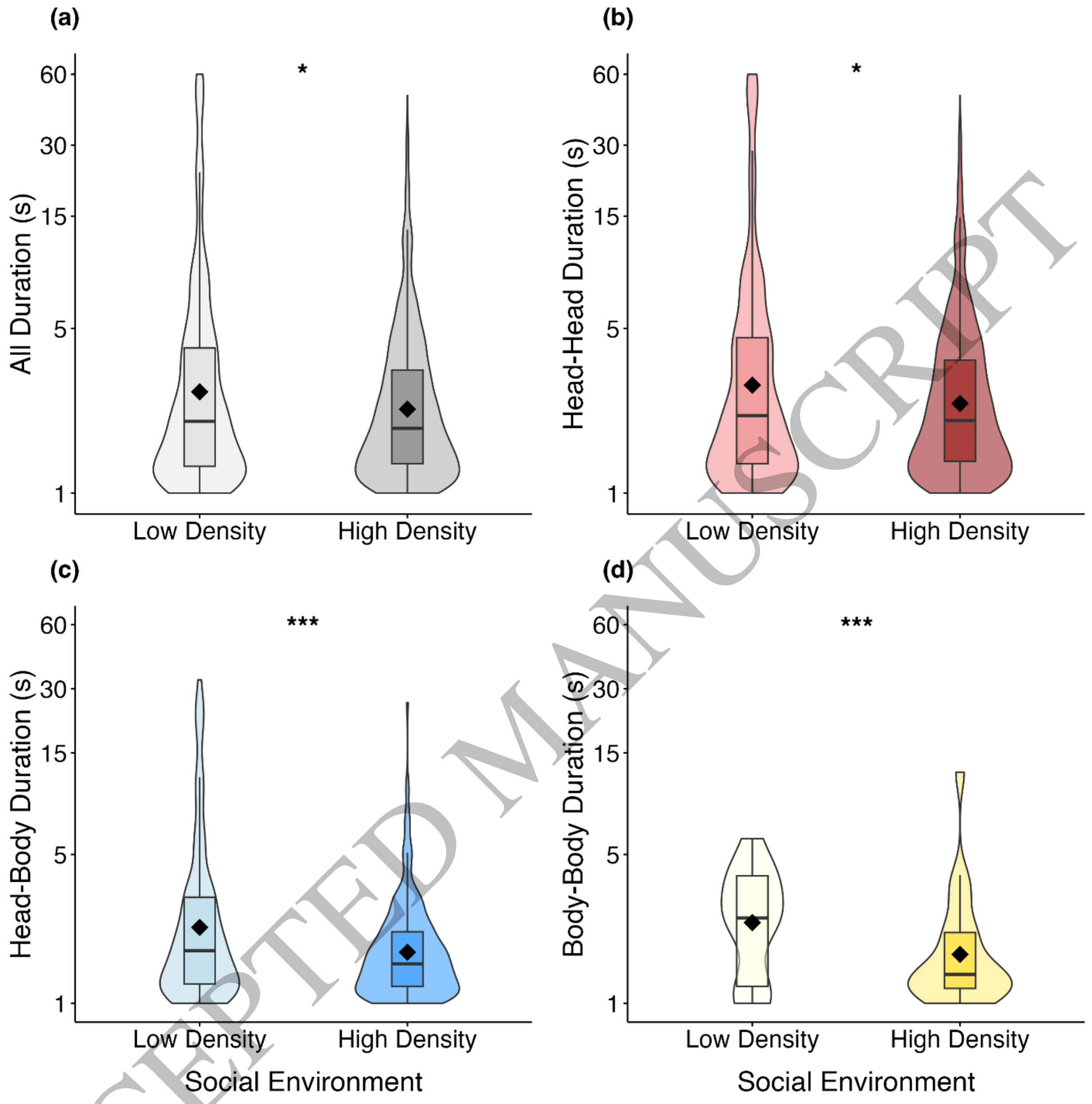

Figure 7
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