

Drone “quality” and caste interactions in the honey bee, *Apis mellifera* L.

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Abstract We investigated the influence of drone size and potential reproductive quality on caste interactions by adding large drones reared in drone cells (DC drones; considered to be of higher quality) and small drones reared in worker cells (WC drones; of lower quality) to two observation colonies and monitoring worker–drone interactions and acceptance by workers. When initially introduced into the colonies more DC drones received trophallaxis, whereas more WC drones received aggression and eviction attempts from workers. Nevertheless, WC and DC drones were equally likely to be accepted by workers. For both drone types accepted individuals had slightly, but significantly greater weights than rejected males. Thus, workers discriminated between drones of different sizes and potential quality upon initial encounter, although these discriminations were not strongly associated with acceptance decisions. After drones were accepted, workers either showed no preference for interacting with WC or DC drones, or if a preference was shown it tended to favor WC drones. Compared to accepted DC drones, significantly more WC drones received grooming for longer periods of time and also spent more time engaged in all interactions with workers combined. DC and WC drones did not differ in the likelihood of receiving trophallaxis or the vibration signal, although for both interactions slightly more WC drones were recipients. Thus, workers may bias some interactions with accepted drones to favor smaller individuals with potential developmental deficiencies,

in a manner that could contribute to the production of a greater total number of competitive males and increased colony reproductive output.

Keywords Caste interactions · Worker–drone interactions · Drone production · Vibration signal · Trophallaxis

Introduction

In highly social insects, interactions among sterile workers and sexual individuals are often major determinants of colony reproductive output, as well as the principal means by which workers gain inclusive-fitness benefits (Hölldobler and Wilson, 1990; Tarpy et al., 2004). Selection acting at the level of both the colony and the individual worker should, therefore, favor caste interactions that contribute to the production of higher quality sexual individuals (Visscher, 1998; Tarpy et al., 2004). In the honey bee, *Apis mellifera*, workers gain reproductive benefits equally through the rearing of virgin queens and reproductive swarming, and the production and mating success of drones (males). Worker–queen interactions and their role in colony reproductive decisions have been well studied (Schneider and DeGrandi-Hoffman, 2003; 2008; Gilley and Tarpy, 2005; Pierce et al., 2007). In contrast, less is known about worker–drone interactions and the role they play in influencing colony reproductive output.

The only function of drones is to mate with virgin queens at drone congregation areas (DCAs), where thousands of drones from many different colonies gather in midair and pursue and then copulate in-flight with virgin queens that fly through the cluster (Koeniger et al., 2005). Larger drones, and those with greater flight capacities and maneuverability,

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may have competitive advantages at DCAs (Coelho, 1991; Berg et al., 1997; Kraus et al., 2003; Jaffé and Moritz, 2010). Larger, heavier drones also produce a greater volume of semen, have higher sperm numbers, fewer sperm abnormalities and increased representation in a queen's offspring (Schlüns et al., 2004; Zaitoun et al., 2009; Couvillon et al., 2010; Gencer and Kahya, 2011). A drone can mate only once, because it dies after ejaculation. Thus, a colony can increase its reproductive success through drones primarily by producing and fielding greater numbers of higher quality, competitive males (Kraus et al., 2003). Any worker interactions that contribute to these outcomes should have strong selective advantages.

Workers build drone comb, rear drone larvae, and adjust the total number of drones raised to colony and environmental conditions (Boes, 2010). After drones emerge as adults, workers interact with them mostly through trophallaxis (mouth-to-mouth exchange of liquid food) and grooming. Trophallaxis provides the nutrients needed for sexual maturation and the development of flight muscles, whereas grooming may contribute to drone hygiene and health (Hrassnigg and Crailsheim, 2005). In addition, workers may communicate with drones by performing vibration signals on them. The vibration signal consists of a worker grasping a recipient with her legs and rapidly vibrating her own body dorso-ventrally for 1–2 s. Vibration signals are performed on drones of all ages, although sexually immature drones (those less than 12 days of age) are vibrated at higher rates (Boucher and Schneider, 2009; Stout et al., 2011). All drones respond to the signal by increasing their rate of contact with workers, which results in significant increases in the amount of time spent receiving trophallaxis and grooming (Boucher and Schneider, 2009). Although drones often engage in trophallaxis and grooming without receiving the vibration signal, workers may use the signal to fine-tune drone care (Boucher and Schneider, 2009; Slone et al., 2012).

Several lines of evidence suggest that drone condition influences caste interactions. First, workers will cannibalize young drone larvae showing developmental abnormalities (Woyke, 1963; Sasaki et al., 2004). Second, drones receiving vibration signals have slightly, but significantly lower thorax weights and thorax-to-body weight ratios compared to age-matched, non-vibrated drones, which suggests that workers may direct signals toward drones with slightly less developed flight muscles (Slone et al., 2012). Because drones respond to the signal with increased trophallaxis, workers may use the signal to help address minor developmental deficiencies in adult drones, potentially contributing to the production of a greater total number of males that are competitive at DCAs (Slone et al., 2012). However, the influence of drone condition and “reproductive quality” on caste interactions is poorly understood.

We investigated the influence of drone quality on worker–drone interactions by introducing into observation hives drones reared in drone cells (hereafter referred to as DC drones) and drones reared in worker cells (WC drones). Because drone cells are larger than worker cells (Winston, 1987), DC drones are larger and heavier than WC drones (Schlüns et al., 2003; Gencer and Firatli, 2005). Given the potential reproductive advantages of larger drones, WC drones are typically considered to be of lower quality (Schlüns et al., 2003; Zaitoun et al., 2009; Couvillon et al., 2010; Gencer and Kahya, 2011). The vast majority of drones produced by a colony are reared in drone cells. Nevertheless, WC drones are produced under some conditions, such as when queenless colonies develop workers that lay unfertilized eggs in worker cells, or when a queen depletes her sperm stores and lays only unfertilized eggs in worker cells. Although WC drones have lower semen volume and sperm numbers, they may not differ from DC drones in-flight speed (Berg and Koeniger, 1990) or sperm viability (Gencer and Kahya, 2011). Berg (1991) estimated that small drones comprise up to 9 % of all drones present at DCAs. Thus, WC drones occur naturally in honey bee biology and can contribute to colony reproductive output.

We examined worker interactions with DC and WC drones during two periods: when first introduced into a colony and then after acceptance into the colony. We hypothesized that when initially introduced, WC drones would experience more aggression from workers and would be more likely to be evicted from the nest than DC drones. We tested three hypotheses for the influence of drone type on caste interactions after drones were accepted into a colony. First, workers would preferentially direct positive interactions (trophallaxis, grooming, and vibration signals) toward DC drones and negative interactions (aggression) toward WC drones, to promote the investment of resources in larger, more competitive males. Second, workers would preferentially direct positive interactions toward WC drones, to foster the development of a greater total number of males that are potentially competitive at DCAs. Third, caste interactions would be unaffected by drone type.

Methods

Colony set-up

Worker–drone interactions were studied in two four-frame observation colonies maintained on the campus of the University of North Carolina at Charlotte during a 5-week period from June to August, 2012. The observation colonies were established by transferring four frames of comb containing brood, food, 6,000–8,000 workers, and a queen from each of two unrelated field colonies. The entrance of each

observation hive was covered with a wire grid (“queen excluder”) through which workers, but not drones could pass, to facilitate monitoring drone eviction and survival. We used queen excluders (following the methods of Currie and Jay, 1988) instead of traps which are often used to collect drones (Henderson, 1994; Zaitoun et al., 2009), because the configuration of the building housing our colonies made it impossible to attach traps to the outside entrances of the observation hives. The runway of each observation hive was equipped with a 9×12 cm wire “feeder” screen located immediately below the bottom frame of comb (see Fig. 1, Gary and Lorenzen, 1980). Each feeder screen was modified to contain a removable section to create an opening through which we introduced drones into the observation colonies. Small baffles were placed at the introduction site to force the drones to crawl onto the bottom frame of comb, where most interactions with workers were initiated.

We obtained DC and WC drones for introduction into the observation colonies as follows. DC drones were collected from frames of sealed drone comb removed from four unrelated field colonies, each with a laying queen. To generate WC drones, virgin queens were raised to adulthood and not allowed to mate, but were treated with carbon dioxide to initiate oviposition (Laidlaw, 1979; Couvillon et al., 2010). Such queens will lay in worker cells, but because the eggs are unfertilized the larvae develop into small drones. Four such treated queens were established in separate four-frame hives maintained in the field and served as the source of WC drones throughout the study. Thus, all drones used in our study arose from queen-laid eggs, but differed in size and potentially in quality depending upon the type of cell in which they were reared.

When the DC and WC drones were ready to emerge, the frames of combs were removed from the field colonies, placed in pre-labeled nylon mesh bags and maintained in an incubator (33 °C and 50 % relative humidity). All drones were collected within 24 h of emergence, such that their age

in days was known throughout the study period. Newly emerged DC and WC drones were placed in separate collection containers, individuals were drawn from the containers randomly and each was weighed to the nearest 0.1 mg to determine initial live weight. Each drone was then marked by gluing to the thorax a plastic tag (Opalithplättchen, Graze Co., Germany) that contained a unique color/number/paint mark combination that allowed for individual identification, as well as identification of drone type (DC or WC). Tagged drones were assigned to an observation colony randomly, such that DC and WC drones from the different source colonies were similarly represented in each study hive to minimize any possible maternal effects associated with the different source queens. To introduce drones into the colonies, the tagged DC and WC drones were placed in a common glass container, the opening of the container was covered with nylon mesh, and the covered opening was then set over the wire feeder screen located at the bottom of each observation colony for 30–60 min to expose the drones to colony odor. After this period, the drones were released into the colonies. Cohorts of newly emerged, tagged drones were added to the observation colonies every 2–3 days depending upon availability, until a total of 494 DC and 281 WC drones had been introduced over a 3-week period. We introduced fewer WC drones than DC drones to mimic the natural condition in which small drones are less abundant in honey bee colonies (Berg et al., 1997). No obvious changes in caste interactions were observed for drones added at different times during the study period, which suggests that any differences in worker–drone interactions were associated with drone condition rather than changes in the external environment as the study progressed.

Determining drone survival and acceptance

Each day throughout the 5-week study period, we conducted a census of all surviving tagged drones in each observation colony. Also, each day we collected all tagged drone corpses at the queen excluder, recorded the identity of each, and stored the bodies at -20 °C for subsequent analysis. Most rejection of drones occurs within 24 h after introduction (Currie and Jay, 1988). Consequently, we classified a tagged drone as rejected if its corpse was found at the queen excluder within 24 h following introduction or if it never appeared in the daily censuses after its initial 24 h in the hive. Drones surviving in the observation hives for more than 24 h were considered to have been accepted by workers. At the end of the study period, the observation colonies were opened and all surviving tagged drones were collected and stored at -20 °C. Subsequently, all corpses collected at the queen excluders and the bodies of all surviving drones collected at the end of the study were dried in

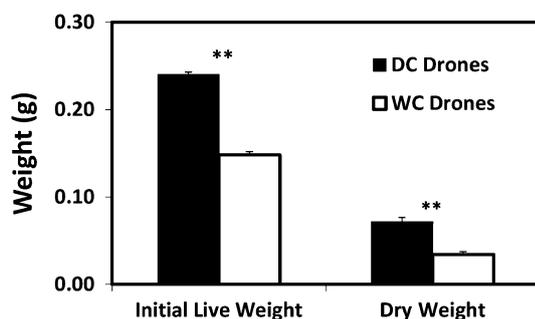


Fig. 1 The initial live weight and final dry weight (mean \pm SE) of the DC drones and WC drones introduced into the observation colonies (** $P < 0.01$)

a desiccator and dry weight was determined to the nearest 0.1 mg. The legs and wings were removed from the drones before determining dry weights, because the appendages can become damaged or detached during freezing and drying.

Monitoring worker–drone interactions

We monitored worker–drone interactions 5 days a week from 9 a.m. to 4 p.m. throughout the study period. During every day of observation, each colony was monitored by a minimum of two observers simultaneously, and the observers rotated between colonies hourly to minimize observer bias.

Two periods of worker–drone interactions were monitored. First, we assessed immediate worker–drone interactions, defined as those occurring during the first 2 h following introduction into the hives. The colonies were scanned continuously throughout the 2 h periods and we recorded the identity and type of each drone observed to receive trophallaxis, aggression, vibration signals and eviction attempts from workers. Aggression was identified following the description provided by Ohtani (1974): a drone was considered to be receiving aggression if workers were biting or rapidly and frantically chewing on the drone’s thorax and abdomen, which typically caused the drone to adopt a “flinching” posture or run away. A drone was considered to be receiving an eviction attempt if it was drug by its legs or wings across the combs or along the bottom of the observation hive toward the queen excluder. At the end of each 2 h introduction period, we determined the total number and proportion of DC and WC drones that received each of the interactions from workers.

Second, throughout the study, we monitored for 20 min each randomly selected focal DC and WC drones that had been accepted into the colonies. During the 20-min observation periods, we recorded each time a drone received grooming, trophallaxis, aggression and vibration signals from workers. Digital stop watches were used to determine the amount of time spent receiving trophallaxis, grooming and aggression. Grooming was distinguished from aggression in that the interaction consisted of gentle nibbling on a drone’s thorax and abdomen and the drone did not show the flinching posture associated with aggression (Ohtani, 1974). At the end of the study period, we determined the total number and proportion of focal DC and WC drones that received each interaction from workers and the proportion of time each drone spent receiving trophallaxis, grooming, aggression, and all interactions combined. In addition, we determined the proportion of vibrated and non-vibrated WC and DC drones that received trophallaxis, grooming and aggression during the 20-min observation periods, to further assess the association between the vibration signal and caste interactions.

Statistical analysis

The live weights of newly emerged drones and the dry weights of drone corpses and surviving drones collected at the end of the study were compared between WC and DC drones using Mixed-model ANOVA (SAS Institute, 1997), with colony as a random factor and drone type as a between-subjects factor. Drone weights were log₁₀ transformed before analysis to normalize the data. Log-linear models (SAS Institute, 1997) were used to compare the proportions of WC and DC drones accepted by the colonies, as well as the proportion that received the different caste interactions during the 2-h periods immediately following introduction and during the 20-min observation periods used to monitor focal accepted drones.

We had large numbers of zero values for the proportion of time accepted drones spent in the different interactions with workers during the 20-min observation periods. Consequently, the data could not be normalized and we used non-parametric Kruskal–Wallis tests (Sokal and Rohlf, 1995) to compare DC and WC drones for the proportion of time spent in the different caste interactions. We first determined if the proportion of time spent in each interaction differed between the two colonies. If not, the data were combined between colonies and we focused our analysis on the main effect of drone type. Otherwise, the data were analyzed separately by colony.

Mixed-model ANOVA was used to compare the proportions of vibrated and non-vibrated WC and DC drones that received trophallaxis, grooming and aggression during the 20-min observation periods. For each interaction, the model used colony as a random factor, drone type and signal status (vibrated or non-vibrated) as between-subjects factors, and generated a drone type-by-signal status interaction. The main factor of signal status assessed the association between the vibration signal and other caste interactions regardless of drone type. The interaction term assessed if the associations were expressed differently between the DC and WC drones. Proportional data were arcsine transformed before analysis to achieve normality (Sokal and Rohlf, 1995).

Results

Drone weights

Live weights were determined for all drones at emergence; dry weights were determined at the end of the study period for a total of 286 DC and 183 WC drones. Compared to DC drones, WC drones had significantly lower initial live weights ($F_{1,773} = 1,015.8$; $P < 0.0001$) and final dry weights ($F_{1,467} = 399.8$; $P < 0.0001$; Fig. 1).

Immediate worker–drone interactions and drone acceptance

Only six drones (5 DC and 1 WC drones) received a vibration signal during the initial 2-h periods following introduction. Consequently, we excluded the vibration signal for our analyses of immediate worker–drone interactions.

DC drones were more likely to receive trophallaxis than WC drones when first introduced into the observation colonies (Log-linear analysis; $X_1^2 = 32.72$; $P < 0.001$; Fig. 2). In contrast, WC drones were more likely to receive aggression ($X_1^2 = 51.13$; $P < 0.0001$) and eviction attempts ($X_1^2 = 3.98$; $P = 0.046$) (Fig. 2). Despite these pronounced differences in immediate worker–drone interactions, there was no significant difference in the number of DC and WC drones that were accepted into the colonies 24 h after introduction ($X_1^2 = 0.76$; $P = 0.385$; Fig. 2). All trends were shown similarly in the two observation hives (for each behavior-by-drone type-by-colony interaction: X_1^2 ranged from 0.10 to 3.05; P ranged from 0.08 to 0.92).

Drone acceptance may have been influenced by drone weight. Accepted drones had slightly, but significantly greater live weights ($F_{1,512} = 12.63$; $P = 0.0004$) and dry weights ($F_{1,465} = 63.85$; $P < 0.0001$; Fig. 3) than did rejected individuals. These trends were exhibited similarly for both DC and WC drones (for live weights: $F_{2,512} = 0.68$; $P = 0.41$; for dry weights: $F_{2,465} = 0.32$; $P = 0.57$). Weight alone could not have been the only factor determining acceptance, because accepted WC drones weighed less than rejected DC drones (Fig. 3). Nevertheless, within each drone type workers may have shown a preference for slightly heavier individuals.

Worker interactions with accepted drones

A total of 181 accepted DC drones and 97 accepted WC drones were monitored for 20-min each. The mean \pm SE

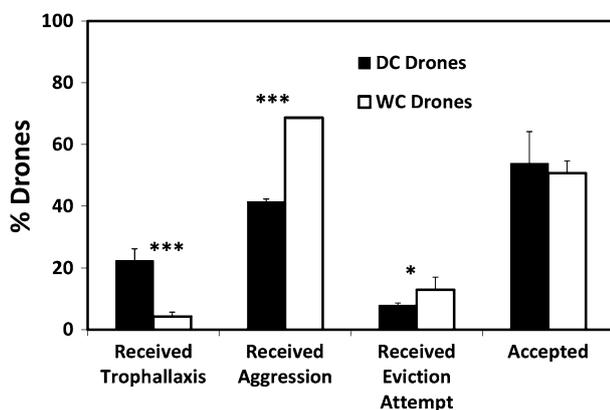


Fig. 2 Percentage (mean \pm SE) of DC and WC drones that received trophallaxis, aggression, and eviction attempts immediately following introduction into the colonies, and the percentage that were ultimately accepted into the two observation colonies (* $P < 0.05$; *** $P < 0.001$)

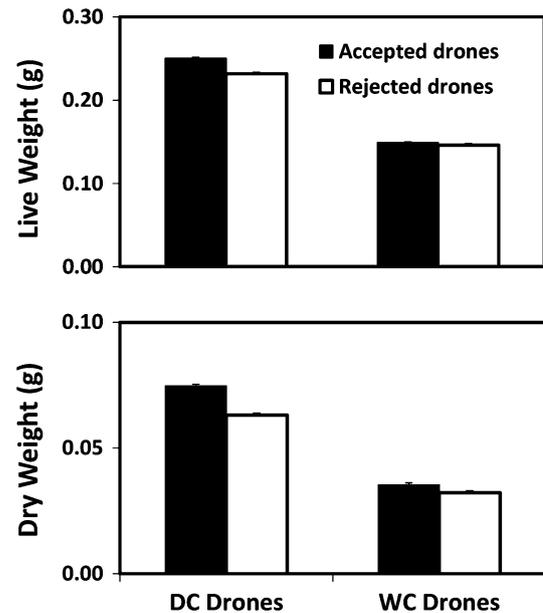


Fig. 3 Live weights and dry weights (mean \pm SE) of accepted and rejected DC and WC drones

age of the monitored drones was 7.0 ± 0.3 days; 89 % of the focal individuals were sexually immature (<12 days old). Only 12 drones (5 DC and 7 WC) received aggression from workers during the 20-min observation periods. We, therefore, excluded aggression from the analyses of focal drone behavior.

A significantly greater percentage of the focal WC drones received grooming during the 20 min observation periods compared to DC drones ($X_1^2 = 7.65$; $P = 0.0057$; Fig. 4). WC and DC drones did not differ in the likelihood of receiving trophallaxis ($X_1^2 = 0.38$; $P = 0.54$) or the vibration signal ($X_1^2 = 1.88$; $P = 0.171$; Fig. 4). However, for all interactions monitored, there was a trend for WC drones to be more likely to receive the interactions than DC drones (Fig. 4).

The trend toward increased interactions with WC drones was also reflected in the percentage of time individual drones spent in the different activities monitored. We found no difference between the two colonies for the percentage of time drones spent in any of the caste interactions examined (for each interaction, X_1^2 ranged from 0.32 to 1.94; P ranged from 0.16 to 0.57). We, therefore, combined data between colonies and focused our analyses on the main effect of drone type.

Compared to DC drones, WC drones spent a significantly greater percentage of time receiving grooming (Kruskal–Wallis test: $X_1^2 = 9.72$; $P = 0.0018$) and engaging in all interactions with workers combined ($X_1^2 = 6.45$; $P = 0.011$; Fig. 5) during the 20-min observation periods. The two drone types did not differ in the percentage of time spent engaging in trophallaxis with workers ($X_1^2 = 0.008$; $P = 0.93$; Fig. 5).

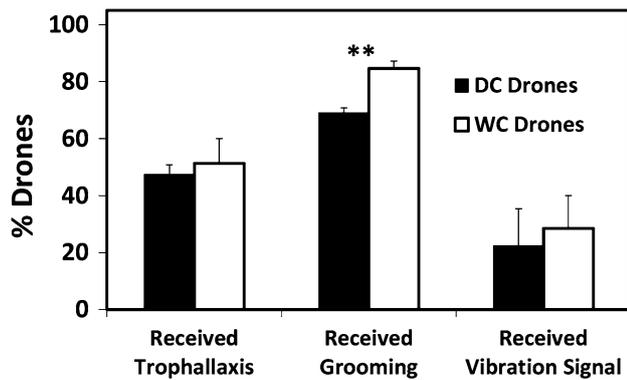


Fig. 4 Percentage (mean \pm SE) of DC and WC drones receiving trophallaxis, grooming, and vibration signals during 20-min observation periods in the two observation colonies (** $P < 0.01$)

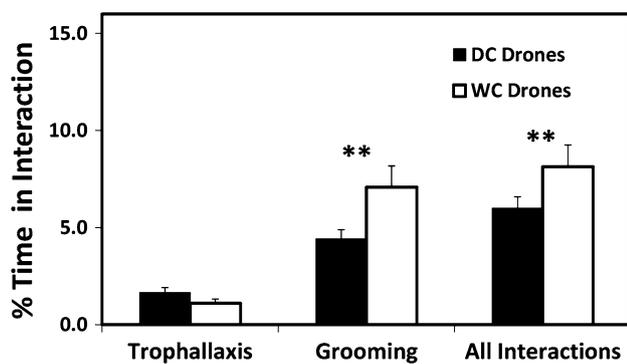


Fig. 5 Percentage of time (mean \pm SE) DC and WC drones spent engaging in trophallaxis, grooming, and all interactions combined during the 20-min observation periods in the two observation colonies ($P < 0.05$; ** $P < 0.01$)

Drones that received vibration signals during the 20-min observation periods were slightly more likely to engage in other interactions with workers than were non-vibrated individuals (Fig. 6). These differences were not statistically significant, but they approached significance for both trophallaxis ($F_{1,6} = 5.49$; $P = 0.0792$) and grooming ($F_{1,6} = 5.74$; $P = 0.0747$; Fig. 6). However, the tendency of vibrated drones to interact with workers did not differ between DC and WC drones for either trophallaxis ($F_{1,6} = 0.94$; $P = 0.39$) or grooming ($F_{1,6} = 0.06$; $P = 0.817$; Fig. 6).

Discussion

Drone physical condition may have influenced caste interactions, both when drones were initially introduced into our observation hives and after they had been accepted by workers. During the introduction periods, workers were significantly more likely to give trophallaxis to DC drones, but to behave aggressively toward and attempt to evict WC

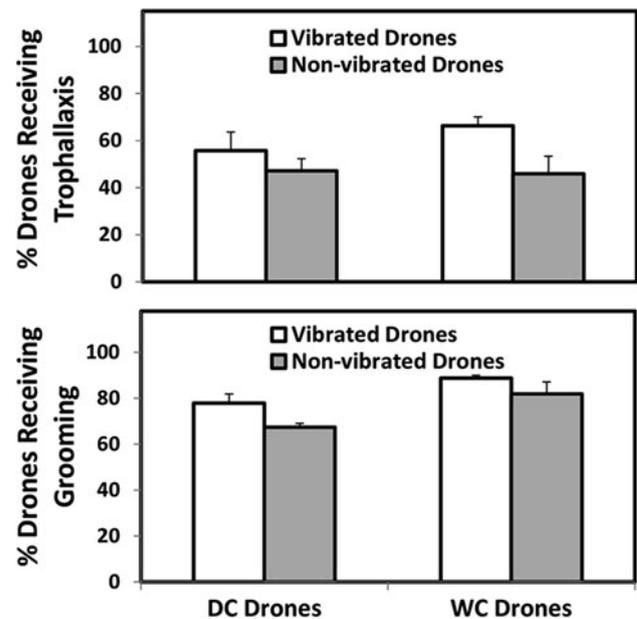


Fig. 6 Percentage (mean \pm SE) of vibrated and non-vibrated DC and WC drones that received trophallaxis (*upper graph*) and grooming (*lower graph*) during the 20-min observation periods in the two observation colonies

drones. Thus, when first encountering drones of potentially differing quality, workers focused positive interactions on DC drones and negative interactions on WC drones, consistent with our hypothesis that immediate caste interactions would favor higher quality males. However, despite the ability of workers to distinguish between the two drone types, they were equally likely to accept WC and DC drones. We cannot fully explain this apparent discrepancy between behavioral discrimination and ultimate acceptance. It is possible that our use of queen excluders at the hive entrances skewed our survival and acceptance data. Because the excluders prevented workers from physically dragging drones from the hives, WC drones that workers attempted to evict may have been abandoned at the queen excluders. If these drones crawled back onto the combs and survived, this could have inflated our estimates of WC drone acceptance. However, we observed a total of only nine tagged drones that were drug to the queen excluders, but eventually gained acceptance into the colonies. Excluding these nine individuals from our analyses did not alter any of our results. The similar acceptance rates for DC and WC drones were therefore unlikely to have been an artifact of our experimental design.

For both the DC and WC drones, accepted individuals were slightly, but significantly heavier than rejected individuals, suggesting that drone weight influenced acceptance by workers. However, the influence of drone size and weight on acceptance decisions is unclear, because rejected DC drones were heavier than accepted WC drones. Taken

together, our results for immediate interactions suggest that workers can discriminate among drones based on physical condition and these discriminations may be influenced partly by drone size. However, none of the factors examined was unequivocally associated with the likelihood of acceptance, nor were they the sole determinants for acceptance decisions.

Several aspects of caste interactions examined for our focal drones (i.e., percentage of drones receiving trophallaxis/20 min; percentage receiving vibration signals/20 min; percentage of time spent receiving trophallaxis) did not differ between DC and WC individuals, suggesting that workers did not discriminate among drones after they were accepted into the colonies. However, whenever differences were observed between the two drone types, the trend was toward increased interactions with WC drones. These differences were especially pronounced for the percentage of drones receiving grooming/20 min, the percentage of time receiving grooming, and the percentage of time receiving all interactions combined. Even for interactions that were not significant, such as the percentage of drones receiving trophallaxis and vibration signals, slightly more WC drones were recipients than DC drones. These trends were consistent with the hypothesis that workers allocate caste interactions to favor smaller drones that potentially have developmental deficiencies, to increase the total number of adult males that will be competitive at DCAs. However, if this hypothesis is correct, it is unclear why we did not find significant differences between WC and DC drones for trophallaxis. Trophallaxis provides virtually all nutrients necessary for drone sexual maturation and flight muscle development (Hrassnigg and Crailsheim, 2005), and is therefore, the most direct way workers can influence drone condition. If the function of caste interactions is to promote the development of a greater total number of competitive males, and if workers can enhance the success of WC drones through increased trophallaxis, we expected to see trophallaxis directed more strongly toward WC drones compared to DC drones. Perhaps we would have observed greater differences between the two drone types if we had monitored more individuals for longer periods. Nevertheless, the available data suggest that when interacting with drones of potentially differing quality, workers tend to preferentially direct at least some beneficial interactions toward smaller males, in a manner that might help to promote development and contribute to increased colony reproductive output. Although we examined pronounced differences in size that arise from males being raised in drone or worker cells, there is also subtler variability in adult drone size that arises from naturally occurring variation in the size of drone cells (Seeley and Morse, 1976). A tendency to direct care toward smaller drones may, therefore, play an important role in increasing total colony reproductive output under typical conditions in which the vast majority of males are reared in drone cells.

For both the DC and WC drones we examined, individuals receiving vibration signals were slightly more likely to engage in trophallaxis and grooming during the 20-min observation periods than were non-vibrated males. These differences were not statistically significant, although they approached significance. In contrast, Boucher and Schneider (2009) reported that vibrated drones spent significantly more time receiving trophallaxis and grooming during 20-min periods compared to age-matched, non-vibrated controls. Direct comparison between our results and those of Boucher and Schneider (2009) may not be warranted because of methodological differences. Whereas, Boucher and Schneider (2009) monitored drones for 20 min after receiving vibration signals, we monitored randomly selected drones, only some of which received vibration signals and then at any time during the 20-min observation periods. The fact that we still found a trend for the vibration signal to be positively associated with increased worker interactions is consistent with the hypothesis that the signal enhances caste interactions in honey bees (Boucher and Schneider, 2009; Stout et al., 2011). We did not find differences in the behavior of vibrated WC and DC drones. Thus, if the vibration signal is associated with increased caste interactions, these associations are not strongly influenced by drone type.

Caste interactions may be influenced by drone larval condition (Woyke, 1963; Sasaki et al., 2004), naturally occurring differences in the thorax-to-body mass ratio of adult drones (Slone et al., 2012), and the gross differences in drone size examined in this study, which resulted from the rearing environment. However, it is unknown how workers might detect variations in drone physical condition. Differences in drone state could be reflected in cuticular hydrocarbon profiles, as has been suggested for some ants and stingless bees (Borges et al., 2012; Smith et al., 2012). It has also been proposed that drones may produce a pheromone that influences caste interactions (Omholt, 1988). Similarly, honey bee queens produce a pheromone blend that varies with mating behavior and reproductive potential, which alters worker-queen interactions (Richard et al., 2007; Kocher et al., 2009). Thus, worker-drone interactions may provide a good system for exploring the assessment and influence of “reproductive quality” on caste interactions in honey bees. Furthermore, studying worker-drone interactions may have several advantages over studying worker-queen interactions. Typically, only 2–12 adult virgin queens are present in a colony for relatively brief periods, which limits the sample sizes that can be obtained for queens experiencing the same colony environment (Gilley and Tarpay, 2005; Schneider and DeGrandi-Hoffman, 2008). Also, relatedness may influence worker-queen interactions, potentially confounding any effects of queen quality on worker behavior (Breed et al., 1994; Schneider and DeGrandi-Hoffman, 2003; Tarpay et al., 2004). In contrast, drones can be produced by the hundreds or

thousands per colony throughout the spring and summer. Because drones develop from unfertilized eggs, they are equally related to all their sister workers. Drones may, therefore, circumvent several of the limitations associated with studying worker–queen interactions and provide a robust system for investigating the role of caste interactions in colony-level reproductive decisions.

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