Introduction

Social hierarchies develop in a variety of different species and are especially important for the functioning of colonies or societies. Social hierarchies can take a variety of different forms, including linear (each individual has a specific rank, such as a pecking order in chickens), despotic (one individual is "despotic" or dominant over all others), and shared (a few individuals share dominance over the others). Most models for the formation of various hierarchies rely on the advanced cognition of individuals to drive social dynamics. However, researchers have observed these hierarchies developed in low-cognition insects. Additionally, while attributes such as size or weight may affect an individual's social status, it seems that these attributes do not account for 100% of dominance outcomes or hierarchy formations (Chase et al. 2011). What are the mechanisms or behaviors which help to form these hierarchies, and how does an individual's behavior affect its social status?

Creatures without advanced cognition such as *Harpegnathos saltator*, commonly known as Indian jumping ants, have been shown to develop a shared hierarchy in the absence of a queen, which determines who takes over reproductive duties for the colony (Sasaki et al. 2016). After a queen is removed from a colony, the non-reproductive workers enter a weeks-long tournament where they compete for dominance, resulting in a few dominant workers and the rest subordinate. The dominant workers, called gamergates, develop reproductive capabilities and take over the egg-laying duties for the colony (Sasaki et al., 2016). Sasaki et al. (2016) have shown that there are three distinct behaviors observed during hierarchy tournaments: antennal dueling, dominance biting and physical policing. Antennal dueling is defined as two or more ants butting heads/antennae (the action is reciprocated by each ant involved) (Liebig 1998). There is no obvious winner; neither ant runs or crouches submissively and it is suspected that dueling results in winner-winner effects where each ant show increased dopamine which is associated with dominance (Sasaki et al., 2016). Dominance biting is defined as a parallel bite followed by one or more downward jerks (Liebig 1998). This is hypothesized to have a winner-loser effect; the biter may increase its social status while the loser may drop in social rank (Sasaki et al., 2016). Physical policing is defined as a perpendicular bite and hold. The biting ant usually jumps to bite its victim (Liebig 1998). It is suspected that subordinate individuals will police more dominant individuals, resulting in a drop in social rank of the victim (Sasaki et al.,2016). While Sasaki et Al. have theorized and modeled the effects of these interactions, there is little known about how these behaviors actually impact the hierarchy formation of *H. Saltator* colonies, and at the individual level, how an individual reproductive status at the end of a hierarchy tournament is influenced by how it interacts with other ants.

The goal of this experiment is to determine how the dominance behaviors (dueling, dominance biting and policing) of workers during the establishment of a hierarchy affect the reproductive status of a worker. We hypothesize that each of these behaviors has some influence over the final reproductive status of a worker. We predict that workers which duel more often are more likely to become gamergates. We also predict that workers which are policed more often are less likely to become gamergates.

We also hypothesize that the ratio between bites received (both dominance and policing) and dueling frequency influences the final reproductive status of a worker. We predict that the higher this ratio (i.e. more bites received and less duels performed), the lower the worker’s likelihood of becoming a gamergate.

Methods

In this experiment, we set up a colony of thirty non-reproductive *Harpegnathos saltator* workers. Each ant was painted with a unique color code so that individuals can be tracked and distinguished from one another. The colony has a 2:1 ratio of inside workers (younger) to outside workers (older). The colony was filmed continuously over several weeks. It was kept at a consistent temperature and was fed regularly.

We measured six different variables during this experiment. For each individual ant, we recorded information about its engagement in each of the following behaviors: antennal dueling, receiving a dominance bite, performing a dominance bite, receiving policing and performing policing. Antennal dueling was recorded when two or more ants butt heads/antennae and the action is reciprocated by each ant. Dominance biting was defined as a parallel bite and jerk motion. The ant being bit receives dominance, and the ant biting performs dominance. Policing was defined as a perpendicular bite with a jump or hold. The ant being bit receives policing, and the ant biting performs policing (Liebig 1998). We recorded the *presence or absence* of dueling or dominance in an individual in *one minute every hour* and recorded the *presence or absence* of policing events in 10 minutes every hour. To do this, we randomly selected one minute from each hour (min 1 to min 50) to observe and code behaviors. We also determined the reproductive status of the ants after filming was complete. We did not measure which partner(s) an ant engages in a behavior with. Also, we did not measure the number of times an ant duels or performs or receives dominance; only the presence or absence of the behavior in one minute each hour.

For analysis, dueling proportion was calculated to estimate the number of antennal dueling events an ant is engaged in compared to the total number of events it is (actively) engaged in. We summed the number of observation periods in which an ant was recorded dueling throughout the duration of filming, which provided an estimate of the amount of times each ant engaged in antennal dueling (duel count). This is a proxy of an ant's dueling behavior; we are not coding behavior over the entire filming period but rather a representative sample. We also summed the total number of observation periods each ant is recorded engaging in each behavior as well (dominance bite performed, dominance bite received, policing performed, and policing received) for use in analysis. We divided the duel count by the total number of recorded behaviors *performed* to estimate the frequency or proportion of time which an ant engages in antennal dueling. This metric, which we will call dueling proportion, is useful in estimating how ants spend their time and whether it has any relationship to their final reproductive status.

In order to determine which ants were gamergates at the end of the recording period, a number of scans were taken over the course of a month in which each ant was observed for a number of different behaviors over a 15-minute period. The scans were taken during a period of social stability during which dominance activity is low. The behaviors observed included positive signals: slow movement, zone and high posture, which are signs of gamergates. During periods of social stability, gamergates stay close to the brood (zone), are relatively inactive (slow) and may walk through the colony with their bodies raised and gaster lifted horizontally (high posture). Non-reproductive workers do the opposite (Fast movement, away from brood, and low posture) (Liebig 1998). We also observed whether the ant had laid an egg or dueled in the observation period; ants holding eggs have recently laid and therefore must be gamergates. These behaviors were coded for *presence or absence* in the 15-minute period. To determine which of the ants were gamergates, I set up testing criteria. If, in a certain scan, it was observed to show at least 2 positive signals, at most 1 negative signal and dueling, OR if it had laid an egg, gamergate status was specified for that scan. For each ant, I summed the number of scans in which it was specified to be a gamergate. I considered each ant that passed the test at least 3 times (3 separate scans) to be a gamergate. Those that did not meet these criteria were considered to be non-reproductive. These categorizations are useful in analyzing the behavior of individuals during the tournament and how that affected their reproductive status.

By separating the data into these two groups, I can analyze the differences in behavior of the two groups during the hierarchy tournament, before they were clearly reproductive or non-reproductive. This may reveal patterns of behavior which future gamergates exhibit, or behavioral criteria for either becoming a gamergate or remaining non-reproductive.

Results

We hypothesized that the dominance behaviors of workers of *Harpegnathos saltator* during a hierarchy tournament influences their final reproductive status at the end of the tournament. To test this hypothesis, we subset the data by reproductive status (gamergates and non-reproductive workers) in order to observe differences between the groups. We predicted that workers which are policed more often are less likely to become gamergates. Figure 1 does not support these predictions; gamergates are not more likely to be policed than non-reproductive workers.

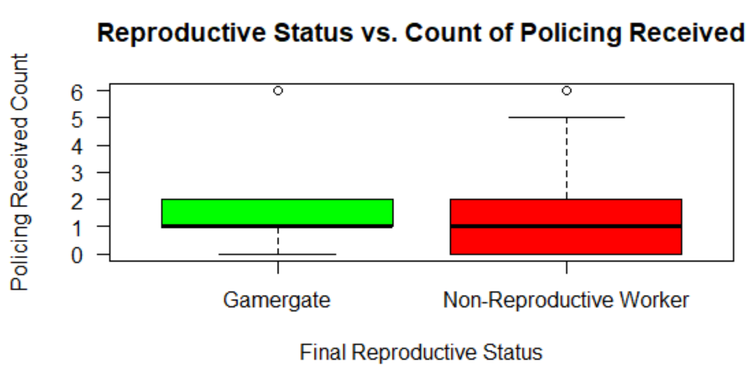

Figure 1: Boxplots of the count of policing received for both gamergates (n=5) and non-reproductive workers (n=25). A Mann-Whitney test (two-sided) did not reject H_0 (p-value of 0.4472, α=0.05); the two distributions are identical.

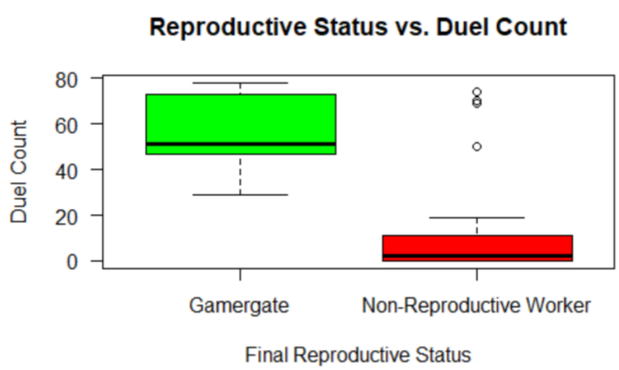

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Figure 2: Boxplots of the count of dueling performed for both gamergates (n=5) and non-reproductive workers (n=25). A Mann-Whitney test (two-sided) reject H_0 (p-value of 0.00456, α=0.05); the two distributions are not identical.

We predicted that workers which duel more often are more likely to become gamergates. Figure 2 shows that future gamergates duel more often than non-reproductive workers, which supports this prediction. We also see that there are a few non-reproductive workers which duel much more often than other non-reproductive workers. This is unexpected and it implies that dueling count is not the only predictor of gamergate status.

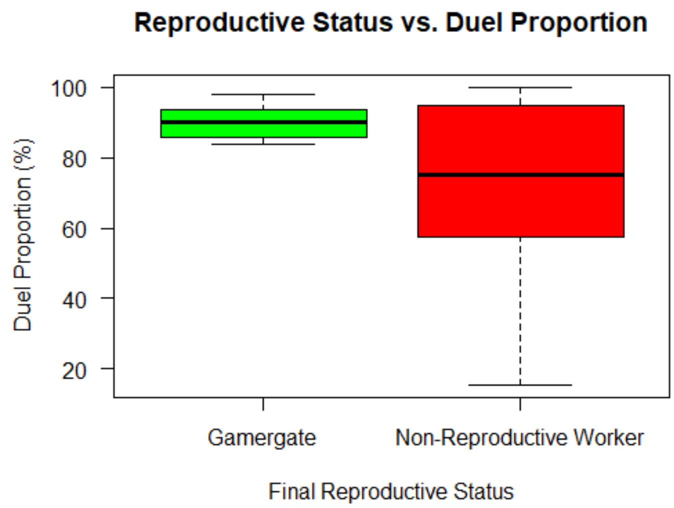
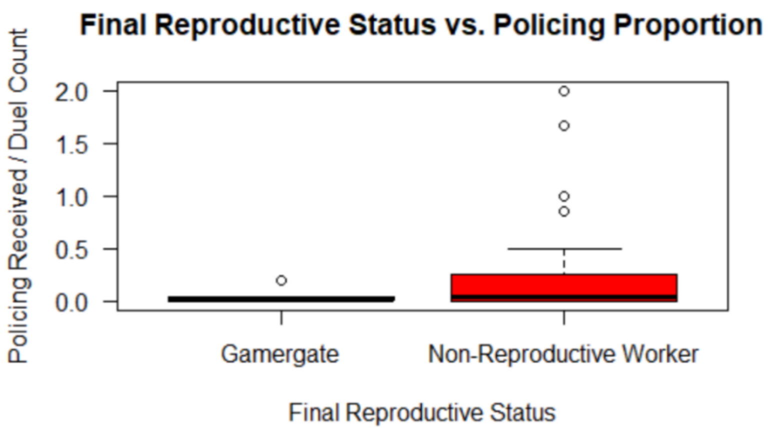
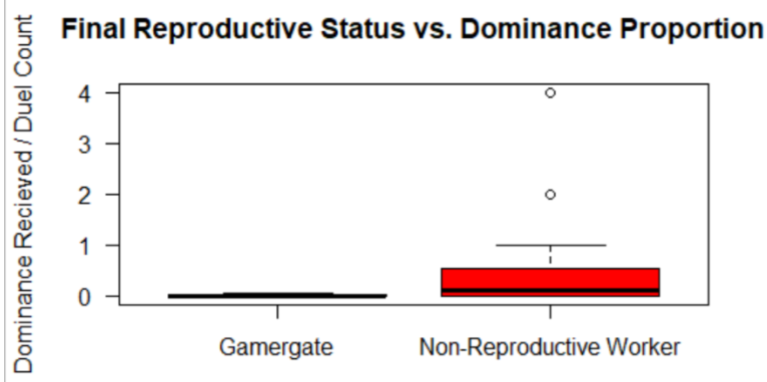


Figure 3: Boxplots of the dueling proportions of both gamergates (n=5) and non-reproductive workers (n=25). A two-mean t-test (unpaired and one-tailed) revealed that the mean duel proportion of gamergates is significantly higher than that of non-reproductive workers, rejecting H_0 (p-value=0.01367, α=0.05).

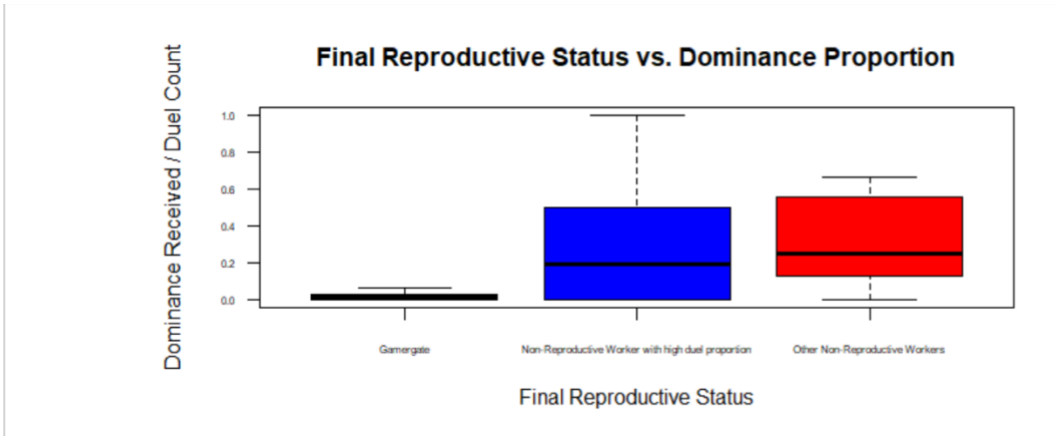
Duel counts may not accurately reflect workers' time budgets, so I plotted duel proportions (displayed in percent) for each group. Figure 3 shows that gamergates spend most of their time dueling, which supports our prediction. It also shows that some non-reproductive workers duel as much as gamergates.

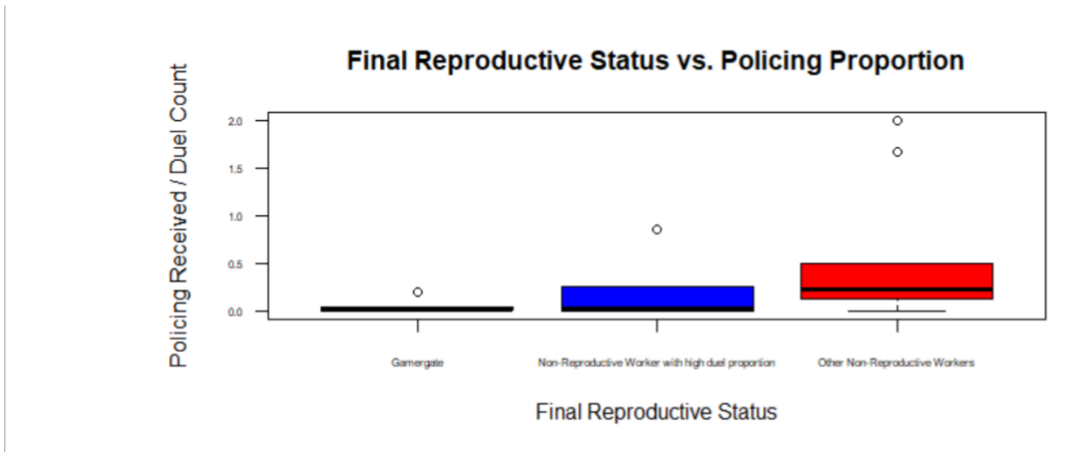




Figures 4 and 5 (left to right): Side-by-side boxplots of the policing and dominance proportions (respectively) of both gamergates (n=5) and non-reproductive workers (n=25). A two-mean t-test (unpaired) revealed that the mean proportions of both metrics is significantly lower for gamergates than for non-reproductive workers (p-value = 0.02782 for policing proportion, p-value = 0.01705 for dominance proportion, $\alpha=0.05$), rejecting H_0.

We had also hypothesized that the ratio between bites received and duel count influences a worker's gamergate status after a hierarchy tournament. We predicted that the higher this ratio, the less likely a worker is to become a gamergate. To calculate these proportions (policing proportion and dominance proportion), I divided the counts of policing and dominance received by duel count for each ant. These data are plotted in figures 4 and 5 respectively. These results support our predictions; the distribution of bite ratios for non-reproductive workers is much more right-skewed than that for gamergates.





Figures 6 and 7 (top to bottom): Side-by-side boxplots of the policing and dominance proportions (respectively) of gamergates (n=5) , non-reproductive workers with high dueling proportion (n=6), and other non-reproductive workers (n=19). A two-mean t-test (unpaired) revealed that the mean proportions of both metrics were not statistically different for gamergates than for high dueling non-reproductive workers (p-value = 0.3715 for policing proportion, p-value = 0.1343 for dominance proportion, α=0.05), not rejecting H_0.

Since there were a few non-reproductive workers with much higher dueling proportions than other non-reproductive workers, I redid the above analysis with those ants in a separate group ("Non-reproductive workers with high dueling proportions"). An individual was considered to be in this group if their dueling proportion was within or above the range of dueling proportions of the gamergate group (dueling proportion >= 0.8392857).

These results imply that workers with high duel proportions, but high dominance and policing received proportions are far more likely to be non-reproductive. This aligns with the hypothesis of Sasaki et al., 2016 that dominance bites result in a loss of social status for the victim.

Discussion

Our results have, for the most part, supported the predictions made. Figures 2 and 3 show that the amount and/or frequency of antennal dueling seems to be strongly related to a worker's gamergate status at the end of a hierarchy tournament. Gamergates were shown to duel more than non-reproductive workers. This may indicate that dueling increases an ant's dominance status, making it more likely to become a gamergate. The non-reproductive distribution in Figure 2 has a few high outliers (workers who duel a lot who are non-reproductive). This may indicate that dueling is not the sole factor which affects reproductive status. However, it could mean that some ants with very high duel counts truly are gamergates but were not observed to pass the gamergate criteria in the scans aforementioned (since we only sampled small time-frames) and instead were identified as non-reproductive workers.

Figure 1 contradicted our prediction that the more policing bites an ant received, the less likely it was to be a gamergate. The median number of bites received (1) is the same for both distributions, and the distributions were not statistically different. The distribution of bites for non-reproductive workers is much more right skewed though. This could suggest that workers can only tolerate a certain number of policing bites before they cannot become gamergates, but the outlier of 6 bites in the gamergate distribution makes this interpretation inconsistent. It seems that there is not a relationship between the count of policing bites an ant receives and its reproductive status.

Figures 4 and 5 also support our predictions. Non-reproductive workers have a higher ratio of policing/dominance bites received to duel count than gamergates, suggesting that the lower this ratio, the more likely an ant is to be a gamergate. It may be that ants which are more active are likelier to be bit, but ants which can "get away" with dueling and avoid bites are much more likely to become gamergates. Another potential explanation is that some ants may tolerate more bites than others, and those who continue to duel after being bit are likelier to become gamergates. However, the data is not detailed enough to confidently conclude this. These data were gathered in an observational study rather than an experiment, so we can only conclude correlations between the variables mentioned. These results support the findings of Sasaki et al. (2016); the behaviors of *H. Saltator* during hierarchy tournaments seem to strongly influence the formation of shared hierarchies in the colony. The simplicity of these behaviors suggests that advanced cognition is not necessary for forming complex social hierarchies.

References

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