AVOIDANCE BEHAVIOR IN TEMNOTHORAX RUGATULUS ANT COLONIES

By

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**ABSTRACT**

Inactivity in social ants is more common than most people suspect. There is no clear answer as to why colonies have so many inactive workers, but it may be that inactive ants are selfishly avoiding work and diverting colony resources toward their own reproduction, rather than contributing to colony fitness. Indeed, it has previously been shown that inactive workers have more developed ovaries than active workers in the ant *Temnothorax rugatulus*. One possible consequence of worker reproduction is that the behavior of active ants and inactive ants towards each other may be altered, such that inactive workers may be avoiding active workers, either to avoid detection or punishment. Active (activity levels is 50% greater than colony average) and inactive (inactivity levels is 50% greater than colony average) workers were then restrained within the nest to determine if active ants are avoiding inactive ants by comparing the distances between restrained ants and their nest mates to the baseline distances established earlier. Results show no significant differences in the distances that active and inactive ants keep from each other. The results mean that the distance between workers is not related to their activity levels.

**INTRODUCTION**

Inactivity is very common among members of social insect colonies. In ant or bee colonies, a high proportion of workers spend most of time motionless (Lindauer 1952; Otto 1958; Herbers 1983; Schmid-Hempel 1990). In fact, workers in *Leptothorax allardycei* spend 55% of their time not doing any recognizable work (Cole 1986). It seems logical to presume that some workers do not do any useful work to increase colony fitness. There have been several reasonable hypotheses that have attempted to explain why a large number of workers spend their
time doing nothing. Inactive ants are often assumed to be a “reserve worker force” (Lindauer 1961; Michener 1964; Evans 2006) for the colony. Lindauer and Michener suggested that the inactive ants serve as a stand by force that can become active if there are unexpected contingencies such as gathering food or protecting the colony from predators. The removal of workers increased the activity of the ants that remained the colony (Winston and Fergusson 1985; Wilson 1986). However, this hypothesis has not been tested further to see if inactive workers are lazy due to receiving less food than active workers. Moreover, another hypothesis pointed out by Oster and Wilson (1978, p. 285) revealed that lack of resources or high proportions of workers is more advantageous for low working tempo than high working tempo by looking into working tempo of individual workers. Also, a similar hypothesis based on foraging dependent mortality by Houston et al. (1988) suggested that an increase in the mortality rate decreased the maximum rate collection of resources so individual workers should perform less work which agrees with empirical data for honey bees (Schmid-Hempel et al. 1985; Seeley 1986; Schmid-Hempel 1987). Another hypothesis is that inactive workers spend their time being inactive due to incapability because inactive ants makes more mistakes in accomplishing their tasks in the colony so the ants are undependable to complete tasks. The reliability theory predicts that a large numbers of working in a large colony can afford to be a little incompetent because of highly redundant in the organizations of tasks (Oster and Wilson 1978; Herbers 1981).

There are inconsistencies with these hypotheses. Oster and Wilson assume that low individual competence cause energy loss and it is possibly be the result of worker’s canceling each other’s work (Schmid-Hempel 1990). Leonard and Herbers agree that a negative relationship between competence and tempo should not hold (Leonard and Herbers 1986). The reliability theory can allow the individual competence to be considered separately (Schmid-
Hempel 1990). The proportion of time spent inactive shows that work load increases with colony size and the proportion of ants that perform self-grooming decreases with colony size (Schmid-Hempel 1990) so there will be a contradiction if one considers inactivity to be a form of incompetence. It will also be difficult to assume inactive ants serve a reserve force when workload increases with colony size (Lindauer 1961; Michener 1964). Also, the hypothesis by Houston et al is based on a model where competition and conflicts over reproduction and work level do not happen (Schmid-Hempel 1990).

The answer to why a high amount of workers are inactive in social insects is not really clear but it may be that inactive workers are selfishly avoiding work and use colonies resources toward their own reproduction rather contributing to colony fitness (Stroeymeyt et al 2007; Schmid-Hempel 1990). Also, there is evidence that workers’ oocyte development positively correlates with inactivity in *Bombus impatiens* (Jandt and Dornhaus 2011). Moreover, there is proof that workers that have high inactivity levels in the ant *Temnothorax rugatulus* have more oocytes in their ovaries which indicates that inactive ants have greater potential to reproduce (Hillis et al in prep).

One possible consequence of worker reproduction is that the behavior of active ants and inactive ants towards each other may be altered, such that inactive workers may be avoiding active workers, either to avoid detection or punishment. Spatial ordering in colonies influences division of labor in many social insects (ants: *Odontomachus brunneus*: Powell & Tschinkel 1999; *Temnothorax unifasciatus*: Sendova-Franks & Franks 1995; *Pheidole dentata*: Wilson 1976; wasps: *Ropalidia marginata*: Robson et al. 2000; bees: *Apis mellifera*: Seeley 1982).

Individuals in social insect colonies keep spatial fidelity zones (Sendova-Franks & Franks 1995) which are areas that is specific to certain individuals. Workers may lessen the distance to move
between tasks by staying in a specific place in a colony (Wilson, 1976 and Seeley, 1982). Workers readopt special fidelity zones even if the whole colony migrates to a new nest (Sendova-Franks and Franks 1994). Workers in the periphery of the nests are more active than those in the center of the nest but the amount of activity changed seasonally (Sendova-Franks and Franks 1995). The workers that are stay on the periphery of the nests can be assumed to be more active than those in the center (Franks et al. 1990). Also, foragers in the *Bombus impatiens* tend to stay in the periphery of the nest (Jandt and Dornhaus). Workers in *Leptothorax unifasciatus* only do tasks that are within their spatial fidelity zone (Sendova-Franks and Franks 1995). Moreover, workers that perform brood care can be found in the center of the nest (ants: *Odontomachus brunneus*: Powell & Tschinkel 1999; *Temnothorax unifasciatus*: Sendova-Franks & Franks 1995; wasps: *Ropalidia marginata*: Robson et al. 2000)

The aim of this study was to find out whether active and inactive ants have specific spatial relationships to each other in *Temnothorax* ants. I test the hypotheses that (0) Distance between workers is not related to related to their activity levels; (1) active ants have closer interaction than inactive ants; (2) inactive ants have closer interaction than active ants. If distances between ants seem to relate to activity level, I will investigate the mechanism for this by testing the following additional hypotheses: (3) neither ant type avoids the other type of worker, but differing distances are the results of spatial fidelity differences among active and inactive ants; (4) active ants avoid inactive ants; (5) inactive ants avoid active ants. I will separately test whether (6) both active ants and inactive ants keep distance away from each other. I will use both unmanipulated colonies and a manipulation in which an ant of known activity status is tied to a fixed place in the nest (thus preventing that individual from avoiding other ants). Table 1 gives the hypotheses and reasons for the predictions.
METHODS

Field Collection and Culture methods

Two colonies of *Temnothorax rugatulus* were collected on 7 September, 2013 from Mount Lemmon (Sta Catalina Mountains, AZ, USA). The two colonies were found under rocks crevices with moss on them. The queen and brood were collected using an aspirator. The two colonies were transferred to lab and were temporarily housed in a cavity made from a pair of large transparent microscopic slides with a small piece of cardboard between the slides. The colonies were given water and honey water upon arriving in lab. The colonies were permanently housed in cavity made from one large transparent microscopic slide (top) and a cardboard (bottom) that was the same size as the large microscopic slide. The internal dimensions of the cavity were 35x50x1mm (width x depth x depth). Tape was used to hold the nest together. Each nest was later on placed in its own container (10 cm x 10 cm) and the sides were coated with fluon to prevent ants from climbing the walls. Nine holes small holes were made in the cardboard in each nest to tie the ants. Green ceramic stones were placed in the container to allow the ants to build a wall around their nests. The temperature in the lab was maintained at 24°C. The ants were fed freeze-killed *Drosophila*, honey solution at least once a week (Dornhaus et al 2009) and water. Each colony had 1 queen. Colony 1 had 25 workers and colony 2 had 31 workers. The rest of the workers were neither active nor inactive. Both colonies contained brood.

Marking and Recording

Individual workers were marked with unique color combinations of lacquer based paint spots, 1 on the head, 1 on the thorax and 2 on the gaster (after the method in Sendova-Franks & Franks 1993). These unique colors helped to differentiate individual workers. Each ant was placed into a slit in a makeup sponge to keep the ant motionless. The individual workers are
marked while the ants were anaesthetized and carbon dioxide was used to knock the ants’ unconscious for about 10-15 minutes while still in the sponge to keep the ants from immediately removing the paints on them. The painted ants were all placed in different container so they would smell the same and be less likely to remove their paints. Colony 1 and Colony 2 were marked on 20 October and 21 October 2013 respectively. White, orange, light blue, dark blue, green and yellow were the colors used to paint the ants.

The two colonies were filmed on 28 October 2013. Ants were filmed in the late afternoon and the cardboard in the nest provided a good contrast and made analyzing the videos easier. The videos were analyzed by keeping track of the time spent by each ant in each of the following behavioral states: building, foraging, brood care, self-grooming, grooming others, other grooming, wandering inside, wandering outside, trophallaxis and inactivity. An ant was said to be building when it is either moving stone or bulldozing and classified to be foraging when it is inside small lid where honey water and drosophila are, or when it is on the cotton for water. Similarly, an ant was counted as doing brood care when the ant starts manipulating the brood and counted as self-grooming when the ant starts grooming itself. An ant was thought to be grooming others when it is grooming other ants and other grooming when another ant is grooming the focal ant. In addition, an ant was classified as wandering inside when it is moving inside the nest and not doing anything. Ants were classified as wandering outside if an ant is outside field of view. Lastly, an ant was scored as doing trophallaxis when it is sharing food with other workers and an ant was considered as inactive when it was immobile and not doing anything for at least 10 seconds. The following tasks counted as active: building, foraging, brood care, self-grooming, grooming other, other grooming, feeding, and trophallaxis.
Ants were determined to be active when their activity levels are 25% greater than colony average and inactive when their inactivity levels are 25% greater than colony average. This means that if the average ant is active 35% of the time, then any ant that is active for more than (35+0.5*35) = 52.5% of the time was counted as active. There were 6 active ants and 9 inactive ants in colony 1 and colony 2 had 9 active ants and 8 inactive ants. Colony 1 had an average activity of 293 and an average inactivity of 673. Colony had an average activity of 312 and an average inactivity of 539.

**Control and Treatment Groups**

The control groups are the colonies that were filmed in October and were unmanipulated. The control groups are basically the baseline videos. The treatment groups consisted of ants form the control group but had a tied ant at any given time. Ants were tied with a thin copper wire between their thorax and abdomen one at a time. A single strand of thread was attached to the wire so that the restrained ant could move a little. Active and inactive ants were both restrained. Both active ants and inactive ants were tied the day before they were recorded. They were recorded at regular intervals at 3 hours, 7 hours, 8 hours, and 24 hours after they have been tied down in the nest. The restrained ants were released if they were alive after 24 hours and the colonies were allowed to rest for 1-2 days after restraining the ants. One active ant and three inactive ants were tied in colony 1. Also, one active and one inactive ant were tied in colony 2. Ants were tied at positions 1, 2, 3, and 4 (Figure 1) in colony 1 and 5 and 6 (Figure 1) in colony 2.
Measuring and Calculating Ant Distances

Baseline activity levels and relative distances to nearest nestmates for all workers in the colony were established using behavioral observations and a semi-automated tracking tool. The distance to nearest neighbor was found by calculating distance of each possible pair type at 1 minute regular interval in each 20 minutes video (i.e. mean distance when A has a nearest neighbor A vs. mean distance when A has a nearest neighbor I vs. mean distance when I has a nearest neighbor A vs. mean distance when I has a nearest neighbor I) in both baseline and treatment videos. Also, the distance between all ants was found by calculating the mean distance of each possible pair type (so not just nearest neighbor) at 1 minute regular interval for each 20 minutes video. This was also done in both baseline and treatment videos. The mean distances of tied ant to the nearest neighbors were compared to the mean distances in the baseline. Furthermore, a random point was picked and was used to find the distance of the tied ants to the nearest neighbor.

Table 1: Hypotheses and predictions. A represent active ants and I represent inactive. The TA stand for tied active ants and TI stand for tied inactive ants.

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<td></td>
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<tr>
<td>$H_0$: Distance between workers is not related to their activity level.</td>
<td>A-A = I-I = I-A = A-I</td>
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<tr>
<td>$H_1$: Active ants have closer interaction than inactive ants.</td>
<td>A-A&lt; I-I</td>
<td>Active ants tend to move so should have more chance of meeting.</td>
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<tr>
<td>$H_2$: Inactive ants have closer interaction than inactive ants.</td>
<td>I-I&lt; A-A</td>
<td>Active ants move often so should have a greater distance as compared to inactive ants.</td>
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<td>PART B</td>
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Neither ant keeps distance from each other because of spatial fidelity differences.

If active and inactive does not move away from each other, the pattern to be unaffected by one ant being tied to a fixed place.

If active ants tend to move and are unable to do so because they are tied, we should expect the distance between TA-I to be less than the distance between A-I. Also, TA-I can be greater or equal to I-A.

If inactive ants avoid active and are unable to do so because they are tied, we should expect the distance between TI-A to be less than the distance between I-A. Also, TI-A can be greater or equal to A-I.

If active and inactive ants avoid each other, the predictions for H_4 and H_5 should hold.

RESULTS

Control Group

The median distance between active ants in the control group was the same the median distance between inactive ants (Wilcoxon signed-rank test, p value=0.63, n=56; Figure 2). This is different from the prediction of hypotheses 1 and 2. The sample size is the total number of ants in both colonies. Also, the median distance between active and inactive ants was the same as the distance between inactive and active (Wilcoxon signed-rank test, p value=0.63, n=56; Figure 2). This means that the null hypothesis cannot be rejected because there is no strong evidence that suggests that the distance between workers is not related to their activity level.

Treatment Group
The mean distance between the tied inactive ants and active ants in the treatment group was also the same as the mean distance between the inactive ants and active ants in the treatment group (Wilcoxon signed-rank test, p value=0.875, n=6; Figure 3). This is different from the prediction of hypothesis 3 and means that the null hypothesis cannot be rejected because there is no strong evidence that suggests that neither ant keeps distance from each other because of spatial fidelity differences. Moreover, the mean distance between the tied active ants and inactive ants was greater than the mean distance between the active ants and inactive ants in the treatment group (Figure 4). The result does not match the prediction from hypothesis 2.

The mean distance for the baseline tied location (no ant tied there yet) was the same as the mean distance between tied inactive ant and active ants (Wilcoxon signed-rank test, p value=0.875, n=6; Figure 5). This is different from what one would expect because the mean distance for the baseline videos to be greater due the lack of awareness of the ants of the tied ants in the baseline videos. Figure 5 suggests that the null hypothesis cannot be rejected because there was no significant difference between the baseline tied location and the distance tied inactive ant and active ants. The mean distance for the baseline tied location (no ant tied there yet) was the same as mean distance between tied active ant and inactive ants (Figure 6). This suggests that the null hypothesis cannot be rejected.
Figure 1: Tied ants location
Figure 2: The median distances between workers in control group do not differ significantly from one another.
Figure 3: Mean distances between pair types do not differ significantly from the treatments. The links compare the mean distance between tied inactive and active ants to the mean distances of inactive and active ants of the same video.
Figure 4: The average distance between tied active ants and inactive ants was more than average distance between active ants and inactive ants. The links compare the mean distance between tied inactive and active ants to the mean distances of inactive and active ants of the same video.
Figure 5: The mean distance for the baseline tied location (no ant tied there yet) was the same as the mean distance between tied inactive ant and active ants. Active ants changed behavior after an ant was tied by moving away or towards the tied inactive ants.
Figure 6: The average distance of baseline tied location was not significantly different from the average distance between the tied active ants and inactive ants at a random point.
DISCUSSION

The results unequivocally supported the null hypothesis that the distances between ants do not depend on activity state. Hypotheses 1 and 2 were refuted because the median distances between pair types in control group were the same and did not differ significantly from one another. Hypotheses 3 and 4 were not supported because the mean distances between pair types was also the same and did not differ significantly from the treatments. Moreover, hypotheses 5 and 6 were refuted because the mean distance for the baseline tied location (no ant tied there yet) was the same as the mean distance between tied inactive ant and active ants and vice versa.

There are several papers that provide evidence that inactive workers selfishly avoid work and use colonies resources toward their own reproduction (Stroeymeyt et al 2007; Schmid-Hempel 1990). There is also evidence that workers’ oocyte development positively correlates with inactivity in Bombus impatiens (Jandt and Dornhaus 2011). However, this study finds that the assumption that the behavior of active ants and inactive ants may be altered is false because inactive and active ants do not avoid active ants to avoid detection or punishments and that activity levels do not influence the distances between ants. The definition of activity and inactivity could have affected the results. In fact, some ants were both active and inactive when ants were determined to be active when their activity levels are 25% greater than colony average and inactive when their inactivity levels are 25% greater than colony average. This could have been due to effect of wandering. An ant was classified as wandering inside when it is moving inside the nest and not doing anything. Ants were also classified as wandering outside if an ant is outside field of view. Ants that were neither active nor inactive were excluded from all calculations.
Although the mean distance between active ants in the control group was the same as the average distance between inactive workers, it has been found in larger colonies that the mean distance between active ants is significantly different from the distance between inactive ants (Charbonneau and Dornhaus 2014 in prep). In that study, 5 minutes videos were taken of ant colonies instead of 20 minutes videos and active ants were classified to be active if they were among the 25% most active ants or inactive if they were among the 25% most inactive ants. More colonies and tied ants are needed to see if distances between ants do depend on activity state.
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