

Examination of Food Preference and Aggressive Behavior in Nest Cohabitation among Ponerines

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ABSTRACT

Environmental stress such as competition is one of the key factors that affecting the behavior of ants. This essay explores food preference and aggressive behavior changes of ponerines (*Harpegnathos venator* and *Diacamma rugosum*) when they were in nest cohabitation. Since there were no variations in stinging time and prey recovery time, our results indicated that cohabitation has no influence on the aggressive behavior of *H. venator*. On the other hand, food preference of *D. rugosum* demonstrated a shift towards fruit, vegetable, and sweet water, with less interest in cherry-red cockroaches and crickets. Although *H. venator* still predominantly prefers cherry-red cockroaches and crickets, their prey contacting time was decreased. These results emphasized the ability of ponerines to adapt their foraging strategy to competition, highlighting the dynamic nature of predator-prey interactions in cohabiting environments.

Introduction

Ants are the preeminent social insects on a global scale, exhibiting unparalleled distribution and diversity (Bourke *et al.*, 2019). Their diet is extensive, encompassing various sources, including but not limited to terrestrial arthropods, larvae of other ant species, plant sap, honeydew produced by insects such as aphids, seeds, and fungi (Hölldobler, 1982; Dejean *et al.*, 1997; Dejean *et al.*, 1999; Blüthgen *et al.*, 2000). Their food foraging can be influenced by both internal factors (nutritional requirements) and external factors (environmental conditions). Specifically, the rapid expansion of ant colonies necessitates the acquisition of high-protein food through predation. Thus, predation behavior represents critical aspect of their survival strategy. In both natural and man-made settings, *H. venator* relies heavily on organisms such as cockroaches and crickets as its primary source of protein for basic survival and larval rearing (Nie *et al.*, 2022). *D. rugosum* is another specie of ponerines with similar body size, and prey on arthropods. However, when encountering interspecific competition, whether their preferred diet will change and lead to temporal resource partitioning, is still unknown (Kronfeld-Schor *et al.*, 1999).

H. venator with venomous stingers is able to paralyze the nerves system of their prey (Maschwitz *et al.*, 1979). This ability was used to evaluate the level of aggressive behavior of ponerine. In addition, the dietary preference shift for both ponerines under different conditions: with competition and without competition, was also measured to evaluate their diet partitioning. An examination of foraging behavior by *H. venator* and *D. rugosum* cohabitation would be a potential revealing example of divergent evolution. Finally, the results of this research could provide reference for artificial cultivation of omnivorous or monophagous ants in an interspecific competitive setting.

Materials and Method

Two colonies of *H. venator* and *D. rugosum* were ordered from the locals in Shaoguan City, Guangdong Province, China. Each colony was introduced into a plaster-acrylic nest with the inner wall coated with a talc powder–ethanol mixture (Ning *et al.*, 2019). The nesting area measured 100*100*25mm, while the active area of ants measured 110*110*85mm. To imitate the cohabitation environment for creating interspecific competition, a common activity area measured 210*110*85mm was placed to connect the two ponerine colonies. Each *H. venator* colony included thirty workers and three queens, while each *D. rugosum* colony consisted of thirty workers. As *D. rugosum* does not have designated queens, each worker is capable of producing offspring (Wheeler *et al.*, 1922). Live cherry-red cockroaches (*Blatta lateralis*) and small crickets (*Gryllus chi-nensis*) were ordered at the local market while apple, cabbage, and sugar were purchased at local supermarket.

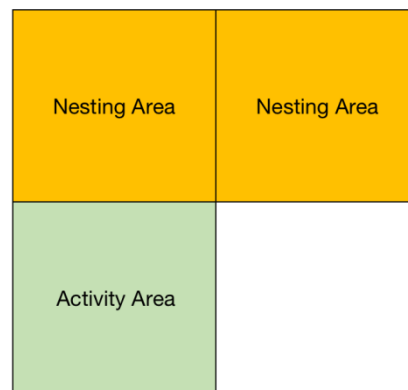


Figure 1. The design of ant nest used in control group.

For control group, *H. venator* and *D. rugosum* colonies were reared in two separate unconnected plaster-acrylic nests throughout the entire experiment, each with 25 individuals (see Figure 1). Four types of food were placed in the activity zones of each nest, namely one cherry-red cockroaches, one small crickets, 5 mL of 10% sugar water, one slice of cabbage and one slice of apple. Video camera (Canon EOS 70D) were placed on top of the nest to record the frequency of contact (measured in second) between the ponerines and the food. The trial was repeated 3 times.

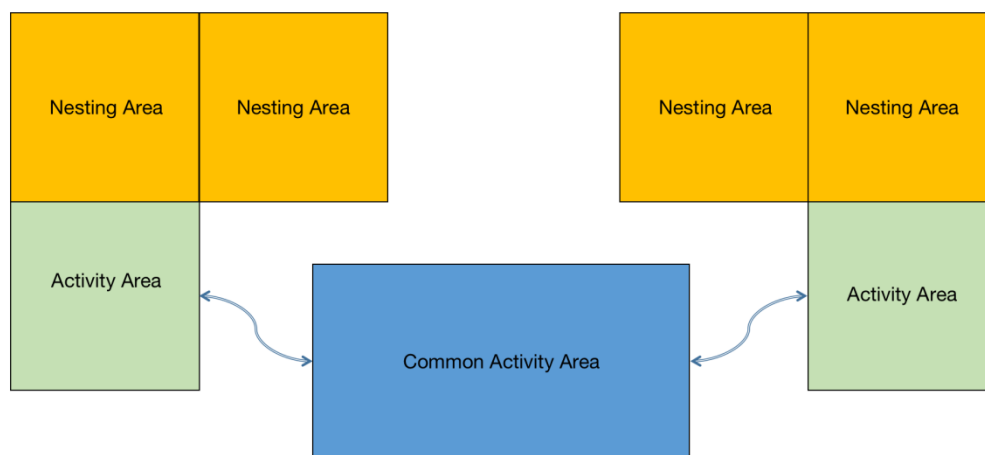


Figure 2. The design of ant nest used in experimental group

For experimental group, *H. venator* and *D. rugosum* colonies were initially placed in a flat plaster-acrylic nests. After one week of settling, the two nest chambers were linked by a rubber tube to a common activity area (see Figure 2), where food was placed to create a cohabiting environment. Four feeding bowls were placed at equal distant locations (2 cm) from the two entrances, containing cherry red cockroaches, small crickets, 10% sugar water, and apple slice. The same amount of food was introduced daily and the number of times the two ant species contacted the food was recorded using video camera. Same treatment as control group was conducted. Prior to introducing the two species of ponerines, three *H. venator* and three *D. rugosum* were placed in a plastic container (without any refuge) to observe whether there was a significant difference in strength between the two species of ponerines and to determine whether the speed of the fatalities caused would allow the experiment to continue.

To determine the level of aggressive behavior among ponerines in isolated and cohabitat environment, two cherry-red cockroaches were placed in the activity area of *H. venator*. After the ponerine captures the cherry-red cockroaches, the timer started to record the *H. venator's* sting time. In the communal nest situation, two cherry-red cockroaches were placed in the communal activity area. After the ponerine captured the cherry-red cockroaches, timing the *H. venator's* sting time. The time difference of stinging measured in two different environments (isolated and cohabitation) was calculated. The trial was repeated thirty times. This part of the research was only done on *H. venator* but not on *D. rugosum* since the latter has no abdominal stinger, thus it cannot capture the prey by stinging.

After all data were measured, the chi square Goodness of fit test (at alpha level, $P=0.05$) was used to examine whether there were significant differences in food choice among ponerines living in cohabiting environments versus those in the control group. For the testing of *H. venator's* aggressive level, *T*-test (at alpha level= 0.05) was used to examine whether there were significant differences in average stinging time among ponerines living in cohabiting environments versus those in the control group.

Results

Observed behaviors of both ponerines in cohabiting environment

Both species of ponerines showed great attention to each other's movements. *H. venator* would quickly shake its abdomen (indicating an impending attack or sensing an upcoming threat) when they noticed the presence of *D. rugosum*, but did not further pounce or attack on *D. rugosum*. Both species exhibited contacting behavior three to four times within five minutes of being placed in a container, but neither attacked. There was no casualty after one day of observation.

Food preference of *H. venator* in isolating and cohabiting environment

Under cohabiting environment, *H. venator* had reduced contact times per individual with cherry-red cockroaches, crickets, and cabbage after the food was introduced for 15 minutes. However, *H. venator* had a slight increase in contact times per individual with 10% sugar water. Apple slices were not touched at all in both environments (Figure 2).

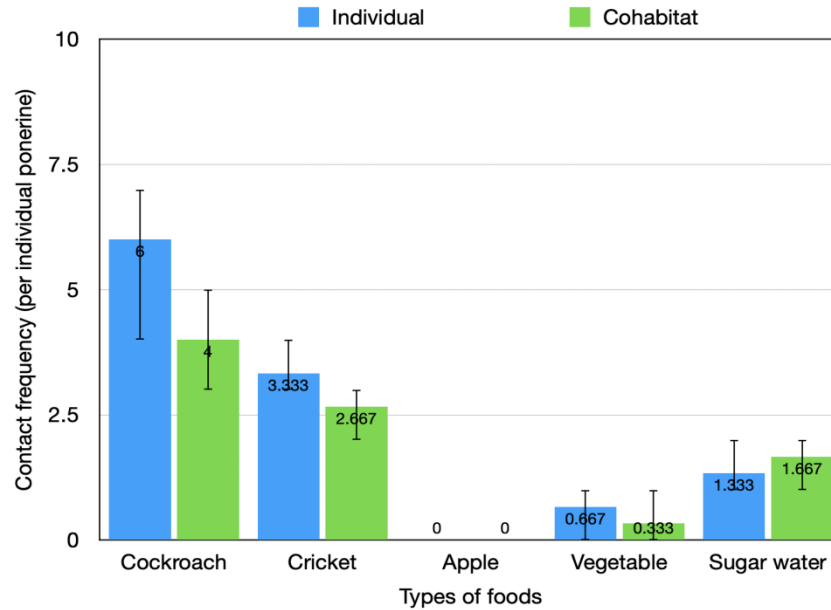


Figure 3. Contact frequency of *H. venator* under isolated nesting and cohabiting environment

Impact of prey preference of *D. rugosum* under cohabiting environment

Under cohabiting environment, *D. rugosum* had significantly reduced contact times per individual with cherry-red cockroaches and crickets. *D. rugosum* had a slight increase in contact times per individual with apple slices and significantly increase in contact times per individual with cabbage and 10% sugar water (Figure 3).

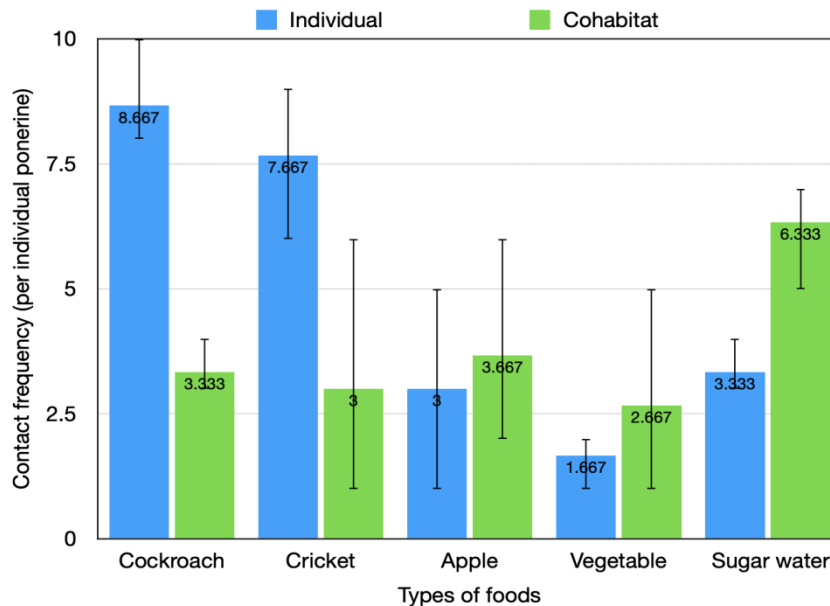


Figure 4. Contact frequency of *D. rugosum* under isolated nesting and cohabiting environment

Impact of stinging time of *H. venator* under cohabiting environment

The stinging time of *H. venator* under isolated nesting environment (5.8 sec) did not significantly differ from that under cohabiting environment (5.7 sec) (Figure 4; Two-sample T-test = 0.3644, $p = 0.7169$).

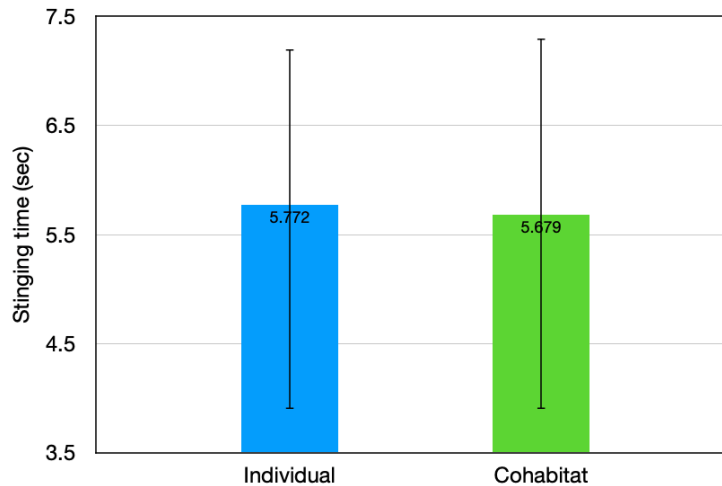


Figure 5. Stinging time of *H. venator* under isolated nesting and cohabiting environment

Discussion

The validity of these experiments is based on the fact that the two ant species do not have a mutually inhibitory relationship. Although *H. venator* possesses an abdominal stinger, it does not have the ability to penetrate the hard armor of *D. rugosum* (according to the observations made the day before the experiment).

The findings of this research demonstrated that in a cohabiting environment, the prey preference of *H. venator* remains unchanged, and the stinging time remains consistent when compared to colonies living alone. *H. venator* still preferred live insects over other food resources, similar to the feeding habits of *H. saltator* (Shivashankar *et al.*, 1989). The lack of prey preference shifts in cohabiting environments indicated that *H. venator* exhibits strong innate foraging instincts or maintains territories that prevent significant overlap with neighboring colonies (Scharffetter *et al.*, 2016). The consistent stinging time could be attributed to the uniform size of cherry-red cockroaches provided. As the amount of venom required to effectively paralyze the prey does not vary significantly based on its size, there is no need for *H. venator* to adjust its stinging behavior and thus avoids wasting additional energy (Helfman, 1990). This consistency suggested the ability of *H. venator* to maintain its ecological niche and adapt to the shared environment.

On the contrary, the prey preference of *D. rugosum* has shifted when cohabitated with *H. venator*. *D. rugosum* shows a greater inclination towards vegetable matter, fruits, and 10% sugar water, other than cherry-red cockroaches and crickets. By shifting its focus towards less contested resources, *D. rugosum* aims to optimize its foraging efficiency and reduce direct competition (Yoshimoto, 2009). These results indicated the ability of *D. rugosum* to adapt its foraging strategy in response to competition, and allowing for niche differentiation (Peiman *et al.*, 2010).

This research offers insights into the intricate mechanisms that govern social insect behaviors. The behavioral dynamics of *H. venator* and *D. rugosum* in cohabitation scenarios broadened our knowledge of social insect interactions. Understanding their adaptations could provide valuable insights into the ecological interactions and coexistence strategies of ant communities. Further research could explore the mechanisms underlying the observed food

preference shift in *D. rugosum* and the observed stability in prey preference and sting time in *H. venator*. Investigating chemical communication, pheromone signaling, and territorial behaviors may help elucidate the specific factors driving these behavioral changes. Additionally, studying the long-term effects of resource competition on the population dynamics and foraging strategies of *D. rugosum* and *H. venator* can contribute to a more comprehensive understanding of divergent evolution.

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