The influence of colony density, temperature and illumination intensity on the aggregation of fire ant, *Solenopsis invicta*

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Abstract: Aggregation plays a basic role in the organization of social insects, and many factors including environment and individual interaction can influence this behavior. In this study, we investigate the influence of changes in individual density, temperature and illumination intensity on the aggregation time of *Solenopsis invicta*. Population density has no influence on the aggregation time until it reached 1.5 individuals/cm^2. Additional results also showed that temperature has negative effect on the aggregation time of fire ant. Along with the fall of temperature, the speed of aggregation increased rapidly. Fire ant required 96.5min to get robust aggregation under strong light intensity (1200lux); which was faster than under low light conditions (10lux). These results revealed that fire ant can shift their cluster behavior under changing environmental conditions.

Key words: fire ant　gather behavior　environment　interaction

1 Introduction

Aggregation is a common phenomenon in many types of animals including birds, fishes and social insects; organisms can get advantages from aggregation in defense, feeding and reproduction (Depickère et al., 2008a). The Japanese honey bee *Apis cerana japonica* can resist attack from predatory hornet *Vespa mandarina japonica* by forming a ball around the hornet (Anderson et al., 2002); clustering is an adaptive behavior of American house dust mites *Dermatophagoides farinae* to help reduce water loss (Glass et al., 1998). *Chlosyne lacinia* larva also can increase survival rate through aggregation behavior (Clark and Faeth, 1997).

The factors which caused cluster behavior in animals involve individual interaction and environment. Devigne et al. (2011) indicated that the inter-attraction among individuals can affect individual preferences in aggregation behavior. Researchers showed a pheromone of *Harmonia* *axyridis* contains long-chai hydrocarbons and pyrazines can cause collective (Brown et al., 2006; Durieux et al., 2012). The effects of environmental factors on animal aggregation behavior also have well documented. *Lasius niger* foragers gather well in total darkness but assemble in small and unstable cluster under red light (Depickère et al., 2004a). Four species of terrestrial isopods (*Philoscia muscorum*，*Oniscus asellus*，*Porcellio scaber*，*Armadillidium vulgare*) individuals clump together more at low RH and high temperature to prevent moisture loss (Hassall et al., 2010). Bee also form a cluster under low temperature, and the lower the temperature, the smaller the cluster size (Wang et al., unpublished).

Many animals have abilities to alter their behavior in response to changes in environmental conditions (Nussey et al., 2005). For alien species, behavioral flexibility makes critical contributions to successful invasion (Luan and Liu, 2011), and ants are good examples. *S. invicta*, a dangerous invasion pest, has inhabited many countries and regions in Asia-Pacific area (Zhang et al., 2007). In its introduced ranges, intraspecific hostile and aggression of fire ant decreased (Holway and Suarez, 1999). Assemble behavior also is a coping strategy when the workers face unsuitable condition. Wilson (1971) indicated the fire ants workers aggregated in a cluster quickly in out of nest. In flooding reasons, the fire ant worker can linked together tarsus-by-tarsus to resist flood, then migrate and colonize new land by water (Mlot et al., 2011), and the colony defensiveness were increased during rafting conditions to reduce their chances of being damage by other animals (Haight, 2006). Aggregation time represent the response speed of ant to environment changes. However, few studies have been done on it. In this paper, we focus on the effect of population density, temperature and illumination intensity on fire ant cluster time.

2 Method and Material

The fire ant collected method referred to Chen (2007). A *S. invicta* nest was collected with mound soil in a plastic box, which was painted with Fluon® to prevent fire ant workers from escaping, from Guangzhou, Guangdong province in May 2012. Ant colonies were kept at room temperature in plastic boxes and provided water and food. After 48 hours, water was slowly dripped into plastic boxes to separate ant from mound soil. Colonies were maintained under laboratory condition at 26℃ and 60%–70% RH, and water, honey and larva of flour weevil were provided. The social form of fire ant was polygyne by observing more than one queen in the colony (Shoemaker et al., 2006).

Collective level was according to Depickère et al. (2004b) and Devigne et al. (2011). Cluster was considered to occur when two or more workers were at a distance less than 0.5cm and touched with each other.

Prior to influence of temperature and illumination intensity experiments, 100 fire ant workers were collected by a soft paintbrush, and slept by carbon dioxide immediately. Then the workers were distributed into bottom of a transparent plastic box (length*width*height=12cm*8cm*8cm) randomly, the inner box was painted by Fluon® to prevent ants escaping. After that the box was put in an environmental chamber (Ningbo Jiangnan instrument factory, Ningbo, Zhejiang); and all experiments were conducted under RH 80%. Simultaneously, we began to time and observed every other 10minute. The observation was done every other 5minute while 60% workers clustered. The accumulation time of fire ant was recorded while 90% workers clustered in container.

2.1 Influence of individual density on fire ant aggregation time

5, 10, 25, 50, 100, and 200 workers were put in a box (length*width*height=12cm*8cm*8cm) respectively, and the population density levels became 0.04 individuals/cm^2, 0.08 individuals/cm^2, 0.19 individuals/cm^2, 0.38 individuals/cm^2, 0.75 individuals/cm^2, 1.50 individuals/cm^2 correspondingly. After that the box was put in an environmental chamber with a RH of 80% at darkness, we began to time and observed every other 10-minute. The observation was done every other 5-minute while 60% workers clustered. The accumulation time of fire ant was recorded while 90% workers clustered in container. Ten replicates were conducted at each density.

2.2 Effect of temperature on *S. invicta* aggregation time

Five temperature levels were studied: 15℃, 20℃, 25℃, 30℃, 35℃, with a RH of 80% at darkness, and the population density is 0.75 individuals/cm^2. Ten experiments for each temperature level were carried out.

2.3 Influence of illumination intensity on aggregation time

There were 10 luxand 1200lux's illumination intensity in this test, with 80% RH at 25℃, and the population density is 0.75 individuals/cm^2. Every treatment replicated ten times.

2.4 Statistical analysis

All statistical data were tested for normal distribution by Shapiro-Wilk test and for homogeneity of variances by Levene's test at first. One-way analysis of variance (ANOVA) using TypeIII sum of squares was used to analyze the data which are normal distribution. When ANOVA results were significant, LSD post-hoc analysis was performed on multiple comparisons of means. The non-parametric Kruskal-Wallis test for comparing the median was performed while the data did not have similar variances. Addition, the Mann-Whitney test (or the two-sample Kolmogorov-Smirnov test) was used to conduct multiple comparisons among the different groups if the results of the Kruskal-Wallis test showed significant differences at the 0.05 significance level.

3 Results

3.1 Influence of the population density on fire ant aggregation time

Population density had strong influence on fire ant aggregation time, (F=2.874, df=5, *P*=0.024, LSD; Figure 1). There was no substantial difference in aggregation time for 0.04, 0.08, 0.19 and 0.75 individual/cm^2 (*P*>0.05). However, the decrease rate became stronger for densities higher than 0.38 individual/cm^2 (see Fig. 1), and it was significantly lower than others (for 0.04 individual/cm^2: t=-2.802, df=18, *P*=0.012; for 0.08 individual/cm^2: t=-2.734, df=18, *P*=0.014; for 0.19 individual/cm^2: t=-2.662, df=18, *P*=0.016; for 0.38 individual/cm^2: t=-2.488, df=18, *P*=0.023) besides 0.75 individual/cm^2 (t=-1.413, df=18, *P*=0.175>0.05). This result revealed that fire ant respond differently to different density.

3.2 Effect of temperature on *S. invicta* aggregation time

Temperature had negative influence on fire ant aggregation (F=91.985, df=4, *P*=0.00, LSD; Figure 2). *S. invicta* needed different time to get robust aggregation in different temperature levels. Along with the rise of temperature, the speed of aggregation decreased rapidly (P<0.05). Fire ant needed 141.5min to get stabilization under 35℃, and 77min in 15℃.

3.3 Influence of light intensity on aggregation time of fire ant

The aggregation speed of fire ant was significantly faster at 1200lux than that at 10lux (t=-2.294, df=13.829, *P*=0.038; Figure 3). The aggregation time is 96.5min at 1200lux. It was faster than 10lux.

4 Discussions

Many successful species respond more rapidly to changes in the environment (Clergeau and

Yésou, 2006). Our studies concluded that the aggregate behavior of fire ant changed with the variation of environment. Fire ant aggregated significantly quickly at lower temperature, and also aggregated significantly quickly at higher worker density; meanwhile, aggregation time had significant difference between weak and strong light condition. The results implied that individual interaction, temperature and light had obviously effect on fire ant behavior.

Depickère et al. (2004c) believed that the number of *L. niger* workers inside a cluster responsible for aggregation, and population density has only a weak impact on it. Our results showed that aggregation time had no significant differences when the fire ant density was from 0.04 to 0.75 individuals/cm^2; about 90% of the ant in those densities were gathered at 85min. It is similar to the results of Depickère et al. (2004c) when *L. niger* colony density from 0.1 to 1.02 individuals/cm^2 which is very close to its natural nest; 80%-100% of *L. niger* workers are clustered at 90min. However, when fire ant population density reached 1.5 individuals/cm^2, the aggregation time decreased sharply, only required approximately 75min. Individual interaction may lead to this result. The more ants were put in box, the more chance they met each other, and this behavior may cause fast aggregation of fire ant. Another reason is that exorbitant population density may means abnormal situation of colony. Rapid aggregation had advantages to keep fire ant away from danger.

Additional results also showed that temperature has negative effect on the aggregation time of fire ant. Along with the fall of temperature, the speed of aggregation increased rapidly. As an arthropod, fire ant is sensitive to changes of surroundings temperature. Lower temperature means more energy lost for fire ant. The cluster may increase the temperature in the middle of group to prevent heat losing. Some animals also overwinter by aggregation, for instance, the multicoloured

Asian ladybird and the firebug (Durieux et al., 2012; Su et al., 2007). The aggregation behavior can lower the energy metabolic rate which is advantageous for their overwintering successfully. Challet et al. (2005) indicated movement speed of ants is positively correlated with temperature, Lu et al. (2012) also showed that the forging activity increased while the temperature was from 12℃ to 25℃. Therefore, along with the temperature rise, the activity of fire ant turned to the active stage, and the aggregation time was also delaying. However, the results are different from the observation of *A. cerana cerana* (Wang et al., unpublished), the aggregation time of bees at 26℃ is four times smaller than16℃. This may contribute to the different behavior and thermal sensitivity of the two species. For instance, *A. cerana cerana* can forge honey in winter while the ambient temperature is over 6.5℃ (Zhou and Xu, 1988); though the fire ants foraged actively until ambient temperature was above 20℃ (Lu et al., 2012). An explanation can be linked to their living condition: *S. invicta* lives in a subterraneous nest with stationary temperature and *A. cerana* cerana lives in a beehive with flexible external environment.

Our results also suggested that fire ant had different aggregation level in different luminosity condition. Fire ant workers under strong light intensity aggregate faster workers under low light intensity, and above 50% of the fire ant population coalesce into a tight cluster under strong light intensity (1200lux), but it showed several cluster and low assembly under low light intensity (10lux). Our results have little differences to previous studies on monogynous and monomorphous ant species, *Crematogaster scutellaris* and *L. niger* (Depickère et al., 2004a; Depickère et al., 2008b). Under total darkness condition, both *C. scutellaris* and *L. niger* workers aggregate well. When the red light was switched on continuously after darkness, the level of aggregation of *C. scutellaris* is not affected by the red light; brood-tenders of *L. niger* also have a high aggregation level, but *L. niger* foragers only aggregate in small and unstable clusters. This can be explained by following reasons. The first one is we use daylight lamp as light source not red lamp in our experiment. *S. invicta* is a soil-dwelling insect, and they spend most of time in darkness or weak light condition. Strong light intensity may expect fire ant workers can received more light wavelengths which they are sensitive. It can make workers aware to their situation. It is may means no shelter to shed or hide in surroundings for them. Flocking together fast may be the best choice to face potential dangerous for fire ant. Depickère et al. (2004a) indicated weak light can induces workers to disperse and forage food. This may be the reason that fire ant needed long time to cluster under low light. The second reason is social form of fire ant. Fire ant has two social forms, polygynous and monogynous form. The two forms have difference in biology and behavior (Kintz-Early et al., 2003). We choose polygynous forms in our experiments, which may cause different results with Depickère et al.'s (2004a, 2008b). Of course, this hypothesis has to be verified by further experiments, and new investigations should be conducted to compare the role of social forms of fire ant in their aggregation behavior.

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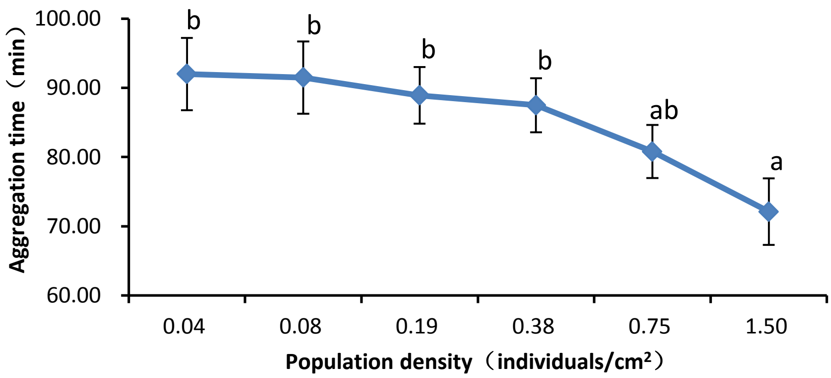

Figure 1 Effect of population density on fire ant aggregation time (average ±SE); the experiment condition was maintained at 25℃ and darkness.

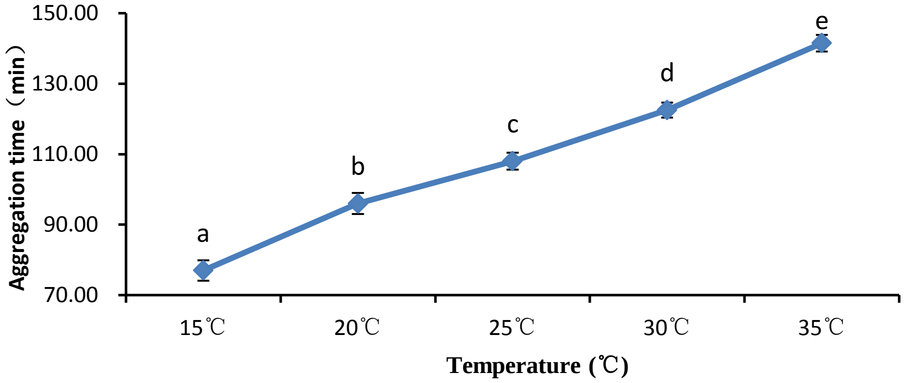

Figure 2 Effect of temperature on fire ant aggregation time (average±SE); the experiment condition was maintained darkness, and the population density is 0.75 individuals/cm^2.

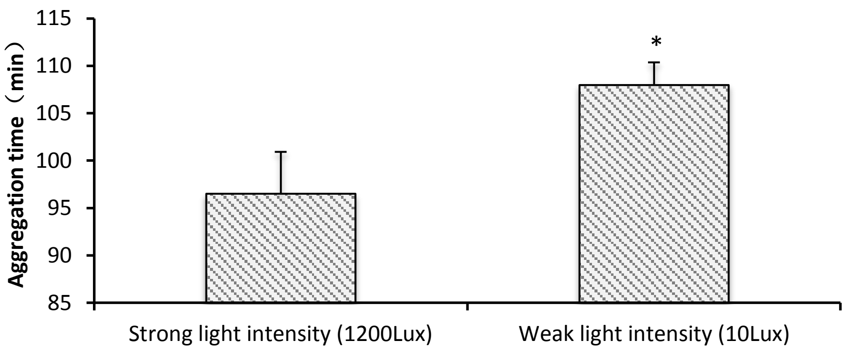

Figure 3 Effect of illumination intensity on aggregation time of *Solenopsis invicta*. Bars represnt means±SE; the experiment condition was maintained at 25℃, and the population density is 0.75 individuals/cm^2.

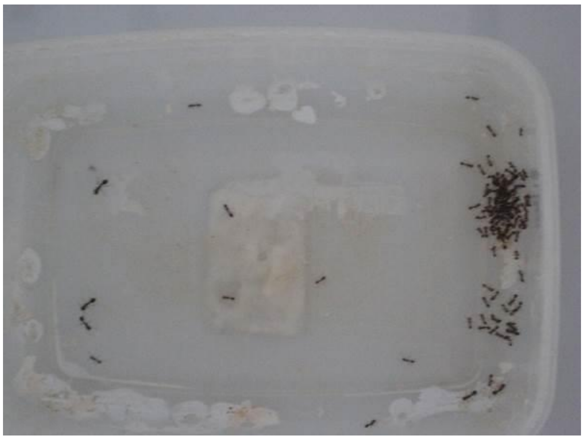

Figure 4 Aggregation situation of fire ant workers under high population density.

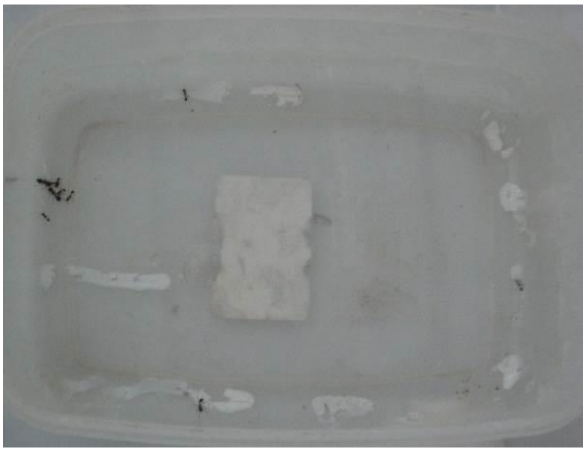

Figure 5 Aggregation situation of fire ant workers under low population density.