

SHORT COMMUNICATION

Aggression of *Solenopsis invicta* towards other ants is not affected by colony rafting

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INTRODUCTION

Rafting is a behavior some ants have evolved to keep it colonies together and survive in flooding (Purcell *et al.* 2014). As a floodplain-dwelling ant species, the red imported fire ant, *Solenopsis invicta* Buren, also can form rafts (Adams *et al.* 2011). When *S. invicta* colonies are exposed to floods and their nests are submerged, they survive by abandoning their nests, forming a raft, floating until they find new land, and then building new nests (Haight 2006; Adams *et al.* 2011).

However, raft formation causes fire ants to face new problems and dangers. For example, rafting colonies are more vulnerable to damage without protection of their nests. Haight (2006) reported that the venom dosage in *S. invicta* increases greatly during colony rafting, which suggests that the aggressiveness of *S. invicta* may increase after rafting along with the increase of venom dosage. To test this hypothesis, this study explores how rafting treatments affect the aggressiveness of *S. invicta* individuals and colonies when confronted with other ants.

MATERIAL AND METHODS

Insects

S. invicta were collected from a suburb of Guangzhou in China, and *Pheidole megacephala* Fabri-

cius were collected from campus of South China Agricultural University. *Pheidole megacephala* was selected because it commonly co-occurs with *S. invicta* in southern China and could be a direct competitor because these species have a similar biology. All *S. invicta* colonies were polygynous. The experiment used 10 fire ant colonies and five *P. megacephala* colonies that were collected a minimum of 50 m apart from one another. During collection, we placed two workers from different colonies together in a petri dish to perform an attack test and determine whether they were from a single supercolony, and aggression between workers was observed in all cases. All of the collected colonies were arranged in plastic boxes, and all four walls were brushed with Fluon to prevent the ants from escaping. The ants were fed 10% honey and live insects (*Tenebrio molitor* L.) and kept at 25 °C.

Rafting treatment

Five fire ant colonies randomly chosen and treated with flooding. Based on the method of Jovenaz *et al.* (1977), the soil that the *S. invicta* colonies were collected with was transferred to the laboratory, with the ants remaining in the soil. The flooding method was then used to separate the *S. invicta* colonies from the soil, while also serving as the flooding treatment. The time at which the ants form a raft on the water surface is then recorded. Individual and colony aggres-

sion experiments consisted of paired experiments between two colonies, comprising one fire ant colony subject to flooding for different time durations, matched with another fire ant or *P. megacephala* colony which was not subjected to the flooding treatment. Five pairs were done in each case (Table 1). A sample of workers was scooped up from rafts and put on dry trays to conduct individual and colony aggression experiments at 0, 3, 6, 12, and 24 h after the initiation of the flooding treatment. The colonies that were not subjected to flooding treatments were assigned a time of 0 h.

Individual aggression experiment

We quantified intraspecific individual aggression between paired colonies using the following behavioral assay (Carlin & Hölldobler 1986). Two medium-sized (averaging 4 mm to 5 mm in length) fire ant workers, one from each colony pair, were placed into a plastic bottle cap (diameter = 1.7 cm, height = 2 cm) using a brush. Aggressiveness tests were conducted for 5 min. Interactions were scored on a scale from 1 to 4, following Rice and Silverman (2013) and adapted for fire ants in this study: ants exhibited no change in direction or posture upon encounter or turned and moved away (Level I), ants made long-term antenna contact (> 1 s) (Level II), ants opened their mandibles or turned their abdomens upward or towards their heads (Level III), both ants attacked one other and were twisted together

or one ant fiercely attacked the other with upper jaw grappling or stinging (Level IV). After five min, the ants' attack score and times were recorded and an aggressiveness index calculated using

the following formula $\frac{\sum_{i=1}^n \delta_i f_i}{T}$ for each trial. In this formula, δ_i and f_i are the interaction score and frequency of each act, respectively, and T is the total interaction frequency, which is defined as the sum of all contacts between ants. We used the same method at the same time to test the interspecific individual aggression between the fire ant workers and *P. megacephala* workers. Five pairs of colonies were tested. Ten trials, each involving different workers, were conducted for each pair of colonies.

Colony aggression experiment

Thirty ant workers varying in size from rafting and non-rafting colonies in each pair of colonies were randomly chosen and placed into a petri dish (diameter = 9 cm, height = 1.5 cm, sides coated with Fluon) using a brush. The ants were mixed intensively to form an ant "ball" and trigger them to attack one another. Mortality was recorded after 3 h. Ants whose bodies were so damaged that they could not stand after the attack were considered dead. Five pairs of colonies were tested, and three trials, each involving different worker samples, were conducted for each pair of colonies (Table 1).

Table 1. Experiment design for intraspecific and interspecific aggression interactions at 0, 3, 6, 12, and 24 hours of rafting.

<i>Solenopsis invicta</i> (SI) rafting treatment	<i>S. invicta</i> (SI) non-rafting		<i>Pheidole megacephala</i> (PM) non-rafting			
Colony ID	Colony ID	Individual aggression experiment replicates	Colony aggression experiment replicates	Colony ID	Individual aggression experiment replicates	Colony aggression experiment replicates
SI-Colony1	SI-Colony6	10 trials	3 trials	PM-Colony1	10 trails	3 trails
SI-Colony2	SI-Colony7	10 trials	3 trials	PM-Colony2	10 trails	3 trails
SI-Colony3	SI-Colony8	10 trials	3 trials	PM-Colony3	10 trails	3 trails
SI-Colony4	SI-Colony9	10 trials	3 trials	PM-Colony4	10 trails	3 trails
SI-Colony5	SI-Colony10	10 trials	3 trials	PM-Colony5	10 trails	3 trails

Statistical analyses

Generalized linear model (GLM) analyses were used to investigate temporal patterns in aggressiveness indices and mortality of workers for interactions between rafting and non-rafting *S. invicta* (intraspecific) and rafting *S. invicta* and non-rafting *P. megacephala* (interspecific). Duration of colony rafting was included in the model as a covariate and colony identity as a random factor. All of the proportional data were analyzed after an arcsine square root transformation. All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The patterns of attack levels were similar at different rafting duration (Fig. 1), and aggressiveness index was unaffected by colony rafting (GLM, intraspecific: $F_{1,244} = 1.57$, $P = 0.764$; interspecific: $F_{1,244} = 0.019$, $P = 0.889$). In the group aggression experiment, the mortality of *S. invicta* workers did not change significantly as the duration of colony rafting increased for interspecific interactions (GLM, $F_{1,67} = 1.785$, $P = 0.186$; Fig. 2). For intraspecific interactions, although the decline in mortality of *S. invicta* workers was marginally non-significant (GLM, $F_{1,67} = 2.995$, $P = 0.053$; Fig. 2); it was possible for workers to consume more energy as the duration of colony rafting increased. There was no significant difference in the mortality (mean $\cong 90\%$) of *P. megacephala* workers across time points (GLM, $F_{1,67} = 2.413$, $P = 0.125$).

The venom dosage of *S. invicta* has been reported to increase after rafting (Haight 2006). However, our data do not show an effect of rafting on *S. invicta*'s aggressiveness as measured using our behavioral index, which may indicate that an increase in venom dosage may not be associated with aggressiveness but rather a response to some other as yet unidentified factors. Ants in rafts may show different aggression levels than ants removed from rafts. However, it is logistically problematic to test the aggression of fire ants directly on rafts, particularly when conducting individual aggression experiments. So ants were tested directly after removal in our study.

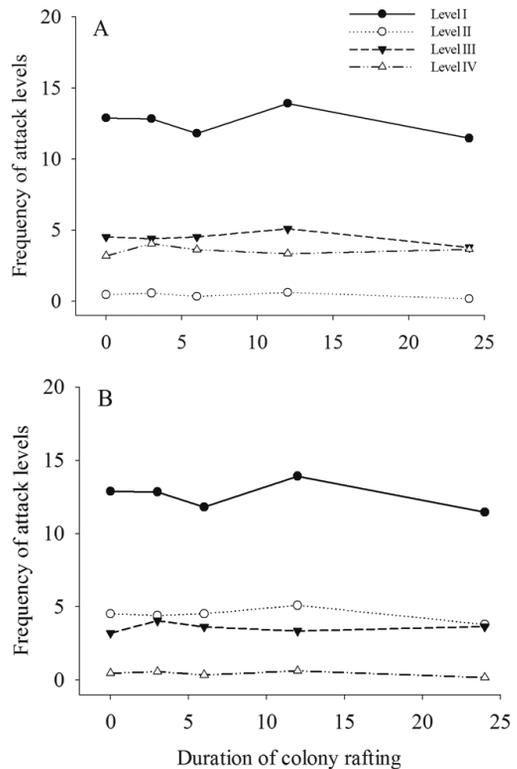


Fig. 1. Effect of various colony rafting durations on the attack levels of *Solenopsis invicta* and *Pheidole megacephala* workers (A) and two colonies of *S. invicta* (B).

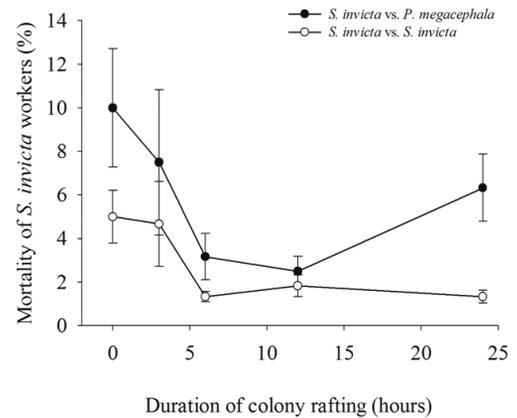


Fig. 2. Effect of colony rafting on the mortality (mean \pm SE) of *Solenopsis invicta* workers in treatments with *S. invicta* vs. *Pheidole megacephala* and *S. invicta* vs. *S. invicta*.

The competitive ability of ants can be evaluated by their aggressive behavior, and ants with greater aggression scores are usually predicted to have a greater competitive advantage (Holway 1999; Fadamiro *et al.* 2009). Previous reports have suggested that this behavior is a species-specific phenomenon, and the mortality caused by *S. invicta* against other ant species varies depending on the species in question (Bhatkar 1988). The aggressiveness index of *S. invicta* did not change significantly as the rafting duration increased, which suggests that *S. invicta* can raft for up to 24 h and still perform against *P. megacephala* as if they had not experienced flooding. Unchanging aggressiveness of *S. invicta* even under bad conditions may help to maintain their competitive advantage over other ants.

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