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Antipredator Behavior Produced by Heterosexual and Homosexual Tandem Running in the Termite *Reticulitermes chinensis* (Isoptera: Rhinotermitidae)

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Abstract

Heterosexual and homosexual tandem running can be observed together in the alate pairings in some species of termites. This study examined the effect of heterosexual and homosexual tandem running in the termite *Reticulitermes chinensis* on the predation risk by a predatory ant, *Leptogenys kitteli*. Results showed that both heterosexual and homosexual tandem running reduced the predation risk of participants. When a male-male tandem encountered a female, the back male had a significant advantage over the front male in winning a female. Moreover, the back males were significantly heavier than the front males. These results indicated that the predation risk of dealates could be decreased by tandem running through the dilution effect. Furthermore, these data suggest that male-male tandem running could induce selection pressure in favor of vigorous males and may play an essential role in indirect sexual selection.

Introduction

Many termite species generally reproduce by annual dispersal of alates that leave the parent colony and found new colonies from bisexual pairs (Bordereau *et al.*, 2002; Peppuy *et al.*, 2004). After the mating flight, individuals may exhibit calling behavior and tandem running (Hanus *et al.*, 2009; Hartke & Baer, 2011). Finally, pairs of dealates look for a suitable nesting site (Hartke & Baer, 2011). The other castes and the nest itself can not protect the imagoes while encountering predators during the period from swarming to colony foundation, which is when they are the most vulnerable (Deligne *et al.*, 1981). However, few studies focus on the role of tandem running in reducing predation risk during mating flights of termites.

Owing to the cryptic nesting habits and short swarming times in subterranean termites, it is very difficult to conduct extensive studies of their pairing behavior and antipreda-

tor behavior in the field. Instead, researchers mainly focus on laboratory simulations and mathematical models to investigate social behavior in termites (Hayashi *et al.*, 2003; Huang *et al.*, 2008; Kenne *et al.*, 2000; Lee *et al.*, 2006; Matsuura & Kobayashi, 2007; Jeon & Lee, 2011). Matsuura *et al.* (2002) demonstrated that homosexual tandem running was an antipredator behavior in the Japanese subterranean termite, *Reticulitermes speratus*. However, how widespread this antipredator behavior is within the Isoptera is still unknown. Thus, it is necessary to further study the effect of tandem running on predation risk in other species of termites.

The termite *R. chinensis* is widely distributed in China, including Beijing, Tianjin, Shanxi and the Yangtze River drainage basin (Wei *et al.*, 2007). This termite species builds nests in the soil and wooden structures, and is an important pest of forest trees and urban buildings (Li *et al.*, 2010). However, knowledge about pairing behavior and antipredator behavior in *R. chinensis* is very limited currently. In this study, we examined the effect of heterosexual and homosex-

ual tandem running in *R. chinensis* on the risk of predation by a sympatric predatory ant, *Leptogenys kittel*, to determine whether dealates of this species might also exhibit antipredator behavior.

Methods and Materials

Insects

The ant *Leptogenys kitteli* was chosen for this study because it is common in the habitat of *R. chinensis* and has been observed preying on it. On March 30, 2011, we collected a colony of *L. kitteli* from the decayed stump of a pine, *Pinus massoniana*, in Wuhan city, China. The *L. kitteli* colony was maintained in a plastic box ($75\times75\times60$ mm³) which was connected by a plastic tube to a clear plastic case ($75\times75\times60$ mm³) used as a foraging arena where the ants were fed on live *R. chinensis* workers every 3 days. On April 20, 2011, alates of *R. chinensis* were collected together with nest wood in Wuhan city just before the swarming season. They were housed in a plastic nest box ($670\times480\times410$ mm³) covered with nylon mesh and were held at 16 °C in a darkroom for 7 days to control the time of flight. Just before starting the experiments, the plastic nest box was transferred to a room with artificial light at 30 °C so that alates emerged from the nest wood and began to fly (Matsuura & Nishida, 2001; Matsuura *et al.*, 2002). The alates or dealates were anesthetized with CO_2 and separated by sex using configuration of the caudal sternites under a stereoscope (Roonwal, 1975). Then, the same-sex imagoes were put together in Petri dishes containing moist filter paper until they shed their wings. Each dealate was used only once, i.e. no dealate was re-used either within or between experiments.

*Effect of Unit Type on Post-encounter Risk*

In this experiment, there were five treatments: single male, single female, male-male, female-female and male-female. Each unit type was selected randomly and placed in the foraging arena. After a single dealate was put in the foraging arena, the entrance to the foraging arena was opened and then shut after an ant entered. Once the ant encountered the single dealate, the capturing situations were recorded. When each pair of dealates began tandem running, the entrance was opened and then shut after one ant entered the foraging arena. Because an ant could only capture one dealate at a time, encounters will result in one of the following three situations: (1) the front dealate is captured, (2) the back dealate is captured, or (3) both dealates escape (Matsuura *et al.*, 2002). Each trial was run until the end of the ant's first attempt to capture a termite. Each treatment was replicated 50 times. Individual capture rates per predatory attack between single and tandem dealates, and between front and back dealates in tandems were compared. Statistical significance was analyzed using Fisher's exact probability test (SPSS Inc., 1989–2002).

Effect of Unit Type on Encounter Risk

As tandem running increased the size of the prey unit, it is easier for ants to find prey. The actual change in the encounter risk could not be evaluated in a laboratory experiment, because dealates tend to run along the perimeter of a container as previously described in *R. speratus* (Matsuura *et al.*, 2002). Therefore, a mathematical model was needed to estimate the encounter risk of tandem dealates relative to a single dealate. The mathematical model of Matsuura *et al.* (2002) was used to estimate the frequency of encounters (R). The parameters w and l show the body width (the biggest diameter of abdomen) and length of dealates for the *R. chinensis*, respectively. The parameter s represents the sensory range of an ant (the range between both antennae).

Effect of Volatiles and Vision on Predator Behavior

If ants search for prey using visual or volatile cues, it is easier to detect tandem dealates than single dealate. Thus, a choice test was tested in a modified T-shaped box. The test is as follows: The apparatus ($200\times20\times20$ mm) was connected to the ant nest by a plastic tube. We used glass or a 60-mesh stainless-steel screen to separate a compartment ($20\times20\times20$ mm) at both ends of the apparatus. Two dealates were put at one end of the apparatus, but there were no dealates at the other end, as a control (Fig. 1). The entrance was shut after an ant entered. The ant was allowed to search for prey for 60 s. Then, the time spent on the dealates and control sides was recorded. Each treatment was replicated 20 times. New white paper was laid in the junction each time to remove the influence of ant trail pheromones. If visual or volatile cues from delalates can attract ants, the ants should spend more time searching on the dealate side than the control side. In addition, we put dealates wrapped in nylon mesh in the foraging arena and observed the reaction of ants, so that we could detect whether ants considered dealates as prey without direct antennal contact
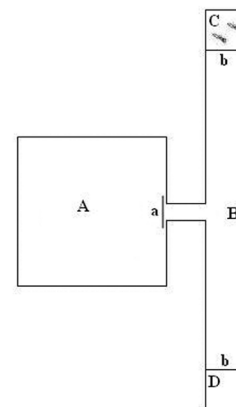
Fig. 1. The experimental apparatus used to detect whether ants were attracted to dealates by visual or volatile cues. A: ant nest; B: search area; C: dealate area; D: control area; a: entrance switch; b: glass

plate or a 60-mesh stainless-steel screen.

Effect of Tandem Position on Pairing Opportunity

Running spee

Results

Effect of Unit Type on Post-encounter Risk

An interesting phenomenon was found in our experiment as described previously in *R. speratus* (Matsuura *et al.*, 2002). When two males **met, they would turn around in circles to compete for the back position**, while such phenomenon did not occur in females. An important question was raised, whether the back position has **dominance in subsequent pairing competition**. Therefore, the following test was performed to examine this possibility. Two dealates of the same sex were chosen randomly and were placed in a 90 mm culture dish. A dealate of the opposite sex was chosen randomly and was put into the culture dish **after the two dealates of the same sex began tandem running**. There will be three results: (1) the front dealate successfully pairs, (2) the back dealate successfully pairs, or (3) triple tandem. Then, the front and back dealates were anesthetized with CO_2 and weighed. Each sex was replicated 20 times.

$p = 0.284$, Fisher's exact probability test).

The post-encounter risk of the back dealate in tandem running was significantly lower than that of a single individual (in male-male tandems: $p = 0.005$; in female-female tandems: $p = 0.003$; in female-male tandems: $p < 0.001$, Fisher's exact probability test). Furthermore, the post-encounter risk of the front dealate in tandem running was also significantly lower than that of a single individual, except in male-male tandems (in male-male tandems: $p = 0.070$; in female-female tandems: $p = 0.027$; in female-male tandems: $p = 0.016$, Fisher's exact probability test) (Table 1). Although the front individual is always captured at a higher rate than the one in the back, regardless of sex or pair type, there were no significant differences in the post-encounter risk between the back dealate and the front dealate (in male-male tandems: $p = 0.412$; in female-female tandems: $p = 0.532$; in female-male tandems: $p = 0.284$, Fisher's exact probability test).

Table 1. Comparison of the post-encounter risk in different unit types. Data in parentheses is the post-encounter risk relative to single dealates. ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Unit type	Capture rate		Escape rate
Single male	0.64		0.36
Single female	0.64		0.36
	Front captured	Back captured	Both escape
Male-male tandem	0.44 (0.69)[ns]	0.34 (0.53)**	0.22
Female-female tandem	0.40 (0.63)*	0.32 (0.50)**	0.28
Female-male tandem	0.38 (0.59)*	0.26 (0.41)***	0.36

Effect of Unit Type on Encounter Risk

The predation risk of tandem dealates relative to single dealates is yielded by multiplying the relative encounter risk and the relative post-encounter risk. The relative encounter risk is $R_2/R_1 = 1.341$ ($l = 4.92$, $w = 1.26$, $s = 5.27$ mm) (Table 2).

The relative predation risk of each position was as follows: male-male tandems, front males: 0.93, back males: 0.71; female-female tandems, front females: 0.84, back females: 0.67; female-male tandems, front females: 0.79, back males: 0.55. Because a value of 1 represents equal predation risk between tandem dealates and single dealates (Matsuura *et al.*, 2002), these results indicate that the total predation risk was reduced by tandem running relative to single dealates.

Table 2. Body size of dealates and running speed of dealates and ants. [†] Data were the average from 20 dealates and 20 ants. [‡] Sensitive width was the interval between the tips of both antennae. [§] Running speed was determined according to the time required to run 20 cm on a white paper at 25 °C.

	Termite alate[†]		Predatory ant[†]
	Male	Female	
Body width w (mm)	1.220 ± 0.009	1.290 ± 0.005	—
Body length l (mm)	4.827 ± 0.030	5.014 ± 0.033	—
Sensitive width s (mm) [‡]	—	—	5.271 ± 0.072

(mm/s) §	29.048 ± 0.974	32.882 ± 0.641

Effect of Volatiles and Vision on Predator Behavior

The differences were not significant in the residence times (visual: df = 19, t = -0.471, p = 0.643, volatile: df = 19, t = 1.344, p = 0.195, paired t-test) (Fig. 2), suggesting that the ants were not attracted to the dealates separated by glass or a steel screen. The supplementary experiment showed that the ants were not interested in dealates wrapped in nylon mesh when they appeared in the foraging arena. These results dem-
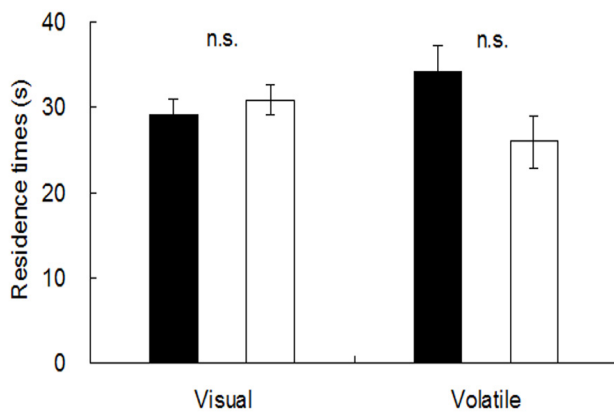

Fig. 2. Differences in the residence times of ants between arms of the apparatus having two dealates and lacking dealates. Error bars represent the standard error. ns, not significant; paired t-test: (■) two dealates, (□) no dealates.

Effect of Tandem Position on Pairing Opportunity

When a male-male tandem encountered a female, the back male had a significant advantage in winning the female over the front male (p < 0.001, two-tailed binomial test) (Fig. 3). The back male won the female 12 times, while the front male only won 2 times. The "triple-tandems" occurred 6 times. These results clearly showed that back males had the superiority over front males in the pairing competition. This advantage was supported further by the weight results. The front males were significantly lighter than the back males in male-male tandems (df = 19, t = -3.133, p = 0.005, paired t-test) (Fig. 4). However, when a female-female tandem encountered a male, there was no significant difference between the front female and the back female in winning the male (p = 0.155, two-tailed binomial test) (Fig. 3).

The front female paired with the male 11 times, and the back female paired 6 times. The "triple-tandems" occurred 3 times. In addition, there was no significant difference in the weights between the front females and the back females in female-female tandems (df = 19, t = -1.371, p = 0.186, paired t-test) (Fig. 4).

Discussion
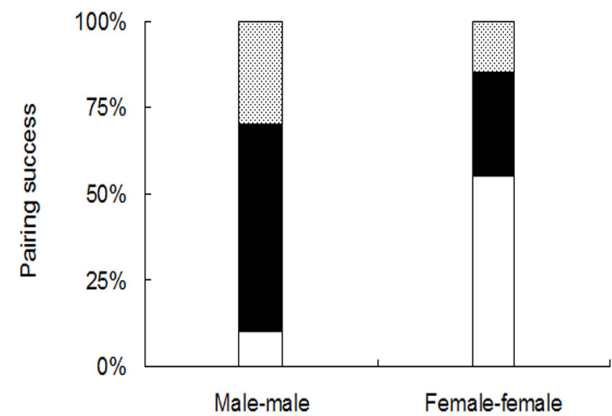
Fig. 3. Pairing success between front and back dealates in male-male tandems and female-female tandems. Two-tailed binomial test: (□) front, (■) back, (▨) triple-tandems.
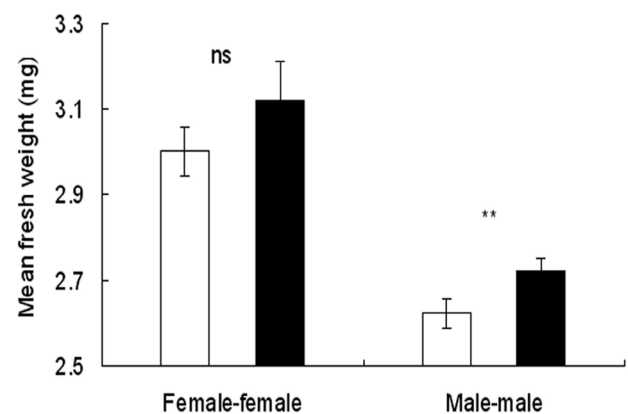
Fig. 4. Differences in body weight between front and back dealates in male-male and female-female tandems. Error bars represent the standard error. ns, not significant; **, p < 0.01; paired t-test: (□) front, (■) back.

The dilution effect could reduce an individual's risk of predation in group-forming animals (Hamilton, 1971; Hall et al., 2009; Marcoux, 2011; Rodgers et al., 2011). Our results suggested that colonies of *R. chinensis* experienced the same dilution effect as described previously in *R. speratus* (Matsuura et al., 2002). The alates of *R. chinensis* need to land and shed their wings in order to search for a mate, so it is easy for them to encounter potential predators during the period from swarming to colony foundation (Bordereau & Pasteels, 2011). Our results suggest that the probability of tandem dealates as the victim of a predator was reduced compared to single dealates. This can be explained by the fact that one ant cannot capture two dealates at the same time. In other words, the escape probability of an individual to each predation attack should be increased through tandem running, because one ant can only capture one dealate in each type of tandem (Matsuura et al., 2002). In summary, the phenomenon

of homosexual tandem before dealates encounter the opposite sex is an adaptive strategy to minimize predation risk. Moreover, heterosexual tandem running had also reduced the predation risk of two participants. This result was different from *R. speratus* in which the predation risk of the following male was larger than that of a single male in female-male tandems (Matsuura *et al*., 2002).

The ant *L. kitteli* did not utilize visual or volatile cues to prey on the dealates in this study, indicating the encounter risk could not be increased by either the enlarged visual image or volatiles. Therefore, we could use the mathematic model of Matsuura *et al*. (2002) to estimate the encounter risk of *R. chinensis* by *L. kittel*. This model estimates that tandem running could reduce the total predation risk to an individual. That is to say, a dealate in a tandem run is safer than a single dealate. Reduced predation risk cannot be explained by reduced encounter rates or reduced post-encounter success of the ant against tandems. Average speed is the same between tandems and single dealates, the ant is apparently not utilizing termite pheromones as localization cues, and the escape rate is the same for both prey types (singles vs. tandems). Rather, ants can only handle one item of prey at a time, with the result that post-encounter predation risk is spread over more individuals. This suggests that the longer "trains" of dealates observed in the field are an extension of this adaptive strategy to reduce individual predation risk, although the limits to this tactic have not been explored.

When two males met, they turned round and round to compete for the back position. The likely interpretation of this phenomenon is that the post-encounter risk of the back dealate in male-male tandem running was significantly lower than that of a single individual. In fact, our results suggested that the ants less often captured back males than front males. In addition, the back males were significantly heavier than the front males in male-male tandems, and the back males have the superiority over front males in the pairing competition. These results suggested that male-male tandem running would induce selection pressure in favor of heavy vigorous males. Tandem running may therefor play a role in indirect sexual selection, if such "dominant" males contribute more to reproductive investment by both direct nutrient transfer and labor in the colony foundation stage (Shellman-Reeve 1990). In female-female tandem, we found that there were no differences in the number of pairing success and vulnerability between the positions in *R. chinensis*, consistent with the results in *R. speratus* (Matsuura *et al*., 2002). In *R. speratus*, the cooperative colony foundation by female pairs was considered as one of the reasons that females do not compete for males as aggressively as males compete for females (Matsuura *et al*., 2004; Matsuura *et al*., 2002; Matsuura & Kobayashi, 2007). However, we need to further investigate whether there also is the cooperative colony foundation by female pairs in *R. chinensis*.

Antipredator effects of heterosexual and homosexual tandem running in termites has been tested only in *R. speratus* and *R. chinensis* until now, although homosexual tandem runs have been seen in many termite species. Austin *et al*. (2004) found that *R. speratus* and *R. chinensis* were close relatives within the genus *Reticulitermes*. Thus, whether the antipredator effect of tandem running exists in only these two species, throughout *Reticulitermes* or possibly beyond requires extensive studies in *Reticulitermes* and in closely related genera such as *Coptotermes* and *Heterotermes*. Our work extends the previous discovery of antipredator behavior in termites, however, the extent to which this is actually antipredator behavior needs to be measured under more realistic conditions. Moreover, the evolutionary significance of termite homosexual tandem runs remains further investigations. Also interesting is that the ants in this experiment were relatively smaller compared to the termites than in Matsuura's experiment, judging from the relative values of *l*, *w*, and *s*, suggesting position dependent predation risk may be generalizable to many termite-ant pairings regardless of the relative sizes of the interactants, although it still remains to be tested with ants that are much smaller than the termites they are preying on.

Acknowledgments

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