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Research article

Effect of group size on the aggression strategy of an extirpating stingless bee, *Trigona spinipes*

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Summary. Group aggression influences communication and defense strategies in many social insect communities. Such aggression plays a particularly significant role in the lives of stingless bees, important native Neotropical pollinators, in which the battle for food resources can be deadly and critical to colony survival. However, the effects of group size on individual aggression levels and the spatio-temporal aggression strategy of communal aggressors have not been fully explored. We therefore investigated how group size affects the aggression levels and the spatio-temporal attack strategy (which body parts, and the amount of time spent in attacking each part) in close combats between *Trigona spinipes* foragers and a natural competitor, *Melipona rufiventris*. In all trials, *T. spinipes* foragers competitively excluded *M. rufiventris* foragers from nearby feeders, exhibiting four levels of aggressive behavior ranging from threat displays to prolonged grappling and decapitation. Surprisingly, aggression levels and spatial strategy corresponded to the size of group attacks. Larger groups of attackers used individually *lower* aggression levels than small groups of attackers. Smaller groups also attacked appendages linked to escape (legs and wings) with greater frequency than larger groups, which focused on vital central body areas (abdomen, thorax, and head). Increased aggression corresponded to increased risks for attackers and the attacked. All combatants engaging at the highest level of aggression died (100% mortality). Thus the dominance style of *T. spinipes* may minimize attack risk and maximize victim harm with finely tuned hostility.

Key words: Group effects, aggression, stingless bees, competition, foraging.

Introduction

Group effects have been defined by Wilson (1971, p. 297) as a form of social facilitation, 'an alteration in behavior or physiology within a species brought about by signals that are directed in neither space nor time' (the latter referring to signals that act over a long period of time without eliciting a directed response). Research on group effects has led to significant insights into the behavioral and evolutionary effects of living in large assemblages (Allee, 1931; Gadagkar, 1997; Alcock, 2001; Blanchard et al., 2001). In particular, the social regulation of aggression plays an important role in competitive interactions among animals (Moynihan, 1998); the structure of arthropod communities (Reitz and Trumble, 2002); invasion ecology (Holway et al., 2002); and the evolution of animal behavior (Maynard-Smith and Harper, 1988). However, questions remain concerning the detailed effects of group size on *individual* aggression during aggressive encounters between groups (Archer 1988), even in primates (Bernstein and Ehardt, 1985; Camperio, 1986; Goodall, 1990; Stanford et al., 1994; Gros-Louis et al., 2003).

In social insects, group aggression plays a vital role in ants, social bees, and social wasps during nest defense and, in some species, during foraging (Wilson, 1971; Michener, 1974; Breed et al., 1990; Hölldobler and Wilson, 1990). For example, Wilson (1971) reported that individual *Acanthomyops claviger* ants increased their sensitivity to alarm odors when in larger groups. Interspecific competition over limited food sources has led to the evolution of aggressive group recruitment systems in stingless bees (Hymenoptera, Apidae, Meliponini), one of the most important native Neotropical pollinators (Johnson and Hubbell, 1974, 1987; Roubik, 1989; Nagamitsu and Inoue, 1997; Slaa et al., 1997; Kevan and Imperatriz-Fonseca, 2002; Slaa, 2003; Nieh et al., 2004). Such aggressive competition may be costly and critical to colony survival, especially during periods of relative food dearth (Roubik, 1982, 1989). Johnson and Hubbell (1974) recorded 63% mortality (1812 dead bees) after a two-day battle between three colonies of *T. corvina* (Lepeletier, 1835) over sucrose solution baits.

However, relatively little is known about the detailed patterns of aggressive behavior (Johnson, 1974) that constitute meliponine dominance styles, the strategies and behaviors that individuals or groups typically employ to attain dominance (Cooper and Bernstein, 2002). Dominance is defined as an asymmetry in the pattern of aggression and submission in agonistic encounters (Bernstein, 1981). Previous analyses have focused on levels of aggression exhibited by different species (Roubik, 1980) and examined the role of fighting as a single behavioral state (Johnson, 1974; Johnson and Hubbell, 1974), but have not determined the dominance style by quantifying the fine scale spatio-temporal strategy of attacks (how bees physically target and allocate time towards attacking different portions of opponents' bodies) or explored the possibility that group effects modulate group attacks. Thus the facilitation of aggression by the presence of nestmates largely remains to be elucidated (Johnson, 1974). Meliponine aggressive behavior can incur costs if the probability of injury correspondingly increases (Johnson, 1974). Given the potentially high cost of escalation (Johnson and Hubbell, 1974; 1987), we predict that the minimal effective force will vary with group size. In the case of individually smaller foragers from an extirpating species fighting larger opponents, aggression may optimally be higher in individual encounters, and lower in group encounters when smaller individuals can use numerical superiority to overcome a larger individual, while avoiding undue risk by taking on individually lower aggression levels. However, the application of minimal effective force may be a good strategy, and we therefore sought to determine if individual aggression levels are density-dependent in such group attacks.

We focus on the aggressive interactions between *T. spinipes* (Lepeletier, 1835), a species that often attacks and harasses other species of stingless bees on floral resources (Cortopassi-Laurino, 1982; Sazima and Sazima, 1989), and *M. rufiventris* (Lepeletier, 1835; Moure, 1975), a moderately aggressive stingless bee that can defend good food sources but generally does not attack or take over food sources occupied by other bees (Rocha, 1970; Souza, 1978). Both species occur in Amazonia, Brazil (Brown and Albrecht, 2001), and we observed *T. spinipes* harassing and attacking *M. rufiventris* on natural food sources at our field site. Our goal was to determine the dominance style of a meliponine group extirpator attacking a larger opponent, a strategy that is widespread among stingless bees and plays an important role in their foraging biology (Roubik, 1980; Johnson and Hubbell, 1987; Nagamitsu and Inoue, 1997; Slaa et al., 1997). We begin by analyzing the spatio-temporal attack strategy of *T. spinipes* to determine (1) overall outcomes, (2) victim responses, (3) the time allocation of attack durations, and (4) the effect of aggression level on spatial attack strategy. We conclude by examining the influence of group size on this spatio-temporal attack strategy and individual *T. spinipes* aggression levels.

Materials and methods

Study site, feeders, and training

We used two colonies of *T. spinipes* (s_1 and s_2, approximately 8000 bees per colony) in trees and two colonies of *M. rufiventris* (r_1 and r_2, 500–700 bees per colony) in hives at the Fazenda Aretuzina, a ranch near São Simão in the state of São Paulo, Brazil during September 2002 and 2003 (colony population estimates based upon nest sizes). We studied the following pairs of colonies: 3 (s_1, r_1); 3 (s_1, r_2); 6 (s_2, r_1); and 6 (s_2, r_2) for a total of 18 trials, using only one type of pairing at any given time.

In order to study aggression during food source extirpation, we trained both species to separate feeders and then observed as *T. spinipes* foragers found and displaced *M. rufiventris* foragers (Nieh et al., 2004). We trained 20 individually marked foragers from each *M. rufiventris* colony (separated by 10 m) to feeders located 2 m east of each colony. We then trained 20 individually marked foragers from each *T. spinipes* colony to identical feeders located 4 m east of each *M. rufiventris* colony. After each trial, we captured and removed all *T. spinipes* and *M. rufiventris* foragers at the feeders (monitoring for 2 hours after the last forager had arrived) and then retrained new foragers from each colony. The *T. spinipes* colonies were respectively located 74 m and 142 m from the center of the axis formed by both *M. rufiventris* colonies. With each *T. spinipes* colony, we used aspirators (Nieh, 1998) to capture all foragers at the feeders and verifying for 3 h that no further foragers arrived at any feeder location before training foragers from the second colony. With *M. rufiventris*, we used wire mesh (applied in the evening after all bees had returned to their nest) to seal the entrance to the colony not under study.

The feeder consisted of a small glass bottle (5 cm diameter, 4.5 cm height, 65 ml) inverted over a 6.7 cm diameter grooved plastic base (von Frisch, 1967). Each feeder contained unscented 2.5 M sucrose solution (Tautz and Sandeman, 2003) and was placed on a 20 cm diameter blue plastic dish supported by a 1 m high tripod. To facilitate forager orientation, we placed a 6.7 cm diameter disk of yellow paper underneath all feeder bases.

We used paint pens to individually mark the thoraces of bees visiting each feeder (Nieh et al., 2003). We verified the identity of the marked foragers by moving the feeder back to each colony and confirming that foragers flew directly from the feeder into the colony entrance. We allowed 20 foragers to visit the feeder, and censused the number of marked training-feeder foragers each 15 min, capturing or releasing marked foragers to maintain a constant number (marked and unmarked bees were captured in separate aspirators so that marked bees could be easily released to maintain visitation by 20 foragers within a 15 min interval).

Attack analysis

To quantify size differences between the two species, we randomly sampled 20 foragers of each species and used an electronic scale and calipers to measure fresh mass, maximum head width (Roulston and Cane, 2000), and the intertegular span (Bullock, 1999).

We filmed with a Canon XL-1 NTSC digital video camera (30 frames per second) positioned above the feeder. We captured the data with an Apple PowerBook G4 computer and used iMovie v3.0.3 to conduct a frame-by-frame analysis of the location and duration of attacks on different parts of the victims' bodies. In order to take a random sample of attacks and to film the attacks at a high magnification sufficient for analysis of the spatial attack strategy, we zoomed the camera to film a 3 cm × 3 cm field of view (N = 67 attack clusters). Attacking *T. spinipes* foragers focused on the body part attacked and did not shift to a different body region or part before the *M. rufiventris* foragers escaped or were killed. Thus we did not perform a transition probability analysis.

We classified all attacks involving direct contact as occurring on one of the following body parts: head, thorax, wings, abdomen, or legs. Attacks on the antennae, mouthparts, or eyes were all included in the head category. Attacks on legs were scored as attacks on metathoracic, mesothoracic, or prothoracic legs. On the abdomen, we also defined coronally demarcated dorsal and ventral divisions. Lateral left and right are defined with respect to a dorsal view of the bee with the head superior. We were also able to clearly distinguish between attacks on the ventral and dorsal sides of the abdomen, and thus we divided the abdomen into these two coronal regions.

Attack intensity was classified following the definitions of Johnson and Hubbell (1974). Level I corresponds to low intensity threats without direct contact in which a bee opens its mandibles while facing its rival, often while spreading its wings at a wide angle. At level II, there is brief body contact such as a brief bite. Level III involves extended biting and pulling of the mandibles and extremities. Finally, at level IV, the attacker and attacked 'wrestle…together, legs gripping the opponent, and mandibles locked or chewing the rival's head or neck' (Johnson and Hubbell, 1974, p. 122). Spatially, aggression at levels II-IV may consist of biting on the appendages and on central parts of the body (head, thorax, or abdomen). This ranking of attack intensities corresponds to the risk of bodily injury, with death most likely at level IV (Johnson and Hubbell, 1974), and corresponds generally to aggression and dominance rankings used in other insect studies (Pabalan et al., 2000; Chen et al., 2002). Johnson and Hubbell (1974) specify that the ventral surfaces of combatants are together in level IV attacks, however, we feel that this definition is too limiting and therefore classify as level IV all attacks that contain the essential (and commonly fatal) aspect of mandibles locked and chewing the head or neck.

In many cases, *M. rufiventris* foragers sought only to escape, not to attack their attackers. We define escape behavior as a forager struggling to walk or fly away while attackers pull or restrain the attacked to keep her from moving away.

Statistical analyses

We use JMP IN v4.0.4 software and Statview v5.0.1 running on a Macintosh PowerBook G4 computer to analyze our results. We use ANOVA to analyze the effect of group size and attack duration on attack strategy (normal transformations applied where appropriate, Sokal and Rohlf, 1981). Multiple pair-wise comparisons were conducted using Student's t-tests. We use the χ^2 test to analyze the overall distribution of attack sites, and use contingency table analysis and the χ^2 test to analyze the effect of aggression level on the spatial attack strategy. All averages are reported as mean ± 1 standard deviation.

Results

Extirpation

Although we trained 20 *T. spinipes* foragers to a separate feeder, the majority quickly found the *M. rufiventris* feeder located 4 m from the *T. spinipes* feeder and attacked and completely excluded all 20 *M. rufiventris* foragers during each 10 min trial. Neither side recruited newcomers during these trials. There was a significant increase in the number of *T. spinipes* foragers (linear regression, $R^2 = 0.60$, $F_{1,26} = 38.9$, $P < 0.0001$), and a significant decrease in the number of *M. rufiventris* foragers (linear regression, $R^2 = 0.88$, $F_{1,26} = 195.3$, $P < 0.0001$) on the *M. rufiventris* feeders (f_{r1} and f_{r2}) over time.

Melipona rufiventris foragers were larger than the *T. spinipes* foragers (on average: 310% heavier, 140% greater maximum head width, and 172% larger intertegular spans). *Trigona spinipes* foragers weighed 21 ± 1 mg and had a maximum head width of 3.7 ± 0.1 mm and an intertegular span of 2.3 ± 0.1 mm. By each of these measures, *M. rufiventris* foragers were significantly larger than *T. spinipes* foragers (in all three Mann-Whitney tests, U = 0.00, Z = –5.41, $P < 0.0001$, $N = 40$).

Overall spatial attack strategy

Figure 1 shows the *M. rufiventris* body parts and regions attacked by *T. spinipes*. Overall, there are no significant differences between the spatial distributions of attacks on different body parts ($\chi^2_4 = 8.23$, $P = 0.08$). However, *T. spinipes* foragers directed 72% of all attacks at the central body (abdomen, thorax, and head) and 28% of attacks at the appendages (wings and legs), and there is a significant difference between the numbers of attacks directed at the central body vs. the appendages ($\chi^2_1 = 24.37$, $P < 0.0001$).

Response of M. rufiventris *foragers*

Trigona spinipes foragers initiated all attacks, and multiple individuals often attacked a single *M. rufiventris* forager. However, we did not observe multiple *M. rufiventris* foragers engaging in counterattacks. In the case of multiple *T. spinipes* attackers, we paired the victim's aggression level with the aggression level of the attacker that the victim directed her mandible gaping or mandible contact towards (when she displayed aggression). Higher levels of aggressive attack may correspond to higher levels of aggressive response by the victim, particularly at aggression levels III and IV. However, 63% of attacked *M. rufiventris* foragers responded with no aggression (Table 1). We observed these foragers struggling to escape. When *T. spinipes* foragers attacked at lower aggression levels (I, II, and III), the majority of *M. rufiventris* foragers did not respond aggressively, only attempting escape. Some *M. rufiventris* foragers (30%) exhibited weak response aggression (levels I and II) when attacked by *T. spinipes* foragers exhibiting level III aggression. Finally,

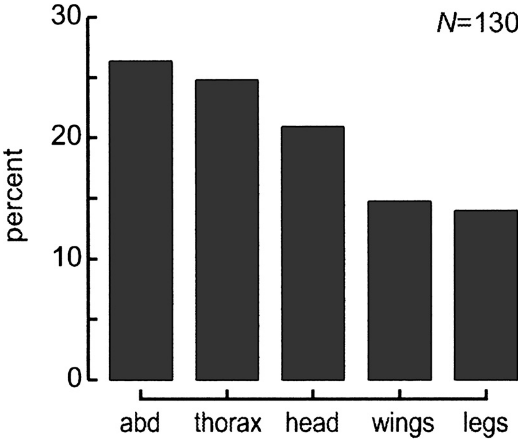

Figure 1. Distribution of *T. spinipes* attack behaviors (total counts) on different body parts (abd = abdomen)

Table 1. Contingency table of *M. rufiventris* aggression levels in response to *T. spinipes* aggression. Counts and row percentages given

T. spinipes aggression level	*M. rufiventris* aggression response level no aggression	I	II	III	IV
I	4 (100%)	0	0	0	0
II	4 (80%)	0	1 (20%)	0	0
III	34 (68%)	11 (22%)	4 (8%)	1 (2%)	0
IV	0	0	0	0	8 (100%)

100% of *M. rufiventris* foragers responded with level IV aggression when attacked at aggression level IV. All level IV attacks ended with the deaths of the *M. rufiventris* and *T. spinipes* foragers involved. No bees died at or around the feeder as a result of level I–III attacks. In level IV attacks between pairs of foragers, both individuals would frequently fall off the feeder and rapidly spin on the ground in circles while buzzing their wings. In level I–III attacks, particularly in the case of multiple attackers and a single attacked bee, the motion of the fighting cluster was reduced, with the attacked bee generally immobilized as attackers arranged around her tugged from different directions.

Time allocation of attack durations

On average, *T. spinipes* foragers spent 4.6 ± 5.1 s ($N = 130$) attacking *M. rufiventris* foragers. *Trigona spinipes* foragers spent roughly equal amounts of time attacking the different body parts (classified into groups), and the ventral or dorsal side of the abdomen ($P \geq 0.33$, Table 2a, b, c).

However, there is a significant difference between the amounts of time that *T. spinipes* allocated to contact attacks on the different legs of *M. rufiventris* (ANOVA, $F_{2,15} = 25.08$, $P < 0.0001$). On average, *T. spinipes* spent 15 times as much time biting the prothoracic legs and three times as much time biting the mesothoracic legs than biting the metathoracic legs (Fig. 2). Thus attackers spent roughly equal amounts of time attacking the different body parts and regions and did not spend more time attacking legs than other body parts (Table 2a). However, when the time spent on leg attacks is analyzed in detail, it is clear that attackers spent increasing amounts of time attacking the progressively anterior legs than the posterior legs (Fig. 2).

There is no significant effect of aggression level on the temporal strategy of *T. spinipes* attackers (Table 2d). *Trigona spinipes* individuals were equally likely to allocate the same amounts of time to attacking at all four aggression levels ($P = 0.84$). There is no significant effect of aggression level on spatial strategy. *Trigona spinipes* foragers attacked the coronal regions of the abdomen with equal likelihood ($\chi^2_2 = 1.19$, $P = 0.55$).

Effect of group size

Figure 3a shows that 25% of attacks consisted of a single *T. spinipes* forager attacking a single *M. rufiventris* forager, with the remaining 75% of attacks consisting of multiple *T. spinipes* foragers attacking a single *M. rufiventris* forager. The most common group attack size consisted of two *T. spinipes* foragers attacking a single *M. rufiventris* forager (37%).

There is no significant relationship between group size and individual attack durations (ANOVA, $F_{1,128} = 0.007$, $P = 0.93$), and no significant effect of group size on the coronal region of the abdomen attacked (ANOVA, $F_{1,34} = 0.222$, $P = 0.64$) or the leg pair attacked (prothoracic, mesothoracic, or metathoracic: ANOVA, $F_{2,15} = 0.363$, $P = 0.70$).

However, there is a significant effect of group size upon the aggression level displayed by individual attackers (Fig. 3b) and the body part attacked (Fig. 3c). First, higher individual aggression levels correspond to increasingly smaller group size (ANOVA, $F_{3,126} = 11.87$, $P < 0.0001$, Fig. 3b). Secondly, group size also affected the *M. rufiventris* body part that *T. spinipes* foragers attacked (Fig. 3c). The central body (abdomen, thorax, and head) was attacked more frequently at larger group sizes and the appendages (wings and legs were attacked more often at smaller group sizes

Table 2. Time allocation of attack durations

Attack duration (s)			*N*	*F*	d.f.	*P*
a) Body part	legs	5.7 ± 5.3	18	1.17	5,124	0.33
	abdomen	5.6 ± 5.1	34			
	head	4.4 ± 6.6	27			
	thorax	4.3 ± 4.1	32			
	wings	3.1 ± 2.9	19			
b) Coronal region	ventral	5.9 ± 4.7	21	0.004	1,32	0.95
	dorsal	5.7 ± 5.9	13			
c) Aggression level	I	5.7 ± 8.6	5	0.29	3,126	0.84
	II	4.1 ± 4.1	13			
	III	4.9 ± 5.3	80			
	IV	4.1 ± 4.4	32			

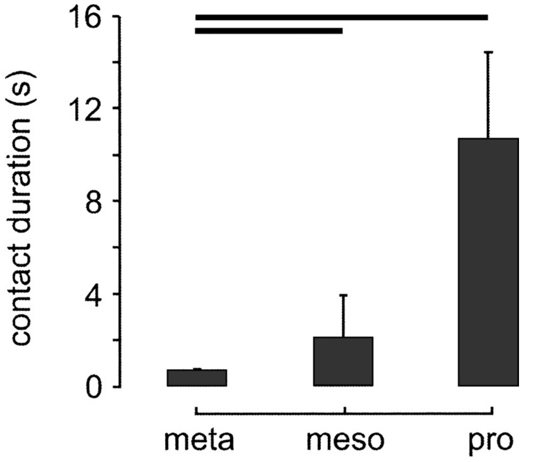

Figure 2. Durations of time that *T. spinipes* foragers spend attacking the different legs of *M. rufiventris* foragers (metathoracic, mesothoracic, and prothoracic legs, $N = 18$). Lines indicate distributions that are significantly different ($P < 0.05$)

(ANOVA, $F_{4,125} = 3.87$, $P = 0.005$). In cases with only one attacker, 13 attacks focused on the appendages and 20 on the central body (no significant difference, $\chi^2_1 = 1.48$, $P = 0.22$).

Discussion

In all trials, *T. spinipes* foragers competitively excluded *M. rufiventris* foragers from nearby feeders, exhibiting aggressive behavior ranging from threat displays to prolonged grappling and decapitation (levels I to IV). Aggression levels corresponded to the size of group attacks, with larger groups of attackers displaying individually lower aggression levels than small groups of attackers (Fig. 3). The majority of *M. rufiventris* foragers (62%) responded with no aggression, attempting only to escape, although all *M. rufiventris* foragers responded with aggression that resulted in the death of both parties when *T. spinipes* attacked with maximum aggression (level IV). Group attack size also influenced spatio-temporal attack strategy of *T. spinipes*, with foragers exhibiting strategic differences in the amount of time allocated to attacking the victim's legs (spending increasingly quantities of time biting legs closer to the victim's head, Fig. 2) and focusing more attention on appendages linked to escape (legs and wings) when attacking individually than when attacking in groups.

As observed by Johnson and Hubbell (1974) in other meliponine species, level IV attacks generally consisted of closely grappling pairs with one forager biting the neck of another forager. Although the neck biter would appear to have advantage, the victim of neck biting also maintained a tight clasp with her legs around her attacker, even in death. At the end of all experiments, we took some of these pairs and gently separated them before either forager had died. When separated at an early stage (<30 s after the start of attacks), both foragers frequently survived and flew off. However, when the attacks had continued for over 1 min, the separated foragers appeared exhausted, moving only slightly, and frequently died without attempting to fly, even when their wings were undamaged.

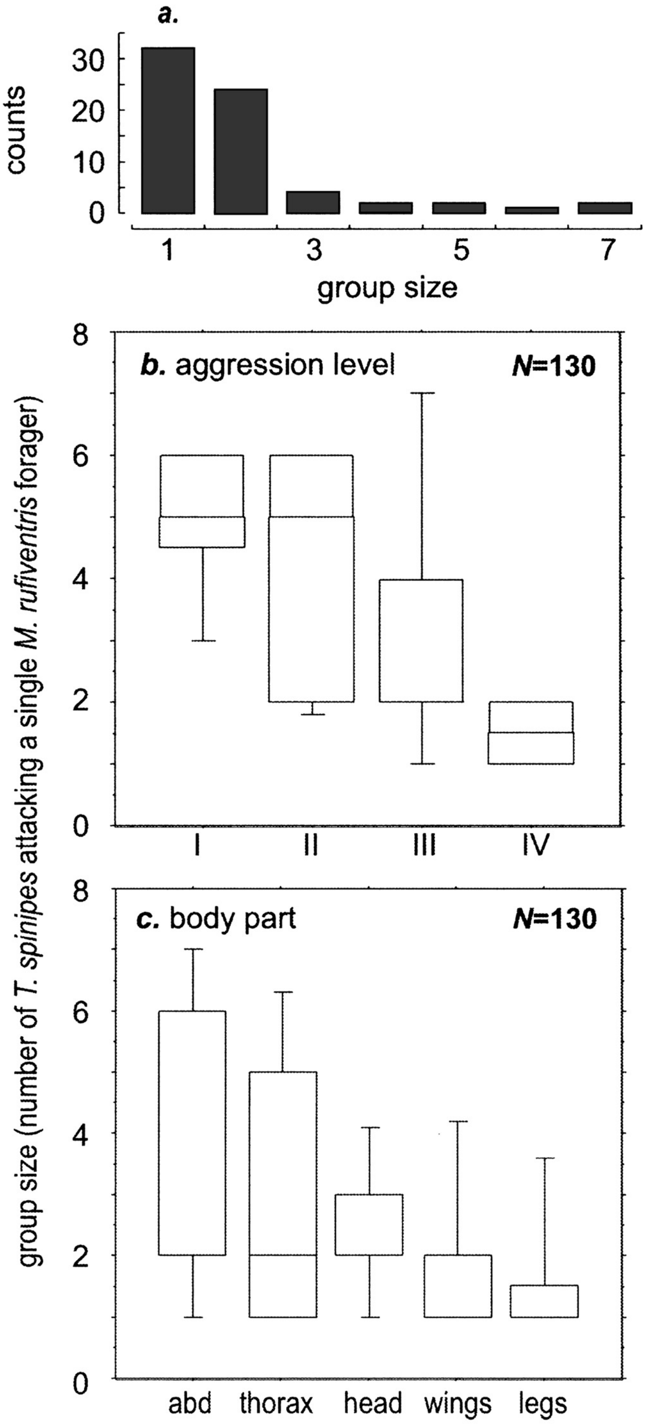

Figure 3. Effect of group size on the attack strategy of *T. spinipes* foragers attacking *M. rufiventris* foragers. **a**. Histogram of group attack sizes. **b**. Aggression level exhibited by *T. spinipes*. **c**. Body part of *M. rufiventris* forager that *T. spinipes* foragers attacked (abd = abdomen)

Spatio-temporal dominance style

In the case of individual attacks (a single attacker and a single victim), the spatial dominance style of *T. spinipes* consisted of assaults that equally targeted the appendages and different regions of the body (Fig. 1). The temporal dominance style consisted of attacking these different areas for approximately equal amounts of time (Table 2a), with the exception of the durations of attacks on the different leg pairs (Fig. 2). It is unclear why attackers should spend more time attacking more anterior legs (Fig. 2). Attacking the prothoracic legs may restrict the reach of the victim's mandibles via body pivoting to a greater extent than attacks on the metathoracic legs.

We did not observation escalation from low to high levels of aggression during the course of individual attacks. Instead, the swiftness and brevity of attacks (4.6 ± 5.1 s duration) resulted in the victims either retreating or immediately committing to a level of aggression. The rapidity of this process has been noted by other investigators (Johnson and Hubbell, 1974; Roubik, 1980) who have scored aggression levels based upon single attacks, not upon a temporal progression of aggression within an attacks. Our results support this approach, at least in cases of minimal resistance to an extirpating species.

Group effects

Group effects played an important role in the attack strategy. Aggression levels corresponded to the size of group attacks, with larger groups of attackers displaying overall lower aggression levels than smaller groups of attackers. The strategy of attacking at a lower level of aggression in larger groups may minimize the risk of individual injury because higher levels of aggression can escalate the risk of injury to both sides (Johnson, 1972; Archer, 1988). In our experiment, death occurred only at level IV and then to both attacker and victim (100% mortality in all level IV encounters), although biting at lower levels of aggression could also have damaged central body areas such as the head, thorax, and abdomen.

Individual strategy

Individual attacks focused on the wings and legs (Fig. 3c) whereas victims of larger groups (two to four attackers) were attacked with greater frequency on their central body (abdomen, thorax, and head) as well as on the wings and legs. This single-attacker strategy may keep the victim from escaping by pinning down its appendages until reinforcements arrive and group attack is possible. Many meliponine species can recruit and it is therefore advantageous to restrain and kill all foragers before they can call for reinforcements (Johnson, 1974). In our experiment, we did not observe either side recruiting newcomers from the nest during the attacks (all bees were individually marked to allow us to determine if recruitment occurred). However, many *T. spinipes* nestmates that had been foraging at the *T. spinipes* feeder located 4 m away from the *M. rufiventris* feeder eventually found the *M. rufiventris* feeder and took part in the group attacks. The ease with which *T. spinipes* took over the feeder and the relatively little resistance and counter-aggression exhibited by *M. rufiventris* (Table 1) suggests that additional recruitment was unnecessary for *T. spinipes* and that *M. rufiventris* generally avoids fighting over food sources or with *T. spinipes* in particular. *Melipona rufiventris* foragers generally flee from feeders that *T. spinipes* has odor marked (Nieh et al., 2004). We worked during a time of a relative, though not complete, food dearth, and thus the relative abundance of natural food sources may have influenced the foraging strategies of both species. Non-aggressive species may behave more aggressively under conditions of extreme food shortage, as predicted in ESS models of forager aggression (Richards, 2002).

Group strategy

During group attacks, there was a small increase in attacks on the head with increasing group size. At the largest group attack sizes (five to seven attackers), *T. spinipes* foragers attacked the central body more frequently than the appendages. The head, thorax, and abdomen are more vital than the appendages because we have sometimes observed *M. rufiventris* bees foraging with missing legs, antennae and damaged wings. Moreover, the generally non-fatal nature of appendage as compared to central body damage has been reported in stingless bees (Johnson and Hubbell, 1974), honeybees (von Frisch, 1967), and ants (Hölldobler and Wilson, 1990). It is interesting that the spatial strategy shifts to the more vital areas while individual aggression decreases in large groups. Relative to the victim, the larger attack groups are potentially more dangerous. To the attacker, larger attack group size may be safer. Thus attack intensity may vary separately of spatial strategy.

Assessment

How could *T. spinipes* foragers detect group size? Slaa et al. (2003) have shown that stingless bees foragers (*T. amalthea* and *Oxytrigona mellicolor*) are attracted by the visual presence of other foragers on food sources (local enhancement). During fights, stingless bees release alarm odors that nestmates can detect and orient towards (Johnson et al., 1985; Roubik, 1989). Moreover, social insects can use olfactory information to assess whether other individuals are nestmates (Wilson, 1971; Breed and Page, 1991; Breed and Stiller, 1992; Suka and Inoue, 1993; Inoue et al., 1999). It remains to be determined whether such mechanisms play a role in *T. spinipes* group size assessment or whether the group size effects arise as emergent properties that do not require group size assessment.

Spatial limitations

Could these negative density-dependent relationships arise because larger groups are spatially blocked from escalating to higher aggression levels or attacking particular body parts? *Melipona rufiventris* is on average 310% more massive and has a 140% greater head width than *T. spinipes*. Given these size differences, up to five *T. spinipes* foragers could spatially fit around to grasp and bite the head and mandibles of a single *M. rufiventris* forager (dorsal, ventral, left lateral, right lateral, superior planes, level IV aggression) and there is sufficient space for more than seven *T. spinipes* attackers to engage in level III aggression (extending biting and pulling of mandibles and extremities). We observed a maximum of seven *T. spinipes* attacking a single *M. rufiventris* forager. Thus higher *T. spinipes* aggression levels are not spatially prohibited by crowding around the victim at the group sizes observed for aggression levels I to III. There is more of a spatial limit to level IV aggression. However, this limit was not reached, because we observed a maximum of two *T. spinipes* foragers engaging in level IV aggression.

With respect to spatial strategy, larger group size should not preclude appendage attacks at the attack sizes observed. The eight appendages of each *M. rufiventris* victim (our classification: legs and wings) can accommodate eight attackers without forcing central body attacks, and we observed a maximum of seven attackers. Thus limits to the spatial packing of *T. spinipes* attackers around a single *M. rufiventris* victim are unlikely to account for our results. It is also possible that motion of an attack cluster makes it difficult for other attackers to engage. However, in larger attack groups the motion of the cluster was generally reduced as bees attacked from different sides around the victim, pulling in opposite directions and bracing themselves against the substrate. Such behavior has been reported in several different meliponine species (Johnson, 1974; see also Fig. 1 in Johnson and Hubbell, 1974).

Aggression with other species

Although we have focused on aggressive interactions between two native species*, T. spinipes* and *M. rufiventris*, *T. spinipes* may generally apply this strategy towards many types of competitors, including Africanized honeybees. *Trigona spinipes* has been shown to attack several species of stingless bees, carpenter bees, and Africanized honeybees (Cortopassi-Laurino, 1982; Sazima and Sazima, 1989). On two separate occasions we observed *T. spinipes* foragers attacking an *Apis mellifera* forager on orange blossoms at our field site. In both cases, a group of six to seven *T. spinipes* foragers were biting the legs and wings of the substantially larger *Apis* and eventually succeeded in severing some wings and legs.

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