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Research article

Jigging in the fungus-growing ant *Cyphomyrmex costatus*: a response to collembolan garden invaders?

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Summary. A conspicuous, rhythmic rocking behavior ("jigging") in the fungus-growing ant *Cyphomyrmex costatus* is described with the help of high-speed videotaping. The ants are known to perform this behavior in their nest, but the cause and function of jigging has so far remained unclear. This study tested one cause of this behavior: if it is triggered by collembolan invaders that feed on the ant's fungal garden, as hypothesized by Weber in 1957. Two experiments were conducted. The first experiment surveyed jigging rates in colonies infected with Collembola and in colonies free of Collembola. The rate of jigging was significantly higher in the colonies with Collembola. In the second experiment, to show causation of jigging by Collembola, a series of single ants were isolated with garden fragments from their colonies and jigging was scored in the presence or absence of Collembola. Again jigging was significantly higher in the presence of the added Collembola, whereas no jigging was observed in the ants kept without Collembola. The experiments therefore support Weber's hypothesis that the presence of Collembola can trigger jigging. However, because jigging was also observed in colonies without Collembola, it cannot be the sole cause of the behavior, and other causes need to be investigated. In addition, the function of jigging in the presence and absence of Collembola also needs to be investigated.

Key words: *Cyphomyrmex costatus*, Collembola, communication, resource defense.

Introduction

The fungal gardens maintained by fungus-growing ants (tribe Attini) provide a concentrated resource that is susceptible to attack by parasites. There are many invaders of the garden including generalized fungivorous pests and specialized competitors for the fungal resource, such as *Escovopsis*, a fungal pathogen (Currie et al., 1999), and ants of the genus *Megalomyrmex* (Solenopsidini), which steal or usurp fungal gardens (Adams et al., 2000). This study examines the behavior of an attine ant species in response to the presence of an arthropod nest invader.

Collembola are an important group of fungal garden pests and are found in nests in natural and in laboratory conditions (Weber, 1957, U.G. Mueller, pers. comm.). In lab colonies, Collembola will sometimes reach high population sizes that infest the ants' nestbox (Weber, 1957, 1964, 1966, 1972; Kweskin, pers. obs.). They are predominately found in the garden chamber of the colony where they consume fungal mycelium, directly competing with the ants for their food source (Weber, 1972; Kweskin, pers. obs.).

Weber (1957) described a peculiar rhythmic rocking behavior in the attine ant *Cyphomyrmex costatus* that he termed "jigging" (Fig. 1), and he hypothesized that this behavior may be stimulated by the disturbance caused by the presence of collembolans or the physical disturbance of a laboratory colony through jarring. Weber also suggested two functions of this behavior: first, as a signal to alert nestmates in response to a disturbance (Weber, 1957), or second to directly drive off pests (Weber, 1972). Jigging also occurs in other lower attine species (e.g. *Myrmicocrypta buenzlii* (Weber, 1972), *C. longiscapus* (pers. obs.), *C. whelleri* (pers. obs.) and *C. muelleri* (pers. obs.)), but not in the higher attine genera *Atta*, *Acromyrmex* or *Trachymyrmex*.

This study tests the relationship between collembolan fungal garden pests and jigging in *C. costatus*. First, jigging behavior is described in detail to differentiate it from other similar behaviors. Then, the correlation between presence of Collembola and occurrence of jigging in colonies was examined, followed by an experimental manipulation to determine if jigging in the ants is a response to the presence of Collembola.

Methods

Experimental colonies of C. costatus

Nests of *C. costatus* were collected May-June, 1998 from the Republic of Panama in Soberanía National Park, Ancon Hill, and Fort Sherman

Military Reservation. Each colony with its garden was transferred to a plaster bottomed laboratory nest box, as described in Schultz (1993). Prior to this study, the nests were kept in the laboratory at room temperature for 18 months. During this period, Collembola reached a high population density in some of the colonies and jigging was common. These factors facilitated the use of this species in the study of jigging. At the time of the experiments, the colonies had a population of 30–100 workers per nest.

Behavioral observations and high-speed videorecording

To aid in the general description and quantification of jigging, video analysis was conducted using high-speed camera footage. The video was recorded at 250 frames per second (FPS) captured through a dissecting microscope. The average rate of jigging was determined by the number of complete jigs counted from high-speed footage representing four seconds of uninterrupted jigging (n = 8 jigging events). The duration of jigging bouts, the average length of time an individual jigged without interruption, was also measured (n = 19). All rates are reported as the mean ± SE.

Experiment 1: Correlation of Collembola presence and jigging

The presence of Collembola varied between laboratory colonies of *C. costatus*. Some of the colonies used for this experiment had an abundant population (approximately 20–100 Collembola) of one species of Sminthuridae Collembola (n = 6 colonies), while other colonies had no Collembola (n = 5 colonies). The amount of jigging in the colonies with and without Collembola was compared. If jigging by ants is related to the presence of pests, higher rates of this behavior would be expected in colonies with Collembola than without.

To compare the level of jigging in the two groups of colonies, jigging rate in each colony was quantified during 30-minute trials. Each trial consisted of 90 5-second observations. During each observation the number of jigging ants was counted. The 5-second observations were separated by 15 seconds of no recording. The rate for the colony was computed as the percentage of the 90 observations where at least one ant jigged. All trials were made in a similar environment- with fluorescent office lighting and on a laboratory bench unbuffered from background vibrations. Observations did not start for at least five minutes following the set up of nests or a change in room lighting; this helped control for the effect of these disturbances. A comparison of jigging frequency between the two groups of colonies (6 nests with Collembola and 5 without) was made using a two-tailed Mann-Whitney test.

Experiment 2: Causation of jigging by Collembola

Based on the results of the first experiment, which showed a correlation between jigging and the presence of Collembola, the causation of jigging by Collembola was tested through experimental manipulations. Four workers from each colony (n = 18) were isolated in 4 separate arenas, each of these replicates consisting of a single ant and a fragment (approximately 5 mm^3) of its fungal garden. Three Collembola were added to two of the arenas using an aspirator and the other two remained without Collembola. Differences in the occurrence of jigging in these two treatments were then compared in a paired experimental design. If Collembola cause ants to jig, the incidence of jigging should be higher in the workers that had Collembola experimentally added compared to their nestmates who did not have Collembola added.

The arenas were plastic Petri dishes (6 cm diameter) that were half-filled with plaster of Paris and then sealed around the sides with a strip of Parafilm™. Workers chosen for the experiment were tending the fungal garden at the time of translocation, because pilot observations had indicated that jigging is most frequently observed in ants that are tending the garden. The source of the transferred Collembola was the original colony (when the source colony had Collembola) (n = 9 colonies) or from a different colony (when the source colony was free of Collembola) (n = 9 colonies). Following the transfer of Collembola, each worker was observed for 15 min twice a day for the following three days. During these observations each occurrence of jigging was scored. All four isolated workers from each colony were observed simultaneously to control for variation caused by background disturbances. The observations of each worker from all six observation periods were then combined, and a worker was categorized as either (1) having at least one bout of jigging during the observations or (2) having not jigged during the observations. A Wilcoxon signed-rank test was used to test the significance of these results. This paired comparison contrasted the number of workers that jigged in the two treatment types from each colony (0–2 of the two workers jigging per treatment) to test the null hypothesis that Collembola have no effect on jigging.

A second possible effect on jigging by the isolated workers was if their original colony had Collembola. This could effect the observed jigging when the workers were isolated, regardless if they have had Collembola added or not. To test this, jigging in isolated ants from colonies with Collembola was compared to colonies without Collembola. This comparison was done without regard to if Collembola were added to their arena or not. For each colony, the number of ants that jigged of the four isolated ants was summed (0–4). The jigging by the two groups of colonies was compared using a Mann-Whitney test. No difference between the two groups would support the null hypothesis that the state of Collembola in the source colony has no effect on jigging by isolated workers.

Results

Jigging

Jigging was observed in queens (both alate and de-alate) and workers. It occurred on the garden as well as on the nestbox's plaster bottom. As observed by Weber (1957), several ants often would jig at the same time. The extent that these ants were coordinated in their jigging was not determined. While jigging, the ant typically kept its middle and rear legs on the substrate while moving its front legs and alitrunk forward and back (Fig. 1). Before an ant started jigging it would be performing typical garden maintenance activities. After it finished, it would return to this activity. There were two anomalous observations of the 34 video recordings of ants jigging. In one case an ant regurgitated while jigging and in a second case an ant was pulling a piece of the fungal cultivar mycelium with its mandibles while jigging. The mean rate of jigging for eight separate jigging bouts was 6.9 ± 0.2 jigs · s^{-1}. Jigging bouts lasted 12.9 ± 2.3 s (n = 19).

A second behavior observed in the colonies of *C. costatus* was a jumping forward by workers. A jumping *C. costatus* worker will quickly (duration of 24.7 ± 2.4 ms, n = 6) jerk forward a short distance (about 2mm), often with its mandibles open. In one case an ant jumped and caught a Collembola in its mandibles, however whether the captured collembolan was killed or was able to escape is unknown. Weber (1966) described this behavior in *C. costatus, Myrmicocrypta spp.* and *Acromyremx octospinosus* colonies.

This jumping behavior is distinct from jigging. In jumping all six of the workers' legs leave the substrate and the ant lands forward of its origin, while in jigging the workers rock back and forth keeping their four hind legs on the substrate for the duration of the jigging. Because this behavior was dis-

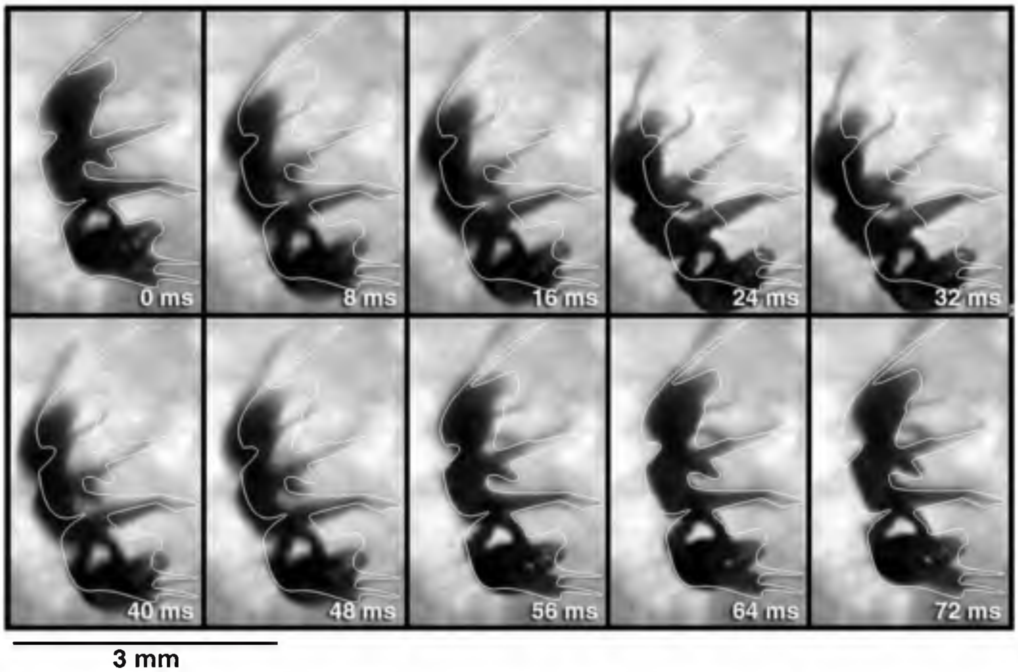

Figure 1. Jigging in the fungus-growing ant, *Cyphomyrmex costatus*. The behavioral sequence shows one "jig" by a worker, starting with all legs on the garden, then raising the front legs, and the return of all legs to the substrate (outline shows original position). The rate of jigging averages 6.9 ± 0.2 jigs/s (n = 8)

tinct from jigging, observations of jumping were not included in the experiments below.

Correlation of Collembola presence and jigging

In the observations of jigging in unmanipulated colonies, there was a significantly higher rate of jigging in those nests with a preexisting Collembola population compared with those nests without (Fig. 2). Those nests with Collembola (n = 6) had a mean of 70.1% ± 13.5% of the 5-second observation periods with jigging, compared to 21.6% ± 7.1% in those colonies without (n = 5), ($p < .02$, Mann-Whitney, $U_A = 5$).

Causation of jigging by Collembola

Jigging was observed in 18 of the 72 workers isolated in separate arenas. Of the 18 colonies sampled, some jigging was seen in isolated workers from 11 of them. In *every* case where an isolated worker jigged, it occurred in a test-arena where Collembola were added (Fig. 3), but never in the absence of Collembola ($P \ll 0.01$, Wilcoxon Signed-Rank test), thus showing that the presence of Collembola is a causal factor that can trigger jigging.

The presence or absence of Collembola in the isolated worker's source colony before the experiments was not a significant effect in the occurrence of jigging. Of the 18 source colonies from which workers were tested, nine had Collembola and nine were free of them. There was a nearly equal distribution of jigging in the workers tested from these two groups (Fig. 4, NS, Mann-Whitney, $U_A = 47$). This supports the null hypothesis in this test, that the occurrence of jigging

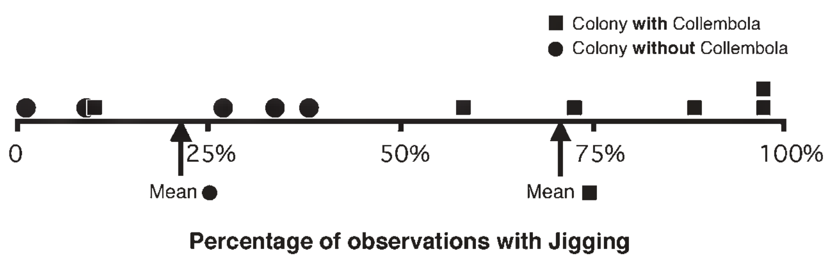

Figure 2. The percentage of the 90 five-second observations over 30 min where any jigging was observed in laboratory *Cyphomyrmex costatus* nests. Nests with Collembola (square) garden pests were significantly more likely to jig than those without Collembola (circle) ($p < 0.02$, Mann-Whitney, $U_A = 5$)

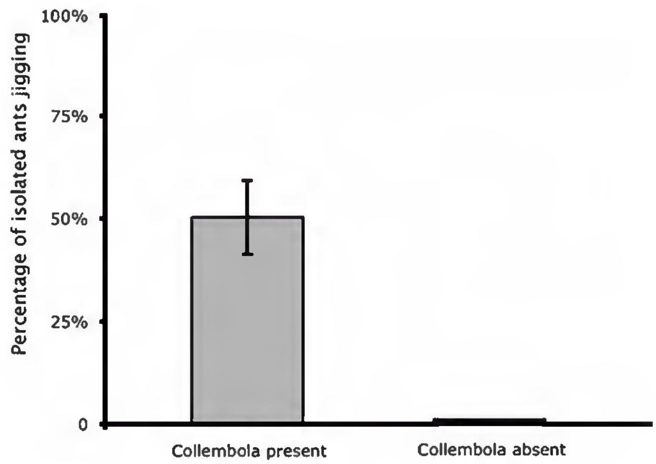

Figure 3. The jigging observed in isolated workers of *Cyphomyrmex costatus* with and without Collembola present during six 15-minute observations. Jigging was only observed in workers with Collembola present, but never in isolated nestmates without Collembola ($P \ll 0.01$, Wilcoxon Signed-Rank test)

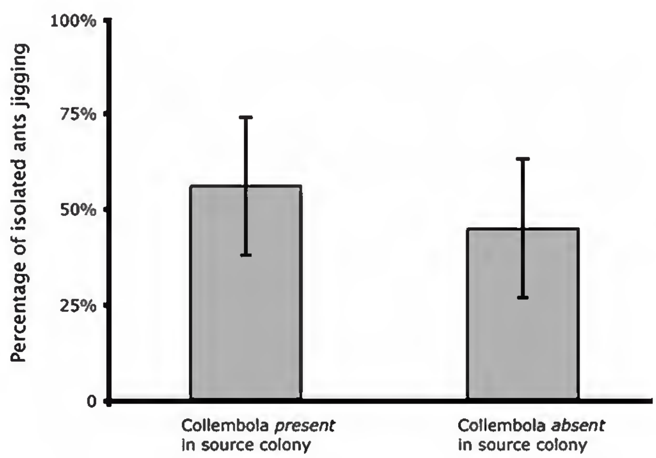

Figure 4. The effect of Collembola presence in the source colony on jigging by isolated workers. There was no colony level effect of jigging that could be attributed to the source colony of the experimental ants either having or not having Collembola (NS, Mann-Whitney, $U_A = 47$)

is independent of the condition of the source colony, and implies that jigging was a response to the local presence or absence of Collembola during the experiments.

Discussion

Jigging behavior in the fungus growing ant *C. costatus* was found to be correlated with and caused by the presence of collembolan fungal garden pests. The correlation experiment showed that jigging was more common in nests with Collembola, however low levels of jigging were also observed in nests without Collembola (Fig. 2), suggesting Collembola were probably not the sole elicitor of jigging. Other arthropod invaders in the garden might be present but not observed (e.g., mites, which can easily be overlooked against the white garden background), or other disturbances (e.g. a change in room lighting) might be responsible for some of the jigging (Weber, 1957). These other causes are worth investigating and can be helpful in elucidating the function of this behavior. In the experiment showing causation, jigging was seen exclusively in isolated workers with Collembola present, however only half of these ants with Collembola jigged. The lack of jigging in all of the workers with Collembola may be due to one or more of the following factors: low encounter rates between the ants and Collembola, the observation period following the manipulation may have been too short, or ants encountering Collembola does not always cause jigging. Detailed observations of ants immediately after encountering Collembola could clarify this.

With one cause of jigging elucidated, the function of this behavior can be investigated. The function might be directly related to defending against pests such as Collembola, perhaps by driving them off, or it might function as a more general response to disturbance. Evidence for the direct effect of jigging on Collembola appears to be minor. Jigging often occurred when no Collembola were in the near vicinity and the behavior was observed in colonies where there were no Collembola. Moreover, Collembola on the fungal garden were sometimes observed walking past an ant that was jigging without the Collembola responding (pers. obs.), suggesting that jigging is not a fully effective behavioral defense against Collembola. Jigging may function as an elicitor of other nest defensive behaviors to nestmates. In contrast, the jumping behavior that was also observed does appear to be directly defensive. It usually occurred just after a Collembola approached the garden, often resulting in the Collembola leaving the vicinity of the ant. In one case an ant was observed jumping and catching a collembolan in it mandibles (pers. obs.).

The function of jigging as a signal to nestmates is a promising area of future investigations. This function could be related to general disturbances and explain the jigging seen in nests without Collembola. Jigging could be a vibrational signal, which are common in ants (Hölldober, 1999) and are known from several species of attines of the more derived genera *Atta* and *Acromyrmex* (Markl, 1965). These ants use stridulation as a signal to nestmates (Roces, et al. 1993; Roces and Hölldobler, 1995), and they might also use it to mechanically aid in leaf cutting (Tautz et al., 1995, but see Roces and Hölldobler, 1996). However, *C. costatus* may be too small to generate sufficient acceleration of the substrate to transmit a vibrational signal through the fungal garden or the nest floor (D. Tautz, pers. comm.). Alternative explanations, such as enhanced dispersion of volatile chemicals through the jigging motion, are more promising (D. Tautz, pers. comm.), and such explanations now need to be investigated to elucidate the purpose of jigging in the presence and absence of Collembola.

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References

Adams, R.M.M., U.G. Mueller, T.R. Schultz and B. Norden, 2000. Agro-predation: usurpation of attine fungus gardens by *Megalomyrmex* ants. *Naturwissenschaften 87*: 549–554.

Currie, C.R., U.G. Mueller and D. Malloch, 1999. The agricultural pathology of ant fungus gardens. *Proc. Natl. Acad. Sci. USA. 96*: 7998–8002.

Hölldobler, B., 1999. Multimodal signals in ant communication. *J. Comp. Physiol. A. 184*: 129–141.

Markl, H., 1965. Stridulation in leaf-cutting ants. *Science 149*: 1392–1393.

Roces, F. and B. Hölldobler, 1995. Vibrational communication between hitchhikers and foragers in leaf-cutting ants (*Atta cephalotes*). *Behav. Ecol. Sociobiol. 37*: 297–302.

Roces, F. and B. Hölldobler, 1996. Use of stridulation in foraging leaf-cutting ants: mechanial support during cutting or short-range recruitment signal? *Behav. Ecol. Sociobiol. 39*: 293–299.

Roces, F., J. Tautz and B. Hölldobler, 1993. Stridulation in leaf-cutting ants: short-range recruitment through plant-borne vibrations. *Naturwissenschaften 89*: 521–224.

Schultz, T.R. 1993. Stalking the wild attine. *Notes from the Underground 8*: 7–10.

Tautz, J., F. Roces and B. Hölldobler, 1995. Use of a sound-based vibratome by leaf-cutting ants. *Science 267*: 84–87.

Weber, N.A., 1957. Fungus-growing ants and their fungi: *Cyphomyrmex costatus*. *Ecology 38*: 480–494.

Weber, N.A., 1964. A five-year colony of a fungus-growing ant, *Trachymyrmex zeteki*. *Ann. Entomol. Soc. Am. 57*: 85–89.

Weber, N.A., 1966. Fugus-growing ants and soil nutrition. *Actas del 1st Coloquio Latinoamericano Biologia del Suelo 1*: 222–256.

Weber, N.A., 1972. *Gardening Ants, the Attines*. The American Philosophical Society, Philadelphia, Penn. 146 pp.