Subcaste composition in responses to alarm and non-alarm pheromones on foraging trails of leaf-cutter ant *Atta cephalotes* (Hymenoptera: Formicidae)

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ABSTRACT

The response of the leaf-cutting ant *Atta cephalotes* to its alarm pheromones from crushed heads were compared to its response to crushed bodies without heads in order to examine the relative subcaste (minor and forager) composition of the responding individuals. The percentage of minor workers responding to the body parts near the foraging trail were recorded and compared with the experimentally determined percentage of minors in the trail traffic. The average percentage of minors in the ants responding to the two treatments (Heads: 44.9±0.72%, Bodies: 38.8±0.77%, N = 1300) both differed (p = .0007, p = .0113, df = 1) from the baseline trail composition (22.3±0.51%, N = 405), but did not differ from each other (p = .382, df = 1). There was a difference in the strength of the reaction over time between the heads and bodies for both minors (p < .0001) and foragers (p = .004), suggesting a difference in stability between the pheromone types. The unexpectedly strong reaction by minors to crushed bodies calls into question the validity of previous conclusions regarding the role of minor workers in foraging trails, especially considering the proportional equality of minor reactions to alarm and non-alarm pheromone sources.

RESUMEN

La respuesta de zompopas *Atta cephalotes* hacia sus feromonas de alarma obtenidas de cabezas maceradas se comparó con la respuesta a cuerpos macerados en orden de conocer la respuesta relativa de sub-casta (mínima y forrajeador). El porcentaje de mínimas respondiendo a las partes corporales cercanas al sendero de forrajeo se compararon con porcentajes experimentalmente determinados de mínimas en el sendero transitado. El promedio de mínimas en respuesta a los dos tratamientos (Cabezas: 44.9±0.72%, Cuerpos: 38.8±0.77%, N = 1300) son diferentes (p= .0007, p= .0113, df= 1) de la línea de base de la composición del sendero (22.3±0.51%, N = 405), pero no difiere una de otra (p = .382, df = 1). Existe diferencia en la fuerza de la reacción entre la cabeza y el cuerpo entre mínimas (p < .0001) y forrajeadoras (p = .004), sugiriendo una diferencia en la estabilidad entre los tipos de feromonas. La inesperada fuerte reacción de las mínimas hacia cuerpos macerados llama a preguntarse sobre la validez de conclusiones previas con respecto al papel de mínimas en los senderos de forrajeo, especialmente considerando la equidad proporcional de la reacción de las mínimas hacia feromonas de alarma y no alarma.

INTRODUCTION

Alarm pheromones are nearly ubiquitous in eusocial insects, although the responses generated by these chemical signals vary considerably between species (Hölldobler & Wilson 1990). They can be broadly characterized as being either aggressive or panic signals, the former involving some or all colony members being attracted to the alarm source with the intention of attacking it and the latter involving colony members fleeing the alarm source or simply running around erratically (Hölldobler & Wilson 1990). Odor trail pheromones also attract nearby workers, but

can be distinguished from alarm attraction by whether or not the workers display certain alarm behaviors, which most commonly include increased movement speeds and wide-ranging movements, but may also include other behaviors such as gaping mandibles or even spontaneous digging (Blum 1969). Many polymorphic social insects demonstrate a caste-based polyethic alarm response, or a difference in reactions to a pheromone source among morphologically distinct subcastes (Roisin *et al.*1990, Hughes & Goulson 2001). Reactions to alarm pheromones can also be affected by contextual factors such as concentration and duration of pheromone signals or current occupation of workers (Wilson 1963, Hölldobler 1981, Hughes & Goulson 2001).

Leaf-cutter ants (Hymenoptera: Formicidae: Attini) of the genus *Atta* are dominant herbivores throughout the neotropics, living in colonies of up to eight million individuals and consuming as much as 17% of total leaf production (Hölldobler & Wilson 1990). Their workers are highly polymorphic, and are generally considered to have four morphologically distinct subcastes: minims, media, foragers, and soldiers (Wilson 1980, Hughes & Goulson 2001). There is no firmly defined morphological distinction between subcastes, as size ranges between them are continuous, but it is generally accepted that ants with a head width of less than 1.4 mm are unable to cut leaves while ants with a head width of more than 1.2 mm seem to be unable to perform many tasks inside the nest, providing a functional separation between "minors" and "foragers" (Hölldobler & Wilson 1990). These subcastes are also highly polyethic, exhibiting a degree of division of labor that separates the subcastes even further behaviorally (Wilson 1980, Hughes & Goulson 2001, Evison *et al.* 2007). It is not surprising, then, that several species of *Atta* have shown polyethism in colony defense and reactions to alarm pheromones (Wilson 1980, Hughes & Goulson 2001), although the exact nature of the polyethism is not yet certain. Typical alarm behavior involves increased movement rate, gaping mandibles, and attacking foreign objects or organisms, all characteristic of a standard aggressive alarm response (Hughes *et al.* 2001b). The primary component of the alarm pheromone in this genus, which is released from mandibular glands in all *Atta* species (Hughes *et al.* 2001b), is 4-methyl-3-heptanone (Moser *et al.* 1967, Blum 1969, Hughes *et al.* 2001a, b). Although it does not appear to be the only chemical component of the alarm signal, it has been noted that no chemical capable of inducing alarm is located in the abdomen or thorax of the body, indicating that the entire or at least the critical component of the *Atta* alarm pheromone is located in the head (Hughes & Goulson 2001).

There is some evidence that minor workers may play a key role in defending the main nest chambers, at least against larger (i.e. vertebrate) enemies (Wilson 1980). Recent studies, however, have suggested that minors may play an important role in trail defense as well. One of these studies found that *Atta capiguara* workers responding to artificial alarm sources (crushed heads) placed near a foraging trail were composed of a much greater than expected percentage of minor (head width < 1.4 mm) workers, as compared to the measured trail composition (Hughes & Goulson 2001). Foragers (head width ≥ 1.4 mm) were less likely to respond to the alarm source, possibly because of a decreased sensitivity to the alarm pheromone. Foragers that were

transporting cargo did not respond at all. They also noted that minors tended to follow a slow, meandering route along the trail in comparison to foragers, suggesting that their role on the foraging trail does not involve fast, efficient movement (Hughes & Goulson 2001). A parallel study noted that crushed bodies without heads also acted as strong attractants to trail workers, but did not generate any alarm behavior (increased movement, gaping jaws, or biting) like the heads (Hughes *et al.* 2001b). However, Hughes 2001b did not determine the subcaste composition of the workers responding to the crushed bodies. These studies concluded, based on the greater than expected percentage of minors responding to alarm pheromones and the meandering route that minors took, that the main function of minors on foraging trails is to patrol the trails for threats, and to be the primary defenders engaging such threats. This conclusion is in part drawn from reports of a distinct "patroller" class of workers in at least two other ant species that demonstrates locomotion and response patterns similar to those found in *A. capiguara* minors (Gordon 1987, Nelson *et al.* 1991 in Hughes & Goulson 2001).

One possibility that was not considered in these studies, however, is that minors respond disproportionately to all pheromones or even all olfactory cues instead of only to alarm signals. Although they established that crushed bodies alone function as olfactory attractants without inducing alarm behavior, the proportion of minors responding to the bodies was never determined, leaving the possibility that the larger than expected percentage of minors responding to the heads was not related to the alarm nature of the pheromones. Although this omission does not invalidate all of the conclusions drawn by these studies, it is premature to assume without this knowledge that the main role of minors on foraging trails is primarily one of patrol and defense. Here that assumption was tested by performing a similar experiment on an *Atta cephalotes* colony in which relative subcaste reactions of foraging trail workers to both crushed heads and crushed bodies (thorax and abdomen) were recorded and compared to an established baseline subcaste composition of the trail. It was predicted, in following with the conclusions drawn previously, that the heads would cause an aggressive alarm reaction with a greater than expected percentage of minor workers (defined as those with head width < 1.4 mm) responding, and that the bodies would have an attractive effect on trail workers without causing alarm behavior and with no difference between the subcaste proportions of responding workers and standard trail composition.

METHODS

Study Site

All work was performed at an *A. cephalotes* nest in the trail system of the University of Georgia Ecolodge and Research Station in San Luis, Puntarenas, Costa Rica. All sampling was done along the main foraging trail one meter from the main nest entrance between 7:30 A.M. and 4:30 P.M. from November 9-17, 2010.

Baseline trail composition

To establish the baseline ratio of minors to foragers on the trail, 405 one-minute counts were taken of the numbers of minors (head width < 1.4 mm) and foragers (head width ≥ 1.4 mm) that passed a specific point in the trail (one meter from nest entrance) during the trial. Differentiation of the subcastes could be reliably performed by eye, and a small length of 1.5 mm diameter wire was placed perpendicularly next to the trail for visual comparison to ensure complete accuracy. These counts were taken consistently throughout sampling periods to accurately reflect the baseline subcaste composition of trail traffic.

Experimental Trials

One hundred experimental trials (50 for each treatment) were performed to compare the responses of ants on the trail to crushed heads and crushed bodies (thorax and abdomen). For each treatment the appropriate body section was removed from a forager with a head width between 2.5 and 3 mm and immediately crushed with a clean metal rod on a small flat stone that had been placed flush with the substrate surface in the center of a raised wire circle 10 cm in diameter. The center of the circle was located 5 cm from the side of the foraging trail and one meter from the nest entrance. The number of foragers and minors inside the circle was recorded at 20-second intervals for five minutes, then at one-minute intervals for ten more minutes. Differentiation was performed by eye, and a small length of 1.5 mm diameter wire was placed inside each circle for comparison to ensure complete accuracy.

RESULTS

Total Subcaste Proportions

The average percentage of minors found in normal trail traffic over the course of the study was 22.3±0.51% (N=405). The percent minors in the composition of responses to the crushed heads and crushed bodies were 44.9±0.72% (N = 1300) and 38.8±0.77% (N = 1300), respectively. The difference in these percentages was significant when comparing either the crushed heads ($X^2 =$ 11.45, df = 1, $p = 0.0007$) or the crushed bodies ($X^2 = 6.45$, df = 1, $p = 0.0113$) to normal trail traffic (Fig. 1), but there was no difference in percent minors between the ants responding to the heads and bodies ($X^2 = 0.765$, df = 1, $p = 0.382$).

Numerical Responses Over Time

The average numbers of minors and foragers responding to the heads rose steadily during the first five minutes of the trials, peaking at seven minutes for both subcastes and then showing a slight decline over the rest of the trial (Fig. 2).The rates of numerical change over time were

equal for the heads in minors and foragers. (t = .4429, df = 2596, p = 0.658; Fig. 2). The bodies showed a similar pattern (Fig. 3), with no difference in slope between numbers of minors and foragers over time (t = 0.175, df = 2596, p = 0.861; Fig. 3). The overall trend over time differed, however, with both subcastes increasing steadily throughout the fifteen minutes of the trials.

Minors responded more quickly to heads than bodies (t = 3.91, df = 2596, p < 0.0001; Fig. 4). Foragers responded more quickly to bodies than heads (t = 2.90, df = 2596, p = 0.004; Fig. 5). Both subcastes showed a peak at the 7:00 minute mark in the crushed head trials and a steady increase in the crushed body trials.

Further Observations

There was a distinct difference between the reactions of ants of any caste to the crushed heads versus the crushed bodies. Ants responding to heads nearly always exhibited alarm behavior: faster locomotion, raised and gaping mandibles, and a tendency to attack any animate or inanimate objects they encountered (except nestmates). They also frequently attempted to climb into any hole in the substrate that they encountered, even if it was too small for them to fit into or simply a small depression. Ants responding to bodies virtually never exhibited any of these behaviors.

There was also a marked difference between the reactions to head and body trials of ants with cargo returning to the nest. In all of the head trials, no ant carrying cargo ever entered the wire circle. If they responded at all to the presence of the crushed heads, it was expressed by increased locomotion speed away from the signal source. In contrast, all unladen workers that showed any reaction to the heads aggressively moved towards it and searched for the source. In the body trials, however, it was not uncommon for ants carrying cargo to enter the circle, although they rarely stayed long.

DISCUSSION

Both crushed heads and crushed bodies served as strong attractants to workers on the foraging trail. The disproportionately high percentage of minors responding to the alarm pheromones as well as the observation that alarm behavior was strongly associated with the heads while absent from reactions to the bodies supports previous research (Hughes & Goulson 2001). The complete absence of laden workers in the response to alarm pheromones that was seen in both this study and Hughes & Goulson 2001b provides even more support to the hypothesis that the degree to which a worker is occupied with a task is the single most important factor in determining whether it will respond to an alarm signal (Moser *et al.* 1991, Hughes & Goulson 2001). The lack of alarm behavior in response to the crushed bodies is predicted by previous research that established submandibular glands as the source of alarm pheromones in *Atta* (Hughes *et al*. 2001a). The non-alarming attractiveness of the crushed bodies is likely due to a variety of trail

and navigation chemicals located in exocrine glands in the thorax and abdomen of workers (Hölldobler & Wilson 1986).

An examination of the change over time in response strength throughout the fifteen-minute trial period shows that despite the increased proportion of minors in both responses, minors and foragers did not differ in the timing of their responses in either of the experimental treatments. Furthermore, the relative subcaste composition of the responders did not change over the course of the trial for either treatment. It is interesting to note, however, that there was a pronounced difference in both subcastes between the timing of responses to the heads and the bodies. While the attraction strength of the bodies did not diminish over the trial period, the heads showed a peak in number of workers present at the 7:00 mark in both the subcastes, followed by a slow decline throughout the rest of the trial. A logical explanation for this can be found in the contrast between the urgency of alarm signals and the necessity for long-lasting effects in trail or navigation pheromones. From a chemical perspective, these functional requirements are reflected in the relatively small, volatile nature of alarm compounds compared to the larger, more stable trail chemicals (Hölldobler & Wilson 1990),

It has been posited that minor workers on foraging trails represented a distinct "patroller" class, their function being general defense of the trail by seeking out and responding to threats (Hughes & Gulson 2001). This observation is based on the behavior of minor workers observed in experiments with *Atta* (slow, indirect movement, no obvious task being performed, and increased response to alarm signals) was very similar to those of other ant species that are known to have a distinct "patroller" class specializing in security and defense (Gordon 1987, Nelson *et al*. 1991 in Hughes & Goulson 2001). However, the validity of conclusions drawn in their study are called into question by the finding that minors respond disproportionately to crushed bodies as well as heads, but do not exhibit any alarm behavior when doing so. That there was no difference in reaction between heads and bodies, in particular, begs an answer to the question of why minors respond so strongly to non-alarm pheromone sources if their primary function is that of patroller and defender.

One potential answer to that question is that their primary function on foraging trails is not, in fact, that of patroller and defender. It is possible that previous work has focused too narrowly on alarm signals, resulting in a premature conclusion that the purpose of minors on the trails is restricted to or focused on trail defense when in fact their role is much broader than supposed. The results presented here suggest that minors may be equally attracted to all olfactory cues, and that their disproportionately strong responses to them are due to a higher sensitivity and lower response threshold than is found in foragers.

This possibility is supported by the recent discovery that minor workers play a major role in the maintenance of pheromone trails (Evison *et al.* 2007), indicating at least one other important role for minors and highlighting the fact that they may have other functions that are still unknown. That the bodies of *Atta* workers contain several navigation related pheromones (Hölldobler & Wilson 1986), however, makes the discovery of minors' attraction to crushed bodies of limited use in guessing what these other roles may be if they exist.

It is important to note that these findings do not necessarily conflict with the idea of minor workers being trail patrollers and defenders, but simply raise questions about the assumptions that have assigned them that role. It is entirely possible, even likely, that if minors do serve several main purposes on foraging trails one of these purposes is a primary role in defense. This would be the most parsimonious explanation for the striking similarity between their behavior and that of known patroller classes in other species (Gordon 1987, Nelson *et al*. 1991 in Hughes & Goulson 2001). The apparent sensitivity of minors to olfactory signals could indicate a tendency to investigate any and all sources of unusual activity in their effort to maintain vigilant.

More investigation is required to fully understand the roles of minor workers on foraging trails and their implications for differences in behavioral responses. The findings presented here call into question the effectiveness of previous hypotheses regarding both of those subjects by demonstrating an equal, consistent, and disproportionately large percentage of minor workers in the responses to crushed heads and bodies.

ACKNOWLEDGMENTS

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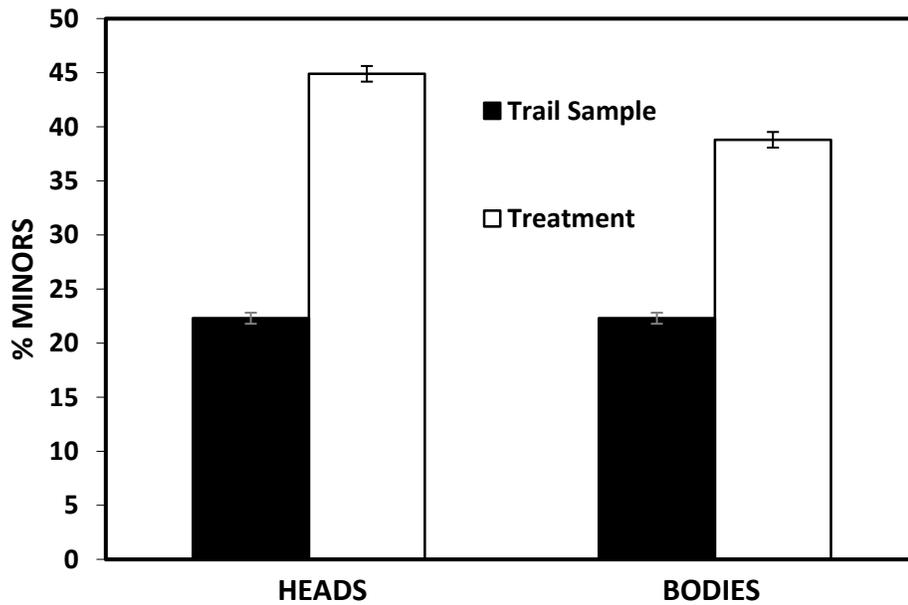
FIGURES

FIGURE 1.   Comparison of overall average percentage of minors in heads and bodies and in trail traffic. Both treatments are significantly different from the trail (Heads: p = 0.0007, Bodies: p = 0.0113, df = 1), but not from each other (p = 0.382, df = 1).
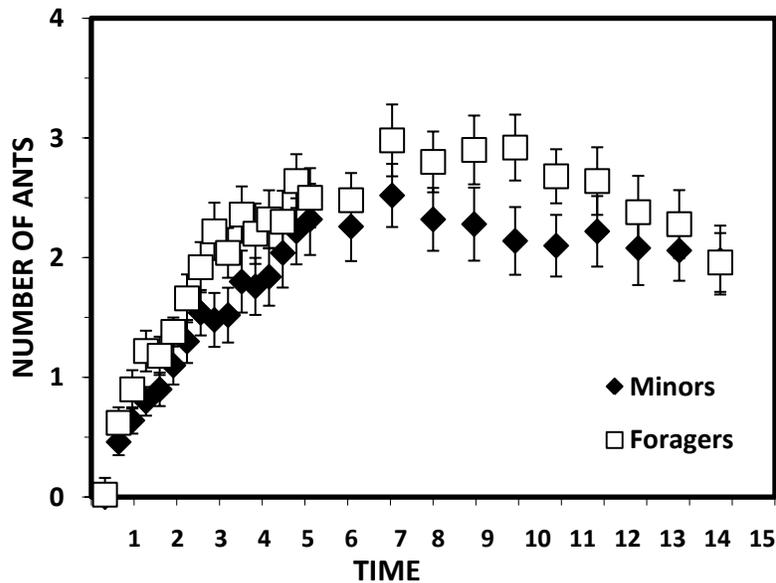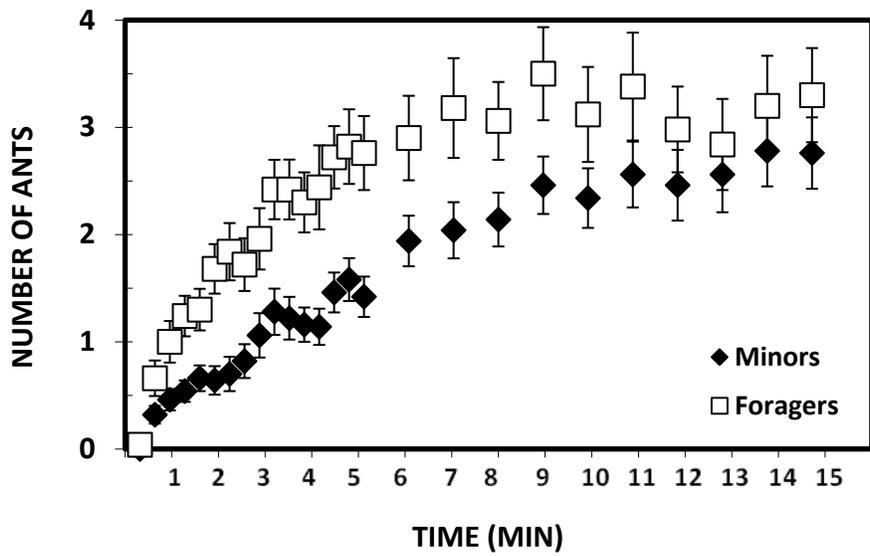

FIGURE 2.   Average number of minors and foragers responding to crushed heads at each time point. Slopes of the two regressions do not differ (t = .4429, df = 2596, p=.658).

FIGURE 3.   Average number of minors and foragers responding to crushed bodies at each time point. Slopes of the two regressions do not differ (t = 0.175, df = 2596, p = 0.861).
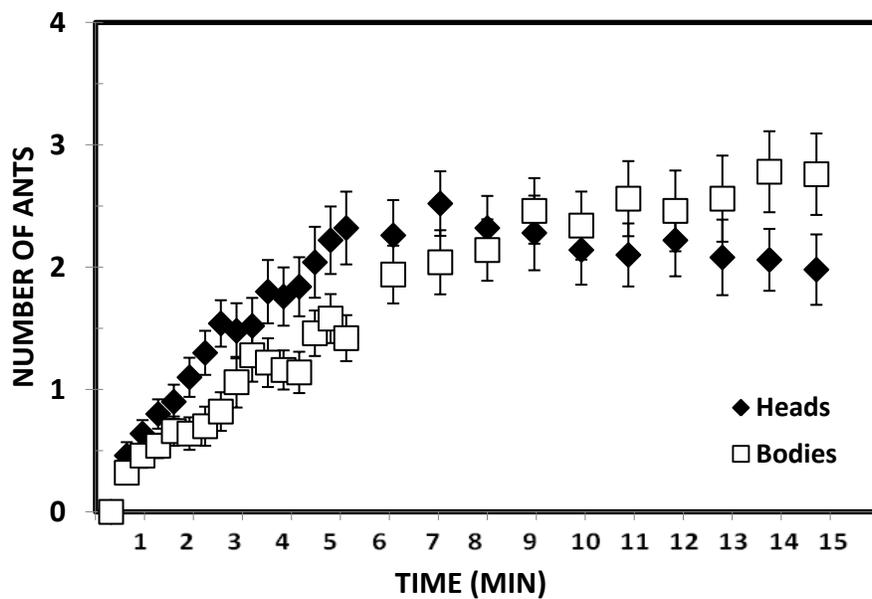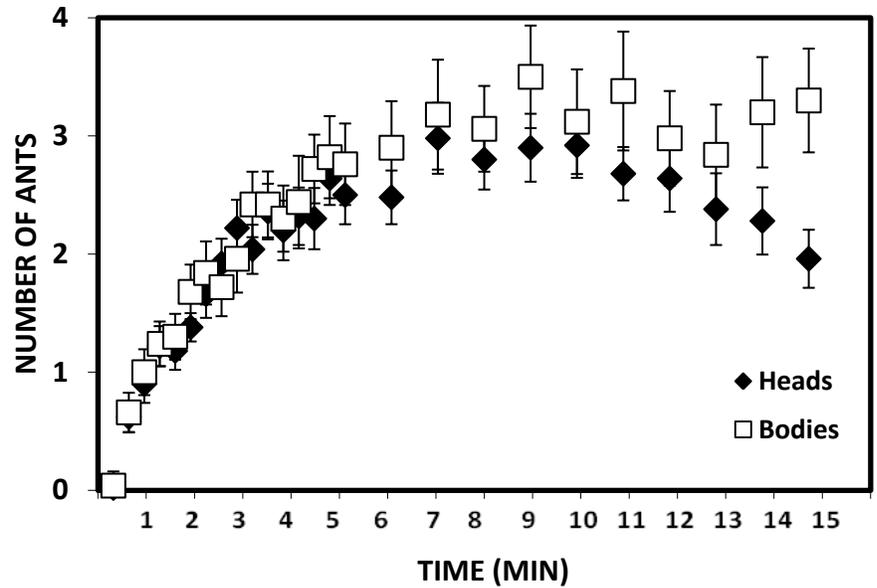

FIGURE 4.   Average number of minors responding to heads and bodies at each time point. Slopes of the regressions differ significantly (t = 3.91, df = 2596, p < .0001).

FIGURE 5.   Average number of foragers responding to heads and bodies at each time point. Slopes of the regressions differ significantly (t = 2.90, df = 2596, p = .004)