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Alarm behaviour in *Eciton* army ants

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Abstract. The effective communication of alarm can be critical for social animals so that they are able to deal with threats posed by predators and competitors. In the case of many of the most ecologically dominant, large-colony ant species, these alarm responses are aggressive and coordinated by alarm pheromones, produced generally from the mandibular glands. In the present study, the alarm behaviour of two Neotropical army ant species is examined, the swarm raiding *Eciton burchellii* (Westwood) and the column raiding *Eciton hamatum* (Fabricius). Both species exhibit aggressive alarm responses in response to crushed heads, suggesting that the alarm pheromone is indeed produced by the mandibular glands in these ants. The most abundant component of the mandibular gland secretion, 4-methyl-3-heptanone (10 μL on a rubber septum), stimulates a substantial alarm response, although this is less than the response to a single crushed head. This suggests that 4-methyl-3-heptanone may be an alarm-stimulating compound in *Eciton*. The alarm response of *E. burchellii* involves more workers than that of *E. hamatum*, although major workers play a much greater role in the response of the latter species. The differences in the alarm response of the two closely-related species may relate to their foraging strategies, with *E. burchellii* relying more on quantity rather than the caste of ants responding and possibly using alarm pheromones for offensive as well as defensive functions.

Key words. Alarm response, caste, foraging strategy, polyethism, social insect.

Introduction

Social groups offer a potential food resource and more cooperative societies have evolved a variety of adaptations to protect against predators (Kraus & Ruxton, 2002). One such adaptation is the use of alarm signals. Although the many individuals within a group make it a greater resource for predators, they also provide it with many opportunities to detect predators. The effective communication of the threat (i.e. alarm) can then enable coordinated responses to escape from, confuse or defend against the predator (Zuberbuhler *et al*., 1997; Hollen & Radford, 2009; Hartbauer, 2010). The nature of both the communication and response may shape, and be shaped by, a trade-off with other life-history traits, such as foraging (Lima & Dill, 1990; Mathis *et al*., 1995; Baack & Switzer, 2000).

Large colonies of densely-packed individuals make eusocial insects a rich resource and, as a result, they have a particular need for effective defence mechanisms against predators. Most social insects have effective alarm behaviours, with alarm generally being communicated, at least in ants, primarily by pheromones produced in the mandibular gland (Blum *et al*., 1968; Blum, 1969; Hölldobler & Wilson, 1990; Vander Meer & Alonso, 1998; Hughes *et al*., 2001b). Although the pheromone may be chemically complex or multicomponent (Bradshaw *et al*., 1975; do Nascimento *et al*., 1993; Hughes *et al*., 2001a; Francelino *et al*., 2006), there are typically one or a few main behaviourally active compounds that are highly volatile, producing a rapid but short-lived behavioural stimulus (Wilson & Bossert, 1963; Blum, 1969). Alarm responses can be broadly categorized into two groups (Wilson & Regnier, 1971). Smaller or more vulnerable societies typically exhibit 'panic' responses, in which individuals run away from the stimulus, either back into the nest or away from the nest when carrying brood or other nonmobile resources. By contrast, larger or better defended societies tend to show an 'aggressive' response, in which individuals are attracted to or arrested at the stimulus and attack any detected threats. The scale and manner of the alarm response may also change depending on the individual and context; for example, with caste in polymorphic species or proximity to the nest (Stuart, 1991; Hughes & Goulson, 2001; Braendle *et al*., 2003).

The *Eciton* army ants are ecologically dominant predators of the Neotropics (Gotwald, 1995; Boswell *et al*., 1998).

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They have large colonies (1×10^4 to 1×10^6 individuals; Hölldobler & Wilson, 1990), which represent a significant potential food resource. Accordingly, most *Eciton* species have specialized major workers, with highly-adapted, long, falcate mandibles, that defend against vertebrate predators. The other workers in the colony are also extremely polymorphic, with workers that are variously adapted for colony tasks ranging from brood care to killing and transporting prey (Gotwald, 1995; Powell & Franks, 2006). As in most ants, the *Eciton* alarm pheromone is produced by the mandibular glands, with the most abundant component of the pheromone of *Eciton burchellii* (Westwood) being 4-methyl-3-heptanone (Brown, 1960; Keegans *et al*., 1993). This chemical is also the main alarm-stimulating chemical in many other ant species (Vick *et al*., 1969; Hölldobler & Wilson, 1990; Hughes *et al*., 2001b). The predatory lifestyle of army ants requires rapid recruitment to overwhelm prey that are often large or mobile. Accordingly, army ants show unusually rapid recruitment compared with other ants, with individuals actively stimulating nestmates to follow pheromone trails deposited from abdominal glands (Chadab & Rettenmeyer, 1975). Alarm pheromones typically stimulate rapid reactions and may potentially serve a dual role in army ants, both recruiting individuals to defend aggressively against threats and also recruiting individuals rapidly to attack prey.

In the present study, alarm behaviour is investigated in two Neotropical army ant species *E. burchellii* and *Eciton hamatum* (Fabricius). Both species are commonly found throughout the Neotropics and exhibit many similar life-history traits, with monogynous colonies headed by a polyandrous queen, polymorphic workers that include majors, nomadic and statary phases of 18–20 days, bivouac construction and epigaeic foraging (Franks & Fletcher, 1983; Kronauer *et al*., 2006; Powell & Franks, 2006; Kronauer & Boomsma, 2007). The species differ in that *E. burchellii* has a more generalized diet and exhibits swarm-raiding foraging on a broad front, whereas *E. hamatum* has column raids focused specifically on social insect colonies (Da Silva, 1982; Franks, 1982; Powell & Franks, 2006). In both species, the response is determined to a natural alarm pheromone and also to the main putative chemical, 4-methyl-3-heptanone. The responses of the different castes are also examined to establish whether these highly polymorphic species exhibit alloethism in their alarm response.

Materials and methods

Experiments were conducted from June to July 2009 on Barro Colorado Island, Panama. Assays compared natural (test and control) and artificial (test and control) sources of alarm pheromone. Twelve replicates were carried out in each case for both *E. burchelli* and *E. hamatum*. Each replicate of each treatment was carried out using a different army ant trail in a different area of the forest. Thus, they were likely to have been on different colonies, although colony migrations and changes in foraging directions meant that this could not be determined for certain. All assays were conducted using active foraging trails with a minimum traffic of 50 ants per min. A 1-min traffic count of each trail was made immediately before the assay. In this and all subsequent ant counts, workers were categorized as majors, submajors or other castes. A flat area (approximately 50 × 50 cm) to either side of the trail was cleared of all debris to facilitate observation. An elevated wire circle (diameter 10 cm) was placed 5 cm to one side of the trail and the alarm source was placed on the ground at the centre of this circle. Natural alarm pheromone assays were conducted using a submajor worker that had been freshly killed by freezing. An *E. burchellii* worker was used for *E. burchellii* assays and an *E. hamatum* worker was used for *E. hamatum* assays. The head was crushed with forceps to release the contents of the mandibular gland (Hughes & Goulson, 2001; Hughes *et al*., 2001b) and then immediately placed on the ground at the centre of the wire circle. In control assays, the head was left uncrushed. The artificial alarm pheromone assays used a rubber septum to which either 10 μL of 4-methyl-3-heptanone (99%; ChemSampCo, Trenton, New Jersey), or ethanol as a control, was applied. The chirality of 4-methyl-3-heptanone in *Eciton* is unknown, and so a racemic mixture was used. In both cases, the chemical was allowed to absorb into the septum and then placed on the ground at the centre of the wire circle. Rubber septa give a slow release of chemicals and, under the experimental conditions (10 μL applied, approximately 30 °C, first 15 min after application), 4-methyl-3-heptanone volatizes from these septa at approximately 17 μg per min (W. O. H. Hughes, unpublished data), which compares with 3–4 μg being released mostly instantaneously by a crushed submajor worker head of *E. burchellii* (Keegans *et al*., 1993).

Observations began immediately after the alarm source was placed on the ground. Snap-shot counts of the numbers of ants within the elevated wire circle centred on the alarm source were made at 20-s intervals for 5 min, as well as at 10 and 15 min. When more than 20 ants were within the circle, their numbers could not be precisely counted and a count of 25 was recorded instead. This was conservative and numbers were probably in the region of 40 ants on some occasions. In addition, the duration of the alarm response (one or more ants with gaping mandibles, increased speed of movement, raised antennae, biting and stinging of the alarm source; Hughes & Goulson, 2001; Hughes *et al*., 2001b) and its maximum effective area (calculated from the maximum distances the alarm response was observed along the axis of the trail and either side of the trail) were recorded for each assay. The proportions of the ants responding that were majors, submajors or other castes were calculated (or estimated where more than 20 ants responded) for 0–300, 300–600 and 600–900 s, as well as for the trail traffic before the alarm response.

Statistical analysis

Trail traffic numbers were log transformed and compared between species with an independent samples t-test. Ant counts were log $(x + 1)$ transformed and analyzed using a repeated-measures analysis of covariance (ANCOVA), with trail traffic included as a covariate. The durations and maximum areas of the alarm responses were analyzed by a one-way ANCOVA, again including trail traffic as a covariate. The proportions of castes present were arcsin transformed and also analyzed with a repeated-measures analysis of variance (ANOVA). Where data failed the assumption of sphericity, the Greenhouse–Geisser correction was used in all repeated-measures ANOVAS.

Results

Eciton burchellii trails had greater traffic than those of *E. hamatum* (mean ± SE: 144 ± 15 and 86.4 ± 7.4 ants min^{-1}, respectively; $t = 3.61$, $n = 24$, $P = 0.001$). Neither species showed any appreciable response to the control stimuli (submajor workers with uncrushed heads or rubber septa with ethanol; Fig. 1a), and the numbers of ants within 10 cm of the sources were significantly lower than in response to crushed heads or 4-methyl-3-heptanone ($F_{1,94} = 103$, $P < 0.001$). Both species showed aggressive alarm responses to the crushed heads and 4-methyl-3-heptanone, characterized by ants with gaping mandibles, an increased speed of movement, raised antennae, biting and stinging of the alarm source. The scale of the response varied from small numbers of ants in the immediate area having raised antennae to extreme responses with many ants biting and stinging the source, and running rapidly across a wide area. In a few cases, the alarm response resulted in the foraging trail splitting or in individuals arresting close to the alarm source. The numbers of ants in both species that were within 10 cm of the alarm stimuli increased over 100 s, and then decreased in *E. hamatum* or stayed approximately the same in *E. burchellii* for the next 200 s (species × time interaction: $F_{4,688} = 2.28$, $P = 0.066$; Fig. 1). However, the increase was much larger in *E. burchellii*, which had more ants involved in its alarm responses overall than did *E. hamatum*, even when controlling for trail traffic (main effect of species: $F_{1,43} = 18.4$, $P < 0.001$). The two species did not differ in the way that they responded to the natural and artificial alarm pheromones, either over time or overall (treatment × species × time interaction: $F_{16,688} = 0.938$, $P = 0.525$; treatment × species interaction: $F_{1,43} = 0.065$, $P = 0.799$). The natural alarm pheromone produced by crushed heads caused a greater increase in the numbers of ants within 10 cm of the alarm sources than did 4-methyl-3-heptanone (treatment × time interaction: $F_{4,688} = 2.74$, $P = 0.033$), and was thus associated with more ants overall (treatment main effect: $F_{1,43} = 8.45$, $P = 0.006$; Fig. 1). The alarm response lasted 10–20 min and the duration did not differ between species ($F_{1,43} = 2.25$, $P = 0.14$), although it was significantly longer in response to crushed heads than to 4-methyl-3-heptanone ($F_{1,43} = 14.4$, $P < 0.001$; Fig. 2a). The maximum area of the alarm response was greater in *E. burchellii*, even when controlling for its greater trail traffic ($F_{1,43} = 8.51$, $P < 0.001$), and was also somewhat greater in response to crushed heads, although not significantly so ($F_{1,43} = 3.27$, $P = 0.077$; Fig. 2b).

Eciton burchellii and *E. hamatum* differed significantly in the proportions of ants that were majors, submajors and other castes on the trails, and responding to the alarm sources (species × repeat-measure interaction: $F_{2,53} = 5.24$, $P = 0.015$; $F_{2,72} = 3.14$, $P = 0.049$; $F_{2,72} = 3.75$, $P = 0.028$ for majors, submajors and other castes, respectively). Majors and, to a lesser extent, submajors made up only small proportion of ants in *E. burchellii*, and this proportion differed little between the trail traffic and the ants responding to the alarm pheromone sources (Fig. 3). The proportions of ants that were majors or submajors were 1.5-fold greater in *E. hamatum* than *E. burchellii* on trails, and up to 28-fold greater at the peak of the alarm response. Furthermore, these proportions differed

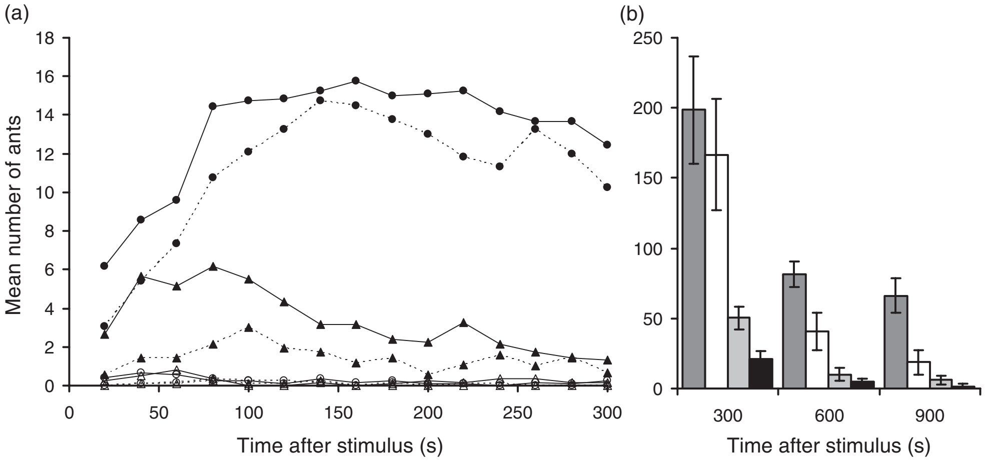

Fig. 1. The number of ants responding during the alarm responses. (a) Mean numbers during the first 300 s of the alarm response for *Eciton burchellii* responding to natural alarm pheromone (produced by a submajor worker with a crushed head; solid circles and lines) and artificial alarm pheromone (4-methyl-3-heptanone; solid circles and dashed line), and *Eciton hamatum* responding to natural (solid triangles and line) and artificial (solid triangles and dashed line) alarm pheromones, or to control natural or artifical stimuli (a submajor worker with an uncrushed head or ethanol respectively; equivalent clear symbols). Error bars are excluded for clarity. (b) Mean ± SE total numbers counted during 0–300, 300–600 and 600–900 s after stimuli were placed for (from left to right): *E. burchellii* to natural and artificial pheromone and *E. hamatum* to natural and artificial pheromone. The numbers responding to control stimuli were negligible and are excluded.

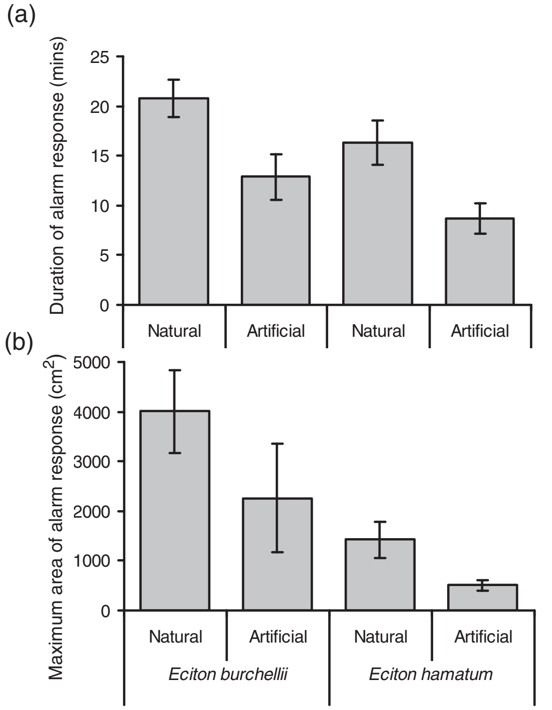

Fig. 2. Mean ± SE (a) duration and (b) maximum area of the alarm responses stimulated by natural alarm pheromone (produced by a crushed head) and artificial alarm pheromone (4-methyl-3-heptanone) for *Eciton burchellii* and *Eciton hamatum.*

between the trail traffic and the ants responding to the alarm pheromones. Only a small proportion of ants were submajors or majors in the trail traffic, although the proportions responding to the alarm pheromones were greater. The increase in proportion compared with trail traffic was particularly marked for the major caste in this species (Fig. 3). There were no differences between crushed heads and 4-methyl-3-heptanone in the proportions of any of the castes on the trails or responding (main effect and all interactions $P > 0.05$).

Discussion

Both *Eciton burchellii* and *E. hamatum* exhibit aggressive alarm responses, with ants accumulating near the source of alarm, either as a result of attraction or arrestment, having raised antennae, gaping mandibles, an increased speed of movement, and biting and stinging of the alarm source. Such aggressive alarm responses are typical of ant species with large colonies (Wilson & Regnier, 1971; Hölldobler & Wilson, 1990). In most ants, however, the alarm response is context-dependent and is less aggressive on foraging trails than close to the nest (Vander Meer & Alonso, 1998; Hughes & Goulson, 2001). The assays were carried out on foraging trails at a distance of many metres from the bivouac itself, so the strength of the aggression that army ants exhibit even in this context is unusual.

The alarm response in both species is greatest to natural alarm pheromone produced by crushed heads, although it is still significant in response to 4-methyl-3-heptanone alone in the manner presented. The alarm pheromones of most ants that have been investigated are produced primarily by the mandibular glands (Blum, 1969; Hölldobler & Wilson, 1990), and the present results confirm that this is also true for *Eciton* army ants. The use of the mandibular glands to produce alarm pheromone may be mechanistically advantageous because pheromone will be released when mandibles are gaped in preparation for biting, and the mandibles also provide a large surface area to facilitate rapid evaporation. 4-Methyl-3-heptanone is a highly volatile ketone that is known to be the main alarm-stimulating component of the mandibular gland secretion of many ants, particularly in the Myrmicinae subfamily (Blum *et al*., 1968; Blum, 1969; Hölldobler & Wilson, 1990). It makes up a large proportion of the secretion in *Eciton* (Keegans *et al*., 1993), and the results of the present study support it as being a major alarm-stimulating compound in these species too, although this conclusion needs to be treated with caution because the quantities and chirality of the synthetic 4-methyl-3-heptanone may have differed from the natural pheromone, which could dramatically affect the response (Riley *et al*., 1974; Mori, 2007). 4-Methyl-3-heptanone is also present in the mandibular glands of *Labidus* and *Aenictus* army ants, as well as their near-relatives, *Cerapachys* (Keegans *et al*., 1993; Oldham *et al*., 1994; Morgan *et al*., 2008), and so may be the main component of the alarm pheromone across the dorylomorph clade. Although this ketone may potentially be the major component, it does not produce quite as great an alarm response in *Eciton* in isolation as the natural alarm pheromone. This may simply be a result of differences in the concentration or chirality of 4-methyl-3-heptanone applied or because other components of the mandibular gland secretion, such as 4-methyl-3-heptanol (Keegans *et al*., 1993), act with it synergistically.

During the course of the fieldwork, single trials were also opportunistically conducted with two other army ants, *Labidus praedator* (Smith) and *Labidus coecus* (Latreille). In both species, the crushed heads of conspecific large workers (a similar size to *Eciton* submajors; taken from the trail and crushed immediately) result in an alarm behaviour similar to that seen in *Eciton*. However, neither species show any response to 4-methyl-3-heptanone (applied in the same way as in the *Eciton* trials). Keegans *et al*. (1993) report that the mandibular gland secretion of *L. coecus* is similar to that of *Eciton*, being composed predominantly of 4-methyl-3-heptanone, whereas that of *L. praedator* lacks this ketone. For two closely-related species to have such different compositions is unusual and further work investigating the chemical ecology of alarm in *Labidus* may prove insightful.

The alarm responses of the two *Eciton* species in the present study are qualitatively similar but quantitatively different. Even allowing for the greater trail traffic of *E. burchellii*, this species shows a much greater, longer lasting alarm response than *E. hamatum*. This difference may relate to the foraging biology of the two species. Unlike *E. hamatum*, which specializes on social insect prey, the foraging strategy of *E. burchellii* relies

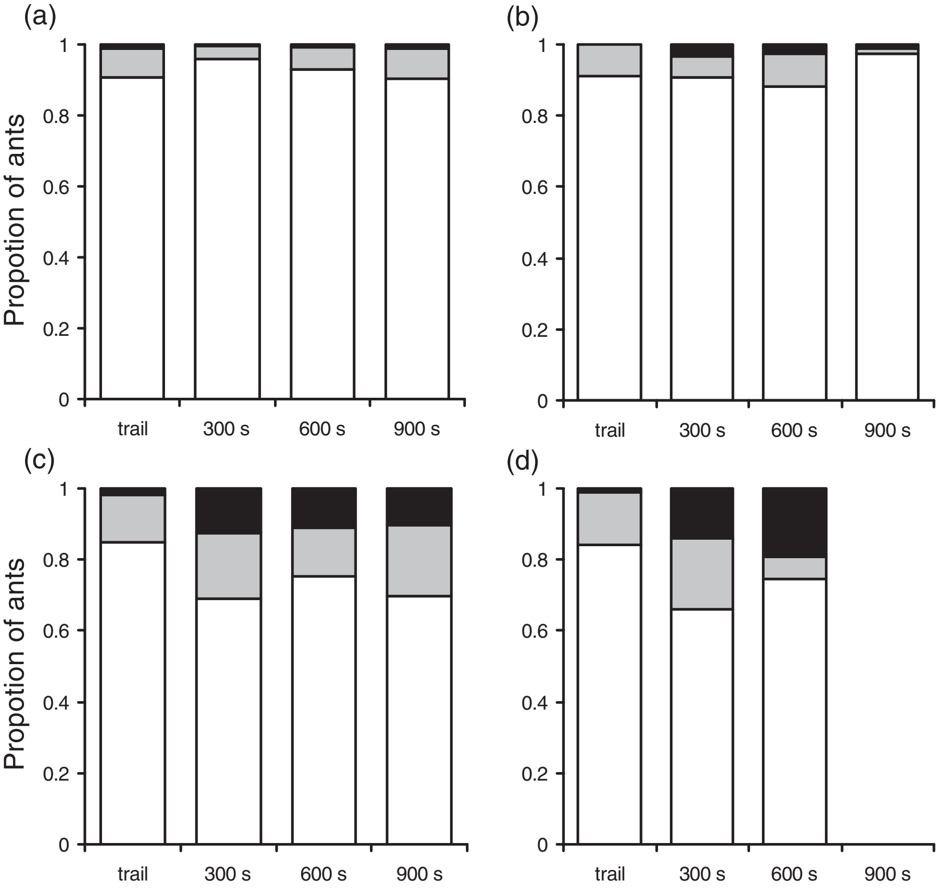

Fig. 3. Mean proportions of ants that were majors (black), submajors (grey) or other castes (white) on the trails or during 0–300, 300–600 or 300–900 s after the alarm stimulus was added, for (a) *Eciton burchellii* responding to natural alarm pheromone, (b) *E. burchellii* responding to artificial alarm pheromone, (c) *Eciton hamatum* responding to natural alarm pheromone and (d) *E. hamatum* responding to artificial alarm pheromone. Error bars are excluded for clarity.

upon rapidly recruiting to, and overcoming, mobile prey, such as large arthropods, by spread-eagling the prey to immobilize it, with other workers stinging and later dismembering the prey (Schneirla, 1971; Gotwald, 1995; Powell & Franks, 2006). Such prey will be likely to escape and army ants accordingly show unusually rapid mass recruitment, which was thought to be based predominantly on trail pheromones (Chadab & Rettenmeyer, 1975). However, a requirement for individuals to leave the prey to deposit a recruitment trail will still risk the prey escaping. Recruitment to the highly volatile alarm pheromone will not suffer from this disadvantage. It may be that, in addition to defence, *E. burchellii* therefore also uses its alarm pheromone in a foraging context to recruit rapidly to mobile prey.

Eciton army ants represent some of the most extreme forms of morphological caste-based division of labour. The results of the present study show that caste influences the response to the alarm pheromone, although this alloethism differs between the two species examined. In both species, only a minority of workers on foraging trails are majors or submajors, and the caste composition of ants responding to the alarm stimuli differs from this only fractionally in *E. burchellii*. However, in *E. hamatum*, a substantially greater proportion of ants involved in the alarm response are majors. This is the case both in response to crushed heads and to 4-methyl-3-heptanone, and at 300, 600 and 900 s into the alarm response. Major workers therefore appear to be more responsive than the other castes to alarm pheromones in at least *E. hamatum* and have a more important role in the alarm response of this species, in keeping with their exclusively defensive function within the colony. The same is true of *Dorylus molestus* army ants (Braendle *et al.*, 2003). The alloethism in *E. hamatum* contrasts with that in leaf-cutting ants, in which minor workers are most responsive to an alarm pheromone outside of the nest and major workers are rare on trails (Hughes & Goulson, 2001; Waddington & Hughes, 2010). This may be because army ants need to protect the food caches that they construct along the foraging trail (Gotwald, 1995). The smaller colony size (Hölldobler & Wilson, 1990) and trail traffic in *E. hamatum* will make their caches more vulnerable, and result in defence based on a number of ants, less effective than in *E. burchellii.* The results suggest that *E. hamatum* consequently relies more on a limited number of majors to defend their trails, whereas the alarm response of *E. burchellii* relies principally on numbers (McGlynn, 2000) or is adapted more for foraging.

The results of the present study show that army ants have aggressive alarm responses and support 4-methyl-3-heptanone being a principal, but possibly not the only, component of this, just as in more phylogenetically derived taxa. The comparison of the two species suggests that life-history, and specifically the foraging strategy, has an important influence on this alarm response. *Eciton burchellii* with its more generalist foraging strategy has a larger-scale alarm response involving very few major workers, whereas the response of *E. hamatum*, with its specialist, more complex foraging strategy, involves fewer workers with the defensive major caste playing a much greater role. Even in two closely-related, biologically similar and aggressive species, therefore, different alarm behaviours may evolve.

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