

Research article

Combat between large derived societies: A subterranean army ant established as a predator of mature leaf-cutting ant colonies

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Summary. Mature colonies of *Atta* leaf-cutting ants are dominant herbivores throughout the Neotropics. Although young colonies have natural enemies, mature colonies, which live in extensive nests containing millions of workers, currently have no recognised predators. New World army ants (Ecitoninae) are specialist social predators of other ants, and the army ant *Nomamyrmex esenbeckii*, a primarily subterranean species, is known to prey upon young *Atta* colonies. Here we present the results of the first long-term study of the predator-prey interaction between *N. esenbeckii* and *Atta*. Our study establishes the army ant *N. esenbeckii* as the only known predator capable of successfully attacking and killing mature as well as young colonies of *Atta* leaf-cutting ants. In natural raids, and experimental tests, *Atta* rapidly recruited their largest workers (majors) as a specific defensive response to *N. esenbeckii* raiders and both taxa used their largest individuals in the frontline of battles. The deployment and behaviour of these large workers demonstrates a size-related division of labour and agrees with the predictions of Lanchester's Linear Law of Combat. Both taxa also used cooperative combat 'teams' to overwhelm large combatants from the other side. The success of *N. esenbeckii* raids varied greatly, such that they were prevented from entering *Atta* nests in the least successful raids, and completely overran *Atta* colonies in the most successful raids. The speed and magnitude of the defensive response of mature *Atta* colonies was key in determining the level of success of *N. esenbeckii* raids.

Key words: *Atta*, *Nomamyrmex*, predation, combat team, Lanchester.

Introduction

Among the main benefits gained by individuals in the evolutionary transition from solitary to social living, are those associated with social predation and social defence. Group predators can tackle prey that an individual cannot handle alone, allowing access to new resources (Oster and Wilson, 1978; Drea and Frank, 2003), and by cooperating, individuals within a group can better recognize and defend against predators (Jerome et al., 1998; Zuberbühler, 2003). The New World army ants (Ecitoninae) are an ancient taxon of nomadic, social predators (Brady, 2003) that specialize in raiding other ants, including many species that live in large, well defended colonies (Rettenmeyer, 1963; Rettenmeyer et al., 1983). Thus, the interactions between army ants and their ant-prey are rare examples of combat between large derived societies, and offer an exceptional context for studying social predation and defence. Furthermore, some of these interactions may be of great ecological importance because army ants are likely to have a significant and previously unrecognized impact on ant taxa that fill key ecological roles.

Leaf-cutting ants in the genus *Atta* are dominant herbivores in many natural and agricultural systems throughout the New World tropics. Consequently, they are of great ecological and economic importance (Cherrett, 1986; Hölldobler and Wilson, 1990; Wirth et al., 2003). Mature colonies are responsible for most of the total herbivory attributed to *Atta*, and each nest can be immense, covering an area as great as 100 m² with a workforce of many millions of individuals (Fowler et al., 1986; Wirth et al., 2003). Furthermore, the workers are highly 'polymorphic', varying considerably in size and relative shape, and display a size-related division of labour (Wilson, 1980a, b; Franks and Norris, 1987). The large polymorphic workforce within mature colonies is important for the scale and efficiency of the fungus-gardening prac-

ticed by *Atta* (Wilson, 1980b), but it is also likely to give them considerable defensive capabilities (Oster and Wilson, 1978). No important predator of mature *Atta* colonies has been identified, to date (Hölldobler and Wilson, 1990; Wirth et al., 2003). However, incipient and young *Atta* colonies, which are orders of magnitude smaller than the mature forms and lack large individuals (Wilson, 1983; Fowler et al., 1986), have a number of natural enemies, including conspecific colonies, armadillos (Fowler et al., 1986), and the army ant *Nomamyrmex esenbeckii* (see Swartz, 1998; Sánchez-Peña and Mueller, 2002 and references therein).

Like most species of New World army ants, *N. esenbeckii* (see Bolton, 1995 for synonyms) is primarily subterranean and rarely seen, relative to the few surface-adapted species (Rettenmeyer, 1963; Schneirla, 1971; S. Powell, pers. obs.). As a result, our understanding of this species is poor and limited to a few descriptive accounts. Descriptions of *N. esenbeckii* raids have recorded a variety of prey taxa (Rettenmeyer, 1963; Rettenmeyer et al., 1983), but notably, most document predation upon young *Atta* colonies (see Swartz, 1998; Sánchez-Peña and Mueller, 2002 and references therein), which has not been verified in any other species of army ant (Rettenmeyer, 1963; Rettenmeyer et al., 1983). *N. esenbeckii* is known from Northeastern Mexico through southern Brazil (Watkins, 1976; Sánchez-Peña and Mueller, 2002), covering most of *Atta*'s range (Hölldobler and Wilson, 1990), and numerous *Atta* species have been recorded as prey, including *Atta mexicana* as far North as Tamaulipas Mexico, *Atta cephalotes* in Costa Rica, and *Atta laevigata* in Goiás, Brazil (Swartz, 1998 and references therein; Sánchez-Peña and Mueller, 2002). Colony size has not been quantified for *N. esenbeckii*, but from the detailed account of an emigration, given by Rettenmeyer (1963), it is possible to calculate that colonies can contain more than a million workers. Much like the workers of mature *Atta* colonies, *N. esenbeckii* workers seem to be polymorphic, and they also have an exceptionally thick exoskeleton, even compared to other New World army ants (Swartz, 1998; Sánchez-Peña and Mueller, 2002; S. Powell, pers. obs.).

Currently, the extent and significance of the predator-prey interaction between *N. esenbeckii* and *Atta* is unclear. Despite the great value of the descriptive accounts to date, they each report on a single raid of a young colony within a particular population, and provide limited or no quantitative data. It is particularly important to note that it is not known if *N. esenbeckii* ever raids mature *Atta* colonies (Swartz, 1998). Predation of mature colonies, which often dominate the habitat they occupy, may have more important ecological implications than predation of young, less established colonies. Furthermore, the large polymorphic workforce of mature colonies represents a significantly greater challenge to a potential predator than the small, incomplete workforce of a young colony. Here we present the results of the first long-term study of the predator-prey interaction between the army ant *N. esenbeckii* and *Atta* leaf-cutting ants, with particular focus on the colony-level impact of raids against mature colonies, and the combat strategies used by both taxa during large-scale battles.

Study site and summary of natural raids

Data were collected on Barro Colorado Island (BCI), Panama. In eighteen months of fieldwork, between June 2000 and August 2003, *N. esenbeckii* was observed raiding *Atta* on nineteen different occasions. Twelve of these raids were against mature *Atta colombica* colonies (mound diameter > 5 m, major-caste present), five were against young *A. colombica* colonies (mound diameter < 1.5 m, no major caste present), and two were against mature *Atta cephalotes* colonies (mound diameter > 7 m, major caste present), which are much less common on BCI than *A. colombica*. Specimens and quantitative data were collected wherever possible (see later sections for details). All raids were discovered as they crossed a heavily compacted trail on the way to an *Atta* nest, or by identifying fighting ants at *Atta* nest entrances, indicating a subterranean raid. All raids were discovered between early morning and late afternoon, but some subsequently extended through the night and even into the next day (see later details).

Atta's initial defensive response: natural raids

Seven of the total nineteen observed raids were discovered before *N. esenbeckii* had gained access to an *Atta* nest. Six of these initial advances were against mature *A. colombica* colonies and one was against a mature *A. cephalotes* colony. In all cases, large numbers of majors were recruited from within the nest, and they advanced rapidly towards the approaching army ants. At the 'frontline' of the battle, defined here as the area where ants from both sides were actively engaging opponents, approximately 90% of the leaf-cutting ants were majors. In all except the raid against the mature *A. cephalotes* colony, the army ants were not successful in gaining access to the nest and capturing brood, and they eventually retreated. In one case, an *A. colombica* colony fought *N. esenbeckii* over a total distance of thirty metres, for approximately two hours, before the army ants disengaged. It is not known if *N. esenbeckii* returned at a later time in any of the aborted raids. In the raid against the mature *A. cephalotes* colony, *N. esenbeckii* successfully entered and overran the nest (see 'Raid impact: *Atta* brood loss' later).

Atta's initial defensive response: staged raids

Methods

The initial defensive response of *Atta* colonies to the presence of army ants was tested experimentally with paired introductions of *N. esenbeckii* and *Eciton hamatum* foragers. Sixteen mature *A. colombica* nests were tested. *E. hamatum* was used as a control because this army ant is a predator of *Acromyrmex* leaf-cutting ants, but not of the closely related *Atta* leaf-cutting ants (S. Powell, unpubl. data). This control species thus allowed the best test of the specificity of any defensive response by *Atta* to army ants. To control for order

effects, half ($n = 8$) of the *Atta* colonies were tested with *N. esenbeckii* first and *E. hamatum* on the following day, and the other half ($n = 8$) were tested *visa versa*. The same nest entrance was used for both introductions to each *Atta* colony. For each trial, a three-minute count was made of the number of majors exiting a main nest entrance that opened to a busy foraging trail (baseline). Thirty-five raiders of the appropriate army ant species were then introduced to the trail approximately half a metre from the nest entrance and left to interact with the *Atta* workers for three-minute. A second three-minute count of the number of majors exiting the nest was then completed (post-introduction), allowing the change in major-traffic from the baseline rate to be calculated. Majors were identified visually on the basis of showing all of the following characteristics: large overall size, a disproportionately large heart-shaped head (see Franks and Norris, 1987), and a colouration difference between the head and the rest of the body. Extensive prior knowledge of the overall size and relative shape of *A. colombica* majors allowed quick assessment of these criteria during experiments, and ants close to the major size-class were seen infrequently, so borderline decisions were rarely necessary. The identification criteria were strictly adhered to throughout the experiments, reducing error between replicates.

Staged raids were conducted between May and August 2002. A replicate was completed whenever adequate numbers of *N. esenbeckii* raiders could be collected from an aboveground raid column not engaged in an attack against *Atta*. *E. hamatum* is common on BCI, so once the *N. esenbeckii* raiders had been collected, similar numbers of *E. hamatum* raiders were readily available for the paired introduction.

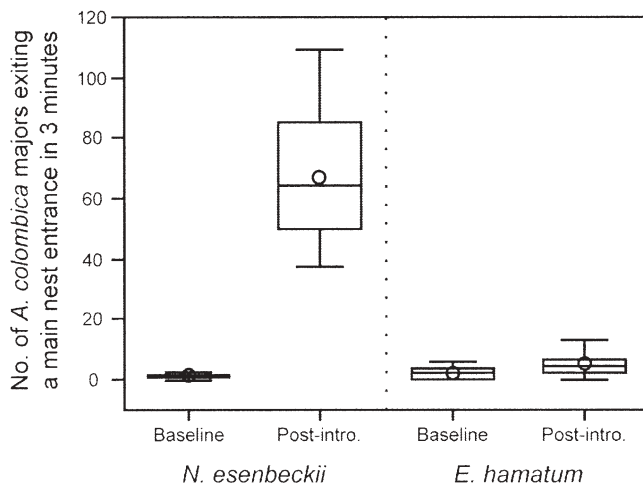


Figure 1. Boxplots showing the recruitment of majors from *A. colombica* colonies ($n = 16$) following the introduction of *N. esenbeckii* and *E. hamatum* army ants. The baseline and post-introduction counts were taken over three min. Each box encompasses the interquartile range, a line is drawn at the median, a circle represents the mean, and whiskers extend to the nearest value within 1.5 times the interquartile range

Results

A. colombica colonies rapidly recruited large numbers of majors following the detection of *N. esenbeckii* raiders, and showed almost no response to the presence of *E. hamatum* raiders (Fig. 1). The difference between the change (post-introduction – baseline) in the number *A. colombica* majors exiting the nest after the introduction of *N. esenbeckii* and after the introduction of *E. hamatum* was highly significant (Two-tailed paired t-test, $t = 11.94$, $df = 15$, $p < 0.0001$). The overall change in *A. colombica* behaviour when *N. esenbeckii* raiders were detected was pronounced. *A. colombica* workers close to the nest-entrance immediately ran back into the nest, while inbound foragers dropped their load and would not pass the army ants, forming a traffic-jam of alarmed ants and a large pile of leaf-fragments. Majors then rapidly exited the nest and fought with the *N. esenbeckii* raiders, but few smaller *A. colombica* workers exited the nest with them. In contrast, the *E. hamatum* raiders initially caused a small disturbance, but the *A. colombica* foragers quickly ignored them, and traffic continued as normal.

Combat strategies

Methods

Thirty-three groups of fighting ants were collected from around nest entrances during a *N. esenbeckii* raid of a small peripheral nest chamber of a mature *A. colombica* colony. Each fighting group was composed of a *N. esenbeckii* raider and an *A. colombica* worker locked head-to-head (hereafter ‘primary combatants’), and most groups had one or more additional *A. colombica* workers (hereafter ‘assistant combatants’) holding onto other parts of the army ant. After preservation in ethanol, the head-to-head individuals remained locked together, maintaining the distinction between the *Atta* primary and assistant combatants. Once all the fighting groups were collected, a shallow excavation revealed a small *Atta* nest chamber that was populated entirely by *N. esenbeckii* raiders, and a large sample of these individuals was taken by haphazardly scooping handfuls of soil and ants into a container. The head width across the eyes was measured for all specimens taken during this raid.

On a subsequent day, a hundred and fifty outbound workers were collected at random from each of three major foraging trails of the previously raided *A. colombica* colony. On each trail every outbound ant that passed a pre-selected point was collected until the total of a hundred and fifty was reached. These ants were used as an estimate of the average size of workers seen outside the nest. The earlier raid was unlikely to have had a significant influence on the overall size frequency distribution of workers found outside the nest, because the *Atta* casualties were few in number and the raid was limited to a small peripheral chamber of the large, mature colony. The fresh wet weight (T) of each *Atta* worker was originally recorded, so to allow comparison with the *Atta* from the raid samples, these data were transformed to head

widths across the eyes (W) using the equation $W = 0.924T^{0.361}$ ($r^2 = 0.997$, $p < 0.0001$, $n = 80$, SD of slope ± 0.0024).

The combat strategies used by *N. esenbeckii* and *Atta* were also observed in the seven natural raids for which the initial advance of the army ants was seen (see ‘*Atta*’s initial defensive response: natural raids’ earlier). The raid against the mature *A. cephalotes* colony was observed in particular detail and photographed. No specimens were collected during these raids, so as not to influence the outcome and subsequent impact of the attacks (see ‘*Atta*’s initial defensive response: natural raids’ earlier and ‘raid impact: *Atta* brood loss’ later).

The combat strategies employed by both taxa suggested that worker size and relative shape were relevant to these interactions, but no studies have quantified the size range and morphology of *N. esenbeckii* workers. To study this aspect of

N. esenbeckii’s biology, specimens were also collected during a colony emigration, when the entire colony relocates, allowing the full size range of workers to be sampled.

Results

Primary combatants

Figure 2 shows that in the fighting groups collected from the raid against a mature *A. colombica* colony, the *N. esenbeckii* primary combatants ($n = 33$) had bigger heads than the general raiders, and this difference was highly significant ($n = 132$; two-tailed t-test, $t = 13.19$, $df = 82$, $p < 0.0001$). Similarly, Figure 2 shows that *A. colombica* primary combatants ($n = 33$) had bigger heads than *Atta* workers found out-

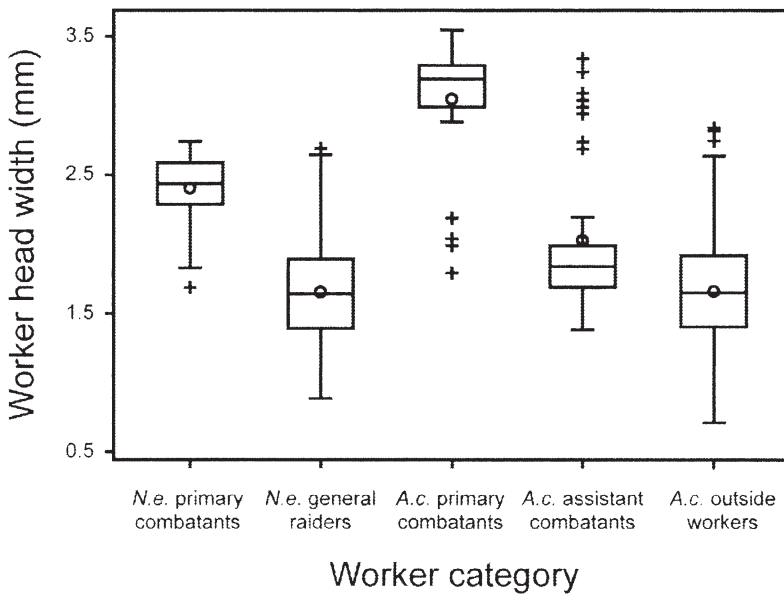


Figure 2. Boxplots for the size of different categories of ants involved in a *N. esenbeckii* raid against a mature *A. colombica* colony, and a random sample of outbound workers from the same *A. colombica* colony. Each box encompasses the interquartile range, a line is drawn at the median, a circle represents the mean, whiskers extend to the nearest value within 1.5 times the interquartile range, and outliers are indicated with plus signs

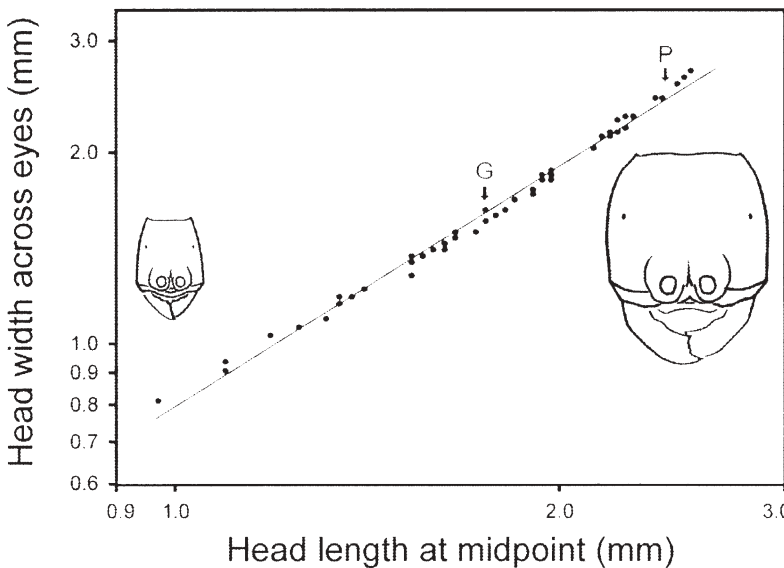


Figure 3. The overall size range and head capsule allometry in the army ant *N. esenbeckii*. The ‘P’ indicates the median head width of *N. esenbeckii* primary combatants and the ‘G’ indicates the median head width of the general raiders from figure 2. The relationship between head width across the eyes (W) and head length (L) is described by the equation $W = 0.793L^{1.26}$ ($r^2 = 0.992$, $n = 53$, $p < 0.0001$ and SD of slope ± 0.0164). The face-view inserts show the overall head shape at the extremes of the size distribution

side the nest ($n = 449$), and this difference was also highly significant (Two-tailed Mann-Whitney U-test, $z = 9.134$, $n_1 = 33$, $n_2 = 449$, $p < 0.00001$). Figure 3 shows that the *N. esenbeckii* primary combatants are among the largest individuals seen in this species, and their head is relatively wider in relation to its length than that of the general raiders, due to a positive allometry across the full size range of workers.

In the seven aboveground raids where the initial advance of *N. esenbeckii* was seen (see 'Atta's initial defensive response: natural raids'), the army ants focussed their attack on a particular nest entrance. The frontline never exceeded one-metre in width and was up to half a metre deep on either side in the largest raids. Almost all of the ants in the frontline of these battles were noticeably large, primary combatants, and they struck at opponents with open mandibles, until one was engaged in a head-to-head duel. These pairings then fought vigorously, with the *Atta* major attempting to lift the army ant and cut through the head capsule, while the *N. esenbeckii* primary combatant tried to sting its opponent.

Cooperative combat strategies

A. colombica assistant combatants were present in twenty-six of thirty-three fighting groups, and they performed markedly different behaviours than the primary combatants. While an *A. colombica* primary combatant remained in a head-to-head grapple with a *N. esenbeckii* primary, the *A. colombica* assistants held legs and other body parts, allowing the group to lift the army ant off the substrate and carry it away cooperatively. Figure 2 shows that *A. colombica* assistant combatants ($n = 51$) were smaller than *A. colombica* primary combatants and generally larger than the workers found outside the nest, and these differences were highly significant (Two-tailed Mann-Whitney U-test, $z = 6.3346$, $n_1 = 51$, $n_2 = 33$, $p < 0.0001$; two-tailed Mann-Whitney U-test, $z = 4.5014$, $n_1 = 51$, $n_2 = 449$, $p < 0.0001$).

In the seven aboveground raids where *N. esenbeckii*'s initial advance was observed, the region behind the frontline, on the *Atta* side, was populated with leaf-cutting ants of mixed size. Whenever an *Atta* primary managed to isolate an opponent in this region, usually by carrying them backwards, ants would assist their larger nestmate, forming the same cooperative combat groups detailed above. This behaviour was photographed in the raid against the mature *A. cephalotes* colony (Fig. 4a).

N. esenbeckii was also observed using a cooperative combat strategy in the aboveground raids. It was harder to clearly identify where the frontline stopped on the *N. esenbeckii* side, because the ants were all relatively large, but the cooperative tactic was only ever seen behind the most intense fighting and when *N. esenbeckii* raiders heavily outnumbered an *Atta*. This behaviour was also photographed in the raid against the mature *A. cephalotes* colony, and Figures 4b–c shows that like the cooperative combat strategy seen in *Atta*, the army ant primary and assistant combatants performed different behaviours.

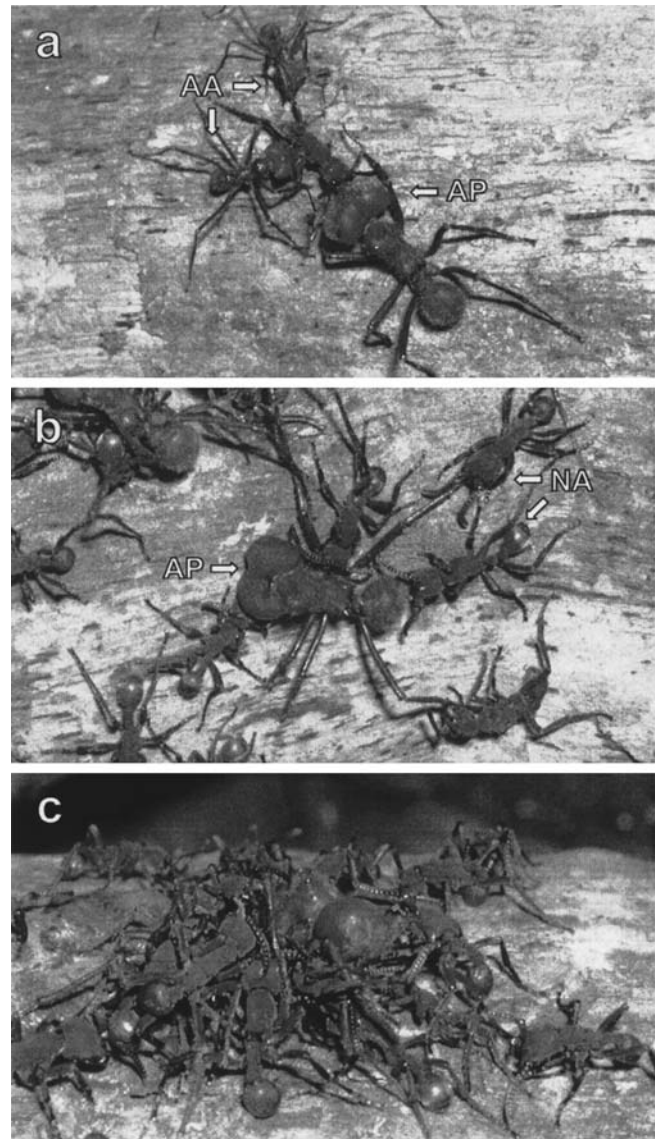


Figure 4. Photographs showing the cooperative combat strategies employed by the workers of a mature *A. cephalotes* colony and *N. esenbeckii* raiders during battle. At the frontline of the battle, large *N. esenbeckii* and *A. cephalotes* primary combatants engaged in head-to-head duels. If an *A. cephalotes* primary combatant (AP) isolated an *N. esenbeckii* opponent behind the frontline, *A. cephalotes* assistant combatants (AA) grasped and pulled the legs of the army ant (a; photo taken approximately 1 m behind the frontline), and attempted to dismember it. If, however, a *N. esenbeckii* primary combatant immobilized an *A. cephalotes* primary (AP) behind the advancing frontline, *N. esenbeckii* assistant combatants (NA) pinned-down the *Atta* (b; *N. esenbeckii* primary obscured beneath *A. cephalotes* primary) and stung it to death in a mob (c; back of *A. cephalotes* head central). Most of the army ants pictured are among the largest in the colony (based on head shape; see Fig. 3), despite looking small next to the generally larger *A. cephalotes* combatants. (Photographs by S. Powell)

Entrance barricading

Atta was seen barricading nest entrances on three occasions, once during a limited raid of a peripheral chamber of a mature *A. colombica* colony, and twice during extensive raids of mature *A. cephalotes* colonies. Workers used soil and organic debris to seal-off the holes. In the limited raid of the *A. colombica* colony, barricading was not seen at the entrance under attack by *N. esenbeckii*, and was restricted to the local area, while foraging continuing from more distant entrances. In the raids against *A. cephalotes*, barricading was seen at many entrances, and all foraging activity ceased. In a fourth raid, the fighting tactics of a mature *A. colombica* colony prevented *N. esenbeckii* from entering the nest, but the following day, leaf-cutting ants were seen barricading the hole from which the army ant raid had originated. Entrance barricading may have occurred on other occasions, but data collection at the raid front made it difficult to inspect the other nest entrances during a battle.

Raid impact: *Atta* brood loss

Methods

The amount of brood retrieved by *N. esenbeckii* foragers was estimated for successful raids against a mature *A. cephalotes* colony and a young *A. colombica* colony. An automated camera setup took pictures of half a metre of raid column every eighteen minutes throughout the *A. cephalotes* raid (allowing photographs to be taken through the night on a single battery) and every two min throughout the *A. colombica* raid. The total number of brood items retrieved in each raid (items) was calculated by multiplying together the average density of laden foragers (items/m), the average speed of laden foragers (m/s), and the duration of brood retrieval (s). For each colony, the average density of laden foragers was calculated by counting all the laden ants in the image sequence and dividing by the total length of trail photographed; the mean running speed of laden *N. esenbeckii* raiders (used for both estimates) was calculated by timing fifty laden ants over half a metre during the *A. cephalotes* raid; and finally, raid duration was calculated from the known start and stop times of each raid. Sixty-three laden foragers were also collected during the raid against *A. cephalotes*. The fresh wet weight and constant dry weight of each brood item were recorded back at the field station for biomass calculations.

Results

In the raid against the mature *A. cephalotes* colony, *N. esenbeckii* retrieved *Atta* brood for thirty-five hours, forty-six minutes. The mean density of laden outbound raiders was twelve (SD \pm 7) per metre and their mean running speed was 0.04 m/s (SD \pm 0.005). Thus, approximately 60,568 brood items were removed by *N. esenbeckii*. The mean wet weight of the *Atta* brood sample was 17.1 mg (SD \pm 16.7), and the

mean dry weight was 3.1 mg (SD \pm 3.0). Thus the brood harvested from the mature *A. cephalotes* colony had an approximate wet biomass of 1036 g and a dry biomass of 188 g. In the raid against the small *A. colombica* nest, brood retrieval lasted three hours forty-seven minutes, with a mean of nine (SD \pm 7) laden foragers per metre, giving a total of approximately 4,903 brood items. The total wet biomass of the brood harvested from this nest was approximately 84 g and the dry biomass was 15 g. These totals are likely to be underestimates because in both cases, brood retrieval was already underway when data collection started.

Raid impact: adult mortality

Methods

After the raid against the mature *A. cephalotes* colony (see previous section), all the dead ants found in the combat area (approximately 0.5 m wide by 3 m long) were collected. These ants were visually sorted into three categories, *N. esenbeckii* workers, *A. cephalotes* workers, and *A. cephalotes* majors. The number of ants in each category was recorded and the head width across the eyes was measured for a random sub-sample of fifty-four ants from the major category. A visual survey of dead *Atta* ants was also made following all other successful raids (n = 12).

Results

Only eighteen dead *N. esenbeckii* workers were found in the combat area, but mortality may have been higher because *N. esenbeckii* raiders carried injured nestmates away from this and other raids. In contrast, the combat area was carpeted with a total of six hundred and thirty-one dead *A. cephalotes* majors (head-width across eyes of random sub-sample, Mean = 4.43 mm, n = 54, SD \pm 0.20), but only twenty-five smaller workers. The cuticle of the dead *A. cephalotes* adults was not damaged, corroborating behavioural observations that *N. esenbeckii* raiders stung *Atta* workers to death.

Majors constituted approximately 90% of the dead *Atta* seen after the other successful raids against mature colonies (7 of 13). The absolute number varied greatly between attacks, ranging from less than fifty majors immediately around a nest entrance to many hundreds scattered along the route taken by *N. esenbeckii*. The difference in the distribution and number of dead majors may reflect the location at which the advancing army ants were first engaged, the intensity of the attack, and whether *N. esenbeckii* successfully entered the nest. No *Atta* adults were retrieved as prey in any of the raids.

Outcome of raids

Table 1 summarises the outcome of all natural raids. *N. esenbeckii* emigrated into the mature *A. cephalotes* colony that

Table 1. The outcome of raids made by *N. esenbeckii* against *Atta* colonies. 'Repelled raid' indicates that *Atta* prevented *N. esenbeckii* from entering the nest. 'Partially overrun' indicates that *N. esenbeckii* was contained in a subsection of the nest, while 'Completely overrun' indicates that *N. esenbeckii* moved throughout the *Atta* nest. Colonies that 'recovered' resumed foraging after the raid, and those that 'died' showed minimal activity days after the raid and no activity thereafter. The asterisk (*) indicated multiple raids made on two colonies: One young *A. colombica* colony was raided twice, while the other was raided thrice and died-off after the last raid

	Repelled raid	Partially overrun & recovered	Completely overrun & recovered	Completely overrun & died	Raid totals
<i>A. colombica</i> Young colony	0	0	4*	1	5*
<i>A. colombica</i> Mature colony	6	5	0	1	12
<i>A. cephalotes</i> Mature colony	0	1	0	1	2
Outcome Totals	6	6	4	3	19

was partially overrun. The emigration occurred after the battle, and it was aboveground for about one-metre before disappearing down a peripheral *Atta* nest entrance. On subsequent days, the *Atta* colony resumed normal foraging from the other nest entrances, and *N. esenbeckii* presumably emigrated to a new site along an underground route. The two young *A. colombica* colonies were approximately ten-metres apart, and all five raids occurred within a one-week period. Most mature *A. colombica* colonies that were partially or completely overrun and recovered deposited fungal substrate onto their external refuse mound at a relatively higher rate, in the days following a raid. One mature *A. colombica* colony also discarded a large number of sexual brood after a *N. esenbeckii* raid.

Discussion

Here, we have reported, and demonstrated experimentally, that mature *Atta* colonies rapidly mobilize large numbers of majors as a specific defensive response to the detection of *N. esenbeckii* raiders. We have also established that both *Atta* and *N. esenbeckii* employ their largest workers in the front-line of battles, demonstrating a size-related division of labour. In addition, both taxa use cooperative combat strategies to overwhelm the large primary combatants from the other side. Finally, we have shown for the first time that *N. esenbeckii* routinely raids mature *Atta* colonies, often resulting in high adult mortality, the loss of many thousands of brood items, and occasionally colony death. These findings establish *N. esenbeckii* as the only known predator capable of successfully attacking and killing mature colonies of *Atta* leaf-cutting ants.

Lanchester's theory of combat has been proposed as a general theoretical framework for understanding the evolution of combat in ants and other animal societies (Franks and Partridge, 1993), and it may help explain the combat behaviours seen in the interaction between *N. esenbeckii* and *Atta*. Lanchester's Square Law predicts that when all combatants can mix freely, in an open combat arena, numerical superiority is relatively more important for victory than the fighting

ability of each individual. Conversely, the Linear Law predicts that when individuals are forced to engage in a parallel series of duels, due to a restricted combat arena, the fighting abilities of combatants becomes relatively more important for victory than the size of the army. The theoretical assumptions of free-mixing for the Square Law and one-on-one duels for the Linear Law are not likely to be met strictly in nature. However, this theory has proved valuable in interpreting combat between opposing sides under artificial conditions that force either free-mixing or one-on-one duels (McGlynn, 2000), and under natural conditions that approximate the free-mixing assumption of the Square Law (Whitehouse and Jaffe, 1996; Wilson et al., 2002).

Battles between *N. esenbeckii* and *Atta* are naturally contained to the relatively small area around a nest entrance, or along the narrow underground passageways of a nest. Under these conditions, the one-on-one assumption of the Linear Law is closely approximated because the combat arena is highly restricted, and only a fraction of combatants can interact at any given time. Accordingly, Lanchester's Linear Law predicts that larger, stronger combatants should be favoured, and we have shown that both taxa do indeed deploy and use their largest individuals at the frontline of battles. By engaging in head-to-head duels, the individual behaviour of the primary combatants also agrees with the predictions of the Linear Law. In support of our observations, Swartz (1998) reported that a 'small' *A. cephalotes* colony (mound 1×1.5 m) under attack by *N. esenbeckii*, recruited many majors and that they played the central role in the colony's frontline of defence. Furthermore, most of the dead *Atta* found around the nest after this attack were majors, as seen in the thirteen successful raids reported here.

Atta majors have long been thought to fulfil a defensive role within mature *Atta* societies, particularly against vertebrate attack (Whitehouse and Jaffe, 1996), and such predators have been implicated in the evolution of this caste (Hunt, 1983). However, majors are first produced when colonies reach a relatively large size (in excess of 10,000 individuals for *A. cephalotes*, see Wilson, 1983), and, to our knowledge, no vertebrates have been verified as predators of colonies that produce majors in significant numbers (Fowler et al.,

1986; Hölldobler and Wilson, 1990), such as those defined as mature colonies in the present study. Furthermore, *Atta* majors only play a minor role in territorial conflicts in *Atta laevigata* (Whitehouse and Jaffe, 1996) and in other *Atta* species (U. Mueller, pers. comm.). In contrast, we have shown *Atta* majors play a pivotal role in defending mature colonies from *N. esenbeckii*. Swartz (1998) also reported that majors played an important defensive role in a much smaller colony, possibly one that had just started producing majors in the transition towards maturity. While other factors may have been important in the evolution of majors in *Atta*, our results demonstrate that *N. esenbeckii* predation is likely to have exerted significant and previously unrecognised selection for this defensive caste. Indeed, existing data suggests that *N. esenbeckii* is the only contemporary predator that *Atta* majors fight with any frequency. Given the importance of majors for defending against *N. esenbeckii*, it is unclear why they are not produced earlier in the colonies lifecycle. However, the early production of majors in ant colonies generally seems constrained by other factors (see Oster and Wilson, 1978; Bourke and Franks, 1995 for overview).

Having considered the importance of large primary combatants in these interactions, we must also consider the significance of the assistants that help them. Interestingly, once a large primary combatant has isolated its opponent behind the frontline, the involvement of the assistants significantly alter the combat conditions. Here, the assistant combatants easily surround and overwhelm the isolated primary combatant, which describes a situation that approximates that of the Square Law. This suggests that both taxa use a two-stage combat strategy: large combatants are employed where their large size is favoured, but once opponents are isolated, the assistants help dispatch with them, thus utilizing the many combatants that cannot be used in the restricted arena of the frontline. The two-stage strategy described here may have also been seen in *N. esenbeckii* by Swartz (1998), who reported *Atta* majors being 'isolated' and then 'encased' by *N. esenbeckii* raiders. Future studies are needed to explicitly test our interpretations based on Lanchester's Laws. However, if it is possible to collect detailed mortality data and even experimentally manipulate the number and size of combatants, such a test may be possible, whilst also allowing the incorporation of recent modifications to Lanchester's original models (Adams and Mesterton-Gibbons, 2003).

Adding further complexity to the combat between *N. esenbeckii* and *Atta*, both taxa show a division of labour within the groups of ants that attempt to kill isolated opponents. An animal 'team' has recently been redefined as an adaptive assemblage of individuals that must perform different subtasks concurrently to complete a task successfully (Anderson and Franks, 2001). The groups of primary and assistant combatants in both *N. esenbeckii* and *Atta* meet this new definition, because while a primary combatant remains locked head-to-head with its opponent, assistants attack the vulnerable areas of the isolated individual and attempt to kill it. Furthermore, the behavioural differences between the primary and assistant combatants of *Atta* teams are reinforced by significant size differences, meeting an older, more

restrictive definition of an animal team (Franks, 1986). To our knowledge, this is the first example of an interaction between two derived social taxa where both parties display team behaviours.

At the broader colony-level, the combat strategies used by both taxa seem pivotal in determining the outcome of a raid and its impact on the *Atta* colony. The instances in which *Atta* repelled *N. esenbeckii* suggest that rapid recruitment of majors can overwhelm the army ants, causing them to abort a raid. In other cases, *Atta*'s defensive efforts were adequate to contain *N. esenbeckii* within a subsection of the nest and limit the amount of brood-loss. In addition to fighting, barricading nest entrances may be vital for physically containing *N. esenbeckii* raiders. Swartz (1998) also reported *A. cephalotes* workers barricading nest entrances, and it is probable that passageways within the nest are closed-off in much the same way as nest access at the surface. Interestingly, Whitehouse and Jaffe (1996) reported entrance 'capping' during *Atta* territorial conflicts, which suggests that barricading nest entrances might be a general response by *Atta* colonies that are under attack from ants that could potentially enter the nest. Finally, when *Atta*'s defences are insufficient for deterring or containing *N. esenbeckii*, colonies are overrun and sometimes killed. High mortality of majors in aboveground raids suggests that massive adult mortality within the nest may be an important factor in those colonies that die. Furthermore, if queen death occurs, as was documented once in exceptional underground film footage (Rettenmeyer et al., 1983), colony death is all but assured.

In addition to suffering high adult mortality, mature colonies also lose tens of thousands of brood items when they are overrun. LaPolla et al. (2002) demonstrated that colonies of the lower fungus-gardening ant *Trachymyrmex arizonensis*, which lives in relatively small colonies, lose as much as 75% of their brood when raided by the army ant *Neivamyrmex rugulosus*. Such accurate calculations cannot be made for the interaction between *N. esenbeckii* and *Atta*, because the colonies are so large that accurate colony size estimates and brood counts are not possible. However, we can estimate that a *N. esenbeckii* colony might have a synchronised brood of approximately 100,000–150,000 larvae. This estimate is based on the fact that *Eciton burchellii* (previously *E. burchelli*, see Bolton, 1995) shares many key characteristics with *N. esenbeckii*, including large colonies of over 600,000 workers, and has synchronized broods of approximately 100,000 larvae (S. Powell, unpublished data). Based on the raid of the mature *A. cephalotes* colony, we can estimate that the number of brood items captured by *N. esenbeckii* can be more than half the number of brood being reared by the army ants. This represents a significant haul from a single prey colony.

Considering the ecological implications of this interaction, *N. esenbeckii* predation is likely to have an important, yet previously unrecognized, direct impact on *Atta* colony growth and lifetime fitness. *Atta* colonies take more than five years to reach reproductive maturity, with particularly slow growth in the first two years (reviewed in Wirth et al., 2003), and repeated raids against young colonies probably con-

tribute significantly to this slow initial growth. In those colonies that reach reproductive size, substantial brood loss, including the loss of sexual brood (queen and male brood), may effectively prevent reproduction until the colony can recover. Sexual production may also be prevented if the sexual brood is damaged in an attack, or if the impact of a raid is so great that a sexual brood becomes too expensive to rear to maturity. This may have been the case for the mature *A. colombica* colony that discarded sexual brood after an *N. esenbeckii* raid. In considering the potential impact of *N. esenbeckii* raids on *Atta* colony growth and fitness, it is important to take their subterranean tendencies into consideration. Of the thirteen successful raids reported here, one was entirely subterranean and the remaining twelve were mostly so. Even though the initial advance of some raids was aboveground (either observed or inferred from the distribution of dead *Atta*) a subterranean route was established wherever possible during brood retrieval. The only visible signs of the entirely subterranean raid were the groups of fighting ants at the nest entrances, and Rettenmeyer et al. (1983) report on an underground attack that showed no signs at the surface. These observations suggest that aboveground raids represent a fraction of *N. esenbeckii*'s total activity and predation upon *Atta* colonies.

N. esenbeckii predation may also have important indirect fitness consequences for *Atta* colonies. Fungi in the genus *Escovopsis* is a virulent pathogen of the symbiotic attine fungus gardens, and it is transmitted horizontally between colonies, possibly by invertebrates that associate closely with Attines and occasionally move between nests (Currie et al., 1999; Currie, 2001). We have shown that nearby *Atta* nests can be raided in quick succession and probably by the same *N. esenbeckii* colony. This suggests that in addition to the small invertebrate associates of *Atta* nests, *N. esenbeckii* could be an important and high frequency vector of *Escovopsis*. *N. esenbeckii* undoubtedly causes massive disturbance to the fungus gardens as they harvest *Atta* brood and this may further promote an outbreak of *Escovopsis*. Indeed, the removal of fungal substrate from the nest at a relatively higher rate following a raid suggests some level of contamination and damage. Colonies of the lower fungus-growing ant *T. arizonensis* also discard significant amounts of seemingly unhealthy fungal substrate after raids by the army ant *N. rugulosus* (LaPolla et al., 2002). This suggests that substantial damage and contamination of fungus gardens, and subsequent reduced garden productivity, might be a general cost incurred by any Attine ant raided by an army ant predator.

Finally, the prevailing view has been that the size and population density of mature *Atta* colonies is limited primarily by bottom-up forces, such as changes in leaf chemistry and toughness, particularly during succession (Jaffe and Vilela, 1989; Nichols Orians, 1991; Farji-Brener, 2001). While top-down regulation from parasitoid Phorid flies and the fungal pathogen *Escovopsis* may also be important (Orr, 1992; Braganca et al., 1998; Currie, 2001), possible regulation by predators has been given little attention. Rao (2000) attempted to address the relative importance of an army ant predator, armadillo predation, and phorid fly parasitoids in

regulating *Atta* populations, but unfortunately this study had a serious methodological problem. The experimental design required a valid army ant predator to be present at some sites and not at others, but the focal army ant was an unidentified species in the genus *Eciton*, which has no member that is a verified predator of *Atta* (Rettenmeyer, 1963; Rettenmeyer et al., 1983; Swartz, 1998): *N. esenbeckii* is the only army ant that has been documented repeatedly as a predator of *Atta*, and it was not reported as present at any of the study sites. The author's conclusions concerning the relative and absolute importance of army ant predation are therefore unsupported. However, considering the significant colony-level impact of *N. esenbeckii* predation on young and mature colonies, and the likelihood that aboveground raids constitute a fraction of the total, *N. esenbeckii* probably exerts significant top-down regulatory pressure on *Atta* populations in natural systems. Furthermore, the overabundance of *Atta* colonies in heavily disturbed and isolated habitat (Vasconcelos and Cherrett, 1995; Rao, 2000; Terborgh et al., 2001), may be explained partly by release from *N. esenbeckii* predation. Terborgh et al. (2001) recently demonstrated that *Atta* populations exploded on small forested islands because of release from top-down pressure from an unknown predator. *N. esenbeckii* is likely to be this key unknown predator, because it probably goes locally extinct in such habitat, like *E. burchellii* (Boswell et al., 1998; Britton et al., 1999), with which it shares key life history characteristics. Further studies are needed to address the dynamics of *Atta* populations in both natural and disturbed habitat, and they must now recognize that predation by the army ant *N. esenbeckii* is likely to be of considerable importance.

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