A dolichoderine ant that constructs traps to ambush prey collectively: convergent evolution with a myrmicine genus

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Azteca brevis Forel, a dolichoderine ant species, builds along the branches of its host plant galleries that bear numerous holes slightly wider than a worker's head. We noted that the workers hide, mandibles open, beneath different holes, waiting for arthropod prey to walk by or alight. They seize the extremities of these arthropods and pull backwards, immobilizing the prey, which is then spreadeagled and later carved up or pulled into a gallery before being carved up. The total duration of the capture ranges from a few minutes to several hours. This ambush group hunting permits the capture of insects of a wide range of sizes, with the largest being 48.71 times heavier than the workers, something that we compared with other cases of group hunting by ants and trap use by other arthropods. A convergence with myrmicine ants of the genus *Allomerus* is shown. Thus, this study also shows that the genus *Azteca* presents the largest panel of group hunting strategies by ants and that there is polyethism related to polymorphism, as hunting workers are larger than their nestmates. We concluded that these gallery-shaped traps correspond to the notion of 'extended phenotype'.

ADDITIONAL KEYWORDS: *Allomerus* – ambush group hunting – ants – *Azteca brevis* – extended phenotype – gallery-shaped trap.

INTRODUCTION

Over the course of evolution, many ant species have developed the ability to live in trees by finding ways to satisfy their need for shelter by building their own nests or becoming hollow-twig dwellers; others are plant-ants that live in association with 'myrmecophytes' (i.e. plants that provide ants with a nesting place in pre-existing cavities). Arboreal ants mostly limit their activities to their host plant, where they exploit energy-rich hemipteran honeydew, extrafloral nectar and/or food bodies. Also, most arboreal ants have evolved different types of group ambushing behaviours adapted to foraging in the host tree crown by optimizing their ability to capture insect prey, many of which are able to escape by flying away, jumping or dropping. A worker that has successfully seized a prey item emits a pheromone to recruit nearby nestmates to help it spreadeagle the prey (Cerda & Dejean, 2011).

This mode of group ambushing has been noted for several species of the Neotropical genus *Azteca* (Dolichoderinae), composed almost entirely of arboreal species, whereas two derived strategies also exist. *Azteca bequaerti* Wheeler, W.M. & Bequaert workers, hidden in the leaf pouches of their host myrmecophyte, react to the vibrations transmitted by a flying insect landing on that leaf. *Azteca andreae* Guerrero, Delabie & Dejean workers lie in wait under the leaf margins of their host trees and use the 'Velcro® principle' to capture large prey (Dejean *et al.*, 2009, 2010).

A more sophisticated technique is used by *Pheidole* sp. (Myrmicinae) workers, which pierce the walls of the leaf pouches of their myrmecophyte host with small, regularly distributed holes and await in ambush under these holes to catch arthropod prey that venture within reach, so that the leaf pouches serve as both lodging and trap (Vogel, 2012). Yet, the most elaborated

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predatory strategy is illustrated by *Allomerus* spp. (Myrmicinae) that collectively ambush prey by building gallery-shaped traps bearing numerous holes. These galleries are constructed from host plant trichomes reinforced by a fungal mycelium. Here, too, each worker lies in ambush under a hole, so that the prey is immobilized with its appendages held in different holes, and recruited workers help to spreadeagle it and carve it up (Dejean *et al.*, 2005).

In Central America, *Azteca brevis* Forel nests have mostly been recorded in the hollow stems of the myrmecophyte *Tetrathylacium macrophyllum* Poepp. (Salicaceae) growing in the primary forest understorey. Furthermore, like *Allomerus*, *A. brevis* workers use fungi to build galleries bearing numerous holes (Schmidt, 2001; Longino, 2007; Mayer, Lauth & Orivel, 2017). We hypothesized that these galleries can also be used as traps.

MATERIAL AND METHODS

In addition to preliminary observations conducted on several trees and treelets, detailed field studies were conducted on four *A. brevis* colonies in Costa Rica around the La Gamba Biological Research Station (08°42′N, 83°13′W) and two other colonies in French Guiana around the Petit Saut field station (03°52′N, 11°31′E). In both areas, the *A. brevis* galleries can be seen on the trunks of several adjacent tall trees of different families up to the crowns (~40 m) and continue
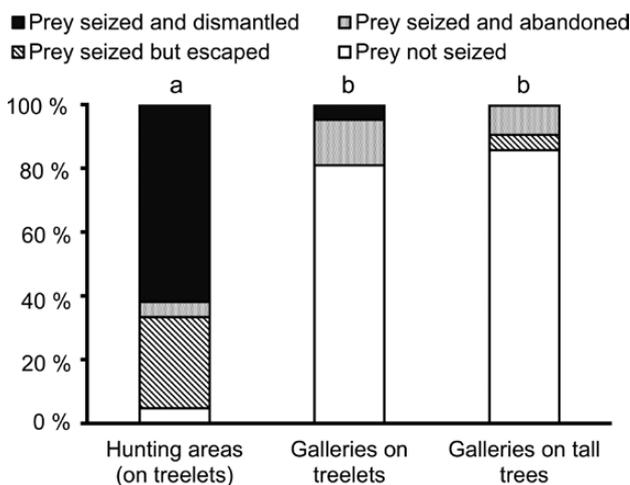
below the leaf litter, interconnecting these trees and understorey treelets (Longino, 2007; A. Dejean, personal observation).

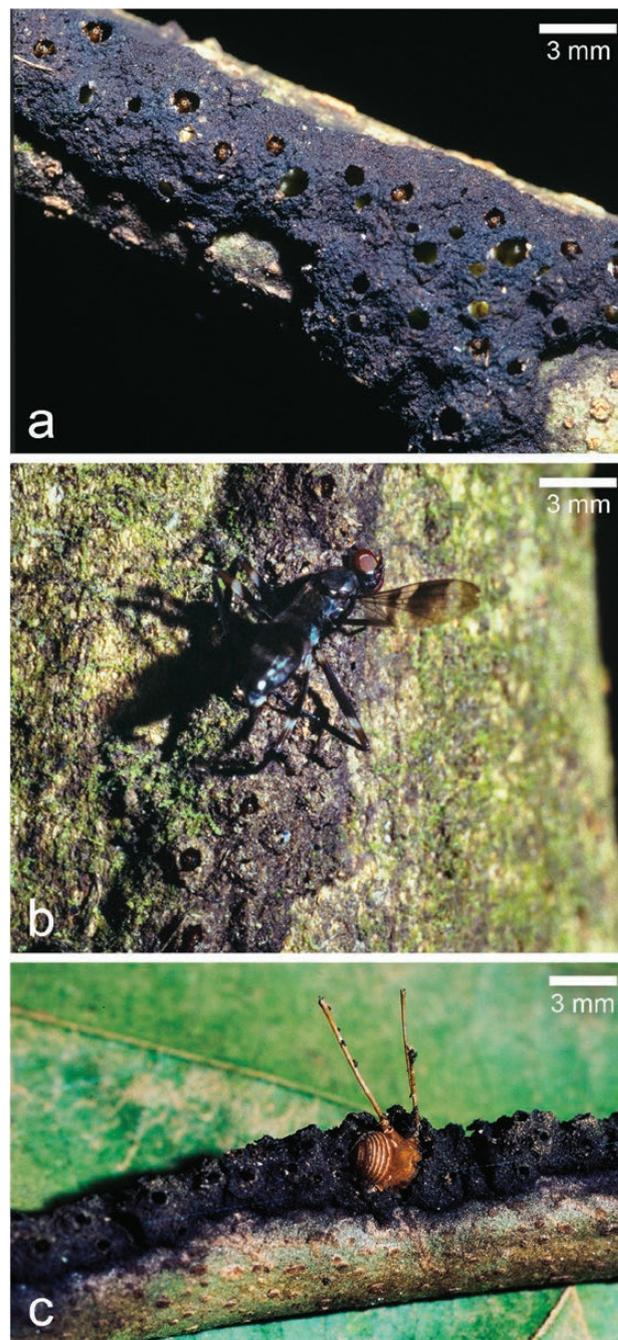To determine whether workers hunt in all parts of the galleries or only in specific areas, we conducted

Figure 1. Testing whether *Azteca brevis* workers hunt in specific parts of the galleries; comparison between three zones ($N = 21$ in each case) and the four situations noted. Statistical comparisons, Fisher's exact test from 2×4 contingency tables and the false discovery rate adjustment for simultaneous comparisons (Past 3.0 software): different letters indicate a significant difference at $P < 0.001$.

Figure 2. *Azteca brevis* workers using their gallery-shaped trap to catch insect prey. A, a gallery-shaped trap with workers hiding under holes with their mandibles open. B, a fly ant trapped on the gallery was later cut up on the spot. C, an insect pulled inside the gallery. (Photographs by M. Schmidt.)

Table 1. A, arthropod taxa captured in natural conditions by *Azteca brevis* workers on their galleries; and B, size and weight of arthropods successfully captured (and legs for taxa losing them through autotomy) during tests, and ratio between these weights and the mean weight of workers (1.201 mg)

A.

Arthropod prey	Number of cases	Size (mm)	Arthropod prey	Number of cases	Size (mm)	Arthropod prey	Number of cases	Size (mm)
Spiders	5	5–8	Winged termites	14	7–9	Flies	1	4–12
Grasshoppers	6	7–12	Caterpillars	2	8–14	Ants (workers)	1	4–6
Heteroptera	3	4–7	Butterflies and moths	3	9–15	Ants (winged sexuals)	6	8
Cockroaches	8	7–10	Tipulidae	1	12	Unidentified	11	4–8
Arthropod legs								
Spiders	2	5–9	Opiliones	2	8–12	Butterflies and moths	4	3–4
Tipulidae	3	5–10	Grasshoppers	5	8–12	Unidentified	8	4–10

B.

Arthropod prey	Size (mm)	Weight (mg)	Ratio to weight of workers	Arthropod prey	Size (mm)	Weight (mg)	Ratio to weight of workers
Spider legs	4	0.45	0.37	*Heterotermes* sp. workers	7	9.8	8.16
Spider legs	6	0.6	0.50	Heteroptera Pentatomidae (nymphs)	8	22.0	18.32
Spider legs	7	0.7	0.58	Butterflies	14	11.3	9.41
Spider legs	9	1.0	0.83	Butterfly legs	5–6	0.3	2.50
Opilione legs	10	0.5	0.41	Hairless caterpillars	11	40.0	33.30
Grasshoppers	9	38.1	31.72	Hairy caterpillars	10	38.4	31.97
Grasshoppers	11	44.5	37.05	Hairy caterpillars	12	40.2	33.47
Posterior legs	11	5.1	4.24	Hairy caterpillars	15	42.0	34.97
Grasshoppers	13	57.2	47.62	Hairy caterpillars	18	46.2	38.47
Posterior legs	12	8.6	7.16	Hairy caterpillars	21	49.9	41.55
Cockroaches	12	43.8	36.47	Hairy caterpillars	22	53.4	44.46
Winged termites	8	13.8	11.49	Hairy caterpillars	25	58.5	48.71

an experiment on the galleries built on eight tall trees and 12 understorey treelets, for which we distinguished the wider parts of the galleries that we hypothesized to be devoted to predation from the other, narrower parts (see details on defense vs. predation in Supporting information, Appendix S1 and Fig. S1). For each type of gallery, we conducted 21 series of tests for which, using forceps, we gently held 3-mm-long male ants so that their extremities just touched the galleries for ~5 s before releasing them. As *A. brevis* workers are polymorphic (Longino, 2007), we gathered some from these three zones of the galleries and transported them to the laboratory, where they were freeze killed and placed in 15 groups of ten individuals from each zone, and we weighed them for comparison using a microscale (Mettler AE 260).

We then studied in detail the predatory behaviour on the parts of the galleries found on understorey treelets where the ants were visibly lying in wait. Using forceps, we proceeded as before, this time using different types of arthropod prey. We noted whether the prey was successfully captured or not and, in the latter case, if it lost a leg through autotomy or not. To obtain a predator–prey ratio, we weighed hunting workers (see above) and the experimental prey (and that of legs for cases of autotomy; mean weight for five to ten individuals or legs).

RESULTS AND DISCUSSION

As empirically observed, the workers hunt mostly, if not always, in the wider zones of the galleries on the understorey treelets (Fig. 1).

The workers gathered from these zones (while hunting) were significantly heavier than those gathered from the galleries on the other parts of the treelets and those from the trunks of tall trees; the latter two categories did not differ significantly from each other (1.201 ± 0.029, 0.952 ± 0.02 and 0.922 ± 0.02 mg, respectively; ANOVA, $F_{2,45} = 42.27$; $P < 0.0001$; Tukey's *post hoc* test, significant differences at $P < 0.001$; Past 3.0 software). Thus, not all workers are specialized in predation, illustrating a case of polyethism related to polymorphism (see also Cerda & Dejean, 2011).

Typically, when hunting, the *A. brevis* workers hide with their mandibles wide open under the holes in certain zones of the galleries and wait for prey (Fig. 2A). When an arthropod walks or lands on these zones, the extremities of several legs are always close to holes; therefore, the workers waiting below seize them, pull backwards and hold tight (Fig. 2B). In trying to escape, the prey moves other legs or antennae close to other holes, resulting in these appendages

being grasped as well. Then, the workers holding the extremity of an appendage weave in and out of holes, moving further and further away until the prey is progressively stretched against the gallery before being carved up or pulled into the gallery before being carved up (Fig. 2C). The stretching and carving up of the prey takes from 20 min to several hours. The principle behind *A. brevis* prey capture is therefore similar to that of *Allomerus*, although the latter move their prey close to a domatium before carving them up (Dejean *et al.*, 2005).

The ratio between the weight of the heaviest prey tested and that of the *A. brevis* workers (Table 1) seems low compared with the maximum noted for *Allomerus* (1:48.71 vs. 1:1800.00, respectively). Yet, in the latter case, this corresponded to very exceptional prey. Compared with ants that do not use a trap, this ratio is similar to that of *Oecophylla longinoda* (Latreille) equipped with hypertrophied adhesive pads (1:50); this ant species, well known for its ability to capture insect prey, is used as a biological control agent (Cerda & Dejean, 2011). It is lower than that of *Daceton armigerum* (Latreille) equipped with trap jaws (up to 1:94.12) and much lower than that of *Az. andreae*, whose workers use the 'Velcro® principle' to capture prey (1:7121; Dejean *et al.*, 2010, 2012). Note that, except for social spiders (1:55.62), all these values are much higher than those known for other arthropods using traps to capture prey (> 1:5.4), probably because they hunt in a solitary manner (Table 2).

Given that *Allomerus* spp. and *A. brevis* belong to two phylogenetically distant subfamilies (Myrmicinae vs. Dolichoderinae; estimated time of separation > 100 Mya; origin of ants 140–168 Mya; Antwiki, 2017 and papers cited therein), the construction and use of gallery-shaped traps represent an example of convergent evolution. Therefore, the genus *Azteca*, composed almost entirely of arboreal species, has evolved the largest panel of predatory techniques in ants, because these gallery-shaped traps are used in addition to other group hunting modes (see Introduction).

By constructing traps, some arthropods increase the probability of capturing prey, including relatively large or fast-moving ones (Scharf, Lubin & Ovadia, 2011). Given that a trap permits a predator (and its genes) to act on its environment beyond the limits of its physical capacity (i.e. mandibles, beak, mouth, legs), it can be considered an 'extended phenotype' of that predator (Dawkins, 1982). Indeed, the quality of these traps, as for bird nests or beaver dams, is correlated with certain alleles of the constructing organisms that are under pressure related to natural selection (Scharf *et al.*, 2011).

Table 2. Largest captured prey, their weight, and the ratio to the mean weight of a hunting individual for arboreal ants that do or do not build a trap (all compared ant species are group hunters) and for other arthropods using traps to capture prey, including a social spider

	Predator weight (mg)	Larger prey	Prey weight (mg)	Ratio of weights	Source
A. Arboreal ants building traps					
Allomerus decemarticulatus	0.225	Hairy caterpillars (Lepidoptera)	405.2	1800.90	Dejean *et al.* (2005)
Azteca brevis	2.202	Hairy caterpillars (Lepidoptera)	58.5	48.71	This study
B. Arboreal ants building traps					
1. Rely on rough leaves from host tree					
Azteca andreae	1.4	*Tinacris albipes*; locust; 65 mm	9920	7121.30	Dejean *et al.* (2010)
2. Rely on powerful adhesive pads					
Oecophylla longinoda	14	Mantodea; praying mantis	700	50.00	Wojtusiak, Godzinska & Dejean (1995) (A. Dejean, personal observation)
Daceton armigerum	17	Orthoptera; locust; 45 mm	1600	94.12	Dejean *et al.* (2012)
C. Arthropods using traps					
1. Web-spinning spiders (Arachnida; Araneae)					
Nephila clavipes	1477	*Tropidacris* sp.; locust; 80 mm	8000	5.40	Zschokke *et al.* (2006) (Y. Hénaut, personal communication)
Cyrtophora moluccensis	1163	Scarab beetle	1000	0.86	Lubin (1980)
Argiope dentata	200	Orthoptera: Tettigoniidae	840	4.20	Robinson & Robinson (1970); Reed, Witt & Scarboro (2004)
Anelosimus eximius (social spider)	8.0	Orthoptera; locust; 34 mm	445	55.62	Guevara & Aviles (2011) (A. Dejean, personal observation)
2. Ant lions (Neuroptera)		(different ant species)			
Euroleon nostras (small)	2.0	*Formica polyctena*	6.0	3.00	Humeau, Rougé & Casas (2015)
Morter sp.	5.0	*Polyrhachis decempunctata*	7.5	1.50	Griffiths (1980)
3. Glow worms (Diptera)		(cave crickets)			
Arachnocampa luminosa	> 5.0*	Orthoptera Rhaphidophoridae	~25*	5.00	Broadley & Stringer (2001)
4. Net-spinning caddifishes (Trichoptera)					
Plectrocnemia conspersa	5.0	Copepods (*Daphnia*)	12.0	2.40	Otto (1985)

*Weight estimated from sizes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Defensive strategy and predatory behaviour.
Figure S1. Fate of *Atta* workers (*N* = 116) placed on myrmecophytic *Tetrathylacium costaricensis* (Salicaceae) colonized by *Azteca brevis* workers. Four scenarios occurred after releasing *Atta* workers: they foraged freely (free), dropped down (dropped), left the tree (abandoned tree) or were caught by *Az. brevis* workers (trapped) (see Schmidt, 2001).