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RESEARCH ARTICLE - ANTS

Ants (Hymenoptera: Formicidae) Associated with Pig Carcasses in an Urban Area

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Abstract

The study of cadaver fauna is the most important aspect of forensic entomology, and it is based on successional patterns of insects that feed on decaying carcasses. Many ant species have scavenger lifestyles, and thus have the potential for use in forensic entomology studies. There are no previous studies of ant fauna as forensic entomology tools in state of Maranhão. Here we characterize ants found on domestic swine carcasses at different stages of decomposition, with the goal of initiating a forensic entomology database in the state. We collected ants between November and December 2012 on two pig carcasses. A third carcass with no ant collection was used as a control to assess the influence of ant removal on decomposition rate. We captured 1,692 Formicidae in total, distributed among 10 genera and including 17 species. The most abundant species was Solenopsis saevissima, with 55.6% of relative abundance. Solenopsis was present at various stages of decomposition either feeding on carcass tissues or exudates, or preying on larval, pupal and newly emerged adult flies. Ants associated with this environment had high species richness and abundance in all stages of decomposition. We suggest that these insects influence the decomposition process either as facilitators (i.e., by laceration and fragmentation of tissue) or as hindering agents (i.e., due to predation upon other scavenging organisms).

Introduction

The most important aspect of forensic entomology is the study of cadaveric fauna, which is based on the successional patterns of insects that feed on decomposing carcasses (Catts & Goff, 1992). The most commonly utilized insects for analysis of a crime scene are flies and beetles (Sousa & Linhares, 1997; Carvalho et al., 2000; Pujol-Luz et al., 2006; Biavati et al., 2010). However, although less studied, ants and other insects also belong to the cadaveric fauna, actively participating in the fractionation and decomposition of vertebrate carcasses (Cruz & Vasconcelos, 2006; Moretti & Ribeiro, 2006). Because some ant species display necrophagous affinities, these social insects have potential to aid in crime solving in forensic entomology studies. Although studies of ants and their relationship with decomposition of cadavers appear among the oldest in forensic entomology in Brazil

(Luederwaldt, 1911; Freire, 1914), studies of their role and importance in the vertebrate decomposition process have been neglected (Payne, 1965; Nuorteva, 1974). The role of the Hymenoptera, especially ants, has taken a secondary position in forensic entomology.

Despite the strong presence of ant species throughout the decomposition process cadavers, few studies in Brazil suggest their use in forensic entomology (Moura et al., 1997; Moretti & Ribeiro, 2006; Moretti et al., 2013). It is also known that these insects can contribute to estimation of the post-mortem interval (Win & Goff, 1997), including those with sarco-saprophagous habits (Cornaby, 1974). Some forensic studies show that ants display omnivorous behavior, feeding as much on decaying cadavers as other associated insects (Carvalho et al., 2004; Cruz & Vasconcelos, 2006). Consequently, their relationship with vertebrate carcasses can vary significantly, from predatory when feeding upon



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eggs, larvae, pupae, or adults of other insects, to scavenging when feeding the decomposing tissues or exudates (Early & Goff, 1986). This behavior influences criminal investigations because scavenging ants can produce spots on the body that are occasionally mistaken for mutilation or injury (Patel, 1994); predatory species can delay cadaver decomposition by decreasing the population of Dipteran larvae active on the body, especially those in families Calliphoridae and Sarcophagidae (Wells & Greenberg, 1994; Carvalho et al., 2004; Moretti & Ribeiro 2006).

Forensic entomology research in Maranhão, Brazil, is still in early stages, with, to our knowledge, only a single study of Dipterans existing in Caxias (Silva, 2012), and no mention of Hymenopteran species. The purpose of this study was to investigate the ant species present at different stages of cadaver decomposition, in order to implement a forensic entomology database for state of Maranhão. We characterized ants occurring on pig carcasses in various stages of decomposition, analyzing species richness, frequency, abundance, and feeding habits.

Material and Methods

Study area

The study took place on São Luís Island (2°31' S, 44°18' W), in Maranhão, Brazil. The climate is hot and humid tropical, with a rainy season (January to June), with an average rainfall of 1,954 mm, and a dry season (July to December) with only 6% of the annual precipitation. The average temperature is approximately 26.1 °C (Carvalho, 2015).

The study area is located on the banks of a secondary vegetation fragment Amazon located within the Federal University of Maranhão campus (02°33'36" S, 44°18'33" W). The environmental characteristics and dynamics result from a high degree of human intervention in the original tropical rain forest vegetation (ICMBio, 2014), which was reduced to a low, secondary growth forest, with a high degree of anthropogenic change due for the installation of an area intended for higher education.

Field procedure

Ants were collected during the dry season of 2012. We collected daily in the first 15 days, and every other day thereafter. Three pigs (*Sus scrofa* L.), average weight 25 kg, were sacrificed by a qualified expert via shot to the head using a pistol. The carcasses were placed roughly 40 meters apart. Ants were collected exclusively from first (P1) and second (P2) carcasses. The third carcass (PC) was used as a control, in which ants were not collected, and instead observations were made for comparison of rates of decomposition. We opted for pig carcasses because they are generally accepted as a model organism for experimental research of forensic insect

fauna, due to similarities to human cadavers with respect to the amount of hair, dermal constitution, and torso/limb relationship (Payne, 1965; Catts & Goff, 1992).

All carcasses were enclosed within metal cages, which allowed the entry of insects and prevented access by medium and large-sized animals. The determination of decomposition stage followed those described by Payne (1965), as follows: Fresh, Bloated, Active decay, Advanced decay, Dried and Remains.

Ant collection and laboratory procedures

We use six pitfall-type traps (for details see Bestelmeyer et al., 2000) placed approximately 1.5 m from the carcasses to capture attracted ants. Collection was aided by use of entomological forceps. Collected ants were fixed and preserved in vials containing 70% ethanol.

Ants were identified at the Myrmecology Laboratory of the Cocoa Research Center (CEPLAC) in Ilheus, Bahia, Brazil. Samples were deposited in the Myrmecology Laboratory collection (CPDC), Convention UESC/CEPLAC at the Cocoa Research Center, Bahia. The project was approved by the Ethics Committee on Animal Use of Federal University of Maranhão (protocol number: 23115.004032/2012-34).

Results

We captured 1,692 Formicids in total, belonging to 17 species and 10 genera (Table 1). The *Camponotus* and *Pheidole* genera had the greatest diversity, with four and three species, respectively (Table 1). *Ectatomma brunneum* Smith and *Solenopsis saevissima* Smith were the most common species (present in 80% of the traps). The most abundant species was *S. saevissima* (54.5% RA), followed by *Dorymyrmex brunneus* Forel (8.5%), *Solenopsis globularia* Smith (8.2%) and *E. brunneum* (8%).

Both pig carcasses (P1 and P2) underwent the five stages of decomposition at roughly the same pace: fresh, lasted for one day; bloated, one day; active decomposition, five days; advanced decomposition, five days; dried and remains, 18 days. Ants were present at all stages of decomposition, though abundance strongly varied.

The two *Solenopsis* species (*S. globularia* and *S. saevissima*) were seen preying on fly larvae and causing injury to carcasses (P1 and P2). *Dolichoderus lutosus* Smith were seen feeding on exudates of the decomposing carcass; we did not observe predation upon other organisms, nor damage to carcasses resulting from this activity. To our knowledge, this is the first report of *D. lutosus* associated vertebrate cadaver decomposition in the Neotropics.

The last three stages of decomposition had the highest species richness in both carcasses, which may be related to the longer duration of these phases. Overall, there was a gradual increase in ant abundance throughout the different stages of decomposition.

Table 1. Absolute and relative frequency of Formicids captured in different stages of pig carcass decomposition in the dry season (November and December 2012) in an urban area in the city of São Luís, Maranhão state, Northeast Brazil. FR: Fresh; BL: Bloated; ACT: Active Decay; ADV: Advanced Decay; DR; Dry and Remains.

		Carcass 1	ss 1					Carcass 2	3.2					Total	
	Ant species	FR	BL	ACT	ADV	DR	n	FR	BL	ACT	ADV	DR	п	z	%
Dolichoderinae	Dolichoderus lutosus (Smith, 1858)			23			23							23	1,3
	Dorymyrmex brunneus Forel, 1908			S	16	64	85	7		33	7	47	59	144	8,5
Ectatomminae	Ectatomma brunneum (Smith, 1858)	30	ю	7		41	81	28		2	2	23	55	136	8,0
	Ectatomma tuberculatum (Olivier, 1792)			4		10	15				_		_	16	1,0
Formicinae	Camponotus blandus (Smith, 1858)					9	9				_		7	∞	5,0
	Camponotus melanoticus Emery, 1894		9	10	9	48	70		4		-	14	19	68	5,3
	Camponotus rufipes (Fabricius, 1775)			9	3	25	34			7	7	24	38	72	4,2
	Camponotus senex (Smith, 1858)			S	1	6	15				3	11	14	29	1,7
Myrmicinae	Acromyrmex rugosus (Smith, 1858)			1	П	∞	10				ω	16	19	29	1,7
	Crematogaster victima (Smith, 1858)				8	7	10			1		11	12	22	1,3
	Pheidole grupo Fallax sp.									3	7	7	12	12	0,7
	Pheidole radoszkowskii Mayr, 1884					1	1					\mathcal{E}	3	4	0,3
	Pheidole synarmata Wilson, 2003				7	13	15			-	-	14	16	31	1,9
	Solenopsis globularia (Smith, 1858)			17	13	44	74			25	2	38	99	139	8,2
	Solenopsis saevissima (Smith, 1855)			28	72	275	375	1	63	66	147	237	547	922	54,5
Ponerinae	Odontomachus bauri Emery, 1892							41					14	14	8,0
Pseudomyrmecinae	Pseudomyrmex schuppi Forel, 1901					7	7							7	0,1
Abundance		30	10	106	117	553	816	45	29	141	177	446	876	1692	100

Discussion

Ant species richness in the current study was the greater than in many other studies of decaying organisms in Brazil (Luederwaldt, 1911; Monteiro-Filho & Penereiro, 1987; Moura et al., 1997; Moretti et al., 2007; Gomes et al., 2007, 2009; Santos et al., 2014). In one forensic study using visual observation only, Luederwaldt (1911) reported nine ant species distributed among nine genera. Monteiro-Filho and Penereiro (1987) found five species, and Moretti et al. (2007) found four genera, three of which were also present in this study (*Camponotus*, *Crematogaster* and *Dolichoderus*). Gomes et al. (2009) highlighted *E. brunneum*, as well as *Pheidole* and *Camponotus* in his study of the insect fauna on pig carcasses.

The high number of species in our study seems to be due to the strong anthropogenic disturbance in the area. It is noteworthy that many of the species recorded here exhibit mass recruitment behavior (see Fowler et al., 1991; Silvestre & Silva, 2001) or some affinity for impacted environments (Brown, 1973; Overal, 1986; Piva & Campos-Farinha, 1999; Fernandes et al., 2001; Barbosa & Fernandes, 2003).

Pheidole and Camponotus are the genera with the greatest geographical distributions, species diversity, and adaptations in the whole Neotropical region, and even in the world (Wilson, 1976, 2003). Furthermore, one of the most abundant species of Camponotus in this study, C. rufipes has already been noted as having medical and legal importance in forensic studies in Brazil (Moura et al., 1997).

The "fresh" period of decomposition hosted predominantly *E. brunneum*, one of the most common species in this study. According to Hölldobler and Wilson (1990), species in this genus show aggressive behavior, and may inhibit the population growth of less aggressive species, or species less adapted for resource competition (Fernandes et al., 2000). The presence of this genus in the remaining stages of decomposition might be linked to the presence of other arthropods, since *Ectatomma* species are largely predacious upon other arthropods (Agosti et al., 2000).

The "active and advanced" stages of decomposition showed a significant increase in the number of ant species compared to previous stages. Liquids associated with decomposition and other carcass resources are exposed in these stages, which with advancing decay diffuses odors at greater distances, acting as an additional attractant for Formicids (Goff, 2009). The majority of ants was collected in the final stages of decomposition (i.e., dry and remains). However, abundance and species richness may have been overestimated in these final stages due to the greater number of days in this stage compared to other decomposition stages in the collection period.

In addition to species richness, abundance was also high and constant in the vicinity of carcasses, which has been observed by other authors (Horenstein et al., 2005; Chin et al., 2009; Bonacci et al., 2011). *S. saevissima* abundance, for example, can be explained by the typical behavioral

dominance of the *Solenopsis* genus. This genus shows massive behavioral recruitment and is capable of rapidly colonizing the exploited resource (Delabie & Fowler, 1995), and further, it behaves aggressively with both other ants and other animal species (Fowler et al., 1991). *Solenopsis* was found at various stages of cadaver decomposition, feeding on carcass tissues or exudates, or preying on larvae, pupae and newly emerged adult flies, behavior previously noted by Oliveira-Costa et al. (2007). The prevalence of *S. saevissima* on our carcasses may have inhibited the proliferation of other ant species. Predation on dipteran larvae and carcass damage caused by *Solenopsis* species in this study suggests that this taxon may influence both corpse decomposition rate and the number of flies that reach adulthood on the corpse. This notion has also been suggested in other studies (Early & Goff, 1986; Stoker et al., 1995).

We report *Dolichoderus lutosus* workers feeding on vertebrate carcasses for the first time in the Neotropical Region. *Dolichoderus* has worldwide distribution, but most species are Neotropical and arboreal (Silvestre et al., 2003). Failure to observe predation on other organisms or damage to carcasses corroborates Moretti et al. (2013), which indicates that Dolichoderinae are generally attracted by the release of carcass exudates. This behavior justifies the classification of these ants as opportunistic scavengers (Early & Goff, 1986) as well as future actions to study them in more detail on the role in and influence upon cadaver decomposition.

Even though few studies exist on the subject in Brazil, Hymenoptera seem to influence vertebrate carcass decomposition. Bees, for example, can be present in all phases of the corpse decomposition and can feed upon exudates (Santos et al., 2014). The wasp Epiponini *Agelaia pallipes* (Olivier) visit *S. scrofa* carcasses to feed on body fluids and collect carcasss pieces to feed their larvae (Gomes et al., 2007). In this same study, the only ant species observed was *Neoponera obscuricornis* (Emery), preying upon larval and adult blowflies.

Our study shows high species richness and abundance of ants associated with pig carcasses. The presence of ants in all stages of corpse decomposition as well as the observed feeding and predatory behaviors suggest that these insects can influence the decomposition process in two opposite ways: as facilitators, through the disruption and fragmentation of tissue, or as hindering agents, through predation of invertebrates (insect larvae, especially) that effectively contribute to decomposition. Consequently, these insects are of great importance for forensic entomology in Brazil, and henceforth should be given more attention in this field.

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