#### **SHORT COMMUNICATION**



# Ants associated with a rat carcass: its implications in forensic entomology with special emphasis on *Carebara diversa* (Hymenoptera: Formicidae)

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#### **Abstract**

The arthropod succession on carrion plays a forensically important role in estimating minimum post-mortem intervals (mPMIs). Among these arthropods, flies and beetles are the major arthropod community associated with carrion decomposition. However, there are other insects that can also be found on carcasses during decomposition. In the current study, activities of ants (Hymenoptera: Formicidae) were observed during the decomposition of a rat carcass, which was placed on the surface of the soil within the vicinity of Universiti Teknologi MARA, Sungai Buloh, Selangor. Ants were observed to directly affect the decomposition process by feeding on the rat carcass. We collected four species of ants from the rat carcass: *Carebara diversa* (Jerdon 1851) (both soldier and worker castes), *Brachyponera luteipes* (Mayr1862), *Anoplolepis gracilipes* (Smith, F. 1857) and *Monomorium floricola* (Jerdon 1851). Among these species, *C. diversa* was observed to cover the whole rat carcass with soil particles, which then subsequently prevented oviposition or colonization by flies and beetles.

**Keywords** Rat carcass · Carebara diversa · mPMI · Malaysia · Formicidae · Forensic entomology

# Introduction

The study of insects and their arthropod counterparts pertaining to legal matters is known as forensic entomology (Hall and Doisy 1993). Although a cadaver is subjected to the intrinsic processes of autolysis and putrefaction, the majority of decomposition process is due to the activity of insects and their immature stages. Insects can arrive on a cadaver within seconds of death (Mann et al. 1990). Blow flies and flesh flies tend to dominate during the early stages of cadaver decomposition for the development of their

offspring. Ants (Hymenoptera: Formicidae) also play an important role in crime investigation (Singh and Bharti 2000). The impacts pose by ants on carrion can intersect with applications of entomology in forensic science through a number of mechanisms. One of the major impacts of ants on carrion is their competition with necrophagous flies, in which the ants interfere with oviposition frequency and larval survival success of these flies. This has a significant implication to forensic entomologists as necrophagous Diptera development or community succession patterns are used to establish timelines, or time of colonization, associated with deaths under investigation (Eubanks et al. 2019).

Ants feeding on carcasses are categorised as carnivorous species in forensic entomological study as they feed on both the corpse and carrion-associated fauna (Smith 1986; Tabor et al. 2005). Ants are capable of capturing adults and removing larvae of both flies and beetles from carcasses, which then could potentially reduce the rate of decomposition (Heo et al. 2009). Furthermore, ants feeding on corpses can cause small punctuate or scratch-type lesions on the skin surface, which could be easily misinterpreted as ante-mortem abrasions or resulting from strong acids (Campobasso et al. 2009).

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However, ants are seldom reported as forensic indicators by crime scene investigators or forensic pathologists (Chen et al. 2014). Heo et al. (2009) observed ants being the first to visit pig carcasses and remained with the carcasses until the skeleton stage of decomposition.

The objective of the present study was to document the diversity and activities of ants during the decomposition of a rat carcass under a tropical climatic condition. We observed that *C. diversa* was covering the whole rat carcass with soil particles, which then subsequently prevented oviposition or colonization by flies and beetles in the field. Overall, these ant species were out competing Diptera and Coleoptera in the ecology of carrion decomposition and therefore, it is important for forensic entomologists to recognize the effects of ant colonization in the determination of mPMI.

### **Materials and methods**

The current study was conducted at the vicinity of the Sungai Buloh campus, Universiti Teknologi MARA, Malaysia (3°13' 21" N 101°35'33" E, 56 m above sea level). A rat (Rattus norvegicus) carcass was procured from the Laboratory Animal Care Unit (LACU) and subsequently placed on the soil surface surrounded by vegetation from 20 February to 2 March 2019. The rat carcass was weight approximate 200 g. The study site was dominated by buffalo grass (Paspalum conjugatum) and tropical carpet grass (Axonopus compressus). Tropical woody bamboos (Bambuseae) were also common at the site. The rat carcass was protected by an anti-scavenging cage to prevent scavenging animals, such as stray dogs or monitor lizards (Varanus sp.). The cage measured 90 cm (length)  $\times$  90 cm (width)  $\times$  45 cm (height). The cage was bottomless, which allowed the carcass to be directly in contact with the ground. During the study period, the average temperature of the study site was  $32 \pm 1$  C and humidity was  $54 \pm 1\%$ , which was recorded on an electronic data logger (Elitech 32,000, UK). The average rainfall throughout the experiment period was 3 mm (data retrieved from www. worldweatheronline.com accessed on 19 June 2019). Daily observations were conducted on the rat carcass between 10: 00to 11:00 h until the carcass reached the dry-remains stage on 2 March 2019. Ants that were visible on the surface and underneath the rat carcass were collected by a spatula, which measured about 3 cm (width) × 10 cm (length). Representative samples of ants were taken from different parts of the rat carcass and then preserved in glass vials, which contained 90% ethanol, for further species identification. Ants were identified using Bolton (2019) and keys provided by Hashimoto (2003). Specimens were observed under a stereomicroscope (Olympus SZ61, Japan) with attached digital camera (Olympus DP22, Japan).



The current study documented the presence of four species of ants during the decomposition of a rat carcass in Malaysia: Carebara diversa (Jerdon1851), Anoplolepis gracilipes (Smith1857), Brachyponera luteipes (Karavaiev1925) and Monomorium floricola (Jerdon1851). In this study, C. diversa workers and soldiers were presence in all stages of carcass decomposition (i.e., fresh, bloated, active decay, advanced decay, and dry-remains stage) from Day 0. Brachyponera luteipes appeared on Day 2 (bloated stage), followed by A. gracilipes on Day 4 (active decay), and M. floricola on Day 6 (advanced decay). Brachyponera luteipes was more abundant than the two ant species that appeared later at the carcass. Overall, among the four ant species, workers of C. diversa were the most dominate on the carcass, while there were only two or three C. diversa soldiers. From the first day of the introduction of the rat carcass, C. diversa workers covered the entire carcass with soil particles. Consequently, oviposition or colonization of flies or beetles were not observed on or around the carcass (Fig. 1), and therefore, fly or beetle larvae were not seen on or around the carcass. When portions of the soil were removed from the carcass with the use of a spatula, we observed flies on the carcass, but they did not lay any eggs. Then by the following day, the ants had the entire carcass covered again with soil particles. During this study, we also observed ants feeding on the blood and tissues of the rat carcass.

In this study, *C. diversa* was the most dominant species observed with the rat carcass, in which most of the individuals were workers. *Carebara diversa* appears to be associated with the various stages of decomposition of carrion from the fresh stage to the skeletal stage. The presence of ants during stages of decomposition indicates their ability to use carrion in different ways (Eubanks et al. 2019). Previous literature indicates workers of *C. diversa* are capable of cutting tissue from carrion with their mandibles (Cornaby 1974; Fonseca et al. 2015). Such behaviour can affect the estimation of the minimum post-mortem interval (mPMI) by forensic entomologists as these ant activities may either reduce the chance or delay fly oviposition on corpses, which could lead to the miscalculation of the mPMI (e.g., underestimate of mPMI) and result in the miscarriage of justice.

Ants have been shown to negatively impact the occurrence and development of forensically significant flies on corpses (Campobasso et al. 2009), which was observed in this study. In addition, we did not observe any beetles on the carcass during decomposition. The presence of ants should be taken into consideration for every case involving mPMI estimation (Fig. 2).

The effects of ants on carrion depend on several factors including the species, species abundance, and the type of habitat. In Malaysia, Heo et al. (2009) observed several ant



Fig. 1 Carebara diversa "buried" the rat carcass during the decomposition process. a Rat carcass was placed on the soil and protected with an anti-scavenging cage. b, c Ants covered the rat carcass with soil particles. d Skeletal stage of the rat carcass



species predating on eggs, larvae, pupae and adults of blow flies on pig carcasses. Chen et al. (2014) observed the occurrence of ants on monkey carcasses. However, they indicated that ants were not a useful indicator for mPMI estimation. In that study, ants were present during all stages of decomposition of the monkey carcasses. Similarly, our current study



Fig. 2 Views of C. diversa. a, b Frontal and lateral view of a soldier. c, d Frontal and lateral view of a worker. Scale bar = 500 µm

demonstrated that *C. diversa* was found throughout all the stages of decomposition and eventually, *B.luteipes*, *A. gracilipes* and *M. floricola* were also present on the carcass.

Goff and Win (1997) suggested that the combined use of forensically important flies and ants should be used to estimate the mPMI. In their study, both ants and flies were used to estimate the mPMI of human remains, which were discovered in a metal box. Ants were used to determine the mPMI by determining the time a new colony starts producing reproductive castes. Preliminary findings also indicate that ants may serve as an indicator of geographic locality, in which this information could aid crime scene investigations in the future. We suggest more studies should be conducted in different ecoregions to map the distribution of ants in order for ants to serve as locality indicators in forensic investigations.

### Conclusion

Four ant species were collected from a rat carcass: *C. diversa*, *B.luteipes*, *A. gracilipes* and *M. floricola*. *Carebara diversa* demonstrated the "burying" behaviour on the rat carcass by covering it with soil particles, which subsequently prevented flies and beetles from colonizing the carcass. Therefore, forensic entomologists should be aware about the effects of ants on corpses and therefore, entomological methods should be used carefully when determining the mPMI from corpses infested with ants.

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# Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical consideration** We procured a dead rat from the laboratory animal care unit of Universiti Teknologi MARA for this experiment. Hence, ethical consideration was not required.

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