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SHORT NOTE

Fortress with Sticky Moats: the Functional Role of Small Particles around *Tetragonisca angustula* Latreille (Apidae: Hymenoptera) Nest Entrance

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Abstract

Many bee species are able to defend themselves against pollen or honey thievery. We herein report the functional role of small sticky particles deposited by *Tetragonisca angustula* Latreille in its nest entrance external side. This strategy was very effective to prevent ants from invading the bees' nest. We reported many dead ants attached to the nest entrance and different ant species easily immobilized after being moved onto the entrance tube containing sticky particles. This is the first description of the functional role played by the nest entrance sticky particles under natural conditions.

Species are usually defined by the physical attributes of their individuals, even though none of them is able to exist without the interactions they establish with other organisms and the environment they live in (Queiroz et al., 2007). These relationships may enlarge the species range or increase its persistence probability over time, as it happens in mutualisms, or the opposite in antagonistic interactions (Stanton-Geddes & Anderson, 2011). Bees and flowers have a longstanding mutualistic relationship established over the evolutionary time; however, by storing pollen and honey provisions, bees create attractive scenarios to antagonist thieves. Other bee species as well as other groups such as ants or vertebrates (Roubik, 1989; Breed et al., 2012) are among the bees antagonists. Some bee species may use their poisoning sting to protect the stored resources and the brood (Roubik, 1989). Moreover, bees from the Meliponini tribe do not have stings. Therefore, they use other defensive strategies to protect themselves against intruders (Rech et al., 2013 and references therein).

Ants are considered the greatest predators in the tropics (Hölldobler & Wilson, 1990) and a threat to bee keeping in this region (Lehmberg et al., 2008; Rech et al., 2013). Some bee species are protected against ant predation by the chemical repellence on their bodies surface (Lehmberg et al., 2008). Moreover, it was already shown that, under lab conditions, *Trigona* bees are able to immobilize attacking ants by gluing small sticky particles (supposedly resin) on them (Roubik, 2006; Grüteret al., 2011). The same small particles are found on the external side of stingless bees' nest entrances. However, their functional role under natural conditions is still unknown (Wittmann, 1985; Roubik, 2006). "Jatay" bees (*Tetragonisca angustula* Latreille, 1811) are known for showing efficient defensive behaviour against flying intruders (Wittmann, 1985). There are soldiers among *T. angustula* that protect the colonies either by flying in front of the nest entrance or by controlling the entrance standing inside tube (Kärcher & Ratnieks, 2009; Grüteret al., 2011).

These soldier bees show particular behaviour, morphology and longevity, thus differing from the other workers (Grüter et al., 2012). The present study increases the set of nest defence strategies known in *T. angustula* by describing, under natural conditions, the functional role played by the small sticky particles deposited on the external side of the nest entrance.

Our observations were performed at a private residential area in São Bartolomeu, a newly urbanized area from Brasília (Brazilian Capital). The local vegetation is a typical Neotropical savannah (Cerrado) from the Central Brazil with the predominance of the locally called Cerradão physiognomy (15˚50'47.70" S; 47˚47'09.10W). The defensive strategy herein described was first observed on August 8th 2014 and recorded in pictures. The observed *T. angustula* colony was located inside a *Qualea grandiflora* Mart. (Vochysiaceae) tree trunk, 20cm above the ground level (Fig 1A). The first ants found stuck to the *T. angustula* nest entrance were *Cephalotes clypeatus* (Fabricius, 1804) dead workers covered by thin layers of the same sticky material used to build the nest entrance (Fig 1B).

Fig 1. Functional role of sticky particles deposited on the external side of *Tetragonisca angustula* nest entrance. A. Entrance tube as it was found by the authors in August 2014, check on the dead ants covered by thin layers of the same material used in the nest entrance, B. close view of *Cephalotes clypeatus* stuck in the nest entrance found in the field, C. Ants (*C. clypeatus*) being experimentally moved to the nest entrance tube for monitoring purpose, D. Fighting behaviour between *T. angustula* and *Atta sexdens* experimentally moved to nearby the nest entrance and the resulting dead ant with a bee attached to its antenna, E. Non-fighting behaviour related to a large ant (*Odontomachus bauri*) partially immobilized by the entrance tube sticky particles.

Two hypotheses were formulated to explain the ants' death: 1. the bees have killed the ants while they were partially immobilized by the sticky particles, or 2. the ants slowly died due to complete immobilization. On March 5th 2015,aiming at investigating which of these hypothesis was the most plausible, we collected *C. clypeatus* (the most frequent species found attached to the nest entrance) healthy workers from the same tree the bee nest was located in and manually moved them to the nest entrance (Fig 1C). We then monitored and recorded in video what happened next. Subsequently, in order to check if the bee response was constant to any species of ant, we manually glued workers of *Odontomachus bauri* Emery, 1892 and *Atta sexdens* (Linnaeus, 1758) to the nest entrance observing and describing what happened.

Behaviour observation in the field revealed that bees completely ignored the living ants artificially stuck in the entrance tube. This behaviour differs from that of honeybees, which are able to recognize ants' odour and promote a ritualized defence (Spangler & Taber, 1970). However, it was clear that the ants were completely immobilized by the sticky particles (Fig 1D). A similar nest entrance of *T. angustula* with dead individuals of *Camponotus* sp. (Formicidae) was also seen in Ribeirão Preto - São Paulo (Barbara Rodrigues, PhD student at USP personal communication), indicating that this behaviour may be more widespread than previously thought.

Moreover, we used *Odontomachus bauri* Emery, 1892 ant workers to check the bee strategy efficiency against larger ants and they were also completely immobilized by the sticky particles (Fig 1E). Similar trials using *Atta sexdens* workers (Linnaeus, 1758) showed that besides the fact that these ants were stuck in the nest entrances, they were actively attacked by bees (Fig 1F). It suggests that the bees' defensive behaviour may vary according to the ants' identity or traits (*e.g.* size or chemical components of the ant's cuticle) (Spangler & Taber, 1970).

Further research should be performed both on the chemical composition of the sticky particles (to clarify what determines, if there is any, toxic effect on ants) and on the variation in the entrances features according to local conditions (e.g. threat levels posed by local ants). The current study described the defensive role played by the sticky particles in the nest entrances of stingless bees, thus confirming the hypothesis assumed by previous studies. The use of sticky particles by *Tetragonisca angustula* workers is an efficient strategy to avoid nest invasion by walking intruders and increase the array of behavioural defensive strategies known in this species and also in social bees.

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