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Natural History Note

Preemptive Defensive Self-Sacrifice by Ant Workers

Adam Tofilski,[1,*] Margaret J. Couvillon,[2,†] Sophie E. F. Evison,[3,‡] Heikki Helanterä,[4,5,§] Elva J. H. Robinson,[6,‖] and Francis L. W. Ratnieks[5,#]

1. Department of Pomology and Apiculture, Agricultural University, 29 Listopada 54, 31-425 Krakow, Poland;
2. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721;
3. Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom;
4. Department of Biological and Environmental Sciences, University of Helsinki, Helsinki FI-00014, Finland;
5. Laboratory of Apiculture and Social Insects, Department of Biological and Environmental Science, University of Sussex, Brighton BN1 9QG, United Kingdom;
6. School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, United Kingdom

ABSTRACT: Worker insects altruistically sacrifice their own reproduction to rear nondescendant kin. This sacrifice reaches its most spectacular level in suicidal colony defense. Suicidal defense, such as when the sting of a honeybee worker embeds in a predator and then breaks off, is normally a facultative response. Here we describe the first example of preemptive self-sacrifice in nest defense. In the Brazilian ant *Forelius pusillus*, the nest entrance is closed at sunset. One to eight workers finish the job from the outside and, in doing so, sacrifice their lives.

Keywords: social insects, colony defense, self-sacrifice, *Forelius pusillus*.

* Corresponding author; e-mail: rotofils@cyf-kr.edu.pl.

† E-mail: mjcouv@email.arizona.edu.

‡ E-mail: bop05see@sheffield.ac.uk.

§ E-mail: heikki.helantera@helsinki.fi.

‖ E-mail: elva.robinson@bristol.ac.uk.

E-mail: f.ratnieks@sussex.ac.uk.

Worker insects normally have little direct reproduction and instead work to rear nondescendant kin (Hamilton 1964; Bourke and Franks 1995). One important worker task is colony defense. In some bees, ants, and wasps, the worker sting has backward-pointing barbs that lodge in an attacker's flesh. The sting then detaches ("sting autotomy") from the worker, who dies (Hermann 1984). Similarly, in some ants and termites, the defending worker's abdomen ruptures to release a sticky fluid that entangles the attacker (Wilson 1971; Maschwitz and Maschwitz 1974). These examples of suicidal defense are facultative because they are deployed only when the nest is under attack. Here we describe a novel form of self-sacrificial nest defense by workers in the Brazilian ant *Forelius pusillus* that is used preemptively to close the nest entrance every evening.

At our field site near São Simão, São Paulo state, Brazil, *F. pusillus* nest entrances were very active during daytime on hot days in January and February, with more than 100 workers per minute (mean ± SD = 136 ± 61, $n = 16$ nests) departing the underground nest via the circular entrance hole (mean diameter ± SD = 3.0 ± 0.4 mm, $n = 16$). These workers (∼2 mm in body length) were mostly transporting excavated soil, which they deposited around the nest entrance to form a flat, elliptical pile (35 ± 9.3 cm × 28 ± 7.7 cm, $n = 16$; fig. 1A). Before sunset, activity ceased and the entrance was closed (fig. 2). The first sign of closing was a gradual reduction of activity. When activity had almost ceased, a few workers remained outside, where they dragged or carried sand and soil particles from the pile toward the entrance. Particles were placed in the entrance tunnel a few millimeters below ground level (fig. 1C, 1D). Initially, workers were able to move in and out of the nest, but 24 ± 22 min (mean ± SD) after closure began, the tunnel became impassable (fig. 1E). This occurred at 1915 ± 0045 hours (mean ± SD; fig. 2); sunset was at 1959 hours.

Most workers involved in entrance closing reentered the nest. However, every time we observed an entrance closure, one to eight workers were trapped outside (fig. 3). These were not late-returning foragers, as reported in *Rossomyrmex* ants (Marikovsky 1974). Entrance closure was
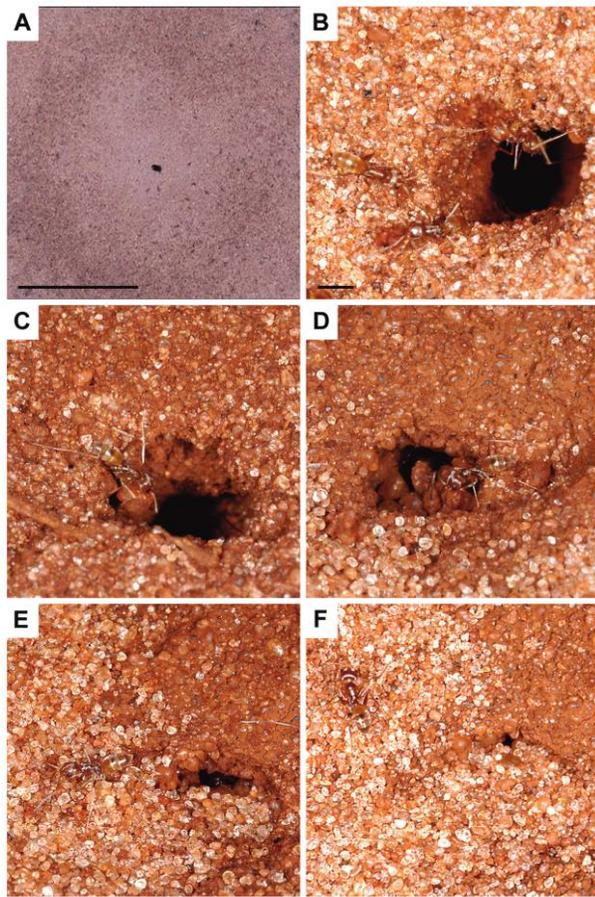
Figure 1: Nest entrances and entrance closing in *Forelius pusillus*. *A*, Typical entrance, showing a flat, circular pile (piles can also be elliptical or kidney shaped) of excavated soil and sand that surrounds the nest entrance and also minimal dumping close to the entrance (scale bar = 10 cm). *B*, Close-up of entrance during daytime activity, showing two workers transporting soil particles out of the nest and one worker returning to the nest (scale bar = 1 mm). *C*, Early stage of entrance closing. *D*, Entrance almost completely closed with large soil and sand particles. *E*, Worker kicking fine sand toward entrance (video of this activity is available in the online edition of the *American Naturalist*). *F*, Worker still kicking sand into an entrance that is almost completely covered with fine sand (video of this activity is available in the online edition). *C–F* are on the same scale as *B*.

the only activity at that time, and there were no ants returning from foraging or soil-dumping trips.

Two lines of evidence indicate that the ants trapped outside were not accidental victims but rather were part of a deliberate strategy of entrance closing. First, the numbers trapped outside differed significantly from a random (Poisson) expectation, with the zero category significantly underrepresented (fig. 3). Second, the ants left outside remained near the entrance for up to 50 min and continued to perform deliberate nest-closing behaviors, including one additional behavior, kicking, rarely seen earlier. During kicking (fig. 1*E*, 1*F*; video of this activity is available in the online edition of the *American Naturalist*), the kicker faced away from the entrance and made rapid leg movements that propelled fine sand toward the entrance. The proportion of total time spent kicking increased from 32% when the entrance first became impassable to 91% 15 min later. Kicking appeared to complete entrance closing. When the entrance first became impassable, the upper part of the entrance tunnel was still clearly visible because the particles blocking the tunnel were below ground level (fig. 1*D*). However, when the ants trapped outside finished kicking, the entrance tunnel was covered with fine sand and was indistinguishable from the surroundings (fig. 1*F*; video of entrance closure is available in the online edition of the *American Naturalist*).

What happened to the ants that were trapped outside, and did they survive the night? After nest closure, these ants eventually left the entrance area, sometimes after being blown away by a gust of wind (video of one such occurrence is available in the online edition of the *American Naturalist*) or a nonfatal encounter with ants of other species but usually by abruptly walking away. The next morning, no *F. pusillus* worker ants were ever seen within 3 m of the entrance location when the entrances were reopened (at 0958 ± 0043 hours [mean ± SD]; fig. 2). Opening always took place from the inside to form a conical depression (video of nest opening is available in the online edition of the *American Naturalist*). Ants from inside the nest immediately exited and began kicking sand away from the nest entrance, presumably to prevent it from rolling into the tunnel.
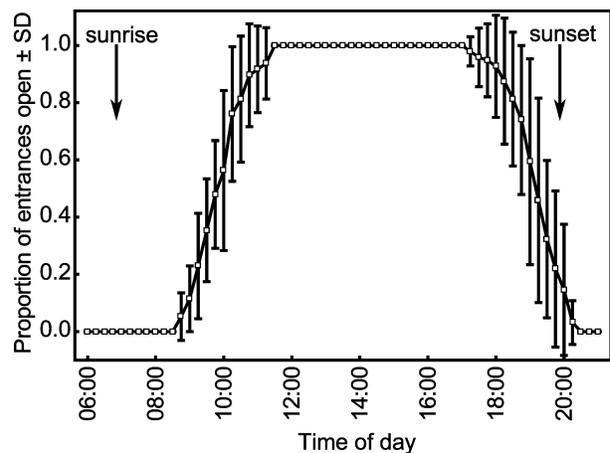

Figure 2: Time at which entrances were opened in the morning and became impassable in the evening. Data from 16 colonies on three days in January 2004 and 16 colonies on three days in February 2005.

The small size of *F. pusillus* workers made it impossible to mark the workers trapped outside with paint dots to recover them the next day, either alive or dead, and so determine whether they had survived being trapped outside. Therefore, we carried out an experiment. In 2006 and 2007, we first modified the entrances of half our study colonies by placing a flat piece of plywood, 7 cm × 7 cm × 5 mm with a 3-mm hole in the center, over each entrance hole. Several days later, many of the colonies were using the hole in the wood as their entrance and had covered the wood in a layer of excavated sand and soil. In the evening, when the ants trapped outside had blocked the entrance tunnel, we carefully lifted the piece of wood plus one or more ants that were closing the entrance into a plastic bowl containing sand from that colony's nest entrance area. This manipulation was unnoticed by the workers, who continued entrance-closing behavior, dragging and kicking sand and soil into the hole in the piece of wood. Of 23 ants transferred in this way, only six (26%) were alive in the bowl the next morning. The bodies of 10 (43%) were found by carefully searching the bowl. The remaining seven could not be found but were presumably dead because nonmoving live ants started to run when the sand or wood near them was moved and so were easily seen. (Ants could not escape the bowls, as each had been covered in a layer of plastic film after the ants were placed inside.) The results of this experiment indicate that a large proportion of the ants left outside had already died by ∼0900 hours the next day, approximately 1 h before the entrances reopened naturally. Combined with the observation that no ants were seen at the nest entrances when these reopened in the morning, this provides strong evidence that most or even all of the ants that closed the entrance from the outside died as a result.

Nest entrance closure occurs widely in social insects (e.g., ants, stingless bees; Roubik 1983; Hölldobler and Wilson 1990), usually when intruders attempt to enter (Hölldobler 1981; Ruano and Tinaut 1999) or in the evening (Couvillon et al. 2008). In some species of ants (Wilson 1974) and termites (Matsuura 2002), large workers with modified heads use their bodies to block the nest entrance during colony defense. *Forelius pusillus* differs from these examples in that the entrance is closed from the outside, not from the inside (Roubik 1989), so that closure results in workers being left outside and sacrificing their lives. In other cases of self-sacrifice by workers, such as with detachable stings or exploding abdomens, the sacrifice is directed at actual intruders. *Forelius pusillus* is unique in that the sacrifice is routine and preemptive. Why do *F. pusillus* colonies go to the additional trouble and cost of sacrificing workers to seal the entrance from the outside? Our research did not address this question, but we hypothesize that closing the entrance and making it level and indistinguishable from the sur-
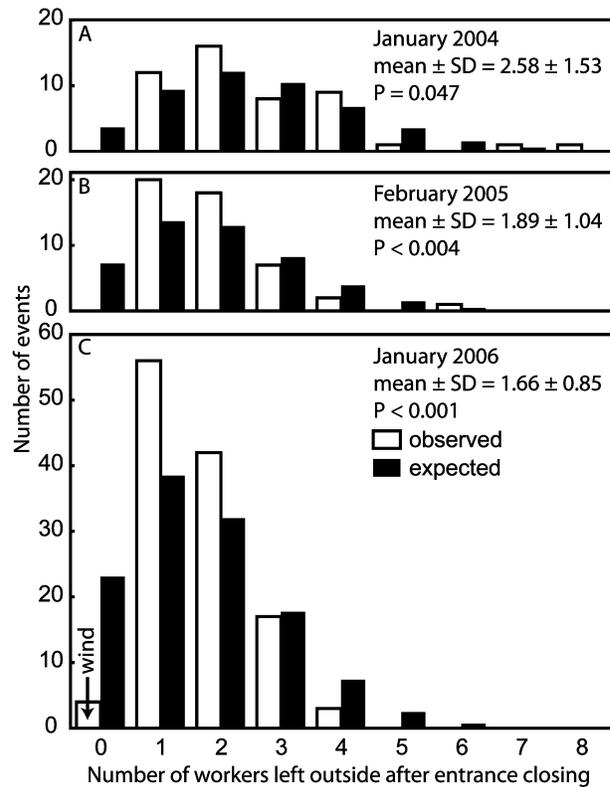

Figure 3: Observed distributions of the numbers of workers trapped outside in January 2004 (*A*, 16 nests on three days), February 2005 (*B*, 16 nests on three days), and January 2006 (*C*, 18 nests on seven days), compared to Poisson (random) distributions. The zero category is significantly underrepresented in comparison to expected values (χ^2 goodness-of-fit test). No ants were seen on only two days in 2006, and on both days strong winds were blowing ants away from the entrance during closing.

rounding area makes it less detectable by some as yet unknown parasite or predator. Nest entrance closing may be especially important for ants living in open habitats, such as *F. pusillus*, because it has been reported from other species with this nest location, including *Cataglyphis*, *Messor*, and *Pogonomyrmex* (Hölldobler and Wilson 1990). The high levels of worker activity we observed during soil dumping show that *F. pusillus* colonies are large, and in other *Forelius* species colony sizes of up to 100,000 workers have been reported (Valone and Kaspari 2005). A few workers sacrificed per day in a large colony would be only a minute proportion of the workforce and is presumably a small cost to pay for the benefit of improved defense of the whole colony (Sherman et al. 1991; Anderson and McShea 2001).

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APPENDIX

Material and Methods

We studied *Forelius pusillus* nests located in a sandy area with almost no vegetation at the edge of a sugar cane field at Fazenda Aretuzina, São Simão, state of São Paulo, Brazil. The distance between neighboring *F. pusillus* colony entrances was 4.7 ± 4.6 m (mean $\pm$ SD, $n = 16$; 2004 data). We observed 16–18 colonies during January and February of three consecutive years (2004–2006). On three days in 2004, starting at 1700 hours, each colony's entrance was video-recorded in turn for 30 s every 15 min until activity ceased. In addition, nest closing and opening were recorded continuously in two colonies. In 2005 on three days and in 2006 on seven days, nest entrances were inspected in the evening every 15 min until nest-closing activity ceased. In 2004 and 2005, the nests were also ob-

served on three days in the morning to determine the time of nest opening. In 2006 and 2007, a piece of plywood with a central hole was placed over the nest entrance so that a sand pile was built on it. In the evening, after the entrance had become impassable but was not fully closed, the piece of wood was transferred, together with the sand pile and the ants trapped outside, to a plastic bowl from which the ants could not escape (fig. A1). The next morning, before the time of nest entrance opening, the death or survival of these ants was determined. In 2006, 25 ants were observed continuously after the completion of nest closure in order to determine their behavior. The ants trapped outside walked actively in the vicinity of the entrance, but they were also seen walking away from their own entrance area. Most of the ants (56%) rapidly moved away from the nest entrance; others wandered off gradually (20%), ran away after being attacked by other species of ants (8%), or were blown away by gusts of wind (8%). A small proportion (8%) was still present at the nest entrance when the observation terminated because of darkness. All the observed workers remained in the entrance area for at least 15 min after it become impassable, but 30 min later only 86% of them were still present within the outer perimeter of the sand pile (fig. A2).

Figure A1: Experimental setup for studying the ability of entrance-closing ants to survive the night outside their nest.
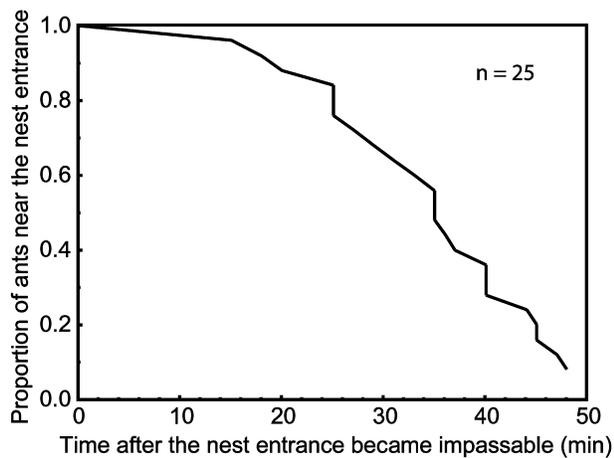
Figure A2: Number of workers within the outer perimeter of the sand pile after the entrance became impassable.

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