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Supplementary Material

***Polyrhachis femorata* (Hymenoptera: Formicidae) habitat and colony defensive immobility strategy**

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SUPPLEMENTARY MATERIAL

Text S1

Choice of terminology for defensive behaviours

Many different terms are applied to defensive immobility behaviours, and views differ among researchers. Sakai (2021) argued that defensive immobility behaviours included freezing (“a state of attentive immobility to avoid detection and to enhance perception” and tonic immobility (“a state of a kind of paralysis caused by profound motor inhibition”). The later definition is different from that of Humphreys and Duxton (2018). The definitions do not make specific assumptions about the particularities of postures, but Chelini *et al.* (2009) suggested that “freezing” was not associated with a stereotype posture for a spider. For the sake of brevity in this paper, we chose the term death feigning to encompass three behaviours that we did not quantify: 1) freezing, where ants stopped moving, crouching slightly; 2) curled up, where ants brought their gaster under their mesosoma, and kept their head down without rolling over (death feigning based on Langen *et al.* 2000, Mbenoun Masse *et al.* 2011; Katayama 2013 and Peters 2021 when accompanied with rolling over); and 3) death feigning involving what we interpreted as a deceptive change in posture (ant on its side or with somewhat contorted body, appearing truly dead to us), without reduced ability to react. We cannot exclude that all three defensive behaviours are submissive or designed to avoid detection, and tonic immobility *sensu* Humphreys and Ruxton (2018) is possibly a suitable term, but our initial interpretation upon opening the first *Polyrhachis femorata* box was that they were dead. The curled-up behaviour could also make handling more difficult for a predator or aggressor (e.g., “death-feigning” behaviour of grasshopper for frog in Honma *et al.* 2006), particularly since *P. femorata* has a spiny petiole.

Text S2

Additional information on set-up and check of nest boxes

The pygmy-possum nest boxes on the Dudley West property were arranged in 12 lines of 6 (i.e., 72). Each box was tied with rope to the tree or tall shrub (e.g., *Melaleuca* spp.) closest to the pre-determined GPS point at chest height. The boxes were separated by 50 m and lines by 100 m. All pygmy-possum cavities on the other properties had one ~ 20-mm entrance, and differed in size, material, and wood thickness. We checked 231 pygmy-possum boxes for 288 cavities in burnt and 181 for 266 cavities in unburnt.

Bat boxes were placed higher on trees with the use of a ladder and checked with a combination of an inspection camera and direct opening. Although bat boxes are more difficult to check than are pygmy-possum boxes, we would certainly have seen at least some *P. femorata* nests if they occurred in bat boxes, but these boxes have larger openings that would be very difficult for the ants to plug.

Text S3

Details of nest box visits at Dudley West from 28 January to 17 June 2022

In the text of our paper, we describe the discovery of the first *Polyrhachis femorata* in Box 22. Box 23, 50 m away, had four individuals of the same species. They did not feign death when the box was opened and inspected. In Box 44, 9 individuals feigned death, and 50 m away from the latter, in Box 45, the single individual feigned death, indicating that whether in groups, nests, or as individuals, *P. femorata* feigns death when facing a potential predator. Two more nests were discovered in Boxes 61 and 68. They behaved in a similar way as the first (in Box 22) did (video B, Petit and Stonor 2022), although in Box 68 several individuals kept moving after one minute, eventually joined by about half of the colony in a three-minute observation. They all feigned death again one minute later (video C, Petit and Stonor 2022).

On the 29th of January, we re-visited one box that had contained a western pygmy-possum (*Cercartetus concinnus*). The pygmy-possum was gone, but two individual *P. femorata* that were exploring the box feigned death when we opened it. We also re-visited Box 22. Fewer

ants occupied the box including one alate individual and some larvae. The colony feigned death as it had done the day before when we opened the lid, some taking ~ 40 s to achieve stillness. A week later, the nests in Boxes 61 and 68 feigned death, the one in Box 68 taking ~ 4 min to be still.

The box check of 14 April 2022 showed that 6 boxes contained *P. femorata* (Table 1). The nest of Box 22 was gone (one individual was in the box), but the nests of Boxes 61 and 68, which behaved similarly as they had previously, remained. The entrances to three boxes had been plugged with organic matter, and every crack of Box 68 was plugged (Table 1). The two nests (Boxes 61 and 68) were revisited 24 April 2022 after dark (20:00 to 21:00). The entrance to Box 61 had a plug with an opening and 8 ants remained in the box, without eggs, larvae, or pupae. One feigned death; after moving for several seconds, the others froze, but did not contract their body shape except for lowering the tip of their abdomen. The plug for Box 68 was intact and most of the ~ 48 ants feigned death after several seconds of movement; some froze, with their abdomen lowered for some at least. Three kept carrying larvae around the box, arousing a few others. On the 27th of April, Box 61 was empty and in Box 68, now devoid of eggs/larvae/pupae, some of the ~ 37 ants feigned death, some froze, and most moved over 73 s of observation. Similar observations took place 17 June, but one box with *P. femorata* contained water, one had a juvenile huntsman spider, and one contained the rotting *E. diversifolia* leaf nest of a pygmy-possum (Table 1). All boxes containing *P. femorata* did not contain other animals or materials until July (Table 1). The most common of the three defensive behaviours was curling up, sometimes with a bend, and with legs and antennae in different positions (e.g., video A, Petit and Stonor 2022).

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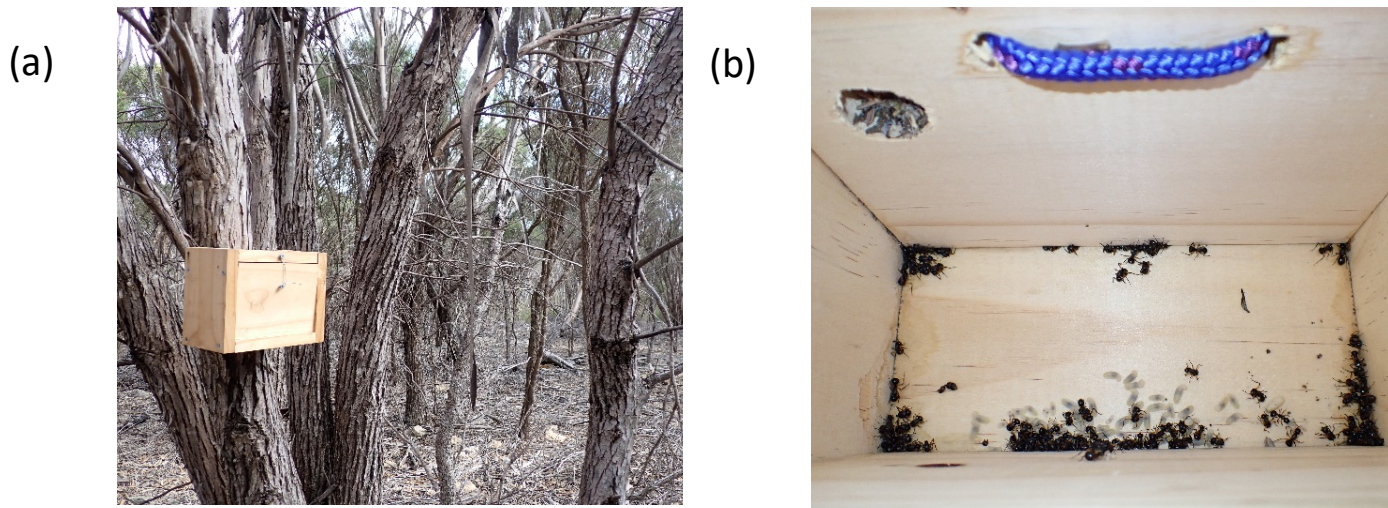


Fig. S1 (a) Pygmy-possum nest box on *Eucalyptus cneorifolia* in Narrow-Leaf Mallee Woodland; (b) nest of *Polyrhachis femorata* feigning death on Kangaroo Island, South Australia