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Report

Extraction of Super Colonies of Crazy Ants from Soil and Wood

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Introduction

Super colonies are formed by ant species that have no distinction between high density, multiple-queen colonies and may occupy large geographic areas (Holway et al. 2002). These colonies usually have an exchange of workers and lack of aggression between neighboring colonies (Holway et al. 2002). *Nylanderia* nr. sp. *pubens* and *N.* nr. sp. *fulva* are invasive crazy ant species that form super colonies in their invaded habitat. *N.* nr. sp. *pubens* has been recently reported in Mississippi (MacGown and Layton 2010) and Texas, where it has become a serious invasive pest (Meyers 2008). Since its initial detection in 2002, *N.* nr. sp. *pubens* now occurs in >14 counties in Texas (Drees et al. 2009, Meyers 2008). *Nylandaria* nr. sp. *fulva* (as indentified by J. Trager) was first reported in Louisiana in 2010 (Hooper-Bùi et al. 2010). The species most similar to it, *Nylandaria fulva* [*Paratrechina (Nylanderia) fulva*] originates in Brazil (Zenner-Polania 1990). *N.* nr. sp. *fulva's* population in Louisiana has grown at a rapid rate and may become just as problematic as the crazy ant. Although the Texas and Mississippi populations of crazy ants are being referred to by different names, the problem of ants that form super colonies is the same.

Nylanderia nr. sp. *fulva* are reddish-brown ants seen in disorganized foraging trails that enter houses and trees. The legs and antennae are usually longer than the body, which is characterized by coarse hairs. They have a one-segmented petiole and twelve-segmented antennae. They do not possess a sting but can spray formic acid. These ants are omnivorous and feed on items such as dead insects and honeydew (Hooper-Bùi et al. 2010). Crazy ant colonies contain numerous queens, are unicolonial, and are not known to excavate soil extensively. Little is known about their natural history; however, early evidence suggests that they can be compared to the Argentine ant (*Linepithema humile*), yellow crazy ant (*Anoplolepsis gracillipes*), and the European garden ant (*Lasius neglectus*), which LaPolla et al. (2010) used as an outgroup for taxonomic studies in determining generic changes to crazy ants.

Little is known about *N.* nr. sp. *fulva* and research is necessary to learn as much as possible about this pest species. There are established techniques for removing fire ants and Argentine ants from the soil, but no techniques for separating crazy ants for laboratory studies. Our objective was to determine a fast and easy way to extract these ants from their substrate.

Methods

We extracted colonies of *N.* nr. sp. *fulva* from soil and debris by modifying flooding techniques used in the extraction of *Solenopsis invicta* from soil (Banks et al. 1981). A large flood chamber was constructed and carried into the field to hold collected crazy ants. The flood chamber consisted of a 55-liter storage container with friction-fitting lid. Before field excursions, the top 7–10 cm of the vertical sides of the

container was painted with Teflon (Dupont, Wilmington, DE) to prevent *N.* nr. sp. *fulva* escape (Fig. 1). Once in the field, debris and soil—such as rotted tree limbs and the top 5-8 cm of soil—containing *N.* nr. sp. *fulva* were collected and placed in the container and sealed for transport back to the lab. The lid was removed and pre-cut wire mesh (6 × 6 mm mesh size) was placed horizontally inside the container so that it fit snuggly against the sides (Fig. 1). The mesh prevented wood and grass from providing a floating safe haven for *N.* nr. sp. *fulva* during flooding. If the Teflon coating was damaged during placement the wire mesh, more Teflon was applied and allowed to dry completely before continuing. Ant harborages or "condos," constructed of covered 118 mL polypropylene cups (4.3 × 7.62 cm) with 1.5 cm of Hydro-Stone (Gypsum Cement, Chicago, IL) covering the floor of the cups were placed on top of the wire mesh. (Fig. 1) Holes were cut near the bottoms of the condos so that the ants could enter (Fig 2). The friction-fitting lids of the condos were perforated to allow air exchange.

Figure 1. Flooding chamber containing debris in which *N*. nr. sp. *fulva* reside. Ant harborage was placed on top of wire mesh to allow the ants to escape the rising water.

Figure 2. *N*. nr. sp. *fulva* workers salvage brood and transfer queens to the ant harborage.

Once the chamber was prepared and the condos were in place, the flooding commenced with the introduction of 500 mL of water. After allowing 30 minutes for *N.* nr. sp. *fulva* to acclimate and move out of the rising water, 1 liter of water was added to the flood chamber every thirty minutes until the debris was inundated. The ants evacuated the debris they had previously inhabited and moved into the condos placed on the mesh in the flooding chamber above the water line. As each condo filled with ants, it was removed and placed in a dry container (arena) with Teflon and a friction-fitting lid that served as the rearing chamber. Empty, replacement condos were placed on the mesh above the water line (Fig. 1). The process was repeated until all ants were collected from the flooding chamber and housed in arenas. Any remaining ants in the flooding chamber were killed using liquid soap to prevent the spread of *N.* nr. sp. *fulva*.

Results and Discussion

In Louisiana, *N.* nr. sp. *fulva* were found nesting and foraging in the first few centimeters of the soil. Their colonies were observed in rotted wood, loose soil, under leaves and debris, and under loose bark. If trash was present, colonies could be found in and under aluminum cans and newspapers. Their extensive and numerous foraging trails (>3 cm wide) extended into large trees. At one location, *N.* nr. sp. *fulva* moved into the air conditioning unit of a home. The ants formed large foraging trails under the doors, through cracks at the edges of the windows and weep holes in brick exteriors to gain access to homes.

Queens, workers, and larvae of *N.* nr. sp. *fulva* and big-headed ants (*Pheidole* sp.) inhabited the wood debris and soil we excavated. Once in the laboratory, *N.* nr. sp. *fulva* were quick to react during flooding. As the water level rose, workers immediately started moving queens and brood to a higher level (not yet in the condo) within the substrate. Occasionally *Pheidole* sp. was found nesting alongside *N.* nr. sp. *fulva* within the same substrate, however *Pheidole* did not survive the flooding procedure. This indicates that our method separates *N.* nr. sp. *fulva* from other ants that share their nests. This is the first report of another ant living in the unicolonial nests of *N.* nr. sp. *fulva*.

After the water level reached about 4 cm in the container, *N.* nr. sp. *fulva* began to move into the condo. We observed that queens and brood were top priority, as the queens moved into the condos first, and then the workers moved the brood. Queens never outnumbered workers within the condo. Each flooding event was conducted with approximately 4–8 liters of soil, wood and other substrate, and most of the ants were removed from the substrate by using our technique. Every time the flooding process was conducted, >10 queens were collected per condo.

Ants fill the condos rapidly as the water rises. Workers first examined the condo before moving the queen and the brood. After about three hours, the queens and brood are moved into the container. *N.* nr. sp. *fulva* formed organized trails on the wire mesh from the substrate to the condo. Even though crazy ants don't often have organized trails, we observed them during the transport of their nest contents from the submerged substrate into the condos. However, if left overnight, *N.* nr. sp. *fulva* were observed to move the queen and the brood out of the condo and return to the unsubmerged substrate floating in the water. When condos contained a few queens and brood, they were moved out of the flooding chamber and into a dry arena. It was necessary to remove them quickly once inhabited; because if left unattended, the crazy ants would vacate the condos as described above. Once *N.* nr. sp. *fulva* were established in the arena, the ants were observed to care for the brood they had salvaged and keep the queens within the condo. The workers readily fed on dead crickets and 20% sugar water.

This passive method resulted in robust colony fragments of *N.* nr. sp. *fulva* that were then reared in the laboratory for aggression experiments. After the ants were removed from the water, very few dead ants were observed in the rearing containers, indicating that this method of ant collection preserves colony integrity.

Using similar methods to those described by Banks et al. (1981), we submerged *N.* nr. sp. *fulva*-infested substrate to remove workers, larvae, and queens for use in laboratory experiments. One difference we noted was that *N.* nr. sp. *fulva* did not appear to raft as *Solenopsis invicta* does in response to rising water. *N.* nr. sp. *fulva* trail out of the substrate to increasing higher ground before they come in contact with the water. Our method results in soil-free large colony fragments of all castes of *N.* nr. sp. *fulva* suitable for behavioral studies in the laboratory.

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