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Protocol

Collecting Live Ant Specimens (Colony Sampling)

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INTRODUCTION

Because of the great diversity of ants, it is difficult to give a single protocol for the collection of live specimens. Ant body size can be very small or extremely large; the ants can be hard or soft, sting or spray toxic chemicals, live in the open or in hard-to-reach places; and colony size can range from tens of individuals to millions. Thus, collection techniques must be tailored to each particular species. In particular, caution must always be taken when dealing with stinging species, and symptoms and basic first-aid measures, especially for the treatment of anaphylactic shock, should be reviewed before beginning fieldwork. Nonetheless, many species are collectable as whole colonies. This protocol reviews some basic techniques for collecting ground-nesting species and describes how to collect whole live colonies (with queens), which are necessary for long-term laboratory studies and addressing questions of social organization and ecology.

RELATED INFORMATION

The protocol presented here has proven very successful for the excavation of several species of harvester ant with very different nesting ecologies, in soils ranging from very sandy to complete clay. If the focal species has nests deeper than one-half meter and lives in very rocky soils, the use of mechanical assistance (e.g., a small backhoe) is recommended. Colony-collection techniques specific to other particular species are described in the literature (Tschinkel 1987, 1993, 1998, 2005; Seal and Tschinkel 2006).

For a more extensive discussion of the background, husbandry, and potential uses of ants as model organisms, see **Ants (Formicidae): Models for Social Complexity** (Smith et al. 2009).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

Fluon

Fluon is an aqueous solution of poly(tetrafluoroethylene) that can coat vertical surfaces and prevent climbing and hence escape of ants.

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Equipment

Aspirator

The collecting tube of the aspirator should be appropriate for the size of the ants to be collected. Some ants spray toxic chemicals (e.g., formic acid) that should not be inhaled. An aspiration bulb can be used in lieu of mouth suction, as can a modified vacuum cleaner.

Cotton balls

Forceps

Use soft (floppy) forceps to collect softer-bodied species.

Hand trowel

Knife or small cement spreader

Shovel

Choosing an appropriate shovel is important. If digging >30 cm, use a shorter-handled shovel with a wide blade. A sharpened blade, beveled on only the top side, increases digging accuracy (e.g., exposing chambers/tunnels).

Tarp

Test tubes

Vials or other collection containers (e.g., plastic boxes), equipped with lids

Wooden planks (optional; see Step 11)

METHOD

Preparation

1. Before going into the field, prepare containers to place the ants in once they have been collected:
 - i. Cover the sides of the containers with Fluon to prevent escapes.
 - ii. Place damp cotton balls in the containers.
This prevents desiccation of the ants after collection but before arrival at the laboratory.
2. Locate the nest entrance(s).
These will serve as a starting point for excavation (e.g., Fig. 1A,B).
3. Clear debris and obstacles (including plants) from near the nest entrance gently in order to maintain the integrity of upper nest chambers.
4. (Optional) Depending on the species, water the nests days or hours before collection.
This can decrease the collection of loose dirt when ants are aspirated and also draws ants toward the ground surface, including larvae/pupae.

Excavating the Top of the Nest

(See Fig. 1A.1-1A.6.)

5. Collect foragers leaving and entering the nest.
Often 10% of the worker population will be foraging. If a complete collection of the population is desired, it is necessary to patiently collect those individuals not physically in the nest. Do not collect foragers away from the nest; these could be from neighboring colonies.
6. Gently excavate the area centered on the nest entrance using a flat-bladed shovel to “peel” away layers of dirt.
In many species, numerous chambers are located just below the surface and can be distributed quite widely around the nest entrance. It is important to explore the surface around the nest fully, collecting ants from the top chambers as each chamber is exposed.
7. Carefully expose the vertical shafts that extend downward from the exposed chambers (Fig 1C,E).
Use a knife (or small cement spreader) so as not to destroy or plug the holes, as can occur when using a shovel or trowel. Blow loose dirt out of the way using the aspirator hose to help expose chamber edges and tunnels.

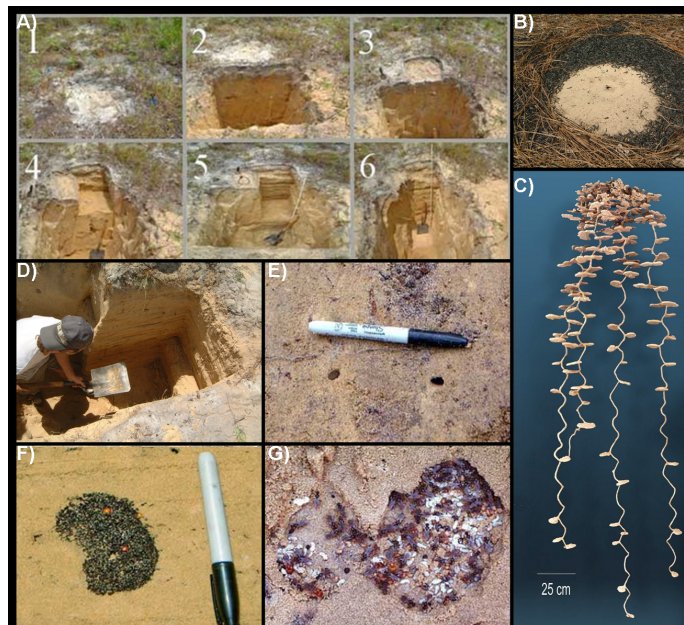


FIGURE 1. An illustrated guide to nest excavation and colony structure for the Florida harvester ant, *Pogonomyrmex badius*. (A, panels 1–6) Sequential stages of the excavation. (B) The nest mound. (C) Plaster cast of the nest's underground architecture. (D) An excavator in the pit works the horizontal surface of the excavation. (E) The openings of two vertical shafts extend downward from the horizontal working surface. (F) A seed chamber. Note the vertical shaft to the left of the chamber. (G) A brood chamber. White objects are pupae and larvae.

8. Lay a tarp down next to a proposed pit location adjacent to the colony.

Piling excavated dirt on the tarp facilitates cleanup and minimizes the footprint of excavation.

9. Dig a pit adjacent to the colony located such that it won't intersect with the nest, but near enough so that all of the exposed chambers can be reached (Fig. 1A,D). The pit should be broad enough to accommodate both you and your equipment.

Generally, the breadth of the nest decreases as it gets deeper. If you are excavating a species known to have deep nests, make the initial pit large enough so that you will be able to dig deeper (i.e., it must be wider than your shovel).

10. Using the shovel, follow the vertical shafts downward:

- i. Dig from the side, removing horizontal layers of dirt to continue exposing the vertical shafts (Fig. 1A,D). Prevent loose dirt and debris from cluttering the horizontal surface.

- ii. Continue collecting ants as you expose more chambers (Fig. 1F,G).

Many ants will fall into the pit if care is not exercised; be diligent about collecting all ants as chambers are exposed.

Excavating the Bottom of the Nest

The queen(s), brood, and younger workers tend to occur at the bottom of the nest (Fig. 1G). Take extra care not to damage the soft-bodied larvae and queen.

11. If digging very deep, reinforce the walls of the pit using wood planks.
12. Widen and deepen the pit as needed to follow chambers and tunnels. Always keep the pit deeper than the working surface (i.e., the exposed chambers), and always keep the working surface horizontal.
This makes it easier to follow the nest downward. Exposing the nest below the bottom of the pit can be dangerous and makes it more difficult to collect the ants.
13. Continue digging beyond the point where chambers and tunnels are no longer found to be certain the complete colony has been uncovered.

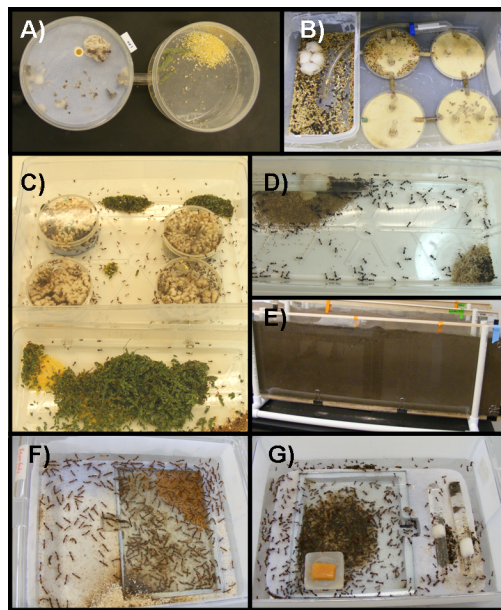


FIGURE 2. Examples of laboratory colony experimental and rearing designs. The species pictured is listed after each nest description. (A) For small nests (e.g., for founding queens), two chambers are connected. One (for living) is filled with plaster, with a watering tube to keep the plaster moist. The other chamber (without plaster) is used for foraging (*Acromyrmex versicolor*). (B) Similar to A, but for a larger colony. Each chamber (Petri dish) can be maintained at a different level of hydration. The dishes are interconnected; only one is connected to the larger foraging area (*Pogonomyrmex badius*). (C) As for B, but the chambers are taller; this is useful for leafcutter ants that grow large fungus gardens (*A. versicolor*). (D) A simple nest with only a test tube as a chamber and an open area where food can be placed (*Pogonomyrmex rugosus*). (E) A traditional “ant farm”: a dirt-filled space between two glass plates (*Pogonomyrmex californicus*). (F,G) Plaster nests covered with a glass plate inside a larger box where food can be provided (F: *Harpegnathos saltator*, G: *Camponotus floridanus*). [(A,C) Photos courtesy of Rebecca Clark; (D,E) photos courtesy of C.T. Holbrook.]

Post-excavation

14. Make sure the collected ants are stored properly and remain cool during transport.
15. Fill in the hole and tamp down the dirt.
Attempt to leave as little of an ecological footprint as possible.
16. Set up the lab colony following a method appropriate for the species.
*For details, see Fig. 2 and the “Sources and Husbandry” section of *Ants (Formicidae): Models for Social Complexity* (Smith et al. 2009).*

TROUBLESHOOTING

Problem: Tunnels cannot be found.

Solution: Carefully using a trowel, scrape the dirt away. Use the aspirator to blow vigorously on the newly exposed dirt. If this does not work, the tunnels might be outside the excavation area. In this case, expand both the horizontal work surface and the pit outward.

Problem: The hole collapses.

Solution: The excavation can usually be salvaged by removing the fallen dirt, but great care must be taken when removing dirt from the horizontal surface of the exposed nest.

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