The absence of *Pheidole* workers at the bait traps near the *Dinoponera* nests and their presence inside the refuse chambers suggests a relationship based on scavenging. The host species could benefit by improved hygiene in the nest. I have observed a different *Pheidole* species inhabiting a *Dinoponera lucida* nest in the wet forest of Espirito Santo in eastern Brazil. Also, C. Roberto Brandão and Jorge Diniz recently collected a different *Pheidole* species inside a nest of *Dinoponera quadriceps* in dry forest near Bahia, in central Brazil.

Stalking the Wild Attine

By Ted Schultz
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In the past year I've been lucky enough to devote four months to field work in Costa Rica, Nicaragua, Brazil, and Trinidad. My mission: to collect as many whole-nest series - including fungus gardens - of attine ants as possible, especially of spedes in the non-leaf-cutting genera. My reasons: 1) to get alates and larvae as sources of untapped characters for systematic study; 2) to get data on the range of variation in nest architecture for later compuison within an evolutionary framework; 3) to get complements of workers for an idea of the size of mature colonies and the size variation and size distribution of workers within colonies; 4) to get fungus gardens for later analysis of substrate; and 5) to get specimens of myrmecophiles (diapriid wasps in particular), virtually an unstudied subject in the attines.

With only a little previous experience excavating attine nests, I learned a lot. My major advisor was trial and error, though I also benefited from the experienced myrmecological advice of Bill Brown, Beta Brandao, Jorge Diniz, Jim Wetterer, Leanne Tenant, Jack Longino, an unamed Curatorial Assistant at the MCZ, and others. With the ulterior but noble motive of encouraging others to collect attine nests, I offer the following description of how I do it. I doubt that attine-tracking methods differ substantially from those used for tracking other ants, and I hope my fellow myrmecologists will add improvements from their own experience.

1. Locating workers. Though not uncommon in tropical rain forests (as well as savannah, cerrado, seashore, and even desert), the so-called "lesser attines" are far less conspicuous than their larger leaf-cutting relatives in the genera *Atta* and *Acromyrmex*. Occasionally one can spot trails of foraging workers of spedes in the genera *Sericomyrmex*, *Trachymyrmex*, and Cyphornyrmex, but for the most part individuals forage singly and are rendered quite cryptic by their dark coloration, dull matte reflectivity, slow movements, and habit of freezing or playing dead when disturbed. Unless one is lucky enough to stumble across a nest by accident, the first step in locating a colony is to locate a forager, and the best way to locate a forager is by baiting. I use barley ("cebada" in Spanish, "cevada" in Portuguese), originally recommended to me by fellow attinophile Jim Wetterer. The whole barley grain must be put through a blender or in some way crushed into pieces in a range of sizes suitable for carrying by the small attine species. The bright white endosperm makes this grain particularly easy to follow while carried in tiny mandibles on a drcuitous journey above and below the litter of the dark forest floor. Baiting can either be a large-scale affair, with many little piles placed at intervals along a trail or transect (6e experimental ecologist method), or carried out on a more localized level (the obsessive systematist method). Mass baiting works well for locating individuals of species with fairly populous nests. On the other hand, it is less rewarding for locating species with small nests and few foragers (*Myrmicocrypta*, for instance), which require the second appro•ch (my favorite), in which a likely spot is chosen and sprinkled liberally with bait, then watched by careful scanning for an extended period of time. Within 15 to 30 minutes, following on the tarsi of the inevitable first wave of Pheidole and Solenopsis, (even some Odontomachus species take this bait!), the slower moving, cryptic attines arrive. I usually collect the first forager and determine through a field lens whether or not it is a species I wish to pursue. If so, I await a second forager (sometimes widening the baiting area), ready to follow it back home.

2. Locating nests. Now it's time for a merry chase. After hours of following an attine worker on its maddeningly tortuous route back to the nest, even the most ardent panselectionist will doubt the optimality aiterion in behavioral evolution (or, alternatively, wonder about selection for altruistic behavior that misleads predatory myrmecologists). Even the highly reflective properties of barley do not always prevent losing the forager under the litter. Aside from the need for stubborn perseverance, I have learned only two things about tracking: 1) It is best not to disturb the leaf litter except when absolutely necessary to relocate the forager, because this can remove a portion of the scent trail and increase the return time geometrically, causing the ant to wander in search mode (which looks no different than "return mode") to relocate the trail. 2) It's helpful to sprinkle more bait every so often under the assumption that the closer to the nest the worker is getting, the more foragers there will be about (whether visible or not), and the more likely it will be that other members of the same colony will pick up bait and head for home.

Unfortunately, locating a spot where multiple foragers are disappearing for a seemingly final time is not necessarily the same thing as locating the nest. In some cases the trail continues into the maze of small rootlets that forms much of the tropical forest litter/soil interface. I can offer no formula for following ants under these conditions except for a careful pruning away of rootlets in the direction indicated by successive baiting. Mostly I attribute what successes I've had under this circumstance to luck.

3. Collecting nests. Nests of attines may be categorized by where they occur: 1) those with fungus gardens constructed between layers of leaf litter, 2) those in which the gardens are built in preformed cavities among roots or in rotten logs, and 3) those with gardens in one or more chambers excavated in the soil. The evolution of nest construction within the attines is unclear, with nest types varying within genera. Thus, information on garden location is very important for future comparison within a historical framework. If the nest is of the excavated type, particulars of the diameter of the opening and presence/absence and shape of an earthen mound should be recorded. In environments where soil-layering patterns are known, the color of the mound can indicate the minimum depth of the nest.

Nests that occur in the leaf litter, under the bark of a hollow log, or in other semi-exposed situations are easy to collect. The major problem is

apprehending escaping ants, especially the queen. I have found that below some aitical . disturbance threshold the queen prefers to remain with the garden. For subterranean-nesting species this threshold is rather high, presumably because there is basically no avenue of escape from the earthen chamber. For species that nest in layers of litter or in cavities in rotten wood the threshold is lower. My strategy with the latter is to try to remove as much of the fungus garden as quickly as possible and with a minimum of disturbance to an appropriate container, then to aspirate up the remainder of the ants, always keeping an eye out for the queen (who, hopefully, will have stayed with the garden during the operation), but a word of warning: queens as well as workers of normally slow-moving species can be surprisingly nimble under these conditions.

In all cases it is best to try to collect as much of the nest population as possible (for reasons given in part 4 below) and to measure the approximate dimensions of the garden.

Soil-dwelling attine nests can be shallow or deep, and have single or multiple chambers. In general, nests of forest-dwelling species are shallower than nests of cerrado and desert-dwellers. There are two approaches to excavation: the blind digging method and the passage-following method. I recommend the blind-digging method only when pressed for time or when informed by prior experience of the approximate location of the chambers). This avoids the risk of missing the chamber completely or breaking into it in such a way that the garden is destroyed and the queen escapes. The negative side of the passage following method is that, for deep-dwelling attines, the procedure can sometimes require more than a day. For example, a nest of *Mycocepurus goeldii* that I excavated in an open area in Amazonas was built in sandy, crumbly soil and had a passage only 3 mm wide that turned frequently and branched into numerous dead-end side passages. Each time I lost the passage I went off and did some general collecting until the ants had reexcavated an opening. The single fungus chamber (discovered on the second day) was so deep (80 cm) and so offset from the entrance at the surface that I never would have located it by blind digging.

To follow the passage prior to digging, I use O.7C mm diameter nylon fishing line, twisting it gently to get it around corners (too much twisting, however, collapses small-diameter passages). When the line is inserted as far as it will go, I anchor the free end out of the way to prevent accidentally puffing it out while working. Idealy, I try to expose a cross-section of the passage, which means that I have to start digging at some distance from and perpendicular to the plane that contains its greater part. I try to enlarge this hole so that it is always deeper than the part of the passage being exposed (so that the loosened soil falls into the hole rather than into the passage) and, to make it possible to see what I'm working on, I extend it outward in a horizontal direction so that it is at least twice as long as it is deep. Aside from the fishing line, useful tools include a spoon and pocket knife (for carefully exposing the passage), small heavy-duty shears (for cutting interfering roots with the least amount of passage-collapsing disturbance), a trowel, and in some cases a large shovel and a pick. A headlamp is useful in the dark forest and even in the open sunlight when working at greater depths.

In some attine species (e.g., *Mycocepurus*, *Trachymyrmex*, *Mycetosoritis*) there is a small (2 cm diameter) first chamber only a few centimeters below the surface. Except in very young nests, this chamber contains no garden, though a number of workers and forage items may be present. This is probably the chamber excavated by the foundress queen and inhabited only until the first generation of workers was able to construct a new chamber deeper down. The passage continues from this chamber, usually from the floor. In this or any case (e.g., a sharp bend) where the fishing line ends and the continuation of the passage cannot be relocated, the best recourse is to await reexcavation by the ants, though I have had occasional success relocating the passage by carefully probing with the fishing line or a wire.

Reaching a fungus chamber is the most exciting moment in this process. This may occur at only a few centimeters below the surface (*Myrmecocrypta*, some *Trachymyrmex*) or at a depth of a meter or more. While the breach in the chamber wall is still small, I excavate around and below it so that soil will fall away from rather than into the chamber, exposing a clean cross-section. During this process I try to aspirate up any escaping workers and to keep a lookout for the queen. As my view is improved I try to notice whether ants are disappearing into passages in the sides or bottom of the chamber, a sure sign that there is at least one more chamber that will require further digging. Some species of *Trachymyrmex* can have as many as five chambers arranged vertically and penetrating to a depth of more than a meter, while *Sericomyrmex* nests can consist of multiple chambers arranged side-by-side.

Once a chamber is exposed, I note down the following information: 1) dimensions, 2) distance from the surface, and 3) whether the fungus garden is pendant (i.e., suspended from rootlets) or sessile (i.e., resting on the floor of the chamber). I take photographs if possible, including a centimeter ruler for scale. I remove the fungus garden by clipping away any roots to which it is attached, scooping it up with a spoon, and placing it temporarily in a fluon-lined plastic container. Sometimes, under conditions in which I believe that the queen may be able to escape through some other opening to the outside, I quickly aspirate up most of the fungus garden and later search for the queen among the aspirator contents.

4. Treatment of prisoners. Once an attine colony is collected, the choices of what to do with them fall into two basic categories: 1) Keep them alive, 2) Kill them. For the latter choice, it is best to pop the entire nest contents into 95% ethanol. This allows for future DNA extraction of both the ants and the fungi. (I am told that 100% ethanol is even better for DNA preservation, but I do not know how well larvae withstand distortion at this concentration; I do know, however, that 95% preserves larval morphology nicely.) Preserved whole-nest series of attines are useful as sources of all life stages (larvae, workers, queens, males) and attinophiles (e.g.,

Coleoptera, parasitic Hymenoptera and Diptera; diapriid pupae can even be identified inside of preserved ant pupae). They also provide information on colony size, on the little -appreciated worker polymorphism of the lesser attines, and on the identities of the fungal substrates employed by different species.

Keeping the attine nest alive preserves all of the above information and, in addition, has the potential of supplying information on behavior, life stages not present in the nest at the time of collection, and myrmecophiles (especially those that are present in the nest only briefly as adults) that may hatch out in captivity. The most critical parameters for successfully maintaining a living nest are humidity, ventilation, and temperature, and for all of these the fungus is more susceptible than the ants. Field conditions in the humid tropics will supply the first two requirements (as long as you keep your nests out of the direct sun), but the third, ventilation, is critical. In the next section I will describe the nest boxes I use for attine colonies in the lab, which are also adaptable for field use.

5. Laboratory nest boxes. I currently maintain in the lab 35 nests of attines in nine genera. Over the past two years I've developed methods for keeping attines that work well for most species, but I am very interested in hearing from others on ways to improve them.

I keep *Atta* and *Acromyrrnex* colonies in "Weber nests." Based on Neal Weber's original design, these consist of large dear polystyrene "refrigerator boxes" (7 3/8" x 5 1/4" x 3 3/4"; available from Tri-State Plastics, see below) connected by tubing (Tygon 1" inner diameter clear flexible tubing fitted snugly into 1 1/8" holes created by a drill hole-saw makes for nice, modular, easily separable units). The entire Weber nest is set inside of a 20"x24" photo-developing tray, in which the ants are allowed to forage freely. They are prevented from exiting by fluon painted onto the sides of the arena. I am still perfecting this system, as *Atta* spp. in particular are fiendishly proficient at navigating across fluon, petroleum jelly, and other barriers effective against other insects. Neal Weber used mineral-oil moats to contain his Attas, a measure I'm currently considering.

Except when they are very young, the *Atta* and *Acronzyrmex* nests do not require moisture sources placed within the nest boxes. A suitable humidity is maintained by the sheer mass of the garden itself, which retains moisture from the substrate (leaves, orange rind, and apple) brought in by the ants. This is not the case with the smaller nests of the lesser attines, where humidity is the most critical parameter. In the bone-dry lab environment here at Cornell, fungus and ants would die out in a matter of days if I did not add water. The nest-box design for these species follows the basic Weber-nest pattern on a smaller scale, but without external foraging arenas. In this case, the boxes (also available from Tri-State) are 2 7/8" x 27/ 8" x 1 1/8 " and the tubing is 3/8" ID Tygon. The box containing the fungus garden is supplied with a poured plaster bottom approximately 3/8" deep; the other boxes, which serve the purpose of foraging and waste disposal chambers, contain iio plaster.

To save space I will simply refer you to the accompanying diagram. All holes in the plastic are drilled with a cutting rather than a conventional drill bit (see supply list), while the water well is drilled into the plaster with a conventional bit. The plaster-bottomed nest chamber is ventilated only by air exchange via the connecting tubes to the satellite boxes and through the incomplete seal around the lid. The satellite boxes, on the other hand, are equipped with ventilation holes in the lids that are covered with wire cloth. This screen is best fused to the lid by melting the plastic with a soldering iron. I have tried various types of glue with poor results.

As shown in the diagram, the plaster-bottomed nest chamber is equipped with a water well in one corner that is directly below a corresponding 5/16" hole in the lid. A short piece of Tygon tubing effectively isolates the water well to the topological "outside" of the chamber, preventing the ants from hanging out in the well (something they like to do). When gardens grow sufficiently large, it is necessary to add additional plaster-bottomed chambers to the first. Though I have not pictured it in the diagram, I usually include a fourth satellite box in the chain beyond one of the food boxes, which is used as a disposal site by the ants. This nest box arrangement may be used in field situations and, when moving from place to place, the satellite boxes may be temporarily removed for compact transport. In this situation the fungus garden should be monitored frequently to insure that it is receiving adequate ventilation. I feed my attines coarsely ground grain (e.g., cream of rice, cream of wheat), small bits of apple (for sugar and moisture), and the white inside of orange rinds (a.k.a. "zest") diced with a razor blade into small bits, and from which the outer orange part (which may contain noxious plant compounds) has been removed. I place the moist and dry foodstuffs in separate satellite boxes. The ventilation hole prevents rapid mold buildup, but it is still necessary to change the moist foods on a two-day cycle, which is also the optimal amount of time between rewettings of the plaster.

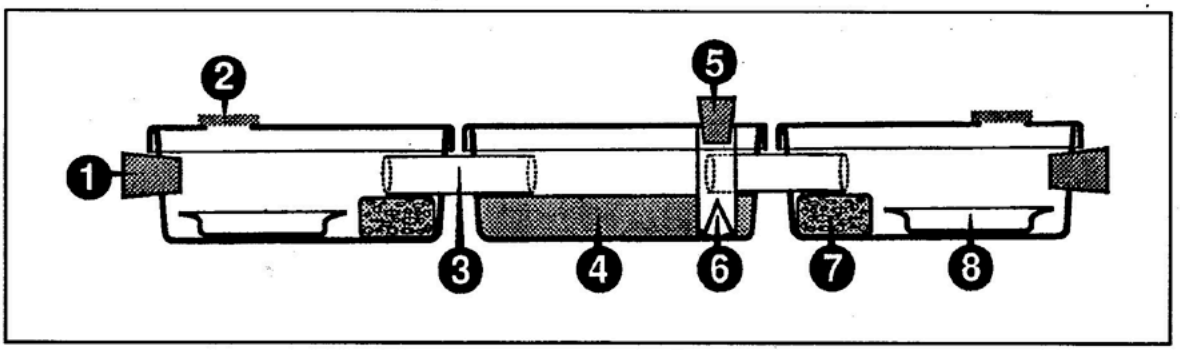
6. Sources of tools and equipment.

Plastic boxes: Tri-State Plastics, P.0. Box 6, U.S. Hwy 41A North., Dixon, KY 42409; 502-639-9142. Supplier of large (7 3/8" x 5 1/ 4" x 3 3/4"; model #179C), small (2 7/8" x 2 7/8" x 1 1/8 ,'; model #TO6C), and other sizes of clear polystyrene boxes.

Drill bits: For drilling small holes in polystyrene, I use a Vermont American 1/8" - 1/2" Drill Tree, Model #13370. The generic term for this type of drill bit is a "multiple step drill bit," and apparently other versions are available, including one from Milwaukee Electric. These bits are used primarily by electricians and auto body repair people, and can be found in stores that supply these professions.

Fluon AD-1: Northern Products, Inc., 153 Hamlet Avenue, P.O. Box 1175, Woonsocket, BI 02895; 401-766-2240/2241. Slippery, expensive stuff that keeps ants from climbing the sides of containers, at least until they inevitably find a way out and swarm into every nook and cranny of your lab.

Fine-mesh stainless-steel screen: Newark Wire Cloth Company, 351 Verona Avenue, Newark, NJ 07104; 1-800-221-0392.



Laboratory Nest Set-Up

1. Neoprene stopper, size 00, closing 7/8" expansion hole for connecting additional chambers.

2. Ventilation hole, -1/2" diameter, covered with 100 x 100 mesh-per-inch,. 0.0045-diameter-wire staii•less steel mesh fused to the plastic by melting with a soldering iron.

3. Tygon tubing connector, 3/8" inner diameter, fitted very snugly into a 7/8" hole. The snugger the fit, the easier it is to move the whole array around as a unit.

4. Plaster bottom in central nest box (location of fungus garden), 3/8" deep.

5. Neoprene stopper, size 0000, sealing a 5/16" opening used for rewetting the plaster.

6. Water well in one corner of the plaster bottom, 15/32" diameter, separated from the nest compartment by a length of 3/8" I.D. Tygon tubing that extends to the lid and fits snugly against it. The tubing is notched at the bottom to facilitate water absorption by the plaster.

7. A little "step" made from a piece of kitchen sponge, to help tiny ants find their way to the floor of the satellite chamber from the connector tube.

8. Food dish, in reality a disposable plastic weighing tray.

Ants of Guana Island, British Virgin Islands

Roy Snelling
Los Angeles County Museum of Natural History

I've now twice had the opportunity to collect ants in the British Virgin Islands, on a small piece of real estate known as Guana Island. My report in SPHECOS 23 last year briefly described Guana and provided a simple map to the collecting areas indicated on my data labels, so I'll not repeat all that, noting only that it's a small (ca. 340 hectares in area), low (highest point 246 m), dry forest island.

The entire month of October 1992 was spent on Guana, except a few day-trips to Anegada, Cooper, Ginger, Tortola and virgin Gorda Islands. In 1991 I was also there during October and collected 11 species of ants. My latest trip added 18 species (asterisked on following list) in addition to those collected last year, so I guess I can say that it was a pretty successful month. There were a few surprises, mostly in the form of range extensions for species known from elsewhere in the Puerto Rico Bank but not previously recorded from the Virgin Islands. Things like *Mycetophylax conformis*, *Trachymyrmex jamaicensis*, *Camponotus* sp. 2, *Discothyrea* sp. and *Amblyo pone* sp.

The last named is represented by two males taken in a malaise trap. Since they are not associated with any workers, there's no way to hang a name on them now. Only one *Amblyopone* is known from this area of the Greater Antilles, *A. falcata*, described last year by John Lattke from Puerto Rico. Most interesting, however, is a single male ponerine also from malaise trap. I thought at first it was a