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Locating, collecting, and maintaining colonies of fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini)

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Abstract

The purpose of this paper is to describe methods developed by generations of attinologists for locating, collecting, and maintaining in the laboratory live colonies of fungus-farming ants. Our goal is to accelerate the study of the poorly known and increasingly threatened non-leaf-cutting species of this fascinating and biologically important group because leaf-cutting ants have historically received the majority of scientific attention. We describe standardized measurement and data collection protocols in three sections: (i) locating fungus-farming ants in the field; (ii) collecting whole colonies of fungus-farming ants; and (iii) maintaining living colonies of fungus-farming ants in the laboratory. We provide lists of necessary equipment and materials, including information on where they can be acquired.

Introduction

The fungus-farming ants (Myrmicinae: Attini: *Atta* species group; herein referred to as "attine" ants, *sensu* Ward et al. (2015) are a monophyletic group, exclusively New World and primarily Neotropical in distribution, consisting of >250 described species as well as many more awaiting discovery and description (Brandão & Mayhé-Nunes, 2001; Schultz & Meier, 1995; Schultz & Brady, 2008; Schultz et al., 2005; Sosa-Calvo et al., 2013). As far as is known, all fungus-farming ants obligately depend upon the cultivation of basidiomycete fungi (Agaricales: Leucocoprineae and Pterulaceae) for food (Barrer & Cherrett, 1972; Chapela et al., 1994; Cruz & Filho, 1993; Dentinger et al., 2009; Fisher et al., 1994; Heim, 1957; Hervey et al., 1977; Möller, 1893; Mueller et al., 1998; Munkacsi et al., 2004; Quinlan & Cherrett, 1979; Weber, 1955). In return, the ants provide their fungal cultivars with nourishment, dispersal, and protection from competing and pathogenic microbes (Currie & Stuart, 2001; Currie et al., 1999b; Fernandez-Marin et al., 2009; Quinlan & Cherrett, 1977). In addition to the ants and their fungal cultivars, the attine symbiosis also includes multiple species of a fungal pathogen (Ascomycota: Hypocreales: *Escovopsis*) as well as symbiotic filamentous bacteria (Actinomycete: *Pseudonocardia*, *Amycalaptosis*, and *Streptomycetes*) that grow on the ants' integuments (Mehdiabadi & Schultz, 2010). New attine microbial symbionts continue to be discovered (e.g., Little & Currie, 2007, 2008; Pinto-Tomas et al., 2009).

Fungus-farming ants are widely distributed in the New World, including the Caribbean, extending from 44° south latitude (Province of Chubut, Argentina) to 40° north latitude (Long Island, New York, and Illinois) (Klingenberg & Brandão, 2009; Mayhé-Nunes & Jaffé, 1998; Sosa-Calvo et al., 2013; Weber, 1966, 1969ab, 1982; Wheeler, 1907). Fungus-farming ants do not occur in Chile (Table 1). Fungus-farming ants have been recorded at various altitudes from sea level to as high as 3500 m elevation (Weber, 1982) and occur in a wide range of habitats, including rain forests, cloud

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forests, savannahs (including grasslands and Cerrado), xeric shrubland and thorn forest (caatinga), seashores, sandy dunes (restingas), semi-deserts, and deserts (Mehdiabadi & Schultz, 2010). Depending on the species, nests and gardens may occur in excavated chambers and preformed cavities below ground, in rotten logs, under objects, inside objects (e.g., palm fruits), and between layers of the leaf litter; they may also occur above ground in, e.g., palm rootlets, bromeliads, tree crotches, and, in a few *Apterostigma* species, on the undersides of leaves (Table 1).

The most conspicuous and well-known fungus-farming ants are the leaf-cutting species in the genera *Atta* and *Acromyrmex*, which use freshly cut plant material as the substrate for their fungal crops, rendering some species important agricultural pests in the New World tropics. Comprising ~20% of fungus-growing ant species, the leaf-cutting ants are in many respects the most highly derived of all fungus-farming ants. Along with two other genera, *Trachymyrmex* and *Sericomyrmex*, the leaf-cutters are part of a clade traditionally known as the higher attine ants. All other fungus-farming ants, a paraphyletic group (Schultz & Brady, 2008), are traditionally referred to as the lower attine ants. This paper focuses mostly on the non-leaf-cutting fungus-farming ants, including the lower attine ants as well as *Trachymyrmex* and *Sericomyrmex*. For information on the leaf-cutting ant genera *Acromyrmex* and *Atta*, including methods for field collecting and laboratory rearing, see: Weber (1937); Fernandez-Marin et al. (2003); Verza et al. (2007); Fernandez et al. (2015); Araújo & Della Lucia (1997); Mariconi (1970); Montoya Lerma et al. (2006); Moreira et al. (2004); Silva Junior et al. (2013); Soares et al. (2006); Della Lucia & Moreira (1993); Forti et al. (2011); Morgan (1991); Della Lucia (2011). Non-leaf-cutting fungus-farming ant species do not cut fresh plant material but instead variously use insect frass, flower petals, seeds, (possibly) insect carcasses, and other organic detritus on which to grow their fungal cultivars. Understanding the biology of these less conspicuous, much more poorly studied species is essential for understanding deeper attine evolution as well as for human welfare. For example, some of the very few chemical studies conducted on lower fungus-farming ants have led to the discoveries of new antibiotics and antimalarials (Carr et al., 2012; Freinkman et al., 2009; Oh et al., 2009; Poulsen, 2010; Poulsen & Currie, 2010; Wang et al., 1999).

Forty-four percent of all fungus-farming ants, including the leaf-cutters, are known solely from the type collections (Mayhé-Nunes & Jaffé, 1998). For these and for the majority of named species as well, nothing is known about nest architecture, fungal association, additional microbial associations, or other aspects of life history. Many critical aspects of attine biology have only been discovered and/or elucidated during the past three decades, including the identities of the various cultivars, the existence of *Escovopsis*, the nourishment of actinomycete bacteria in integumental crypts, and the molecular phylogenetics of the ants and fungi (Chapela et al., 1994; Currie et al., 2006; Currie et al., 1999ab; Hervey & Nair, 1979; Hervey et al., 1977; Muchovej & Della Lucia, 1990; Mueller et al., 1998; Schultz & Brady, 2008; Sosa-Calvo et al., 2013). New species and even genera continue to be described even as the habitats of many attine species are increasingly threatened (Brandão & Mayhé-Nunes, 2001, 2008; Klingenberg & Brandão, 2009; Mackay & Serna, 2010; Mayhé-Nunes & Brandão, 2006, 2007; Rabeling & Bacci, 2010; Rabeling et al., 2007a; Sosa-Calvo et al., 2009; Sosa-Calvo & Schultz, 2010; Sosa-Calvo et al., 2013). For example, only 20% of the Cerrado biome, an important habitat for many attine ant species (Leal & Oliveira, 1998, 2000; Leal et al., 2011; Vasconcelos et al., 2008), remains intact and only 6.2% is protected (Myers et al., 2000). It is absolutely urgent, therefore, to document the biology of all attine species; to preserve for posterity in both traditional and in cryostorage collections ants, fungal cultivars, and other symbionts; and to maintain in the lab living nests of as many attine species as possible.

By collecting and maintaining in live culture entire nests of fungus-farming ants, attinologists can: (i) obtain all life stages and castes (larvae, pupae, workers, gynes, males) necessary for morphological study; (ii) document nest architecture, enabling comparisons between species and genera; (iii) obtain fungus gardens, enabling analyses of host-cultivar symbiotic associations, the identification of fungal parasites, and the identification of useful compounds, e.g., antibiotics; and (iv) obtain rarely collected myrmecophiles known to inhabit fungus-farming ant nests, including diapriid wasps, pyrgodesmid millipedes, mites, psychodid and milichiid flies, beetles, cockroaches, pseudoscorpions, and others.

In the following three sections, we describe how to locate, collect, and maintain in the lab living colonies of fungus-farming ants. The first two sections predominantly focus on the collection of soil-dwelling ants because colonies and fungus gardens of soil-dwelling attine ants are much more difficult to locate and collect than those of non-soil-dwelling attines.

1. Locating fungus-farming ants

In this section we describe how to locate nests of fungus-farming ants, with an emphasis on the less conspicuous non-leaf-cutting ants. Leaf-cutting ant species (genera *Acromyrmex* and *Atta*) are easily encountered due to the large size of individual foraging workers, their generally conspicuous foraging columns, and their conspicuous nest entrance mounds. Unlike the leaf-cutter species, the non-leaf-cutting fungus-farming ants are generally far less conspicuous in the field. Workers of most species forage individually, are small, move slowly, and feign death when disturbed. They are almost uniformly cryptic, with dark color and dull, matte integuments. Some non-leaf-cutting species in the genera *Sericomyrmex* and *Trachymyrmex*, in which colonies tend to be slightly larger than those of most lower fungus-farming ants, forage in noticeable columns, albeit columns that are much sparser than those of leaf-cutters. The nest entrances of some non-leaf-cutting fungus-farming ant species can be very distinctive, e.g., taking the form of turrets or crater-like or crescent-shaped mounds (Mueller & Wcislo, 1998; Schultz et al., 2002; Solomon et al., 2011; Weber, 1969b), but the majority of nest entrances consist of a single, small hole in the ground lacking any accompanying turret, mound, or noticeable accumulation of excavated soil (Sosa-Calvo et al., 2013). For this reason, the usual first step in locating a colony of fungus-farming ants is to locate a foraging worker. In turn, the best way to locate a foraging worker is through the use of bait. Several foods have been employed as baits for ants in general, e.g., canned tuna or sardines and Keebler Sandies™ pecan cookies, but these are not particularly effective for attine ants. Attine-ant-specific baits include barley, farofa (toasted manioc flour) mixed with orange peel, and mixtures of fruits and dry cereal, among others (e.g., Leal & Oliveira, 1998, 2000; Schultz, 1993; Weber, 1966, 1972c; Wheeler, 1907). We have found Cream of Rice™ Cereal (B&G Foods, Inc., Parsippany, NJ, USA) to be universally effective because the grains are small enough for even the smaller species to carry and because the bright white color contrasts well with the background colors of most soils and leaf litters, making it easy to follow the forager as it traverses its sometimes long and frequently circuitous route back to the nest.

Upon arriving at a location where we expect a targeted species to occur, we broadcast around 4 tablespoons of bait across an area of about 1-2 square meters and then watch and wait. If the area is covered by a dense layer of leaf litter, then, prior to baiting, we may choose to clear away the majority of the leaf litter to expose patches of bare soil. Clearing away the litter disrupts ant pheromone trails so, when this has been done, it is often best to broadcast bait on the cleared area and return a few hours later when pheromone trails have been reestablished. Otherwise, the return trip of a bait-laden forager to the nest may be significantly longer and significantly more circuitous than it would otherwise be when guided by a pheromone trail. In any case, either immediately or after an appropriate time span (i.e., 30 minutes to 2 hours, depending on the genus), we visually monitor the area until we observe fungus-farming ant foragers, which can be very small (e.g., ~3 mm long) and cryptic, taking and carrying the bait back to their nests.

When a likely worker is observed to take the bait, we pick it up and examine it to confirm its identity using a Hastings Triplet 20x magnifier (Bausch & Lomb, Rochester, NY, USA). Whether or not it is a species of interest, that first specimen is usually preserved in 95–100% ethanol. If it is a species of interest, the next worker that takes the bait is followed in order to locate the nest entrance. Very rarely, a second worker may not be encountered and the investigator may need to move to a new location. Following the worker as it makes its way above and below layers of leaf litter on the forest floor usually requires considerable patience, concentration, and a good headlamp (we favor the Petzl Myo RXP™ headlamp, Petzl, ZI Crolles, France). Careful removal of dead leaves and sticks may at times be necessary to relocate the worker when it disappears for more than 5-10 minutes. Such disturbances may cause the worker to briefly feign death, but after a short while it will recover and, provided its pheromone trails have not been disrupted, continue to its destination.

Another way to locate nest entrances is by direct inspection of the forest floor after rain, when most ground-nesting fungus-farming ants will reopen their nest entrances and remove soil particles present within their tunnels, rendering their nest entrances temporarily conspicuous (Fig 1a). These piles of excavated soil may persist for a few hours or even for a day, but, for reasons that are not understood, some attine ant species do not allow soil pellets to accumulate in the vicinities of their nests, whereas others do.

2. Excavating and collecting entire colonies of fungus-farming ants

Once a nest entrance has been located, excavation can commence. In most cases, however, and especially when we are excavating a species for which the natural history, including nest architecture, is unknown, we mark the location of the first colony encountered with flagging tape and search for additional colonies. It is always desirable to excavate multiple colonies of the same species, but, especially in cases where nest architecture is unknown, locating multiple colonies serves as insurance in a learning process in which the first one or two excavations may fail to locate the nest chamber(s).

Prior to excavating the nests of soil-dwelling species, information about the nest entrance is recorded, including: i) diameter of the nest opening, ii) presence or absence of a turret, earthen mound, or other accumulations of soil, and iii) the shapes and sizes of such features, when present, and of any other conspicuous features of the entrance. Creating a photographic record of the nest entrance is ideal; such photographs include a ruler or other reference for scale (Figs 1a–b). These guidelines obviously do not apply to non-ground-nesting species, for which there is seldom a distinct nest entrance.

We begin an excavation by digging a rectangular trench at least 0.5 meter deep and a variable distance from the nest entrance. The distance separating the trench from the nest entrance is determined by two factors: (i) the expected depth of the deepest nest chamber (the deeper the chamber, the greater the distance) and (ii) the expected lateral deviation of the nest chambers from a vertical axis descending through the nest entrance. For species in which nest architecture is entirely unknown, we might initially choose to make the trench 1 m deep and 1.0 m distant from the nest entrance. For digging, our primary tool is a sturdy and dependable folding shovel (we strongly prefer the folding shovels made by Gerber Gear, Portland, OR, USA) (Table 2). Once the trench has been excavated, we extend it horizontally, using the shovel to carefully

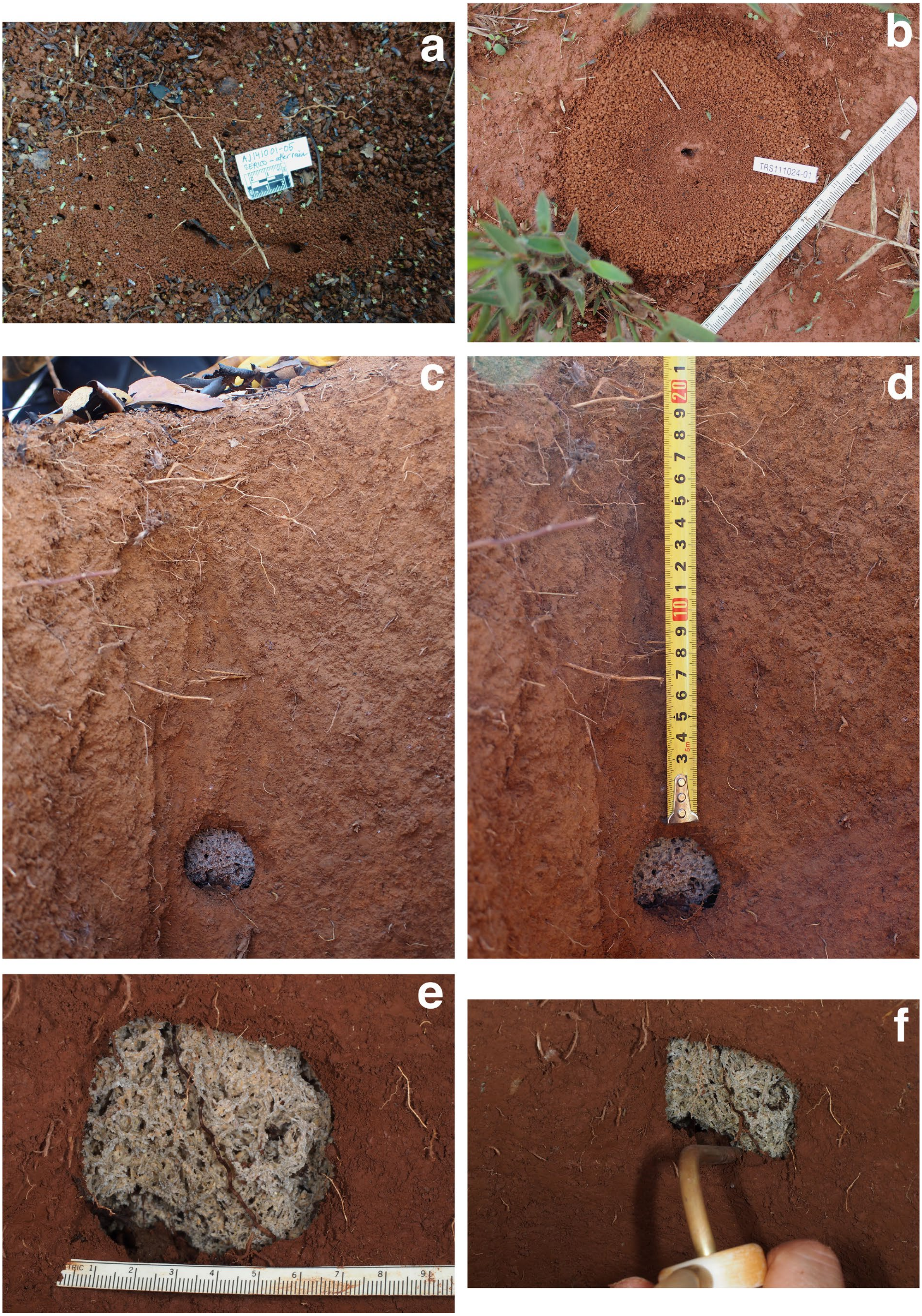

Fig 1. Nests of ground-nesting non-leaf-cutting fungus-farming ants. **a.** Nest entrance of *Sericomyrmex* sp. (AJ141001–05) with multiple entrances after rain. **b.** Nest entrance of *Myrmicocrypta* sp (TRS111024–01) with small single entrance surrounded by circular dirt mount. **c.** Subterranean subspherical garden chamber of *Myrmicocrypta* sp. **d.** Subterranean garden chamber of *Myrmicocrypta* sp. with depth record (see text). **e.** Subterranean chamber of *Apterostigma* sp. **f.** Subterranean chamber of *Apterostigma* sp. demonstrating collection of ants with receptacle aspirator.

shave soil from the vertical face of the trench in the direction of the nest entrance, periodically removing accumulated soil from the bottom of the trench. When they are encountered, we remove large roots with a foldable pruning saw and small roots with a pruning shears or a knife (Table 2). As the vertical wall of the trench approaches the nest entrance, shaving becomes increasingly cautious in order to avoid missing or accidentally destroying chambers and tunnels. If the trench has intersected the soil beneath the nest entrance and chambers or tunnels have still not been encountered, the trench is deepened. At this point it is usually necessary to expand the trench horizontally in the direction away from the nest entrance as well, because more room is required at greater depths, both to maneuver as well as to expose and observe, preferably at eye level, deep chambers. We always use a headlamp while digging, which particularly facilitates the discovery of nest chambers in dark forests and/or at greater depths.

The depth and number of chambers varies considerably across species of fungus-farming ants. In what may be the primitive condition in the attines, the nests of quite a few soil-dwelling species consist of one or less frequently two shallow chambers (<50 cm from the surface), e.g., soil-dwelling *Apterostigma* spp., most *Myrmicocrypta* spp., *Mycetarotes* spp.*, Mycetophylax* spp., soil-dwelling *Cyphomyrmex* spp., and a few *Trachymyrmex* spp. (e.g., *T. bugnioni*). A few *Sericomyrmex* species have shallow nests with multiple chambers. In the remainder of fungus-farming ants nests may consist of a few to dozens of chambers >50 cm deep and, in exceptional cases (e.g., *Mycetagroicus* spp.), 3-4 meters deep (Fig 2a), including *Cyatta abscondita*, *Kalathomyrmex emeryi*, *Myrmicocrypta camargoi*, *Mycocepurus* spp., *Mycetosoritis asper*, and most *Trachymyrmex* and *Sericomyrmex* spp. (see Table 1 for details and Fig 3 for a generalized schematic representation of nesting behaviors in fungus-farming ants). Some species of *Myrmicocrypta* and most soil-dwelling *Cyphomyrmex strigatus*-group species have very small (~2 cm diameter) single chambers and nest quite close (~2-5 cm) to the surface; such chambers can easily be missed during excavations. In many soil-nesting attine species, a shallow, small, upper chamber is present that does not contain a fungus garden and that is connected to the lower chamber(s) by a vertical tunnel. This upper chamber is probably the chamber initially excavated by the foundress queen.

Prior to collecting ants and fungus gardens, we flame-sterilize, using a lighter, a number of tools including a spoon, soft forceps, and pocket knife. This prevents cross-contamination, by *Escovopsis* and other microbes, of successively excavated nests. When a chamber is encountered, we carefully expose it by progressively scraping away surrounding soil with the flame-sterilized spoon and/or knife until the opening is large enough to collect the chamber contents (Figs 1c, e). During this process we collect any escaping ants with the flame-sterilized soft forceps or a receptacle aspirator (Table 2; Figs 1f, 2e) and keep careful watch for the queen and alates. We try to work rapidly because, in nests with more than one chamber, individuals (especially the queen) of some species will attempt to escape through tunnels to other chambers or, more rarely, to the tunnel leading to the surface. Some encountered chambers may be entirely empty or else may contain ants but no garden. If a garden is present, we extract it by inserting the spoon under the garden and lifting it out while trying to keep it intact. If the garden is interlaced with rootlets, these can be cut prior to extraction with a knife, scissors, or pruning shears, trying not to disturb the garden. The fungus garden is placed temporarily in a clean plastic container without a plaster bottom. Large gardens may require multiple plastic containers. Once all visible garden fragments and ants have been extracted, we examine the collected contents to determine whether or not the queen is present. At some point in this collecting process, either when the garden is still in the chamber or else once the chamber has been emptied, we photograph the chamber, including in the photograph a ruler or other measurement for scale (Figs 1d–e, 2a–b).

We immediately preserve garden fragments and a series of workers in 95–100% ethanol in 2 ml or 8 ml vials (Table 2). Information about the chamber is recorded (Fig 4), including: (i) depth from the surface, measured from the ceiling of the chamber to the surface (Figs 1d); (ii) chamber height (the maximum distance from the ceiling to floor of the chamber), chamber width (the maximum horizontal distance parallel to the plane of excavation), and chamber depth (the maximum horizontal distance perpendicular to the plane of excavation); (iii) chamber contents (garden, workers, brood, queen, alates, commensals); and (iv) when a garden is present, whether it is pendant (suspended from the ceiling of the chamber directly or by attachment to rootlets) or sessile (resting on chamber floor). In nests containing multiple chambers, we record this information for each chamber and ultimately record (i) the total number of chambers encountered, (ii) the relative distances between chambers, (iii) and the chamber in which the queen was encountered (typically the deepest chamber). Such information about nest architecture is often best recorded in the form of a field sketch. In addition to the above, we record standard field data including GPS coordinates, collectors' name(s), date, and habitat type.

Over the next 24-48 hours, field-collected ant colonies are allowed to restabilize their gardens, which are usually disturbed during nest excavation and subsequent transport. During this time the ants will remove soil particles and reconnect garden fragments (Fig 2c). Restabilized colonies are then transferred to the containers in which they will be kept for the duration of the field trip using a sterile forceps and spoon and, where necessary, pieces of index cards formed into makeshift spatulas (Fig 2d). Constant humidity is maintained in the containers by lining them with poured plaster or with a plug of cotton thoroughly saturated with water. Many sorts of plastic containers may be used, including plastic Petri dishes,

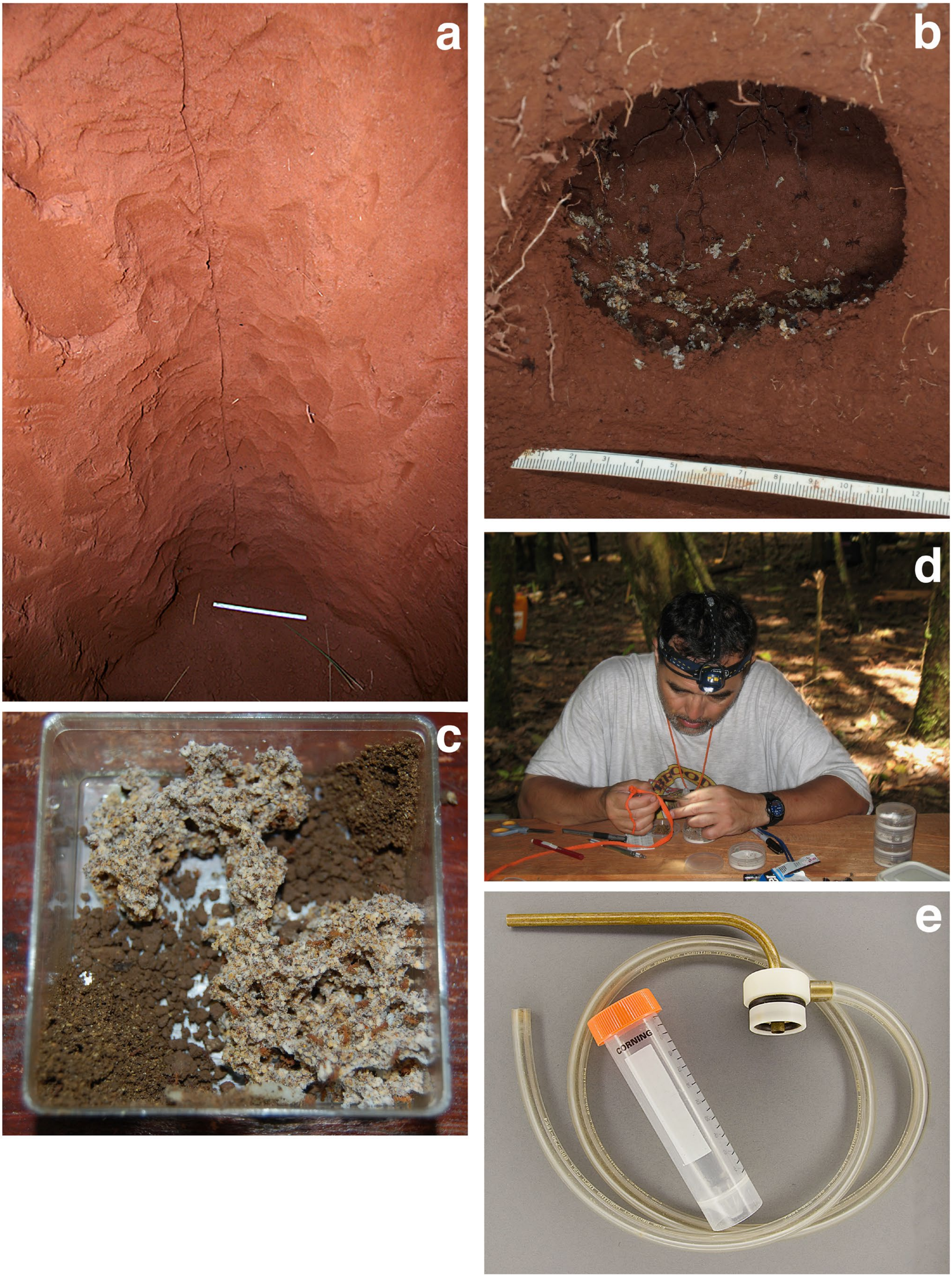

Fig 2. Field collection and care of fungus-farming ants. **a.** Vertical tunnel and empty chamber of *Mycetagroicus cerradensis.* **b.** Empty garden chamber of *Apterostigma* sp. with garden remnants on chamber floor. **c.** Restabilized fungus garden of *Sericomyrmex* sp. after ants have separated garden and soil particles. **d.** Daily care of fungus-farming ants in the field. **e.** Rose Entomology "Bug Vac" receptacle aspirator and 50 ml clear plastic centrifuge tube with cap.

Table 1. Known distribution of the fungus-farming ant genera with information of nesting behavior and nesting architecture.

	America					Nest architecture		
Genus	**N**	**C**	**S**	**CI**	**Nesting behavior**	**Number of chambers**	**Nest entrance (ground-nesting species)**	**References**
Acromyrmex	x	x	x	x	Underground, under rotten logs, superficial depression in soil covered by mound of dry litter and debris, above ground (tree crotches or lianas)	1-15 chambers	Mound builders: Mound formed of grass, litter, and/or soil	(da Silva Júnior et al., 2013; Silva et al., 2011; Verza et al., 2007; Weber, 1941, 1945, 1946, 1947, 1969b, 1972b)
Apterostigma	–	x	x	–	Underground, in and under rotten logs, under rocks, between layers of leaf litter, semi-exposed locations (tree-trunks or undersides of palm leaves), in rotten fruits	1 chamber	Inconspicuous hole, some with crescent-shaped crater	(Delabie et al., 1997; Pitts-Singer and Espelie, 2007; Weber, 1941, 1945, 1946, 1969b, 1972b, 1982)
Atta	x	x	x	x	Underground.	From 1 to >7000 chambers	Mound of craters, >1000 nest entrances	(Autuori, 1942; Moreira et al., 2004; Weber, 1941, 1945, 1946, 1947, 1966, 1969b, 1972b, 1982; Wheeler, 1907)
Cyatta	–	–	x	–	Underground.	Up to 8 chambers	Inconspicuous hole.	(Sosa-Calvo et al., 2013)
Cyphomyrmex	x	x	x	x	Underground, in rotten logs, in rotten fruit husks, in preformed cavities in soil, fissures in rocks, under rotten logs, under animal feces, in dead snail shells, in curled-up dead leaves, under coconuts, in roots or pseudobulbs of epiphytes, in decaying tree limbs, in tree-trunk mosses, in debris accumulated in tree crotches, hanging from tree trunks, clay banks	1 chamber	Inconspicuous hole, broad funnel-like auricles, small opening surrounded by circular or crescent-shaped crater	(Adams and Longino, 2007; Dejean and Olmsted, 1997; Klingenberg et al., 2007; Mueller and Wcislo, 1998; Ramos-Lacau et al., 2012; Schultz et al., 2002; Weber, 1941, 1945, 1946, 1947, 1969b, 1972b, 1982; Wheeler, 1907)
Kalathomyrmex	–	–	x	–	Underground.	Up to 2 chambers	Inconspicuous nest mound.	(Bucher, 1974; Klingenberg and Brandão, 2009)
Mycetagroicus	–	–	x	–	Underground; very deep nests up to 371 cm deep	2-4 chambers	Single entrance hole, in some cases surrounded by mound of excavated soil	(Jesovnik et al., 2013; Solomon et al., 2011)
Mycetarotes	–	–	x	–	Underground.	Up to 2 chambers	Single hole in ground, nest entrance surrounded by exhausted fungal substrate, small crater-shaped mound or turret made from excavated sand or clay pellets	(Mayhé-Nunes and Lanziotti, 2004; Mayhé-Nunes and Brandão, 2006; Solomon et al., 2004)
Mycetophylax	–	–	x	x	Underground.	1 to 3 chambers	Circular crater formed by accumulation of grains of soil; sand turret	(Bucher, 1974; Diehl-Fleig and Diehl, 2007; Klingenberg and Brandão, 2009; Klingenberg et al., 2007; Weber, 1941, 1945, 1969b, 1982; Wheeler, 1907)
Mycetosoritis	x	x	x	–	Underground.	1 to 2 chambers	Small mound, single entrance	(Mueller et al., 2010; Wheeler, 1907) AJ, TRS, JSC unpublished.
Mycocepurus	–	x	x	x	Underground, up to 105 cm deep.	1-21 chambers	Inconspicuous hole in ground, irregular crater formed by accumulation of grains of soil	(Fernandez-Marin et al., 2005; Rabeling et al., 2007b; Weber, 1941, 1945, 1969b, 1982; Wheeler, 1907)
Myrmicocrypta	–	x	x	–	Underground, under rocks, and in rotten logs. Underground colonies ranging from 2cm up to 100 cm deep	1 chamber	Inconspicuous hole in ground or small heap of excavated soil surrounding nest entrance.	(Weber, 1941, 1945, 1946, 1969b, 1972b, 1982) JSC unpublished.
Paramycetophylax	–	–	x	–	Underground.	1 chamber	Conspicuous, conical mound, single entrance at top	(Bucher, 1974)
Pseudoatta	–	–	x	–	Underground (parasite of *Acromyrmex*)	N/A	N/A	

N= North, C= Central, S= South, CI= Caribbean Islands.

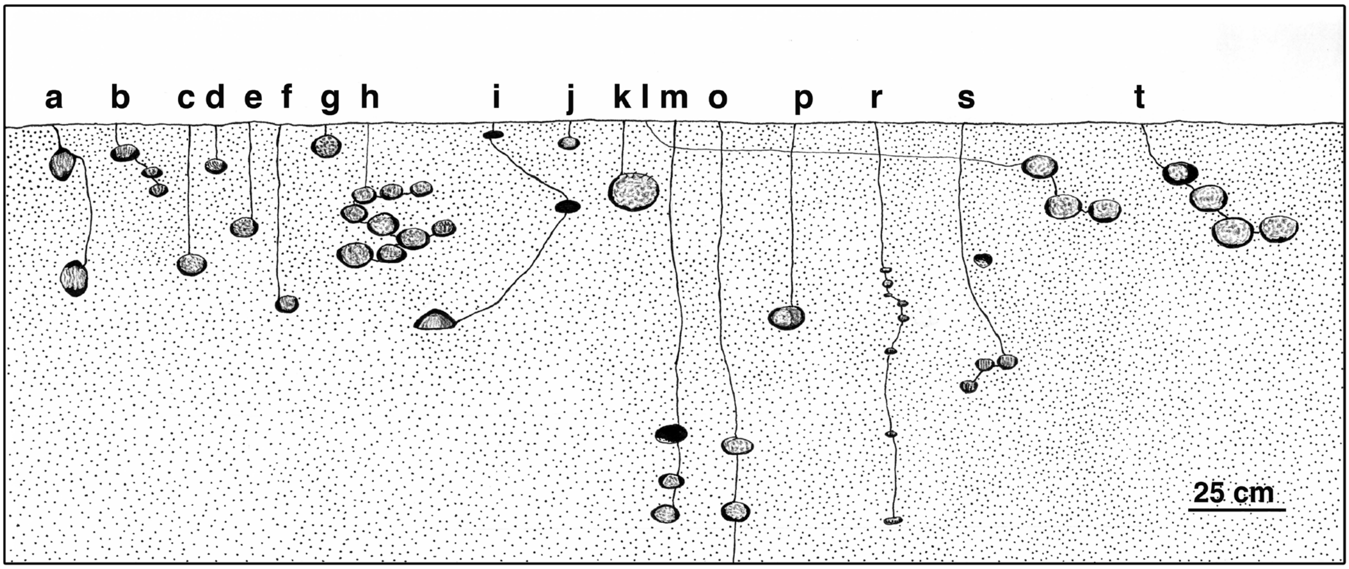

Fig 3. Schematic representation of nests of various ground-nesting fungus-farming ants. **a**. *Trachymyrmex septentrionalis* (Tschinkel, 2003). **b**. *Cyphomyrmex* cf. *rimosus* (CTL110918–01). **c**. *Myrmicocrypta* sp. (JSC080924–05). **d**. *Myrmicocrypta* sp. (JSC100417). **e**. *Trachymyrmex* sp. (TRS111004–05). **f**. *Kalathomyrmex emeryi* (TRS121007–02). **g**. *Mycetarotes acutus* (TRS120802–4). **h**. *Sericomyrmex* cf. *harekuli* (AJ111125–08). **i**. *Mycetophylax simplex* (Klingenberg, 2007). **j**. *Cyphomyrmex cf. strigatus* (CTL110926–02). **k**. *Apterostigma sp.* (JSC111007–06). **l**. *Sericomyrmex sp.* (AJ121026–02). **m., o**. *Mycetagroicus inflatus* (Jesovnik, 2013). **p**. *Mycetosoritis asper* (AJ141020–02). **r**. *Cyatta abscondita* (Sosa-Calvo et al., 2013). **s**. *Mycocepurus smithii* (Rabeling, 2007). **t**. *Sericomyrmex* sp. (AJ120801–03).

but Petri dishes have two problems: (i) they are constructed of thin, easily breakable polystyrene and (ii) the inner surfaces of the lids have ribs that separate the lid from the bottom to permit air exchange, creating a space through which most smaller ants can escape. Those ribs must be filed down in order to make Petri dishes suitable for field nest boxes. We prefer flexible polypropylene field nest boxes with snap tops (see below).

After nests have stabilized, additional garden fragments and ants are collected and preserved in 95–100% ethanol and/or RNAlater (Qiagen Inc., Valencia, CA, USA) for morphological and molecular work (Fig 2d). All preserved material is stored at the coldest possible temperatures, although in extreme field conditions cold storage obviously may not be available. Isolations from gardens that have been stabilized by the ants may be transferred to standard Potato Dextrose Agar (PDA) plates in order to obtain living monocultures of fungal cultivars as well as *Escovopsis* and other microbes. Although collecting, maintaining, and, through a series of daily transfers, isolating sterile cultures of attine fungus gardens and other microbes is an important field endeavor, it is beyond the scope of this paper to describe this process.

Collection of colonies of leaf-cutting ants (*Atta* and *Acromyrmex*) may be carried out using the above described methods, but the large sizes of many leaf-cutter nests and the presence of a defensive soldier caste with formidable mandibles makes excavation of mature colonies a nightmare. In contrast, incipient (~1-year-old) leaf-cutter nests are quite easy to collect, since most nests at this stage consist of a single garden chamber containing all the workers, brood, and queen, but lack a soldier caste.

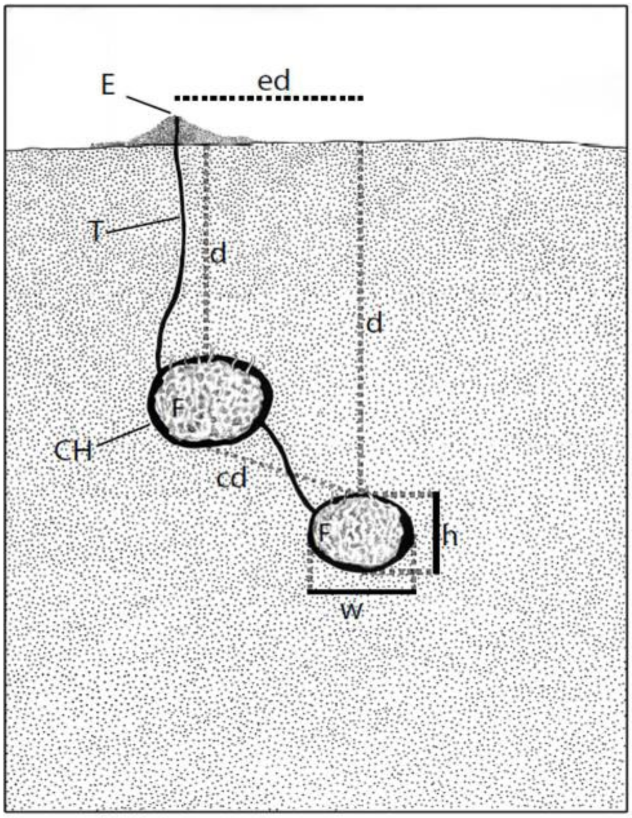

Fig 4. Cutaway view of a subterranean fungus-farming ant nest indicating standardized measurements. E = nest entrance; T = tunnel; CH = chamber; F = fungus garden; d = chamber depth; w = chamber width; h = chamber height; cd = distance between chambers; ed = offset distance from the entrance (measured for chambers that are not directly below the nest entrance).

Colonies and gardens of non-soil-dwelling attine ants are generally far easier to collect than those of soil-dwellers. In species that nest in or under rotten logs or between layers of the leaf litter, a distinct nest entrance may not be present, and gardens may fill irregularly shaped wholly or partly preformed cavities. In colonies nesting in rotten fruits, fungus gardens may occupy hollow cavities corresponding to the vanished endo- and mesocarp.

For maintaining most colonies for extended times in the field, we construct nest boxes from round, flexible, snap-top, polypropylene containers (diameter: 2.65 inch, height: 0.935 inch) (ESD Plastic Containers, Yorba Linda, CA, USA; Table 2, Figs 5a, c, e). For air exchange, we use a "drill-tree" 1/4-inch-to-3/4-inch step-drill bit (e.g., Irwin #3 Unibit 10233) to create a hole ~0.7 cm in diameter in one side of the container (Fig 5e). This hole is covered with fine-mesh, stainless-steel wire screen (Newark Wire Cloth Company, Newark, NJ, U.S.A.) (Table 2), which is permanently fused to the plastic box by melting the plastic in contact with the screen with a soldering iron. Dental stone is poured into the bottom of the container to a thickness of ~0.5 cm (Figs 5a, c, e). Larger colonies are maintained in the field in the same square polystyrene containers that we also employ in the lab (Figs 5b, d, f). See below for details.

Colonies are cared for in the field using the same techniques we employ in the laboratory (see below) except that, in the lab, we connect foraging and refuse auxiliary boxes to the nest box. This is infeasible in the field,

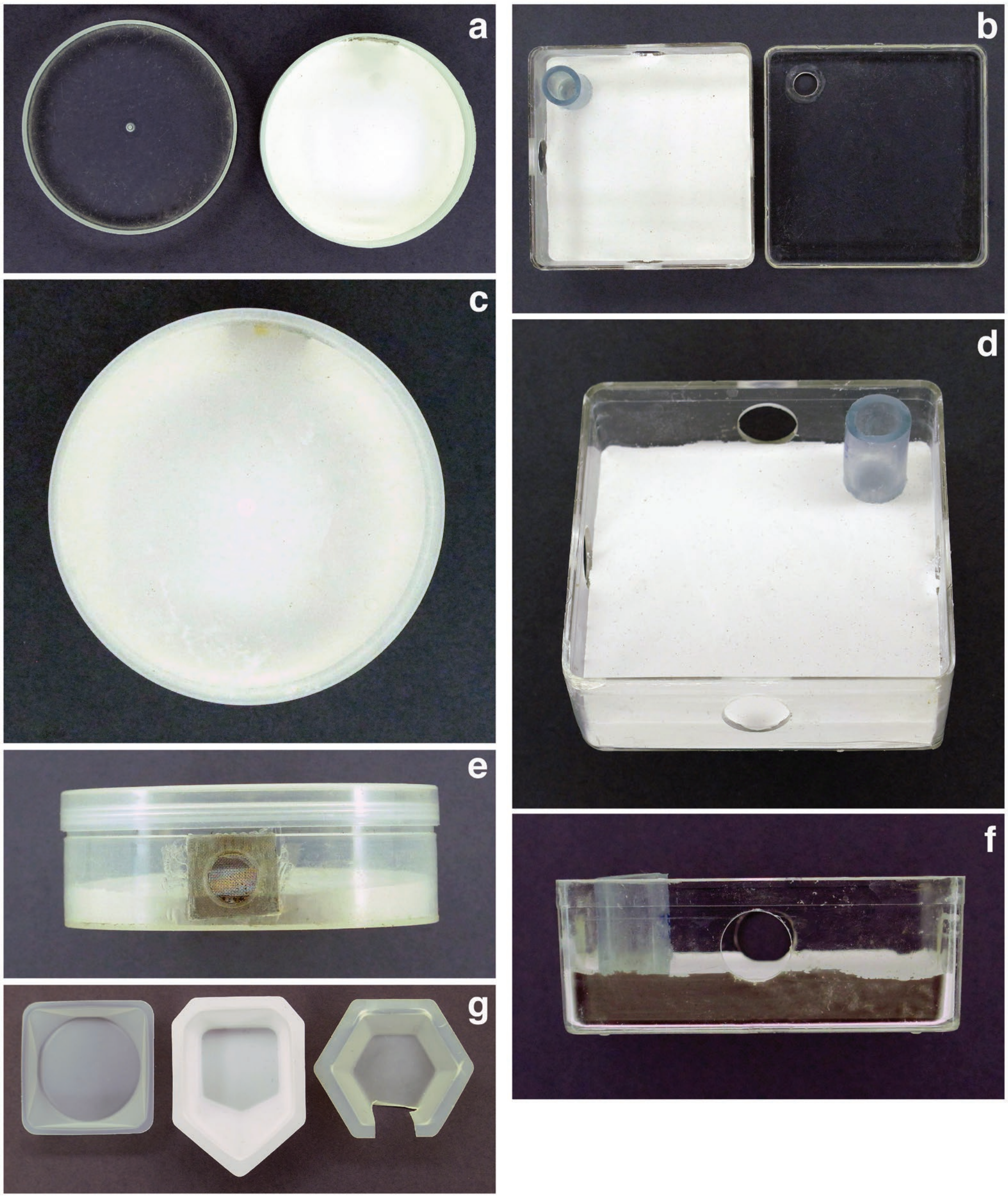

Fig 5. Nest boxes used in the NMNH Ant Lab. **a.** Round, flexible, snap-top polypropylene nest box with plaster, seen from above, with lid. **b.** Small, square, polystyrene nest box with plaster, seen from above, with lid. Water well and tube in upper left corner. **c.** Round polypropylene nest box with lid affixed, seen from above. **d.** Square nest box, oblique view. Water well in upper right corner. **e.** Round nest box, lateral view, with ~0.7 cm diameter hole covered with fine-mesh, stainless-steel wire screen for air exchange. **f.** Square nest box, lateral view, with ~1 cm diameter hole for connections with other boxes. **g.** Various weigh boats used for serving food in auxiliary foraging boxes and also used as platforms to separate fungus gardens from the plaster nest box floor.

however, unless we are in the same location for weeks. As a result, in the field nests must be cleaned of waste and refurnished with food on a daily basis. The plaster or cotton must be checked and resaturated as necessary. In the field we use Cream of Rice™ cereal for food and, if available, insect frass. Colonies are inspected regularly to remove soil or other refuse that the ants have isolated from the garden (Fig 2d).

3. Maintaining colonies of fungus-farming ants in the lab

Starting in 1991 as a graduate student (TRS) and subsequently in the AntLab in the National Museum of Natural History (NMNH), Smithsonian Institution, we have maintained hundreds of colonies of every genus of fungus-farming ant taken from a large number of localities in the Neotropics and the United States. In so doing we have relied on modified versions of methods originally developed by Weber (1972b) and Schultz (1993) based on non-attine-specific methods utilized by previous generations of myrmecologists (summarized in Hölldobler & Wilson, 1990).

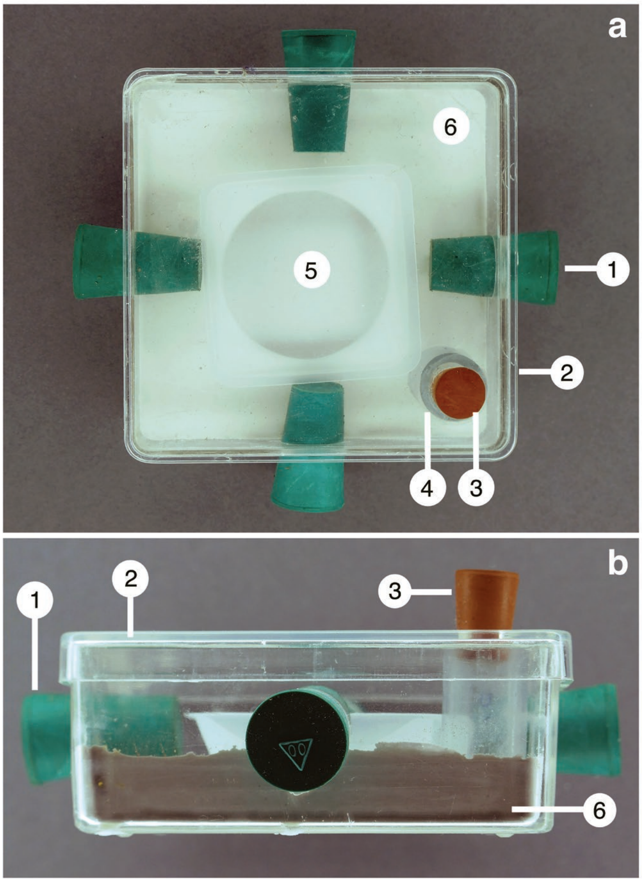

Fig 6. Garden nest box. **a.** Garden box as seen from above. **b.** Garden box as seen from the side. **1.** Neoprene stopper No. 00; **2.** Square polystyrene nest box; **3.** Small, stopper No. 0000; **4.** Short piece of Tygon tubing connecting the water well and the small hole in the lid, used to moisten the plaster without allowing the ants to escape. **5.** Weigh boat used as a platform to separate the fungus garden from the plaster. **6.** Dental stone (plaster) poured into the bottom of the box to a depth of ~0.5 cm.

Most colonies of non-leaf-cutting ants are maintained in the lab in square polystyrene nest boxes with plaster bottoms (Figs 5b, d, f; 6a, b) connected by tubing to other such nest boxes, as needed, and to non-plaster-lined auxiliary boxes that serve as separate foraging and waste disposal chambers (Fig 7). The boxes are made from 2 7/8 inch x 2 7/8 inch x 1 1/16 inch polystyrene plastic containers (Tri-State Plastics, Latonia, KY, USA) with ~0.7 cm thick poured-plaster bottoms (slow-setting lab plaster, Die Stone, Robert Forrester Co., Oreland PA, USA) (Figs 5b, d, f). In each of the four sides of the nest box, a ~1/2 inch diameter hole is drilled using a "drill-tree" 1/4-inch-to-3/4-inch step-drill bit (e.g., Irwin #3 Unibit 10233) (Figs 5b, 6a). These holes serve to connect the nest box to other boxes and, when not in use, are plugged with shortened #00 or #0 neoprene stoppers (Figs 6, 7).

The plaster in the bottom of the nest box is equipped with a water well in one corner (Figs 5d, 6a), carefully drilled into the plaster with a 15/32 inch drill bit. The water well is located directly below a corresponding 5/16 inch hole in the lid drilled with a "drill tree" 1/8-inch-to-1/2-inch step-drill bit (e.g., Irwin #1 Unibit 10231) (Figs 5b, 6–7). A short piece of 3/8 inch inner diameter x 1/2 inch outer diameter Tygon tubing is inserted into the the water well and cut to fit snugly against the hole in the lid (rendering it ~2.6 cm long), effectively isolating the water well to the topological "outside" of the chamber and preventing the ants from entering the well (Figs 5d, 6a). A small #0000 stopper plugs the hole in the lid (Fig 6–7). Notches are cut in the end of the tube that contacts the plaster, facilitating the absorption of water by the plaster when water is introduced into the top of the tube. This arrangement allows the plaster to be wetted without opening the lid of the nest box.

The plastic nest box is connected to other nest boxes and to auxiliary boxes by tubing of 3/8 inch inner diameter, 1/2 inch outer diameter, and 1/16 inch wall thickness (Nalgene™, Thermo Fisher Scientific Inc., Waltham, MA, USA) (Fig 7), which fits snugly in the pre-drilled holes (Figs 5d, 6a). The auxiliary nest boxes lack plaster bottoms and serve, separately, for foraging or for disposal of waste (e.g., spent fungus garden, dead ants) (Fig 7). In order to reduce the humidity in the auxiliary containers and thereby reduce mold growth on food and waste, and to deter the ants from using the auxiliary boxes as garden chambers, a 5/16 inch (~1 cm) diameter hole is drilled into the corner of the lid using a "drill tree" 1/8-inch-to-1/2-inch step-drill bit (e.g., Irwin #1 Unibit 10231) and the hole is covered with fine-mesh, stainless-steel, wire screen (Newark Wire Cloth Company, Newark, NJ, U.S.A) fused to the plastic by melting the plastic at the edges of the screen with a soldering iron (Fig 7a). Food is served in the food/foraging box in a small weigh boat (Figs 5g, 6a, 7, 8a), which facilitates introduction of new and removal of old food (Fig 8a). To facilitate access to the food by smaller ant species, notches are cut into the side of the weigh boat (Figs 7a, 8a). To make it easier for ants to reach the floor of an auxiliary chamber from the connecting tubing, platforms are

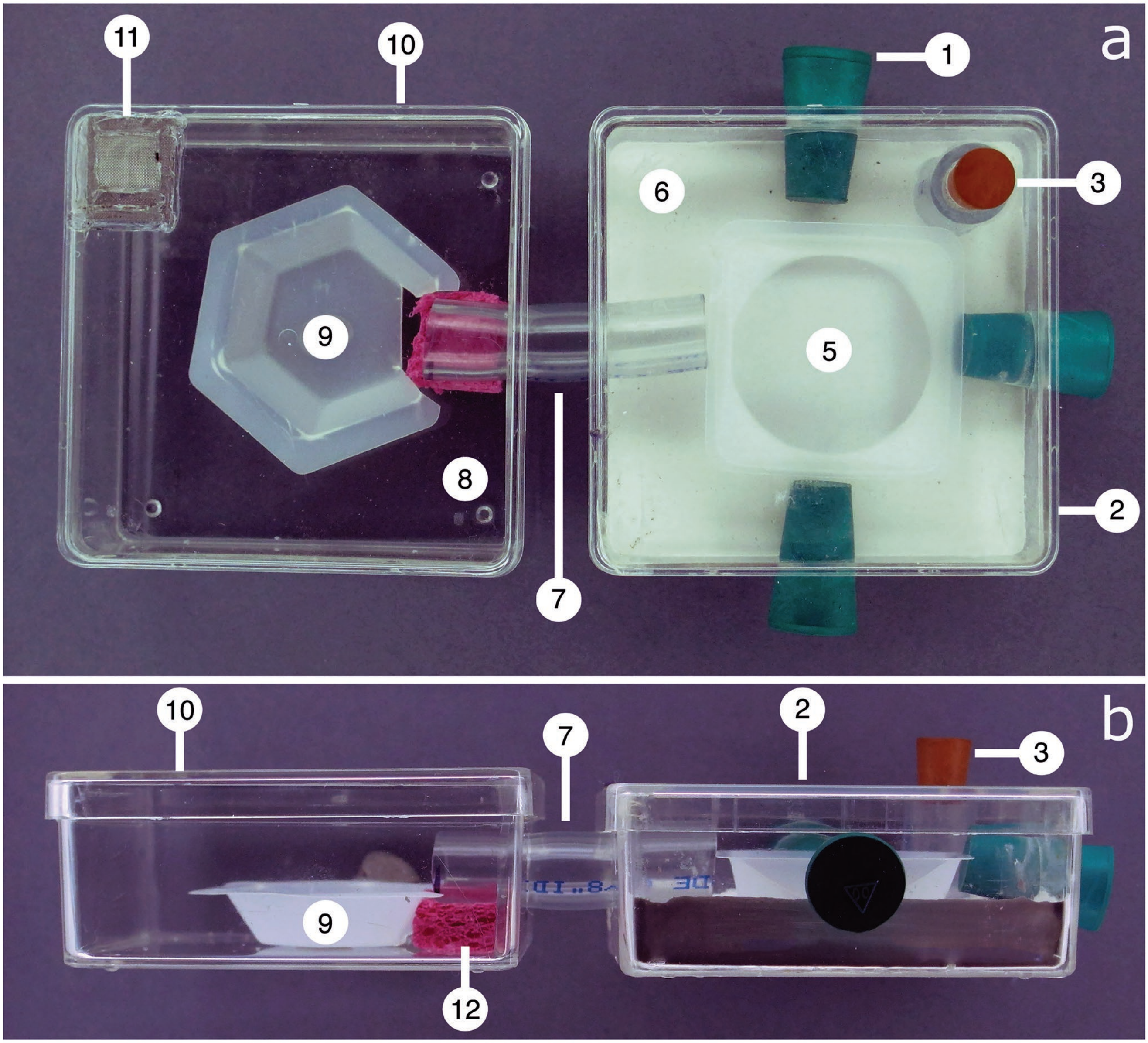

Fig 7. Nest box laboratory set up. **a.** Garden nest box (right) and auxiliary box (left) as seen from above. **b.** Garden nest box (right) and auxiliary box (left) as seen from the side. **1.** Neoprene stopper No. 00; **2.** Square polystyrene garden nest box; **3.** Small stopper No. 0000; **5.** Weigh boat used as a platform to separate the fungus garden from the plaster. **6.** Dental stone (plaster) poured into the bottom of the box to a depth of ~0.5 cm. **7.** Tygon tubing connecting fungus nest box to the auxiliary (foraging) box. **8.** Bottom of auxiliary box without dental stone (plaster). **9.** Weigh boat used to serve the food for the ants. Food in the lab consists mostly of Cream of Rice, caterpillar frass, oak catkins, finely diced inner white part of orange peel, and small pieces of apple. **10.** Auxiliary (feeding/foraging) box. **11.** Ventilation hole (~1 cm) in lid of auxiliary box, covered with fine-mesh, stainless-steel, wire screen fused by melting to the lid. **12.** Small piece of sponge serving as step for ants entering and exiting via the connecting tube.

constructed of small pieces of sponge (Figs 7, 8a). We supply food, re-saturate the plaster as needed, and remove waste a minimum of three times a week. Different attine ants prefer to forage on different food items. We supply colonies with Cream of Rice^{TM} (also used as bait in the field), small pieces of apple (from which the ants directly imbibe juice), caterpillar frass (collected from the NMNH Insect Zoo), the mesocarp (white inside) of orange peel finely diced in a food processor, and oak catkins, collected fresh, sifted, and dried (Fig 8a).

Larger colonies, e.g., those of *Atta*, *Acromyrmex*, and some *Sericomyrmex* species, are maintained in plastic boxes identical to those used by pioneering attinologist Neal Weber (see photographs published in Weber, 1972a, b, c). Large nest boxes are constructed from 7 7/16 inch x 5 5/16 inch x 3.75 inch polystyrene refrigerator boxes (Tri-State Plastics, Latonia, KY, USA) (Fig 8b). Holes ~3 cm in diameter are drilled in both ends of the box ~1.75 cm from the bottom in two steps, first using a “drill-tree” 1/4-inch-to-3/4-inch step-drill bit (e.g., Irwin #3 Unibit 10233), then following with a “drill-tree” 3/4”-1 3/8” step-drill bit (e.g., Irwin #5 Unibit 10235) until the holes reach the desired diameter of ~3cm. Attwood® (1 1/2 inch) Thru-Hull hose connectors are inserted into the holes and boxes are connected to one another with Tygon™ (1 inch ID x 1 1/4 inch OD x 1/8 inch Wall) tubing (Saint-Gobain Performance Plastics, Akron, OH, USA) (Fig 8b). Because humidity is generally fairly stable in larger fungus gardens, plaster bottoms are usually not necessary in large nest boxes. However, when smaller gardens are introduced into a large nest box, humidity is controlled in two ways: (i) a wad of cotton or an open plaster-bottom box is placed in the larger nest box and the cotton or plaster is resaturated frequently and (ii) a cylindrical/conical plastic carry-out food container is inverted over the garden (Fig 8b). For large auxiliary containers, a rectangular hole 7.0 x 7.5 cm (or 7.5 x 13 cm) is cut in the lid using a 10-inch (25.5 cm) hacksaw. The hole is covered over by an 8.5 x 8.5 cm (or 8.5 x 14 cm) fine-mesh wire screen, which is fused to the plastic by melting the plastic at the edges of the screen with a standard soldering iron. Food for leaf-cutters (or, more properly, for their fungi) consists mostly of almost any kind of fresh leaves collected

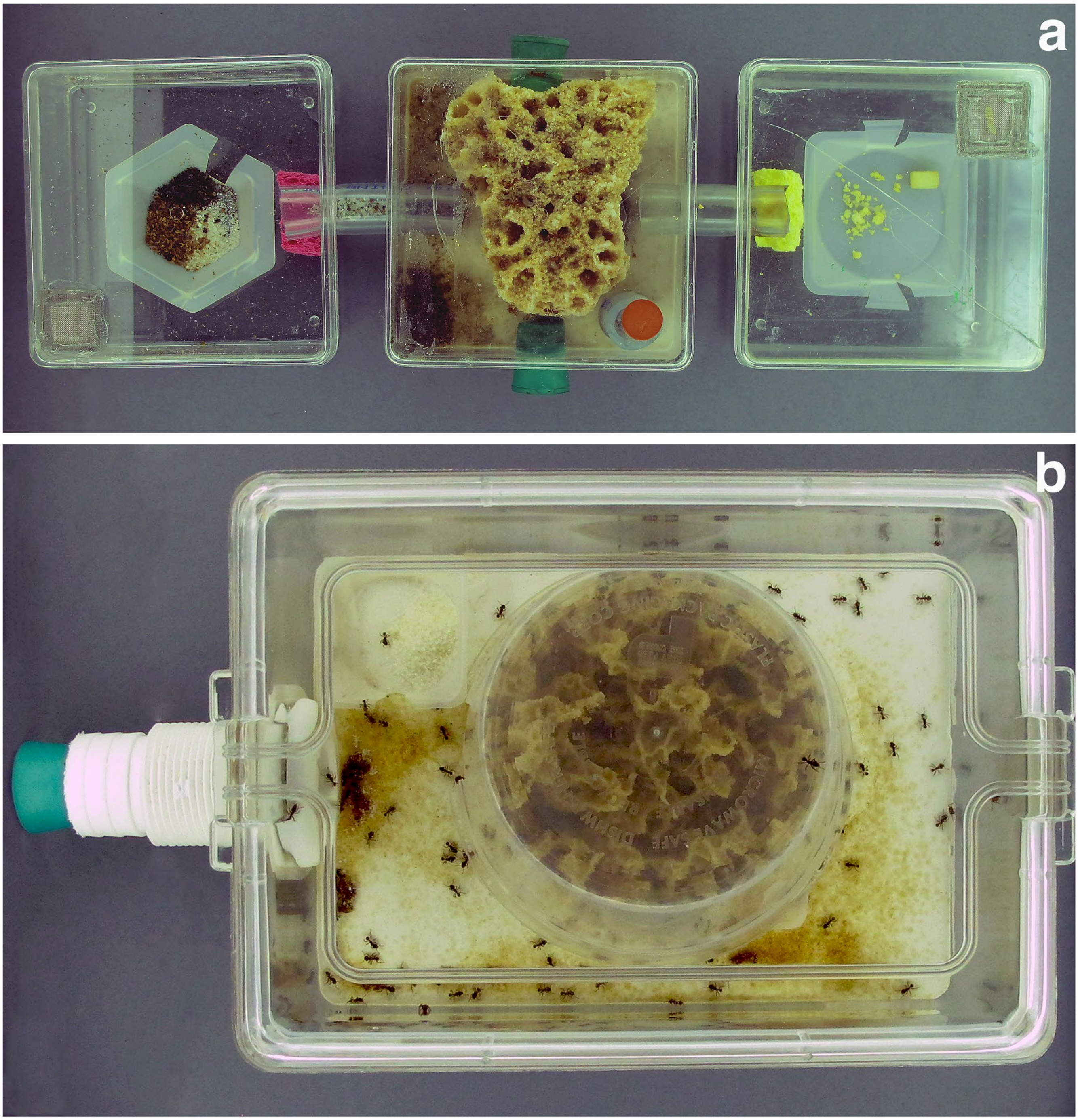

Fig 8. Examples of live colonies maintained in the NMNH Ant Lab. **a.** Nest of *Trachymyrmex* cf. *bugnioni* (TRS141003–04). **b.** Nest of *Sericomyrmex* sp. (AJ141001–05) in large polystyrene nest box with a plaster bottom. To maintain constant high humidity, the fungus garden is temporarily covered by a plastic food storage container.

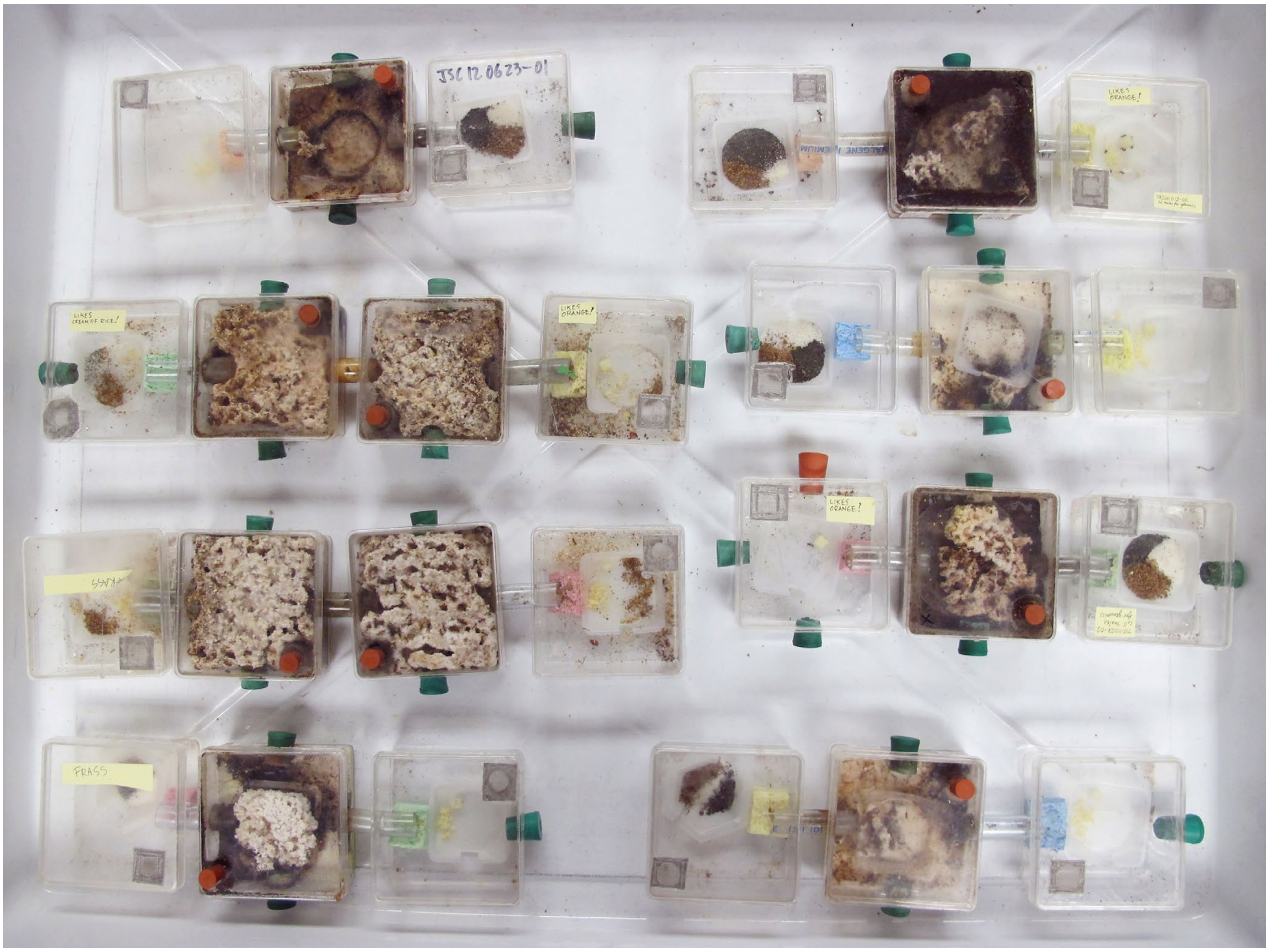

Fig 9. Non-leaf-cutting fungus-farming ant colonies maintained in the NMNH Ant Lab. The nests are arranged in a 20 inch x 24 inch photo-developing tray.

throughout the summer and frozen for use during the winter months. In addition to leaves, the ants also receive pieces of apple and, when conditions in the nest box are overly humid, oatmeal rather than leaves.

For efficiency in space and handling, the nest boxes of both non-leaf-cutting and leaf-cutting ant colonies are organized into groups inside of multiple 20 inch x 24 inch photo-developing trays (Fig 9), which are in turn stored on specially constructed laboratory shelves.

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Table 2. Materials and tools for collecting fungus-farming ant colonies.

Materials	What we use	Where we get it	Comment/explanation
Bait	Cream of Rice, barley, or farofa	Most grocery stores	
Magnifying glass	Hastings Triplet 20x magnifier	http://www.opticsplanet.com/ausch-lomb-triplet-magnifiers.html http://www.bausch.com	Cat No. 816181, focal distance: 1.3cm, diameter: 8.3mm. Bausch & Lomb, 1400 N. Goodman St., Rochester, NY 14609, USA
GPS	GPSMAP 60CSx, GPSMAP 60Cx, GPSMAP 62St Handheld GPS Navigators	www.garmin.com	Garmin International, Inc., 1200 E. 151st St., Olathe, KS 66062-3426 (Kansas City Metro Area), USA
Collecting tools			
Basic aspirator tubing	Nalgene™ 180 PVC Tubing, ¼ ID x 3/8 OD x 1/16 in Wall	http://www.thermoscientific.com	Product No. 8000-4060
Basic aspirator glass tubing	PYREX® Tubing, 7mm OD x 48 in LG Standard Wall Tubing	http://www.corning.com	Product No. 234070, Corning INC, One Riverfront Plaza, Corning, NY 14831, USA.
Basic aspirator mesh	Cotton Organdy Fabric	http://www.voguefabricsstore.com	To prevent specimens from entering collector's mouth
Basic aspirator filter	ACE Fuel filter, fits ¼ in or 5/16 in tube	http://www.amazon.com or http://www.acehardware.com	Cat No. 71903
Basic aspirator filter	Maxpower precision parts, fuel filter, fits ¼ in tube	http://www.amazon.com or http://www.elawngarden.com	Cat No. 334283
Receptacle aspirator	Bug Vac, inlet tube: inside diameter= 5.6mm, outside diameter= 6.3mm. Inlet tube is 14 cm length.	http://www.roseentomology.com	Rose Entomology, P.O. Box 1474, Benson, AZ 85602, USA
50 ml clear plastic centrifuge tubes	Corning® Self-standing Centrifuge Tubes, Polypropylene, sterile	https://us.vwr.com/store/catalog/product.jsp?catalog_number=21008-777	For the aspirator above. VWR International, 2039 Center Square Rd, Bridgeport NJ 08014, USA. Cat No. 21008-775 or -777
Large vials	8ml, 57 x 16.5 mm, flat base, Polypropylene (PP), with enclosed O-ring	https://www.sarstedt.com/en/	8ml vial [60.542.007], screw cap [00.500.744]
Small vials	2ml Micro tube, PP, flat, with attached cap	https://www.sarstedt.com/en/	2ml Micro tube [72.694.100]
Whirl Pak bags	24 oz., Write-on style, 3mil	http://www.fishersci.com	For collection of very large nest series, Catalog No.: 01-812-1A
Soft forceps	Featherweight Forceps, Narrow Tip	https://www.bioquip.com	Cat No. 4748, Narrow, squared point 0.05 in (1mm); blade length 2-1/8 in (54mm); overall length 4-1/2 in (115mm).
Soft forceps	Featherweight Forceps, Wide Tip	https://www.bioquip.com	Cat No. 4750, Wider, rounder tip 1/8 in (3mm); blade length 1-1/4 in (32mm); overall length 4-1/4 in (108mm).
Watchmaker's forceps	Forceps, Swiss, #4A Stainless Steel	https://www.bioquip.com	Cat No. 4523, very fine yet extra strong tips (33 mm), 4-3/8 in (111 mm) long.

Table 2. Materials and tools for collecting fungus-farming ant colonies (Continuation).

Materials	What we use	Where we get it	Comment/explanation
Soft forceps	Live Insect Forceps, Sharp	http://www.finescience.com	Cat No. 26029-10, Tip dimensions: 0.9 x 0.15mm, length: 10cm, Stainless Steel.
Soft forceps	Live Insect Forceps, Blunt	http://www.finescience.com	Cat No. 26030-10, Tip dimensions: 2.3 x 0.3mm, length: 10cm, Stainless Steel.
Ultra fine forceps	Moria Ultra Fine Forceps	http://www.finescience.com	Cat No. 11370-40, Tip dimensions: 0.01 x 0.005mm, length: 11cm, style: MC40.
White plastic for groundcloths	Premium shrink wrap	https://dr-shrink.com	When cut into 1m x 2m sheets, ideal for reducing exposure to ticks and chiggers when sitting or lying on the ground. Dr. Shrink, Inc., 315 Washington Street, Manistee, Michigan 49660, USA. 12 ft wide x 149 ft long, 1,788 square feet [3.66m x 45.42m, 166.24 square meters]. Cat No. DS-127149
Headlamp	Petzl Myo RXP headlamp, Tikka® XP headlamp	http://www.petzl.com	Petzl, Cidex 105A – ZI Crolles 38920 Crolles - France
Flagging tape	Flagging 60 ft Length	http://www.fishersci.com	To mark nest entrances
Nylon fishing line	0.70 mm diameter	Any fishing store	For following nest tunnels
Folding shovel	Gerber folding shovel	http://www.gerbergear.com	Folding spade, serrated. Item No. 30-000075. Gerber Gear, 14200 SW 72nd Avenue, Portland, OR 97224, USA
Folding pruning saw	Corona RS 4040 Razor Tooth Folding Pruning Saw, 6-1/2 in Blade	http://www.amazon.com	For cutting away large roots
Knife	Gerber Paraframe I– Ti-Grey Serrated	http://www.gerbergear.com	Item No. 22-48445. Gerber Gear, 14200 SW 72nd Avenue, Portland, OR 97224, USA
Pruning shears	Corona BP 6250 Forged Aluminum Bypass Pruner 1 in	http://www.amazon.com	For clipping small roots
Sharpening tool	Corona AC 8300 Sharpening Tool	http://www.amazon.com	
Measuring tape		http://www.amazon.com	
Small ruler		http://www.amazon.com	To provide scale in photographs of nest entrances and chambers.
Small ruler	6 in Vinyl evidence photo scales/rulers	http://stores.crimetech.net	To provide scale in photographs of nest entrances and chambers. CrimeTech, Inc., 10950 San Jose Blvd, Ste 60-200, Jacksonville, FL 32223, USA. Pack of 10 rulers. Cat No. 66-3810.
Adhesive ruler	20mm adhesive photo scales	http://stores.crimetech.net	To provide scale in photographs of nest entrances and chambers. CrimeTech, Inc., 10950 San Jose Blvd, Ste 60-200, Jacksonville, FL 32223, USA. Roll of 250 stickers. Cat No. AM-20MM.
Stainless steel spoons	Teaspoons and table spoons	Any supermarket	For lifting the fungus garden out of the chamber

Table 2. Materials and tools for collecting fungus-farming ant colonies (Continuation).

Materials	**What we use**	**Where we get it**	**Comment/explanation**
Disposable lighter		Any supermarket	For flame-sterilizing spoons and forceps to prevent cross-contamination by microbes from one excavated nest to the next.
Field book	Rite in the Rain, all-weather horizontal line No. 390N	http://www.riteintherain.com	
Label paper	WAUSAU® Bright White Papers, 65lb, 216 x 279 mm, acid- and lignin-free	http://www.neenahpaper.com	Neenah Paper, Inc., 3460 Preston Ridge Road Suite 600, Alpharetta, GA 30005, USA.
Label paper	Byron Weston Paper Company, white, linen ledger, 100% cotton, acid-free, 36lb paper	http://minutebooks.com/record.php	Lautzenhiser's Stationery, 1802 Eastman Avenue #109 Ventura, CA 93003, USA
Pencils		Any supermarket	
Labeling pens (insoluble in alchohol)	Zig Millenium 005mm Archival Pen	http://www.dickblick.com/items/21304-2002/	
Labeling pens (insoluble in alchohol)	Sakura® Pigma® Micron® 005 (0.20mm line-width), black archival ink.	http://www.sakuraofamerica.com	Cat No. XSDK005-49
Labeling pens (insoluble in alchohol)	Sakura® Pigma® Micron® 01 (0.25mm line-width), black archival ink.	http://www.sakuraofamerica.com	Cat No. XSDK01-49
Labeling pens (insoluble in alchohol)	Sakura® Pigma® Micron® 02 (0.30mm line-width), black archival ink.	http://www.sakuraofamerica.com	Cat No. XSDK02-49
Sharpies®	Fine Point Permanent Marker	http://www.sharpie.com	
Sharpies®	Ultra Fine Point Permanent Marker	http://www.sharpie.com	
Nest boxes	Square or round containers	See Table 3	Without plaster, to allow colony to reconstitute their garden after collection and before to being transferred to wetted nest boxes
Storage container	Food storage container ~9.4 x 9.4 x 2.2 in (24 x 24 x 6 cm)	http://www.amazon.com	To sort ants and garden fragments that are mixed with the soil.
Cotton		Any supermarket or pharmacy	To maintain humidity in nest boxes without plaster bottoms
Water bottle			For wetting the nests
Soil thermometer	Chaney Soil Thermometer	http://www.amazon.com	For taking soil and air temperature of the nest surroundings
RNAlater	RNAlater RNA Stabilization Reagent (250 ml)	http://www.qiagen.com	Cat No. 76106. QIAGEN Inc., 27220 Turnberry Lane, Suite 200, Valencia, CA 91355, USA
95% ethanol			For preserving ants and garden

Table 3. Materials and tools for maintaining colonies of fungus-farming ants in the laboratory.

Materials	What we use	Where we get it	Comment/explanation
Square container	Tri-State Plastics 006-C, 2 7/8 in x 2 7/8 in x 1 1/16 in Cap. 23 oz.	http://www.tristateplastics.com	Nest boxes; Tri-State Plastics 4306 Boron Ave., Latonia, KY 41015, USA
Round container	Nucon 2-piece, lid design: flat solid lid; Diameter: 2.65 in Height: 0.935 in	http://www.esdplasticcontainers.com	Nest boxes; Cat No. 260900. ESD Plastic Containers, 24895 East La Palma Ave., Yorba Linda, CA 92887, USA
Large container	Tri-State Plastics 079-C, 7 7/16 in x 5 5/16 in x 3.75 in Cap. 80 oz.	http://www.tristateplastics.com	Nest boxes; Tri-State Plastics 4306 Boron Ave., Latonia, KY 41015, USA
Large tubing	Tygon™ R-3603 Clear Laboratory Tubing, 1 in ID x 1 ¼ in OD x 1/8 in Wall (ACC00062)	http://www.fishersci.com	Saint-Gobain Performance Plastics, 2664 Gilchrist Road, Akron, OH 44305, USA
Small tubing	Nalgene™ 180 PVC Tubing, 3/8 in ID x ½ in OD x 1/16 in Wall	http://www.thermoscientific.com	Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02451, USA. Product No. 8000-4120
Plaster	Dental lab plaster, slow set, 30lbs	http://www.ebay.com/usr/plasterguys?_trksid=p2047675.l2559	Robert Forrester Co., PO BOX 25, Oreland PA 19075, USA
Plaster	Dental stone casting material	http://tritechforensics.com/store/product/dental-stone-casting-material/	Tritech Forensics, 4019 Executive Park Blvd., SE Southport, NC 28461-8026, USA
Plastic weigh boats (small)	Feeding dishes for ants	http://www.fishersci.com	Fisher Scientific, 300 Industry Drive, Pittsburg, PA 15275, USA. Cat No. 70040; Plastic micro boat 50-278-83
Neoprene stoppers	Fisherbrand™ Solid Neoprene Rubber Stoppers	http://www.fishersci.com	Fisher Scientific, 300 Industry Drive, Pittsburg, PA 15275, USA. Different sizes, including No. 00, 0, 1, 2, 3, 4. Catalog No. 14-141A–14-141E. Recommended where rubber would be attacked or deteriorated
Connector large containers	Attwood® 1-1/8 in - 1-1/4 in ID Hose Thru-Hull Connector	http://www.attwoodmarine.com/store/product/thru-hull-plastic-resins	Attwood® Corporation, 1016 N. Monroe Street, Lowell, MI 49331, USA
Step drill bit	Vermont America, Industrial Duty, Drill tree titanium step drill bit, 1/8 in –1/2 in	http://www.homedepot.com; http://www.amazon.com	To open holes in small nest boxes ½ in (~1.3 cm) for connectors and 1/3 in (~0.9 cm) for water
Step drill bit	Irwin #3 Unibit 10233 ¼ in-3/4 in step-drill bit	http://www.homedepot.com; http://www.amazon.com	To open holes in small nest boxes ½ in (~1.3 cm) for connectors and 1/3 in (~0.9 cm) for water
Step drill bit	Irwin #5 Unibit 10235 ¾ in-1 3/8 in step-drill bit	http://www.homedepot.com; http://www.amazon.com	To open holes in larger nest boxes ~3 cm for connectors and foraging area
Mini saw	Stanley mini hack saw, with 10-inch steel hack saw blade	http://www.amazon.com	For sawing roots that may be present, Catalog No.: 01-812-1A
PVC hose barb connector	Inserts 90º Elbow, insert x insert, 1 in Lasco® D-2609	http://www.lascofittings.com	LASCO Fittings, Inc., 414 Morgan Street, P.O. Box 116, Brownsville, TN 38012, USA. Part No. 1406010
PVC hose barb connector	Inserts Tee, Insert x Insert x Insert, 1 in Lasco® D-2609	http://www.lascofittings.com	LASCO Fittings, Inc., 414 Morgan Street, P.O. Box 116, Brownsville, TN 38012, USA. Part No. 1401010
PVC hose barb connector	Inserts Coupling, Insert x Insert, 1 in Lasco® D-2609	http://www.lascofittings.com	LASCO Fittings, Inc., 414 Morgan Street, P.O. Box 116, Brownsville, TN 38012, USA. Part No. 1429010
Fine-mesh stainless-steel wire screen	Newark Wire Cloth Company: 100 x 100 meshes per linear inch, 0.0045 diameter wire	http://www.newarkwire.com	Newark Wire Cloth Company, 351 Verona Ave., Newark, NJ 07104, USA
Soldering iron	X-Acto Precision soldering iron and hot knife tip, X73780, 25W	http://www.amazon.com	To fuse wire cloth to plastic
Trays	Cescolite Heavy-Weight Plastic Developing Tray (White) - for 20x24 in Paper	http://www.bhphotovideo.com	To organize nest boxes
Trays	Doran Plastic Ribbed Developing Tray - for 20x24 in Paper	http://www.bhphotovideo.com	To organize nest boxes