

# How to Study Ants

## Collecting Ants

Collecting ants is simple and straightforward and anyone can do it very easily. We routinely place specimens in 80 percent ethanol or isopropyl alcohol; the latter substance is especially useful because it can be obtained as rubbing alcohol in many parts of the world without a prescription. (An unusual but workable approach was taken by the late astronomer and amateur myrmecologist Harlow Shapley, who used to preserve ants in the strongest spirits of the country he visited. A worker of *Lasius niger* he placed in vodka while dining with Stalin in the Kremlin is now in the Museum of Comparative Zoology at Harvard.)

The vials we favor are small and slender, 55 millimeters long and 8 millimeters wide, dimensions that allow many to be kept in a small storage space or carried in a pocket or field pack. They are closed with neoprene stoppers, which permit preservation of "wet" material for many years. A few wider bottles, 55 millimeters long and 24 millimeters wide, are carried to accommodate the largest ants.

Workers should be collected whenever possible. You can mix them, as to both colonies and species, if you find the ants foraging singly (this fact should be noted on the label). If you discover the colony, however, you should put together a sample of at least 20 workers in a vial, along with up to 20 queens, 20 males, and 20 larvae if these can be captured. In emergencies, when the supply of vials is running low, you can place several nest series (that is, members of several colonies) in the same vial and separate them from one another with tight plugs of cotton. Up to four nest series can thus be accommodated in a typical, 55 X 8 millimeter vial. In small but clear letters, write a label of the following kind with a sharp pencil or indelible ink:

Florida: Andytown, Broward Co.

VII- 16-87. E. O. Wilson. Scrub hammock,

nesting in rotting palm trunk.

To pick up the ants, use stiff narrow forceps with pointed (but not needle-sharp) tips. A pair of very sharp watchmaker's forceps, for example Dumont No. 5, can be carried for use with exceptionally small ants.

A rapid, efficient method is to moisten the tip of the forceps with alcohol from the vial and touch it to the ant; this procedure fastens the specimen to the forceps long enough to transfer it to the liquid in the vial. Fine, flexible forceps can also be carried for the collection of live specimens, if these are needed for behavioral observation.

To conduct a general survey of a particular locality, continue collecting until you have encountered no previously unseen species for a period of several days. Work primarily during the day, but search through the same area at night with a flashlight or headlamp to pick up exclusively nocturnal foragers. A good

collector can obtain a virtually complete list of the fauna in an average site of 1 hectare (about 2½ acres) within one to three days. Habitats with dense, complex vegetation, however, such as those in tropical rain forests, are likely to take much longer and require special techniques such as arboreal fogging with insecticides.

For ordinary arboreal collecting, rake branches and leaves back and forth with a strong sweep net. Then break open hollow dead twigs on bushes and trees. This technique will reveal colonies of species, especially those with nocturnal habits, not readily discovered in any other way.

Often it is possible to make rapid, clean collections by snapping the inhabited twigs into short segments (3 to 6 mm long) and blowing the contents into the vial. An aspirator can also be used to suck up ants rapidly, particularly when the nest has just been broken open and the inhabitants are scattering. Exercise care in using this technique, because many ants produce large quantities of formic acid, terpenoids, and other volatile toxic substances. The unwary collector is in danger of contracting formicosis, a painful but not fatal irritation of the throat, bronchial passage, and lungs.

For terrestrial species, collect workers foraging on the ground both during the day and at night. It is necessary to look closely for certain species that are small and slow-moving and hence difficult to see.

A favorite technique of ours in sampling forest faunas is to lie prone, clear the loose leaves from a square meter of ground to expose the soil and humus, then simply watch for up to a half-hour for the most inconspicuous ants.

Another is to put out baits of pieces of tuna or cake crumbs and track the laden workers back to their nests.

In open terrain look for crater nests and other excavations, and with a gardener's trowel dig down into them in search of colonies. Turn over rocks and pieces of rotting wood on the ground to seek the species specialized for nesting in such protected sites. Tear open rotting logs and stumps, looking with special care beneath the bark for the small, inconspicuous species that abound in this microhabitat. Spread a ground cloth (a sheet of white cloth or plastic, 1 to 2 m on the side), and scatter leaf litter, humus, and topsoil over it. Break up rotting twigs and small tree branches buried within the litter. Where the humus and litter are relatively thick and moist, they often harbor a large part of the ant fauna and contain many inconspicuous and still poorly studied species.

The following technique has proved effective for collecting whole colonies that nest in small rotting logs and branches lying on the ground.

Pick up a fragment of the decaying wood (say about 50 cm long), hold it above a photographer's developing pan or similar shallow-walled container, and strike the fragment with a trowel several times to shake out portions of the colony. Small pieces of the wood will also fall into the pan, but it is still much easier to locate and collect the ants, including entire colonies, this way than by ordinary excavation.

Slower but more thorough collecting of terrestrial ants can be accomplished with the aid of Berlese-Tullgren funnels, named after the Italian entomologist A. Berlese, who invented them and the Swede A. Tullgren, who modified and improved them.

In simplest form the apparatus consists of a funnel topped by a wire-mesh screen onto which soil and litter are placed. As the material dries out, possibly aided by a light bulb or some other heat source placed

above, the ants and other arthropods fall or slide down the smooth funnel sides into a collecting bottle partly filled with alcohol and suspended tightly under the lower spout of the funnel.

## Preparing Specimens for Museum Work

Ants can be stored indefinitely in alcohol, but it is best to prepare part of the nest series as pinned, dried specimens for convenient museum work.

This step is especially important if the ants are to be given to a taxonomist for identification. It is also the best way to store them in museums as voucher specimens, to serve as references for field or laboratory research (all such studies should be taxonomically verifiable with voucher material).

The standard method for preparing dried specimens is to glue each ant on the tip of a thin triangle of stiff white paper. The tip should approach the right side of the ant and touch her ventral body surface beneath the coxae of the middle legs and hindlegs.

The droplet of glue should be small enough and placed so as not to obscure any other part of the body except a portion of the coxae and ventral alitrunk surface—which have relatively few features of taxonomic importance. Prior to this "pointing" procedure, an insect pin should be inserted through the broad ends of two or three of the paper triangles, so that two or three ants from the same colony can be mounted one to a triangle on each pin.

A rectangular label with the locality data goes beneath the mounted ants, so that when you read the label, the triangles point to the left and the ants point away from you. An effort should be made to get a maximum diversity of castes on each pin: for example, queen, worker, and male, or major worker, media worker, and minor worker.

In the case of large ants, it may be possible to mount only one or two ants to a pin; and in the case of very large ants, it is sometimes best simply to pass the insect pin directly through the center of the thorax.

## Culturing Ants

The culture and study of ants in the laboratory is a relatively simple operation. For many years we have used an economical arrangement that serves for both mass culturing and behavioral observation of a majority of species.

The newly collected colony is brought into the laboratory (preferably with the queen and some of the original nest material) and placed in plastic tubs of a size to accommodate the size of the ants and the number of workers in the colony. For example, fire-ant colonies (*Solenopsis* species) with populations up to 20,000 are readily maintained in tubs about 50 centimeters long, 25 centimeters wide, and 15 centimeters deep.

In order to prevent the ants from escaping, we use various means depending on the humidity of the room in which the ants are to be kept. The sides of the tub are coated with petroleum jelly, heavy mineral oil, talcum powder, or, preferably, Fluon (Northeast Chemical Co., Woonsocket, Rhode Island), a water-based

material that is both effective (providing a silky smooth surface) and long-lasting (but unsatisfactory under humid conditions).

The colony is allowed to settle into test tubes (15 cm long with inner diameters of 2.2 cm) into which water has been poured and then trapped at the bottom with tight cotton plugs, leaving about 10 centimeters of free air space from the plug to the mouth of the tube. The 10-centimeter segment is surrounded by aluminum foil to darken the air space and encourage the ants to move in (most do so promptly).

It can be removed later to allow behavioral studies; most ant species adapt well to light at ordinary room intensities, carrying on brood care, food exchange, and other social activities in an apparently normal manner.

The tubes are stacked at one end of the tub prior to insertion of the colony, leaving most of the bottom surface of the tub bare to serve as a foraging arena.

The nest tubes can also be placed in closed plastic boxes, making it easier to keep the ambient air of the foraging arena moist and hence better suited for forest-dwelling species. The following dimensions are roughly correct for ant species with different-sized workers:

Small. 11 X 8.5 centimeters on the side and 6.2 centimeters deep. Very small ants such as *Adelomyrmex*, *Cardiocondyla*, *Leptothorax*, small *Pheidole*, and *Strumigenys*. These species can also be cultured readily in small, round petri dishes (10 cm diameter, 1.5 cm depth).

Medium. 17 X 12 centimeters on the side and 6.2 centimeters deep. For example, *Aphaenogaster*, *Dorymyrmex*, and *Formica*. Smaller colonies of *Camponotus*, *Messor*, and *Pogonomyrmex*.

Large. 45 X 22 centimeters on the side and 10 centimeters deep. For example, larger colonies of *Pheidole*, *Pogonomyrmex*, and *Solenopsis*.

Variations on the elementary test-tube arrangement can be adapted to ant species with unusual nesting habits. Colonies of arboreal stem-dwelling ants such as *Pseudomyrmex* and *Zacryptocerus* can be induced to move into glass tubes 10 centimeters long with diameters of 2-4 millimeters, the latter dimensions varied according to the size of the workers.

The tubes are closed at one end with cotton plugs. The plugs can be kept moist, but in many cases this is not necessary, because stem-dwelling ants are often adapted to dry nest interiors, and a small dish of water placed nearby is an adequate source of moisture. Each set of tubes containing a colony is then placed in a tub of the kind just described. Or the tubes can be bound horizontally in rows on a rack or potted plant, in order to simulate the natural environment.

Colonies of small fungus-growing ants can be maintained easily in moistened tubes in tubs. Large fungus growers, such as leafcutter ants of the genera *Acromyrmex* and *Atta*, are better kept via a technique developed by the American entomologist Neal Weber.

Newly inseminated queens or incipient colonies are collected in the field and transferred to a series of closed, clear plastic chambers each about 20 X 15 centimeters and 10 centimeters deep (ordinary refrigerator food receptacles with transparent sides serve very well).

The chambers are connected by glass or plastic tubes 2.5 centimeters in diameter, allowing the ants to move readily from one chamber to the other.

Foraging workers are permitted to collect fresh vegetation (possibly supplemented by dry cereal) from empty chambers, from an open tub whose walls are lined with Fluon, or from a tub surrounded by a moat containing water or mineral oil.

As the colony expands in size, the ants fill one chamber after another with the characteristic spongelike masses of processed substratum, through which the symbiotic fungus grows luxuriantly.

Except in the driest laboratory environments, no special water supply is needed, because the ants obtain all the moisture required from the vegetation.

Leaves from a wide range of plant species are accepted by the ants. In the northeastern United States we have most frequently used basswood (linden), oak, maple, and lilac; the last two are especially attractive to the foragers.

The colonies deposit the exhausted substrate in some of the chambers, which can be removed and cleaned from time to time.

## Transporting Colonies

Colonies can be kept for days or weeks at a time in bottles or other tight containers, provided that certain elementary procedures are followed.

The first, absolute rule is that the ants must be given a moist area into which to retreat—not soaking wet, with films or drops of water that might entrap the ants, but an environment with a moist surface and saturated ambient air. The ideal retreat is part of the nest material itself, placed directly in the container, preferably with a portion of the colony in it.

A large piece of moistened (but not dripping wet) cotton wool or paper toweling should be added as backup. The rest of the container can be filled with nest material or loose-fitting paper toweling or other neutral material to prevent the colony from being knocked about excessively in transport.

The colony should be uncrowded, in no case occupying more than 1 percent of the container volume. The lid of the container should be tightly fitted. Unless the colony is unusually active or aggressive, it is not necessary to punch holes in the lid to aerate the interior; in fact, this procedure risks excessive drying.

Once or twice a day the lid can be removed and the container waved gently back and forth to freshen the air. The colony can be given drops of sugar water and fragments of insects or other foods if the duration of the journey is more than several days.

If ants appear dead after remaining in a closed container too long, they may just be narcotized by carbon dioxide. Expose them to the open air for a few hours to see if they can recover.

Because many countries have restrictions on the importation of live insects, it is prudent to check with the appropriate government agencies before collecting live colonies abroad.

In the United States, for example, the U.S. Department of Agriculture (Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Plant Importation and Technical Support) must furnish a permit, which has first to be approved by the appropriate state officials.

The entire procedure usually requires six to eight weeks. The permit must be presented to the appropriate customs officer upon reentry into the United States.

An increasing number of countries restrict the export of preserved and living specimens, including insects, and a special export permit may be required. Local regulations should always be consulted and respected.