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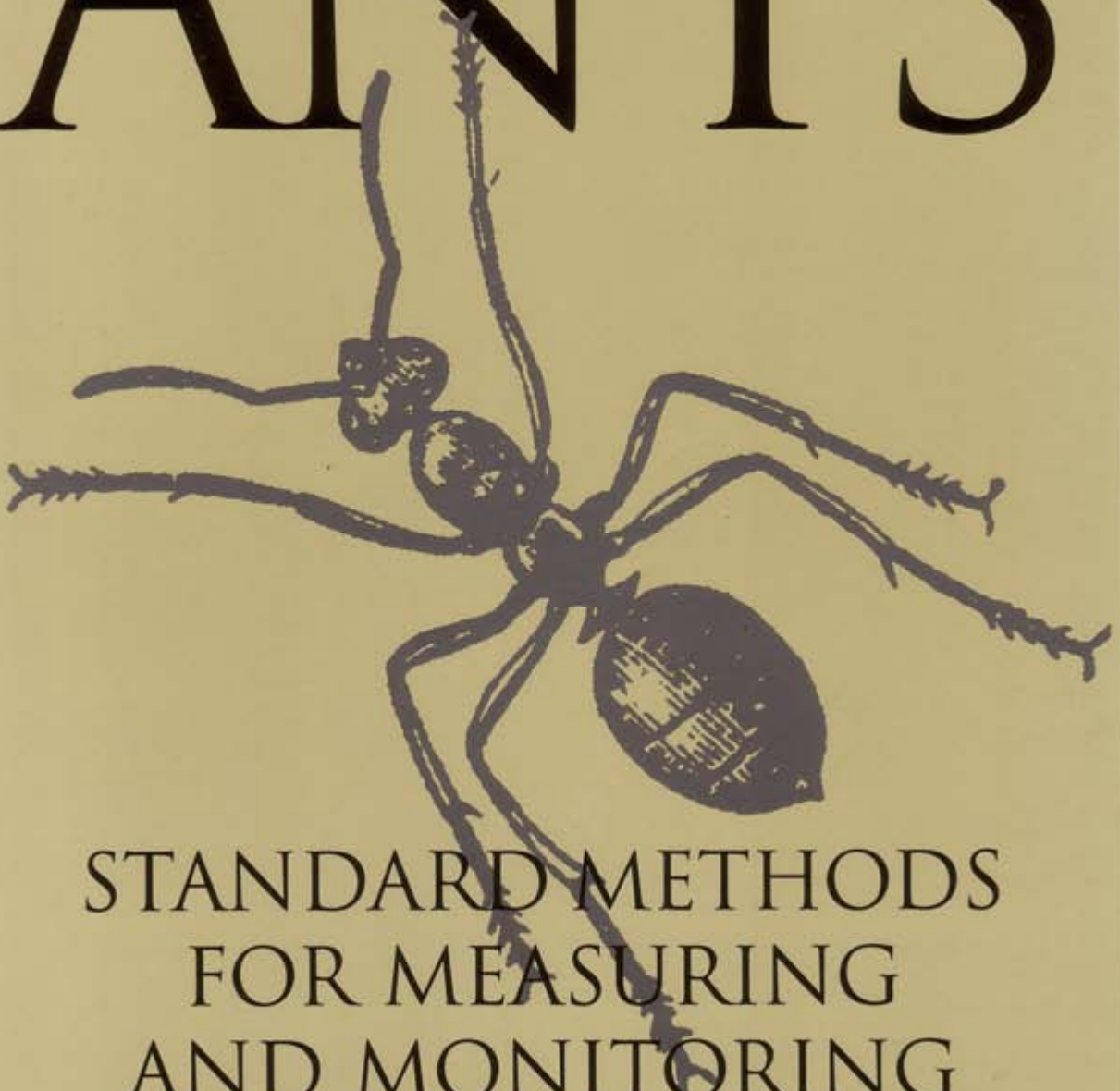

STANDARD METHODS FOR MEASURING AND MONITORING BIODIVERSITY

EDITED BY DONAT AGOSTI, JONATHAN D. MAJER,
LEEANNE E. ALONSO, AND TED R. SCHULTZ

FOREWORD BY EDWARD O. WILSON





ANTS

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Biological Diversity Handbook Series

Series Editor: Don E. Wilson

This series of manuals details standard field methods for qualitative and quantitative sampling of biological diversity. Volumes focus on different groups of organisms, both plants and animals. The goal of the series is to identify or, where necessary, develop these methods and promote their adoption worldwide, so that biodiversity information will be comparable across study sites, geographic areas, and organisms, and at the same site, through time.

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This book is dedicated to the memory of William L. Brown Jr.,
with affection, respect, and gratitude. For the inspiration you provided,
for the firm foundation you built for ant systematics, and especially
for your generous soul and irreverent good humor, we will never forget you, Bill.

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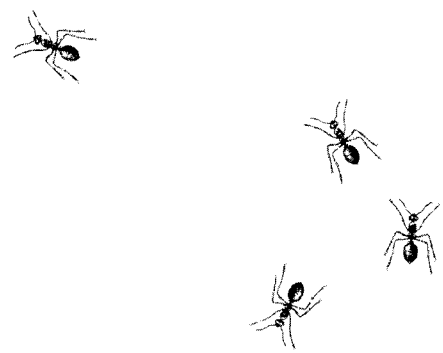

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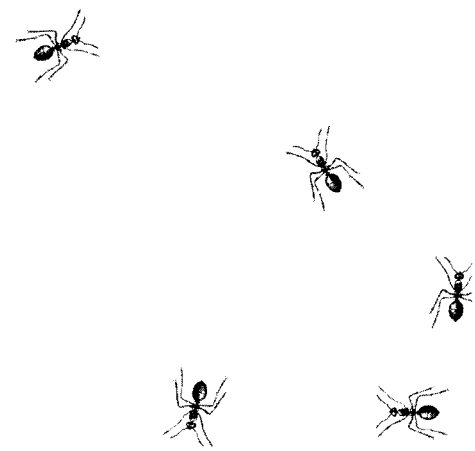
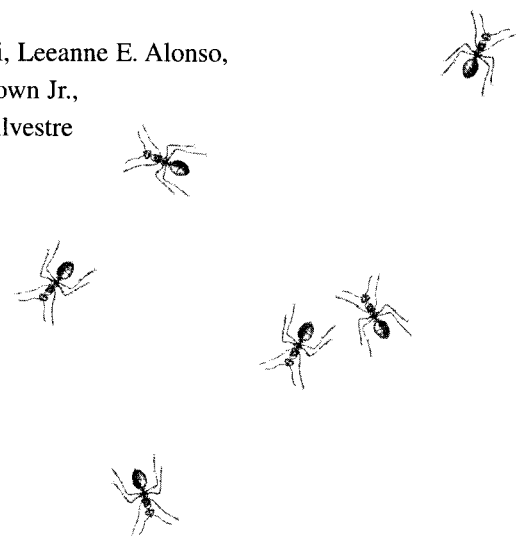


Chapter 9

Field Techniques for the Study of Ground-Dwelling Ants

An Overview, Description, and Evaluation

Brandon T. Bestelmeyer, Donat Agosti, Leeanne E. Alonso,
C. Roberto F. Brandão, William L. Brown Jr.,
Jacques H. C. Delabie, and Rogerio Silvestre



The precise nature of the methods used to estimate the abundance and composition of organisms in biodiversity assessment is of critical importance. Owing to the inevitable limitations of field methods, these estimates are often biased; that is, some species in a given habitat are either over- or underrepresented relative to their true abundances. The estimates obtained from different sampling techniques or from variations in the execution of a particular technique may bias the data in different ways. This fact, in conjunction with differences in sampling design or analytical procedures between studies of a particular system, impedes the direct comparison and integration of data. Integrated data sets based on sound and repeatable methodologies are essential for long-term ecological monitoring as well as for developing a general understanding of patterns of biodiversity.

In this chapter we describe seven field techniques that are used to study ground-dwelling ants and recommend a set of protocols for the use and execution of these techniques. We discuss the special considerations required for each method, and the utility and limitations of each technique for different kinds of research questions and habitats. Our goal is to provide a set of standard, repeatable methods that (1) can be adapted to different research programs and logistical situations, (2) will provide data that are as accurate as possible, and (3) will produce results that are comparable between studies and researchers.

An Overview of Ant Sampling: Challenges and Opportunities

In spite of the abundance and ease of collection of ants in most ecosystems (see Chapter 1), several features of ant biology complicate their sampling. First, ants are variably and nonrandomly distributed on several spatial scales. Individual ants are aggregated into colonies on small scales, and colonies are often regularly dispersed across the landscape owing to competition (Wiernasz and Cole 1995; Crist and Wiens 1996). Thus caution should be exercised with sampling designs and statistical procedures that require the assumption that the subjects are randomly distributed. Second, ants may be studied and sampled both as populations of individual foragers (ignoring colony membership) and as populations of colonies. Forager-based studies often emphasize ecological or functional relationships to the environment (Greenslade 1973; Andersen 1991a), whereas colony-based studies emphasize population or genetic structure (e.g., Herbers and Grieco 1994).

The relationship between the activity and abundance of foragers and colony abundance and distribution varies greatly between species, so that forager- and colony-based comparisons of communities may not be equivalent. For example, given equal colony density, the foragers of highly active ant species with large foraging distances from the colony will be sampled more frequently than those of sedentary species that forage near the colony (Andersen 1991b). Finally, the diversity of behavior and habitat selection exhibited in ants results in different sampling probabilities between species and methods. An obvious example is that arboreal ants are seldom found in leaf litter samples.

The first of the challenges just outlined is of special relevance to the sampling design and analytical procedures used in studies of ant communities and is discussed in Chapter 10. The remaining points also have implications for the field techniques used to census ants. Different methods should be used if one's focus is on ant colonies rather than ant foragers. Different methods of ant collection are required to sample in the different habitats that ants occupy. Furthermore, the several methods that may be employed for a given research question in a particular habitat each have biases owing to practical limitations and differences in species behavior. These biases must be recognized in order to interpret and compare field data correctly.

Two broad categories of questions are commonly addressed in biodiversity assessment: those related to evaluating differences in communities between habitats or sites (e.g., to assess environmental degradation or restoration) and those concerned with species inventory within sites (Heyer et al. 1994; Chapter 13). For purposes of monitoring or comparing ant communities, several features can be examined that respond to environmental variation: richness, species composition, forager abundance, foraging behavior, and colony density. Furthermore, different kinds of ants and ants occupying different microhabitats (litter-dwelling, ground-dwelling, and arboreal) will reflect this variation in unique ways (e.g., Ward 1987). Thus, many different techniques can be used to compare ant communities as long as they provide the desired data, are applied consistently between the sites, and are logistically feasible. On the other hand, the primary goal of a species inventory is to record as many of the species present at a site as possible. For a given habitat, a certain set of complementary techniques will best achieve this objective (Majer, 1996).

See Chapter 2 for more information on how the biology of the ant species under study can influence the choice of sampling methods.

Field Techniques

In the sections that follow, we provide detailed descriptions and evaluations of seven field methods that are commonly used to study ant communities. Some techniques—such as colony, intensive, and direct sampling—overlap slightly with respect to methodology and the kinds of data collected, but we separate the techniques here because of their distinct objectives. Some of the suggestions we make regarding the choice or execution of certain techniques should be evaluated and modified in the course of pilot studies in a given ant community. For example, the abundance and activity of ants will influence the duration of pitfall trapping or quadrat sampling. If too few ants are sampled the research question will remain unanswered, and if too many ants are sampled the investigator may be unable to sort and identify all of the specimens.

The techniques we describe may be divided into two broad classes: passive and active sampling. Passive sampling methods—including pitfall trapping, baiting, and quadrat sampling—are easy to replicate and rely on ant activity at sample stations to obtain data. Active sampling methods—such as direct sampling, colony counts, and intensive sampling—require that investigators seek out ants over the study area and are difficult to replicate precisely between investigators. In general, passive techniques suffer from biases because of differences in the behavior of different ant species in different habitats or alterations in the natural foraging behaviors of the ants. Each technique will systematically miss some ant species. Active sampling techniques introduce bias through differences in the effectiveness of researchers in different habitats, through differences in the detectability of ant species, and through variations in the execution of the sampling techniques. The resulting lack of comparability of samples will hinder spatial or temporal comparisons, such as those in long-term monitoring. Litter extraction is subject to both active and passive sampling biases because the techniques used by investigators vary widely and also depend upon the reactions of ants to behavioral stimuli.

In addition to these broad characteristics, each technique has a particular set of challenges and advantages that investigators should keep in mind when choosing their methods. Here we outline the types of questions that are best addressed by each technique and summarize important criticisms of the data generated by them.

General Materials and Methods

Materials

General preparation for all techniques will require several items, including vials or plastic twirl bags (small plastic bags with a wire-twist closure), ethanol solution, card stock or paper tags, pencils, field notebook, and materials for setting up study plots, including meter tapes, a compass, a random number table or calculator, flagging, stakes, and field tags. See Appendix 1 for a complete list of materials and sources.

Methods

In all of the techniques, ants are collected in the field or afterwards into vials or twirl bags filled with alcohol. Vials should have tight-fitting caps to retard the evaporation of the alcohol. The vials should be filled with at least 75% ethanol, and preferably 90% for long-term storage. Every sample receptacle (e.g., cup, twirl bag, vial) should be clearly labeled with either a temporary or a permanent label. The proper label is a thick paper or card stock tag with the sample code written in pencil. The label should be placed inside the receptacle whenever possible. The sample code should also be recorded in a field book with appropriate information about the identity of the sample (e.g., location, date and time, habitat; see below).

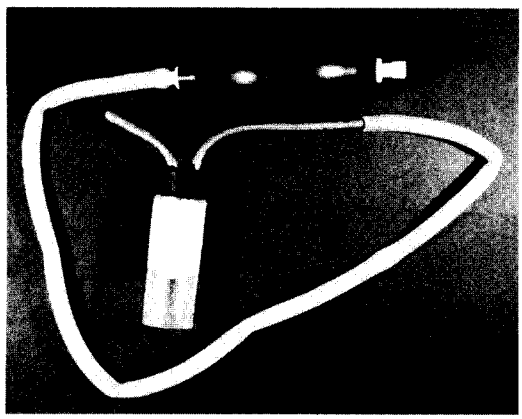


Figure 9.1. An aspirator. Note that the black gas-collecting bulb can be removed and that a vacuum can be created with the mouth. Photo by Brandon Bestelmeyer.

For general ant collecting, forceps and aspirators are required (see Appendix 1). Featherweight forceps are preferred in order to minimize damage to delicate ants. Aspirators are very useful for collecting small or fast-moving species (Fig. 9.1). When air is drawn through the aspirator and the tip of the aspirator is held close to an ant, the ant is drawn into the collecting vial. A small screen prevents ants from being sucked back out of the vial. Air can be sucked through the aspirator with the mouth, but volatile compounds released from several formicine and dolichoderine ants may be irritating to the lungs or cause formicosis. The use of a gas-collecting bulb (see Fig. 9.1) will eliminate this hazard. Studies should be conducted along transects or within plots of standardized area. Metal tags and sturdy metal or wooden stakes may be called for in long-term studies.

General Collection Data

Regardless of collection method, ant specimens are most valuable when accompanied by the fullest possible collection information. Data documentation involves several levels: regional, local, and sample.

REGIONAL. At the regional level one must note the country and lesser political subdivisions, such as state, county, district, or national park. Additional regional information may include geographic features, such as a watershed, peninsula, mountain range, or valley.

LOCAL. Local information includes type of habitat or vegetation in which the collection is being carried out, for example, lowland humid forest, dry forest or scrubland, and altitude. Given the varied names of habitats and ecological communities, it is best to choose a system of nomenclature already in print for ease of comparison. Use the most specific vegetation classification that is available.

At a finer level, microgeographic characteristics of the collection site can be described, including slope, aspect, the presence of gullies or bluffs, soil type, and so forth (see Appendix 2). This information can be especially useful in characterizing the ecological preferences of ant species.

SAMPLE. Each sample—be it from a leaf litter sack, pitfall trap, or individual nest collection—carries its own individual record. It receives a unique collection number that goes into the field notebook. The sample code is the only certain means by which multiple specimens may be recognized as coming from the same colony, or by which trap sample specimens may be linked to a specific sample and to data entries. Codes may be created to reflect the hierarchical structure of a sampling design; for example, SBE2-9 could indicate a pitfall trap sample from site "S," habitat "BE," transect 2, and point 9.

Other collection data at this level can include a brief description of the microhabitat at the sample location, such as a rotten log, under a stone, in a bromeliad, beneath the bark of tree (specify type)—specific data that may help determine microhabitat preferences of ant species. All of the field data we describe should

be recorded as soon as possible; the sooner it is recorded the less chance there is of forgetting a detail that may later prove important, or of mixing up information. Errors should never be erased but crossed out.

Materials

Maps should be of geological survey or cartographic quality, with geographic or Universal Transverse Mercator (UTM) coordinates and elevation contour lines. Road maps are frequently used in lieu of higher-quality maps, but their standards are poor and they are of no use for precisely delimiting areas. Good maps for a particular area however, may be unavailable. Tactical maps used by the U.S. Air Force cover the entire globe and can be purchased by the general public.

Global positioning system (GPS) receivers have dramatically fallen in price over the last few years, and a good-quality receiver can be purchased for $200 or even less in the United States. Given adequate reception conditions a GPS can furnish accurate latitude, longitude, and altitude data for a site.

Suitable notebooks for recording field data are those used by engineers or surveyors. If a notebook with neutral-pH paper is available, then it is highly preferable to ensure the permanence of the data. A sample data sheet is provided in Appendix 2.

Writing materials should include No. 2 or HB pencils or leads, as well as pens or markers with indelible ink (see Chapter 11).

Baiting

Objectives

Baiting uses food substances to attract foraging ants to points where they may be collected or observed. Tuna or sardine baits are the most common (Fig. 9.2), but foods that are richer in carbohydrates—such as fruit jelly, cookie crumbs, honey, peanut butter, or sugar solutions—are also used alone or in combination with proteinaceous baits. Live or dead insects and seeds are employed for special applications.

This technique is commonly used to estimate the composition and richness of the active ground-foraging ant fauna, to examine ant activity and behavior patterns in studies of community structure, and to estimate the contributions of particular ant species to ecosystem processes such as seed redistribution or scavenging. The abundance of ant foragers at baits may help to measure ecological and behavioral dominance and provides a general measure of ant foraging efficiency (Greenslade and Greenslade 1971). Baits can be set out in different microhabitats at different times and can provide information on habitat use, biotic interactions, and activity patterns on very fine scales (Bestelmeyer 1997).

Many factors influence the species composition and abundance of ants at baits. The species most likely to visit baits are trophic generalists. Ant species with marked preferences for particular items (such as leaf-cutting ants or specialist predators) may not visit artificial baits, but dietary generalists represent a significant proportion of ant faunas worldwide and can be used to examine patterns in ant communities. More specialized groups can be targeted for study by using the appropriate bait; grass seeds, for example, can be used to attract desert harvester ants (Davidson 1977a, 1977b).

Baits are used on the surface of the soil or litter as well as in vegetation and underground. Because the activity of different ant species varies with microclimate, daily and seasonally, baiting performed at different times of the day (and night) or year in the same area will attract foragers of different species or of the same species in varying abundance. Because ants may occupy nest sites for long periods, repeated collections in the same location may attract individuals from the same colonies, and temporal variation in colony activity may be exam-

Figure 9.2. A tuna bait monopolized by *Solenopsis xyloni* in a desert grassland in New Mexico, USA. Photo by Brandon Bestelmeyer.

ined. Furthermore, ant species that differ in foraging behavior and behavioral dominance tend to discover and occupy baits at different times after the baits are placed; submissive and rapidly moving species find baits early but are often later displaced by dominant but slower-moving species (e.g., Fellers 1987). Thus repeated observational samples of a bait over time can reveal behavioral dynamics.

Materials

A bait substance, bait platforms (made of paper, cardboard, plastic, or leaf), vials or twirl bags, ethanol, forceps, an aspirator, and a timer (optional).

Methods

Baits that are pastes or solids are usually preferred because they tend to be more difficult for ants to remove than liquids or particles; thus ants will be present to be collected or observed for longer periods. Tuna or other fish baits, honey mixed with lard, and peanut butter are frequently used bait substances, and they can be deployed in pieces of about 1–2 cm^3. Tuna should be well mixed and should not contain excessive amounts of oil. The bait may be set directly on the ground or on a piece of paper to make the attracted ant species more readily visible. Graph paper is useful for distinguishing some ant species in the field because the squares provide a reference with which to compare worker sizes. The bait placed on a paper platform tends to attract more dominant ants, while the oil around and under the paper will attract smaller and/or less aggressive species.

Alternatively, an impermeable bait platform (e.g., plastic) will restrict ant activity to the bait above the platform, and it will be less likely to blow away in windy conditions. Baits can be placed on the ground, in shrubs and trees, and underground in small containers that are perforated to allow the ants to enter (a string can be attached to the container to allow the buried trap to be recovered; Quiroz-Robledo and Valenzuela-González 1995). The amount of time the bait is observed will vary depending upon the objectives of the study. Care should be taken to avoid disturbance of the baits and foraging trails during observations. If the vegetation must be disturbed, allow at least a day before baiting so that foraging trails may be reestablished.

To collect ants attending baits in a single-sample or "snapshot" fashion, investigators may collect the bait and platform (and also the leaf litter around the bait, when necessary) into a coded plastic twirl bag. When cleaner samples are desired, baits may be quickly placed into a plastic tub and the ants removed with forceps or an aspirator from the bottom of the tub. Fluon or petroleum jelly around the walls of the tub will prevent the escape of fast-moving species. Our observations suggest that 60–90 minutes is usually sufficient time for dominant ant species in an area to discover and recruit to the baits. A small amount of ethanol may be injected into the bags to allow the ants to be separated from the bait and debris under a microscope afterwards; ants may then be transferred to ethanol-filled vials. Acetone can be used to remove tuna oils from the ant specimens. Brandão and Silvestre (unpubl. data) found that circa 1800 bait samples were needed to record 90% of the fauna visiting baits in a Brazilian cerrado site.

For behavioral studies, it is imperative to leave the bait undisturbed for the duration of observations. Baits can be observed repeatedly (e.g., every 20 minutes for 2 hours) to study behavioral dynamics over time (see Fellers 1987). Voucher specimens of unrecognized ant species may be collected from around the bait with forceps. It is important to collect these ants, because some ant species may leave the bait before the end of the observation period. It is best to target individuals that are separated from foraging groups so that other ants will not be alerted by chemical signals released by the victim. It is convenient to place several previously labeled vials on the ground next to baits in advance, so that specimens can be collected in sequential samples. It is important to collect representatives of the different castes of polymorphic species, especially majors of such species as *Pheidole* and *Solenopsis,* which facilitate species identifications. Majors are often more timid than minors, and they may take more time than minors to recruit to baits.

While ants are present at baits, behavioral interactions between individuals of different species and the numbers of individuals of the various species attending the baits can be recorded. Small baits, such as cookie crumbs, can be used to follow ants back to their nests. As noted earlier, baiting at different times of the day or year in a locality can reveal the influence of abiotic features on ant activity and interactions between species. In general, ant activity levels tend to vary from the cooler hours of the day, to the warmer hours (especially in full sun), and at nightfall. Bestelmeyer (1997, unpubl. data) found that soil surface temperatures of 37–40°C represent an important transition between the activity of different species at baits in both North American and South American arid-zone ant communities.

In studies of the impacts of ants on ecosystem processes, dead insects can be used as baits to measure the consumption rates of scavenging or predaceous ants (see Jeanne 1979; Fellers and Fellers 1982; Seastedt and Crossley 1984; Retana et al. 1991; Olson 1992), and seeds can be used to examine the impact of granivorous ants on seed removal and redistribution (see Crist and Wiens 1994). In both cases, the size and density of the items presented may influence the species of ants that will remove them.

Data Output

The data produced by this technique may include richness, composition, relative abundance of ant foragers at individual baits, frequency of occurrence of species at sets of baits, frequency and nature of behavioral interactions, timing and duration of forager activity, and rate and distance of removal of food items.

Evaluation

Baiting is the most common of the techniques used to study ant communities, no doubt

because it is very simple and inexpensive and can be deployed rapidly and extensively. Baiting is ideal for work involving behavioral questions. Baits may, however, seriously bias descriptions of community composition (Greenslade and Greenslade 1971). Baits are dietarily selective and may systematically exclude some components of an ant fauna from samples. Because baits tend to be monopolized by dominant, mass-recruiting species (e.g., *Solenopsis;* Fig. 9.2), subordinate and single-foraging ants may be underrepresented at baits relative to their abundances. This problem may be alleviated in part by using several baits at a sample point (Culver 1974). Because ant activity at baits varies with time after bait placement, daily, and seasonally, multiple observations at baits will ensure a more complete representation of the species and the factors that affect ant foraging.

There is evidence that the preference of some ants for proteinaceous or carbohydrate baits may vary seasonally (Stein et al. 1990), although it is unclear how this may affect the data. Brandão and Silvestre (unpubl. data) compared the species composition of ants that visited sardine or tuna and honey-water baits, which mimic protein/fat and sugar sources, respectively. Their results indicate that the nutrient composition of the baits does not significantly affect the composition of species at the baits. Canned sardine or tuna bait may be transported easily along with collecting gear and stored for indefinite periods.

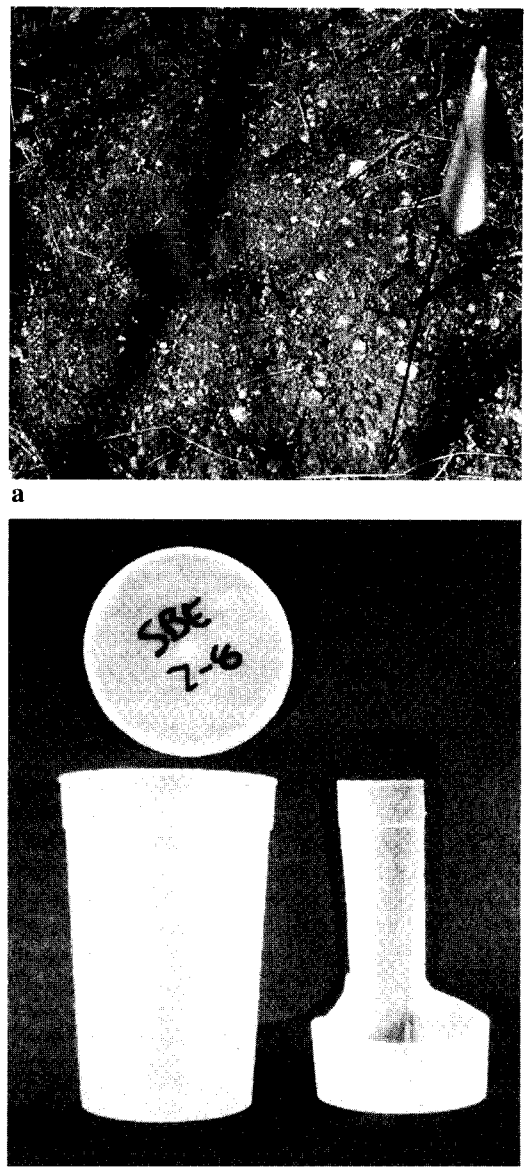


Figure 9.3. (a) A pitfall trap placed in desert soil. (b) A polypropylene sample container used as a pitfall trap and a pitfall-trap scoop (right) made from the same container, used to catch and remove debris that falls into the trap while it is being set. Photo by Brandon Bestelmeyer.

Pitfall Trapping

Objectives

Pitfall trapping involves the placement of open containers in the ground (Fig. 9.3a). Surface-active animals fall unwittingly into these traps and are either killed and preserved in a liquid or "dry-trapped" and allowed to survive after a census.

This method is used to estimate the abundance and species composition of ground surface–active ants in an area. As in baiting, the abundances of ants in pitfall traps provide a measure of species importance in a community by integrating both forager attributes and colony dispersion patterns (Greenslade 1973). Pitfall trapping may be used to census ants foraging on soil or leaf litter. It may be very difficult to use pitfall traps on rocky surfaces. Pitfall trapping may be performed for short (days) or long (continuously) durations.

Materials

Pitfall traps (containers), pitfall trap scoop, hand trowel or shovel, killing and preserving agent, detergent, and a tea strainer or muslin cloth with additional containers (optional).

Methods

For ants, it is best to use a killing agent in the pitfall trap; otherwise, captured ants will dismember one another and the specimens will be damaged. Several killing agents can be used. Propylene glycol (available in the United States as "environmentally friendly" automobile antifreeze) is an ideal choice because it is slow to evaporate (even when soil surface temperatures exceed 60°C) and is reputed to be nontoxic to vertebrates. The more common antifreeze, ethylene glycol, may also be used, but it is toxic to vertebrates. Ethanol solution may serve as a killing agent, but a few drops of glycerol should be added to retard evaporation. The addition of ethanol to propylene or ethylene glycol may kill ants more quickly and reduce the chance of escape. A drop of unscented detergent added to the killing agent breaks surface tension and may prevent ants from escaping the trap. Ideally the killing agent should not attract or repel ants (or at least not attract or repel species differently); otherwise estimates and comparisons of forager densities may be biased. Ethanol/glycerol (Greenslade and Greenslade 1971) and propylene glycol (Abensperg-Traun and Steven 1995) are believed neither to attract nor to repel ants; other substances await examination in this regard.

Traps may be plastic or glass containers, such as jars or drinking cups. Polypropylene sample containers (see Appendix 1) make ideal traps because they are durable and flexible and have tightly fitting lids, enabling them to be used to transport and temporarily store the specimens after trapping. Metal containers should be avoided because rust produces a rough surface in the trap that ants can use to escape. In general traps should have clean and smooth interiors (Luff 1975). The diameter of the mouth of the trap has been shown to affect the efficacy of pitfall traps for ants (Abensperg-Traun and Steven 1995). Traps with a very small diameter (18 mm) may bias against larger ant species and collect fewer of the species present in an area than larger traps. A 42-mm-diameter trap was found to perform as well as traps 86 and 135 mm in diameter. Smaller traps (40–70 mm in diameter) are easier to use and best for studies concerned solely with ants. Larger traps may be called for if other taxa are to be trapped.

Traps should be placed so as to minimize the disturbance of the surface around the trap because surface texture conditions may affect ant capture rates. A hand trowel that is only slightly bigger than the trap should be used to dig the hole. Surface features should be returned to normal by hand (e.g., coarse sand, stones, or leaf litter should be replaced).

When possible, traps should be allowed to settle for about a week (with the lids on or the trap inverted) before they are opened, in order to avoid the "digging-in effect" (Greenslade 1973). One manifestation of this effect is an abnormally high capture rate of ants when traps are placed in the ground and opened immediately thereafter. Causes include the penetration of nest galleries in the course of placing the trap and the exploration of novel habitat features by

the ants. A settling period ameliorates this effect as the ants become accustomed to the disturbance. The return of natural surface characteristics (e.g., a soil crust) with settling is also desirable.

Traps should be placed in the ground with the lip of the trap flush with the soil or leaf litter surface or a few millimeters below the surface. If the lip is even slightly above the surface, small and/or wary ant species may be undersampled. Soil or leaf litter should completely cover the lip. When setting the trap, a tight-fitting scoop made from another trap container (Fig. 9.3b) may be used to catch soil and litter that falls into the trap so that they can be removed. This will result in cleaner pitfall samples and reduce the time needed to sort. Alternatively, one cup may be placed into the ground and act as a sleeve for a second cup that may be placed and removed easily. Over the trap settling period and especially after precipitation or wind, the surface may fall below the lip. If the soil around the trap is packed well this effect will be minimized. Immediately before trapping (and during trapping, for long-term applications), the condition of the trap lip should be checked.

The killing agent is placed after the trap is set, and it should fill about 25% of the cup's volume. In situations in which soil or litter is likely to fall or blow into the trap, more liquid may be necessary. If rain is likely to flood the trap, a cover may be suspended over it. In ecological studies, covers should not extend beyond the trap circumference in order to avoid changes to the microclimate. Traps placed in depressions or drainages may also flood.

The duration that the traps are left open will depend on the objectives of the study and on logistics. Traps left open longer will collect more ants and more of the species occupying an area. Traps left open for very long periods may deplete populations of foragers or alter foraging paths around the traps (Greenslade 1973). In general, 2 or 3 days seems to be sufficient to capture the ants foraging around the trap and provide a measure of forager abundance. Temperature and humidity have profound effects on ant activity, and in cooler, drier weather a longer duration of trapping may be required. When traps are collected, they may be capped and removed for short-term storage when polypropylene sample containers are used. In other cases, the contents may be poured through a tea strainer to remove excess liquid. The tea strainer is then inverted and the contents washed into a container using 90% ethanol solution. In the case of large pitfall traps, which may collect large quantities of animals, the contents may be poured through a piece of muslin cloth, the cloth tied into a tight ball, and the ball stored in ethanol. In these cases, washing the strained specimens with water before storing may be desirable in order to remove the propylene glycol and debris that may stick to the ants.

Data Output

The data produced by pitfall traps include richness and composition, relative abundance of ant foragers in traps and sets of traps, and frequency of occurrence of species in sets of traps.

Evaluation

The greatest advantage of pitfall traps is that they take little time to place and operate by themselves. Most epigaeic ants are well represented in pitfall traps, especially in open habitats. Andersen (1991b) found that the results obtained from pitfall traps were comparable to those from the relatively unbiased but time-intensive quadrat method (discussed in the next section).

If one wishes to use ant capture frequencies or abundances in pitfall traps as a measure of ant populations, the estimates may be biased by differences in locomotion among ant species (Greenslade 1973; Andersen 1983). Fast-moving species (e.g., *Forelius, Iridomyrmex*) will

be overrepresented relative to slower-moving species (e.g., *Crematogaster*) in forager abundance studies. Ant species may differ in their ability to climb on trap walls or in their wariness of traps, and this may bias estimates of activity. More importantly, the physical structure of the ground surface may affect ant capture rates (Greenslade 1964; Adis 1979). Heavy litter or numerous stones will reduce ant captures and can confound between-habitat comparisons of forager populations. Ant species may also differ in their deliberate avoidance of pitfall traps (Marsh 1984). Certain castes of ant species needed for identification (e.g., *Pheidole* majors) are often not recorded in pitfall traps, and additional nest collections are required. Because pitfall traps collect only surface-active ants, they are believed not to provide an adequate sample of most leaf litter ants (Olson 1991; Majer 1996).

Quadrat Sampling

Objectives

In this technique, ants are sampled directly by the investigator from within a quadrat-delimited sample area. Like pitfall trapping, this method is used to estimate the abundance and species composition of surface-active ants in an area (Andersen 1991b). Quadrats are best used in open-ground situations at different times of the day or year.

Materials

A prefabricated quadrat, vials, ethanol, forceps, an aspirator, a timer, and data sheets.

Methods

This method is similar to techniques used to measure ground-layer vegetation (see Bonham 1989). A fixed, transportable quadrat made of wood or plastic (e.g., polyvinyl chloride [PVC]) pipe is used to delineate the observation area. The quadrat may be raised slightly off the ground by pegs or nails so as not to interfere with ant movements. Species of ants seen inside the quadrat or entering the quadrat over a fixed time interval (e.g., 2 minutes; Andersen 1991b) are counted, collected, or both. As with baiting, ant activity in quadrats varies throughout the day, and quadrats can be sampled several times, for example, in the morning, at midday, and at night.

Although a quadrat of 0.5×0.5 m is generally small enough that it can be viewed effectively by a single researcher, if the level of ant activity is high a smaller quadrat may be desirable. Data may be collected in two ways: (1) all the ants in the quadrat may be collected, or (2) the number of ants may be tallied by species onto data sheets. If all the ants are collected, the researcher must be adept with forceps or use an aspirator to remove ants to a vial. When the level of ant activity is very great, it may be impossible to collect all the ants in a quadrat. Furthermore, subsequent samples in the same quadrat may be affected by the loss of ant foragers. If the ants are to be tallied, the researcher must be able to distinguish on sight the ants present in the study site. This will require a considerable amount of preparation for researchers who are unfamiliar with ants. A few representatives of unknown ants may be collected to a vial for identification in the laboratory and given a provisional code in field notes. It is important not to disturb other ants during collecting because natural foraging behaviors may be altered; for example, ants may swarm into the quadrat. During periods of high activity, it may be useful to record ant counts using an abundance scale (e.g., 1, 2–5, 6–20, >20 ants; Andersen 1991b) rather than absolute numbers.

Data Output

The data produced by quadrat sampling include richness and composition, relative abundance, frequency of occurrence in sets of quadrats, and time and duration of activity.

Evaluation

Quadrats provide information that is similar to that of pitfall traps and represent the densities of epigaeic ant foragers more accurately than pitfall traps. The densities of the foragers recorded in quadrats are not influenced by the differential tendency of ants to be trapped (Andersen 1991b). Furthermore, quadrats can be used to examine hourly and daily activity patterns, whereas pitfall trapping sums activity over time. Of course this level of detail comes at the cost of a considerable investment of time in the field and in preparing to identify the ants by sight. Quadrat techniques may be difficult to implement at night when small, yellowish-colored ants are difficult to see. Observations can also be difficult when levels of activity are very high.

Litter Techniques

Objectives

In the two techniques described in this section, a quantity of moist leaf litter (usually all the litter and humus present under a 1 × 1-m quadrat) is collected and placed in an extraction apparatus. The apparatus compels mobile ants, through disturbance to the litter or through changes in microclimate, to migrate from the litter into a collecting receptacle.

These techniques are designed to measure the abundance and composition of ants inhabiting a volume of leaf litter. Whole colonies of ants nesting in the litter as well as ants foraging in the litter from colonies outside the litter sample are collected. These methods are especially appropriate for use in forest and woodland habitats, where many ant species inhabit the litter layer. However, this method is not particularly successful during very dry periods. When the leaf litter is dry, ants move their nests deep into the soil or up into the vegetation.

Winkler Extraction

In this technique the collected litter is first sifted to remove large leaves and twigs from the sample. The sifted litter is then placed in the Winkler sack to process. During this time, ants from within the litter sample migrate out of the litter as a behavioral response to disturbance of their habitat and eventually fall into a container (see Besuchet et al. 1987; Fisher 1998).

Materials

Winkler extraction requires a litter sifter, a Winkler sack, a quadrat, a ground cloth, large plastic sample bags, plastic cups, twirl bags, vials, ethanol, and in some cases a machete.

Methods

First, the litter sample is collected within a quadrat placed on the ground. The wooden or plastic quadrat should have movable joints (use bolts with wing nuts) and be able to open at one corner so that the frame can be placed around shrubs or trees. The litter should be scooped from the edge of the quadrat toward the center and be removed by hand into the opening of the *sifter* (Fig. 9.4a). Gloves should be used to prevent stings and bites. The litter should be removed from the top of the litter pile to the bottom and put quickly into the sifter. Twigs and clods should be broken open; decayed logs can be minced with a machete to expose and disturb ant nests within them (Fisher 1998). The sifter should be held immediately adjacent to the litter sample to minimize the loss of ants from the sample. Water-soaked litter should not be collected.

The sifter consists of an open-ended sack with a metal ring and attached handle at the top end, a mesh screen and handle located at about one-third the length of the sack from the top, and a bottom end that may be tied shut. The sifter should be long enough so that part of the sack is supported by the ground while being held. Prior to filling the sifter, its bottom end should be tied with two knots, a single and a shoestring knot, so that the sack does not open during the sifting process. The upper part of the

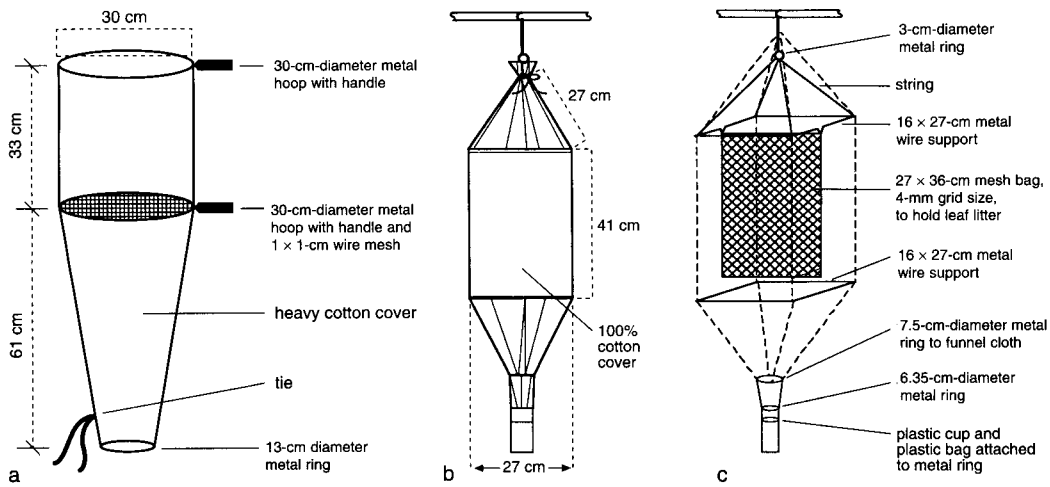


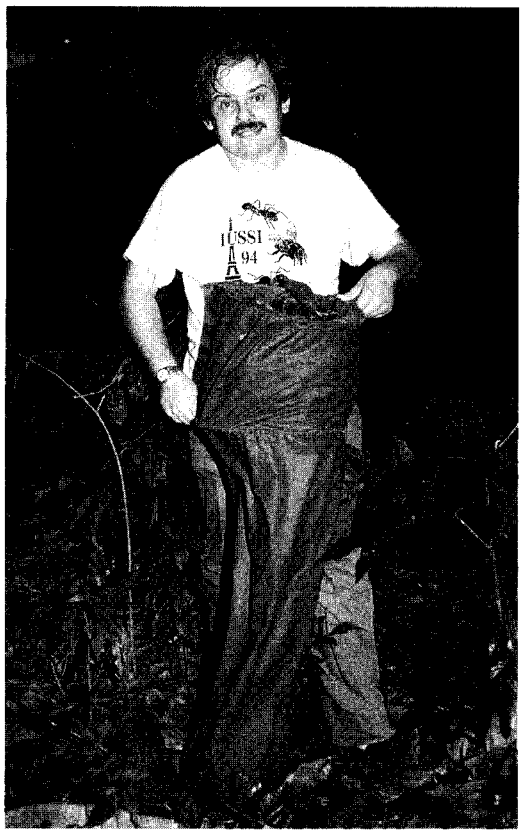
Figure 9.4. (a) Construction of the litter sifter. (b) External dimensions of the "mini-Winkler" sack. (c) Construction of the "mini-Winkler" sack (Fisher 1999a).

sifter is filled with litter that should not quite reach the upper margin. Holding both handles, the worker shakes the sifter to separate detritus and smaller invertebrates into the bottom of the sack while retaining coarse material above the mesh.

The sifter should be shaken thoroughly both laterally and vertically (Fig. 9.5a). The litter in the upper section should be turned over several times in the process. When the litter is very dry it should be shaken briefly, because most of the animals will fall through the mesh quickly and extended shaking will only add more debris to the sample. When the litter is moist, it should be shaken longer so that ants that are stuck to wet leaves may fall through. The sifting process may need to be repeated a number of times for a 1-m^2 sample. After the sample has been sifted, the top of the sifter sack should be twisted (twice) shut to ensure that animals do not escape through the top.

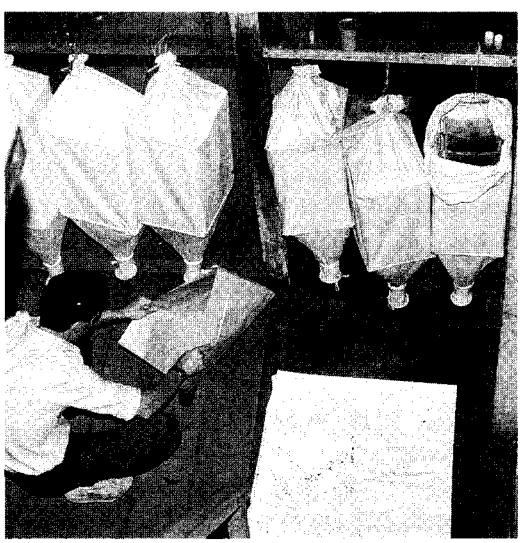
The *sample bag* should be large enough to hold a single litter sample, and the sample code should be written on it. The bag should be porous and made of synthetic material (e.g., nylon) to prevent rot. The contents of the sifter sack are poured into the sample bag through an opening in the bottom of the sifter. Close the sample bag using a tight knot. If the bags are to be stored for days in dry conditions, a little water can be used to moisten the sample. The sample bags are now removed to be processed in the *Winkler sack.*

The Winkler sack (Figs. 9.4b,c and 9.5c) consists of a metal box frame that supports a covering made of canvas or cotton. Litter from each sample bag is separated into one or more 4-mm *mesh inlet sacks,* which are suspended inside the Winkler sack. Ants in the litter migrate out of the inlet sacks and are collected in a receptacle tied to the bottom. The inlet sacks should have stitches in their centers that allow the sacks to maintain a flattened shape, which accelerates the migration of ants from the litter. The receptacle may be a twirl bag or a cup partially filled with ethanol solution. The first step in using a Winkler sack is to find a protected site where it can be mounted. A sack can be suspended from a nail in a wall, a beam in a shed, a pole under a tarp in the field, or a tree branch



a

b



c

Figure 9.5. Leaf litter extraction using the Winkler extractor. (a) Sifting leaf litter. (b) Transferring sifted litter into a mesh inlet sack that will be placed inside the Winkler sack. (c) Winkler sacks hanging from support beams, with researcher collecting excess debris from sacks. Photo by Donat Agosti.

at sites where rain is unlikely. It is important to find a location where the sack will not be tossed about by the wind or bumped by passersby, since any vibration or shock causes additional debris to fall into the receptacle. In preparation for loading inlet sacks, attach a dry receptacle to catch falling debris. Label each Winkler sack according to the sample it is to receive.

The next step is to distribute the contents of the sample bag into one or more inlet sacks (Fig. 9.5b). Prior to filling the inlet sacks, place a large white plastic cloth on the ground, pre-

pare the inlet sacks, and have a vial or two on hand in which to place escaping ants. Open the inlet sacks, pour some material onto the cloth, and immediately fill each inlet sack by hand. Hold the inlet sacks over the leaf litter so that escaping animals fall back to the litter. As each sack is filled, occasionally and gently shake the sack to settle the material. Air spaces in the litter may hinder migration from the sack. Because ants crawl to the top of the litter column before falling out, it is most effective to fill each inlet sack as completely as possible such that only the last sack is partially filled, if need be. Ensure that the inlet sacks are kept flat by the stitching.

After the inlet sacks have been filled, hang them inside the Winkler sacks (Fig. 9.5c). This should be done as quickly as possible. The "mini-Winkler" (Fisher 1999a) shown in Figs. 9.4b,c holds one inlet sack; the standard Winkler sack (see Appendix 1) will accommodate up to four sacks. The inlet sacks should not touch the walls of the Winkler sack. Pour the material that remains on the ground cloth into a cup and place in an inlet sack. Next, pour the material that has fallen into the collecting receptacle into an inlet sack. Add the ethanol solution to the cup and reaffix it to the Winkler sack. Finally, tie the top of the Winkler sack with a single and a shoestring knot to prevent animals from escaping.

The Winkler sack should be allowed to run for at least 24 and preferably 48 hours. Leaf litter from Brazilian Atlantic rainforest that was allowed to process for 1 day collected about 90% of the species and 70% of the individuals that could be extracted from the sample, and in 2 days about 95% of the species and 85% of the individuals were collected (Delabie et al. 2000). The length of time that each Winkler sack can be allowed to process will depend upon the duration of one's stay in the field, the number of samples to be processed, and the number of Winkler sacks available (see Chapter 10). The rate of extraction of ants from litter samples can be increased by removing the litter to a polyethylene bag and shaking it once during every 24 hours of processing. When the litter is shaken gently and returned to the inlet sack, ants that have settled down in the center of the litter are again agitated, begin to move, and eventually fall out. After 4 days, Delabie and do Nascimento found that samples that were agitated once per day yielded 15% more species and 70% more individuals than unagitated samples. On conclusion of the processing period, remove the collecting receptacle and rinse the contents with ethanol into a labeled vial.

The Berlese Funnel

In the Berlese funnel technique, a quantity of leaf litter is placed directly into one or more of the funnels to process. The funnels are then placed under a lamp or in the sun. As the upper portions of the litter column dry, mobile invertebrates are driven down the litter column to the bottom of the funnel and fall into a collecting receptacle (Southwood 1978). Berlese funnels can be purchased (see Appendix 1), fabricated, or modified from other kinds of funnels, as described later in this section.

Materials

Berlese funnels, a quadrat, large plastic sample bags, plastic cups, a ground cloth, supports for the funnels, vials, ethanol, and a light source (optional).

Methods

Litter is collected as described previously for Winkler extraction.

This section describes a simple, portable, and inexpensive version of the Berlese funnel process. The Berlese funnel may be fabricated from 0.7-mm-thick acetate sheeting. The funnel is constructed such that it may be opened and carried flat or rolled up for transport. The general outline of the funnel pattern is shown in

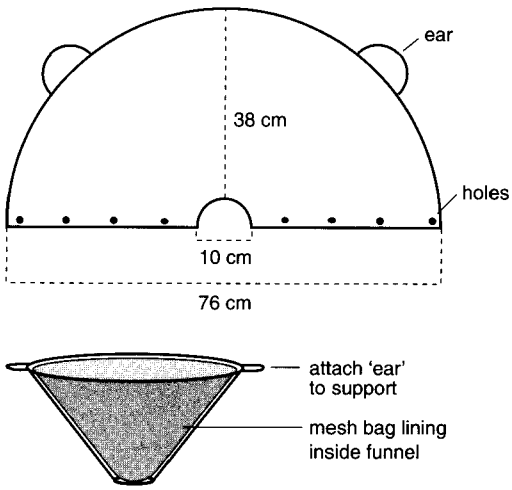


Figure 9.6. The pattern used to create a Berlese funnel (above) and the appearance of the assembled funnel (below).

Fig. 9.6. For setup, the funnel is formed by bringing the two straight edges of the sheet together to overlap slightly, so that the four to five pairs of punched holes are aligned. Paper fasteners are then placed through the holes and spread.

A bag to support the litter in the funnel is fabricated from plastic netting (available at fabric stores) with a mesh size of about 3 mm. The bag is made by attaching the edges of a circular piece of mesh to the upper edge of the funnel with paper clips. The two ears at the outer edge of the funnel are bent and may be used to affix the funnel to a support, such as two chairs or the rungs of a wooden, straight-sided ladder that is supported horizontally by sawhorses or benches (the ladder may hold five or six funnels). Alternatively, a Berlese funnel may be constructed from oil or kitchen funnels, perhaps with some minor modifications (e.g., cutting the tip off the oil funnel).

Before filling the mesh bag in the funnel, place a white plastic cloth or dry cup under the tip of the funnel to catch material that falls through the mesh. As with Winkler extraction, break up sticks, clods, and decayed logs before placing them into the funnel. After filling, make sure the opening at the funnel tip is not clogged. Next, add material that fell through the mesh to the top of the sample. The litter should be circa 10–12 cm deep in the middle of the funnel. Place a collecting receptacle filled with ethanol under the funnel tip.

If an electric light with a reflector shade (or improvised aluminum foil reflector) is available, suspend the light about 2 cm above the surface of the litter. To reduce fire hazard, do not allow the bulb to touch the litter. A screen may be added to the top of the funnel to keep out flying insects that may be attracted to the light at night. If no electricity is available, place the funnel in bright sun and away from wind and other disturbances. The sample should be allowed to process until the litter is dry or for 2–4 days, depending on the condition of the litter, the temperature, and the humidity. The alcohol should be checked daily and replenished as needed. Do not disturb the funnel to minimize dirt in the sample. After the processing is complete, place the contents of the receptacle into a labeled vial or jar for storage. Add some fresh ethanol to the vial if needed.

Data Output

Both litter techniques produce the following data: richness, composition, relative abundance, and frequency of occurrence among litter samples.

Evaluation

Litter sampling techniques have been relatively little used for ants, and therefore the ants that inhabit litter microhabitats remain largely unknown (Olson 1991; Agosti et al. 1994). Both the Winkler and Berlese methods sample the abundant and diverse leaf litter ant fauna, which is severely undersampled using other methods owing to the ants' cryptic habits and small foraging ranges (Greenslade and Greenslade 1971;

Majer 1996). In ant surveys, Winkler sacks may contribute a relatively large proportion of unique species compared with pitfall traps (Olson 1991). Many individuals and species are often represented in a single litter sample, and Winklers or Berleses are the most efficient way of obtaining extensive samples in litter microhabitats. Nonetheless, larger, active, epigaeic ant species tend to be undersampled because they escape litter samples, and extensive litter sampling may require considerable effort and cost (especially for Winkler extraction).

Delabie and do Nascimento (unpubl. data) have shown that several species of litter ants in Brazilian Atlantic rainforest are undersampled in Berlese funnels relative to Winkler sacks. They attribute these differences to the sifting procedure used with Winkler sacks, which compels the ants to migrate relatively quickly, and to the possibility that some sensitive ants may desiccate and die before leaving the litter in Berlese funnels.

Colony Sampling

Objectives

In colony sampling, ant colonies in a defined area are identified and counted to estimate colony density and monitor changes in populations. Frequently colonies are also mapped so that demographic processes and spatial relationships among colonies (Herbers 1994) and between colonies and environmental features (Crist and Wiens 1996) may be studied. This technique can be used to sample species of ants that nest in an area and are detectable to the investigator in several kinds of habitats.

Materials

A white sorting tray (in litter microhabitats), vials, ethanol, forceps, an aspirator, and materials for mapping (optional).

Methods

In open habitats such as deserts (Schumacher and Whitford 1976; Whitford 1978; Bernstein and Gobbel 1979) some ant colonies may be identified to species by the characteristic structure of the aboveground portion of the nest or by observing worker ants at the nest. Such identification will of course require a knowledge of the local ant fauna prior to using the technique. Nests are identified by searching the sample area, usually along belt (rectangular) transects (e.g., 1×50 m [Wisdom and Whitford 1981] or 20×1800 m [Johnson 1992]) or by searching a marked study plot using overlapping belt transects (e.g., Chew 1995) and marking every nest encountered within the transects. The width and length of the transects and the size of the plots will depend on the colony densities and the conspicuousness of the nests. Often these studies are limited to common, active, and large-bodied ant species because other species have secretive habits, inconspicuous nest entrances, or both.

In forests with dense leaf litter, most nests are inconspicuous, and the sample area must be searched by excavating the litter and soil. Ants are found nesting in the soil; between rotting leaves; in all sizes of rotting twigs, branches, and logs; in nuts such as acorns; and under rocks. Quantities of litter and soil that are removed from the ground are searched in a white pan to help find the ants (see also the section on intensive sampling). Partial or entire nests may be collected into vials, and the location where the nest was found may be flagged for mapping. This technique is destructive, so temporal patterns may be examined by sampling in nearby plots (e.g., Herbers 1989). A nondestructive alternative is to place baits systematically in plots and follow the trails of ant foragers to locate a subset of the nests in the plot (Herbers 1985; see also the section on intensive sampling). This method is easier to execute but, in some cases, may only sample a subset of the nests because baits are monopo-

lized by nests of dominant species or colonies and because some species are less attracted to the bait than others. Obviously the spatial extent of sampling is limited by the methodology used. For example, Herbers (1989, 1994) studied forest ant communities in 25-m^2 plots by searching leaf litter and soil for nests. Crist and Wiens (1996) mapped the large and conspicuous colonies of the western harvester ant, *Pogonomyrmex occidentalis,* in shortgrass steppe in areas of up to 146 ha using low-level aerial photography and geographic information system software within which photographs were digitized.

For many studies addressing ecological questions, researchers may consider each nest as an independent sample unit. However, researchers should not confuse nest entrances with nests; a single nest may have several crater- or conelike entrances at variable distances from one another (e.g., *Lasius,* some *Pheidole*). In some population studies, it is important to recognize that the number of nests may overrepresent the number of genetically distinct colonies. This is because some species of ants are polydomous, with individuals of a single colony occupying two or more distinct nests. This challenge may be addressed by transplanting individuals among nests that are suspected to represent a single colony. The introduced workers may be distinguished by dusting them with fluorescent powder (Snyder and Herbers 1991). Agonistic interactions (e.g., threat displays, chasing, biting, swarming) between the transplant and the occupants of the nest indicate that the nests represent distinct colonies. The relationship among aggressiveness, nest relatedness, and polydomy can be quite variable and complex and may require more detailed study (see Banschbach and Herbers 1996).

Data Output

The data produced by colony sampling include richness, composition, colony density, and colony location.

Evaluation

Colony sampling can provide a population-based perspective on ant communities by focusing on the genetically distinct individuals rather than on the foraging components of the colonies. As noted previously, forager-based sampling methods may misrepresent patterns in population structure. Ant colonies provide unique information about the ecological setting because the location of the colony reflects the responses of the foragers as well as the response of the queen, especially through her selection of a nest site (see Johnson 1992). Colony sampling would best serve a single-species study and is essential to develop conservation strategies for endangered ant species. For community studies, researchers should recognize the potential bias against ants that have inconspicuous nests.

Intensive Sampling

Objectives

The primary goal of intensive sampling is to gather data on the total number of ants in an area by searching for and collecting all the ants within fixed plots. This approach allows precise estimation of the number of ant colonies and ant species per unit area, as well as an estimate of the total species richness of the site.

As with some kinds of colony sampling, entire ant colonies may be collected to obtain data on the number of workers, queens, male and female reproductives (alates), and pupae per colony. These "nest series" provide information on the life history characteristics of the ant colonies (e.g., colony size, reproductive status) and are of great use to both taxonomists and ecologists. This method can be used in all habitat types; it is particularly appropriate for structurally complex habitats with abundant leaf litter.

Materials

A plot frame or flagging, a white sorting tray and sifting tray, a bait substance, a shovel and

sample bags (for soil samples), vials or twirl bags, ethanol, forceps, and an aspirator.

Methods

Samples are usually taken in 1-m^2 plots set in a linear transect. As in quadrat sampling, the plot can be measured using a meter stick and marked at the corners with small flags, or the plot outline may be delimited with a premeasured 1-m^2 quadrat made of PVC tubing.

Ants are sampled by carefully inspecting all the litter in the plot (see the section on colony sampling). After the litter has been inspected, it is placed in a sifting tray, which consists of a wire mesh screen embedded into the center of a shallow tray and then placed over a deeper tray that measures at least 30 × 30 cm. A metal colander can be used in place of the wire screen. After accumulating some litter in the tray, the worker shakes the tray so that ants and other small animals fall through the screen into the tray below. This additional sifting method ensures that no ants were overlooked while inspecting the litter. Whenever an ant nest is found, the entire nest—including workers, queen(s), alates, and pupae—is collected and preserved in a vial of ethanol. To save time in the field, any ant colonies found in twigs, litter, or logs can be placed into resealable plastic bags and labeled by plot and nest. The ants are later collected from the bags in the laboratory and preserved in one or more vials of ethanol. Ants present in the plot but not associated with a nest are also collected and placed together in a vial labeled "strays." Nest series and strays must be preserved in separate vials so that ants that nest in the plot can be distinguished from ants that may nest outside the plot.

After all litter has been removed, the plot can be baited. Light-colored cookie crumbs are an ideal bait because the crumbs are very visible against the dark leaf litter. As in colony sampling, the cookies, or other bait, are crumbled over the plot to help locate any ant nests in the soil. After 15–60 minutes, the plot is inspected and any ants carrying cookie crumbs are followed to their nest. If a nest is located inside the plot, the nest should be collected, which would involve digging up the nest if it is in the soil. If the nest is located outside the plot, the ants are collected as strays. Some cryptic genera, such as *Basiceros* or *Trachymyrmex,* will feign death if alerted and are nearly invisible until they begin to move again (Romero and Jaffe 1989). If the study plot is undisturbed for 10–15 min, the ants resume activity and may be collected. After the sampling is complete, it is best to return the sorted litter to the plot so that ants and other organisms can recolonize the area.

Several unique ant species may dwell entirely below the soil, and their abundance may vary with soil depth (Harada and Bandeira 1994). Soil-dwelling ants can be censused by retrieving blocks of soil (e.g., 20 × 20 × 30 cm) to sample bags and sectioning the blocks along the vertical axis into subsamples (e.g., 5-cm increments) in order to examine variation in ant composition and abundance with soil depth (Harada and Bandeira 1994). The subsamples may then be searched by hand in a white tray or separated from the soil using flotation techniques (Chapter 11).

Data Output

The data produced by intensive sampling include richness, composition, the abundance of colonies and foragers, and the frequency of occurrence of colonies and foragers in sets of plots.

Evaluation

Unlike the other methods, intensive sampling is able to provide a complete representation of the ant fauna in a sample plot. Colonies within and foragers from outside the plot can be recorded and distinguished from one another. Because the method relies upon visual inspection to record nests, there is a great potential for inves-

tigator bias. This method also requires more time than other methods. Although it is very useful for small-scale studies, the extensiveness of sampling may have to be compromised. For faunal surveys, Romero and Jaffe (1989) found that intensive sampling was unlikely to record many species that were not recorded by simpler methods. Some fast-moving foragers may escape the plot while nests are being searched. The destructive nature of the sampling (as with some kinds of colony sampling) may interfere with temporal comparisons.

Direct Sampling

Objectives

Direct or hand sampling involves searching for and collecting ants in different microhabitats within an area. Unlike intensive sampling, in which the objective is to provide a precise estimate of colony or forager density in a relatively small area, direct sampling may be spatially extensive, and the primary goal is to record the number of species inhabiting an area. A minimum of material is needed in this technique, although some experience with ants is required.

Materials

Vials, ethanol, forceps and an aspirator, a white cloth or tray, and a timer.

Methods

Several microhabitats in which different kinds of ants nest and forage should be searched, with searches carried out on bare ground, in leaf litter, on twigs and nuts, under and on shrubs and trees, in epiphytes, at the base and in the roots of grass clumps, under stones, and in decaying logs (especially under bark; see also the sections on intensive sampling and additional techniques for arboreal and herbaceous strata). Twigs, nuts, and logs should be broken open (over a cloth or tray) during the search. A favorite method of Hölldobler and Wilson (1990) in forests is to clear loose leaves from small plots to expose soil and humus and watch the plot for 30 minutes in order to find small or cryptic ants. When ants are discovered, forceps, an aspirator, or both may be used to collect the ants into vials. As in intensive sampling, nest series should be collected into separate, labeled vials; stray foragers may be collected into a single vial.

As with other techniques, samples taken at different times of the day will include different ant species. To better standardize collecting effort for comparative purposes, the area searched may be delimited and the time taken for the search recorded using a stopwatch. When a colony is discovered and collected (which may take several minutes), the timer should be paused. Searches may be stratified by variables such as habitat or microhabitat type and investigator identity (Longino and Colwell 1997). Hölldobler and Wilson (1990:630) note that an experienced collector can obtain a "virtually complete list of the fauna of a 1-ha site within 1 to 3 days."

Data Output

The data produced by direct sampling include richness, composition, and the frequency of occurrence of species in plots.

Evaluation

Many of the species inhabiting an area can be recorded in relatively little time through direct sampling (Romero and Jaffe 1989). Direct sampling may be spatially extensive, and several microhabitats in an area may be sampled simultaneously. Direct sampling is especially useful for short-term faunal inventory. Abundance, however, is difficult or impossible to record with this technique (although frequency may be used as a surrogate). Considerable expertise is required for this technique to be efficient. Variability in the competence and technique of researchers, as well as differences in habitat

structure between areas (Greenslade and Greenslade 1977), reduces the comparability of samples, and direct sampling alone is inappropriate for long-term monitoring. Direct sampling is frequently used as a supplement to other techniques, such as pitfall trapping (e.g., Andersen and Reichel 1994), and may significantly augment the number of species recorded.

Additional Techniques for Arboreal and Herbaceous Strata

Although arboreal and herbaceous microhabitats are used extensively by ants and harbor considerable ant diversity (Wilson 1987), we will not cover in detail the techniques used to sample these strata. For arboreal habitats, insecticidal fogging (Erwin 1983; Majer 1990) or malaise traps (Longino and Colwell 1997) may be used to sample ants high in the forest canopy. To sample ants from the lower parts of trees and herbaceous strata the following techniques may be employed:

1. Sweeping vegetation with insect nets to collect ants (Lynch 1981).
2. Beating vegetation with a stick to dislodge ants onto sheets or trays (Andersen and Yen 1992; Majer and Delabie 1994; Perfecto and Snelling 1995).
3. The use of sticky traps to capture ants on tree trunks or limbs (Majer 1990).
4. Collecting ants by hand, especially by breaking open twigs and branches and searching epiphytes (see the section on direct sampling). Tree-falls after storms offer a good opportunity to collect arboreal species.

Southwood (1978) and Clements (1982) provide some additional discussion of these techniques, and Basset et al. (1997) provide a review of several techniques.

Environmental Covariates

Information on the environment at the localities where ants are collected contributes greatly to the value of any specimen and is necessary for ecological studies. Both regional and local information (see Appendix 2), as well as GPS coordinates, should be recorded whenever possible.

Researchers should consider recording environmental variables that are known to covary with or affect ant distribution and activity (see Appendix 2). These include the following:

1. A habitat classification by vegetation type or dominant plant species, including slope, aspect, and elevation.
2. Information on the type of ant nests.
3. Soil-surface and air temperatures, relative humidity, insolation levels, and wind speed and direction.
4. The percentage ground cover of bare ground, litter, vegetation, rocks, logs, and other potential ant nest sites (measured with a point frame; see Bonham 1989).
5. The depth of the leaf litter, measured with a wire marked off in 0.5-cm units or some other measuring device.
6. Soil type and texture.
7. Vertical vegetation profiles (or foliage height profiles), measured as the number of touches of vegetation on a thin rod at different height intervals above the ground.
8. The amount of overhead canopy cover, estimated using a densitometer or by eye.

Measurements such as canopy cover or vegetation profiles may be made at several points around each ant sampling point. The characteristics of the sample should also be recorded, including the litter volume and density sampled, quadrat sizes, size of the pitfall trap, and bait type. This information will provide a more mechanistic understanding of comparisons that

Table 9.1 Relative Efficacies of Field Techniques Used to Study Ants[a]

Technique	Total Species Richness	Epigaeic Ants	Litter Ants	Forager Abundance	Behavior	Population	Ease	Comparability
Baiting	0	0	0	–	+	–	+	+
Pitfall trapping	0	0	–	0	–	–	+	+
Quadrat sampling	0	+	–	+	+	–	–	0
Litter techniques	0	–	+	–	–	0	0	+
Colony sampling	0	0	0	–	–	+	–	0
Intensive sampling	+	0	+	0	–	0	–	–
Direct sampling	+	+	+	–	–	–	0	–

[a]Entries are based on a subjective 3-point scale: +, good; 0, moderate; –, poor.

Table 9.2 Percentage of Species Recorded Uniquely by Different Techniques in Single Communities[a]

Technique	Percentage Unique	*n* (area, m^2)	Duration (hours)[b]	Reference
Quadrats	15	12 (0.25)	1–4[c]	Andersen (1991b)
Pitfall traps	30	20	48	
Direct sampling	18.6	1020	nr	Cammell et al. (1996)
Baits	nr	10	na[c]	
Pitfall + direct + baits + Berlese/Winkler	40-82	na	na	Majer (1996)
Pitfall alone	nr	610	168	Majer (1996)
Winkler extraction	46	60 (1.5)	na	Olson (1991)
Pitfall traps	13	40	48	
Beat sheets	38	20		Perfecto and Snelling (1995)
Baits	24	50	na	
Intensive sampling	38	3 (25)	nr	Quiroz-Robledo and Valenzuela-Gonzáles (1995)
Bait traps (in soil)	30	18	72	
Pitfall traps	25	18	72	
Bait traps (on ground)	20	18	72	
Pitfall/bait traps	16	20	48	Romero and Jaffe (1989)
Direct sampling	14	nr	4[c]	
Intensive sampling	3	3 (7.5)	4	

[a]Abbreviations: *n*, sample size per site or season; na, a measurement is not applicable for a given technique (e.g., area for pitfall sampling); nr, the percentage of unique species was not reported for a particular method.

[b]The duration of sampling events per site or season is given where appropriate.

[c]Sampling duration was divided among different times of day.

are made between the ants of different habitats and ecosystems. Which variables are measured and the degree of detail in the measurements will depend on the importance of those features in the habitat that is sampled. In Appendix 2 we offer a generalized format for a data collection sheet that could be used at each sampling point. This format would be useful when a variety of techniques are used in ant inventories.

Conclusion

Table 9.1 summarizes the utility and disadvantages of the techniques presented in this chapter for several different research foci and practical considerations. Each technique is useful for a particular research priority, and a combination of techniques is usually best. For example, 56% of 75 published studies on ant communities that we reviewed employed two or more methods. Several studies have compared the relative efficacies of different techniques in maximizing the number of unique species records at a site (Table 9.2). Unfortunately these comparisons suffer because (1) the results are confounded when different techniques are spatially separated owing to small-scale changes in ant richness and composition (Andersen and Reichel 1994), and (2) it is difficult to match either the intensity or the extent of sampling in applications of the different techniques, even when the sample sizes are similar (but they are often not similar; see Table 9.2). Conclusive comparisons of the relative efficacies of different methods for recording different kinds of ants await further study.

The use of species-accumulation curves (Chapters 10 and 13) can aid in standardizing comparisons. Nonetheless, it is clear from Table 9.2 and other studies that the use of several techniques adds considerably to the number of species recorded at a site. Baiting, pitfall trapping in open microhabitats, the use of Winkler sacks or Berlese funnels for litter-dwelling ants, and direct sampling are an ideal set of techniques for biodiversity monitoring programs and together form the basis for the ALL Protocol (Chapter 14). This combination of methods will ensure both the comparability of samples and as complete a representation of the ant fauna as can be expected. The success of any sampling program will necessarily depend on the sampling protocol used alongside each method, as well as a careful interpretation of the data that accounts for the limitations of the methodology.

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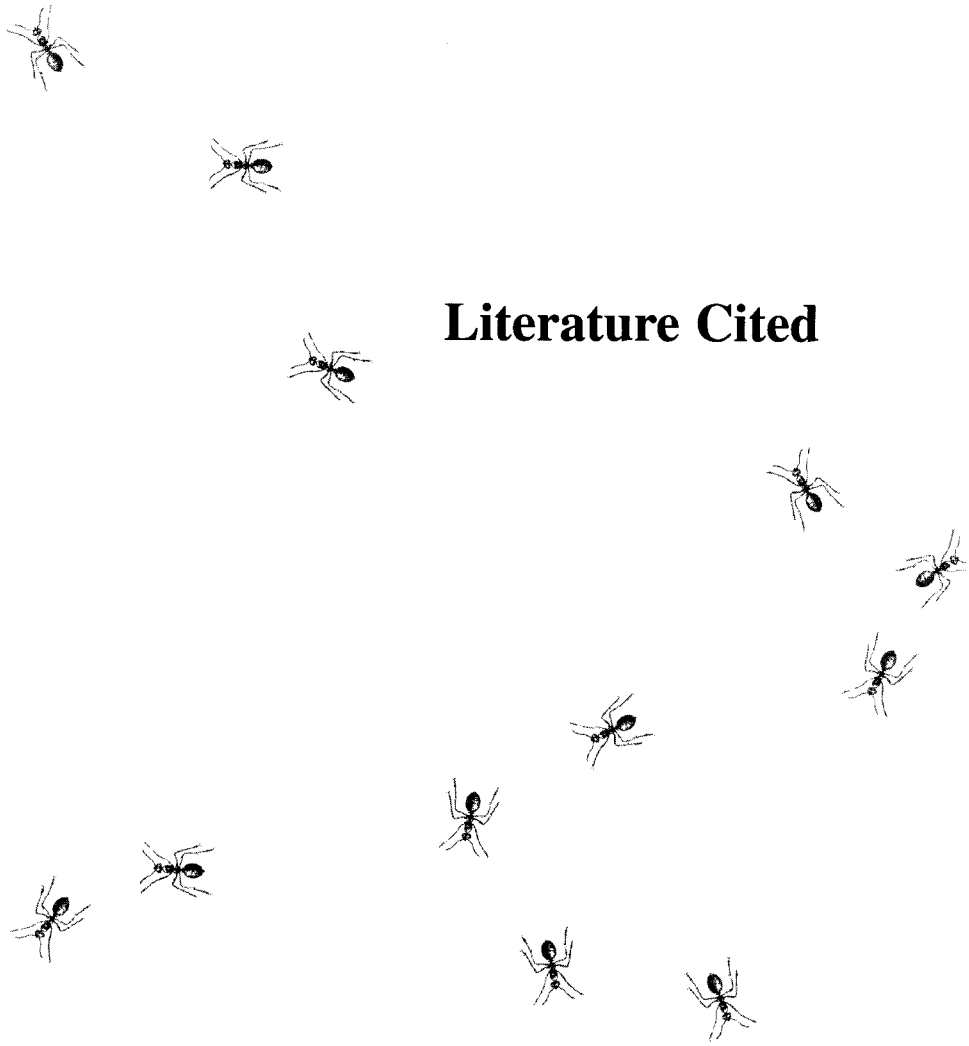

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