ANTS
STANDARD METHODS FOR MEASURING AND MONITORING BIODIVERSITY
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.
ANTS

STANDARD METHODS FOR MEASURING AND MONITORING BIODIVERSITY

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

SMITHSONIAN INSTITUTION PRESS
WASHINGTON AND LONDON
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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Biodiversity Studies, Monitoring, and Ants: An Overview

Leanne E. Alonso and Donat Agosti

The goal of this book is to encourage and enable anyone involved in conducting biodiversity inventories, monitoring programs, or both to include ants among their focal organisms. The information provided here should be sufficient to guide principal investigators, station directors, natural resource managers, technicians, and graduate and undergraduate students in the study of ant diversity patterns.

Although biodiversity inventories should seek to sample as many taxa as possible, logistical constraints dictate that only a subset of all organisms can be sampled. Effective biodiversity studies may do best to focus on organisms that constitute a diverse group, make up a large proportion of the biomass in the area, and perform important or diverse ecological functions in the ecosystem. Ants meet all these criteria and therefore should be given serious consideration for inclusion in biodiversity studies.

Nevertheless ants and other invertebrates are usually not included in such studies, despite their high diversity, numerical dominance, and ecological importance (Wilson 1987). Some of the reasons for this include

1. The high diversity of most invertebrate groups (particularly in tropical areas).
2. The poor status of taxonomic studies of many invertebrate groups (New 1987).
3. The lack of understanding and recognition of the ecological importance of invertebrates in ecosystem functioning.
4. The small size of invertebrates, which causes them to be overlooked.
Such negative perceptions make the incorporation of invertebrates into biodiversity programs more difficult, but not impossible. As this book demonstrates, with proper training and background information, it is possible for any biodiversity program to include invertebrates, and specifically ants. This book is an attempt to make the process as smooth and straightforward as possible, with the hope that data on ant diversity will be included in a network of biodiversity programs throughout the world. Such a network would build a substantial database and enable analysis of global patterns of ant diversity.

To facilitate the inclusion of ants in biodiversity studies, this chapter provides a general overview of the process of sampling and monitoring ants, with reference to the appropriate chapters of this book for more information.

Reasons for Including Ants in Biodiversity Programs

Ants have numerous attributes that make them ideal for biodiversity studies (Table 1.1). These attributes include high diversity, numerical and biomass dominance in almost every habitat throughout the world (e.g., Fittkau and Klinge 1973; Agosti et al. 1994; Fig. 1.1), a fairly good taxonomic knowledge base (Chapters 5 and 12), ease of collection, stationary nesting habits that allow them to be resampled over time, sensitivity to environmental change (Chapters 3 and 7), and important functions in ecosystems (Chapter 2), including interactions with other organisms at every trophic level (Chapter 4).

Ground-dwelling ants, in particular, are the focus of this book because they represent a subset of ants that can be fairly completely sampled using only a few target methods. Ants living in vegetation and tree canopies are more difficult to sample effectively. The ground-dwelling ant community is a good candidate for use in biodiversity inventory and monitoring programs owing to its relative stability, moderate diversity, and sensitivity to microclimate.

Using Ants in Investigative and Management Programs

Before any biodiversity inventory or monitoring program is started, the questions and goals of the study must be clearly defined. For example, for a biodiversity inventory: What is the purpose and scope of the inventory? Will a record of the number of ant species in the area or a list of morphospecies be enough information? Or is the goal to compare the ant fauna of one site with those of other sites, requiring that species names be compiled?

Likewise, monitoring programs must state clear objectives. These objectives in turn will dictate which organisms will provide the best answers to monitoring questions. Are ants the most sensitive indicators of the environmental factor under study? What types of data are needed to provide answers to the monitoring goals? How long must the study run before patterns are revealed?

Knowledge of the diversity of ants in an area can provide a great deal of useful information for conservation planning. First of all, an inventory of the species of ants in an area will provide data on their distribution and will document the presence of any rare, threatened, or ecologically important species, such as introduced species or those found only in particular habitat types. The number and composition of ant species in an area can indicate the health of an ecosystem (Chapter 7) and provide insight into the presence of other organisms, since many ant species have obligate interactions with plants and other animals (Chapter 4).

Data on the species richness and composition of ants provide the baseline needed for using ants to monitor environmental change or recovery. Although many ant species are capable of living in a wide range of nesting sites, many
Table 1.1 The Importance of Ants

**Biomass**
- Ants constitute up to 15% of the total animal biomass in a Central Amazonian rainforest (Fittkau and Klinge 1973).
- Of the more than 750,000 described species of insects, some 9500 are ants (Arnett 1985).
- Of all insect specimens collected in the celebrated forest canopy fogging samples in Peru, 69% are ants (Erwin 1989).
- Some 5300 individual ants were enumerated in 1 m² of tropical lowland forest soil near Manaus, Brazil (Adis et al. 1987).

**Diversity**
- In 20 m² of leaf litter and rotting logs in Malaysia, 104 ant species representing 41 ant genera were collected (Agosti et al. 1994).
- A single tree in Peruvian tropical lowland forest yielded 26 genera and 43 species of ants (Wilson 1987).
- In 250 m² on a cocoa farm in Ghana, 128 species and 48 genera of ants were reported (Room 1971).
- In approximately 5 ha of Peruvian tropical lowland forest, 365 species from 68 genera of ants were found (Tobin 1994).
- In 18 km² of semi-arid South Australia, 248 species from 32 genera of ants were documented (Andersen and Clay 1996).
- In 5.6 km² in temperate Michigan, 87 species from 23 genera of ants were observed (Talbot 1975).

**Biology**
- All ants are social. Their nests are perennial and thus can be collected all year round.
- There is little variation in ant abundance between rainy and dry seasons (Adis et al. 1987).
- Fragmentation affects ground-dwelling ants (see Chapter 15).
- Together they turn more of the soil than do earthworms in New England (Lyford 1963).
- The density of leaf cutter ant (*Atta sexdens*) nests is up to 20 times greater in secondary forest than in primary forest (Nepstad et al. 1995).
- Leaf cutter ants are the dominant herbivores in tropical forests: the ground volume occupied by a single 6-year-old nest of *A. sexdens* weighed approximately 40,000 kg, and this young colony was estimated to have gathered 5892 kg of leaves (Wilson 1971).
- The seeds of 35% of all herbaceous plants are estimated to be dispersed by ants (Beattie 1985).
- Ants rank among the principal granivores in the southeastern United States (Davidson et al. 1980).

**Systematics**
- A catalogue of all described ant taxa exists and lists 9538 species (Bolton 1995b).
- An illustrated key to the ant genera of the world is available (Bolton 1994).
- The taxonomy of ants is based on the ubiquitous worker cast.

**Leaf litter ant surveys are cost efficient**
- A statistically representative sample of the ant diversity of a given area can be completed in one week.
- In comparison, other taxonomic groups require:
  - Sampling and identification of tree species in 1 ha in the Atlantic Forest, Brazil: 4 person-years (Thomas, pers. comm.).
  - The number of new tree species (DBH > 10 cm) in an Amazonian rainforest still readily increases after 4 ha have been sampled (Ferreira and Prance, 1998).
  - Representative sample of snakes in the Brazilian Amazon: >1000 km walked (Zimmerman and Rodriguez 1990).
  - Representative sample of frogs near Manaus, Brazil: >350 person-hours (Zimmerman and Rodriguez 1990).
  - Representative sample of birds in Western Amazonia: >800 catches; 1.2–8 catches per day using mist nets (Robinson and Terborgh 1990).
  - Representative sample of butterflies in Ecuadorian rainforest: >1000 catches (specimens) over one year (De Vries et al. 1997).
  - Representative sample of ithomiine butterflies in Cartago, Costa Rica: 4 days (Beccaloni and Gaston 1995).
others have specific requirements and can therefore be used as indicators of habitat change or restoration success (Chapter 7). On the flip side, there are several ant species in all parts of the world that are well adapted to living in disturbed areas and are the first to colonize these areas. The presence of these ant species is a reliable indicator of habitat disturbance. Since most ant species live in stationary colonies and do not move readily between habitats, they are ideal for monitoring because they can be resampled repeatedly over time using the same methods, providing information about how vegetation structure, prey abundance, soil quality, or predator density may be changing over time (Chapter 7).

The First Steps: How to Begin Incorporating Ants into a Study

In order to get the most useful information from an inventory, it is best to begin with a good knowledge base about the organisms under study. Therefore, the first step, for those who are not particularly familiar with ants, is to read the chapters in this book that cover basic ant biology and ecology (Chapters 2–5 and 8). The most comprehensive and readable overview of ants can be found in *The Ants* by Hölldobler and Wilson (1990). One should also read the chapters on how ants can be used in monitoring programs, as indicators of diversity or environmental change (Chapters 6 and 7), to evaluate whether ants are appropriate for the goals of the biodiversity project in question. Ants are sensitive to many types of environmental disturbance, but other organisms might be better suited for particular challenges.

The next step is to learn some basic ant taxonomy (see Chapters 5, 11, and 12 for basic taxonomic information and additional references).

What Sampling Involves: Resources, Time, and Helpful Hints

Once the necessary background in ant biology has been acquired, the standardized sampling protocol for ants, the ALL (Ants of the Leaf Litter) Protocol (Chapter 14), can be implemented with ease. As with all research projects, the first step in conducting field work is to gather all equipment needed (Appendix 1). Second, the sampling site must be chosen. The placement of the sampling transect should be determined based on the research objectives. For example, a transect may be placed randomly if an objective overview of ant diversity in the
habitat is desired, or it may be positioned so that it traverses several microhabitats within the sampling area, thus collecting ants from a variety of habitat types. Alternatively, the transect may be placed in the same areas where mammal or reptile surveys have been carried out, in order to draw some comparisons between taxa. Sampling need not be limited to only one transect per site; several transects can be utilized at each site.

The ALL Protocol, described in Chapter 14, is very simple to implement. The entire process can be carried out in less than 3 days if all the necessary equipment has been obtained. The standard protocol relies on two principal sampling methods: (1) leaf litter samples, which are extracted through mini-Winkler sacks for 48 hours, and (2) pitfall traps, left in the ground for 48 hours (see Chapter 9 for descriptions of these methods). The number of available mini-Winkler sacks will usually be the limiting factor to the efficiency of this sampling method. The ALL Protocol recommends taking 20 samples. This implies that 20 mini-Winkler sacks will be needed to process all the samples at the same time. If 20 mini-Winkler sacks are available and can be run at the same time, then all samples can be processed in just over 48 hours. If fewer than 20 sacks are available, samples may be extracted one after the other. This will prolong the sampling process, since for every set of Winkler sacks used, 48 hours is needed for litter extraction.

Alternatively, a Berlese or Tullgren funnel (Chapter 9) may be used for extracting ants from the leaf litter, or the litter samples may be sorted by hand. Extraction using Berlese or Tullgren funnels should take the same length of time as that using Winklers, and hand sorting should also be completed in 48 hours.

During this 48-hour period, it is a good idea to do some general hand collecting in the area near the sampling transect, in order to collect a greater number of ant species. General collecting is not standardized and therefore should not be part of a monitoring program, but it can be a valuable addition to an inventory. General hand collecting includes inspecting rotting logs, branches, and twigs on the ground; scraping soil; and visually searching for ants (see Chapter 9 for more details). When doing general collecting, one should be sure to record as much data as possible about where the specimens were collected, particularly distinguishing between ground and vegetation collections. The standardized protocol restricts sampling to ant species that live or forage in the leaf litter or on the ground. General collecting can add additional ant species from the vegetation.

At the end of the 48-hour period, the ants must be collected from the pitfall traps and Winkler sacks. This process may take from 2 to 4 hours for two people, depending on the ability of the researchers to distinguish tiny ants in the bottom of muddy cups. This is an important step, and it should be performed with great care so as not to miss any ants, some of which are nearly microscopic. Ant specimens should be placed in vials of alcohol and completely labeled with such information as the type of collection method, trap or sample number, date, and collector's name (Chapter 11). The steps involved in processing the specimens to prepare them for identification are covered in Chapter 11.

**Beginning the Identification Process**

Perhaps the most difficult part of incorporating ants into biodiversity programs is the identification process. Few people in the world are able to identify ants to species level, largely because of the lack of training and the poor state of tropical ant taxonomy. However, it is not impossible, and identification to genus and morphospecies can be done by most people after a little instruction and a lot of practice.
Is It an Ant?
The first step is to sort the ants from the other organisms collected in the pitfall traps and Winkler sacks (Chapter 11). Make sure that all organisms identified as ants really are ants (for help, see Chapters 5 and 11). All ants are classified into one family, the Formicidae, which is in the order Hymenoptera along with bees and wasps.

Ant Subfamilies and Genera
The next steps are to identify the ant specimens to subfamily and then to genus. There are 16 subfamilies of ants, with 296 genera and more than 9000 described species (Bolton 1994). Only a subset of these subfamilies, genera, and species are found in each biogeographic region of the world. Dichotomous taxonomic keys to ant subfamilies and genera are available in Hölldobler and Wilson (1990), Bolton (1994), and several other publications (Chapter 12). If these references are not available, consult the social insects Web site (http://research.amnh.org/entomology/social_insects/) for general pictorial keys and information on how to obtain these publications. These keys are fairly technical and require some knowledge of insect morphology. However, with practice and a little background reading on insect morphology, most researchers should be able to identify ants to genus. A more user-friendly, pictorial guide to ant genera of the world is currently being prepared.

Before attempting to identify an ant specimen to genus or species, it is best to become familiar with the taxonomic keys, the body parts of an ant (Fig. 5.1), and the morphological characters that are most frequently used to identify ants (Chapters 5 and 11).

Species Identifications
Identifying ants to species is much more difficult because taxonomic keys to species are scattered throughout the literature, many keys are out of date, and there are no keys for many regions of the world, particularly tropical areas (Chapter 12). The first step in species identification is to separate the ant specimens into morphospecies, or units that look different from one another (Chapter 11). Each morphospecies should be assigned a number so that specimens sorted later can be associated with similar previously encountered specimens. Morphospecies designations should be based on the traditional morphological characters used in ant taxonomy (Chapters 5 and 11).

It is unlikely that identification of all ant species at a site can be completed without some assistance. However, attempts should be made to identify as many of the specimens as possible, using publications that contain information on the ants of the area, especially those publications that contain taxonomic keys (see Chapter 12 for sources).

For a biodiversity inventory, the number of species (based on morphospecies) may be information enough. However, without knowing the scientific names of the ant species in an area, little can be inferred about the presence of particular species or the patterns of their distribution or diversity. Environmental management and monitoring studies require species identifications so that the presence of exotic species, rare or threatened species, or species specialized on particular climatic conditions can be recognized (Chapters 6 and 7). In addition, comparisons between sites, both locally and globally, require species identifications if the studies are not conducted by the same researchers.

Essential Collaboration with Ant Taxonomists
After identifying the ant specimens as far as possible, the next step is to contact local entomologists, some of whom may be familiar with ants, to provide taxonomic training or assist with identifications.
If no entomologists are available in the area, scientists trained in ant taxonomy may be able to help. The major ant collections of the world are listed in Chapter 12; most of these collections have ant taxonomists on staff. The social insects Web site also has lists of and links to ant taxonomists. Some ant taxonomists may be available to visit a site and provide training in collecting methods and ant identification. This approach is highly recommended—especially if funding can be provided for the taxonomist to travel to the site—since it can provide valuable training for the project participants and enable local researchers to identify ants on their own in the future. If training is neither desired or financially feasible, ant taxonomists can likely provide species identifications if specimens are sent to them. When seeking assistance from any of the taxonomists, be sure to contact them well in advance (at least two months) of sending the specimens for identification. It is important to keep in mind that these taxonomists are busy and that species identifications take considerable time. Allow at least three to twelve months for identifications to be made, depending on how many specimens must be identified.

The Value of Information Exchange

The exchange of ant collections and taxonomic expertise between local researchers conducting biodiversity inventories and ant specialists can be beneficial for both parties. Field researchers receive assistance with species identifications, enabling them to learn more about their local fauna. Biological information about particular species may also be provided by the ant specialists, and this helps the local researchers better understand and utilize the patterns of ant diversity in the management of their areas. Ant taxonomists also benefit by receiving ant specimens from taxonomic groups and geographic areas of interest to them. The specimens and associated biological information that specialists receive from field projects contribute to systematic studies and taxonomic revisions, thus adding to our understanding of the biology and evolutionary history of ants and furthering our ability to identify particular species.

What to Do with Specimens

The ants collected in biodiversity studies are potentially valuable to taxonomists and local researchers, so they should be handled with care. A reference collection of the species from the site should be established at the local field station, university, or research institution. If possible, a few representatives from each species should be pinned and housed in a cool, dry collection case (see Chapter 11 for details). The pinned specimens will serve as a reference for future ant identifications. The remaining specimens may be stored in vials of alcohol.

Ant specimens should also be sent to those ant taxonomists who are working on particular groups of ants, regardless of whether their taxonomic assistance is needed (see Chapter 12 or the social insects Web site). These specimens may prove valuable in a taxonomic revision by providing needed material on poorly known species or additional data on geographic distributions.

Additional specimens should be deposited in major ant collections (see Chapter 12 for a list). Depositing ant specimens in national collections allows other researchers to examine them for taxonomic comparisons.

Data Output: How Best to Utilize the Information

Collecting and identifying ants provides data that can be used in furthering the goals of the biodiversity project. How the data are processed after collection is perhaps the most important
part of the entire study. Careful consideration should be given to which methods of data analysis will best address the questions of each particular study. Several possible analytical methods are described in Chapter 13.

Analytical tools can be used throughout the study to determine the ultimate sample size needed to collect representatives of all ant species in an area (Chapter 10), to determine how long a monitoring program need be run, or to make adjustments to management practices.

The Importance of Training

Depending on their background knowledge of insects and ant biology, researchers involved in biodiversity studies may or may not feel qualified to carry out the standard protocol for ants on their own. Although this book should provide enough detail for almost anyone to utilize the methodology, some may feel that they need more direct training or assistance. As mentioned earlier, on-site training is recommended if the project has sufficient funding to bring in ant specialists. Such training provides a lasting knowledge base that will enable the project to carry out future inventories and follow through with ant identifications. It also enhances the researchers’ ability to train others in the ant collection process.

The standard protocol for ants is also being taught as part of the multitaxa inventory and monitoring approach of the Smithsonian Institution/Monitoring and Assessment of Biodiversity (SI/MAB) training courses throughout the world. For more information on becoming a part of the Smithsonian network, readers should contact the SI/MAB Biodiversity Program of the Smithsonian Institution, 1100 Jefferson Drive SW, Suite 3123, MRC 705, Washington, DC 20560; www.si.edu/simab.

In addition to receiving training themselves, many researchers will eventually be required to train others to implement the protocol. Standardized protocols, such as that described in this book, are an invaluable part of biodiversity programs, and promotion of their use will enable data from biodiversity studies worldwide to be put into broader geographic and global contexts.

Promoting Ants in Biodiversity Programs

We hope that this book will help facilitate the incorporation of ground-dwelling ants into biodiversity programs throughout the world. The advantages of their inclusion should be clear from this chapter as well as several others in this book (e.g., Chapters 2, 4, and 6–8). Ants are a key taxon for biodiversity studies and a valuable addition to multitaxa programs. Their incorporation into biodiversity programs in diverse habitats and geographical regions will provide a global database of ant diversity that can be used by taxonomists, ecologists, and natural resource managers in any country. Knowledge of global patterns of ant diversity and responses of ant communities to local and global environmental change will assist with conservation planning worldwide.

Further Information and Assistance

The contributors to this book have joined together to form the ALL Group, a team of ant taxonomists and ecologists from throughout the world dedicated to the promotion of ants in biodiversity programs. Readers should feel free to contact the ant experts of the ALL Group via the social insects Web site (http://research.amnh.org/entomology/social_insects/) for help with incorporating ants into biodiversity programs. We will try to arrange assistance in training, obtaining equipment, identifying specimens, or other matters. Be sure to contact the Group well in advance of target dates. Further information about the ALL Group and ants in general is available on the social insects Web site.
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