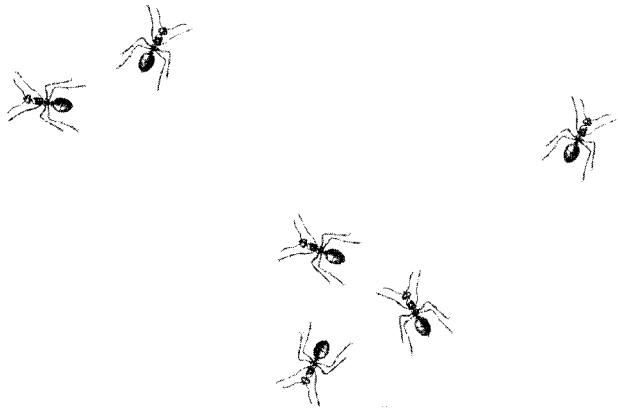


The ALL Protocol

A Standard Protocol for the Collection of Ground-Dwelling Ants

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There is no single best method to sample the ground-dwelling ant fauna. The objectives of each study will determine the appropriate methods and sampling intensity. For example, a wide variety of methods and a greater effort are needed to obtain a thorough inventory of the ants of an area and to collect as many species as possible. In contrast, a rapid assessment of the ant fauna using a few standardized methods and less sampling effort would allow for comparisons between different habitats and would establish a baseline for a longer-term monitoring program.

However, the use of standardized methods that can be reliably repeated in different habitats, at different times of the year, and by different researchers is beneficial. Using the same basic methodology, individual studies can be analyzed in relation to others and can thus be

put into a larger, global context. Here we present a standard protocol for the collection of ground-dwelling ants, the Ants of the Leaf Litter (ALL) Protocol. We expect that this protocol will stimulate further research on ant diversity and that it will be used by a variety of researchers in a diverse array of sites.

The ALL Protocol starts with a minimal configuration, utilizing two of the ant collecting methods that have been proven to sample the largest component of the ground- and leaf litter-inhabiting ant fauna: the mini-Winkler extractor and pitfall traps (Chapter 9). This method is rapid; sampling can be completed in a total of 3 days per site if desired. The sample size, 20 1-m² samples of leaf litter and 20 pitfall traps, has been found to be sufficient to sample at least 70% of the ant fauna (Chapter 10).

However, we suggest that researchers start with 50 samples during the first survey to practice the techniques and to determine the actual number of samples needed to collect the desired percentage of ant species (Chapter 10). Depending on the study objectives, other complementary methods can be added to the standard protocol in order to sample a wider range of ant species (Chapters 1 and 9).

The ALL Protocol

Basic Setup

- 200-m transect (at least one)
- 20 sampling points at 10-m intervals
- 48-hour time period
- 1–2 people (2 people recommended)

Methods Employed at Each Sampling Point

Standardized, Repeatable Techniques

- Collect leaf litter, 1 m²
- Sift litter
- Extract ants from litter using mini-Winkler
- Place 1 pitfall trap

Optional Techniques to Collect More Species

- Inspect dead wood
- Scrape soil (15 × 15-cm area at 1-cm layers down to 10 cm)
- Direct collecting by hand

Overview of the ALL Protocol

The most important points in implementing the ALL Protocol are outlined in this section, along with references to chapters of this book that contain more information. See Appendixes 1 and 3 for complete lists of equipment needed for the sampling methods and specimen processing.

Transects

Before choosing a particular transect, it is worthwhile to walk through the area to get an impression of the overall environmental variation. Chapters 1 and 9 provide guidance on transect placement.

Ecological Data

In addition to the standard collection information (Chapter 11), ecological data must be

recorded. For each transect, the following minimal set of parameters should be described: name of collector, date, choice of transect, locality, habitat, season, soil type, temperature, and microhabitat. See Chapter 9 for a complete list of relevant ecological information and explanations of these parameters.

Labeling Field Samples

It is of the utmost importance to label all samples adequately. Most of the labeling can be done prior to the commencement of field work. Vials used for collecting ants by hand or from logs should preferably be pre-labeled as well. See Chapters 9 and 11 for more details.

Pitfall Traps

Pitfall traps should be placed 1 m from the transect line on the opposite side of the transect from where the leaf litter samples were taken. Any plastic drinking cup with smooth sides can be used, but it is best to use cups with openings of the same diameter consistently, in order to standardize the samples. Twenty cups are needed. See Chapter 9 for more information on how to set and collect pitfall traps.

Leaf Litter Samples and Mini-Winkler Extraction

See Chapter 9 for complete instructions on how to collect the 1-m² leaf litter samples and extract ants using mini-Winkler sacks. The ALL Protocol requires at least 20 mini-Winkler extractors and one sifter.

Sorting Samples in the Laboratory

Ant specimens and other invertebrates can be separated from debris using the salt water extraction method (Chapter 11). After separation the samples should be washed with ethanol.

Identifying Morphospecies

Ants from each sample should be separated from other invertebrates and housed in a separate vial.

Procedures for sorting and identifying specimens to morphospecies are given in Chapter 11.

Labeling Samples and Specimens

All samples must be labeled immediately with proper labels (Chapter 11).

Time Requirements

A minimum of 3 days is needed to use the standard ALL Protocol. The leaf litter collections should be run through the mini-Winkler extractor for a 48-hour period. Pitfall traps should also be left out for 48 hours. Both the mini-Winkler extractor and the pitfalls can be left running for a longer time if desired, but samples should be collected from both at 48 hours in order to obtain the standard sample. More time may produce additional species, but the benefits should be weighed against the advantages of running more transects instead.

For inventories, it is recommended that more than one transect be run and the species-accumulation curves be plotted by sample and transect (Chapter 13). This approach will provide a review of the fraction of the ant fauna sampled and will help determine if additional transects are needed. Additional sampling methods—such as the inspection of dead wood, soil scraping, and direct sampling—may be added in order to maximize the diversity of ants sampled (Chapter 9).

Timetable

An estimate is given in this section for the amount of time required for one person to carry out the standard ALL Protocol. It is recommended that two people carry out the protocol together, to provide assistance with leaf litter gathering, sifting, and other tasks. The estimated total time needed to sample, process, and identify ant specimens from one transect is 161 working hours for a single professional.

Field Work

All times are in hours.

	One Person	Two People
DAY ONE		
Early morning		
1. Mark transect	1.5	1.0
2. Dig in pitfall traps	1.5	1.0
3. Collect Winkler samples	5.0	3.0
Afternoon		
1. Fill in Winkler apparatus	3.0	2.0
Late afternoon/early evening		
1. Direct collecting	<u>1.0</u>	<u>1.0</u>
Total	12.0 hours	8.0 hours
DAY THREE		
Morning		
1. Collect one log	1.0	1.0
2. Direct collecting	1.0	1.0
3. Scrape soil	1.0	1.0
Afternoon		
1. Analyze soil samples	2.0	1.0
2. Collect pitfall traps	2.0	1.5
3. Collect Winkler samples	2.0	1.5
4. Check all labeling	<u>0.5</u>	<u>0.5</u>
Total	9.5 hours	7.5 hours

Laboratory Work, Identification, and Analyses

Mounting, labeling, and identifying specimens from Winkler samples	60
Mounting, labeling, and identifying ant specimens from pitfall traps	60
Mounting, labeling, and identifying ant specimens from other samples	10
Entering and analyzing data	<u>10</u>
Total	140 hours

Data on ant diversity collected using this protocol can be compiled and compared, thereby providing the context needed to begin looking at truly global ant diversity patterns. We encourage researchers who use the ALL Protocol to provide their data to the social insects Web site (http://research.amnh.org/entomology/social_insects/) for inclusion in a global database on ant diversity.