# TROPICAL INSECT DIVERSITY - HOW TO SAMPLE IT

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## Contents





## Summary

Insects are a hyperdiverse element of tropical ecosystems. Estimates of diversity are needed, and quantitative sampling programs are required to obtain them. Although lack of taxonomic knowledge limits the study of many insect groups, butterflies, ants, and dung beetles are seeing increasing use in biodiversity assessment. Insect sampling methods can be divided into "volumetric" samples, which provide data on absolute abundance per unit of substrate, and "individual-based" methods which are less able to provide abundance data. The former include direct visual inspection of samples, Berlese samples, Winkler samples, branch bagging, and canopy fogging. The latter include netting, sweeping, beating, Malaise traps, flight intercept traps, pitfall traps, light traps, and baiting. Ecological diversity includes both number and relative abundance of species. Diversity indices include species richness, Fishers Alpha, Shannon, and

Simpson. Estimates of diversity are usually dependent on sample size, and pooled-quadrat plots reveal at what sample size estimates stabilize. Species richness is one of the most important yet most difficult characteristics to measure. Examining species accumulation as a function of number of samples provides information about species density, but species accumulation as a function of number of individuals is a better method for investigating species richness of a whole community. Estimating species richness for an area or community can be done by extrapolating species accumulation curves, fitting parametric abundance distributions, or using various non-parametric methods. However, for hyperdiverse tropical insect communities none of these methods are satisfactory. Species accumulation curves tend to increase indefinitely, without approaching an asymptote, and richness estimates do not stabilize with sample size. Recommendations for measuring and comparing tropical insect diversity are (1) using diversity indices such as Fishers Alpha that stabilize at small sample size, and (2) using sample-based rarefaction to compare observed species richness based on a common number of individuals sampled.

## 1. Introduction

Four large groups of multicellular organisms form the macroscopic substance of terrestrial ecosystems: vascular plants, fungi, vertebrates, and arthropods. Arthropods are animals with a jointed exoskeleton and include mites, spiders, centipedes, millipedes, crustaceans, and insects. Insects are the six-legged arthropods, a subset of arthropods that has been very successful in freshwater and terrestrial habitats. Insects include dragonflies, mayflies, grasshoppers, cockroaches, termites, stoneflies, true bugs, flies, beetles, butterflies and moths, ants, bees, and wasps. Throughout this chapter the word "insects" will be used to refer to all terrestrial arthropods. Among terrestrial animals, vertebrates dominate the size range from frogs to elephants. Insects dominate the next tier down, ranging in size from a pinhead to the size of a human fist.

Insects form a high proportion of the animal biomass in terrestrial ecosystems, equaling or surpassing vertebrate biomass. They have major roles in ecosystem function, facilitating decomposition and biomass conversion, and acting as major components of food chains. They have an enormous variety of forms and lifestyles, far surpassing vertebrates in both species and functional diversity.

For most organisms the tropics have an elevated diversity compared to the temperate zone, and insects are no exception. Tropical insect communities are extraordinarily diverse, and insect diversity strongly declines with increasing latitude. Higher diversity in the tropics occurs at all spatial scales: from 1 $m^2$ plots to continent-scale areas. A square kilometer in lowland Washington State, USA, may contain 30 species of ants. A square kilometer in lowland Costa Rica may contain 450 species. Aquatic insects do not show the same degree of change in diversity with latitude. Methods for studying aquatic insect diversity do not differ between tropical and temperate zones and are not considered further here.

### 1.1. Why Sample Insects?

The average insect has a shorter lifespan than the average vertebrate or plant, and their small size allows them to specialize on small microhabitats. Populations can fluctuate rapidly and across small spatial scales. Investigators have found insect sampling to be informative in addressing basic questions of ecology and evolutionary biology because they can be done over small temporal and spatial scales. The individuals in a community of trees have experienced environmental processes operating over a scale of centuries. The individuals in a community of insects have been shaped by processes operating over a scale of months. Biotic response to climate change and other forms of human-induced habitat change are more rapid for insects than for larger, longer-lived organisms. In practical terms, insects are typically sampled from the field and identified later, and voucher specimens can be kept in perpetuity in museums. This increases the reliability of identifications and permits future verification should the need arise.

### 1.2. ATBI versus ABTI

When studying insect diversity, two approaches can be taken and there is a tradeoff between them. The opposite ends of the spectrum are the All Taxon Biodiversity Inventory (ATBI), in which a large number of insect taxa is measured in one or a few areas. The other is the All Biodiversity Taxon Inventory (ABTI), in which one or a few taxa are measured over a large area or many areas. These approaches have inevitable tradeoffs in personnel and expertise. The first requires many taxonomic experts and a relatively small investment per person. The second requires fewer taxonomic experts but a larger commitment per person (and a larger travel budget). The first approach may be appropriate when the goal is monitoring change in one or a few sites, or comparing biodiversity value of a few sites. The second approach is appropriate when investigating landscape or global-level patterns of diversity.

### 1.3. The Taxonomic Impediment

A limitation to measuring insect diversity is the poor state of taxonomic knowledge for many groups, which has been called the "taxonomic impediment". Many groups of insects and other arthropods have never received much taxonomic research. Many species do not have names and in many cases it is not even clear how to recognize species boundaries. Many insect species are subtly distinct and it takes practice to learn how to differentiate species of a particular group. Novices who sort insect samples into "morphospecies" often are recognizing generic level differences. This is not surprising, since characteristics which are relatively conspicuous are often used to define genera. Species-level characters are often small differences in shape, color, distribution of setae, and details of genitalia. Insects also have large numbers of "cryptic" species, which are species that are genetically distinct but exhibit few or no differences in external morphology. The advent of rapid DNA sequencing is confirming the fact that there are many cryptic species of insects. Finally, many insects are so small that labor-intensive specimen preparation is required before identification can be done.

### 1.4. Favorite Taxa

#### 1.4.1 Butterflies

As a result of these impediments to identification, a few groups of insects have emerged as favored subjects: butterflies, ants, and dung beetles. Butterflies are a group of day-flying Lepidoptera in several families. Butterflies are conspicuous to humans because they are brightly colored, day-flying, and can be seen at a distance. Even though they are among the most noticeable of all insects, they are also among those with the lowest density. A 2 mm long brown beetle with the density of the average butterfly would either be unknown to science or considered extremely rare. Two methods are commonly used to sample butterflies: netting and trapping at baits. Netting involves moving through the habitat with a net and attempting to capture every butterfly that is seen, or at least capturing an example of every species encountered, during a predetermined length of time. In the tropics, a second method is trapping butterflies by baiting with rotten fruit or similarly odiferous material. This attracts a guild of fruit-feeding butterflies that are large and long-lived as adults. Baiting has the advantage of being more quantitative and less dependent on the skills of individual researchers in the field, but the disadvantage of sampling only a small part of the butterfly fauna. The advantages of studying butterflies are (1) they are popular with the general public and have been studied by amateurs and professionals for centuries, and thus the taxonomy is better known and more accessible than for any other insect group of comparable size; and (2) species-level and population-level genetic differences are often revealed by color patterns on the wings, which can be readily observed on specimens without recourse to special preparation or microscopic examination. The disadvantages are (1) butterflies are large, brightly colored, and very low density, which makes them atypical compared to most other insects; (2) the presence of adults may be strongly seasonal, requiring sampling at multiple times of year; (3) both common sampling techniques are subject to high interspecific variation in catchability, which can bias abundances.

### 1.4.2. Ants

Ants comprise a single insect family, the Formicidae. They are all social, forming colonies of genetically related individuals, some of which are sterile workers and some of which are reproductive females. In low to mid-elevation terrestrial habitats throughout the tropics they are abundant and diverse, filling the environment from subsoil to the tops of trees. About 60% of the ant fauna lives on the forest floor, in or just under the leaf litter and in rotten wood. The remaining 40% are arboreal species, living up in the vegetation column. Terrestrial and arboreal ants form two distinct communities with relatively little spatial overlap. Three common methods for sampling ground ants are baiting, pitfall traps, and Winkler samples (see section 3). Baiting is easy and inexpensive, but because it relies on a behavioral response and attraction, it samples mainly the larger generalist omnivores, which are only a small subset of the ground ant fauna. Observed abundance at baits is not a good measure of true density because it is also affected by diet preference and recruitment ability. Pitfall traps are easy and inexpensive to install, and compared to baiting they are less affected by diet preference and recruitment ability. But they still introduce strong variation in trapability among ground ant species. They favor large species that are active foragers on the surface. Small, slow-moving species that live in or under the litter are undersampled. Winkler samples involve chopping a square meter of the forest floor with a machete, sifting the chopped material, and extracting the arthropods from the sifted material. It requires more equipment than other methods (sifter, extraction bags) but is less prone to differential capturability of species and thus gives a better measure of abundances of most species in the ground ant community. Arboreal ants are more difficult to sample quantitatively, although it can be done with Malaise traps or canopy fogging.

The advantages of studying ants are (1) they are ubiquitous and abundant; (2) they can be sampled with methods that extract all individuals from a sample of the environment, instead of methods that rely on the hunting skills of a researcher or behavioral attraction of individuals to a trap; (3) the taxonomy of the group is moderately well known; and (4) colonies are sessile (for the most part) and perennial, which results in few occurrences of long-distance migrants and weak affects of season. Disadvantages of ants are (1) sociality confounds abundance measures because the number of workers in a sample is caused by a combination of colony density and numbers of workers in colonies; (2) specimens can be difficult to identify to species because many interspecific distinctions are subtle; and (3) specimens must be point-mounted for proper identification, which is a labor-intensive step.

### 1.4.3. Dung Beetles

Dung beetles comprise one subfamily of the scarab beetles, the Scarabaeinae. They are common in lowland habitats in many parts of the tropics. They are easily and inexpensively sampled with baited pitfall traps. Commonly used baits are feces of large mammals (e.g. humans, cows, pigs) and carrion. The advantages of studying dung beetles are (1) they are relatively large organisms that do not require point-mounting or other labor-intensive preparation; (2) the taxonomy is moderately well known; (3) dung beetles show strong habitat affinities; and (4) sampling methods are quantitative, easily replicated, and inexpensive. The disadvantages of studying dung beetles are (1) similar to baiting for ants, sampling methods rely on attraction to baits and introduce strong trapability variation among species, resulting in biased abundance measures; and (2) adults may respond strongly to season, requiring sampling at a particularly favorable season or multiple times during the year.

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## Biographical Sketch

**John T. Longino** is a professor at The Evergreen State College in Washington State, USA, where he divides his time between teaching and research. His research interests include (1) taxonomy of Neotropical ants, (2) community ecology and biodiversity patterns of ants, and (3) methods of measuring and comparing biodiversity. From 1979-1983 he carried out his doctoral research in Corcovado National Park, Costa Rica, on insect-plant relationships. While observing ants at extrafloral nectaries of passionflower vines he became interested in ant diversity. Since 1984 his primary focus has been on the ant fauna of Costa Rica, and since 1996 he has been developing the Ants of Costa Rica Website. From 1992-1995 he was co-Director (with Rob Colwell) of Project ALAS, a major arthropod survey project at La Selva Biological Station. He is currently initiating a new project on the leaf litter arthropods of Mesoamerica (Project LLAMA), a joint project with Robert Anderson of the Canadian Museum of Nature. Project LLAMA will sample ant and weevil diversity on elevational gradients from Nicaragua to southern Mexico. Longino has 53 scientific publications and was the recipient of the American Society of Naturalists' 2006 E. O. Wilson Naturalist Award.