

# ANTS

## STANDARD METHODS FOR MEASURING AND MONITORING BIODIVERSITY

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# Applying the ALL Protocol

## Selected Case Studies

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Although the ALL Protocol put forward in this book was only recently developed and is published for the first time here, it is derived from the experiences of many myrmecologists and from numerous studies of ant diversity over the years. Before the development of this protocol, studies of ant diversity utilized a wide variety of methods, as described in Chapter 9. The ALL Protocol is the result of an extensive evaluation of these methods in different countries and under a variety of conditions. The studies by Delabie et al. (Chapter 10) provided the strongest data for choosing methods for the ALL Protocol, but a number of other key studies were also influential in its development.

Brief descriptions of these key studies, from diverse parts of the world (Madagascar, Malaysia, India, and Brazil), are provided here to

illustrate how the two principal collecting methods of the ALL Protocol (leaf litter extraction using the Winkler technique and pitfall traps) compare, and how they have been used successfully in a variety of biodiversity studies, particularly to measure ant diversity and to detect habitat change. Full descriptions of these studies are presented in Agosti et al. (2000).

### Madagascar

A series of studies in Madagascar by Fisher and colleagues (Fisher 1996a, 1996b, 1998, 1999a, 1999b; Fisher and Razafimandimby 1997; Fisher et al. 1998) provides the best insight into the usefulness of the ALL Protocol and the comparative value of its two principal methods, Winkler extraction and pitfall traps. In one

particularly informative study, Fisher surveyed ground-dwelling ant diversity at elevation zones separated by 400 m at four rainforest localities: the Reserve Naturelle Integrale d'Andohahela, the Reserve Naturelle Integrale d'Andringitra, the Reserve Speciale d'Anjanaharibe-Sud, and the Western Masoala Peninsula in eastern Madagascar.

At each site, 50 pitfall traps were used and 50 leaf litter (Winkler) samples were taken, in parallel lines 10 m apart along a 250-m transect. Pitfall traps were placed and leaf litter samples gathered every 5 m along the transect. Pitfall

traps consisted of test tubes (18 mm internal diameter by 150 mm long), partly filled to a depth of about 50 mm with soapy water and a 5% ethylene glycol solution, inserted into polyvinyl chloride (PVC) sleeves, and buried with the rim flush with the soil surface. Traps were left in place for 4 days. Ants were extracted from samples of leaf litter using Winkler extractors over a 48-hour period (Chapter 9; Fisher 1998).

The observed and predicted number of species sampled by the Winkler, pitfall, and combined methods for 10-, 20-, 30-, 40-, and

**Table 15.1** Observed Number of Ant Species Evaluated at Different Sample Sizes for Winkler Sacks, Pitfall Traps, and Both Methods for Each 800-m Zone Site in Madagascar<sup>a</sup>

Methods	Observed Species Richness after:					Estimated Species Richness <sup>b</sup>		
	10 Samples	20 Samples	30 Samples	40 Samples	All (50) Samples	ICE	Jack-knife	M-M
800 m Andohahela								
Winkler	39.6 (59.3)	49.5 (74.1)	55.3 (82.8)	59.5 (89.1)	63 (94.3)	80.0	79.7	66.8
Pitfall	14.8 (43.6)	20.5 (60.3)	24.3 (71.5)	27.4 (80.6)	30 (88.3)	48.4	43.7	34.0
Both methods	44.5 (59.6)	55.5 (74.4)	62.1 (83.2)	66.9 (89.7)	71 (95.2)	90.3	90.6	74.6
785 m Andringitra								
Winkler	52.2 (66.8)	63.3 (81.0)	68.8 (88.1)	72.8 (93.1)	76 (97.2)	87.5	90.7	78.2
Pitfall	10.2 (45.6)	14 (62.4)	16.4 (72.9)	17.6 (78.6)	19 (84.7)	23.4	24.9	22.4
Both methods	53.8 (68.6)	64.1 (81.8)	69.8 (89.0)	74.0 (94.4)	77 (98.2)	88.2	91.7	78.4
825 m Andringitra								
Winkler	43.4 (67.6)	51.7 (80.6)	56.4 (88.0)	60.5 (94.4)	64 (99.8)	78.8	79.7	64.1
Pitfall	8.7 (39.0)	12.2 (54.9)	14.9 (67.0)	17.0 (76.7)	19 (85.6)	32.1	27.8	22.2
Both methods	44.9 (66.5)	53.3 (79.1)	59.4 (88.1)	63.5 (94.1)	67 (99.3)	82.6	83.7	67.4
825 m Masoala								
Winkler	62.2 (57.1)	79.8 (73.3)	91.1 (83.7)	99.3 (91.3)	106 (97.4)	139.84	136.38	108.81
Pitfall	8.9 (38.2)	12.7 (54.5)	15.8 (67.7)	18.27 (78.6)	20 (86.0)	33.05	27.84	23.25
Both methods	62.5 (55.4)	81.2 (71.9)	93.4 (82.7)	102.4 (90.6)	109 (96.5)	141.7	139.4	113.0
875 m Anjanaharibe-Sud								
Winkler	54.4 (58.4)	69.1 (74.1)	78.9 (84.7)	86.7 (93.1)	92 (98.8)	117.17	114.5	93.2
Pitfall	8.5 (37.8)	11.7 (52.1)	14.6 (65.0)	17.4 (77.2)	20 (88.8)	89.2	32.7	22.51
Both methods	56.2 (57.6)	71.9 (73.7)	82.7 (84.7)	91.1 (93.3)	97 (99.3)	126.7	122.5	97.6

<sup>a</sup>Number of species represents the mean of 100 randomizations of sample pooling order.

<sup>b</sup>ICE, incidence-based coverage estimator; jackknife, first-order jackknife estimator; M-M, Michaelis-Menten asymptote (the percentage of the M-M asymptote is given in parentheses in the first five columns).

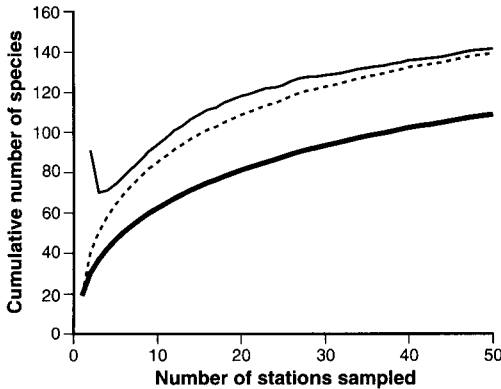


Figure 15.1. Assessment of leaf litter ant sampling technique at 825 m on the Masoala Peninsula, Madagascar. The lower species-accumulation curve (thick line) plots the observed number of species as a function of the number of stations sampled. The upper curves display the nonparametric first-order jackknife (dashed line) and the incidence-based coverage estimator (ICE; solid line), estimated total species richness based on successively larger numbers of samples from the data set. Curves are plotted from the means of 100 randomizations of sample accumulation order.

50-sample sizes are shown in Table 15.1. Within the area of the survey, the Winkler technique collected the majority of ants foraging and living in the leaf litter. Most species collected by pitfall traps were also sampled by Winkler extraction, indicating that whereas pitfall samples in the same area would most likely add additional species, these species would probably have already been obtained by the Winkler method. Although this may hold true for most rainforest sites, it may not apply to all habitats. For example, Fisher and Razafimandimby (1997) found that in dry forest habitats, which contain more areas of open or bare ground, pitfall traps may collect a greater number of unique species.

Species-accumulation curves for the 825-m site on the Masoala Peninsula, the most species-rich site (Fig. 15.1), indicate that within the area

of the survey the techniques employed collected the majority of ants foraging and living in the leaf litter in the area encompassed by the 250-m transect, and that with increased sampling effort using the same methods in the same area, only marginal increases in species richness would be attained. Although additional collecting methods, or a survey in a different area or season at the same elevation, would most likely collect additional species, these results show that the ALL Protocol provides sufficient sampling for statistical estimation and comparison of species richness, and for comparison of faunal similarity and species turnover.

## Malaysia

Studies of ant diversity in the Pasoh Forest Reserve of West Malaysia by Malsch provide interesting data on the effects of plot size sampled in ant diversity studies. Situated in Negeri Sembilan, West Malaysia, about 140 km southeast of Kuala Lumpur (2 59'N, 102 19'E), the Pasoh Forest Reserve is a typical example of a Southeast Asian ever-wet lowland rainforest, with primary lowland dipterocarp forest situated between 75 and 150 m above sea level.

A total of nine leaf litter plots (each 25 m<sup>2</sup>) were investigated. Each plot comprised a 5 × 5-m<sup>2</sup> area with an additional 3 × 3-m<sup>2</sup> area nested in the middle of the plot. Each of the two nested areas (16 m<sup>2</sup> and 9 m<sup>2</sup>) was sampled separately, and the sum of the two equaled a 25-m<sup>2</sup> area. This approach enabled the comparison of all nested areas within plots. Ants from the leaf litter were extracted by the Winkler method after 24 hours and then again after an additional 24 hours (Chapter 9).

The nested sampling area design revealed that the size of the leaf litter sample (plot size) can influence the number of ant species collected. The number of species collected per square meter for each 9- and 25-m<sup>2</sup> plot is shown in Table 15.2. On average, one more species was

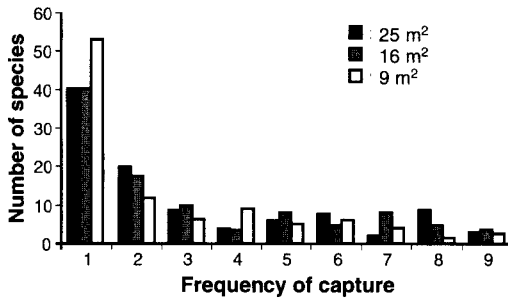


Figure 15.2. Frequency of capture for species in nine 9-m<sup>2</sup>, 16-m<sup>2</sup>, and 25-m<sup>2</sup> plots in Malaysia.

found per m<sup>2</sup> on the 9 m<sup>2</sup> plots compared to the 25 m<sup>2</sup> plots. The similarity in ant species composition was also affected by plot size, with Sorensen's similarity values of the 25 m<sup>2</sup> plots ranging from 37.5% to 63.8%, and those of the 9 m<sup>2</sup> plots from 28.5% to 66.7%. The mean values are 52.7% for the 25 m<sup>2</sup> plots and 43.0 % for 9 m<sup>2</sup> plots. These differences are highly significant ( $P < 0.001$ , Mann-Whitney U-Test). The higher species turnover in the 9 m<sup>2</sup> plots resulted from more single captures and a smaller number of repeated captures (Fig. 15.2).

The species-accumulation curves for each plot size reveal that none of the plot sizes samples all ants of the area since none of the curves

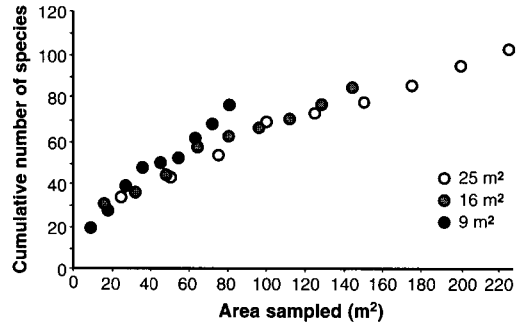


Figure 15.3. Species-accumulation curves for the three plot sizes of 9 m<sup>2</sup>, 16 m<sup>2</sup>, and 25 m<sup>2</sup> in Malaysia.

levels off (Fig. 15.3). In addition, each plot size produces a different estimation of the overall ant species richness. This is an important consideration when comparing the results from studies using different plot sizes; it is not possible to compare them directly.

These results emphasize the importance of using a consistent, standard plot size across studies in order to make comparisons. The 1-m<sup>2</sup> litter plot of the ALL Protocol provides this standardization.

## Western Ghats, India

Ant diversity was investigated using a variety of sampling techniques in the state of Karnataka, Western Ghats, India (Gadagkar et al. 1990, 1993). A total of 36 1-ha plots from 12 habitat types were sampled in sites representing elevations from sea level to 600 m in forested habitats, in three monoculture plantations, and in a forest that was regularly harvested to produce leaf manure. At each of these sites, sampling was carried out in three 1-ha plots.

Five sampling methods for ground-dwelling ants were employed at each site: vegetation sweeps, pitfall traps, light traps, scented traps, and direct (hand) collecting. Light traps use a luminescent light source to attract insects that

**Table 15.2** Number of Ant Species per Square Meter for 9- and 25-m<sup>2</sup> Plot Samples in Malaysia

Plot	9 m <sup>2</sup>	25 m <sup>2</sup>
P1	2.22	1.36
P2	2.44	1.16
P3	2.22	1.44
P4	2.22	1.64
P5	1.55	0.92
P6	2.00	1.24
P7	3.11	1.52
P8	3.22	1.76
P9	3.22	1.68
Mean	2.47	1.41

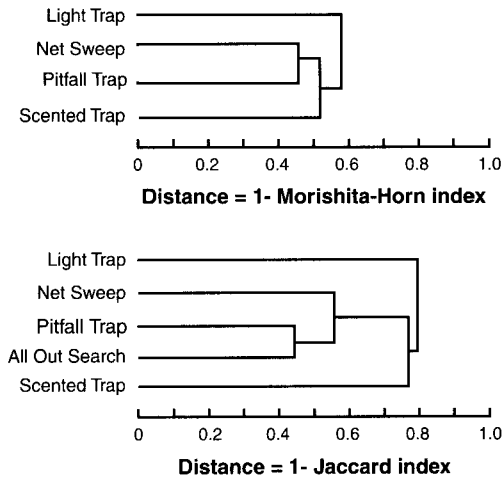


Figure 15.4. Dendrograms comparing different sampling methods by ant species trapped in India. Data pooled from 36 plots for each sampling method.

are active at night. Although light traps are typically used to sample flying insects, they can occasionally be useful for attracting flying alate ants and some nocturnal ant species. Scented traps are essentially a combination of two standard ant sampling techniques (pitfall traps and baits); in this study they consisted of 2.5-liter plastic jars that were baited with unrefined sugarcane and hung at about 1 m from the ground on wooden pegs. Intensive hand collecting was performed in each 1-ha plot to collect representatives of as many species of ants as possible. Two persons made the search for 1 hour between 1400 and 1500 in every case.

In addition to providing the first estimates of ant diversity and abundance for any forest locality of India, the results of this study provide informative comparisons of five different methods of ant sampling. The combination of the four trapping methods used was somewhat more successful than hand collecting, yielding 120 species from 31 genera while hand collecting yielded 101 species from 27 genera. More significant is the fact that the traps and hand col-

lecting yielded different species; while 78 species were obtained by both methods, the traps yielded 42 unique species and hand collecting yielded 20 unique species. It appears, therefore, that in spite of the efficacy of the traps, a combination of trapping and hand collecting may be desirable if a more complete list of ant species at a site is desired.

Of the four trapping methods used, pitfall traps sampled the most species, followed by vegetation sweeps, scented traps, and light traps in that order. The fact that pitfall traps and vegetation sweeps were more successful is not surprising; indeed the fact that scented traps and light traps yielded as many ants as they did is surprising. Not only did the scented traps and light traps yield more ants than expected, they yielded an ant fauna rather different from that obtained by the other methods (Fig. 15.4).

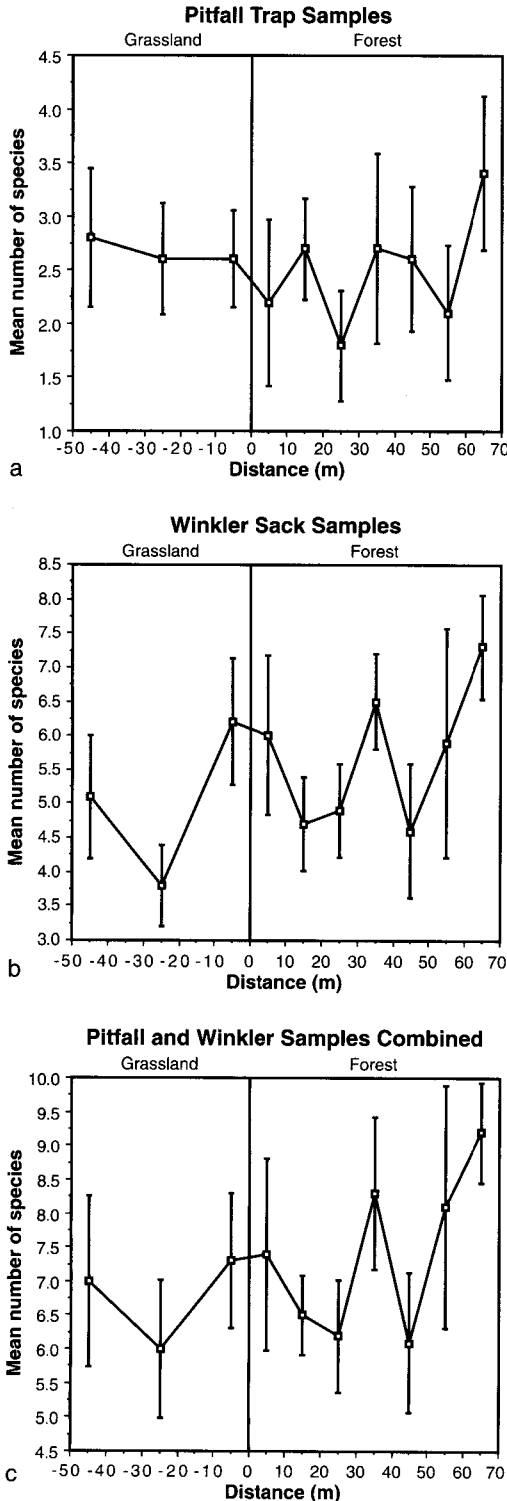
Had this study included leaf litter extraction as in the ALL Protocol, many more ant species would likely have been collected. However, the combination of several sampling methods used in this study, including hand collecting, illustrates that different techniques usually collect different components of the ant fauna. Therefore, if a more thorough inventory of ground-dwelling ants is desired, it is recommended that a few additional methods be used along with the ALL Protocol.

## Brazil

Two studies in Brazil, one in the highly fragmented Atlantic rainforests of Bahia and the other in the Brazilian Amazon, reveal the utility of the ALL Protocol as a means of detecting habitat disturbance.

### Atlantic Forests

In Bahia, ten 110-m transects were established from the center of a botanical reserve of secondary rainforest in the Centre for Cocoa Research, Itabuna, Bahia (Majer et al. 1997).



Transects were marked at 10-m interval points and were sited so that seven points extended into the reserve, one occurred in the middle of the planted edge 5 m outside the forest, and two were in an adjacent field. The ALL Protocol was followed, with leaf litter samples collected along the transect and extracted using Winkler sacks (for only 24 hours) and pitfall traps set out along the transect and left out for 48 hours.

Figures 15.5a–c illustrate the variation in the mean number of ant species collected by pitfall traps, by Winkler sacks, and by both methods along the transects. The mean number caught by pitfall traps ranged from 1.8 to 3.4, and there was little apparent trend in numbers along the transects, although the maximum richness was encountered at the point farthest into the forest (Fig. 15.5a). The mean number of species extracted by the Winkler sacks ranged from 3.8 to 7.3. It is noteworthy that the highest density was once again reached at the point farthest into the forest and that the lowest density was encountered at the point 25 m into the field (Fig. 15.5b).

The results of this survey reveal an abrupt differentiation in forest and field ant community composition. Five assemblages of ant species were distinguished along the transect. The largest grouping contained species that were ubiquitous along the transects or that were ubiquitous except at points outside the forest. The second group comprised ants that showed a tendency to occur around the outer forest margin, while the third and fourth groups contained ants that were generally found in deeper forest. The fifth group contained 12 species that were found

Figure 15.5. Mean number of ant species (and standard error) sampled by (a) pitfall traps, (b) Winkler sacks, and (c) both methods combined along ten transects extending from the field into the rainforest in Bahia, Brazil. The vertical line indicates the position of the fence around the forest reserve.



only in the field or planted edge. These ant assemblages can be used to monitor ant communities in these different land uses and to detect further changes, even if fairly subtle, in habitats and their microclimates.

### Amazon

In the Brazilian Amazon, ground-dwelling ants were collected in three 1-ha forest fragments, in three 10-ha fragments, in two 100-ha fragments, and in one continuous forest area. In each of these nine fragments a 1-ha plot was delimited and, within this, a total of 36 sampling points, distributed at intervals of 20 m, were established. Three methods of ant sampling were used: litter extraction, pitfall traps, and soil samples.

Of the three methods, litter sampling was the most efficient in terms of the number of species collected. The mean number of species collected per plot was significantly greater in the litter than in the pitfall traps and soil, whereas the number of species collected in the pitfall traps was greater than that in the soil (ANOVA,  $F_{2,16} = 29.87$ ,  $P < 0.001$ ; Table 15.3). Although the number of species recorded per fragment was greater in the litter samples than in the pitfall traps, the total number of species recorded by each of these two methods in all nine forest plots studied was quite similar, and both yielded greater numbers than collections from the soil samples (Table 15.3). Litter sampling was also the best method to predict overall ant species richness (number of species collected using the three methods combined) in each of the study plots.

The number of species that were unique to each method ranged from 20 to 43 species, a number that usually represented more than 20% of all species collected by that method (Table 15.3). This observation indicates that these methods are complementary. Their use in combination, therefore, better characterized the ant fauna of the fragments. Species of Cerapachy-

**Table 15.3** Number of Ant Species Collected Using Three Different Sampling Methods in Forest Fragments near Manaus, Brazil

Subfamily	Litter Samples	Pitfall Traps	Soil Samples
Myrmicinae	96	82	53
Ponerinae	33	36	33
Formicinae	11	12	12
Dolichoderinae	3	5	0
Ecitoninae	1	5	1
Cerapachyinae	2	0	5
Pseudomyrmecinae	1	2	0
Leptanilloidinae	0	0	2
Total (unique)	147 (43)	142 (39)	106 (20)
Mean $\pm$ SD	54.2 $\pm$ 11.6	45.7 $\pm$ 7.9	30.3 $\pm$ 5.6

inae, for instance, were recorded mostly in the soil samples, whereas those in the Ecitoninae were mostly recorded in the pitfall traps. On the other hand, many Myrmicinae were only recorded in the litter samples (Table 15.3).

No consistent changes in species diversity were found in response to variations in fragment area. It must be stressed, however, that these results reflect a lack of relationship between the *density* of ant species (number per unit area) and forest area, not in overall species *number* and forest area, as the latter relationship is clearly positive and significant. Within two of the three sites studied, the density of ant species increased as forest area increased, whereas in the third site the opposite trend was found. Differences in the history of fragment isolation (resulting in different matrix habitats) may have accounted, at least in part, for these conflicting results (Vasconcelos and Delabie 2000).

Ordination of the study plots according to their similarities in species composition indicated that forest fragmentation does affect the composition of the ground-dwelling ant community. A "site effect" on species composition was also detected, indicating some degree of

heterogeneity in species distribution among the three sites studied, even though these sites were only 10–25 km apart.

The results of this study strongly suggest that forest fragmentation affects the structure of ground-dwelling ant communities. The diversity and composition of the ant community would thus be useful to include in a monitoring program of forest fragments to follow and predict future changes.

## Conclusion

These five case studies, plus those conducted by Delabie et al. (Chapter 10), provided the comparative basis for selection of methods for the ALL Protocol. They also illustrate the application of the ALL Protocol to address a wide range of research and applied conservation

questions in a variety of locations. We hope that these studies will inspire and guide the use of the ALL Protocol, and the inclusion of ground-dwelling ants, in biodiversity studies across the globe.

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