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Optimizing Diversity Assessment Protocols for High Canopy Ants in Tropical Rain Forest

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ABSTRACT

The canopies of tropical rain forests support highly diverse, yet poorly known, animal and plant communities. It is vital that researchers who invest the time needed to gain access to the high canopy are able efficiently to survey the animals and plants that they find there. Here, we develop diversity assessment protocols for one of the most ecologically important canopy animal groups, the ants, in lowland dipterocarp rain forest in Sabah, Malaysia. We design and test a novel trap (the purse-string trap) that can be remotely collected, thus avoiding disturbance to ants. We compare this modified trap with two other methods for surveying canopy ants: precision insecticide fogging and baited pitfall trapping. In total, we collected 39,351 ants belonging to 173 species in 38 genera. Fogging collected the most individuals and species, followed by purse-string trapping with baited pitfall trapping catching the fewest. Fogging also resulted in samples with a different species composition to purse-string trapping and baited pitfall trapping, which were not different from one another. Using a 'greedy algorithm', which guides the selection of inventory methods in order to maximize new species discovered per researcher-hour, we show that projects allocating fewer than 132 researcher-hours to canopy ant collection and identification should sample exclusively using fogging. Those with more time should use a combination of methods. This prioritization technique could be used to accelerate species discovery in future rapid biodiversity assessments.

Abstract in Malay is available in the online version of this article.

Key words: fogging; Formicidae; greedy algorithm; *Parashorea*; pitfall trap; purse-string trap; rapid biodiversity assessment; rope access technique.

Despite the high diversity of animals and plants they support, the canopies of tropical rain forests remain relatively poorly explored. Advances in canopy access techniques have meant that an increasing number of scientists are able to access the canopy, although they are by no means in the majority. There remain many canopy species yet to be described, mainly belonging to invertebrate groups (Lucky *et al.* 2002, Sorensen 2004), although the canopy is a likely stratum for the discovery of new vertebrates as well (see Edwards *et al.* 2009 for birds). It follows that there is a need to develop rigorous inventory techniques for canopy biota, because valuable time spent sampling in rain forest canopies needs to be utilized as efficiently as possible.

We chose to test different inventory techniques on ants, one of the most abundant and ecologically important animal groups in rain forest canopies (Hölldobler & Wilson 1990, Lach *et al.* 2010). We suggest that diversity assessments should focus on ecologically important taxa where possible, because these are most likely to reflect the proper functioning of the ecosystem. Ants cultivate symbiotic relationships with a range of animals (Blüthgen *et al.* 2003, Davidson *et al.* 2003) and plants (*e.g.*, Treseder *et al.* 1995, Edwards *et al.* 2010), disperse seeds (*e.g.*, Gove *et al.* 2007), act as predators (Jeanne 1979) and hosts for parasites (Andersen *et al.* 2009), turn over soil (Whitford 2000), and play roles in nutrient cycling (Bestelmeyer & Wiens 2003). Canopy ants in southeast Asian rain forest are threatened by logging (Widodo *et al.* 2004) and conversion to oil palm plantation (Brühl & Eltz 2010, Fayle *et al.* 2010), and consequent species losses have the potential to affect the rates of ecosystem processes such as nutrient redistribution (Fayle *et al.* 2011). Therefore the optimization of biodiversity assessment techniques for this group and others is an urgent priority (Turner *et al.* 2008).

Although it is possible to inventory the canopy biota entirely from the ground, these methods are often either inefficient, like ground-based fogging, which collects five to ten times fewer arthropods than fogging from within the canopy (Dial *et al.* 2006), or time consuming, for example cutting down trees to make collections (NGBRC 2010). It should be noted, however, that it is possible to hoist a fogging machine into the canopy without researchers having to access the canopy themselves (Gering & Crist 2000, Yanoviak *et al.* 2003). While a range of different methods have been used to access canopies (Lowman & Rinker 2004), rope access techniques (Perry 1978) are the most widely used, mainly because they are both relatively inexpensive and highly mobile. We will focus on ant collecting techniques that can be used in conjunction with rope access.

Available nondestructive methods for collecting ants while in the canopy include fogging, with both fogging pans and fogging

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machine in the canopy of the tree to be sampled (Ellwood & Foster 2002, Dial *et al.* 2006) and baiting, either using baited pitfall traps (Kaspari 2000; note these were inserted from ground level) or using traditional baiting methods (Kaspari & Yanoviak 2001). Although no comparison of sampling methods has yet been conducted for arboreal ants, it has been found that for litter ants combinations of methods give the largest number of species (Delabie *et al.* 2000). Two important questions, however, still remain for ants from all parts of a habitat: (1) for a given amount of time available for sampling and processing ants, what is the best combination of methods to use? And (2) should effort be spread equally between methods, or should particular methods be prioritized?

Here, we modify standard ground-based baiting methods for use in the canopy and test their efficiency. We then compare this modified 'purse-string' trap with fogging and baited pitfall trapping in terms of the number of ant individuals, number of species and species composition. Finally, we use a sampling method selection algorithm to optimize sampling combinations under scenarios with different amounts of available researcher time.

METHODS

STUDY SITE.—Sampling was conducted during the periods 13 September 2007–29 February 2008 and 28 March–20 August 2008 in lowland dipterocarp rain forest in Danum Valley Conservation Area and the Ulu Segama Forest Reserve, Sabah, Malaysia (Fig. S1; 5°01′ N, 117°49′ E).

SAMPLE DESIGN AND FIELD MEASURES.—Ants were collected from the high canopies of 20 dipterocarp trees of the genus *Parashorea* ranging in height between 35 m and 60 m (two species: *Parashorea tomentella* (Symington) Meijer and *Parashorea malaanonan* (Blanco) Merr.).

SAMPLING METHODS.—We first tested the effects of modifying traditional ground-based baiting (*standard baiting*) with a modified design (*purse-string trapping*) that allows the researcher to collect the baits without stepping onto the supporting branch. We then compared the purse-string trap with two other commonly used methods for sampling ants in the canopy: *baited pitfall trapping* and *fogging* (Fig. S2).

STANDARD BAITING.—For bait, we used both tuna and sugar water, because different species of ant are attracted to protein and to carbohydrate (Yanoviak & Kaspari 2000). Approximately 30 g of tuna were placed in a 50 mL bottle without a lid. The bottle was placed on its side and attached to a 20.3 cm × 20.3 cm plywood board using thumb-tacks. The sugar water solution (1 part sugar to 10 parts water) was poured into a 50 mL bottle with a water-tight cap into which a hole was drilled and a cotton wick inserted. The two bait types were placed on separate boards. On each tree, one set of baits was nailed lightly on lateral branches adjacent to the main trunk and one set nailed 7–15 m away from the main trunk. The baits were left for 1 h (1000–1100 h) before the board and the bait were retrieved into plastic bags. This use of bait located on a bait platform and directly collected to provide a snapshot of ant activity is a standard one commonly used on the ground (Bestelmeyer *et al.* 2000).

PURSE-STRING TRAPPING.—We modified the standard baiting design to create a 'purse-string' trap by adding two 20.3 cm × 10.2 cm and two 20.3 cm × 5.1 cm pieces of thin board to the main board (Fig. 1). These were attached with hinges on both sides. A mesh cloth bag was placed on the branch beneath the trap. This had a string sewn into the edge, tied to which were four additional strings which were in turn tied to a main collecting string. The trap was then nailed lightly to the branch in the same location as the standard bait had been. The extra wood

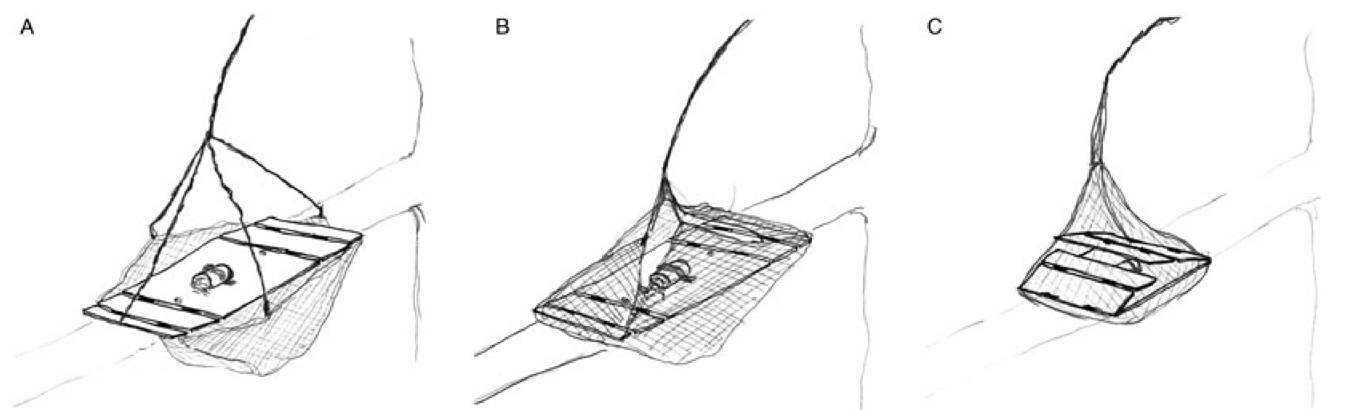

FIGURE 1. The purse-string trap in action. (A) A thin string is looped around the edge of a fine mesh cloth, which is then placed under a hinged board upon which the bait is located. Four more strings are attached to the thin string at the corners of the mesh. These four strings are tied to a single collecting string that is looped over a branch higher on the tree. The dimensions of the entire board (including folding parts) are 20.3 cm × 50.9 cm. (B) To collect the trap the trailing end of the collecting string is pulled, causing the mesh cloth to envelope the board along with any foraging ants. (C) The board sections fold up and the whole trap is put in a plastic bag.

panels were necessary in order to provide an easy route for ants to access the bait over the top of the mesh. Traps were retrieved by pulling the end of the collecting string causing the trap to collapse into the purse-string bag (Fig. 1). The whole trap was then placed in a sealed plastic bag. We compared standard baiting and purse-string trapping across nine trees (1000–1100 h only), with purse-string traps placed in the same locations on the tree as the standard baits (see above). Purse-string trapping was carried out on the same trees as standard baiting, but 3 months later. When comparing purse-string trapping to fogging and pitfall trapping, we carried out trapping on 20 trees during both the day (1000–1100 h) and the night (1900–2000 h).

FOGGING.—Fogging was carried out a maximum of 1 month after purse-string trapping using an Igeba TF 35 fogging machine (Igeba Geraetebau GmbH, Germany) with synthetic pyrethrum insecticide (active compound: Alphacypermethrin with synergist 2.27%) that was diluted in diesel by a ratio of 15:1 (0.14% active compound in final mixture). The fogger was started on the ground and hoisted into the canopy and run for 10 min allowing 360° rotation spreading fog throughout the crown. Running the fogging machine in the canopy rather than on the ground is likely to result in more complete coverage of fog and consequently higher capture rates (*e.g.*, Gering & Crist 2000, Yanoviak *et al.* 2003). Ten circular 1 m^2 fogging trays were hoisted into the crown of the target tree and fogging was carried out at 0600 h to avoid losing ants to drift caused by wind. Trays were left for 3 h before collection of ants.

BAITED PITFALL TRAPPING.—Arboreal pitfall traps comprised two plastic cups 8 cm in diameter tied together with a piece of string and hung over a branch. Tuna and sweet (strawberry and orange-boiled sweets) baits were suspended in mesh bags from the center of a wire across the top of the cups. Water containing a small amount of detergent was placed in the bottom of the cups to retain insects. Four pairs of pitfall traps (each pair having only one bait type, with two pairs of each type per tree) were placed in each of the 20 trees at the same time as the purse-string traps were laid and left for 24 h before collection.

CANOPY ACCESS METHODS.—We inserted lines into trees using a pole catapult (Bigshot, supplier: http://www.proclimber.co.uk) and fishing line with attached small throwbag. This allows line insertion up to 90 m. Climbers were attached to two ropes during ascent into the canopy: a main climbing line and a safety line. A solo climber ascended first and redirected the safety line to a different branch. Climbers moved within the canopy using arborist-style technique, with a single, looped line and a friction hitch (Dial *et al.* 2004). All trees were fixed with semi-permanent lines that were left out for the whole fieldwork trip to allow easy access to trees.

ANT IDENTIFICATION.—All specimens were identified first to genus using Bolton (1994) and Hashimoto (2007). Specimens within genera were split into morphospecies, which were assigned species names using a range of keys, online image data bases (Bolton 1974, Brown 1978, Rigato 1994, Schödl 1998, Fisher 2009, Pfeiffer 2009), and the reference collection at University Museum of Zoology, Cambridge. All analyses are based on worker castes.

STATISTICAL METHODS.—Univariate analyses were carried out on the abundance and species density (species per tree) of the ants collected using the different trap types. To test the effects of adding a purse-string apparatus to standard baits, paired *t*-tests were used. To test for differences across the three trap types (fogging, purse-string trapping and pitfall trapping) general linear models (GLMs) were used, with Tukey's pairwise comparisons used as *post-hoc* tests for those GLMs that showed a significant effect of trap type. Ant abundances were $\log_{10}(x)$ transformed to meet the assumptions of normality. All univariate analyses were conducted in Minitab 14.

Species accumulation curves with 95% confidence intervals were also plotted to illustrate species turnover between trees. Incidence-based species-richness estimators were calculated to estimate the total number of ant species present in the forest canopy and hence to assess the completeness of sampling. Accumulation curves and richness estimators were calculated in EstimateS 7.52 (Colwell 2009). Rates of species-richness accumulation did not stabilize, so values presented here should be considered to be minimum estimates of total species richness.

Ordination analyses on square-root transformed ant abundances were carried out to assess differences in species composition between trapping methods (R package 'vegan', functions *decorana, cca*, *anova.cca*). Unconstrained detrended correspondence analyses showed that axis lengths were greater than four, indicating high levels of turnover between samples. Consequently, unimodal canonical correspondence analyses were used (Leps & Smilauer 2003). CCA models were built up by adding factors sequentially, with those explaining the most variance (largest *F*-ratio/smallest *P*-value under permutation tests) being added first. One thousand permutations were used for all tests. For both univariate and multivariate analyses, tree identity (1–20) was included as a random factor.

RECOMMENDED SAMPLING TECHNIQUES IN RELATION TO RESOURCES AVAILABLE.—We accumulated ant samples using a 'greedy algorithm' that progressively selects the sample giving the most new ant species per researcher-hour. This included time taken to insert lines into trees, access the canopy, conduct sampling and sort and identify material (Table 1). This way of selecting sampling techniques differs from the combined-curves method of Longino and Colwell (1997) in that it explicitly allows different intensities of sampling with different methods. It also enabled us to take into account the fact that once a tree was rigged for one method, the expected rate of species accumulation per unit time was reduced for other methods.

RESULTS

COMPARISON OF STANDARD BAITING AND PURSE-STRING TRAPPING.—Across the nine trees, a total of 4557 ants from five subfamilies

TABLE 1. *Mean time taken in researcher-hours to conduct each of the different sampling methods on a single tree. Shaded figures are those that apply when fieldwork needs to be carried out only once per tree for those kinds of sampling. When a particular tree had already been sampled using one method, then the number of hours required for the other methods was reduced, because lines had already been inserted. Note that purse-string trapping and baited pitfall trapping can be conducted during the same climb, although neither may be carried out at the same time as fogging. (See Appendix S1 for the implementation of the greedy algorithm.)*

Activity	Fogging	Purse-string trap	Baited pitfall trap
Throw-line insertion	16 h 00 min	16 h 00 min	16 h 00 min
Climbing line insertion and canopy ascent/descent	7 h 20 min	7 h 20 min	7 h 20 min
Sampling	32 h 40 min	12 h 30 min	4 h 30 min
Sorting and identification	10 h 00 min	7 h 00 min	3 h 00 min
Total (for a new tree)	66 h 00 min	42 h 50 min	30 h 50 min

(18 genera, 52 species) were collected (Table 2). Ten times as many ants were collected in the purse-string traps compared with standard baiting (Paired *t*-test: $t_8 = 3.91$, $P = 0.004$, Fig. 2A). Purse-string traps also collected over twice as many species per trap (species density) than standard baiting ($t_8 = 3.67$, $P = 0.006$, Fig. 2B). Across trees, ant species were accumulated at a faster rate in purse-string traps than on standard baits (Fig. 2C). There was no difference in the composition of the communities sampled by the two trapping methods (CCA, $F = 1.54$, number of permutation = 1000, $P = 0.055$, Fig. S3). Standard baiting was abandoned for the remainder of sampling and is not included in the analyses below.

PURSE-STRING TRAPPING COMPARED WITH FOGGING AND BAITED PITFALL TRAPPING.—In the canopy, by using all three trapping methods on 20 trees, we collected a total of 39,351 ants from six subfamilies (38 genera, 173 species, Table 2). In terms of overall abundance, the subfamily Myrmicinae made up 47.1 percent (18,521 individuals), followed by Dolichoderinae with 42.8 percent (16,325 individuals) and Formicinae 10.5 percent (4142 individuals). However, the Formicinae were the most diverse, representing 45.7 percent of the total species sampled (79 out of 173 species). *Polyrhachis* was the most diverse single genus of ant collected with 40 species. Three other genera made up 72.6 percent of the total abundance: *Dolichoderus, Vollenhovia* and *Crematogaster* (Table 2).

Overall, there was a difference in ant abundance across all three sampling methods (GLM: $F_{2,38} = 12.29$, $P < 0.001$, Fig. 3A) with fogging catching greater numbers of ants than pitfall trapping (Tukey simultaneous tests [TST] $t_{2,38} = 4.63$, $P < 0.001$) but not more than purse-string trapping (TST: $t_{2,38} = 0.80$, $P = 0.705$). Purse-string traps caught more ants than did pitfall traps (TST: $t_{2,38} = 3.83$, $P = 0.001$). There was also a difference in species-richness across all three sampling techniques (GLM: $F_{2,38} = 53.98$, $P < 0.001$, Fig. 3B) with fogging catching more species than either purse-string trapping (TST: $t_{2,38} = 7.09$, $P < 0.001$) or pitfall trapping (TST: $t_{2,38} = 10.12$, $P < 0.001$). Purse-string traps also caught more ant species than did pitfall traps (TST: $t_{2,38} = 3.03$, $P = 0.012$).

All three methods combined caught 173 ant species (Table 3). As a single collecting method, fogging gave the most species (154), followed by purse-string trapping (84) and then pitfall trapping (51). For combinations of two trapping methods, fogging and purse-string trapping caught the most species (171), followed by fogging and pitfall trapping (160), with purse-string trapping and pitfall trapping combined catching many fewer species (96). Species-richness estimators indicated that the total number of species in the canopies of the *Parashorea* was between 216 and 233, with 74–80 percent of the species present having been collected during sampling (Table 3).

The species composition of ants collected by fogging differed from those collected by the two baiting methods (CCA: $F = 2.64$, number of permutations = 1000, $P < 0.001$), which did not differ from each other (CCA: $F = 1.21$, number of permutations = 1000, $P = 0.169$, Fig. S4).

RECOMMENDED SAMPLING TECHNIQUES IN RELATION TO RESOURCES AVAILABLE.—Species accumulation on the basis of prioritizing samples giving more species per hour of research time indicates that projects with up to 132 h available should focus on fogging (approximately two trees, Fig. 4). Projects with between 132 and 275 h available should conduct a mixture of fogging and purse-string trapping, while those with more than 275 h should use a mixture of all three techniques.

DISCUSSION

The purse-string trap caught more individuals and more species of ant than standard baiting in the canopy. Consequently this novel trap should be used in situations where canopy ants need to be sampled in a manner similar to traditional ground-based baiting, because it avoids the problem of disturbing the ants before collection. Of course, this is not an issue when baits are placed close to the main trunk (*e.g.*, Kaspari & Yanoviak 2001: 32 species at 40 baits in Neotropical forest), but this approach does not allow the whole canopy of the tree to be sampled. Standard baiting and purse-string trapping did not sample different species pools of ants, which is not surprising because they both use the

TABLE 2. *Abundances of ants collected using the different sampling methods. Note that standard baiting was conducted on only nine trees while the other three methods were conducted on 20 trees. We conducted statistical comparisons between standard baiting and purse-string trapping using the subset of data from the nine trees on which both methods were carried out. Species names are given only for those morphospecies for which we have confirmed identifications.*

Subfamily	Genus	Spp.	Species names	Standard baiting (n = 9)	Purse-string trapping (n = 20)	Pitfall trapping (n = 20)	Fogging (n = 20)
Aenictinae	*Aenictus*	1	*gracilis* Emery	0	0	0	3
Dolichoderinae	*Dolichoderus*	7	*magnipastor* Dill, *thoracicus* (Smith)	77	1843	828	13,051
	Tapinoma	5		0	28	3	32
	Technomyrmex	7		0	144	4	479
Formicinae	*Camponotus*	30	*camelinus* (Smith), *festinus* (Smith), *gigas* (Latreille), *spenceri* Clark, *saundersi* Emery	19	1198	407	396
	Echinopla	2	*lineata* Mayr, *tritschleri* Forel	0	3	0	6
	Gesomyrmex	1		0	1	0	2
	Nylanderia	1		0	76	0	215
	Paraparatrechina	1		0	0	0	2
	Plagiolepis	3		0	246	2	40
	Polyrhachis	40	*armata* (Le Guillou), *bicolor* Smith, *cryptoceroides* Emery, *equina* Smith, *lepida* Kohout, *proxima* Roger, *striata* Mayr, *ypsilon* Emery	124	353	100	1094
	Prenolepis	1		0	0	1	0
Myrmicinae	*Cardiocondyla*	2	*wroughtonii* (Forel)	26	30	0	46
	Carebara	1		0	2	0	3
	Cataulacus	2	*horridus* Smith, *praetextus* Smith	0	2	1	8
	Crematogaster	12		82	841	399	1840
	Dilobocondyla	1		0	0	0	1
	Lophomyrmex	3	*bedoti* Emery, *longicornis* Rigato,	0	2	1	5
	Meranoplus	1	*castaneus* Smith	0	7	0	0
	Monomorium	10	*floricola* (Jerdon)	20	382	719	1277
	Myrmicaria	4		1	178	95	1078
	Paratopula	2		0	1	0	3
	Pheidole	2		0	185	4	6
	Pheidologeton	1		0	1	1	11
	Rhopalomastix	1		0	0	0	2
	Strumigenys	3		0	4	1	23
	Tetramorium	5		0	1301	55	41
	Vollenhovia	5		71	6998	1068	1705
	Vombisidris	3		0	105	15	74
Ponerinae	*Anochetus*	1	*princeps* Emery	0	0	0	13
	Diacamma	1		1	0	1	1
	Hypoponera	1	*pygmaeus* Smith	0	0	0	1
	Leptogenys	1		0	0	0	19
	Odontomachus	1		0	0	0	3
	Pachycondyla	1	*tridentata* Smith	0	2	0	1
	Platythyrea	1		0	3	0	0
	Ponera	1		0	0	0	1
Pseudomyrmecinae	*Tetraponera*	8		0	12	4	212
	Total	173		421	13,948	3709	21,694

same baits and were deployed at the same locations in the canopy. In summary, the purse-string trap catches many more ants than standard baiting, but from the same pool of species.

Although fogging collects the most ant species per tree, the two baiting methods sampled a different species pool, and so are also worthwhile conducting. Purse-string trapping caught fewer

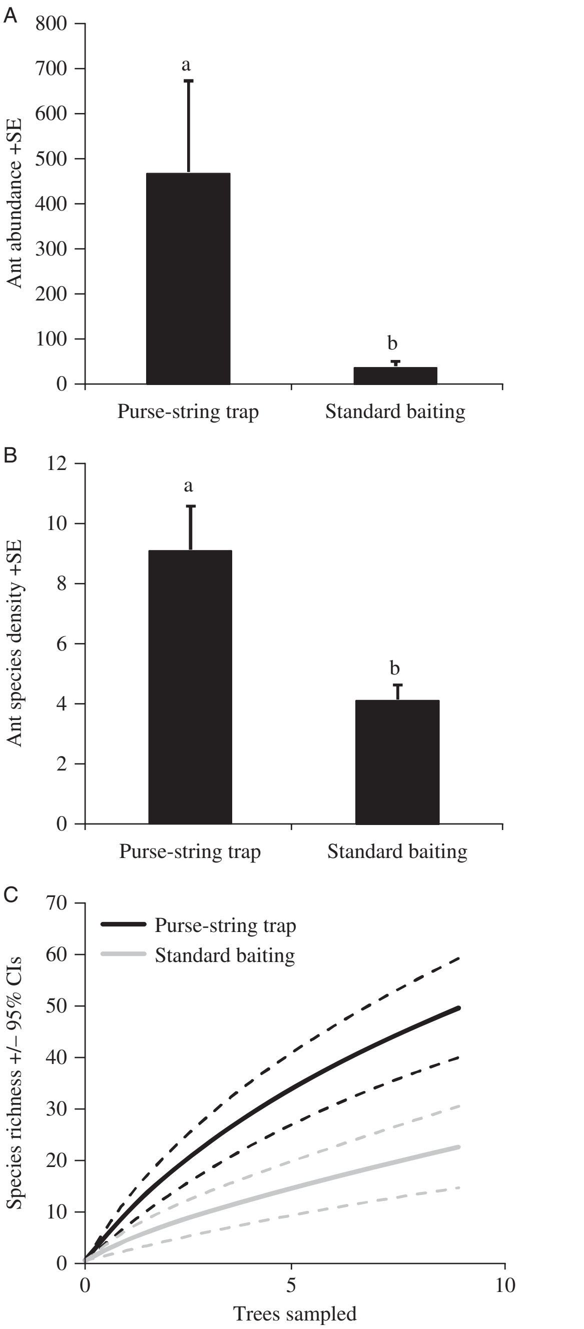

FIGURE 2. Differences in ant trapping efficiency between standard baiting and purse-string trapping in terms of (A) abundance; (B) species density (species-richness per sample); and (C) rate of species accumulation. For panels A and B, significant differences are denoted by different letters (see text for statistics). For panel C, broken lines indicate 95% confidence intervals.

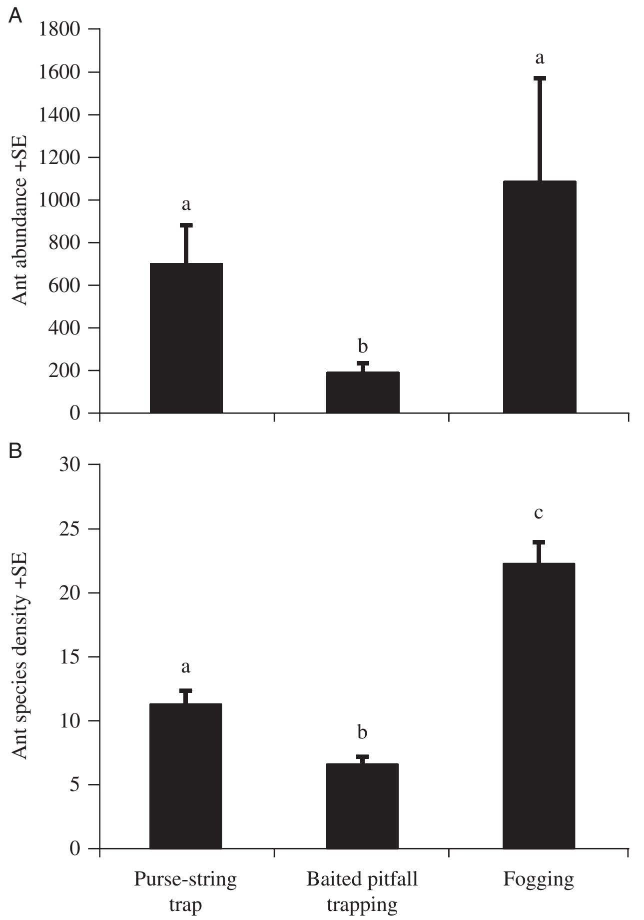

FIGURE 3. Efficiency of purse-string trapping compared with fogging and baited pitfall trapping for sampling canopy ants in terms of (A) abundance and (B) species density (species-richness per sample). Significant differences are denoted by different letters (see text for statistics).

ants belonging to fewer species than fogging, but more individuals and species than baited pitfall trapping. The species pool sampled by purse-string trapping was the same as that sampled by baited pitfalls. Again this was probably because both are baiting methods using similar bait types. It might be expected, however, that baited pitfall trapping would catch a different range of species, because species active throughout a 24-h period can be caught, although only those that are clumsy enough to fall off the bait. The lack of any difference in composition indicates that the two runs of purse-string trapping at different times of day (1000–1100 h and 1900–2000 h) were sufficient to sample the majority of the ants active on the tree, and that there was little bias in terms of which species fall into the pitfall traps, relative to those feeding on the baits. Fogging sampled a different pool of species, which includes those not attracted to baited traps, as well as those confined to the very tips of branches. Species not well sampled by fogging probably comprise those that mainly live inside the abundant litter-trapping epiphytes in the canopy (Ellwood *et al.* 2002, Yanoviak *et al.* 2003, Fayle *et al.* 2009, Turner & Foster 2009) as well as in other suspended soils (Longino & Nadkarni 1990), and in myrmecophytes that provide domatia (Edwards *et al.* 2010).

The total number of species that we collected in the canopies of the two *Parashorea* species (173) is similar to that reported in other studies of rain forest canopy ants. The most diverse canopy ant fauna to date is that of Floren *et al.* (2001), who collected

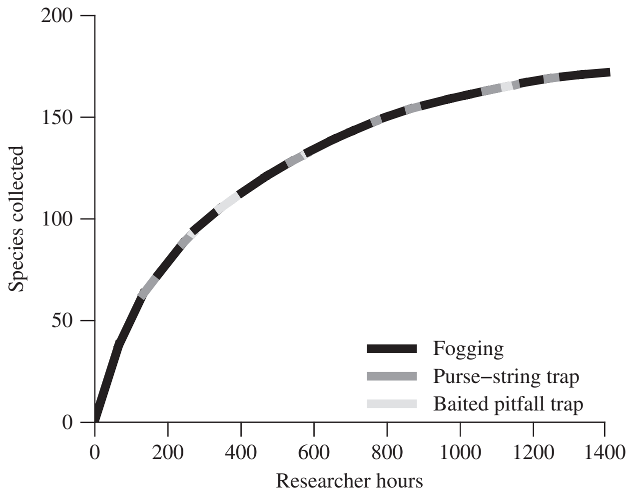

FIGURE 4. Recommended combinations of sampling techniques and resulting rates of species accumulation in relation to number of fieldworker hours. Samples are selected on the basis of a 'greedy algorithm' that prioritizes samples that give the greatest number of new species per unit time invested. Note that as a result of this, the rate of species accumulation is faster than it would be for a randomly selected sample order.

TABLE 3. *Numbers of species caught using the different trapping methods across the canopies of all 20 trees (Observed S) and estimated total number in the species pool sampled (incidence-based richness estimators Chao 2 and ICE). Percentages in brackets refer to the estimated completeness of sampling for that combination of methods.*

Method	Observed S	Chao 2	ICE
Purse-string trap	84	133 (63%)	136 (62%)
Baited pitfall trap	51	71 (72%)	84 (61%)
Fogging	154	206 (74%)	237 (65%)
Purse-string trap+baited pitfall trap	96	137 (70%)	149 (64%)
Baited pitfall trap+fogging	160	203 (79%)	230 (70%)
Purse-string trap+fogging	171	223 (77%)	240 (71%)
All methods	173	216 (80%)	233 (74%)

195 ant species by fogging 19 trees of three species in Sabah, Malaysia. Longino *et al.* (2002) collected 190 species using a combination of fogging and malaise trapping on 24 trees in Costa Rica, indicating that southeast Asian and Central American rain forest canopies support a similar diversity of ants. Other studies report smaller numbers of species, for example, Fayle *et al.* (2010) found 137 species by fogging the canopy and dissecting epiphytic ferns. This may be because fogging was carried out from the ground, meaning that fog did not reach the upper canopy of the sampled trees. The importance of spatial and temporal scale of sampling are demonstrated by the fact that Widodo *et al.* (2004) collected 112 species from only three individual trees by sampling them very intensively (50 m^2/tree) and on four occasions over the course of 2 yr. Studies using only traditional ground-style baiting or baited pitfall traps tend to report many fewer species (Kaspari & Yanoviak 2001 [baiting]: 32 species, Eguchi *et al.* 2004 [baiting]: 22 species, Oliveira-Santos *et al.* 2009 [pitfall traps]: 31 species, this study [pitfall traps]: 51 species, this study [baiting]: 22 species).

Using our greedy algorithm, we were able to determine the most time-efficient way of inventorying the canopy ant fauna. After finishing sampling using a particular method on one tree, the algorithm chose the tree and method giving the most new species per researcher-hour. All three sampling methods were required to give the highest species discovery rate for all amounts of researcher time over 275 h. Although there is a hierarchy in terms of numbers of individuals and numbers of species caught (fogging>purse-string trapping>baited pitfall trapping, Fig. 3), the same hierarchy exists for the amount of time taken to conduct sampling. The outcome of this is that when sampling is carried out on the basis of maximizing the number of new species discovered in each new sample, all three methods are used (Fig. 4). This is also due to the fact that once lines are already in place in previously fogged trees it becomes much less time consuming to ascend again and conduct baiting. In addition, both types of baiting can be conducted simultaneously. This explains why pitfall trapping is included in the optimized protocol, despite the fact that only two species were uniquely found in the pitfall traps. As a rough guideline, projects that have a relatively limited number of researcher-hours available for the collection of canopy ants (4 d, with four fieldworkers working for 8 h/d) should concentrate on fogging, and those with longer should use fogging plus a combination of baiting methods, with an emphasis on purse-string trapping. The need for using multiple different methods to increase rates of species accumulation for making inventories of ants has been demonstrated previously (Longino & Colwell 1997, Fisher 1999, Yanoviak *et al.* 2003, Vasconcelos *et al.* 2010), however, this is the first time that the relative effort dedicated to different sampling techniques has been assessed. It should be noted that trapping efficiency and turnover rates between different samples and different methods will vary between forest types and geographical areas, so these figures represent only a guideline for optimal sampling.

In addition to its use in rapid biodiversity assessments, purse-string trapping may be of utility in the study of ant ecology. Although purse-string trapping catches fewer ants and fewer species than fogging, it is a powerful method when it is required that ants be collected on very small spatial and temporal scales, for example in the study of ant mosaics (Blüthgen & Stork 2007). This is not possible with fogging, which, even when conducted within a single tree, will sample ants from multiple branches, nor with baited pitfall traps, which need to run for longer periods of time to catch reasonable numbers of ants, and so are unable to sample over short temporal scales.

To our knowledge, this is the first time that different collection methods have been compared for canopy ants, and the first time for any group that this prioritization technique has been used to determine the optimal balance of different sampling methods. We envisage that this approach will prove useful for other taxa where time or resources are limited and rapid biodiversity assessment is required.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

APPENDIX S1. Implementation in R of the greedy algorithm used to determine the optimal order of sample accumulation for maximum rate of discovery of new species. See Table 1 for times taken to conduct different sampling methods.

FIGURE S1. Locations of the trees sampled in the primary forest in the Danum Valley Conservation Area and the Ulu Segama Forest Reserve.

FIGURE S2. Mixed photography of sampling in the high canopy for ants.

FIGURE S3. Unconstrained Detrended Correspondence Analysis (DCA) ordination of species composition for samples resulting from purse-string trapping and standard baiting.

FIGURE S4. Unconstrained Detrended Correspondence Analysis (DCA) ordination of species composition for samples resulting from purse-string trapping, baited pitfall trapping and fogging.

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