OUTLINE OF PROTOCOLS FOR BASELINE SURVEYS – CTFS ARTHROPOD INITIATIVE - BCI

This is a general outline intended for the Barro Colorado Island (BCI) permanent plot. These protocols will be used for baseline survey and as pilot studies to develop the proper monitoring program. Each protocol will be specifically detailed later on. Protocols for mosquitoes are developed by EPA.

1. OVERALL PROTOCOL

1.1. Overview:

- Light traps: 5 traps, 3 replications within one survey (one night), 4 surveys per year, total 60 samples
- Butterfly transects: 10 transects of 500m, 3 replications within one survey, 4 surveys per year, total 120 samples
- McPhail traps: 10 traps, 4 replications within one survey (one week), 4 surveys per year, total 160 samples
- Trap nests: 50 traps, one replication (two months and half), two surveys per year, total 100 samples
- Euglossine baits: 10 McPhail traps, one replication (one week), 4 surveys, total 40 samples (only in the Neotropics)
- Winkler: 10 transects of 25m, each with 5 samples, one replication, one survey per year, total 50 samples
- Termite transects: one transect of 400m including 40 samples, outside the permanent plot, one survey per year, total 40 samples

1.2. Constraints while working within the CTFS and BCI plots:

Pitman (2005) has discussed the possible long-term bias introduced by trampling seedlings within CTFS plots. Three situations may be applicable, depending on local plot management: (a) transects and traps can be run without spatial limitations within the study plot; (b) transects and traps need to be run on trails within the study plot; and (c) transects and traps need to be run outside but near the study plot. For the BCI permanent plot, the BCI managing committee would prefer that the CTFS Arthropod Initiative performs all protocols on existing trails within the BCI permanent plot. Hence this document refers to situation (b), above. The protocols detailed below should be modified in case situations (a) or (c) are more suitable. For BCI and as detailed below, several protocols are best performed along 500m walking trails. Thus, we considered 10 walking transects of ca 500m each, near and within the permanent plot, as follows (Figs 1, 2):

10 Trail sectors of ca 500m each:

1. Balboa1 (BAL1; Rotation)
2. Wheeler1 (WHE1; Fixed)
3. Wheeler2 (WHE2; Rotation)
4. Drayton1 (DRA1; Fixed)
5. Armour1 (ARM1; Rotation)
6. Armour2 (ARM2; Rotation)
7. Armour3 (ARM3; Fixed)
8. Armour4 (ARM4; Fixed)
9. Zetek1 (ZET1; Rotation)
10. Zetek2 (ZET2; Fixed)

For definitions of 'Rotation' and 'Fixed' see section 1.6.

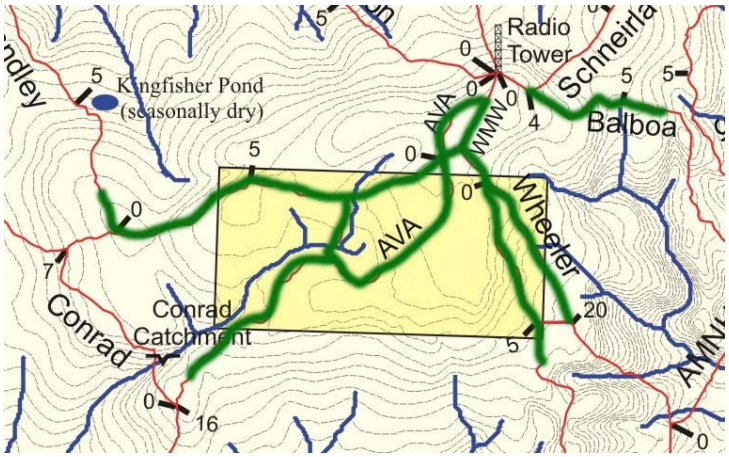

Fig. 1. Outline of the 10 trail sectors near and within the CTFS plot on BCI.

Totally within the CTFS plot = ZET1, ARM3
200m or more of trail within the plot = ARM4, ARM2, DRA1, WHE2, WHE1, ARM1
Less than 200m of trial within the plot = ZET2
Totally outside the plot = BAL1

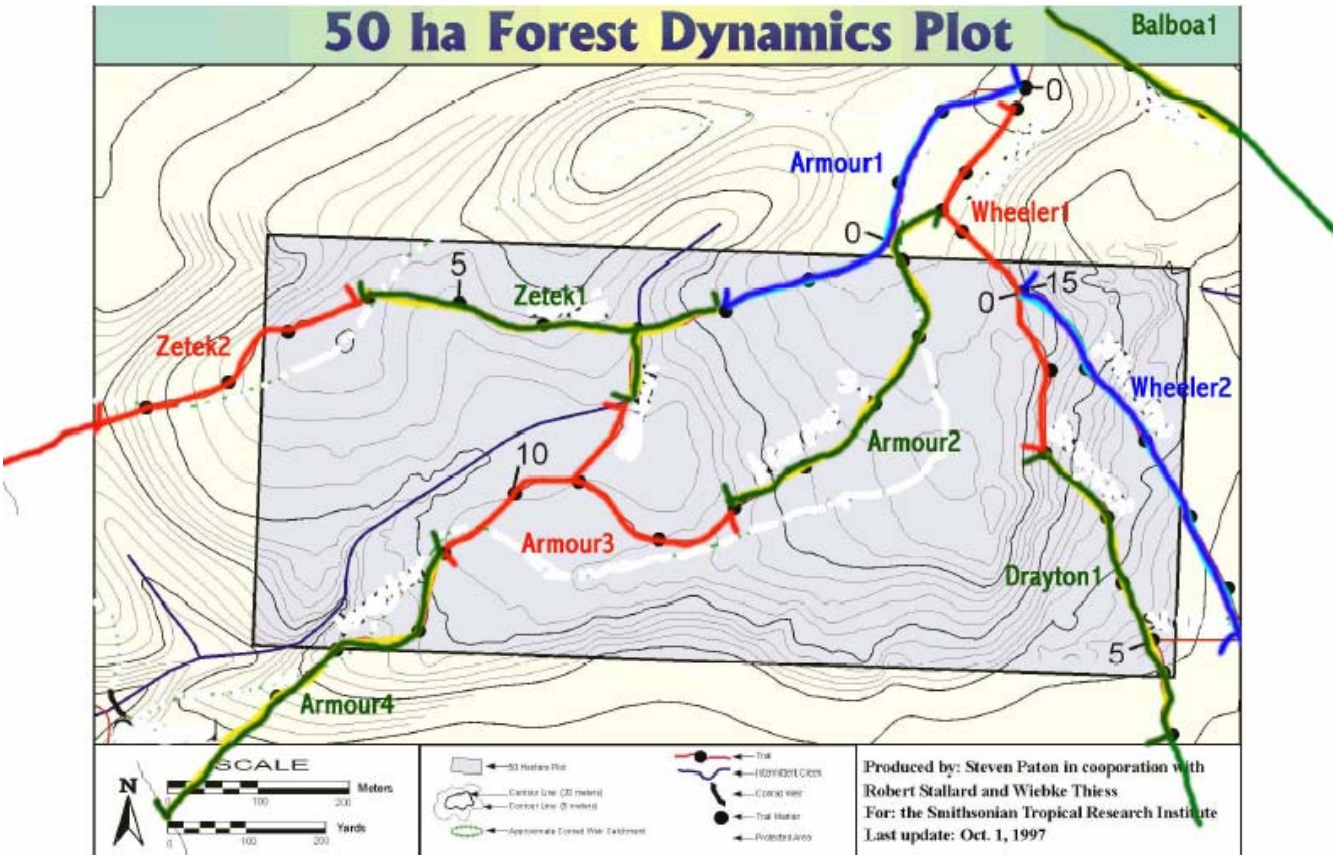

Fig. 2. Details of the 10 trails sectors near and within the BCI plot.

Joe Wright set up 200 seed traps within the plot. On average, there is one trap on either side of the existing trail system, every 35-40m. The seed traps are obvious large PVC frames in the forest. Two meters away from the traps are seedling plots, marked by a red flag in their centers (1mx1m). Any arthropod trap should be set up at least 4m away from the seed traps, so that traps can be surveyed without trampling seedlings. In practice, traps should be set up 4m away from seed traps near existing trails, and 6m off the trails, inside the forest.

1.3. Common sets of transects/traps and inter-taxa correlations

There are different strategies to reduce trampling and disturbance. In general, we could use a common set of transects and traps to survey and monitor priority assemblages. This would allow a more powerful interpretation of data and, specifically, inter-taxon correlations (Magnusson *et al*. 2005). Interference between different traps or transects may be neglected as protocols target specific arthropod groups and the action modes of traps are anyway different.

1.4. Stratified random sampling

Full randomization is most likely to be impractical because of logistical constraints. Instead, transects will be established along ten sectors of existing trails (since most of our transects or trap replicates are in multiple of ten). Sampling locations (head of transect or trap location) will be chosen with stratified random sampling within this study system, with the constraint that transects/traps should be located > 25m from the plot border (so that local vegetation data are available for the radius of each sampling location; i.e. restricting sampling into a core area of 950m x 450m in a 50ha plot). Depending on protocols stratified random sampling will be either single (choose randomly one out of three sampling locations: two furthest location or one intermediate within the sector; most protocols) or double (in addition to the previous procedure, choose randomly one out of four cardinal directions; Winkler transects; see Fig. 3).

1.5. Spatial and temporal effects

To evaluate the relative importance of spatial and temporal effects in this baseline survey, but also to optimize its spatial coverage, the location of half of transects/traps will be fixed among surveys, and the location of the other half of transects/surveys will be randomly chosen among surveys. To implement stratified random sampling, we will assign five trail sectors to be 'fixed sectors' (i.e., sectors where transect/trap locations are fixed), and five trail sector to be 'rotation sectors' (i.e., sectors where transect/trap location are randomly rotated among surveys). Sampling locations in fixed sectors will be chosen as to optimize spatial coverage. Note that the location of three light traps will be fixed, the location of the others being random (with the extra constraint that they should be located within the plot). For a simulation of the overall strategy, see Fig. 3).

1.6. Practical example: BCI, baseline study, 2008-2009

To illustrate our protocols, we consider a simulation applied to the BCI permanent plot (Fig. 3). We consider the ten 500m trail sectors as described in section 1.2. For each of these sectors, we consider three sampling locations (two furthest and one intermediate locations along the trail), coinciding with some of the markers along the trails. To optimize spatial coverage in fixed sectors, we assign five sampling locations as furthest North and West, central, furthest South and East, furthest West and South and furthest North and East (i.e., more or less in the four corners of the plots and in its center). We randomly assign five other sampling locations in rotation sectors as a single stratified random sampling.

These yield 10 sampling locations for McPhail traps, butterfly transects, trap nests and bee baits, as in Fig. 3a. We then randomly assign five new sampling locations in rotation sectors for each of the three subsequent surveys (Fig. 3b-d).We further assign randomly the cardinal direction to each of the ten 25m-Winkler transects (double stratified random sampling), which are performed during a single survey (Fig. 3b, this information is detailed for September 2008, but will be performed sooner, in July 2008). The three fixed light traps are assigned to as furthest North and West, central, furthest South and East locations (see Fig. 3). The two remaining traps are randomly assigned to locations chosen for other protocols from the five other rotation sectors (Fig. 3, enforcing the choice of two different sectors and locations).

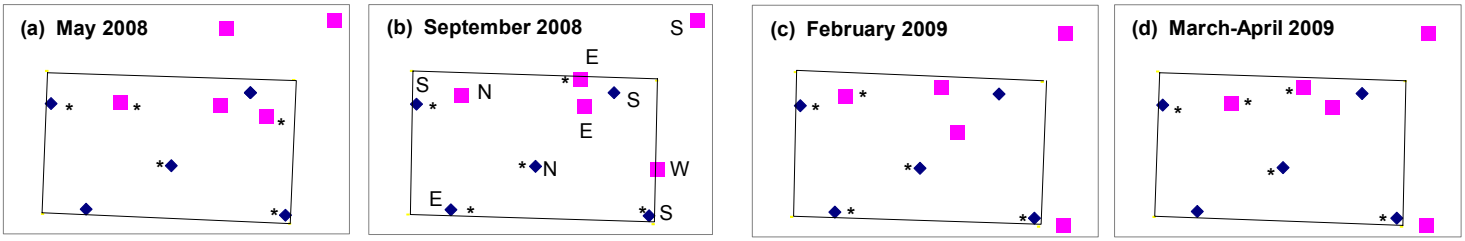

Fig. 3. Simulation of the choice of sampling locations for the first four surveys of the first study year, within the BCI plot. (a) May 2008, (b) September 2008, (c) February 2009 and (d) March-April 2009. Each sampling location (blue diamond = fixed location; pink square = stratified random sampling) will include one McPhail trap, a butterfly and nest census transect, one group of trap nests and one location for bee baits. Asterisks indicate the locations of the five light traps. Winkler transects will be performed once and the direction of each transect is indicated by N, E, S or W in Fig 3(b). Sampling locations are restricted to existing trails within the plot.

Selected key references:

Magnusson, W. E., Lima, A. P., Luizão, R., Luizão, F., Costa, F. R. C., Volkmer de Castilho, C. & Kinupp, V. F. (2005) RAPELD: a modification of the Gentry method for biodiversity surveys in long-term ecological research sites. *Biota Neotropica,* **5,** 1-6.

Pitman, N. C. A. (2005) A feast for census. A review of E. C. Losos & E. G. Leigh, Jr. (eds.). 2004. Tropical Forest Diversity and Dynamism: Findings from a Large-Scale Plot Network. *Tropinet,* **16,** 5-6.

2. PROCESSING OF MATERIAL AND BARCODING

Focal assemblages will be isolated from the collected material by parataxonomists/technicians, who will either spread (moths and butterflies), pin (beetles, bees) or store specimens in absolute ethanol and freezer (flies, other focal taxa and unsorted non-focal taxa). Most of focal assemblages will be pre-sorted and morphotyped using local reference collections,

databased with unique specimen codes, before final identification of subsets of this material by taxonomists. Additionally, the hind leg of ten specimens of each morphospecies will be torn off and stored in an individual vial with the corresponding specimen code in freezer, for subsequent barcoding analyses. Vouchers will be deposited at different institutions. Specifically, the insect material will include the following categories:

Cat. A: focal moths and butterflies are pinned, spread, dried and put in collection with a specimen code. Later, moths are pre-sorted and eventually morphotyped and databased. For ten specimens of each morphospecies: tear off one hind leg and keep in a vial dry (without ethanol) in freezer with the same code. In freezer, vials are organized by boxes.

Cat. B. These taxa are isolated and stored in absolute alcohol (or 95 deg. alcohol) in freezer, for further examination, if possible. Later, at least Cerambycidae and Scolytinae may be pinned, morphotyped and databased. As for Cat. A, a hind leg will be pulled off and kept dry in freezer with the same specimen code. In freezer, vials are organized by boxes.

Cat. C: non-focal Lepidoptera are counted, pinned (pin through, no spreading) and dried. Smaller Lepidoptera which cannot be pinned are thrown away. Keep pinned specimens dry, in cheap boxes (eg. Schmidt or BioQuip boxes). No further action, unless somebody has interest in this material.

Cat. D. The remaining material is sorted by family/order and counted, and discarded (unless of intrest for a general reference collection).

3. LIGHT TRAPS

***Focal taxa*:** selected Lepidoptera: Arctiidae, Geometridae and subfamilies Pyraustinae and Spilomelinae of Pyralidae; Isoptera; Cerambycidae; and Scolytinae, Platypodinae. Other focal taxa such as Apoidea and Tephritidae. Possibly also alates of Ecitoninae and Attini (Formicidae).

Methods: 12W black light traps of the automatic bucket-type model, running on 12V DC batteries (12 Ah sealed battery), fitted with intercept panes and a roof protecting catches from rain (Kitching et al. 2001). A commercial timer automatically switches on the trap at 18h00. The battery provides enough power for one night trapping when re-charged slowly (about ten hours). Traps are filled with crumpled paper to provide surface to hold moths and other insects so that they do not loose most of their scales. Insects are collected dry and killed by a commercial liquid insecticide dispensed in the trap.

Spatial replication: at each plot, five traps: three at fixed positions, two with random locations among surveys (see first section). Traps are set in the understorey, in the middle of a rope tied between two trees at DBH height. Traps are secured on the rope by cloth pegs.

Temporal replication: simultaneous set up of five traps and their batteries late in the afternoon, survey early in the next morning. Three single nights of trapping per trap on different days (=three samples) per survey and 4 survey during the year. Total 5 traps x 3 nights x 4 surveys = 60 samples per year. Trapping is performed when black moon (consult local moon phases at http://stardate.org/nightsky/moon/). Avoid extreme weather. If a catch for one trap appear unusually small (e.g., related to battery failure), then light trapping should be repeated next night. Timing of trap surveys for BCI and Agua Salud: February; March-April; May; and September.

Attraction range: < 3-5m (125W bulb, Baker & Sadovy 1978), < 20-30m (125W bulb, K. Fiedler, pers. comm..) but < 50m for larger moths such as sphingids (125W bulb, Beck & Linsenmaier 2006). In any case, < 50m, particularly because our traps will be equipped with 15w bulbs.

Likely disturbance: walking inside the plot, to set up and survey five traps at three fixed and two random locations, twenty-four times a year.

Processing of material (see section 2 for categories):

Category A: Arctiidae, Geometridae (or restrict to Geometrinae, most of green geometrids), and subfamilies Pyraustinae and Spilomelinae of Pyralidae (also known as Pyraustinae in the old, broader sense).
Category B: Isoptera, Cerambycidae, Scolytinae, other focal taxa: Apoidea.
Category C: non-focal Lepidoptera.
Category D: other taxa.

Field equipment: Five traps similar to the following models: BioQuip´s, Roger Kitching´s and Anthony Gonzaga-Rhett Harrison´s (Fig. 4). A sixth trap for replacement. Other equipment needed: sealed batteries and carrying bags, batteries chargers (laboratory), timer, boxes to collect catches, insecticide, ropes, cloth pegs, etc.

Selected key references:

Baker, R.R. & Sadovy, Y. 1978. The distance and nature of the light-trap responses of moths. *Nature*, **276**, 818-821.

Beck, J. & Linsenmaier, K.E. 2006. Feasibility of light-trapping in community research on moths: Attraction radius of light, completeness of samples, nightly flight times and seasonality of Southeast-Asian hawkmoths (Lepidoptera: Sphingidae). *Journal of Research on the Lepidoptera* **39**, 18-37.

Brehm, G., and J. C. Axmacher. 2006. A comparison of manual and automatic moth sampling methods (Lepidoptera: Arctiidae, Geometridae) in a rain forest in Costa Rica. *Environmental Entomology* **35**, 757-764.

Hilt, N. & Fiedler, K. (2005) Diversity and composition of Arctiidae moth ensembles along a successional gradient in the Ecuadorian Andes. *Diversity and Distributions,* **11,** 387-398.

Holloway, J. D., Kirk-Spriggs, A. H. & Chey, V. K. (1992) The response of some rain forest insect groups to logging and conversion to plantation. *Philosophical Transactions of the Royal Society of London, Series B,* **335,** 425-436.

Kitching, R. L., D. Li, and N. E. Stork. 2001. Assessing biodiversity 'sampling packages': how similar are arthropod asemblages in different tropical rainforests? *Biodiversity and Conservation* **10**, 793-813.

Kitching's model

Gonzaga-Harrison's model

BioQuip model

Fig. 4. Proposed models of light traps.

4. BUTTERFLY WALKING TRANSECTS

***Focal taxa*:** 'Rhopalocera' (butterflies).

***Methods*:** Walking transects of 500m, timed to about 30 minutes (similar to Caldas & Robbins, 2003). Ideally, transects should be walked relatively fast so that weather is not so changing. Ideally the observer should restrict his/her attention to 2m wide (KHC) across the transect and up to 5m height. The observer is timing his/her transect to last about 30 min. Two people walk the transect, one primary hunter, and one recorder. Butterflies not captured are recorded in a field form with a confidence index related to its identification (1= highly confident, 2 = intermediate, 3 = low confidence). The establishment of a reference collection is primordial to help with transect work. Most records of lycaenids, riodinids, hesperiids and certain nymphalids will probably have to be databased with low levels of confidence.

Spatial replication: for each 'survey' walk 10 different transects of ca 500m and 30 minutes (one full day of work for two persons). Walk each transect 3 times on different days during the whole survey (total sample 30 transects). For the baseline survey, perform perhaps a couple of transects at the edge of the forest, to get a better idea of the local fauna.

***Temporal replication*.** Four such surveys within a year, total 10 transect x 3 days x 4 surveys = 40 transects (samples) per year and plot. Transects should be walked in rainless weather, from 9h00 to 14h00. Surveys on BCI and Agua Salud: in February; March or April; May; and September.

***Likely disturbance*:** survey walk of 10 transects within the plot, twelve times a year

Processing of material: Category A (see section 2). Specimens not collected are databased with different levels of confidence.

Field equipment: butterfly nets, glassine envelopes, record sheets, field notes, etc.

Selected key reference:

Caldas A. & Robbins R. K. (2003) Modified Pollard transects for assessing tropical butterfly abundance and diversity. *Biological Conservation* **110**, 211-219.

Spitzer, K., Novotny, V., Tonner, M. & Leps, J. (1993) Habitat preferences, distribution and seasonality of the butterflies (Lepidoptera, Papilionoidea) in a montane tropical rain forest, Vietnam. *Journal of Biogeography*, **20**, 109-121.

5. McPHAIL TRAPS

***Focal taxa*:** Tephritidae breeding in fruits.

Methods – all but Neotropical plots: At each site, use 10 McPhail traps (Fig. 5), with separate baits:

2 traps baited with methyl eugenol (plug)
2 traps baited with cue lure (plug)
4 traps with protein (torula yeast / borax tablets dissolved into water)
2 traps baited with other baits: Africa = 2x trimedlure; Asia = 1x cue lure, 1x methyl eugenol)

Prepare the liquid protein solution as 2 tablet per trap with a bit of water. Exact solution: 20 tablets in 1.6 liters for 10 traps. Prepare this solution three days in advance so that the mix is active before setting up traps in the field. Traps baited with baits other than proteins are left without collecting fluid (flies collected dry), with the insecticide trip at the bottom of the trap. Traps with liquid protein baits do not need insecticide strips. This sort of standardization is necessary across the network, even if some of the traps will have a rather low efficiency.

Methods – Neotropical sites, including BCI:

In the Neotropics, use 8 traps baited with liquid protein, one baited with liquid protein and methyleugenol, and one trap baited with liquid protein and cuelure. Use a solution with three tablets per trap, i.e. 30 tablets in 1.6 liters (possiblky add to this solution 40g of urea, pilot study pending).

Spatial replication: ten traps per site. Stratified random sampling, with half of the traps at fixed location, the other half with randomly varying locations between surveys, see first section. Set up all traps at 3-4m height, with the help of a hook on traps and telescopic pole used to clean windows or paint walls (Fig. 6). Ensure that the trap hook is blocked in position by extra wire, so that rotation the trap do not get it stuck on the branch. Do not set up traps in direct sunlight. No need to spread insect glue on trap hooks.

Temporal replication: for each survey, traps will be active for a month, but need to be surveyed every week, changing the liquid protein but not the methyleugenol or cuelure pheromones, which stay active for a month. There will be four surveys in a year and 10 traps x 4 weeks x 4 surveys = 160 samples per year. Two protein tablets per trap x 160 samples = at least 320 protein tablets are needed per site and per year (more for Neotropical sites). As far as possible, survey periods will target fruiting peaks (different for each plot). However, in Panama, the best months for collecting fruitflies are June, July, August and September (C. Korytkowski, pers. comm.). Hence, the four surveys for BCI and Agua Salud plots will be performed in the above months.

***Attraction range*:** protein baits: 6-7m (D.C. Robacker, pers. comm.): other baits < 100-200m (Cunningham & Couey 1986), but a small proportion (<1% of flies) may be attracted up to 700m (Meats & Smallridge 2007; most probably this rather accounts for random foraging; D.C. Robacker, pers. comm).

Likely disturbance: walk within the plot to set up ten traps (five at fixed locations, five at random locations) and survey them one week later, four times during the year.

Processing of material:

Survey : use a strainer and insect pliers to collect flies into vials filled with ethanol and relevant labels. Collect the protein liquid into a large mouth recipient and discard this liquid far away from the traps! Category B (see section 2). Isolate fruit-flies into labeled vials with absolute alcohol, and store vials into freezer, organized in boxes. Vials are later sent to taxonomic authorities helping with this project. Other insects may be sorted by order, counted and stored in labeled vials with absolute ethanol in freezer.

Field equipment: Suppliers. McPhail traps: Biobest (www.biobest.be), Baits and protein: Scentry, Montana (www.scentry.com). Latex gloves to manipulate baits, DDVP fumigant strips (or alternative) to kill flies inside traps, telescopic pole, strainer and insect pliers, water containers, field notes, etc.

Selected key references:

Clarke, A. R., Balagawi, S., Clifford, B., Drew, R. A. I., Leblanc, L., Mararuai, A., McGuire, D., Putulan, D., Romig, T., Sar, S. & Tenakanai, D. (2004) Distribution and biogeography of *Batrocera* and *Dacus* species (Diptera: Tephritidae) in Papua New Guinea. *Australian Journal of Entomology,* **43,** 148-156.

Copeland R.S, Okeka W., Freidberg A., Merz B. White I.M., De Meyer M., Luke Q. 2005. Fruit flies (Diptera, Tephritidae) of Kakamega Forest, Kenya. *Journal of East African Natural History* **94**, 247–278.

Cunningham, R.T. & Couey H.M. 1986. Mediterranean fruit fly (Diptera: Tephritidae): distance/response curves to trimedlure to measure trapping efficiency. *Environmental entomology*, **15**, 71-74.

Meats, A. & Smallridge, C.J. 2007. Short- and long-range dispersal of medfly, *Ceratitis capitata* (Dipt., Tephritidae), and its invasive potential. *Journal of Applied Entomology*, **131,** 518-523.

Novotny, V., A. R. Clarke, R. A. I. Drew, S. Balagawi, and B. Clifford. 2005. Host specialization and species richness of fruit flies (Diptera: Tephritidae) in a New Guinea rain forest. Journal of Tropical Ecology **21**, 67-77.

International Atomic Energy Agency. 2003. Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency, Vienna.

Fig.5. Detail of McPhail trap (ca 20cm tall, left) and as set up in the field (right).

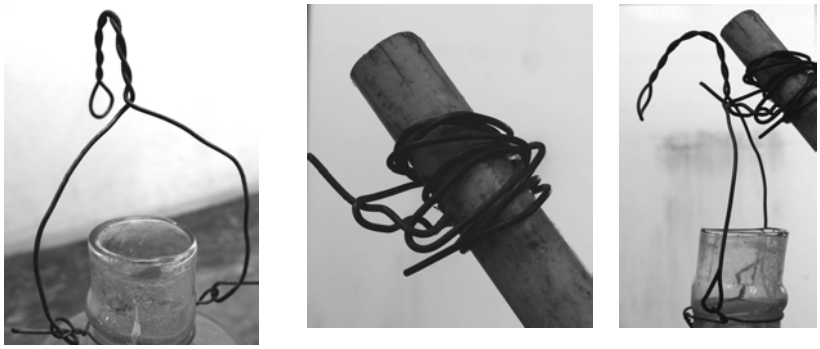

Fig.6. Details about the hook of the trap (glass model) and apex of telescopic pole.

6. TRAP NESTS

***Focal taxa*:** Solitary nesting bees (Apoidea), wasps (Vespidae, Sphecidae) and associated parasitoids (varia).

Methods: artificial trap nests targeting nesting bees (Tylianakis et al. 2005; Roubik & Villanueva-Gutierrez, MS; Fig. 6). At each site, 50 wooden block trap nests of 4 x 14 x 16cm (1 ½ x 5 ½ x 6 inches) wood drilled with 15 holes (five inches in length) of three different sizes (five of 1/8, 3/8 and 5/8 inches in diameter), hang on vegetation at about 2m height, tied by wires and protected by tangle foot glue. Leave them for two months and a half in the field and harvest them. Rear in greenhouse, with Eppendorf tubes attached to holes and with insect mesh to protect traps from outside parasitoids (Fig. 7). After bees emerge from blocks, open the nests with a wood chisel and carefully empty and label the contents.

Spatial replication: five traps spread among each of the ten transects (50 traps).

Temporal replication: exposure two months and a half twice a year, in mid February and then in early May. Total 50 x 2 = 100 samples.

Likely disturbance: walking to set up/survey 50 traps nests, four times a year.

Processing of material: Category B (see section 2). Contents of trap nests are stored in labeled vials with absolute ethanol in freezer.

Field equipment: wood blocks (untreated dry pine wood or equivalent), wire, driller, ropes, tangle foot glue, bags to carry the blocks.

Notes*:* In New Guinea, since there will be megachilids (*Ceratina, Megachile, Nomia, Amegilla*), we should also be using nesting holes of 12mm in diameter (D. Roubik, pers. comm.).

Selected key references:

Frankie, G.W., Vinson, S.B., Newstrom, L.E. & Barthel1, J.F. 1998. Nest Site and Habitat Preferences of *Centris* Bees in the Costa Rican Dry Forest. *Biotropica*, **20**, 301-310.

Roubik, D.W. (2001) Ups and downs in pollinator populations: when is there a decline? *Conservation Ecology* **5(1),** 2. [online] URL: http://www.consecol.org/vol5/iss1/art2/.

Roubik, D.W., Villanueva-Gutierrez, R. Diversity begets neotropical pollinator stability: pollen and population analysis of solitary bees after invasive *Apis*, drought and hurricanes. Manuscript for *Ecological Letters*.

Tscharntke, T., Gathmann, A. & Steffan-Dewenter, I. 1998. Bioindication using trap-nesting bees and wasps and their natural enemies: community structure and interactions. *Journal of Applied Ecology*, **35**, 708-719.

Tylianakis, J. M., A.-M. Klein, and T. Tscharntke. 2005. Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology* **86**, 3296-3302.

Trap nest in the field

Trap nests in the greenhouse

Detail

Fig. 7. Trap nests.

7. EUGLOSSINE BAITS

***Focal taxa*:** Euglossinae (Apidae, orchid bees).

Methods: cineole baits for Euglossinae, dispensed in McPhail traps (Neotropics only; Ackerman et al. 1982; Roubik 2001; Fig. 8). Ten traps, each baited with 7ml cineole and 100ml of commercial ethyleneglycol (car coolant).

Spatial replication: Ten traps within the plot. Stratified random sampling, with half of traps at fixed locations, the other half randomly assigned between surveys.

Temporal replication: Traps ran for a week during each survey, four surveys per year. Total 10 x 4 = 40 samples. Surveys in February; March-April; May; and September.

Attraction range: 1km (Ackerman et al. 1982; Ackerman 1983; D.W. Roubik, pers. comm.).

Likely disturbance: walking to set up traps at ten locations within the plot, four times a year.

Processing of material: Category B (see section 2).

Field equipment: cineole, car coolant and McPhail traps.

Notes: Cineole dispensed on paper attract a wide range of species (including larger ones) than cineole dispensed in McPhail traps. However, the former involves netting all bees, which can be difficult, time-consuming and dependent on weather conditions. McPhail trap efficiency can be dramatically improved by adding a piece of tissue for bees to climb inside the traps. However, this collects a large amount of bees. Bee diversity collected by McPhail traps tend to be rather restricted to smaller species.

Selected key references:

Ackerman, J.D. 1983. Diversity and seasonality of male Euglossine bees (Hymenoptera: Apidae) in Central Panama. *Ecology*, **64**, 274-283.

Ackerman, J.D., Mesler, M.R., Lu, K.L. & Montalvol, A.M. 1982. Food-foraging behavior of male Euglossini (Hymenoptera: Apidae): vagabonds or trapliners? *Biotropica*, **14**, 241-248.

Roubik, D.W. (2001) Ups and downs in pollinator populations: when is there a decline? *Conservation Ecology* **5(1),** 2. [online] URL: http://www.consecol.org/vol5/iss1/art2/.

Roubik, D. W. & Ackerman, J.D.. 1987. Long-term ecology of euglossine orchid-bees (Apidae: Euglossini) in Panama. *Oecologia* **73**, 321-333.

Fig. 8. Euglossine bees attracted to cineole dispensed on a pad (right) or in a McPhail trap (left).

8. WINKLER SAMPLES

***Focal taxa*:** litter ants (Formicidae), wood-lice (Isopoda), millipedes (Diplopoda)

Methods: Concentrate and extract litter arthropods with mini-Winkler eclectors (Besuchet et al., 1987; Fig. 9) from a 0.25m^2 sample of leaf litter. The litter is picked up from within a 0.25m^2 frame, concentrated with a litter sifter and stored into a cloth bag, carried back to the laboratory.

Spatial replication: at each plot, 10 transects with double stratified random design, see first section. One transect consists of 5 replicates of 0.25m^2 plots, each separated by 5m (n= 5 replicates, overall n= 50), starting at 5m distance from the trail. Transect length is 25m. Possibly only 30 samples needed at Agua Salud, but test this with 50 samples at BCI.

Temporal replication: once a year, in July (2008 for BCI, 2009 for Agua Salud). Climate criteria = "no rainfall 6 h previous". Ideally done by two assistants in 15+ days in cycles of collecting (sifting) and extracting:

Example: 50 samples on BCI obtained in July 2008, as follows:

Day1: sift 10 samples (two people)
Day 2: extraction
Day 3: extraction
Day 4: sift 10 samples (sum = 20 samples)
Day 5: extraction
Day 6: extraction
Day 7: sift 10 samples (sum = 30 samples)
Day 8: extraction
Day 9: extraction
Day 10: sift 10 samples (sum = 40 samples)
Day 11: extraction
Day 12: extraction
Day 13: sift 10 samples (sum = 50 samples)
Day 14: extraction
Day 15: extraction

Likely disturbance: walking within the plot and removal of 50 samples of 0.25m^2 of leaf litter, once a year, spread over ten transects.

Processing of material: Each replicate (sample) is calibrated with a 400ml cylinder randomly scooped up and hung in a mini-Winkler. Set up the net bag into the Winkler with the mouth open over a dissecting tray. Scoop up any debris in the dissecting tray and set up the Winkler with an empty, dry, collecting vial. Change the collecting vial to one including ethanol and pour back the contents of the empty one into the net bag. This procedure reduce the amount of falling debris into the collecting vial at the onset of the extraction. Extraction of material lasts for 48 hours. After the first 24 hours, the sample is re-mixed and extraction proceeds for an additional 24 hours. Thus, two separate samples are obtained for each Winkler sample, after 24 hours and after 48 hours of extraction. Focal taxa are sorted and stored in labeled vials with absolute alcohol, in freezer (Category B in section 2).

Field equipment: 0.25m^2 frame, litter sifter, cloth bags and larger bags to carry them, measuring tapes, gloves, note books, etc.

Selected key references:

Agosti, D., Majer, J. D., Alonso, L. E. & Schultz, T. R. (2000). *Ants. Standards Methods for Measuring and Monitoring Biodiversity*. Washington, Smithsonian Institution Press.

Besuchet, C., Burckhardt, D. & Löbl, I. (1987). The "Winkler/Moczarski" eclector as an efficient extractor for fungus and litter Coleoptera. *Coleopterists Bulletin* **41**, 392-394.

Kaspari, M. (1996) Litter ant patchiness at the 1-m^2 scale: disturbance dynamics in three Neotropical forests. *Oecologia,* **107,** 265-273.

Leponce, M., Theunis, L., Delabie, J.H.C. & Roisin, Y. (2004). Scale dependence of diversity measures in a leaf-litter ant assemblage. *Ecography* **27**, 253-267.

Removal and concentration of litter

Arthropod extraction

Fig. 9. Winkler protocol

9. TERMITE TRANSECTS

***Focal taxa*:** Isoptera

Methods: one transect of 400m, including 1 quadrat of $5m^2$ searched for 30 minutes, every 10m (total 40 samples; Roisin et al., 2006). Smaller samples of 15x15x10 cm are used to search for termites in the top soil. The method is destructive (Fig. 10) and will be used only for baseline survey, not for monitoring. The method is further very dependent on the observer. Thus, it will be best to ask experts to perform the baseline study at study plots.

Spatial replication: one single transect, including 40 samples, performed outside (but near) the study plot.

Temporal replication: once a year, at the beginning of the wet period. May-August 2009: one transect on BCI (could be parallel to the longest distance of the 50 ha plot), four transects at Agua Salud.

Likely disturbance: destructive (wood needs to examined, etc.), will not be performed inside the plot.

Processing of material: Category B in section 2 (labeled vials with absolute ethanol stored in freezer).

Field equipment: quadrat of 2.25x2.25m made out of rope and four camping pegs; ax (Husqvarna style: light and robust); machete; robust knife; soft insect pliers and pre-labeled vials; plastic containers or plastic plates, frontal light useful when the understorey is dark; GPS, measuring tape, compasses, note books, digital camera, etc.

Notes:

Y. Roisin & M. Leponce to instruct how to perform these transects, perhaps with a video.

Selected key references:

Jones D.T. and Eggleton P. 2000. Sampling termite assemblages in tropical forests: testing a rapid biodiversity assessment protocol. *Journal of Applied Ecology* **37**, 191-203.

Roisin, Y., Dejean, A., Corbara, B., Orivel, J., Samaniego, M. & Leponce, M.. (2006) Vertical stratification of the termite assemblage in a neotropical forest. *Oecologia* **149,** 301-311.

Roisin, Y., & Leponce, L. (2004) Characterizing termite assemblages in fragmented forests: a test case in the Argentinian Chaco. *Austral Ecology* **29**, 637-646.

Fig. 10. Termite transects are rather destructive as requiring manual search and wood fragmentation.

Yves Basset, version 13 July 2008