TROPICAL ECOLOGY, ASSESSMENT, AND MONITORING (TEAM) INITIATIVE

BUTTERFLY MONITORING PROTOCOL

DRAFT
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Introduction

The potential value of butterflies as ecological "indicator" taxa is due in part to their varied ecological requirements over the course of their life histories. Adults rely on nectar, fruits, dung, and carrion for energy, and on the presence of correct host plant species on which to lay eggs. Among species, caterpillars range from oligolectic to extremely specific, and the presence of host plants in turn requires certain ecological conditions. Moreover, their various life history stages render them vulnerable to the extremes of different types of environmental conditions. As a taxon, butterflies are highly sensitive to microclimatic heterogeneity and disturbance (Kremen 1994; Sparrow et al. 1994; Daily and Ehrlich 1996; Brown 1997), thus making them potentially extremely useful as indicators of habitat quality (Brown 1997). Since butterflies have a short generation time relative to many of the plants and animals which have been the subject of a majority of tropical research, their sensitivity to subtle ecological changes may be reflected relatively rapidly in their community composition (Brown 1997). Additional advantages to the use of this taxon for monitoring for change include the practical aspects of taxonomy: butterflies are well known and have been studied, catalogued, and collected for centuries across the globe, their taxonomy is relatively stable, and they are readily identifiable in the field or lab with the aid of photographic guides alone.

Over the long term, an examination and comparison over time in butterfly community composition may reveal trends in habitat quality that are occurring due to more widespread combined effects of climate and land use change. For example, desiccation resulting from rising temperatures and/or longer dry seasons in some areas already appear to be having a measurable effect on flowering plant geographic range (Parmesan and Yohe 2003) and phenology (Root et al. 2003) among others (Walther et al. 2002). The susceptibility of different species of butterflies to variations in microclimatic conditions, host plant geographical range, and phenological state may be reflected by shifts in their relative abundances and community composition relatively quickly. Such communities may have the ability to detect these changes with greater sensitivity, and earlier, than measures of those variables themselves, and may act as an "early warning system" for the effects of such widespread global change on biodiversity (Pollard 1977; Pollard and Moss 1995; Brown 1997).

A monitoring program for butterflies focused on global tropical forests designed to act as an "early warning system" for losses of biodiversity must accomplish a number of things: establish the baseline for expected natural fluctuations in butterfly communities by sampling at regular intervals, maximize sensitivity to change by sampling from different habitats and different regions, discern directional trends in composite indices of diversity and community composition occurring against the background fluctuations and any shifts or switches in communities between habitats within a region sampled, and provide correlational data on climate and microclimatic fluctuations over the same period of time.

Specific questions

By monitoring butterflies, we will address the following questions:

1) What is the baseline level of fluctuation in fruit feeding butterfly communities in tropical forests? How does that baseline differ across regions?
2) Are there trends in indices of species richness or relative abundance over time? If so, do those trends correlate with trends in climate data, tree growth rates, phenology/litterfall, or measures of land-use change? How do the trends vary among the two strata? At what spatial scale do trends occur over? Are similar trends occurring across regions?
3) Is community composition changing over time? If so, in what way? (e.g., Are species being lost or gained? Are there invasions of exotics occurring? Is community structure, i.e., dominance and sub-dominance shifting? Are there compositional shifts occurring between strata in the forest? Are species which are relatively restricted in geographic range more vulnerable to population changes?)

Methods for monitoring butterflies

Though there have been only a few long-term monitoring studies of butterfly communities (e.g., Pollard and Yates 1993; Daily and Ehrlich 1995; Brown 1997; DeVries and Walla 2001) the methods employed have been similar to those used in general studies of butterfly communities. Transect methods largely consist of observers walking replicate lines of given width and length in a given area and identifying butterflies that are in flight or at rest which are seen along the transect (Pollard 1977). Individuals whose identity cannot be identified are either caught or recorded as unidentified, and in some tropical studies, certain difficult to identify families are excluded altogether from the censuses (Hill et al. 1995; Brown 1997; Hamer et al. 1997). This method has proven to be extremely useful in at least one long-term temperate monitoring study, yielding not only useful data on species composition and relative abundances, but also in outreach and capacity building efforts among local communities and park managers (Pollard and Yates 1993).

The transect method has been used in tropical rainforests (e.g., Hill et al. 1995; Hamer et al. 1997; Ghazoul 2002), but has proven to be of limited utility, especially in studies that attempt to make interspecific comparisons across-site comparisons. Differences in visibility between sites, and interspecific differences in activity patterns are cited in at least one study to have contributed to the less than 50% rate of identification of individuals to species level(Walpole and Sheldon 1999). Across multiple sites, in a large scale monitoring program such as the TEAM Initiative, the potential for large differences in observer bias and skill are great, and such differences could obscure important patterns when making intersite comparisons.

An alternative to using the transect method which has been used extensively is the use of fruit bait traps to assess community composition (Pinheiro and Ortiz 1992; Sparrow et al. 1994; Daily and Ehrlich 1995; DeVries et al. 1997; Hughes et al. 1998). Traps are baited with fermenting fruit, thus exploiting the fruit feeding habits of some sub-families of the Nymphalid butterflies. The globally distributed family contains all of the fruit feeding butterflies (DeVries et al. 1997); though they belong to several sub-families, they comprise an ecologically as well as taxonomically cohesive unit. For monitoring, this is the ideal situation since it provides a reliable and distinct ecological guild to survey for change, while ensuring that results from different parts of the globe will be comparable due to the single evolutionary history, and thus shared taxonomy of the group. Finally, though trapping captures only a taxonomic subset of the species the transect method has the potential to capture (Kremen 1994), it largely eliminates any sampling artifacts that may arise due to differences in observer skill, provides a highly repeatable method, and samples from the family of butterflies that is the most diverse and most often captured in transect surveys in any case (Hill et al. 1995; Wood and Gillman 1998)

One long-term study of tropical butterfly communities (DeVries and Walla 2001) indicates that the forest understory and canopy have distinct faunas, and that dynamics between the canopy and understory are extremely complex. Therefore, to understand the baseline fluctuations occurring in the forest, it would be ideal to study at least these two strata. By hanging traps in both the canopy and understory (DeVries 1988; DeVries and Walla 2001) we will be able to sample from a stratum of the forest which is excluded from transect methods, and which may respond differently or at different rates to causal factors affecting the landscape. Permanently placed traps will not be deliberately placed to include light gaps, but some treefalls will undoubtedly over time occur next to traps, thereby including in the sampling a component of habitat heterogeneity important to butterfly diversity (Brown 1997).

Spatial layout

Each array will have 16 fruit bait traps, placed in eight pairs, with one of each pair in the understory, and the other one in the canopy above it. Traps are to be placed in the array and will be left in place permanently, unless they need to be replaced due to a tree or branch fall. All arrays will have the same spatial arrangement of traps (Figure 1). Trap placement is such that pairs are equidistant from each other and are evenly placed across the array. Trap pairs are identified according to the grid square they fall in, and grid squares are identified by the coordinates of the lower of each number. For example, grid square (01, 300) means the trap is placed in the grid square between (01,300) and (02, 400). Trap pairs are to be placed in the following grid squares:

(01, 300)
(01, 900)
(04, 00)
(04, 600)
(07, 300)
(07, 900)
(10, 00)
(10, 600)

Traps can be placed anywhere within the grid square, and canopy traps should be placed first since the availability of suitable locations from which the canopy line can be shot and suitable branches for the trap to hang from will limit the placement of traps. The understory trap should be placed within five meters of the canopy trap location on the ground. To avoid confusion, flagging for different groups of variables is color coded. All insect sampling locations and/or entrance points from the trail should be marked with *blue flagging only*.

Equipment

(see Appendix 1 for a list of equipment suppliers)

Per trap with dimensions 25 cm diameter, 1 m length:

1 m^2 nylon mosquito netting (white or off-white color)
2 rustproof wire hoops 25 cm diameter (80 cm wire each)
25 cm velcro
35 x 35 cm plywood base with a hole drilled in each corner (Do not use chemically treated wood.)
plastic cup (approximately 7-8 cm depth, 10 cm diameter)
>60 m nylon cord (for canopy trap) **or** 1 m (for understory trap)
1 m nylon string to attach base to cylinder, cylinder to hanging loop, and to attach to bottom of trap to put the trap down with.
Nylon thread for stitching
strong transparent plastic sheet
Trapping adhesive paste to exclude ants (e.g.Tanglefoot or Tangle-trap)

For use with all traps:

wrist slingshot
100 m spool of 7 –10 kg test strength monofilament fishing line
1 oz. (25 -30 gram) fishing weights
blue flagging

For field sampling:

200-250 small bananas (monthly)
10 liter plastic container with lid
glassine envelopes
featherweight forceps
Waterproof pens for marking butterflies
waterproof notebooks
binoculars
Kestrel 3000 Pocket Weather Station

For specimen processing, identification, and data entry:

Featherweight forceps
2 Spreading boards
3000 insect pins (#2 and #3)
insect storage boxes
napthalene or paradichlorobenzene crystals
any available illustrated guides to butterflies of the region
digital camera
printer

Methods

Trap construction

Figure 3 illustrates the fruit bait trap design. Each trap consists of a cloth cylinder made of mosquito netting with a metal ring frame at the top and bottom. The netting should cover the top of the cylinder also so that it is completely closed. A piece of transparent plastic sheeting should be placed on top of the cylinder also to keep rain out of the bait cup. The diameter of the plastic should be slightly larger than the cylinder diameter with holes cut around the string fastening position. The cylinder should have an opening running along the length of it and fastened with Velcro through which butterflies can be taken out of the trap, and unwanted insects can be released. Suspended from the bottom ring of the cylinder is a square piece of plywood hung about 5 cm below the opening of the cylinder. Place a small plastic cup on the plywood base and fasten in place with a small piece of Velcro attached the base and the cup. Cup should be about 7-8 cm in height such that the top of the cup is above the bottom ring of the cylinder. That is, the mouth of the cup extend inside the cylinder. Tie a 30 m nylon string to the bottom of the platform to use for pulling the trap down. The main cord used to suspend the trap should have a 4-5 cm long, 4-5 mm thick ring of Tanglefoot above the trap. This will prevent ants from getting into the bait, and should be replenished as needed.

Trap placement

<u>Understory</u>: Hang trap 1-1.5 m above ground using cord to suspend it from a low branch. If a low branch is not available, tie a short piece of rope between two trees and hang the trap from that. (Figure 2).
<u>Canopy</u>: Traps should be hung so that the opening to the bait is within the upper canopy. In some forests there may be a sub-canopy tree layer as well, but suspend traps so that they are not sampling the sub-canopy. (Figure 2).

Enter the grid square location from a main trail approximately mid-way between the two stakes. Walk in at least 20 meters and begin looking for a branch in the canopy that is horizontal enough to hold the line in place, high enough that the trap opening will be in the main canopy level, and with a clear enough area around it such that the weight and fishing line will not get entangled in leaves. The space below the where the trap will hang should not have such a dense understory that the trap cannot be pulled through it.

Using the slingshot, shoot monofilament line with a lead fishing weight attached to the end over a tree limb. It helps to tie a small piece of blue flagging to the lead weight so that you can see it if it gets tangled in the branches. Make sure that the tree limb does not have wasp nests and such. It may take several tries to get the line in the desired position. Tie the end of the monofilament to the nylon cord that will be attached to the trap, and pull the trap up to the desired height. It should be suspended within the canopy layer. Tie end of rope to a tree. There should be enough rope so the trap can be lowered. Once the trap is hung, exit the grid square via the main trail and tie a piece of blue flagging along the trail where you should enter to reach the trap.

Traps stay in place permanently unless they are broken by a branch or tree fall. If this happens, replace them within the same grid square of the array, and record the new information for the trap in the database.

Field sampling

Field work will require two people, for 8-10 hours a day, a minimum of 6 days per month, 12 months a year. If travel time between arrays prohibits being able to check all arrays in one day, traps may take 12-18 days per month to check.

During the same five days every month, traps are baited for sampling, once on the day before the five day sampling period, and replenished once on day 3. All traps in a plot should ideally be running simultaneously so that trap sites are sampling the same time of the month in every location. If this is not possible, a minimum of two arrays should be running simultaneously so that it does not take more than 18 days to sample all arrays, including setting the baits out on the first day.

Two days prior to putting baits out, mash bananas and allow the to ferment for 48 hours in a large covered plastic container. One day prior to actual collection of butterflies, the traps should be set. This consists of putting the cups, filled about ¾ full with fermented banana bait, on the center of the platform and raising the canopy traps to their desired heights. Leave the bait in place for the duration of the following five days. Add more banana bait to the cups on the third day unless they are empty before that.

Check the traps for butterflies every day for the next five days, alternating the order in which traps and arrays are checked. An average estimate over the year of number of samples is 2-3 individuals per trap per day, thus be sure to take enough glassine envelopes with you. Using featherweight forceps, remove all butterflies from the trap cylinder by firmly grasping them at the base of the wings with the entire length of the forceps, not just the tips. With the wings folded, put the butterfly into a glassine envelope. Put all the envelopes into a box so that they stay flat. The box can be put into the freezer or into a cyanide killing jar to kill the butterflies. Data on the envelope should include:

Date
Array
Trap ID (grid coordinates)
Stratum (canopy or understory)

After the first one or two years of sampling, once the butterfly fauna of the area is well documented and known, individuals will not be collected but instead their identity will be recorded and they will be marked with a Sharpie pen on the forewing ventral surface, and released. The mark will prevent recaptures from being counted more than once, as some species' individuals learn the trap locations and may return to them for several days. A previously marked butterfly found in the traps should be counted as a "recapture". If butterflies are marked with unique numbers, their movements can be tracked from trap to trap.

Ecological data

At the time of hanging the trap, and once per month after that, a relative measure of canopy cover should be recorded. The categories are "closed/semi-closed" or "open/gap." "Open/gap" will apply only if there is a treefall gap larger than 10 m^2 within five meters from the tree on which the trap is hanging. If this is not the case, the canopy is considered “closed/semi-closed”.

At the time of checking the traps, air temperature, relative humidity, and wind speed should be measured using the handheld weather device (Kestrel 3000 Pocket Weather Station.)

Specimen processing and identification

Specimen mounting will take the remainder of the month (two weeks) to complete by an experienced person. Identification of each month’s specimens can also be done during this period by an expert. In year one, all specimens will be collected and identified by an expert in butterfly taxonomy. Representatives of each species will be photographed with a high resolution digital camera, and these images will form the basis for field guides, thus eliminating the need for destructive sampling in subsequent years.

Field data forms, database forms, and data entry

The field notebook should contain the following data, collected when the traps are checked, as well as notes on any other pertinent observations:

Collectors: names
Date: dd-mm-yr
Time of day: HH:MM
Array: 1-6
Grid coordinates:
Canopy cover (once a month per grid): closed/semi-closed vs. open/gap
Air temperature: degrees C
Relative humidity: %
Wind speed: km per hour
Bait refill: yes or no
Stratum: canopy vs. understory

Data should be entered into the TEAM database as soon after field work as possible. In the database, fields are often constrained to a pulldown menu of only a given set of available choices. For example, there are only eight possible grid locations for a trap, so entering the trap location will be a matter of choosing one of the eight choices. Stratum of the trap will either be “canopy” or “understory”. The following fields will be included in the database, along with the possible choices and their definitions. The types of data fields that appear on the forms is of four types: trap information, ecological information, specimen information, and species information. The data forms in the database are divided somewhat differently, but the fields that appear are listed.

Information about the trap and its location

FIELD	CHOICES	DEFINITION
Array ID	1-6	Identity of the array
Trap Coordinates	(01, 300) (01, 900) (04, 00) (04, 600) (07, 300) (07, 900) (10, 00) (10, 600)	Grid square where trap is located
Stratum	Canopy Understory	Vertical stratum of forest where a particular trap is placed
Date of first baiting	dd/mm/yr	Date the trap was first used. This will only change if a trap is relocated due to a treefall or breakage, etc. If it is relocated, there will be a field for a second date of first use entry.
Height of trap platform at first time of use	meters	Approximate height that the trap location is sampling from.

Information about ecological conditions at time of sampling

FIELD	CHOICES	DEFINITION
Date	dd/mm/yr	Date of sampling (5 dates/month)
Names of collectors	Name1 Name 2	Names of field workers
Air temperature	XX.X° C	Air temperature next to/underneath trap during trap checking
Relative humidity	XX.X %	Relative humidity next to/underneath trap during trap checking
Wind speed	XX.X kph	Wind speed next to/underneath trap during trap checking
Date of bait preparation	dd/mm/yr	Date the bait being checked was started
Date of bait set/refill	dd/mm/yr	Date the bait being checked was refilled
Canopy cover	Closed/semi-closed Open/treefall gap	forest cover surrounding trap (see text for details)

Information about specimens sampled

Genus and species	(list of possible species, including morphospecies A,B, etc)	Identity of each specimen collected. If a species is encountered that does not appear on the list, you must first enter the species' information in the species table below. It will then automatically appear in the pulldown list.
Sex	M F undetermined	Sex of specimen, if known
recapture	Yes No	Was the specimen previously marked at a bait and not captured?
Date determined	dd/mm/yr	Date of the identification of the specimen
Determined by	Name 1 etc	Name of person who identified the specimen

Information about species

FIELD	**CHOICES**	**DEFINITION**
Order	Lepidoptera	Only one order sampled
Family	Nymphalidae Other	Nymphalidae only is the target group but others may occasionally be encountered
Sub-family	Brassolinae Charaxinae Ithomiinae Morphinae Nymphalinae Satyrinae Other	These are the fruit feeding subfamilies, but others may occasionally be encountered.
Genus and species		Each species, even morphospecies, needs a new entry before being able to record its specimens
Species authority		Name of the author who first described the species

There are many more fields for species specific information that is necessary for taxonomists and museum collections, but for the purposes of this protocol, what is listed above is sufficient.

Data analysis

See Avian Protocol for a discussion of analytical methods of species richness estimators. Further analyses will include ordination methods of diversity indices along axes that describe climate and vegetation measures; examination of differences in community composition between strata, between years, and between regions using rarefaction methods (Gotelli and Graves 1996), similarity indices, and ordination (Kremen 1992; Kremen 1994).

Acknowledgements

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Appendix 1

Equipment list and Suppliers in U.S.

Per trap with dimensions 25 cm diameter, 1 m length:

1 m^2 nylon mosquito netting (white or off-white color)
2 rustproof wire hoops 25 cm diameter (80 cm wire each)
25 cm velcro
35 x 35 cm plywood base with a hole drilled in each corner (Do not use chemically treated wood.)
plastic cup (approximately 7-8 cm depth, 10 cm diameter)
>60 m nylon cord (for canopy trap) **or** 1 m (for understory trap)
1 m nylon string to attach base to cylinder, cylinder to hanging loop, and to attach to bottom of trap to put the trap down with.
Nylon thread for stitching
strong transparent plastic sheet
Trapping adhesive paste to exclude ants (e.g.Tanglefoot or Tangle-trap)

For use with all traps:

wrist slingshot
100 m spool of 7 –10 kg test strength monofilament fishing line
1 oz. (25 -30 gram) fishing weights
blue flagging

For field sampling:

200-250 small bananas (monthly)
10 liter plastic container with lid
glassine envelopes
featherweight forceps
Waterproof pens for marking butterflies
waterproof notebooks
binoculars
Kestrel 3000 Pocket Weather Station

For specimen processing, identification, and data entry:

Featherweight forceps
2 Spreading boards
3000 insect pins (#2 and #3)
insect storage boxes
napthalene or paradichlorobenzene crystals
any available illustrated guides to butterflies of the region
digital camera
printer

Many of the items on the list are readily available in most cities. Some catalogue or web order suppliers for items which may be difficult to find in some areas are listed below.

For trapping adhesive, glassine envelopes, featherweight forceps, waterproof pens, waterproof notebooks, specimen processing and identification tools, and any other general entomological equipment:

BioQuip Products
2321 Gladwick Street
Rancho Dominguez, CA 90220
USA
Telephone: 310-667-8800
Fax: 310-667-8808
e-mail: bioquip@aol.com

web: www.bioquip.com

For wrist slingshot:

SlingshotDepot.com
2895 Olde Town Park Drive
Norcross, GA 30071
USA
Telephone: 404-918-6139
Fax: 770-216-1735
web: www.slingshotdepot.com

For wrist slingshot, fishing line, fishing weights, binoculars, general outdoor gear:

Cabela's
One Cabela Drive
Sidney NE 69160-9555
USA

Telephone (U.S. and Canada) :	800-237-4444
Telephone (International):	308-234-5555
Fax (U.S. and Canada):	800-496-6329
Fax (International):	308-254-2200
Web:	www.cabelas.com

For trapping adhesive, flagging, waterproof notebooks, binoculars, pocket weather station, and other general field supplies:

Forestry Suppliers, Inc.
Post Office Box 8397
Jackson Mississippi 39284-8397
USA

Telephone (USA):	800-647-5368
Fax (USA):	800-543-4203
Telephone (International):	601-354-3565
e-mail for international customers:	int@forestry-suppliers.com
Web:	www.forestry-suppliers.com

Ben Meadows Company
PO Box 5277
Janesville WI 53547-5277
USA

Telephone (USA & Canada):	800-241-6401
Telephone (Worldwide):	608-743-8001
Fax (USA & Canada):	800-628-2068
Fax (Worldwide):	608-743-8007
E-mail for international customers:	export@benmeadows.com
Web:	www.benmeadows.com

Figures

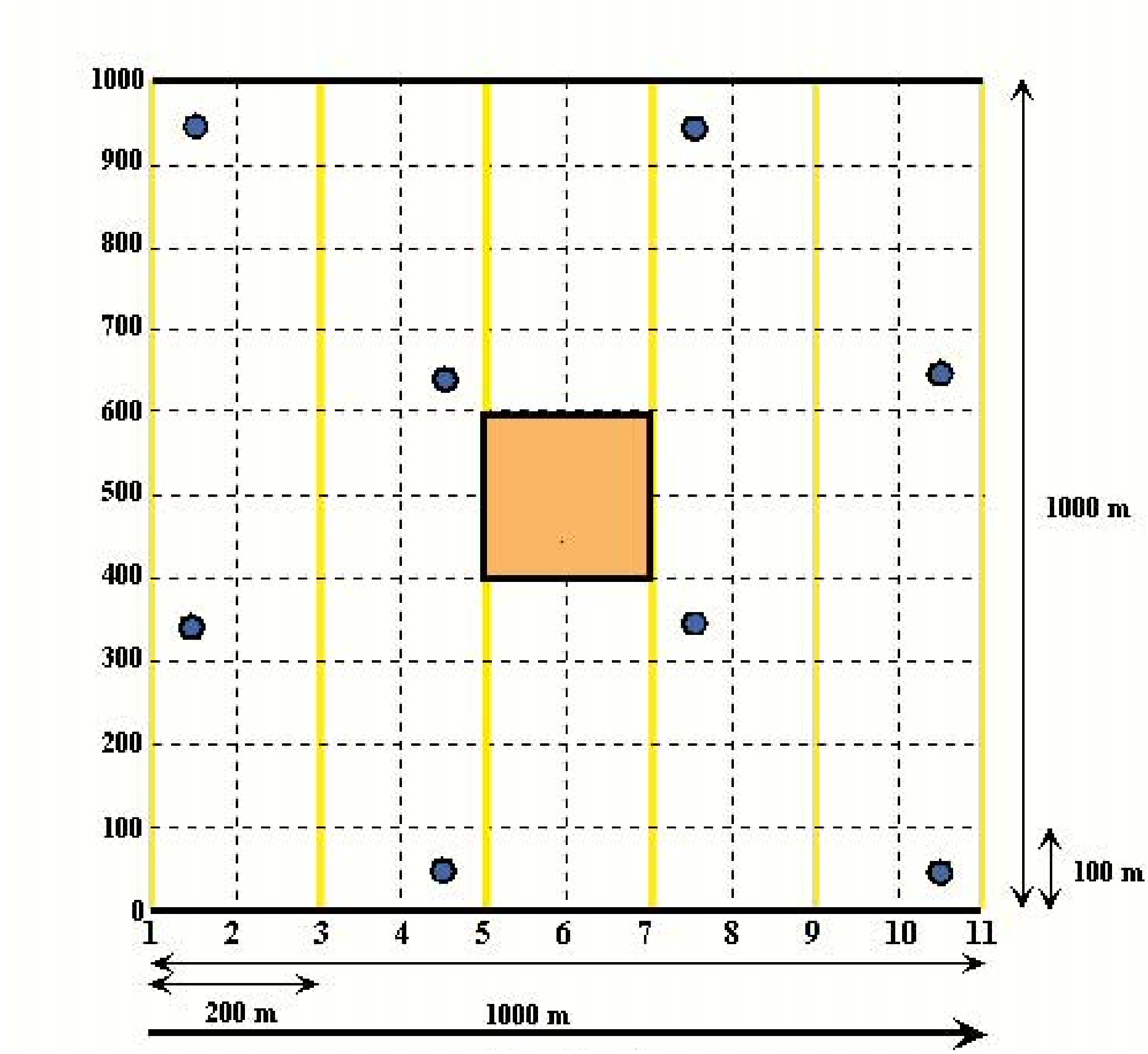

Figure 1. Placement of butterfly fruit bait traps in 1 km^2 array. Circles indicate paired traps.

Figure 2. Understory fruit bait trap (left photograph) and canopy fruit bait trap (right photograph, circled) approximately 20-22 m above the ground.

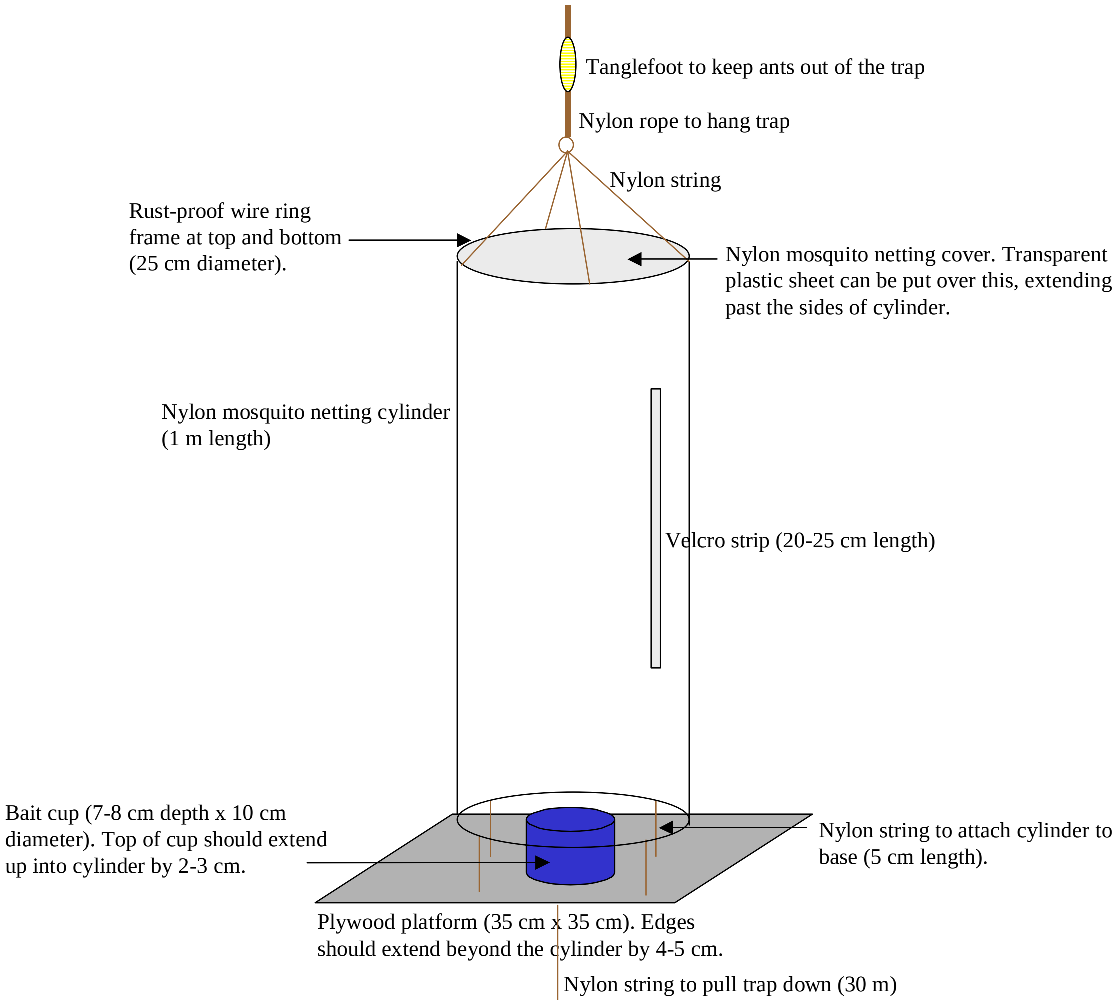

Figure 3. Butterfly fruit bait trap design.