Methodology for Faunal Biodiversity Assessment:

Insects

Papua New Guinea National Forest Inventory

BINATANG RESEARCH CENTER

Vojtech Novotny and Pagi Toko

The New Guinea Binatang Research Center

Contact:

V. Novotny, novotny@entu.cas.cz, Ph (+675) 71893875

P. Toko, pagi.sione@gmail.com, Ph (+675) 70472127, 79399892

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Introduction

This analysis builds on the plans for the botanical survey produced for the PNG National Forest Survey (NFS) by the PNG Forest Authority and the analysis of vertebrate component by Ringma et al. (2015, University of Queensland). Please refer to these materials for the general introduction of PNG forests as well as the aims and scope of the plant and vertebrate surveys.

Focal taxa for the zoological component of the survey

The NFS provides an excellent, and cost-effective, opportunity to combine the plant survey with a zoological component. In particular, the main challenges of the botany surveys are three-fold: (i) botanical expertise, (ii) logistics needed for reaching numerous, often remote sites, and (iii) the necessity to negotiate with and obtain permission for research from numerous landowner groups. The zoological component can benefit from combining the logistics, and landowner negotiations, with the botanical survey, providing thus additional data at relatively low additional costs.

The environmental assessment of forests would greatly benefit from a zoological component. In particular, botanically well preserved forests with severely impoverished fauna are becoming widespread in many tropical countries. The disappearance of fauna, while conservation problem by itself, has also long-term effects on the vegetation dynamics as most tropical trees depend on animal dispersal, and are also impacted by herbivores and pollinators.

The selection of focal taxa for zoological surveys is difficult since individual animal taxa, and guilds, respond often idiosyncratically to vegetation change, and each requires a specific sampling protocol. An ecologically comprehensive and taxonomically balanced survey would be therefore logistically unfeasible, particularly for such large-scale enterprise as the NFS.

Arguably, vertebrates and insects represent, functionally, as well as in terms of biodiversity and conservation importance, the two key taxa in tropical forests so that a survey protocol should combine elements from both vertebrates and insects. Vertebrates represent flagship species for conservation, and their generally low population sizes make them vulnerable to extinction. Insects, with their large population sizes, short life span, multiple overlapping generations and rapid reproduction, and numerous coexisting species, are able to respond rapidly to environmental changes by species composition and abundance and are thus an excellent taxon for rapid and sensitive bio-indication.

The vertebrate selection has been addressed by Ringma et al. (2015), suggesting birds as the focal group, surveyed by a protocol based largely on point-counts. Based on our experience with vertebrate surveys, we support this decision and provide more detailed comments on the protocol elsewhere. Here, we focus on the selection of focal taxa of insects.

Our recent survey in Panama (Basset et al. 2012, *Science* 338, 1481-1484) estimated local diversity of arthropods – mostly insects – at 25,000 species coexisting in 6,000 ha of rainforest. This study, has taken 8 years of insect analysis by >100 specialists and thus illustrates the need for a few well selected focal taxa for the NFS, rather than comprehensive sampling. The ideal focal taxa should have the following attributes:

- well known taxonomically and easy to identify
- species-rich and distributed across different forest types and altitudes
- responding to environmental conditions and thus of high bio-indicative value
- representing a mix of ecological roles and trophic levels in forest communities
- important for forest ecosystem dynamics, forestry and/or agriculture

● frequently used by other surveys and thus with large baseline information

● quantitative methods, comparable across forest types, available for rapid sampling

● sampling independent from weather and season, and requiring no trained experts in the field

Unsurprisingly, no insect taxon satisfies all these requirements. Here we discuss our proposed focal taxa: (i) moths from Geometridae, Sphingidae and Pyraloidea, (ii) ants (Formicidae), and (iii) fruit-flies (Tephritidae), in the context of the above criteria.

General methodological considerations

The key requirement of NFS, viz. that the insect sampling be completed within one day so that the logistics could be coordinated with the botanical team, has placed some constraints on the selection of sampling methods and focal taxa. Further, the large number of study sites, while being a highly exciting feature of the sampling protocol, also limits the range of insect taxa that can be sorted, identified and analysed for multiple sites within a feasible time frame.

These constrains exclude certain sampling methods – such as intercept or Malaise traps, or Pollard butterfly transects – as 1-day sampling would require large number of replicates difficult to set–up in the field. For instance, butterflies, often used as a focal taxon, are unsuitable for the present study. They are well known taxonomically, good indicators and of high conservation value, but quantitative sampling by Pollard transects or fruit traps is (i) sensitive to weather, (ii) takes at least several days, and (iii) in case of Pollard transects requires trained experts to be present in the field. The scale of the survey also excludes insect taxa that are too difficult to sort into species and identify, including many Hymenoptera and Diptera families.

Our selection of focal taxa includes (i) moths from Geometridae, Sphingidae and Pyraloidea (GSP moths), (ii) ants (Formicidae), and (iii) fruit flies (Tephritidae), representing three major insect orders (Lepidoptera, Hymenoptera and Diptera), two key herbivorous groups attacking respectively leaves (moths) and fruits (fruit flies), one key group of predators (ants), and a significant biodiversity in total (4,000 - 5,000 species in PNG). Further, these taxa could be quantitatively surveyed within one day and night of sampling by a team on 3 people.

The selected taxa are moderately to well known taxonomically and BRC has sufficient in house and collaborative expertise for their analysis. BRC has already built extensive DNA barcoding libraries for the focal taxa, in collaboration with the Smithsonian Institution (S. Miller) and the Guelph University (P. Hebert). These efforts have put Papua New Guinea amongst the world's 5 or 6 top countries in the number of barcodes available for its fauna from our focal taxa:
ants (http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=685),
Geometridae moths (http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=525),
Pyralidae moths (http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=689).
We will use these libraries together with newly obtained DNA information for rapid identification of species (Fig. 2A-D).

1. Lepidoptera: Geometridae, Sphingidae and Pyraloidea

We propose to use 10 automatic battery-operated light traps (Fig. 1A) used for one night of sampling per site. The traps can be transported in the field by carriers, battery recharged using portable generator, and traps suspended individually in the forest canopy, using a rope positioned by a slingshot. The traps will be spaced by approximately 100 m, and placed within the 300 x 300 m area sampled for botany. The traps use UV light to attract and collect moths. They will be placed in sampling position by 18.00 (before sundown) and collected, together with their samples, on the next day morning. The sampling is relatively insensitive to weather, except that several days around the full moon should be avoided. The entire sample, preserved in the field, will be sorted in the laboratory and three families used for the analysis (Fig. 1E).

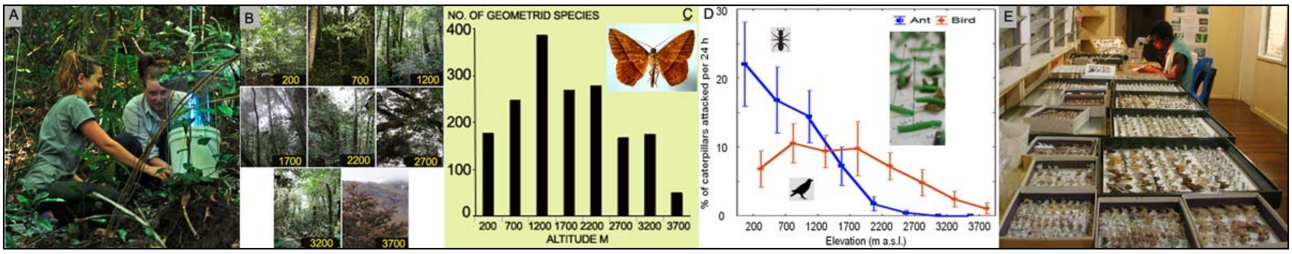

Fig. 1. Automatic light trap for moth sampling (A), insect survey along altitudinal forest gradient at Mt. Wilhelm (B) documented peak diversity of geometrid moths at mid-elevations (Toko, 2011, MSc Thesis, UPNG) (C), and showed that ants and birds were the dominant insect predators in respectively lowland and montane forests (Sam et al. 2014, *Ecography* 37, doi:10.1111/ecog.00979) (D), sorting of moths at BRC (E).

A. Geometridae: Species-rich family (probably more than 2,500 species in PNG, many of them unknown to science) that is often used for bio-indication and ecological studies in the tropics. We have studied Geometridae extensively in PNG (Fig. 1B-C) and the combination of BRC experts (P. Toko) with overseas collaboration (S. E. Miller, J. Holloway) ensures our ability to sample and analyse this group. We are also working on the *GONGED: Geometridae of New Guinea Electronic Database* project (Holloway et al. 2009, *Spixiana* 32:122-123) that will provide description, genitalia dissection and DNA barcoding for all type material from PNG and thus form a solid taxonomic foundation for the study of this family. The family has good indication value accross different elevations (Fig. 1C) and tends to be locally highly diverse, while still manageable for species sorting, particularly with our DNA barcode libraries.

B. Sphingidae: Relatively species-poor family of easily identifiable species (78 species in New Guinea, Beck et al. 2006, *Biol. J. Linn. Soc.* 2006, 89, 455–468). Detailed information on the geographic distribution of individual species is available across the tropics, allowing us comparisons with neighboring countries. Its low diversity makes the family unsuitable as an indicator on its own but useful when used in combination with other families.

C. Pyraloidea (Pyralidae and Crambidae). Another species-rich taxon, forming a suitable counterpart to geometrids by its higher host plant specificity, and thus more sensitive response to the vegetation composition (Fig. 2E).

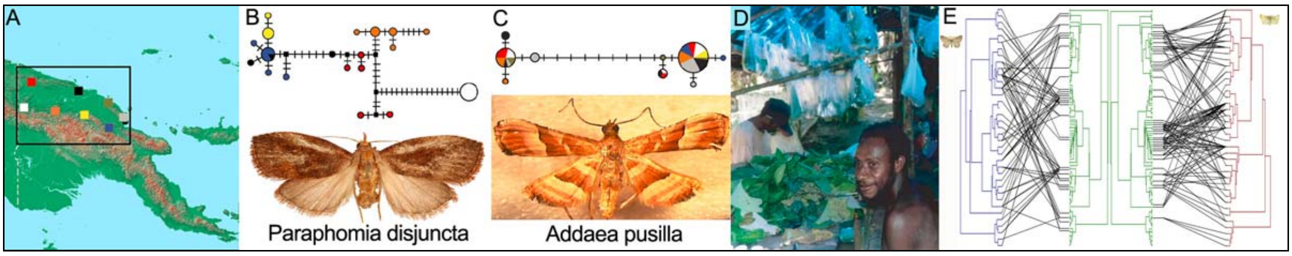

Fig. 2. Examples of moth research at BRC: Genetic diversification of Lepidoptera in a 500 x 150 km rainforest area in PNG (A) was explained by geography and by host plant species. The mtDNA haplotype networks (colours represent the geographic origin of specimens, as shown in A, diameter is relative to the number of specimens, and lines denote genetic distances between haplotypes; each mark represents a single nucleotide substitution) are shown for *P. disjuncta* (B) exhibiting geographic differentiation, and *A. pusilla* (C) unresponsive to geography (Craft et al. 2010, *PNAS* 107, 5041-5046). The specimens were reared in field camps as in (D). The food webs showing host plants for moth species can be partially structured by phylogeny, illustrated here for plant species (green) and geometrid (left) and pyralid (right) moths (Segar et al., in preparation) (E).

2. Ants (Formicidae)

Ants are among the most often used indicator taxa in insects, for their immense importance as predators and mutualists on tropical vegetation. For instance, they are, together with birds, the most important predators of insects in PNG forests (Fig. 1D). BRC has an active program in ant studies and we are also maintaining the web site Ants of New Guinea (http://www.newguineants.org/) that collates all available taxonomic information for PNG ant fauna, comprising approximately 800 species (Fig. 3C).

We propose to sample ants using 20 tuna + cordial baits (proteins plus carbohydrates) spaced by 10 m along a 200 m transect. The bait will be placed on the ground (10 baits) and on tree trunks at 130 cm height (10 baits), checked after 1 hour of exposure and ants at the baits sampled (Fig. 3A). Further, we will sample ants from forest floor and understorey (up to 2 m height) by hand search within the four circular plots with 5 m radius used for botanical surveys of plants with DBH > 5 cm at each study site (Fig. 3B). The ant sampling could be completed in approx 4 hours per site by 2 persons.

We have used these methods extensively in PNG (e.g., Klimes et al. 2011, *Ecol. Entomol* 36, 94–103; Klimes et al, 2015, *PLoS One* 10(2): e0117853). The tuna bait method captures only a subset of ant species, but these are the most active and dominant species on the forest vegetation, and as such a suitable subset for ecological analysis. The hand collection is more efficient in recovering also other arboreal and epigeic species. We have decided not to use litter samples, targeting species rich ant communities in the soil and litter layers, because their processing and sorting of ants to species would be too time consuming for the project of the current size.

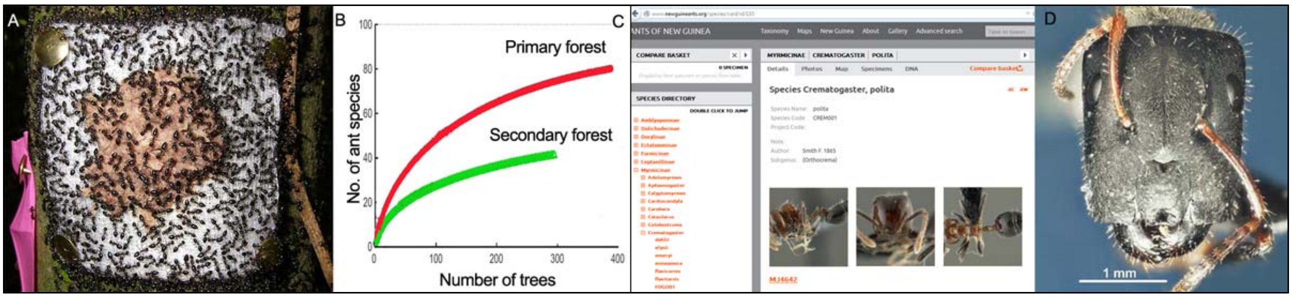

Fig. 3. Tuna bait in a primary forest (A), complete census of ant nests in 1 ha primary and 1 ha secondary rainforests in PNG by hand search (Klimes et al. 2012, *J. Anim. Ecol.* 81, 1103–1112), example species page in our Ants of New Guinea web database (C, http://www.newguineants.org), and *Camponotus wanangus*, a newly described PNG species from our studies (D, Klimes & McArthur 2014, *Myrmec. News*, 20, 141-158).

3. Fruit flies (Tephritidae)

Agricultural research of fruit flies in PNG is very active, led nationally by the PNG National Institute of Agriculture (Dr Sim Sar, http://www.spc.int/lrd/country-profiles/papua-new-guinea), and from Australia by the laboratories od R. A. I. Drew (Griffith University) and A. R. Clarke (Queensland University of Technology). BRC has established collaborations with all three teams and has thus access to the taxonomic expertise and extensive information available for PNG fruit flies. This group is moderately diverse (~250 species in PNG) and economically important as it includes numerous existing and potential pest species. However, fruit flies, including agricultural pest species, also attack numerous rainforest fruits and are thus an important component of forest communities (Fig. 4C-D). Our study will be thus of interest both to forest ecology and agriculture.

Fruit flies can be collected using simple Steiner traps (Fix. 4A) baited with cuelure, methyl eugenol or zingerone; these lures attract males of approx. 75% of fruit fly species in PNG, and these will be targetted by the survey. We plan to expose 10 Steiner traps, each combining all three lures, in the forest canopy, as 100 m intervals. Each trap will be paired with the light trap and thus not contributing further to the sampling logistics. These traps provide clean samples of fruit flies which will be preserved in the field and further sorted in the laboratory. The method thus does not require the presence of a fruit fly specialist in the field. We have used this method in the past (Novotny et al. 2005, *J. Trop. Ecol.* 21, 67–77) and it has been very effective (Fig. 4B).

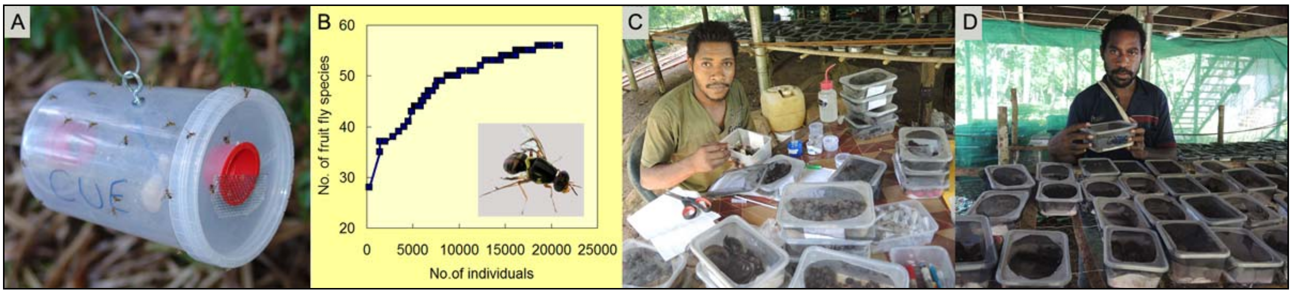

Fig. 4. Steiner trap for fruit fly sampling (A), species accumulation curve for Steiner trap sampling in a Madang rainforest (Novotny et al. 2005, *J. Trop. Ecol.* 21, 67) (B), current BRC work on rearing fruit flies from rainforest fruits (C, D).

4. Facilities

The proposed surveys will be based at the New Guinea Binatang Research Center (BRC) in Madang, one of the best-equipped institution for biological research in PNG (Fig. 5). The Center comprises a team of 25 researchers and research technicians with expertise in botany, entomology, ornithology and herpetology, and also hosts 8 postgraduate students. The facilities includes four buildings with 250 m^2 of air-conditioned laboratory space, accommodation for 35 people, a 50 kVA diesel electric generator, autonomous water supply, a network of four VHF radios, four 4WD Toyota Landcruiser vehicles, a wireless computer network with broadband internet access via satellite link, macro-photography equipment with image auto-montage, stereomicroscopes with fiber-optic lights, an insect rearing facility, insect and plant drying ovens, and freezers for sample storage. Further resources include reference collections of plants (~1,000 species) and insects (~3,500 species) with storage space for plant and insect specimens (capacity 15,000 plant and 50,000 insect specimens), and extensive on-line access to biological literature. The Center also operates a field research station at the Wanang study site, comprising three buildings with 70 m^2 of laboratory space and accommodation for 15 people. It is equipped with solar and diesel electric generation, a VHF radio, stereomicroscopes and computers. The Wanang field site includes a 50-ha forest dynamics plot, developed in collaboration with the PNG Forest Research Institute, monitoring 280,000 individual plant species. The plot is a member of the Center for Tropical Forest Science (CTFS) network of plots.

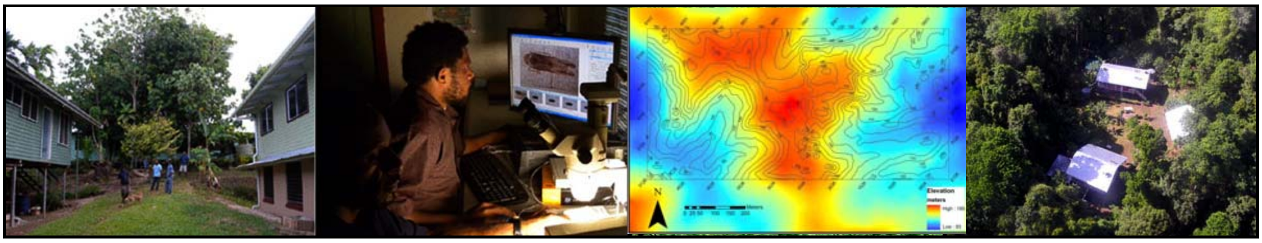

Fig. 5. BRC facilities include laboratory space and herbarium and insect collections equipped with digital imaging, as well as a CTFS 50-ha plot with a field station in the Wanang Conservation Area.