

DNA profiling of insect herbivory in tropical forests

Brief description of the project.

The huge species richness of insects in tropical forests is widely attributed to coexistence and coevolution with an equally puzzling diversity of angiosperm host plants. Numerous hypotheses have been proposed as the causes for this mutual proliferation of diversity, including the higher tropical hostplant diversity, higher host specificity and niche partitioning, and greater turnover among local assemblages. While none of them are fully conclusive, they invariably rely on empirical tests of diet breadth which require time-consuming observations of host use either *in situ* or in the laboratory.

Studies of herbivore – host interactions require, first, that the taxa and their phylogenetic relationships are known and, second, that host records can be established with high reliability. Our recently developed DNA-based technique will solve the dual problem of imprecise taxonomy and incomplete host plant data in a single step. We established that specimens of folivorous leaf beetles (Chrysomelidae) contain a 'molecular record' of their feeding source in the form of ingested plant material (Jurado-Rivera et al., Proc. Roy. Soc. 2009; Pinzon-Navarro et al., Ecol. Entomol. 2010). Consequently, host plant DNA can be PCR amplified from a standard DNA extraction of herbivorous insects and identified against existing taxonomic DNA databases, i.e. the host associations can be established without the immediate context of the host plant from insects caught in flight or traps. This opens up entirely new approaches to the study of tropical herbivore-hostplant interactions, by studying the interactions without the need to witness them.

Herbivore-hostplant interactions in tropical forests operate in a three-dimensional space along the horizontal and vertical stratification of the forest that additionally is structured by the patchy distribution of most host tree species. Existing estimates of insect diversity have focused on individual trees, e.g., classical fogging experiments by Erwin (1982) or feeding trials in a set of tree species (Novotny et al., 2006), but these approaches ignore the spatial complexity of the interactions. Here, the described technique of identifying the feeding source from the herbivore DNA preparations will be applied to the study of host use in the Barro Colorado Island (BCI, Panama) 50 ha plot mapped for all trees (>10 cm diameter). A uniform sampling regime will be used to obtain herbivores at various distances and heights from the ground to test the structuring of the insect communities and their diet in regard to spatial patterns and host availability. This will produce a greatly improved picture of the widely assumed, yet poorly understood, spatial complexity and stratification of tropical insect communities.

Specific objectives.

1. A three-dimensional sampling regime of herbivores. Flight intercept traps will be set up throughout the 50 ha plot suspended from host trees at various heights (understory, mid-canopy, upper canopy). Sites will be spaced at three spatial scales ($10^{1.5}$, $10^{2.0}$, $10^{2.5}$ meters), in order to assess beta-diversity with regard to spatial patterns *per se*. Permanent traps (a total of 27 traps at 3 heights = 81 traps) will be surveyed regularly.

2. Species delimitation and genetic variation of herbivores. Individuals of Chrysomelidae will be selected for DNA extraction and sequencing of mtDNA (COI), to assess distribution and turnover of herbivores, at the genotype and species level. Species delimitation from DNA data will be using a Yule-coalescent model developed by the host group and university supervisor (Pons et al., Syst. Biol. 2006; Lahaye et al, PNAS 2009). Depending on overall sampling success, selection of specimens will focus on subgroups (morphospecies and genus-level clades) to increase comparability across traps.

3. DNA-based identification of diet. Chloroplast DNA (cpDNA) of the host plant will be amplified from the beetle DNA extraction. Identification of plants will be against currently established plant 'barcode' markers (Lahaye et. al. PNAS 2009; CBOL Plant Working Group, PNAS 2009). A database of the >10 cm trees is in progress at CBOL but here we will generate a complementary data set for understory plants using the expertise of the co-supervisor for identification and specimens in the rich NHM botanical collections from Mesoamerica.

4. Correlating map position of trees and beetle diet. The 1000 x 500 m plot includes >208,000 mapped trees in 299 species against which the distribution of herbivores can be assessed. Spatial assessment will be separately for beetles (mtDNA) and for the diet (cpDNA). The design will

permit the assessment of turnover with the among-sites distance and stratigraphic layer, testing if these distributions are correlated with tree distribution. An attempt will be made to use this map of trees as an error structure in modelling the beetle and/or diet distributions (separated for the three stratigraphic levels) to enable quantitative measures of patterns in three dimensions and comparisons among layers. This will reveal clustering and turnover (beta-diversity) of both host use and herbivore species distribution, at each of the three stratigraphic levels. The analysis will provide a unique measure of the complexity of the tropic interactions that can potentially be extended to the genotype level using the DNA data.

5. Testing for reduced complexity in disturbed forest. Additional sites outside BCI of various disturbance levels from the Agua Salud Integrated Ecosystems Services project (in PN Soberania on the Pipeline Road to the W of BCI) will be compared as an example of a disturbed, potentially more homogeneous tropical forests. How does disturbance, through its impact on stratigraphic and spatial complexity, affect ecosystem ecosystem function? Unlike previous studies assessing species composition with the difficulty of establishing causative changes, we here address the degree of complexity. A quantitative approach to this problem has been lacking.

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