Interactions among Neotropical beetles (Coleoptera) and host plants: from feeding niche and host specificity to food web structure

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I. INTRODUCTION

Tropical forests are well known for their great biodiversity (Wright, 2002), and one of the most enduring challenges in ecology is to understand the processes generating and maintaining this biodiversity (McKenna and Farrell, 2005; Okuyama and Holland, 2008). Another challenge in ecology is to identify the nature or pattern of species interactions within communities (Lewinsohn *et al.*, 2006). Plant-herbivore interactions viewed as ecological networks may encompass 40% or more of total terrestrial biodiversity, much of it located in the Tropics (Price, 2002). High levels of terrestrial diversity may have been generated by evolutionary and ecological interactions resembling “arms races” between different organisms (Ehrlich and Raven 1964; Coley and Barone, 1996). This reciprocating process, one of the most important shaping biodiversity is called “coevolution” (Thompson, 1999). The “interface” between plants and herbivores may be a major zone of interaction responsible for generating much of terrestrial biodiversity (Ehrlich and Raven, 1964). Phytophagous beetles (Coleoptera) and especially hispine beetles (Chrysomelidae, Cassidinae) that feed on angiosperms represent an extraordinary and well-known model to study coevolution and high rates of diversification in the Cretaceous and early Cenozoic (McKenna and Farrell, 2005, 2006). Emerging 3 to 4 million years ago and joining North and South America (Webb, 1976), the Isthmus of Panama represents a “hotspot” of diversity an extraordinary opportunity to study the influence of both ecological and historical factors influencing levels of biodiversity (more than 10,000 varieties of plants and except insects, 125 of them are endemic species; Leigh, 1999).

The Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971) suggests that high species diversity and its maintenance (and organization) is the combined result of two mechanisms: parent-centered dispersal patterns and negative density dependent mortality of plants and their offspring. In other words, contagiously distributed plant species are likely to suffer greater attack and hence lower survival (decreasing fitness) than rare and highly

dispersed species. It seems likely that these two mechanisms are driven by natural plant pests, including herbivorous vertebrates, insects and fungal pathogens (Wright, 2002). Tropical phytophagous insects are extraordinarily diverse and have been shown to play a critical role in regulating the distribution, abundance, and perhaps even the species diversity of tropical plants (Leigh *et al*., 2004; Fine, 2004). Nevertheless, the role that phytophagous insects (and other pests) play in maintaining plant diversity in tropical forests remains controversial (Wright, 2002). There is little controversy, however, regarding the importance of host specificity (defined as diet range), whether it is by insects, or other pests. The structure of tropical plant-phytophagous insect communities, directly addresses the degree of host specificity exhibited by tropical insects.

Long-term feeding interactions among plants and herbivores determines the nature of food web structure and community diversity (Coley and Barone, 1996). Recent large-scale studies suggest relatively low levels of host specificity for phytophagous insects, including beetles, feeding on trees in both temperate and tropical forests (Novotny *et al*. 2002, Novotny and Basset 2005). Moreover, host specificity has been reported to vary depending on guild membership with specificity decreasing along a series from endophagy, exophagy, xylophagy to rhizophagy (Novotny and Basset, 2005). Nevertheless, other studies report high host specificity of herbivorous insects in the Tropics, at different taxonomic levels (e.g., plant family or species; Coley and Barone, 1996; Barone, 1998), and a significant increase in host specificity with decreasing latitude (Dyer *et al*., 2007). Differing levels of host specificity may act locally to determine food web structure (Ferry-Graham *et al*., 2002). The “escape and radiate” model of diversification (Ehrlich and Raven, 1964; Farrell *et al*., 1991; Agrawal, 2006) suggests that once plants evolve a novel defensive trait they may expand into a wider range of habitats, which with sufficient time and environmental change can be followed by allopatric speciation. The new plant species will then present continuing opportunities for pest species to radiate, and so the arms race continues. This process explains the origin of diversity. The host specificity

of herbivorous beetles and other pests in different tropical communities is a key assumption of the Janzen-Connell hypothesis explaining the regular spacing and maintenance of high levels of plant diversity in the Tropics ("negative density dependence"; Janzen, 1970; Connell, 1971; Wright, 2002).

There are several reasons why herbivorous insects may not be randomly associated with their host plants. The geographical ranges of potential host plants are independent and may only overlap in certain areas. The local abundance of each of the acceptable plant species can vary greatly across habitats (Southwood, 1960; Fowler and Lawton, 1982). Further, the intrinsic characteristics of different host plant species (Bernays and Chapman, 1994) can vary geographically due to complex factors, thereby affecting the preferences of herbivore species. The first part of this thesis focuses on describing interactions between hispine beetles (Coleoptera, Chrysomelidae, Cassidinae) and their monocotyledonous host plants. The second part of this study brings together host plant data from a range of beetle families and subfamilies in Central America (e.g., Cerambycidae, Buprestidae, Bruchinae; Panama and Costa Rica) and uses standardized food web analysis techniques to determine levels of host specificity and community "architecture" (or structure).

Hispine beetles are widely recognized as a basal group of endophagous and cryptophagous Cassidinae feeding largely on monocots (but including several tribes of dicot-feeding leaf miners) distributed worldwide (Jolivet, 1997), containing approximately 170 genera (Staines, 2002). New World hispine beetle species exhibit diverse life histories that include three larval feeding guilds (e.g., exploiting similar host tissues in a comparable way): rolled-leaf feeders (tribes Arescini, Cephaloleiini), "open leaf" surface scrapers (Hemisphaerotini, Prosopodontini, Delocraniini) and leaf miners (Chalepini, Sceloneoplini) (Strong, 1977a, 1977b and 1983; Jolivet, 1997). These beetles are intimately associated (oviposition, larval and adult feeding, pupation, mating; Windsor *et al*., 1992) with five families (three

orders) of Monocotyledonous plants, the Heliconiaceae, Marantaceae and Costaceae (Zingiberales), the Poaceae (Poales), and the Arecaceae (Arecales) (Jolivet, 1997; Hespenheide and Dang, 1999; McKenna and Farrell, 2005 and 2006). Moreover, the Cephaloleiini (Neotropical rolled-leaf hispine beetles), a well-known tribe of ecological specialists (Ferry-Graham *et al*., 2002), has figured prominently in the development of community ecological theory (Strong, 1982; McKenna and Farrell, 2006).

We use cluster analysis in our first step to define the different groups of hispine species associated with host plant species (Legendre and Legendre, 1998; Rott and Godfray, 2000). Host plant species were chosen based on their abundance, their hispine species richness, their "easy" identification in the field and their habitat. Surveyed plants included both gap-dependent and understory (or shade-tolerant) species, which can differ greatly in terms of defensive characteristics (secondary metabolites, toughness, etc.) and thus suffer significantly different losses to herbivores (Coley and Barone, 1996). Further, host specificity can be estimated by several metrics, such as effective specialization (Novotny *et al*., 2002) and generality (Bersier *et al*., 2002; Tylianakis *et al*., 2007). A second and complementary method used in this study to determine feeding preferences was the use of feeding trials (choice and non-choice tests). At a realistic scale (number of species tested), the trials allowed us to define the potential (or fundamental) and realized feeding niches (Futuyma and Moreno, 1988; Ferry-Graham *et al*., 2002) of hispine beetles. The potential niche is the "manifestation of the species genotype within the environment" (Ferry-Graham *et al*., 2002). The realized niche "reflects how extrinsic factors affect a species diet", which is frequently narrower than the potential niche (Ferry-Graham *et al*., 2002). Feeding trials have the potential to identify unlikely or erroneous associations observed in the field.

Plant-herbivore interactions can be viewed as food webs or ecological networks that represent feeding relationships among organisms within a community (Allaby, 2003). The recent food web approach offers a

systematic tool for summarizing the multitude of interactions possible among herbivores and plants. Food web analysis provides statistics and descriptors, which allow us to both describe and objectively compare complex communities. Moreover, structure analysis is used to define and understand (i) the structural properties of present-day plant-beetle community and (ii) the host specificity of herbivorous insects. Characterizing plant-beetle food web structure can highlight consistency with the known phylogenetic relationships of studied organisms, such as hispine feeding clades (McKenna and Farrell, 2005, 2006). Finally, it can provide insights into the maintenance of biodiversity and the stability of ecological systems, among other things (Blüthgen *et al*., 2008).

Food webs are representations of interacting species, made of nodes (species) and links (interactions/associations). Basically, there are two types of food web analysis: the qualitative approach, based on binary matrix (presence/absence; field sampling results), using unweighted descriptors, and the quantitative approach, based on relative abundance matrix, using weighted descriptors (Bersier *et al*., 2002; Tylianakis *et al*., 2007). The qualitative approach is more convenient because it allows comparisons among independent studies, to deeply define the food web structure (nestedness analysis; Bascompte *et al*., 2003; Guimarães and Guimarães, 2006; Nielsen and Bascompte, 2007; correspondence analysis; Lewinsohn *et al*., 2006; modularity analysis; Guimerà and Amaral, 2005; Fortuna *et al*., 2010). On the other hand, the quantitative approach is less sensitive to sampling bias by giving less weight to uncertain associations (Bersier et *al*., 2002; Tylianakis *et al*., 2008).

Conventional wisdom suggests that ecological networks can present four different structures: gradient, nested, modular (or compartmented) and combined (Lewinsohn *et al*., 2006; see Methodological overview). Moreover, antagonistic networks, such as plant-phytophagous insect food webs mainly present a modular structure. Further, it is widely assumed that hispine beetles are monophagous. In order to better understand how host specificity

influences food web structure and its ecological and evolutionary implications, we have explored nestedness and modularity for this plant-hispine food web, and have defined the taxonomic level of host specificity exhibited by hispine beetles.

The last part of this thesis is a meta-analysis dedicated to investigate more globally the food web structure and the host specificity of three Neotropical superfamilies of Coleoptera coming from a single region, lower Central America (Costa Rica and Panama). The seven data sets include four different families (Chrysomelidae, Cerambycidae, Buprestidae and Curculionidae) and four feeding guilds within the Chrysomeloidea superfamily alone. The food web approach allows us to find structural regularities among Neotropical plant-beetle food webs and other network types (e.g., mutualistic vs. antagonistic or resource-consumer networks; Bascompte *et al*., 2003; Thébault and Fontaine, 2008). Moreover, we examine whether these Neotropical beetle subfamilies and feeding guilds have narrower host ranges than other insects from other regions (host specificity metric used: effective specialization; Novotny *et al*., 2002).

II. OBJECTIVES

The present thesis consists of two major parts and follows four main objectives. The first part mainly covers the field collecting of plant-hispine associations and experimental procedures. The second part draws on the food web approach.

I). Starting with field sampling, the first objective is to identify groups of hispine beetle species (assemblages) associated with their host plants in lowland Central Panama. Hispine beetles are ecological specialists and are known to be highly specialized to their host plant species. We hypothesize that hispine assemblages should differ according to host plant families and habitats.

II). The second objective is to define both realized and potential feeding niches of hispine species. This allows us to determine their diet range and their taxonomic level. Within this aim, we perform two types of feeding trials: non-choice and choice tests. Moreover, this approach helps us to eliminate erroneously recorded host associations.

III). Plant-hispine beetle interactions can be viewed as a food web. In this part, our hypothesis is that several factors, such as hispine host specificity, its taxonomic level and plant habitats should influence the "architecture" of a food web. Therefore, we apply both quantitative and qualitative approaches to characterize structure and complexity. Moreover, several indices and descriptors emerging from these analyzes allow us to estimate hispine beetle host specificity and niche overlap.

IV). Within the framework of the Janzen-Connell hypothesis and the controversy over host specificity of tropical phytophagous insects, we examine the food web structure and estimate the host specificity level of four different phytophagous beetle families in Central America (Costa Rica and Panama). We hypothesize that high host specificity of Neotropical

phytophagous beetles determines the food web structure, and determine how food web structure differs from other locations or other types of ecological networks, such as mutualistic networks. Moreover, the level of host specificity exhibited by these beetles may represent an important selective pressure on plant species, and thus may play a major role in maintaining high levels of tropical plant diversity.

The host specificity and food web structure of phytophagous insects has been the subject of several recent studies and debates in ecology, all with the aim to better understand coevolutionary processes (Bascompte *et al*., 2003; Lewinsohn *et al*., 2006; Fortuna *et al*., 2010), to "describe how ecosystems respond to global change (Fortuna *et al*., 2010)" as well as to obtain general insights into the ecology and evolution of organisms (Gómez *et al*., 2010).

III. METHODOLOGY OVERVIEW

3.1. Community and species assemblage

Defining pattern in species assemblages is an enduring quest in community ecology. Common patterns detected among plant-beetle communities (e.g., hispine beetles associated with their host plants) can indicate similar coevolutionary processes, and levels of community stability and diversity (Lewinsohn *et al*., 2006; Bascompte and Jordano, 2007). Coevolution can be difficult to study as several extrinsic and intrinsic factors may act simultaneously. Plant-beetle interactions represent an extraordinary model to study coevolution because life history traits are well-known, feeding records are becoming increasingly thoroughly documented and phylogenetic studies are covering greater numbers of taxa. But the first step is to identify what the insect eats.

A species assemblage can be defined as: “a collection of plant and/or animal species characteristically associated with a particular environment that can be used as an indicator of that environment (Allaby, 2003)”. In this study, the concept of “species assemblage” is used to describe the set of hispine species closely associated with a particular plant species (cluster analysis result, details in chapter 1, Core section).

Biologically, a community is defined as “any grouping of populations of different organisms that are found living together in a particular environment; essentially, the biotic component of an ecosystem. Organisms interact and give the community structure (Allaby, 2003)”. Nevertheless, communities can be viewed and defined based on structural qualities revealed by food web analysis. Qualitatively, a community is defined as a subset of nodes (species) within the graph (network) such that connections (plant-beetle interactions) between the nodes are denser than connections with the rest of the network. That is, each node has more connections within the community than with the rest of the graph. Or alternatively, the sum of all degrees

(number of associations) within the community is larger than the sum of all degrees towards the rest of the network (Radicchi *et al*., 2004).

3.2. The "food web approach"

For decades, ecologists have tried to understand natural communities as ecological networks, such as plant-herbivore communities (Fig. 1). Ecological interaction networks such as those occurring between plants and hispine beetles can be viewed as a food web. The structure is composed of nodes (interacting species) and links (associations or plant-beetle interactions) (Bascompte *et al*., 2003). Analysis may reveal patterns of community organization, structure and complexity in trophic associations, including measures of host specificity (Blüthgen *et al*., 2008). Food web structure can be useful in predicting functional responses of ecosystems to structural change, including the effects of invasive species on species interactions (Dunne *et al*., 2002; Tylianakis *et al.*, 2008). Further, with at least a network size of 50 interacting species (Bascompte *et al*., 2003), the approach allows comparison among different types of networks (mutualistic vs. antagonistic networks or food web), and/or different taxa. Food web analysis includes two different types of approaches: qualitative and quantitative (Bersier *et al*., 2002; Blüthgen *et al*., 2008), which depend on the type of data set available (chapter 3 and 4, Core section).

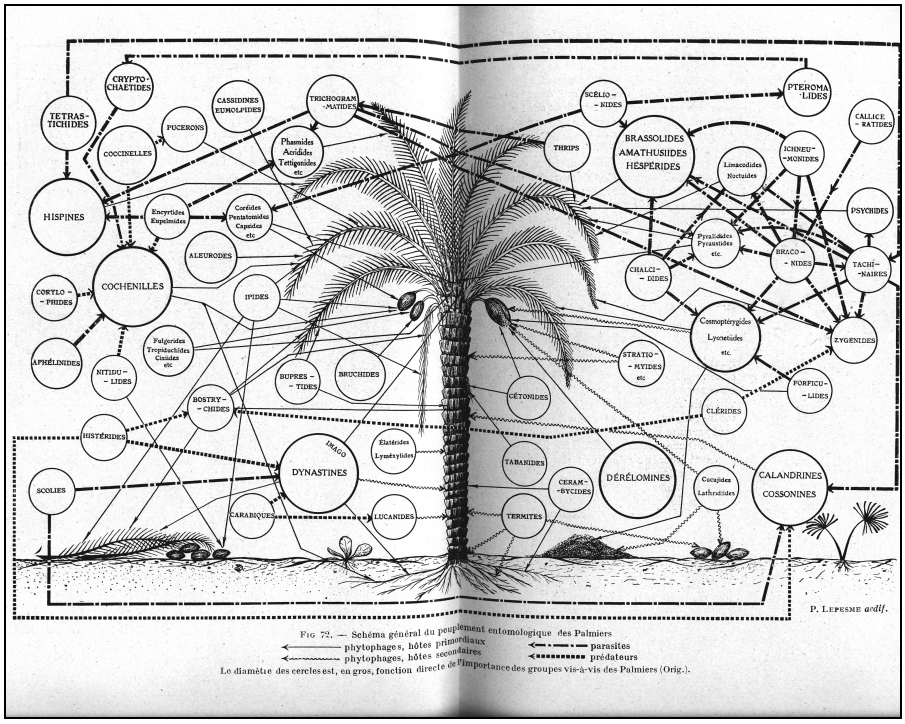

Fig. 1. Illustration of entomological fauna on palms; "old school multi-trophic network" (Lepesme, 1947).

Qualitative approach based on a binary matrix (represented as bipartite graph: two-mode network consisting of two sets of nodes or species with interactions between sets, where size of node can be proportional to number of interactions; Fig. 2; Bascompte and Jordano, 2007) with plant species in rows and beetle species in columns (1 = association recorded between plant species *i* and insect species *j*; 0 = no association) uses different unweighted descriptors.

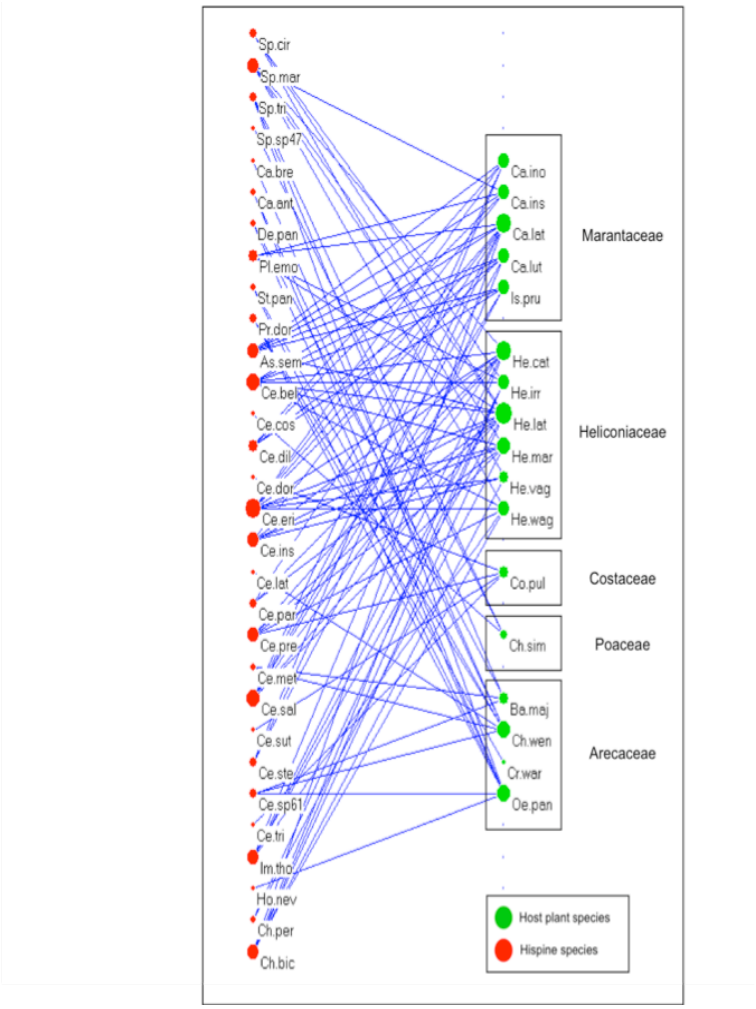

Fig. 2. The qualitative bipartite food web for Panamanian hispine beetles and their hispine herbivores (Meskens et al., 2008; see Core section 3 for species codes); left red circles represent hispine species; right green circles represent plant species; blue lines joining two species represent recorded interactions; circle sizes are proportional to the number of interactions for each species; right boxes represent plant families. The graph was computed using PAJEK (Batagelj and Mrvar 2003; http://pajek.imfm.si/doku.php).

Some of the most important descriptors that emerge from analyses are: connectance (the proportion of possible links that are actually realized in a food web; Blüthgen *et al*., 2008), linkage density (or interaction richness), nestedness (Atmar and Patterson, 1993; Bascompte *et al*., 2003; Guimarães and Guimarães, 2006), and compartmentalization (or modularity; Guimerà and Amaral, 2005; Olesen *et al*., 2007) (see details on computation for these descriptors in Bersier *et al*., 2002; Tylianakis *et al*., 2007; Blüthgen *et al*., 2008). The nestedness metric computed by ANINHADO 3.0 (Guimarães and Guimarães, 2006), originating from studies of island biogeography, characterizes a structural pattern, where, specialists are predominantly interacting with species most often selected by generalists (Bascompte and Jordano, 2007; see Fig.1 in chapter 4, Core section). Two major properties arise from this particular structure. First, the network is highly cohesive; a few species within the network may drive the selective forces, which can "provide alternative routes for system responses to perturbations (Bascompte and Jordano, 2007)". Second, the structure reveals "asymmetric specialization" where specialists only interacting with generalists (Blüthgen *et al*., 2008). This particular structure provides pathways for the persistence of rare species (Bascompte *et al*., 2003). Besides the nestedness analysis, Lewinsohn *et al*. (2006) provide another type of analysis to define network structure: correspondence analysis. Moreover, an alternative option to detect and quantify the modularity of a food web is available (Olesen *et al*., 2007; Fortuna *et al*., 2010). The software using this new algorithm is called NETCARTO (Newman, 2004; Guimerà and Amaral, 2005). More than quantifying the modularity (or compartmentalization) of a food web, NETCARTO identifies the different modules (or compartments) and defines the interacting species within each module (chapter 3, Core section). Analysis is restricted to networks must containing at least 50 species (plant + beetle species) (Bascompte *et al*., 2003).

The results of these analyzes allow us to characterize three simple structures (gradient, compartmented/modular and nested) and one combined model (Fig. 3). Food webs displaying a gradient pattern (Fig. 3 A

and E) feature a relatively constant number of beetle interactions per plant species. Compartmented or modular food webs (Fig. 3 B and F) feature relatively non-overlapping subsets of associated beetle and plant species (e.g., host groups with small phylogenetic distances within subsets; Novotny et al. 2006). A nested food web (Fig. 3 C and G), from the beetle perspective, is organized in such a way that the most host specific beetles feed only on plant species eaten by a large array of both generalist and specialist beetles. Beetle species with decreasing levels of host specificity feed on the most eaten plant species, and also on plant species attacked by specialist beetles. Bascompte and Jordano (2007) called this pattern of interactions "asymmetric specialization". Fortuna *et al*. (2010) showed that both nested and modular structures can occur simultaneously in different types of mutualistic networks and in food webs exhibiting low values of connectance, which is the proportion of possible links actually observed in the network excluding any cannibalistic interactions or interactions within the same trophic level (Fortuna *et al*., 2010). Finally, food webs displaying a combined model (Fig. 3 D and H) feature multiple nested compartments within the same community.

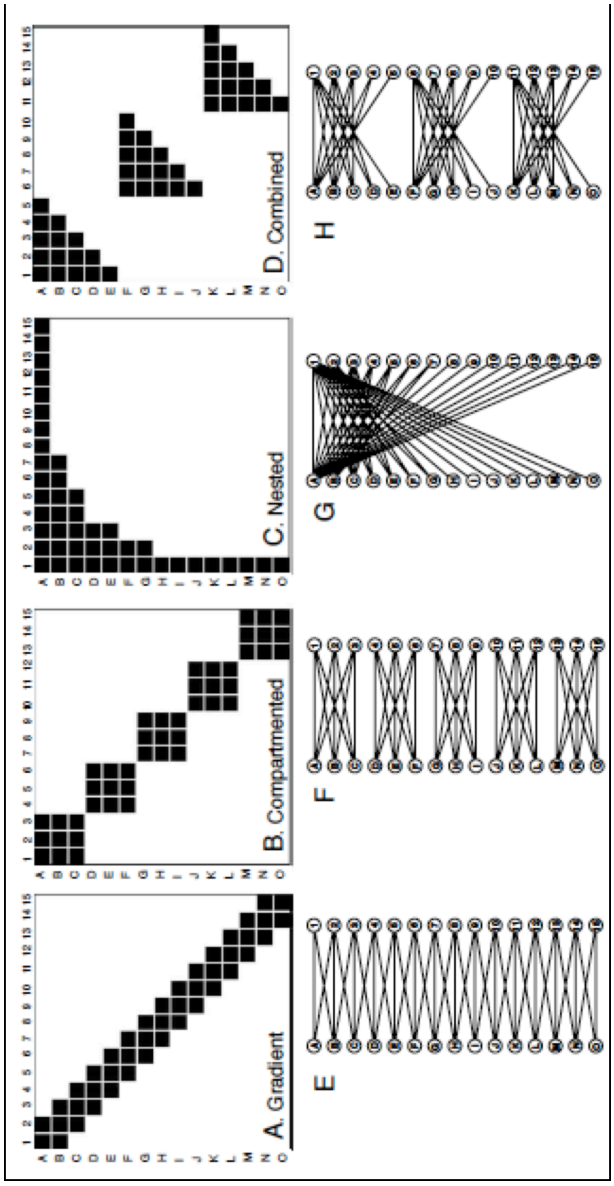

Fig. 3. Plant-animal matrices (left) and bipartite graphs (right) illustrating three simple structures and one combine model; numbers represent animals species; letters represent plant species; black boxes (in matrices) and black lines (in bipartite graphs) represent recorded interactions or associations (see Fig. 1 in Lewinsohn et al., 2006).

The quantitative approach used in this study, is based on the relative abundance matrix (occurrence data of hispine species on host plant species). A quantitative food web can be represented as a weighted network that includes information on the "intensity" and complexity of the interactions among species (Bascompte and Jordano, 2007; Fig. 4). Most of the metrics obtained here (weighted descriptors) have their counterparts in the qualitative approach (Tylianakis *et al*., 2007), such as weighted connectance, weighted generality and vulnerability (Bersier *et al*., 2002). To perform the quantitative analysis, we used the open-source R-package *bipartite* (R Development Core Team, 2008; Dormann *et al*., 2008). This software allows us to visualize quantitative food webs, and includes all recent network analysis indices (Dormann *et al*., 2009). The major advantage of the quantitative approach is that it is less sensitive than qualitative approaches to sampling bias because the analysis gives less weight to uncertain plant-beetle association (Bersier *et al*., 2002).

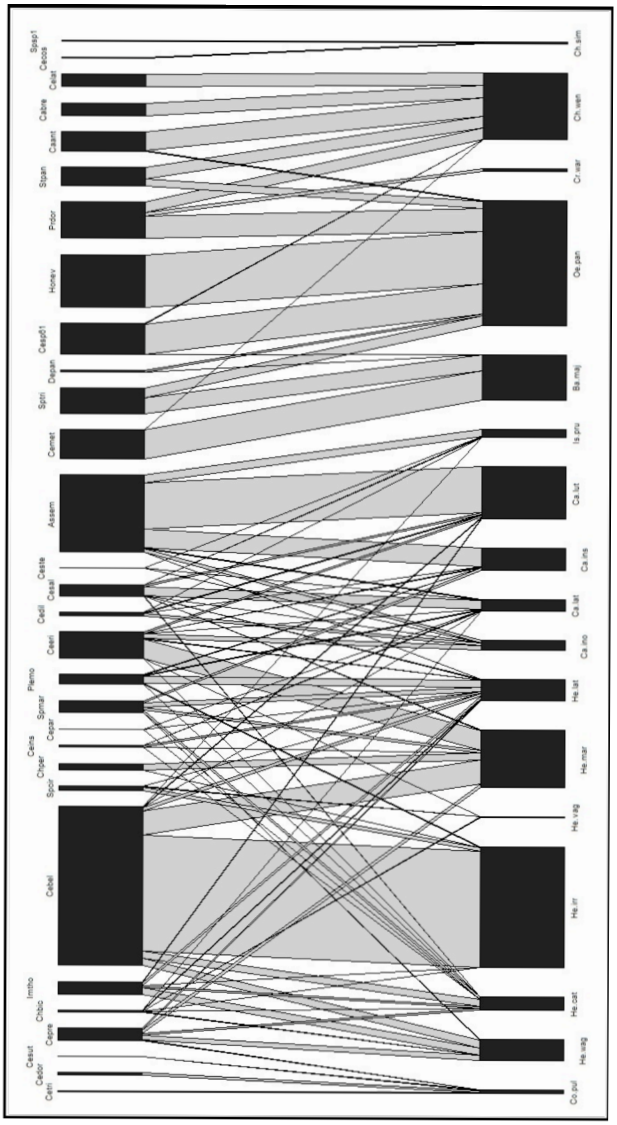

Fig. 4. ***Quantitative plant-hispine food web (function plotweb in R-package bipartite); lower and upper blocks represent respectively plant and hispine species (Meskens et al., 2008; see Core section 3 for species codes); linkage width indicates frequency of each plant-beetle trophic interaction.***

A complete flowchart synthesizing both complementary approaches is detailed above (Fig. 5).

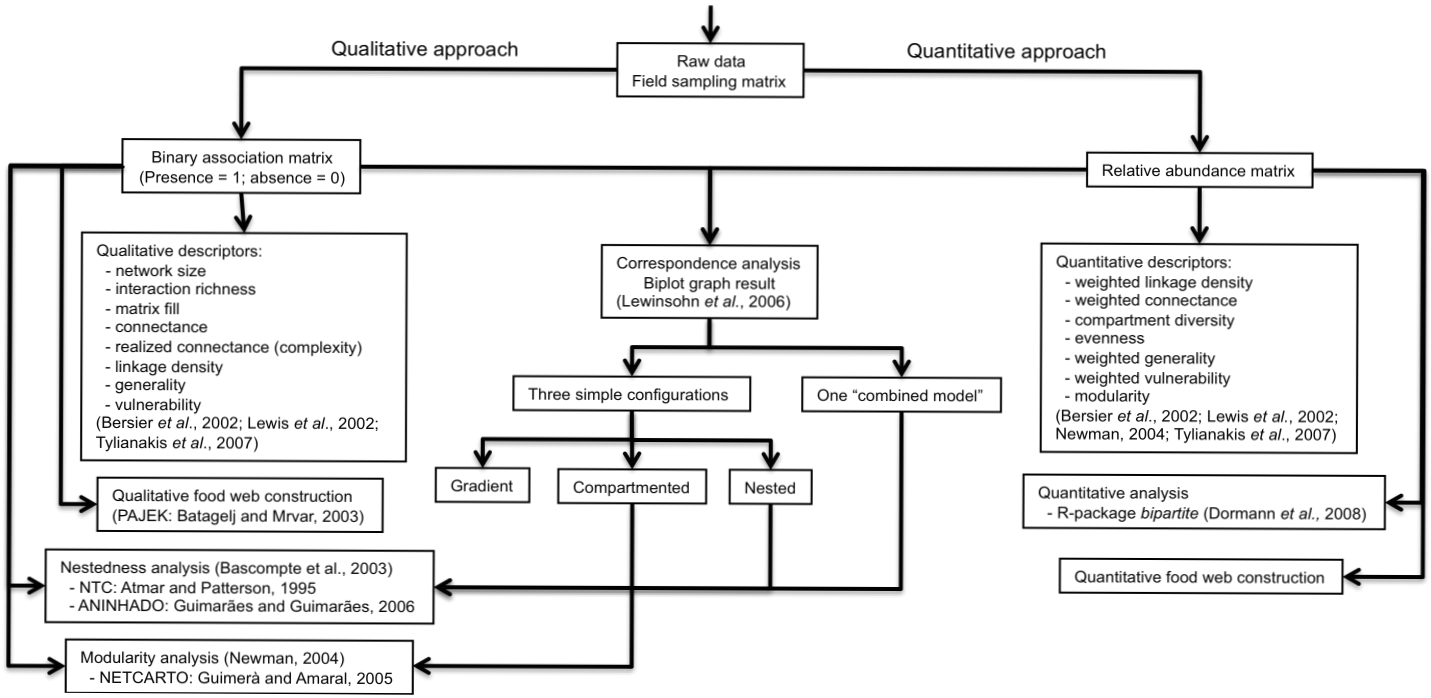
Raw data
Field sampling matrix
Qualitative approach
Quantitative approach
Binary association matrix
(Presence = 1; absence = 0)
Relative abundance matrix
Qualitative descriptors:
- network size
- interaction richness
- matrix fill
- connectance
- realized connectance (complexity)
- linkage density
- generality
- vulnerability
(Bersier et al., 2002; Lewis et al., 2002; Tylianakis et al., 2007)
Correspondence analysis
Biplot graph result
(Lewinsohn et al., 2006)
Quantitative descriptors:
- weighted linkage density
- weighted connectance
- compartment diversity
- evenness
- weighted generality
- weighted vulnerability
- modularity
(Bersier et al., 2002; Lewis et al., 2002; Newman, 2004; Tylianakis et al., 2007)
Three simple configurations
One "combined model"
Gradient
Compartmented
Nested
Qualitative food web construction
(PAJEK: Batagelj and Mrvar, 2003)
Quantitative analysis
- R-package bipartite (Dormann et al., 2008)
Nestedness analysis (Bascompte et al., 2003)
- NTC: Atmar and Patterson, 1995
- ANINHADO: Guimarães and Guimarães, 2006
Quantitative food web construction
Modularity analysis (Newman, 2004)
- NETCARTO: Guimerà and Amaral, 2005

Fig. 5. Food web analysis flowchart summarizing both quantitative and qualitative approaches with the most common used descriptors, indices and analyzes, and including the four possible network structure.

3.3. Host specificity

One of the most critical questions in ecology is the understanding of global species richness (Barone, 1998). Estimating the host specificity of tropical herbivore insects would be a way to evaluate species diversity (Novotny *et al.*, 2002). Host specificity defines here the diet range of phytophagous insects, and it allows to classify them in term as, generalists or specialists (narrow diet range). The taxonomic level targeted is an important factor to define this host specificity, e.g., a family specialist is used for a beetle species feeding on a single plant family (Novotny and Basset, 2005) (chapter 3, Core section). There are several metrics and methods to quantify host specificity (Dyer et al., 2007), and there is no unifying metric allowing us to compare different studies of several growth forms (Ferry-Graham *et al.*, 2002; Novotny and Basset, 2005). Therefore, we use "effective specialization" (F_t) as a metric for host specificity, which is widely applied for plant-phytophagous insect interaction studies (Ødegaard, 2000; Novotny *et al.*, 2002) (chapter 4, Core section). F_t is defined as B/L (Ødegaard 2000, Novotny et al. 2002), where B equals the number of beetle species, and L (interaction richness) represents the number of plant-beetle associations encountered during the sampling.

IV. Material

4.1. Localization

4.1.1. Republic of Panama

The Isthmus of Panama emerged 3 to 4 millions years ago joining North and South America for the first time in approximately 70 millions years (Coates *et al*., 1992). The emergence of the "Isthmus" gave rise to "the Great American Interchange" (Webb, 1976), the mixing of terrestrial flora and fauna, which has played a role in generating high levels of regional biodiversity (more than 10,000 varieties of plants and except insects, 125 of them are endemic species; Leigh, 1999). At the same time, the emergence of the Isthmus served to isolate the marine communities of the Caribbean and Eastern Pacific (Knowlton *et al*., 1993). Located between 7° and 10° north, Panama has borders with Costa Rica (west), the Caribbean Sea (north), Colombia (east) and the Pacific Ocean (south) (Fig. 6). This location provides unique opportunities to study evolutionary and ecological processes; furthermore it is an important site for migratory birds (e.g., more than 3.1 millions migratory raptors were observed in 2005; STRI.org).

The climate on the Isthmus is classified by Leigh (1999) as a "Tropical Monsoon Climate" with 2-4 m annual rainfall and a dry season containing 2-5 months with less than 150 mm per month. Panama's wet season lasts from May though December in most years.

Except along the central Cordillera, most of the Panamanian forests are "Tropical Lowland Moist Forests" (Croat, 1978; Dogget, 1999). The elevation of the Cordillera increases from east to west, near the Costa Rican border, reaching 3,478 m.

Panama has a surface of 75,990 sq. km. and a highly mixed population of 3,309,679 people (July 2008; CIA.gov).

4.1.2. The Smithsonian Tropical Research Institute

The Smithsonian Tropical Research Institute (STRI) (formerly known as the Canal Zone Biological Station) began studies on Barro Colorado Island (BCI) in 1923 surrounded by Gatun Lake and the waters of the Panama Canal. The station has developed as the only bureau of the Smithsonian Institution based outside of the United States. It is now one of the leading research institutions on the world with 38 international staff scientists, 11 stations within Panama (Fig. 6) and projects around the world (Asia, Africa…) dedicated to understanding biological diversity. The Institute welcomes annually more than 900 scientists worldwide (STRI.org).

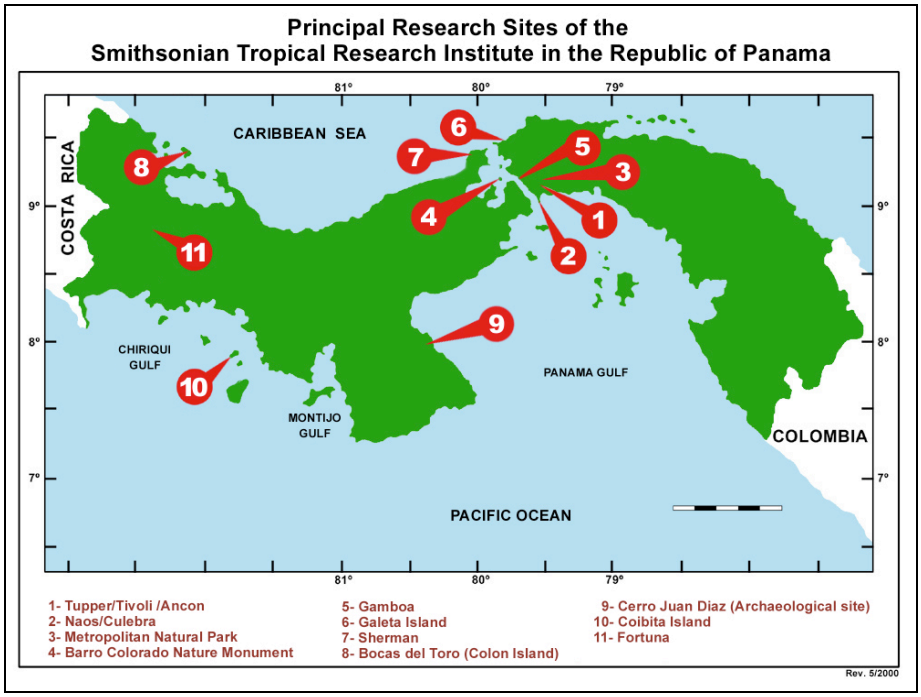

Fig. 6. Map of Panama with STRI principal research sites (STRI.org).

4.2. The host plants

Although Chrysomelidae are known to feed on Bryophyta, Cycadophyta (Windsor *et al*., 1999), Gymnospermas and Angiosperma (Jolivet, 1997), the majority of both Paleotropical and Neotropical hispoid Cassidinae (hispine beetles) are specialized feeders on Monocotyledonous angiosperms (Maulik, 1937; Kalshoven, 1957; Staines, 2004). The present study addresses the Neotropical hispine beetles associated with representative families within the three major orders of Commelinid monocots in the New World tropics: the Arecales, the Poales and the Zingiberales (Staines, 2002; Janssen and Bremer, 2004; Soltis *et al*., 2005; Windsor D. pers. comm.).

4.2.1. The Arecales

This order consists of a single family, the Arecaceae (Palmae) (Soltis *et al*., 2005). The monophyletic family of Arecaceae represents more or less 2,000 species placed in 190 genera, widespread in the tropics and subtropics (Hahn, 2002; Soltis *et al*., 2005). Due to the height of some plant species, the Arecaceae is the only monocot family able to compete with dicotyledonous trees in the forest, e.g., up to 20 m tall for *Oenocarpus panamanus* Bailey (Croat, 1978) and 25 m for *Dictyocaryum lamarckianum* H. Wendl. (Henderson *et al*., 1995).

The developing apical leaf of palms forms a relatively compact sword-like structure, which offers shelter for rolled-leaf hispine beetles for a short period of time as the leaflets of the new leaf grow and expand (Fig. 7).

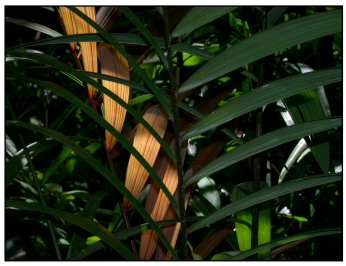

Fig. 7. Young (brownish purple) and expand mature (dark green) leaves of *Oenocarpus panamanus*.

The 4 species of Arecaceae examined in this study occur throughout Central America (the genus *Chamaedorea* Willd. is primarily distributed throughout Central America (Henderson *et al*., 1995)) and into northern South America (*Bactris major* Jacq.; Croat, 1978).

4.2.2. The Poales

The order of Poales contains 17 families (Soltis *et al*., 2005). The present study investigates one of them: the Poaceae. This family consists of 650 genera and 9,700 species distributed worldwide (Croat, 1978; Soltis *et al*., 2005).

These woody shrub- or vinelike herbs (like bamboos) occur in clearings (along trails, anthropic disturbed areas like roadsides…) and are annual or perennial. Sometimes armed, stems arise from stolons or rhizomes and have 2-ranked petiolate leaves. Stems can be up to 25 m in length, e.g., *Chusquea simpliciflora* Munro in our study (Croat, 1978).

Hispine beetles (Sceloenoplini and Cephaloleiini) are feeding associates of Bromeliaceae, a family within the Poales (Soltis *et al*., 2005) at higher elevations (Windsor D. pers. comm.).

4.2.3. The Zingiberales

This well-defined clade consists of 8 families: Musaceae, Strelitziaceae, Lowiaceae, Heliconiaceae, Zingiberaceae, Costaceae, Cannaceae and Marantaceae. Most of the 2,000 species are native to the tropics worldwide (Berry and Kress, 1991; Soltis *et al*., 2005). The genus *Musa* L. is cultivated as a food crops while the Heliconiaceae and Costaceae have horticultural value ("the birds-of-paradise") (Berry and Kress, 1991).
The Zingiberales can be easily recognized by morphological characteristics: young leave blades rolled into a tube, large leaves with long petioles (banana-like leaves) and colorful bracteate inflorescences (Fig. 8) (Berry and Kress, 1991; Soltis *et al*., 2005).

Fig. 8. The inflorescences and leaves of *Heliconia latispatha* Benth.

This study investigates 3 families of Zingiberales: the Heliconiaceae, the Costaceae and the Marantaceae. These families are rich in major classes of flavonoids but concentrations vary greatly between them (Williams and Herborne, 1977).

I. The Heliconiaceae

This monogeneric family (*Heliconia* L.) was at first classified as a subdivision of the Musaceae, but now includes 250 species among which 180 have been described (1 to 6 m tall). Most Heliconia species are native to the Neotropics, from Central Mexico to South America (Berry and Kress, 1991;

Soltis *et al*., 2005). The Heliconias have 2 types of colorful inflorescences (pendent or erect) (Fig. 9) and 3 types of leave arrangements or shoot growth habits: cannoid, musoid and zingiberoid (Berry and Kress, 1991).

Fig. 9. The erect inflorescence of *Heliconia vaginalis* Benth.

Some species have a waxy-white appearing stem, e.g., *H. catheta* R.R. Smith (Berry and Kress, 1991; pers. obs.). The liquid-filled flower bracts (plant-produced and rainfall) (Bronstein, 1988) attract a large diversity of insects. The young unfurled leaves ("rolled-leaves") provide cover to a large diversity of organisms: including rolled-leaf hispine beetles, katydids (Orthoptera, Tettigoniidae), Carabidae, frogs or even Spix's disk-winged bats (*Thyroptera tricolor*) (C. Meskens and D. Dechmann, *personal observations*).

II. The Costaceae

The family of Costaceae contains four genera, which combined include more than 150 species. Their distribution is circum tropical (Asia, Africa, America). Two of the major identification characters are the well-developed stem and the staircase-like spiral of leaf arrangement (Berry and Kress, 1991). Reaching up to 6 m height, the genus *Costus* L. is the most diverse genus in the family with more than 100 species distributed primarily throughout the American tropics (Croat, 1978; Berry and Kress, 1991).

III. The Marantaceae

With 30 genera and up to 500 species, the Marantaceae is the second largest family in the Zingiberales. Most species of the Marantacaea are found in the New World tropics. Within this family, the Neotropical genus *Calathea* G. Meyer contains more than three-quarter of all Marantaceae species. The swollen "pulvinus" on the stem below the leaf blade of all Marantaceae immediately distinguishes this genus from *Heliconia* (Fig. 10) (Croat, 1978; Berry and Kress, 1991).

Fig. 10. Detail of the swollen "pulvinus" for *C. inocephala* Kenn. and Nic.

The common name of the family is the "prayer plant family" stemming from the fact that the petioles stand up straight during the night (Berry and Kress, 1991).

The hispine beetles associated with two genera (*Calathea* and *Ischnosiphon* Koern.) are included in the present study. Species within these genera occur in a wide range of habitats (tree-fall gaps, along trails, understory…). Some species reach 3 m height, e.g., *C. lutea* Schult. (Croat, 1978).
A detail description of surveyed plant species characteristics is given in chapter 1 and 3 (Core section).

4.3. The hispine beetles (Cassidinae): a general overview

4.3.1. The Chrysomelidae family

On Earth, one of the most diversified lineages is the order of the Coleoptera with more than 350,000 species; witch the “Phytophaga” represent 40% of all known species (Gomez-Zurita *et al*., 2007). The Chrysomelidae, this common worldwide herbivorous family is composed by 5 sections (Eupoda, Camptosoma, Cyclica, Trichostoma and Cryptostoma) and contains 40,000 known species (approximately 9.000 genera) distributed among twenty or so subfamilies (Jolivet and Hawkeswood, 1995; Jolivet, 1997; Chaboo, 2007).
The Chrysomelidae or “leaf beetles” can be characterized by a particular five-segmented structure of the tarsi and most of the time, by an antenna composed by 11 segments (Jolivet, 1997). Their form, size and colour vary largely within the different sub-families, e.g., the size of the Cassidinae has a range from a few mm to 45 mm (Staines, 2002).

Feeding on Bryophytes, Gymno- and Angiosperms (well-known feeding damages on leaves of the latest Cretaceous and early Eocene; McKenna and Farrell, 2006), their worldwide distribution, and their huge diversity of parasitoids, the “specialized” Chrysomelidae (from polyphagous to monophagous) are an ideal tool to study the mechanisms of adaptive evolution (Fernandez and Hilker, 2006).
Their importance in agriculture is quite undeniable as pests, e.g., the “Colorado Potato Beetle”, *Dicladispa armigera* Olivier feeding on *Oriza sativa* L. (rice), but also, as biological control agent for several weedy plants (Fernandez and Hilker, 2006; Chaboo, 2007).

4.3.2. The Cassidinae subfamily

I. Classification

More than 6,000 species placed in 312 genera and 42 tribes compose the Cassidinae subfamily (Cassidinae sensu lato) that belongs to the Cryptostoma. This monophyletic subfamily (Chaboo, 2007) contains two forms: the hispoid Cassidinae (hispine beetles, Hispinae Gyllenhal sensu stricto) and the truth Cassidinae (tortoise beetles, Cassidinae Gyllenhal sensu stricto) (Chen, 1940; Staines, 2004). These two forms were merged into one subfamily according to morphology-based character and phylogenetic analysis (Hsiao and Windsor, 1999; Staines, 2004; Chaboo, 2007). This subfamily is one of the most food-specialized Chrysomelidae subfamilies (Turelli *et al*., 2001).

Different studies (Farrell, 1998; Hsiao and Windsor, 1999; Chaboo, 2007) suggest that there is an evolutionary progression from endophagous (broad sense of cryptic larval feeding habits) to exophagous larval feeding, and from solitary to gregarious larva with subsocial habits. Within this progression from ancestral hispine beetles to tortoise beetles, there are some intermediate ("transitional") forms such as the Hemisphaerotini tribe (*Spaethiella* spp) among which the larvae are exophagous and have a fecal shield (Fig. 11).

Fig. 11. The fecal shield of *Spaethiella marginata* Champion.

II. Biology – Ecology

Our study is focused on hispine beetles that represent 3,000 species worldwide placed in 170 genera and 24 tribes, mainly found in the Tropics (below 2,000 m high) (Jolivet, 1997). There are no tribes or genera common to both Old and New Worlds (Staines, 1997).

Cassidinae sensu lato show 7 groups according to their larval feeding modes and microhabitats: leaf-shelter builders, stem borers, rolled-leaf feeders, bract scrapers, leaf miners, open leaf-scrapers, and petalophagous (Chaboo, 2007). The hispine beetles can be ecologically placed into four groups based on larval habits (called feeding guilds) (Jolivet, 1997): “open leaf” surface scrapers; rolled-leaf feeders; leaf miners and stem borers.

A large majority within this form feed on Monocotyledonae and the adaptation to the Dicotyledonae is probably really recent (Jolivet, 1997; Wilf *et al*., 2000). Many species are monophagous or narrow oligophagous (86% at the generic level) (Jolivet and Hawkeswood, 1995). Different plant orders support breeding populations of hispoid Cassidinae among with the tree principals in Central America are the Zingiberales, the Arecales and the Poales (Staines, 2002). But most of hispine beetles literature is focused on the Zingiberales and especially on the genus *Heliconia* and their associated Cassidinae richness (rolled-leaf hispines). This highly specialized plant-insect interaction between rolled-leaf hispines and Zingiberales (Fig. 12) has been maintained during more than 66 million years in the New World (Wilf *et al*., 2000; McKenna and Farrell, 2006).

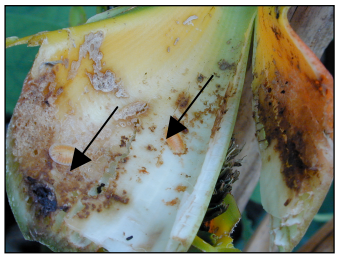

Fig. 12. Larvae of *Cephaloleia pretiosa* Baly (black arrows) on bract of *Heliconia wagneriana* O.G. Petersen.

Eggs hatch within 20 days after being lay on tender, wet tissue of the host plant. A large part of the eggs (up to 50%) is parasitized by different Hymenoptera and Diptera families (Cuignet et al., 2008). The other major factors of mortality are ants (*Crematogaster* species) and desiccation (Strong, 1983; Jolivet, 1997).

The larvae fall into 4 groups as discussed before. Their morphology varies widely in size, color and habit. The number of larval instars can be up to 9 (holometabolous cycle) and the development time can reach 8 months (Jolivet 1997; Chaboo, 2007). The parasitic rate for the larval stage, mainly attacked by Diptera (Tachinidae), is lower (Strong, 1983). Desiccation is a major factor of mortality during the search of a new rolled leaf. For different species such as *Cephaloleia pretiosa* Baly (Fig. 12), the larva presents a cryptic mimicry (Jolivet, 1997; pers. obs.).

The pupae are very similar morphologically to the larvae but more “brownish” and can be found stuck on different parts of the host plant. This phase lasts 20 days and the parasitic rate is as high as for the eggs (40%) (Strong, 1983).
In the New World Tropics, three groups are reported in the literature (Jolivet, 1997; Staines, 2004).

1. The rolled-leaf hispines feed inside the watertight immature leaves produced from the axil of the plant (*Chelobasis bicolor* Gray; Fig. 13), between two stuck mature leaves (*Cephaloleia belti* Baly; pers. obs.) and/or within the bracts (*Cephaloleia pretiosa*; pers. obs.). The adults are waxy, flat and spineless. This group contains some of the largest species: the Alurnini tribe (25-45 mm), and it is a well-studied model in tropical insect-plant associations, especially on Heliconiaceae (A. Richard) (Chaboo, 2007).

Fig. 13. *Chelobasis bicolor*

2. The "open leaf" surface scrapers among which the larvae feed from open leaves. This group can be split into two subgroups: the ancestral hispine beetles (e.g., *Aslamidium semicircularum* Baly; Fig. 14) and the intermediate ("transitional") form, such as *Spaethiella marginata* Champion of which the larva hold a basket-like fecal shield (Fig. 11) or *Delocrania panamensis* Champion with an exuvial shield. The pronotum and elytra edges of the adult that have a round body are well developed which consists as a protection against predators.

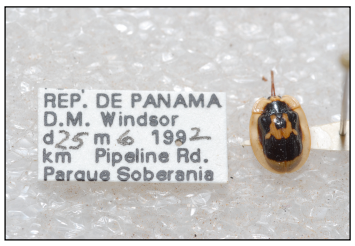

Fig. 14. *A. semicircularum*

3. The leaf miners dig mines into host plant tissues as larvae (*Platocthispa emorsitans* Baly; Fig. 15). The mine may contain one or several larvae depending on species. The pupation happens within the mine. The adult presents an elongated body.

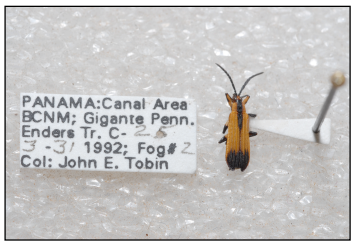

Fig. 15. *P. emorsitans*

V. CORE SECTION

Chapter 1

« *A comparison of hispine beetles (Coleoptera; Chrysomelidae) associated with three orders of monocot host plants in lowland Panama* »

Context

The first chapter of this thesis represents the main field part of our study. Hispine beetles are well-known ecological specialist and highly host specific. Previous studies have been mainly focused on *Heliconia*-hispine associations. But, data including other plant families and/or orders were limited. Based on field collecting plant-hispine beetle associations (or records) in lowland forests (Panama Canal Area, Central Panama), our aim is to define hispine species assemblages associated with 17 plant species within three orders (Arecales, Poales and Zingiberales) and five families (Arecaceae, Poaceae, Costaceae, Heliconiaceae and Marantaceae) occurring in a wide range of habitats from primary forest to disturbed area.

Our sampling results (*occurrence* data) covered more than 5,000 individual hispine records on 4,760 host plant individuals, and included 30 hispine species within mainly the feeding guild of rolled-leaf feeders, but also, “open leaf” surface scrapers, leaf miners.

In this first paper, we performed a cluster analysis that allowed us to characterize hispine species assemblages. Moreover, we defined and discussed the principal factors influencing these assemblages.

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A comparison of hispine beetles (Coleoptera; Chrysomelidae; Cassidinae) associated with three orders of monocot host plants in lowland Panama

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Abstract. The feeding traces in fossil ginger leaves and the conserved phylogenetic relationships we see today in certain clades of hispine beetles on their Monocot hosts point toward a long and intimate plant-insect evolutionary relationship. Studies in the 1970's and 80's documented the rich fauna of rolled-leaf hispine beetles and their association with the Neotropical Monocot family, Heliconiaceae in Central America. In the report below we expand the taxonomic breadth of these early studies to include species in the families, Marantaceae, Poaceae, Arecaceae and Costaceae, all with species occurring sympatrically with Heliconiaceae in lowland Panama. Additionally, we widen the analysis to include open-leaf scraping and internal leaf-mining clades of hispoid Cassidinae. Our censuses add

more than 5,080 Cassidinae herbivore occurrence records on both open and unfurled new leaf rolls of 4,600 individual plants. Cluster analysis reveals that while many Hispinae species tend to group with plant species in only one of the three Monocot orders, 9 of the 16 Hispinae species on Zingiberales hosts were recorded in substantial numbers on both Heliconiaceae and Marantaceae, indicating an underlying pattern of feeding flexibility at the host plant family level.

Keywords**:** *Cassidinae, Arecales, Poales, Zingiberales, hispine beetles, species assemblages*

Introduction

Interactions among plants and phytophagous insects play a key role in community function and evolution. Trophic relationships provide useful insights into understanding important community dynamics (Chave, 2004). Relationships with host plants and food-specialization that evolved in the past may shape contemporaneous communities and species assemblages (Novotny et al., 2002). Diet and degree of food-specialization are poorly characterized for many tropical phytophagous insects, including many species of hispine beetles (Coleoptera; Chrysomelidae; Cassidinae), a group distributed worldwide containing approximately 3,000 species distributed among 24 tribes and 170 genera (Staines, 2002). The relationships between selected groups of host plants and their phytophagous insects can provide insights into the evolution of feeding associations and their phylogenetic constraints (Dobler, 1996). Such an approach requires sampling a large number of potential host plant species during consecutive seasons for an entire guild of phytophagous insects. Our study compares the assemblages of hispine beetle species (Coleoptera; Chrysomelidae; Cassidinae) associated with five families of Monocotyledonous plants, the Heliconiaceae, Marantaceae and Costaceae (Zingiberales), the Poaceae (Poales) and the Arecaceae (Arecales) in a single geographical area, the lowland Neotropical forests of Central Panama.

The diverse New World hispine beetles include eleven tribes, among which, the Cephaloleiini or “rolled-leaf hispines” have cryptophagic larvae and are largely associated with three orders of plants, the Poales, Arecales and Zingiberales (Jolivet, 1997). Feeding grooves in fossilized ginger leaves of late Cretaceous age suggest that the Cephaloleiini clade of hispine beetles may have had a long and intimate plant-insect evolutionary relationship with their Monocot hosts (Wilf et al., 2000). The hispine tribes, Chalepini and Sceloenoplini have endophagic or “leaf-mining” larvae and are associated with a diverse set of both monocotyledonous and dicotyledonous host plant families (Hespenheide and Dang, 1999). The Hemisphaerotini, a relatively

small tribe of putative hispines, have exophagic larvae and are associated principally with the New World monocotyledonous orders: Pandanales (Cyclanthaceae) (DW, pers. commun.), Poales (Bromeliaceae) (DW, pers. commun.), Arecales and Zingiberales. Aspects of the natural history and ecology of these three groups are discussed in Jolivet (1997), Strong (1977, 1983), Chaboo and Nguyen (2004) and Chaboo (2007). Thus, with the exception of some genera of leaf mining hispines, the vast majority of taxa are associates of basal angiosperms in the Monocotyledonae (Soltis et al., 2005). A basal position for several hispine taxa within the Cassidinae subfamily was indicated in an early phylogenetic reconstruction using a fragment of the 12S ribosomal mtDNA gene (Hsiao and Windsor, 1999) and in a subsequent morphological study (Chaboo, 2007). With only minor exceptions, both adult and larval hispines feed on the same host plant species (Jolivet, 1993). Previous studies of hispine beetles examined communities associated with a single monocot genus, *Heliconia* (Seifert, 1976 and 1982; Staines, 2002; Strong, 1977a, 1977b, 1982 and 1983). The actual host plants of more than 70 % of hispine genera are still unidentified or poorly supported (Jolivet, 1997). In this paper, we present additional data on the assemblage of hispine species associated with *Heliconia* species and we compare those to the hispine assemblages occurring on other species of Monocots in the same habitat. Because the term “community” when referring to a collection of hispine species can be misleading or ambigous (Strong, 1977b and 1982), we use the term “species assemblage” (Allaby, 2003) in this paper to refer collectively to all hispine species occurring on a specified set of host plants.

The summary trees of commelinid phylogeny (Soltis et al., 2005; Janssen and Bremer, 2004) indicate that the Arecaceae is the older plant family in our study, splitting off from the core monocots before the Poaceae and the Zingiberales. The Heliconiaceae branched from Zingiberales stock earlier than the Costaceae and these earlier than the Marantaceae. Janssen (2004) showed that these three phylogenetically separated families of Zingiberales often co-occur in the understory of Neotropical forests. Substantial

differences in flavonoid content between these three Zingiberales families are reported by Williams (1977). Marantaceae and Heliconiaceae are the most abundant Zingiberales in many lowland areas of Central America (Seifert, 1976 and 1982; Strong, 1977a, 1977b and 1982; Flowers and Janzen, 1997). Our study examines the hypothesis that hispine beetles will share structurally similar species of plants occurring in the same or nearby habitats. An alternative hypothesis is that hispine beetles will share plants having similar histories of diversification. That is, flowering plants may have shaped beetle diversification leaving host plant associations at the family level as an enduring record of this process. In particular, older plant families such as the Arecaceae (Soltis et al., 2005) are expected to support more diverse faunas and more basal taxa than are found on younger families such as the Marantaceae. Our study also examines Strong's contentions that broadly distributed Zingiberales food plants will have more associated hispine species than narrowly distributed ones and that a plant species will have a constant number of associated hispines throughout its range but that the particular species may change according to location ("geographical replacement") (Strong, 1977b and 1982).

Materials and methods

Study area, host plants and sampling method

Observations were made within forests along the east bank of the Panama Canal including a site near Gamboa in Parque Natural Soberania (9°7' N, 79°42' W), along the trail system on Barro Colorado Island (9°9' N, 79°51' W) and within the Parque Metropolitano (8º59' N, 79º33' W; near Panama City). Observations were made during 18 total months within both dry and wet seasons between May 2002 and July 2006.

Our study is based on observations recorded from a total of 17 species of monocot host plants (Table 1). Twelve of these species are within the order, Zingiberales and included six species of Heliconiaceae, five species of

Marantaceae and 1 species of Costaceae. Species within these families, all native to the Neotropics (Croat, 1978), are characterized by large and colourful bracts and by young leaves rolled into long tubes opening after periods from a few days to a few weeks into large mature leaves with transverse venation, long petioles and high flavonoid content (Berry and Kress, 1991). The scroll-like immature leaves are the typical habitat for "rolled-leaf" hispoid Cassidinae (Strong, 1983; Jolivet, 1997). This study additionally includes four species of Arecaceae whose young leaflets initially form relatively compact sword-like structures, which subsequently begin to open and provide habitat for hispines as the leaf expands to full size. Finally, the study includes one species of Poaceae (*Chusquea simpliciflora* Munro) whose new leaves are rolled at the base.

Ecologically, these five families occur in a range of habitats from old forest to human disturbed areas. We classified each species into one of 7 habitat categories according to information given by Croat (1978) and Kress (1990) (Table 1). Host plant species were chosen because of their abundance in the study sites (Croat, 1978; pers. obs.) and the presence of a diversified hispoid Cassidinae fauna (Strong, 1977; Staines, 2004; DWindsor pers. commun..). Thus, the study lacks data from rare host plant taxa and those taxa that are rarely or never fed upon by hispines. While the host plant species are all part of the native flora of Panama (Croat, 1978), all of them occur elsewhere in Central and South America (Table 1).

We recorded the number of leaves unrolled or expanded for each individual plant that was sampled and the number of species and developmental stage (egg, larva and pupa) for hispine beetles observed or collected. Plants occurring along trails were surveyed inside young (60-120 year old) and old (400 year old) forest on BCI and along trails in mainland forests (Parque Soberania and Parque Metropolitano). Hispine species were identified based on the plates in the Biologia Centrali-Americana (Baly, 1885-1894), descriptions and key given by Staines (1996 and 2002) and by comparison to identified specimens in the STRI insect collection. Beetles species were

normally easily identified in the field, but were collected when their identification was in doubt, as with leaf-miner larvae which were reared in the lab to obtain and identify the adults. Study sites were visited once a week during the sampling periods. Because leaf-rolls and mined leaves had to be destructively removed, each individual plant was surveyed only once a field season to minimize disturbance to beetle assemblages. The scroll-like immature leaves were only included in the study if they were closed at the bottom (watertight) thereby maintaining a high degree of humidity, which appeared to be an important factor for “rolled-leaf” Cassidinae communities (Strong, 1977a). A set of voucher specimens was deposited in the collections of the Smithsonian Tropical Research Institute in Panama.

Table 1. Host plant characteristics and numbers of each plant species sampled

Host plant family and species	Range[a]	Habitat[b]	Size[a]	No.
Arecaceae				
Bactrtis major Jacq.	Panama	Understory (forest)	8 m	172
Chamaedorea wendlandiana Hemsl.	Nicaragua to Panama	Understory (forest, near streams)	Up to 5 m	358
Cryosophila warscewiczii Bartl.	Belize to Colombia	Understory (younger forest)	Up to 10 m	196
Oenocarpus panamanus Bailey	Costa Rica and Panama	Understory (forest)	8-20 m	289
Costaceae				
Costus pulverulentus Presl	Mexico to Ecuador	Old tree-fall gap	0,5-3,5 m	63
Heliconiaceae				
Heliconia catheta R. R. Smith	Panama	Tree-fall gap	3-5 m	156
H. irrasa R. R. Smith Lane	Mexico to Colombia	Understory (forest)	1,5-2 m	242
H. latispatha Benth.	Mexico to South America	Disturbed area	1,5-3 m	+/- 740
H. mariae Hook	Belize to Panama	Understory (forest)	3-6 m	82
H. vaginalis Benth.	Mexico to Ecuador	Tree-fall gap	1-3 m	125
H. wagneriana O. G. Petersen	Honduras to Panama	Clearings	Up to 6 m	39
Marantaceae				
Calathea inocephala Kenn. & Nic.	Mexico to Peru	Understory (forest)	2-4 m	831
C. insignis O. G. Petersen	Mexico to Peru	Tree-fall gap	2-3 m	227
C. latifolia Klotzsch	Venezuela to Panama	Disturbed area	1-2 m	452
C. lutea Schult.	Mexico to Peru	Tree-fall gap	2-3 m	51
Ischnosiphon pruinosus O. G. Petersen	Belize to Panama	Tree-fall gap	2-3 m	247
Poaceae				
Chusquea simpliciflora Munro	Guatemala to Panama	Forest	Up to 25 m	+/- 400

Range = distribution area; No. = number of visited individuals per host plant species in the present study.
[a] = Croat (1978); [b] = Berry and Kress (1991) and Croat (1978)

Statistical analysis: species richness and community analysis

Hispine species accumulation curves for each host plant species were generated using 5 different estimators: Chao 1, Chao 2, Jacknife 1, Jacknife 2 and Bootstrap (Species Diversity & Richness, Version 3.0, Pisces Conservation Ltd, 2001). The sampling of hispine species was considered exhaustive when the species accumulation curve for that plant species approached an asymptote. The faunas of two species, *Costus pulveruventus*

Presl and *Heliconia vaginalis* Benth. did not reach an asymptote indicating further sampling is warranted for these species.

To determine whether factors proposed by Strong (1977a and 1977b) had a significant impact on Cassidinae species richness and average abundance per plant, we performed a Kruskal-Wallis One-Way Analysis of Variance (Systat, Version 10, SPSS Inc, 2000). This analysis was applied independently to 6 matrices (3 factors: plant size (height: < 3 m or > 3 m), plant habitat (forest and understory or disturbed), plant range (Central America or Central America plus South America); 2 variables: richness, average abundance). The sampling effort was not constant for each plant species, therefore we normalized herbivore species occurrence data by dividing the number of hispine individuals observed on a particular host plant species by the number of individuals of that plant species surveyed. To define the hispine species assemblages associated with each host plant species; we applied "average linkage" and Bray-Curtis' distance clustering to normalized field data, procedures widely used in ecological studies with species abundance. The cluster analysis allows identifying a set of groups (hispine assemblages), which both minimize within-group variation and maximize between-group variation (Dufrêne and Legendre, 1997; Legendre and Legendre, 1998; Clustering, Community Analysis Package, Version 2.0, Pisces Conservation Ltd, 2001).

Results

More than 4,670 host plant individuals (17 species) were inspected (Table 1) and 5,080 hispoid Cassidinae individuals were collected or identified in place (1,013 on five species of Marantaceae, 1,072 on six species of Heliconiaceae, 11 on one species of Costaceae, 36 on one species of Poaceae and 2,948 on four species of Arecaceae). The host plant species were classified into 7 habitats and had ranges varying from Mexico to South America (Table 1). The Cassidinae observed in this study belonged to 30 species distributed among 11 genera (Table 2). Two Cassidinae species are

apparently undescribed and were assigned provisional morphospecies names (*Cephaloleia* sp61 and *Spaethiella* sp47).

Hispine species richness per plant species ranged from 1 to 12 species (median number of hispine species per host = 5) (Fig. 1). The plant species with the richest assemblages of hispines were *Heliconia latispatha*, *Heliconia catheta* and *Calathea latifolia* with 12, 10 and 9 species, respectively. The smallest assemblages were found on *Cryosophila warscewiczii, Chusquea simpliciflora* and *Heliconia vaginalis,* with 1 (*Prosopodonta dorsata*), 3 and 3 species, respectively. At the family level, the average richness of hispines was 7.8 for Heliconiaceae, 5.8 for Marantaceae, 4 for Costaceae, 3 for Poaceae and 2.5 for Arecaceae. The numbers of hispine species associated with Heliconiaceae and Marantaceae species were not significantly different (Mann-Whitney U = 19.0, p = 0.457, df = 1).

Table 2. The list of hispine species observed in the present study (n = 30), species abbreviations used in the cluster analysis (Fig. 2), and species feeding habits and larval morphologies (Strong, 1977a, b, 1983; Staines, 1996, 2002, 2004; D. Windsor pers. commun.)

			Larvae		Adult
Hispine species	**Code**	**Habitat**	**Shape**	**Dorsal shield**	**Habitat**
Arescini					
Chelobasis bicolor Gray	Ch.bic	Rolled Leaf	Onisciform	None	Rolled Leaf
Chelobasis preplexa Baly	Ch.per	Rolled Leaf	Onisciform	None	Rolled Leaf
Cephaloleiini					
Aslamidium semicircularum Olivier	As.sem	Open Leaf	Onisciform	None	Open Leaf - Upper Side
Cephaloleia belti Baly	Ce.bel	Rolled Leaf	Onisciform	None	Rolled Leaf
C. costaricensis Uhmann	Ce.cos	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
C. dilaticollis Baly	Ce.dil	Rolled Leaf	Onisciform	None	Rolled Leaf
C. dorsalis Baly	Ce.dor	Rolled Leaf	Onisciform	None	Rolled Leaf
C. erichsonii Baly	Ce.eri	Rolled Leaf	Onisciform	None	Rolled Leaf
C. instabilis Baly	Ce.ins	Rolled Leaf	Onisciform	None	Rolled Leaf
C. lata Baly	Ce.lat	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
C. metalescens Baly	Ce.met	Rolled Leaf	Onisciform	None	Rolled Leaf
C. partita Weise	Ce.par	Rolled Leaf	Onisciform	None	Rolled Leaf
C. pretiosa Baly	Ce.pre	Rolled Leaf	Onisciform	None	Rolled Leaf
C. sallei Baly	Ce.sal	Rolled Leaf	Onisciform	None	Rolled Leaf
C. stevensi Baly	Ce.ste	Rolled Leaf	Onisciform	None	Rolled Leaf
C. suturalis Baly	Ce.sut	Rolled Leaf	Onisciform	None	Rolled Leaf
C. trimaculata Baly	Ce.tri	Rolled Leaf	Onisciform	None	Rolled Leaf
C. sp61 (Meskens 2005)	Ce.sp61	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
Imatidium thoracicum Fabr.	Im.tho	Open Leaf	Onisciform	None	Open Leaf - Lower Side
Homalispa nevermanni Uhmann	Ho.ner	Rolled Leaf	Onisciform	None	Rolled or Open Leaf
Chalepini					
Platocthispa emorsitans Baly	Pl.emo	Leaf Miner	Onisciform	None	Open Leaf
Delocraniini					
Delocrania panamensis Champion	De.pan	Open Leaf	Onisciform	Detritus	Open Leaf - Lower Side
Hemisphaerotini					
Spaethiella circumdata Boheman	Sp.cir	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. marginata Champion	Sp.mar	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. tristis Boheman	Sp.tri	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. sp47 (Meskens 2005)	Sp.47	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
Imatidiini					
Stilpnaspis panamensis Borowiec	St.pan	Rolled or Open Leaf	Onisciform	None	Open Leaf
Prosopodontini					
Prosopodonta dorsata Baly	Pr.dor	Open Leaf	Onisciform	Urogomphus	Open Leaf
Spilophorini					
Calyptocephala antennata Spaeth	Ca.ant	Open Leaf	Onisciform	Exuvial Shield	Open Leaf
Calyptocephala brevicornis Boheman	Ca.bre	Open Leaf	Onisciform	Exuvial Shield	Open Leaf

"Onisciform" larvae are flattened with extended segmental margins contacting the substrate, approximately oval in shape when viewed from above; while "eruciform" larvae are circular in cross section, their mass largely supported by their legs and they are tubular shape.

Neither host plant habitat, host plant range nor the average size of host plants had a significant affect on hispine richness (host plant habitat: Mann-Whitney U = 29.5, p = 0.53, df = 1; plant range: Mann-Whitney U = 47.5, p = 0.26, df = 1; plant size: Mann-Whitney U = 49.5, p = 0.19, df = 1) or on

hispine abundance (host plant habitat: Mann-Whitney U = 42.0, p = 0.56, df =1; plant range: Mann-Whitney U = 51.0, p = 0.15, df = 1; plant size: Mann-Whitney U = 30.0, p = 0.56, df = 1).

Ten well-defined hispine species groups (containing a total of 30 species) were arranged by Cluster analysis (Average Linkage method; Bray-Curtis' distance) (Fig. 2). The first dichotomy in the output separates the hispine species clusters associated with the Zingiberales from those associated with the Poales + Arecales. The next branching within the Zingiberales separates the hispines associated with the three Zingiberales families, Marantaceae, Heliconiaceae and Costaceae. The second branching of the Poales + Arecales separates these two orders. Each hispine species fell within only one cluster group (Table 4). Two hispine species, *C. stevensi* and *D. panamensis*, clustered only with themselves and diverged early in the statistical output.

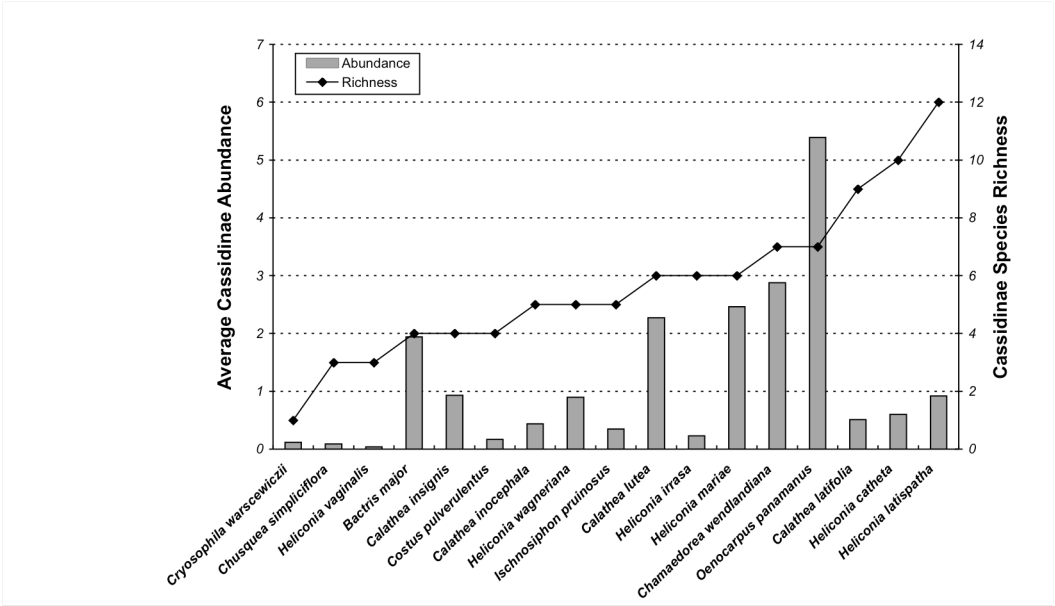

Figure 1. Hispine species abundance (the average number of hispine individuals observed per plant) and richness (number of hispines species per plant species) on 17 species of monocotyledonous host plants

Discussion

This study represents the first large-scale survey of hispoid Cassidinae on the Isthmus of Panama. Thirty species of hispoid Cassidinae were encountered on leaves of more than 4,670 individual plants in five Monocotyledonous families (Table 3). When normalized by number of individuals per host plant species, the occurrence data suggest that strong preferences exist within the hispoid Cassidinae at the host plant ordinal level but less so at the familial and generic levels. Only 1 hispine species (*Spaethiella tristis*) was found on more than one plant order (Poales and Arecales), while 12 hispine species were found on more than one plant family. No hispine species were found to be randomly distributed among host plant families, genera or host plant species (Fig. 2).

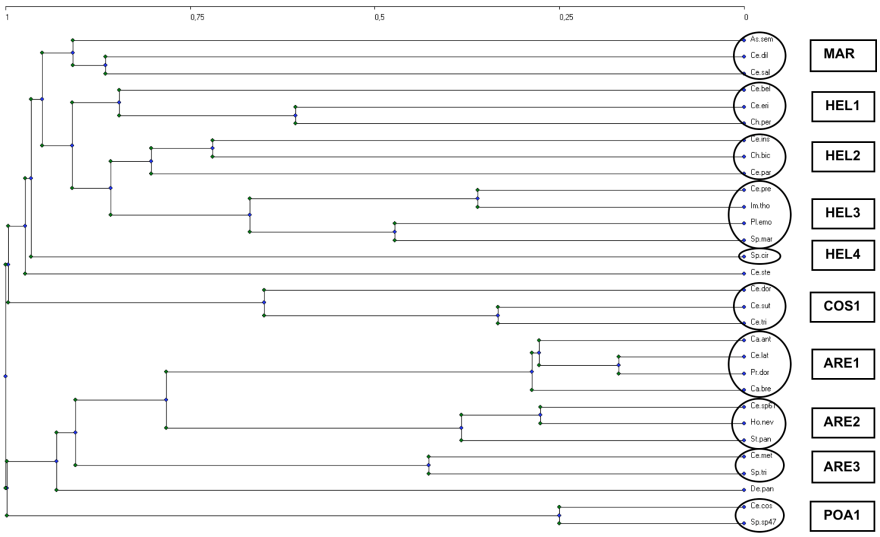

Figure 2. Cluster analysis: dendrogram of hispoid Cassidinae species (average linkage method; Bray-Curtis' distance)**.** ARE1, first cluster group of Arecaceae; ARE2, second cluster group of Arecaceae; ARE3, third cluster group of Arecaceae; HEL1, first cluster group of Heliconiaceae; HEL2, second cluster group of Heliconiaceae; HEL3, third cluster group of Heliconiaceae; HEL4, fourth cluster group of Heliconiaceae; COS1, cluster group of Costaceae; MAR1, cluster group of Marantaceae; POA1, cluster group of Poaceae

While several factors have been suggested to influence hispoid Cassidinae richness on particular host plant species (Strong 1977a, 1977b), our study does not support either host plant size, range, or habitat type as factors significantly affecting either richness or abundance. However, it is possible that some or all of these factors working in combination could explain the high richness of the hispine assemblage associated with *Heliconia latispatha*. This species has 12 hispine species (4 open-leaf scrapers, 1 leaf-miner and 7 "rolled-leaf" hispines), has the largest range of the 17 plant species in the study, and is of moderate to large size, frequents forest margins and occurs in dense patches. Moreover, each of the 3 richest plant species (*Calathea latifolia, Heliconia catheta and H. latispatha*) occurs in disturbed areas and tree-fall gaps (Fig. 1). Differences among species in major chemical content at the family level as described by Williams (1977) could also contribute to the differences in hispine richness we observed among plant species. Seasonality in the production of rolled leaves is another factor that could affect species richness. Heliconiaceae (Croat, 1978) produce young rolled leaves throughout the year while other plant families may be more seasonal in the production of rolled leaves. Heliconiaceae thus may offer a more stable habitat than the other understory species and thereby attract and retain more "rolled-leaf" hispine colonists (Fig. 1). The inclusion of only four Arecales species in the study, two of which had very low hispine richness and two of which had moderate to high hispine richness, makes any conclusion about the importance of the phylogenetic "age" of this family premature.

Strong (1977a, 1977b and 1982) recorded 12 "rolled-leaf" hispoid Cassidinae species occurring on *Heliconia* spp in different areas in Central America while we found 9 "rolled-leaf" species in Panama (Table 3). However, only 3 of these species (*Cephaloleia instabilis*, *Chelobasis bicolor* and *C. perplexa*) occur on the same host plant species (Table 3). The difference in these faunas (Table 3) does suggest that considerable geographical replacement of hispine species in *Heliconia* communities may occur across Central America.

Table 3. Results of cluster group analyses (dendrogram; average linkage method; Bray-Curtis' distance).

Code for cluster	Included host plant species	Habitat	Hispine species*
ARE1	*Chamaedorea wendlandiana*	Understory (forest, near streams)	Ca.ant, Ce.lat, Pr.dor, Ca.bre
ARE2	*Cryosophila warscewiczii*	Understory (younger forest)	Ce.sp61, Ho.nev, St.pan
	Oenocarpus panamanus	Understory (forest)	
ARE3	*Bactris major*	Understory (forest)	Ce.met, Sp.tri
COS1	*Costus pulverulentus*	Old tree-fall gap	Ce.dor, Ce.sut, Ce.tri
HEL1	*Heliconia mariae*	Forest	Ce.bel, Ce.eri, Ch.per
HEL2	*H. catheta*	Tree-fall gap	Ce.ins, Ch.bic, Ce.par
	H. vaginalis	Tree-fall gap	
	H. wagneriana	Clearings	
HEL3	*H. latispatha*	Highly disturbed area (anthropic)	Ce.pre, Im.tho, Pl.emo, Sp.mar
HEL4	*H. irrasa*	Understory (forest)	Sp.cir
MAR1	*Calathea inocephala*	Understory (forest)	As.sem, Ce.dil, Ce.sal
	C. insignis	Tree-fall gap	
	C. latifolia	Disturbed area	
	C. lutea	Tree-fall gap	
	Ischnosiphon pruinosus	Tree-fall gap	
POA1	*Chusquea simpliciflora*	Forest	Ce.cos, Sp.sp47

Habitat, plant species habitat (Berry and Kress, 1991; Croat, 1978); ARE1, first cluster group of Arecaceae; ARE2, second cluster group of Arecaceae; ARE3, third cluster group of Arecaceae; HEL1, first cluster group of Heliconiaceae; HEL2, second cluster group of Heliconiaceae; HEL3, third cluster group of Heliconiaceae; HEL4, fourth cluster group of Heliconiaceae; COS1, cluster group of Costaceae; MAR1, cluster group of Marantaceae; POA1, cluster group of Poaceae.
* See Table 2 for hispine species abbreviations.

The results of our study also suggest that host plant data are under reported in the literature, possibly a product of sampling a narrower range of plant families and hispine tribes (Jolivet, 1997; Fernandez and Hilker, 2007). Laboratory feeding trials will be essential to know just how accurately occurrence data as presented in this study reflect actual feeding habits of hispines (Descampe et al., 2007, in press).

Table 4. Hispine occurrence data from the present study and that reported by previous studies

Hispine species	Host plant in present study	No.	McKenna[a]	Staines[b]	Strong[c]
Aslamidium semicircularum	*Calathea inocephala*	113	-	Zingiberales	-
	Calathea insignis	180			
	Calathea latifolia	32			
	Calathea lutea	102			
	Heliconia latispatha	1			
	Ischnosiphon pruinosus	79			
Calyptocephala antennata	*Chamaedorea wendlandiana*	286	-	-	-
	Oenocarpus panamanus	10			
Calyptocephala brevicornis	*Chamaedorea wendlandiana*	197	-	-	-
Cephaloleia belti	*Calathea latifolia*	4	*Heliconia* sp. – PA	*Heliconia imbricata* – CR	
	Calathea lutea	2	*H.* sp. – CR	***Heliconia latispatha***	
	Ischnosiphon pruinosus	1		*Heliconia pogonantha*	
	Heliconia catheta	47		***Heliconia mariae***	
	Heliconia irrasa	5		*Heliconia tortuosa*	
	Heliconia latispatha	101			
	Heliconia mariae	85			
	Heliconia wagneriana	13			
C. costaricensis	*Chusquea simpliciflora*	12	-	-	-
C. dilaticollis	*Calathea inocephala*	86	***Calathea lutea*** – PA	***Calathea insignis*** – CR	-
	Calathea insignis	4	*Calathea* sp. – PA		
	Calathea lutea	2	*Calathea* sp. – EC		
	Ischnosiphon pruinosus	1	*Renealmia* sp. – EC		
C. dorsalis	*Costus pulverulentus*	7	*Renealmia* sp. – EC	*Costus* sp. – CR	-
C. erichsonii	***Calathea inocephala***	186	*Calathea gymnocarpa* – CR	*Calathea gymnocarpa* – CR	-
	Calathea insignis	1	*Calathea* sp. – CR	***Calathea inocephala***	
	Calathea latifolia	4	*Heliconia* sp. – CO	*Calathea leucostachys*	
	Calathea lutea	3	*Calathea* sp. – PA		
	Heliconia catheta	1			
	Heliconia latispatha	5			
	Heliconia mariae	70			
	Heliconia vaginalis	1			
C. insignis	*Calathea latifolia*	1	*Heliconia wagneriana* – CR	***Heliconia latispatha***	*Heliconia platystachys* – PA
	Heliconia catheta	1		*Heliconia imbricata*	*Heliconia curtispatha* – PA
	Heliconia latispatha	38		*Heliconia wagneriana*	***Heliconia latispatha*** – PA
	Heliconia mariae	2			***Heliconia latispatha*** – CR
					Heliconia wagneriana – CR
C. lata	*Chamaedorea wendlandiana*	195	*Chamaedorea tepejilote*	-	-
C. metalescens	*Bactris major*	215	Unknown – CR	-	-
	Chamaedorea wendlandiana	4			
C. partita	*Calathea latifolia*	2	***Heliconia latispatha*** – PA	*Heliconia* sp.	-
	Heliconia catheta	2	*Heliconia* sp. – PA		
	Heliconia latispatha	1			
C. pretiosa	*Costus pulverulentus*	1	*Heliconia* sp. – CO	-	-
	Heliconia catheta	12			
	Heliconia latispatha	50			
	Heliconia mariae	12			
	Heliconia wagneriana	8			
C. sallei	*Calathea inocephala*	1	*Heliconia irrasa* – CR	*Heliconia* sp. – CR	-
	Calathea latifolia	168		*Renealmia strobilifera*	
	Calathea lutea	4			
	Ischnosiphon pruinosus	4			
	Heliconia catheta	1			
	Heliconia latispatha	11			
	Heliconia mariae	1			
C. stevensi	*Calathea inocephala*	2	*Calathea micans* – CR	*Heliconia* sp. – CR	-
	Calathea latifolia	1			
	Ischnosiphon pruinosus	1			
C. suturalis	*Costus pulverulentus*	1	*Costus* sp. – CR	-	-
C. trimaculata	*Costus pulverulentus*	2	*Renealmia* sp. – PA	Costaceae – CR	-
C. sp61	*Bactris major*	1	-	-	-
	Chamaedorea wendlandiana	1			
	Oenocarpus panamanus	376			

Table 4. *Continued*

Hispine species	Host plant in present study	No.	McKenna[a]	Staines[b]	Strong[c]
Chelobasis bicolor	*Calathea latifolia*	1	-	*Heliconia* sp. – CR	*Heliconia curtispatha* – CR
	Heliconia catheta	1			*Heliconia imbricata* – CR
	Heliconia irrasa	1			***Heliconia irrasa*** – CR
	Heliconia latispatha	10			***Heliconia latispatha*** – PA
	Heliconia vaginalis	2			***Heliconia latispatha*** – CR
	Heliconia wagneriana	1			*Heliconia metalica* – CR
					Heliconia platystachys – PA
					Heliconia wagneriana – CR
Chelobasis perplexa	*Heliconia irrasa*	1	*Heliconia pogonantha* – CR	*Calathea insignis* – CR	*Heliconia imbricata* – CR
	Heliconia mariae	23		*Heliconia imbricata* – CR	*Heliconia latispatha* – CR
Delocrania panamensis	*Bactris major*	1	-	-	-
	Oenocarpus panamanus	20			
Homalispa nevermanni	*Oenocarpus panamanus*	659	-	-	-
Imatidium thoracicum	*Calathea lutea*	3	-	Arecaceae – CR	-
	Heliconia catheta	11		Zingiberales	
	Heliconia irrasa	1		*Calathea insignis* – CR	
	Heliconia latispatha	83			
	Heliconia wagneriana	12			
Platocthispa emorsitans	*Calathea insignis*	8	-	*Calathea* sp.	-
	Calathea latifolia	17		*Costus* sp.	
	Heliconia catheta	1			
	Heliconia irrasa	11		Piperaceae	
	Heliconia latispatha	221			
Prosopodonta dorsata	*Chamaedorea wendlandiana*	170	-	Arecaceae	-
	Cryosophila warscewiczii	24			
	Oenocarpus panamanus	287			
Spaethiella circumdata	*Heliconia irrasa*	37	-	-	-
	Heliconia latispatha	1			
	Heliconia vaginalis	2			
Spaethiella marginata	*Calathea insignis*	19	-	-	-
	Heliconia catheta	16			
	Heliconia latispatha	159			
	Heliconia mariae	9			
	Heliconia wagneriana	1			
Spaethiella tristis	*Bactris major*	117	-	-	-
	Chusquea simpliciflora	4			
	Oenocarpus panamanus	122			
Spaethiella sp47	*Chusquea simpliciflora*	20	-	-	-
Stilpnaspis panamensis	*Chamaedorea wendlandiana*	180	-	-	-
	Oenocarpus panamanus	83			

Country codes are: PA, Panama; CR, Costa Rica; CO, Colombia; EC, Ecuador; species in bold, species common to both this study and previous reports.
[1] McKenna and Farrel (2005, 2006).
[2] Staines (1996, 2002, 2006).
[3] Strong (1977a,b, 1983); Strong *et al.* (1984).

Cluster analysis shows that hispine assemblages (Fig. 2 and 3) are primarily determined by host plant affiliation (order and family) and secondarily by plant habitat. *Heliconia latispatha* and *Calathea latifolia*, which occur in similar habitats, present clearly distinct beetle assemblages (Table 3 and 4). Table 3 clarifies the cluster nodes (Fig. 2) according to the host plant species' habitats. However, the fit of some hispine species into these nodes are not clear-cut. For example, *Aslamidium semicircularum*, is an "open-leaf

scraper" species that seems to be a "family-specialist" (Barone, 1998; McKenna and Farrell, 2005 and 2006), actually occurs on many Marantaceae species (Table 3). This species does not to our knowledge feed on other monocot families, including the Arecaceae as indicated by Staines (2002). Conversely, *Cephaloleia sallei* seems to be strongly specialized on a single plant species: *Calathea latifolia*, much as *Homalispa nevermanni* occurs on only *Oenocarpus panamanus* among the four palm species we examined. If we follow the feeding concepts of Barone (1998), *Cephaloleia sallei* and *Homalispa nevermanni* could be termed "species-specialists". In our study areas, *C. sallei* is a feeding specialist on primarily one species of Zingiberales (Table 3), contrary to other accounts that suggest it is a generalist (McKenna and Farrell, 2005).

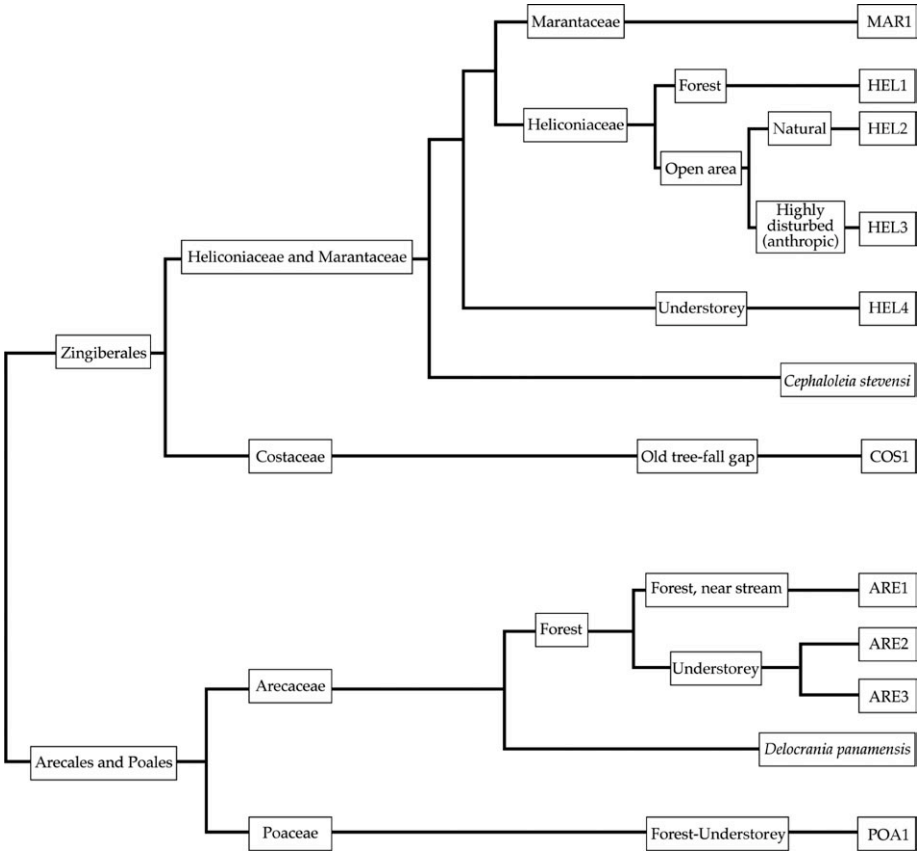

Figure 3. Synthesis of the relationships between plant family, habitat and hispoid Cassidinae assemblage composition based on cluster analysis results (average linkage method; Bray-Curtis' distance)**.** ARE1, first cluster group of Arecaceae; ARE2, second cluster group of Arecaceae; ARE3, third cluster group of Arecaceae; HEL1, first cluster group of Heliconiaceae; HEL2, second cluster group of Heliconiaceae; HEL3, third cluster group of Heliconiaceae; HEL4, fourth cluster group of Heliconiaceae; COS1, cluster group of Costaceae; MAR1, cluster group of Marantaceae; POA1, cluster group of Poaceae

Taxonomy can complicate the comparison and interpretation of results from this study and previous studies. For example, the polymorphic leaf-miner, *Platocthispa emorsitans*, is intimately associated with *Heliconia latispatha*, a common resident in shady anthropically disturbed areas such as like roadsides and power transmission line cuts. However, this hispine was also occasionally reared from *Calathea insignis, C. latifolia* and *Heliconia irrasa*. Future genetic studies will be needed to test whether individuals reared from *Calathea* and *Heliconia* mines belong to the same or different species. An

additional example is provided by *Cephaloleia vicina* Baly and *C. belti* whose morphologies are extremely similar and occur on the same species of Heliconiaceae in different areas. *Cephaloleia belti* occurred in this study primarily on Heliconiaceae but also in smaller numbers on Marantaceae. According to a preliminary molecular phylogenetic analysis (Descampe and Meskens unpubl. data), the *C. belti* individuals collected in this study appear to belong to a single species. Another taxonomic problem is presented by the genus *Nympharescus* Weise, which Strong (1982) records from the northern Panama Canal area, but both Blackwelder (1946) and Staines (2002) indicate is absent from Central America but present from Colombia to Peru. It now appears that this species, which was recorded as a common resident in *Heliconia mariae* scrolls, is a morphological variant of *Chelobasis perplexa*, which closely resembles some *Nympharescus* species.

Based on occurrence data presented in this study and feeding trials (Descampe et al., 2007, in press; Meskens unpubl. data), we conclude that most hispines feed on more than one related plant species, often on several genera and families but rarely cross to feed on different orders of monocot plants. The feeding flexibility, which we have documented, is best exemplified by *C. belti* which occurred in the leaf scrolls of 8 species, 3 genera and two families of Zingiberales.

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Chapter 2.

« *From potential to realized feeding niches: feeding flexibility of Neotropical hispine beetles (Chrysomelidae; Cassidinae)* »

Context

Indirect feeding observations, such as *occurrence* data previously collected (chapter 1, Core section) may lead to obtain a plant-hispine matrix with uncertain plant-beetle associations, such as “tourists”. More than allowing to exclude uncertain field records of associations, feeding trials permit to define the potential and realized feeding niches of hispine species. The realized feeding niche is an exhaustive list of plant species on which the beetles feed in the field. The potential, also called fundamental feeding niche is “the manifestation of species genotype within the environment (Ferry-Graham *et al*., 2002)”.

In the second chapter of this thesis, we performed two types of feeding trials (choice and no-choice tests) on 15 hispine species (mainly rolled-leaf feeders). Only adults were tested, which did not allow us to define larval host range, and to verify a complete development cycle.

Results of both tests permit to define locally nonbiased host range (and its taxonomic level) for hispine species and are essential to better understand plant-herbivore evolutionary relationships.

Plant-Insect Interactions

Potential and Realized Feeding Niches of Neotropical Hispine Beetles (Chrysomelidae: Cassidinae, Cephaloleiini)

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Abstract. Accurate descriptions of feeding habits are essential to understanding the evolution of dietary preferences and the high levels of diversification within the Chrysomelidae. Both primary observations and summaries suggest that the cassidine beetle tribe, Cephaloleiini, is a species-rich group of feeding specialists on monocot hosts. However, accurate host ranges are poorly defined for most hispine beetle species. To better document occurrence and feeding, we censused the Cephaloleiini associated with rolled leaves of five species of Marantaceae and six species of Heliconiaceae (Zingiberales) in lowland Central Panama. Additionally, we

conducted choice and no-choice feeding tests on a subset of both the plants and beetles encountered in the censuses. Both types of data suggest that most species of Cephaloleiini feed on a greater variety of related plant species than has previously been reported.

Keywords**:** *Hispinae, Cassidinae, Zingiberales, feeding niches, trophic selection*

Introduction

Summaries of tropical insect feeding based on field observations generally conclude that most species are restricted to a narrow set of host plants (Erwin 1982, Barone 1998, Odegaard 2000). However, more recently, Novotny et al. (2002) have reported that many tropical insects are less specialized than was previously thought. There are several reasons to believe that field observations of insect feeding associations, which ressect the realized feeding niche (Futuyma and Moreno 1988), could be biased toward specialization. First, the geographic range of a particular insect species can be quite broad, and within this range, the composition of plant communities may change dramatically. As a consequence, the list of host plants an insect species actually uses across its entire range may not be adequately sampled by regionally restricted field observation, leading to an underestimation of the actual number of host plants used by a particular insect. This will be a more serious bias for species with specific associations that occur in only certain regions and not others. Second, factors such as plant habitat, plant structure, and predator and parasitoid pressures may constrain diet to a subset of plant species on which the insect is capable of feeding and developing successfully (Fox and Morrow 1981). Third, insects visit plants not only to feed, but also to rest or find mates, and if such activities are not separated from feeding, they may tend to skew host plant lists. For all these reasons, simple field observations and records may not give an accurate indication of the diet range of an herbivorous insect and mass records are essential to increase the probability of obtaining the most complete description of host plant-insects associations.

In the unrealistic case of a completely exhaustive and nonbiased list of plant-phytophagous associations, we would observe what is called the realized feeding niche (Futuyma and Moreno 1988). Thus, the results of choice and no-choice tests by presenting potential food items may provide a method to estimate feeding flexibility of insects without the biases inherent in the field observations. Moreover, because the insects are not exposed to extrinsic

factors that can narrow their choice, their feeding response can thus be associated to the fundamental feeding niche, which is more the result of genetic properties of the species (Futuyma and Moreno 1988). Nonbiased dietary information and comparison of realized and fundamental feeding niches are essential to accurately interpret the nature of plant-herbivore evolutionary relationships.

Our knowledge of trophic selection in the “rolled-leaf” hispine beetles (also called “hispoid Cassidinae”; principally the tribe, Cephaloleiini), is based on field observations and collecting notes made by investigators with differing priorities and access to well identified botanical and entomological collections (Strong 1977, Maes and Staines 1991, Windsor et al. 1992, Staines 1996, 2004, Flowers and Janzen 1997, Hespenheide and Dang 1999, McKenna and Farrell 2005). Most of these studies conclude that species of hispoid Cassidinae feed within a single family of host plants and that nearly all used plant families fall within the Monocotyledonae (Jolivet 1997, Jolivet and Hawkeswood 1995). Furthermore, the list of hispine species used by McKenna and Farrell (2005) indicates that the majority of Cephaloleiini are associates of Zingiberales, whereas a smaller number of species at the base of the phylogeny is associated with Arecaceae. The extent to which Cephaloleiini species feed on related plant species within or even among those families is less well documented.

Our aim was to better define the range of host plants accepted by cassidine leaf beetle species in the tribe Cephaloleiini, by recording their presence on monocot hosts, and by conducting feeding tests in the laboratory. The tribe Cephaloleiini contains >380 Neotropical species placed in 18 genera, which are best differentiated from other Cassidinae by their flat “onisciform” larval stage (Seeno and Wilcox 1982, Jolivet and Hawkeswood 1995, Staines 2002). The majority are considered “rolled-leaf hispines” and are found in tightly rolled apical leaves of monocots. Adults and juvenile forms of some taxa are principally found in leaf axils of palms (e.g., *Cephaloleia lata* on *Chamaedorea* spp.) or are surface feeders on the fully expanded leaves of

some *Calathea* species (Marantaceae) and the newest expanded, nongreened leaves of several palm species (e.g., *Demotispa* sp. on *Oenocarpus mapori*). The host plants of many Cephaloleiini species remain poorly documented because they have only been identified to genus or family, and fertile vouchers have rarely been deposited (Flowers and Janzen 1997).

Materials and methods

Field sampling

Observations were made within forests bordering the Panama Canal area (Fig. 1) including a site near Gamboa (Pipeline Road) in Parque Natural Soberania (9°7' N, 79°43' W), along the trail system of the field station of the Smithsonian Tropical Research Institute (STRI) on Barro Colorado Island (BCI; 9°9' N, 79°51'W) and within Parque Nacional San Lorenzo (9°17' N, 79°58' W). Sampling was performed during December 2001, from May to September 2002, from March to June 2003, and from August to September 2003.

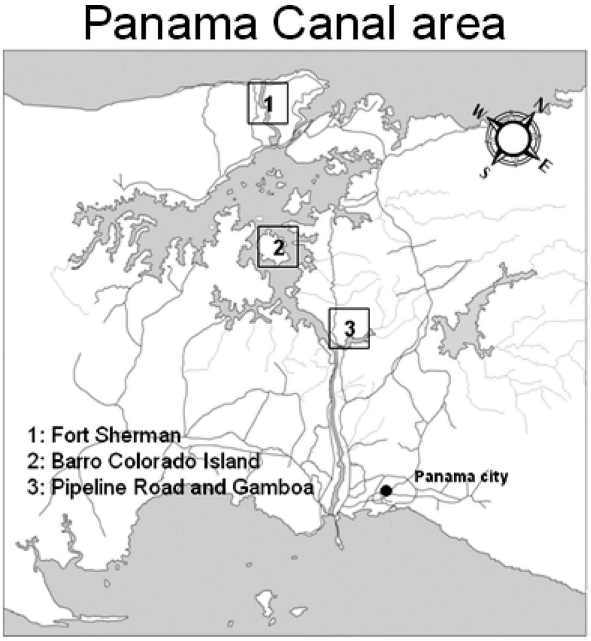

Figure 1. Sampling sites of hispine beetles in areas bordering the Panama Canal area.

Our study is based on observations recorded from Heliconiaceae and Marantaceae (Zingiberales) families containing 8 and 11 species, respectively, in the lowland areas of the Isthmus (Croat 1978). We surveyed hispoid Cassidinae on six Heliconiaceae and five Marantaceae species, chosen because of their greater abundance in the study sites (Croat 1978, D.W. and C.M., unpublished data) and because they were known to have a diversified hispoid Cassidinae fauna (Strong 1977, Staines 2004). Native to the tropical regions (Croat 1978), the Marantaceae and Heliconiaceae are characterized by large and colorful bracts and by young leaves rolled into long tubes opening after periods from a few days to a few weeks into large mature leaves with transverse venation and long petioles (Williams and Harborne 1977, Berry and Kress 1991). The scroll-like immature leaves are the typical habitat for numerous species of “rolled-leaf” Cassidinae (Strong 1977, Jolivet 1997, McKenna and Farrell 2005). These two host plant families occur in a range of habitats from old forest to highly disturbed areas (Croat 1978, Berry and Kress 1991). All of the host plant species are native

to Panama (Croat 1978), but most occur elsewhere in Central and South America as well.

For each individual plant that was surveyed, a single mature or rolled leaf was carefully inspected, the number and species of adult hispine beetles were recorded, and the beetles were returned to the plant. Beetles were collected without replacement only when their identification was in doubt. Plants occurring along trails were surveyed inside young (60-120 yr old) and old (400 yr old) forest on BCI and in mainland forests (Parque Soberania and Fort Sherman). Hispine species were identified based on the plates in the Biologia Centrali-Americana (Baly 1885); descriptions and key given by Staines (1996 and 2002) and by comparison with identified specimens in the STRI insect collection.

During the sampling periods, sites were partially visited once a week. Because leaf-rolls had to be destructively removed from plants, each individual plant marked with flagging tape was surveyed only once in a field season to minimize disturbance to beetle assemblages.

The scroll-like immature leaves (rolled leaves) were only included in the study if they were closed at the bottom (watertight), thereby maintaining a high degree of humidity, which seems to be an important factor for “rolled-leaf” hispoid Cassidinae communities (Strong 1977).

Feeding tests

The seven most frequent Cephaloleiini species in this study were *Cephaloleia belti* Baly, *C. sallei* Baly, *C. dilaticollis* Baly, *C. erichsonii* Baly, *C. instabilis* Baly, *C. pretiosa* Baly, and *Aslamidium semicircularum* Borowiec. Larvae of six of these species fed within the unexpanded leaves of Zingiberales, mainly on Heliconiaceae and Marantaceae (Strong 1977). The seventh species, *Aslamidium semicircularum,* an open leaf scraper, fed on open mature leaves of various species of Marantaceae. Eight plant

species were selected for the feeding trials, each belonging to the Heliconiaceae (*Heliconia latispatha* Bentham, *H. mariae* Hooker, *H. vaginalis* Bentham, *H. irrasa* R.R. Smith, *H. catheta* R.R. Smith) or the Marantaceae (*Calathea latifolia* Klotzsch, *C. inocephala* H. Kenn. and Nicolson, and *Ischnosiphon pruinosus* Petersen). These species are known to shelter some species of Cephaloleiini (Strong 1977) and were the most abundant species of Marantaceae and Heliconiaceae in the study area.

Adult insects and the fresh plant leaves used during feeding trials were collected daily near the town of Gamboa. Individuals were maintained in the laboratory, at 24°C, on the host plant on which they were initially collected within 3-liter plastic bags. Adult beetles were placed in a petri dish containing a moistened filter paper and two circular leaf discs (one facing upward and the other downward) of each of the plant species in the choice test. A cleaned, 12-mmdiameter cork borer was used to punch disks from the outer margin of the internal side of rolled leaves or from mature leaves, according to hispine species feeding habits. For no-choice tests, the same procedures were followed but with only one plant species per petri dish (two disks from open leaves and two from rolled leaves, one disk facing upward, the other downward). After 24 h without food, beetles were introduced and allowed to feed for 24 h. After the tests, the area (mm2) of the disk consumed was quantified using Scion Image software after picture transformation (Adobe Photoshop 5.0 LE) in binary color. Consumption was measured by calculating the eaten area of leaf disk such as suggested by Peterson and Renaud (1989), which minimizes biases caused by autogenic changes during the test.

A qualitative feeding response, accepted or rejected, was scored for each disk. When only bitten once, disks were considered rejected because this slight damage results from probing or tasting behavior, which is the first step of the trophic-selection process (Schoonhoven 1991). Each test was replicated at least 10 times with naïve beetles.

Statistical analysis

Quantitative Analyses. Statistical analyses were performed using SAS Enterprise Guide v 8.02. Mean areas consumed by each hispine species were tested for homogeneity of variance with Levene's test before performing analysis of variance (ANOVA). Welch's correction was used in cases of non homogeneity.

Because of the design of choice tests, the factor *hispine individual* must be taken into account. Because individual physiological state could influence the feeding response differentially between replications, the factor *hispine individual* was crossed to *consumed plant species* thus making a two-way ANOVA. Although there was only one replicate for each level of the factor *hispine individual,* this has little impact because we were only interested in testing the significance of the *consumed plant species* factor.

No-choice tests were analyzed by one-way ANOVA, with *consumed plant species* as fixed factors.

Qualitative Analyses. χ^2 tests (Yates correction for 1 df) were used in qualitative analyses where responses were limited to plant accepted or plant rejected.

Voucher specimens were deposited in the insect collection of the Unité d'Écologie et de Biogéographie of the Université catholique de Louvain (Belgium) and in the voucher collections of the Smithsonian Tropical Research Institute in Panama.

Results

Field sampling

A total of 2,045 individuals belonging to 19 hispine species were found on 2,399 surveyed plants (Table 1). The more abundant hispine species were *Cephaloleia belti, Aslamidium semicircularum, C. erichsonii,* and *C. sallei. C. belti* and *C. erichsonii* were each found on 9 of the 11 plants species in the study, whereas *C. dilaticollis, C. pretiosa,* and *C. instabilis* were found on 4 plant species. *A. semicircularum* and *C. dilaticollis* were mainly present on species of Marantaceae, *C. pretiosa,* and *C. instabilis* on Heliconiaceae and *C. erichsonii, C. belti,* and *C. sallei* on both families, but not always on all species of each plant family.

Table 1. Field sampling results of hispine beetles on plants of Marantaceae and Heliconiaceae

Hispoid Cassidinae species	Plant species	Number of inspected plants[#]	Number of hispine adults found
Aslamidium semicircularum	*Calathea inocephala*	726	68
	C. insignis	182	149
	C. latifolia	286	22
	C. lutea	33	101
	Ischnosiphon	225	75
Cephaloleia belti Baly	*C. latifolia*	123	42
	C. lutea	3	2
	I. pruinosus	22	2
	Heliconia catheta	37	121
	H. irrasa	27	5
	H. latispatha	103	289
	H. mariae	74	85
	H. vaginalis	25	2
	H. wagneriana	12	34
C. dilaticollis Baly	*C. inocephala*	105	103
	C. insignis	5	4
	C. lutea	3	2
	I. pruinosus	22	1
C. erichsonii Baly	*C. inocephala*	105	220
	C. insignis	5	1
C. erichsonii Baly	*H. catheta*	37	1
	H. latispatha	103	5
	H. mariae	74	70
	H. vaginalis	25	2
	H. wagneriana	12	1
	C. latifolia	123	5
C. instabilis Baly	*H. catheta*	37	8
	H. latispatha	103	44
	H. mariae	74	2
C. pretiosa Baly	*H. catheta*	37	26
	H. latispatha	103	53
	H. mariae	74	12
	H. wagneriana	12	9
C. sallei Baly	*C. inocephala*	105	1
	C. latifolia	123	200
	C. lutea	3	4
	I. pruinosus	22	7
	H. catheta	37	2
	H. latispatha	103	12
	H. mariae	74	1
	H. vaginalis	25	1

[#] The no. plant species observed corresponds to the no. of inspected plants with rolled leaves for rolled leaf hispines and to the no. plants with only open leaves for *A. semicircularum,* which is an open leaf scraper.

Feeding trials

Choice tests. Definite feeding responses (more than one bite) were recorded for all beetle species except *A. semicircularum,* which responded weakly to all plant species. The maximum percentage of individuals of a particular species feeding on a host plant species was 73% (*C. instabilis* on *H.*

latispatha) during choice tests.

The average area of leaf disks eaten did not vary significantly among plant species for six of the seven beetle species tested (Table 2). Only *C. instabilis* consumed significantly different areas of leaf (F = 3.36; df = 5,50; P = 0.01), preferring the leaf disks of *H. latispatha* and *H. irrasa* above the other six plant species. Although heterogeneity in *C. pretiosa* feeding responses was not significant, average acceptance of Heliconiaceae was higher than Marantaceae (χ^2 = 14.83; P < 0.0001). Moreover, Marantaceae were preferred by *C. dilaticollis,* which did not feed on Heliconiaceae. There was no significant difference in the average acceptance of *C. belti, C. sallei, C. instabilis,* and *C. erichsonii* on Marantaceae and Heliconiaceae.

Table 2. Results of choice tests

		Calathea inocephala	*C.latifolia*	*Ischnosiphon pruinosus*	*Heliconia catheta*	*H.irrasa*	*H.latispatha*	*H.mariae*	*H.vaginalis*	n[§]	P-val[£]	Favourite family[$]
Aslamidium semicircularum	mm^2 ± sd	0.09 ± 0.3	0.30 ± 0.8	0	0	0	0	0	0	10	0.23 NS	M
	eaten disks[#]	1	2	0	0	0	0	0	0			
Cephaloleia belti	mm^2 ± sd	20.28 ± 33.3	4.79 ± 7.2	0	0.66 ± 1.3	13.49 ± 15.0	13.80 ± 21.4	2.69 ± 4.0	4.63 ± 7.5	10	0.13 NS	M+H
	eaten disks[#]	3	4	0	3	7	5	4	3			
C. dilaticollis	mm^2 ± sd	19.63 ± 36.9	8.77 ± 22.6	5.42 ± 16.4	0	0	0	0	0	14	0.21 NS	M
	eaten disks[#]	8	2	3	0	0	0	0	0			
C. erichsonii	mm^2 ± sd	28.75 ± 51.9	9.35 ± 22.0	0	2.21 ± 6.0	0	0	33.28 ± 48.8	1.97 ± 5.0	12	0.07 NS	M+H
	eaten disks[#]	5	2	0	2	0	0	5	2			
C. instabilis	mm^2 ± sd	0.24 ± 0.8	6.95 ± 15.8	0	1.94 ± 5.0	13.28 ± 16.6	18.91 ± 17.6	0	4.90 ± 9.8	11	0.01 **	M+H
	eaten disks[#]	1	5	0	3	6	8	0	5			
C. pretiosa	mm^2 ± sd	0.81 ± 2.5	0	0.47 ± 1.5	4.41 ± 5.0	*10.14 ± 14.0*	7.07 ± 10.4	10.14 ± 13.5	10.09 ± 17.7	10	0.10 NS	H
	eaten disks[#]	2	0	1	7	6	5	6	4			
C. sallei	mm^2 ± sd	1.79 ± 5.0	19.24 ± 39.8	4.36 ± 9.4	0	0	0.22 ± 0.7	9.88 ± 17.0	17.42 ± 28.1	11	0.18 NS	M+H
	eaten disks[#]	2	6	3	0	0	1	4	5			

The mean quantitative (mm^2 ± SD) and qualitative consumption (eaten or not) of each hispine species on each plant species tested using choice test (ANOVA II, consumed plant species and individual as factors). #: Number of eaten disks represents qualitative answers; £: calculated for consumed plant species factor; §: n = number of replication; $: M = Marantaceae, H = Heliconiaceae; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant

No-choice tests. The performance of three hispine species was evaluated in no-choice tests, *A. semicircularum, C. belti,* and *C. sallei,* and each accepted a slightly broader array of food items than in choice tests (Table 3). *A. semicircularum* included *Ischnosiphon pruinosus* in its diet under no-choice conditions. Similarly, *C. sallei* fed on *H. catheta* and *H. irrasa,* and *C. belti* on *I. pruinosus* only during no-choice tests. The maximum percentage of individuals of a particular hispine species feeding on a host plant species was 80% for *C. sallei* on *C. inocephala.*

There were significant differences among plants in the mean areas *C. sallei* consumed under no-choice tests (ANOVA, $F = 2.21$; df = 7,76; $P = 0.04$). Neither *A. semicircularum* nor *C. belti* preferred any particular plant species ($F = 0.96$ and $F = 2.20$; df = 3,36 and df = 7,81; $P = 0.42$ and $P = 0.06$, respectively). No-choice tests also revealed that *C. sallei* consumed Marantaceae more often than Heliconiaceae ($\chi^2 = 4.56$; $P = 0.03$). Similar results were seen with *A. semicircularum* which gave only one positive response on Heliconiaceae. In contrast, *C. belti* consumed equally the two plant families ($\chi^2 = 1.84$; $P = 0.17$).

Table 3. Results of no-choice tests

	Calathea inocephala	*C.latifolia*	*Ischnosiphon pruinosus*	*Heliconia catheta*	*H.irrasa*	*H.latispatha*	*H.mariae*	*H.vaginalis*	P-val[£]	Favourite family[$]
Aslamidium semicircularum	1.03 ± 2.6	0.60 ± 2.0	0.64 ± 2.1	0	0	0	0	0.027 ± 0.1	0.40 NS	M
Cephaloleia belti	10.15 ± 32.9	1.48 ± 6.8	0.30 ± 1.0	5.15 ± 17.6	3.66 ± 7.6	11.60 ± 32.4	3.43 ± 8.1	18.47 ± 45.7	0.11 NS	M+H
C. sallei	9.26 ± 13.0	18.60 ± 31.6	1.80 ± 3.4	0.09 ± 0.3	1.00 ± 3.6	5.00 ± 20.6	12.39 ± 28.5	3.25 ± 7.1	0.01 **	M

The mean quantitative (mm^2 ± SD) and qualitative measures of the amount of leaf area consumed by each hispine species when given leaf disks of a single plant species (ANOVA II, consumed plant species and leaf type as factors, $n = 10$). £: calculated for consumed plant species factor; $: M = Marantaceae, H = Heliconiaceae; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant

Discussion

The eight species of Cephaloleiini recorded in monocot leaf scrolls in Panama were associated with from 4 to 9 of the 11 plant species included in this study. Furthermore, three of the eight beetle species were recovered from hosts in both of the families (Zingiberales) included in this study. Both of these observations suggest that the list of hosts used by Cephaloleiini is larger than has typically been reported in the literature and may well be even larger if all Heliconiaceae and Marantaceae occurring regionally had been included. Earlier reviews of occurrence data focusing on Heliconiaceae feeding beetles have generally concluded that Cephaloleiini are associated with a small number of host plant species within a single family (Staines 2004). By including host plants from more than one family, we are able to see that some Cephaloleiini species include numerous related host plant species in their diet and that hosts may come from more than one plant family (Table 4).

Although previous reports indicated *Cephaloleia belti* occurs on five *Heliconia* species, we found it associated with 9 of the 11 species in the survey and in our choice experiments. Morrison and Strong (1981) have suggested that hispines could be strongly specialized explaining that "*C. consanguinea* (*Hispinae),* [which] *is a monophagous herbivore of H. imbricata* (*Zingiberales: Heliconiaceae) in wet lowland of eastern Costa Rica."* Later, Staines (2004) redescribed *C. consanguinea* as *C. belti,* which is one of the least host specific species in lowland Panamanian forest. Another example is *C. erichsonii,* which is reported from four plant species (Staines 2004), whereas we found it on nine host plant species.

The preferences expressed during the feeding tests (Tables 2 and 3) by *A. semicircularum* and *C. dilaticollis* for Marantaceae and by *C. pretiosa* for Heliconiaceae correspond to "first-degree oligophagy" as defined by Jolivet (1998). The absence of obvious specific preferences corresponds to Jolivet's

"second-degree oligophagy." We found no indication of monophagy among any of these nine beetle species. The results of choice and no-choice feeding tests at the plant family level corresponded generally to preferences observed in the field. However, there were greater differences in the abundances of hispines observed on different host plants in the field. *C. erichsonii* was mainly found on *C. inocephala* and *H. mariae, C. sallei* on *C. latifolia,* and *C. instabilis* on *H. latispatha,* whereas other hispine species were more equally distributed among plant species (Table 1). However, choice tests revealed no such preferences. Defining the host range of hispines requires a large and time consuming survey, which may nevertheless underestimate host ranges as shown in Table 4. The review of Cassidinae associations with Zingiberales by Staines (2004) seems to considerably underestimate actual numbers of host plant species used by hispine beetles as indicated by our studies in Panama. Furthermore, feeding tests as a complementary tool may provide an even better indication of the range of acceptable hosts. Indeed, specialization can vary spatially and temporally in nature as a function of food availability and other extrinsic factors such as predators and parasitoids pressure (Fox and Morrow 1981). The larger diets recorded in no-choice tests, where tested beetles fed on one more plant species than in choice tests, is a good example. As suggested by Barone (1998), a broader range of feeding in no-choice tests could be the consequence of starvation, where individuals do not have the possibility to focus on preferred rather than secondary host plants (Zwölfer and Harris 1971). Essentially, this is analogous to a different habitat with different host-species composition, which would also affect insect choice. In this way, feeding tests can be useful in defining the potential or fundamental feeding niche, whereas field observations correspond to the realized niche (Futuyma and Moreno 1988).

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Supplementary data

Additionally to the results presented in chapter 2 (Core section), we performed in 2006, feeding trials focused on hispine species mainly associated with Arecales (Meskens *et al*., 2008). The experimental and analysis procedures were similar to those described in Descampe *et al*. (2008). There are two procedure differences: (i) during the tests, hispine individuals were maintained in outside conditions and not in laboratory conditions (24°C), and (ii) the constant replication number for each tested species (see N in Table 1 & 2). In total, we tested 15 hispine species. The entire set of results for all hispine species are detailed in Table 1 for choice tests and in Table 2 for no-choice tests.

Table 1: choice test results (ANOVA II)

Hispine species tested	Host plant species	Host plant species consumed	Average surface consumed (cm^2)	N	Pval	Significant degree	F	DF
As.sem	Ca.ino	Ca.ino	0,009	10	0,2268	NS	1,68	1
	Ca.lat	Ca.lat	0,03	10				
	Is.pru							
	He.cat							
	He.irr							
	He.lat							
	He.mar							
	He.vag							
Ce.bel	Ca.ino	Ca.ino	0,2028	10	0,1253	NS	1,76	6
	Ca.lat	Ca.lat	0,0479	10				
	Is.pru	He.cat	0,0066	10				
	He.cat	He.irr	0,1349	10				
	He.irr	He.lat	0,138	10				
	He.lat	He.mar	0,0269	10				
	He.mar	He.vag	0,0463	10				
	He.vag							
Ce.dil	Ca.ino	Ca.ino	0,1963	14	0,2072	NS	1,67	2
	Ca.lat	Ca.lat	0,0877	14				
	Is.pru	Is.pru	0,0542	14				
	He.cat							
	He.irr							
	He.lat							
	He.mar							
	He.vag							

Hispine species tested	Host plant species	Host plant species consumed	Average surface consumed (cm^2)	N	Pval	Significant degree	F	DF
	Ca.lat	Ca.lat	0,0935	10				
	Is.pru	He.cat	0,0221	10				
	He.cat	He.mar	0,3328	10				
	He.irr	He.vag	0,0197	10				
	He.lat							
	He.mar							
	He.vag							
Ce.ins	Ca.ino	Ca.ino	0,0024	11	0,0108	**	3,36	5
	Ca.lat	Ca.lat	0,0695	11				
	Is.pru	He.cat	0,0194	11				
	He.cat	He.irr	0,1328	11				
	He.irr	He.lat	0,1891	11				
	He.lat	He.vag	0,049	11				
	He.mar							
	He.vag							
Ce.pre	Ca.ino	Ca.ino	0,0081	10	0,1016	NS	1,88	6
	Ca.lat	Is.pru	0,0047	10				
	Is.pru	He.cat	0,0441	10				
	He.cat	He.irr	0,1014	10				
	He.irr	He.lat	0,0707	10				
	He.lat	He.mar	0,1014	10				
	He.mar	He.vag	0,1009	10				
	He.vag							
Ce.sal	Ca.ino	Ca.ino	0,0179	11	0,1811	NS	1,59	5
	Ca.lat	Ca.lat	0,1924	11				
	Is.pru	Is.pru	0,0436	11				
	He.cat	He.lat	0,0022	11				
	He.irr	He.mar	0,0988	11				
	He.lat	He.vag	0,1742	11				
	He.mar							
	He.vag							
Ca.ant*	Ba.maj	Ch.wen	0,0322	10	0,0679	NS	3,14	2
	Ch.wen	El.ole	0,0062	10				
	Cr.war	Oe.pan	0,011	10				
	El.ole							
	Ge.cun							
	Oe.pan							
Ce.lat*	Ba.maj	Ch.wen	0,1575	10	_	_	_	_
	Ch.wen							
	Cr.war							
	El.ole							
	Ge.cun							

Hispine species tested	Host plant species	Host plant species consumed	Average surface consumed (cm^2)	N	Pval	Significant degree	F	DF
	Oe.pan							
Ce.sp61*	Ba.maj	El.ole	0,0004	10	0,2354	NS	1,57	2
	Ch.wen	Ge.cun	0,0021	10				
	Cr.war	Oe.pan	0,0121	10				
	El.ole							
	Ge.cun							
	Oe.pan							
Pr.dor*	Ba.maj	Ch.wen	0,13	10	0,0016	**	9,41	2
	Ch.wen	El.ole	0,0138	10				
	Cr.war	Ge.cun	0,0099	10				
	El.ole							
	Ge.cun							
	Oe.pan							
St.pan*	Ba.maj	Ch.wen	0,0279	10	_	_	_	_
	Ch.wen							
	Cr.war							
	El.ole							
	Ge.cun							
	Oe.pan							
Ca.bre*	Ba.maj	Ch.wen	0,01	10	_	_	_	_
	Ch.wen							
	Cr.war							
	El.ole							
	Ge.cun							
	Oe.pan							
Sp.tri*	Ba.maj	Ba.maj	0,01	10	0,4143	NS	0,73	1
	Ch.wen	Oe.pan	0,0054	10				
	Cr.war							
	El.ole							
	Ge.cun							
	Oe.pan							
Im.tho*	Ba.maj	Ca.lat	0,009	10	0,9519	NS	0,001	1
	Ch.wen	He.lat	0,01	10				
	Cr.war							
	El.ole							
	Ge.cun							
	Oe.pan							
	Ca.ino							
	Ca.lat							
	He.lat							

See chapter 1 (Core section) for hispine and host plant species codes (Ge.cun: *Geonoma cuneata* H.Wendl., Palmae); ˣ: additional species to Descampe *et al.* (2008); NS: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; _: not applicable

Table 2: no-choice test results (ANOVA I)

Hispine species tested	Host plant species	Host plant species consumed	Average surface consumed (cm^2)	N	Pval	Significant degree	F	DF	Levene's Test
Ca.ant*	Ba.maj	Ch.wen	0,0073	20	0,0607	NS	2,94	2	NS
	Ch.wen	Ge.cun	0,0024	20					
	Cr.war	Oe.pan	0,0004	20					
	El.ole								
	Ge.cun								
	Oe.pan								
Ce.lat*	Ba.maj	Ch.wen	0,1283	20	_	_	_	_	_
	Ch.wen								
	Cr.war								
	El.ole								
	Ge.cun								
	Oe.pan								
Ce.sp61*	Ba.maj	Cr.war	0,0012	20	0,2698	NS	1,33	3	NS
	Ch.wen	El.ole	0,0006	20					
	Cr.war	Ge.cun	0,0021	20					
	El.ole	Oe.pan	0,0043	20					
	Ge.cun								
	Oe.pan								
Pr.dor*	Ba.maj	Ba.maj	0,0102	20	0,0004	***	4,99	5	0,0058
	Ch.wen	Ch.wen	0,215	20					
	Cr.war	Cr.war	0,0002	20					
	El.ole	El.ole	0,073	20					
	Ge.cun	Ge.cun	0,081	20					
	Oe.pan	Oe.pan	0,0789	20					
St.pan*	Ba.maj	Ch.wen	0,0421	20	_	_	_	_	_
	Ch.wen								
	Cr.war								
	El.ole								
	Ge.cun								
	Oe.pan								
Ca.bre*	Ba.maj	Ch.wen	0,0432	20	0,0015	**	7,32	2	0,0013
	Ch.wen	Cr.war	0,0008	20					
	Cr.war	El.ole	0,0048	20					
	El.ole								
	Ge.cun								
	Oe.pan								
Sp.tri*	Ba.maj	Ba.maj	0,0067	20	0,5949	NS	0,29	1	NS
	Ch.wen	Oe.pan	0,0098	20					
	Cr.war								
	El.ole								

Hispine species tested	Host plant species	Host plant species consumed	Average surface consumed (cm^2)	N	Pval	Significant degree	F	DF	Levene's Test
	Oe.pan								
Im.tho*	Ba.maj	Ca.lat	0,009	20	0,8936	NS	0,02	1	NS
	Ch.wen	He.lat	0,01	20					
	Cr.war								
	El.ole								
	Ge.cun								
	Oe.pan								
	Ca.ino								
	Ca.lat								
	He.lat								
As.sem	Ca.ino	Ca.ino	0,0103	10	0,6843	NS	0,51	3	NS
	Ca.lat	Ca.lat	0,006	10					
	Is.pru	Is.pru	0,0064	10					
	He.cat	He.vag	0,0003	10					
	He.irr								
	He.lat								
	He.mar								
	He.vag								
Ce.bel	Ca.ino	Ca.ino	0,1015	10	0,0056	**	9,71	7	0,0231
	Ca.lat	Ca.lat	0,0148	10					
	Is.pru	Is.pru	0,003	10					
	He.cat	He.cat	0,0515	10					
	He.irr	He.irr	0,0366	10					
	He.lat	He.lat	0,116	10					
	He.mar	He.mar	0,0343	10					
	He.vag	He.vag	0,1847	10					
Ce.sal	Ca.ino	Ca.ino	0,0926	10	0,0763	NS	2,11	7	NS
	Ca.lat	Ca.lat	0,186	10					
	Is.pru	Is.pru	0,018	10					
	He.cat	He.cat	0,0009	10					
	He.irr	He.irr	0,01	10					
	He.lat	He.lat	0,05	10					
	He.mar	He.mar	0,1239	10					
	He.vag	He.vag	0,0325	10					

See chapter 1 (Core section) for hispine and host plant species codes (Ge.cun: *Geonoma cuneata* H.Wendl., Palmae); *: additional species to Descampe *et al.* (2008); NS: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; _: not applicable

Chapter 3.

« *Host plant taxonomy and phenotype influence the structure of a Neotropical hispine beetle-host plant food web* »

Context

Coevolution is one of the major processes that generate species richness and interaction diversity within communities. To understand coevolution, a central question in ecology is to define community structure. Interactions among plant and phytophagous insects can be viewed as an ecological network (or food web). Several factors may influence the food web structure, such as spatial distribution of hosts (and habitats; shade-tolerant vs. gap-dependent host species), host specificity (and its taxonomic level). The recently developed tool, the food web analysis, allows us to characterize the structure of interactions among Neotropical hispine beetles and their monocot host plants.

In this paper, we applied on field collected plant-hispine *occurrence* data, both quantitative and qualitative approaches (using several descriptors and indices) to depict this food web. We identified the structure as modular (or compartmented) and not significantly nested, and the absence of hispine niche overlap. These results are essential to understand the feeding clades found by McKenna and Farrell (2005) within the phylogeny of rolled-leaf hispine genus *Cephaloleia*.

Host plant taxonomy and phenotype influence the structure of a Neotropical host plant-hispine beetle food web

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Abstract

1. Past studies of plant-phytophage communities have encountered both nested and modular/compartmented food web structures revealing potentially important differences in the nature of interactions.

2. We analyzed qualitatively and quantitatively a taxonomically resolved plant-hispine beetle food web from Panama (in lower Central America) to characterize its structure. The study included all beetles in the subfamily Cassidinae (Chrysomelidae) found feeding on monocotyledonous plants common in the Tropical forest understory and edge habitats.

3. Nestedness, a structural property common to many food webs, was not observed in this study, however we found strong evidence for modularity.

4. Our findings suggest no niche overlap and that module boundaries in the hispine beetle food web are defined by plant ordinal and familial limits, and by certain key plant attributes, such as young leaf types.

5. We conclude that modularity in this food web is largely a product of narrow, discrete hispine beetle host specificity for larger phylogenetically related plant taxa.

Keywords: *food web, nestedness, modularity, modular structure, niche overlap, Chrysomelidae, hispine beetles, host specificity*

Introduction

Plants and the phytophagous insects that consume them comprise approximately 40% of global biological diversity (Price, 2002). Insects have a long history of intimate, specialized and often deeply coevolved trophic interactions with plants (Labandeira *et al.*, 1994; Wilf *et al.*, 2000; Farrell & Sequeira, 2004; McKenna *et al.,* 2009). We know from contemporary observations that these interactions are rarely random. Instead, insect feeding is usually restricted to plants within the same family, genus, or species (Strong *et al.*, 1984; Novotny *et al.*, 2002), and is often mediated by complex ecological, biochemical, and historical factors (Ehrlich & Raven, 1964; Gómez-Zurita *et al.*, 2007).

Documentation of the numbers and kinds of trophic interactions between insects and plants permits characterization of the antagonistic networks comprising plant-insect food webs. Food webs can be summarized and analyzed in two ways: quantitatively using relative abundance data (Tylianakis *et al.*, 2007), or qualitatively using binary matrices (presence/absence data; Lewinsohn *et al.*, 2006). Both approaches are complementary and can be used to summarize data, e.g., numbers and kinds of interactions within networks (Bersier *et al.*, 2002; Tylianakis *et al.*, 2007). Recent advances in food web complexity and structure analysis now permit the comparison of food web structure among diverse faunal assemblages (Lewis *et al.*, 2002; Bascompte et al. 2003, Lewinsohn *et al.*, 2006; Tylianakis *et al.*, 2007).

A recently developed qualitative approach to studying food webs (Atmar & Patterson, 1993) allows characterization of food web structure using the concept of nestedness (Bascompte *et al.*, 2003; Guimarães & Guimarães, 2006; Lewinsohn *et al.*, 2006; Nielsen & Bascompte, 2007). A high degree of nestedness can indicate particular ecological and evolutionary processes shaping interspecific interactions. A plant-beetle food web with a nested structure is characterized by specialist beetles that interact with host plants

(e.g., more frequently consumed) to form perfect subsets of the species with which generalist beetles interact (Bascompte & Jordano, 2007). Studies of nestedness have focused primarily on mutualistic networks (Bascompte *et al.*, 2003; Guimarães *et al.*, 2007; Rezende, 2007), while antagonistic interaction networks (food webs or trophic networks), such as those between plants and herbivores, have received less attention.

A nested structure has been reported for some antagonistic networks, including interactions between plants and phytophagous insects (e.g., Sugiura et al. 2007, Thébault & Fontaine, 2008). However, other network structures are possible. Low values of nestedness may indicate compartmented (or modular), gradient or combined network structures (Bascompte *et al.*, 2003; Lewinsohn *et al.*, 2006). A gradient structure features relatively constant numbers of beetle interactions per plant species while a combined structure features multiple nested compartments within the same community. A more complete description of these food web structures can be found in Lewinsohn *et al.* (2006). A modular structure is characterized by subsets of species (termed compartments; Prado & Lewinsohn, 2004) within which interactions are numerous but among which interactions are few. Such interactions are known to be fairly common between insects and their host plants (Prado & Lewinsohn, 2004; Lewinsohn *et al*., 2006). A recently developed qualitative metric, “modularity”, evaluates the degree to which networks are compartmented (or modular; Newman, 2004; Newman & Girvan, 2004; Guimerà & Amaral, 2005; Olesen *et al*., 2007) and estimates how clearly modules are delimited (Gómez *et al*., 2010). It has been showed that food webs (trophic networks) tend to have higher modularity (Thébault & Fontaine, 2010). These module boundaries may reflect how phytophagous insect species are specialized to their hosts especially when this specificity has a phylogenetic basis (Lewinsohn *et al*., 2006). In plant-phytophagous insect networks, the high degree of host specificity is mainly responsible for network modularity, driven by divergence in plant key attributes (Prado & Lewinsohn, 2004), such as young leaf type, leaf secondary chemistry, nutrient content or leaf physical characteristics

(Strong, 1983). Fortuna *et al*. (2010) showed that both nested and modular structures can occur simultaneously in different types of mutualistic networks and in food webs exhibiting low values of connectance.

Quantitative approach based on relative abundance matrix has been developed recently to gain ecological information on food web, and to give less weight to small proportions (Bersier *et al.*, 2002; Tylianakis *et al*., 2007). Moreover, this approach is well-known to be more conservative when comparing habitats (Tylianakis *et al*., 2007). Several weighted indices (quantitative metrics) are available to estimate the complexity and ecological impacts within food webs or other ecological networks, such as weighted generality, weighted vulnerability and interaction evenness (Blüthgen, 2010).

Here we apply qualitative structure analysis and estimate quantitative metrics to field collected host occurrence records for hispine beetles and their host plants in lowland tropical forests of Central Panama (Meskens *et al.*, 2008), to clarify patterns of food web organization, and heterogeneity in their trophic associations (Blüthgen *et al.*, 2008). The resulting data are then used to determine whether this host plant-hispine beetle food web exhibits a modular structure typical of other plant-consumer food webs (Prado and Lewinsohn, 2004; Lewinsohn *et al*., 2006). Finally, we discuss factors potentially influencing plant-hispine beetle food web structure (Bascompte *et al.*, 2003; Cattin *et al.*, 2004; Lewinsohn *et al.*, 2006; Bascompte & Jordano, 2007).

Hispine beetles (Chrysomelidae: Cassidinae) are distributed worldwide. There are approximately 3,000 described species in 24 tribes and 170 genera (Staines, 2002). While most hispine species are known to feed on multiple plant species, a tendency for their hosts to occur within the same plant order and plant family has been well-documented (Strong *et al.*, 1984; Novotny *et al.*, 2002; Descampe *et al.*, 2008; Meskens *et al.*, 2008). The hispine beetle species included in our study fall within one of three larval feeding guilds: “rolled leaf” feeders (tribes Arescini, Cephaloleiini), “open

leaf" surface scrapers (tribes Hemisphaerotini, Prosopodontini, Delocraniini) and leaf miners (tribe Chalepini) (Strong, 1977a, b, 1983; Jolivet, 1997).

Materials and methods

Panamanian hispine beetles

We performed walking transects of forested areas east of the Panama Canal along the first four km of the Camino Oleoducto (Parque Natural Soberania), along six trails on Barro Colorado Island, and along the "Monotiti" trail within the deciduous forest of Parque Natural Metropolitano. We recorded the number and identity of adult hispine beetles occurring within the rolled or partially expanded young leaves of each plant encountered. A total of 17 species of native Neotropical monocots (three orders, five families; Table 1) were sufficiently common to include in the study. Plant species were identified by reference to Croat (1978), Berry and Kress (1991), Correa *et al*. (2004) and by comparison to herbarium specimens archived at the STRI-Tupper Research and Conference Center, Panama City, Panama. Sampling procedures are further described in Meskens *et al*. (2008). The resulting food web involved 30 hispine beetle species within seven tribes (Table 2). We recognized two distinct species in the genus *Chelobasis* Gray (*C. bicolor* Gray and *C. perplexa* Baly), and we combined similar forms (*Cephaloleia belti* Baly, *C. luctuosa* Guérin, *C. vicina* Baly) (Staines, 1996) in the "*Cephaloleia betli* complex" into a single species (*C. belti*).

Table 1. Plant species characteristics and numbers of sampled individuals in Panama

Host plant order, family and species	Code	Range[a, c]	Habitat[b,c]	Size[a,c]	No.
Arecales					
Arecaceae					
Bactrtis major Jacq.	Ba.maj	Panama	Understory (forest)	8 m	172
Chamaedorea wendlandiana Hemsl.	Ch.wen	Nicaragua to Panama	Understory (forest, near streams)	Up to 5 m	358
Cryosophila warscewiczii Bartl.	Cr.war	Belize to Colombia	Understory (younger forest)	Up to 10 m	196
Oenocarpus panamanus Bailey	Oe.pan	Costa Rica and Panama	Understory (forest)	8-20 m	289
Zingiberales					
Costaceae					
Costus pulverulentus Presl	Co.pul	Mexico to Ecuador	Old tree-fall gap	0.5-3.5 m	63
Heliconiaceae					
Heliconia catheta R. R. Smith	He.cat	Panama	Tree-fall gap	3-5 m	156
H. irrasa R. R. Smith Lane	He.irr	Mexico to Colombia	Understory (forest)	1.5-2 m	242
H. latispatha Benth.	He.lat	Mexico to South America	Disturbed area	1.5-3 m	+/- 740
H. mariae Hook	He.mar	Belize to Panama	Understory (forest)	3-6 m	82
H. vaginalis Benth.	He.vag	Mexico to Ecuador	Tree-fall gap	1-3 m	125
H. wagneriana O. G. Petersen	He.wag	Honduras to Panama	Clearings	Up to 6 m	39
Marantaceae					
C. inocephala Kenn. & Nic.	Ca.ino	Mexico to Peru	Understory (forest)	2-4 m	831
C. insignis O. G. Petersen	Ca.ins	Mexico to Peru	Tree-fall gap	2-3 m	227
C. latifolia Klotzsch	Ca.lat	Venezuela to Panama	Disturbed area	1-2 m	452
C. lutea Schult.	Ca.lut	Mexico to Peru	Tree-fall gap	2-3 m	51
Ischnosiphon pruinosus O. G. Petersen	Is.pru	Belize to Panama	Tree-fall gap	2-3 m	247
Poales					
Poaceae					
Chusquea simpliciflora Munro	Ch.sim	Guatemala to Panama	Forest	Up to 25 m	+/- 400

Code: species code used in qualitative association matrices; [a]**:** Croat (1978); [b]: Berry & Kress (1991), and Croat (1978); [c]: tropicos.org (Missouri Botanical Garden, USA); No.: number of individuals sampled in Panama

Network analysis

I. Qualitative approach

We employed two different qualitative approaches to analyze plant-hispine network structure (Bascompte *et al.*, 2003; Lewinsohn *et al.*, 2006; Olesen *et al.*, 2007). These approaches permitted us to characterize the configuration of interactions, facilitated comparisons of networks, and ultimately helped clarify community-shaping factors.

We analysed a binary matrix of all possible plant x beetle interactions (HISP) and characterized the structural organization of the resulting food web using an index of matrix nestedness (N) (Bascompte *et al.*, 2003). Under this index, nestedness is characteristic of asymmetric specialization, whereby "specialists interact with proper subsets of the species interacting with generalists" (Fortuna *et al*., 2010). We estimated matrix temperature (T) with the computer software ANINHADO 3.0 (BANGU) (Guimarães & Guimarães, 2006). This statistic describes the symmetry in the distribution of unexpected absences and presences, respectively, on both sides of the perfect nestedness boundary line (Almeida-Neto *et al.*, 2007). Values of T can range from 0° to 100°. The nestedness value (N) was calculated as: (100 – T)/100, with a range from 0 to 1 (1 = maximum nestedness). Values of N close to 1 indicate a high degree of nestedness. We chose the null model CE in ANINHADO because it was the most biologically realistic for the studied communities and it had the smallest type-I errors (Nielsen & Bascompte, 2007). This null model CE means that "the probability of a cell a_{ij} shows a presence is $\left(\frac{P_i}{C}+\frac{P_j}{R}\right)\Big/2$, in which P_i is the number of presences in the row i, P_j is the number of presences in the column j, C is the number of columns and R is the number of rows" (Guimarães & Guimarães, 2006). To test whether the observed value of N for the different networks was higher than the random expectation, each network was compared with 1,000

replicates generated by the null model CE. We used ANINHADO 3.0 to obtain graphical output of the packed network matrix, which characterizes network structure (Lewinsohn *et al.*, 2006). However, the index N only permits determination of whether network structure is nested.

An additional algorithm (NETCARTO software; Guimerà & Amaral, 2005) detects compartmentalization and identifies modules (or compartments: group of species sharing similar host use) within qualitative food webs. This algorithm gives an index called modularity (M), which defines whether the network is organized into well-defined modules. Moreover, the significance level of modularity is computed by comparison to the mean modularity value of 100 similar-sized random networks. Modularity ranges from 0 to 1. Values above 0.3 suggest a strongly compartmented structure. Values higher than 0.7 are rare (Newman & Girvan, 2004). The output files allow us to compute and visualize the network using PAJEK (Batagelj and Mrvar, 2003).

Connectance (C) ranging from 0 to 1, provides a descriptor of network structure (Table 1) that is the proportion of possible links actually observed in the network excluding any cannibalistic interactions or interactions within the same trophic level (Fortuna *et al*., 2010).

II. Quantitative approach

The quantitative approach allowed us to analyze relative abundance matrices (weighted food webs). Quantitative indices are more robust to sampling differences (Tylianakis *et al.*, 2007) and give less weight to small proportions corresponding to possible uncertain plant-beetle field associations (Bersier *et al.*, 2002) or rare species (Blüthgen, 2010). To perform the quantitative analysis, we used the open-source R-package *bipartite* (R Development Core Team, 2008; Dormann *et al*., 2008). This software allows us to visualize quantitative food webs, and includes all recent network analysis indices (Dormann *et al*., 2009). To better understand the structure of this plant-hispine food web, we calculated several indices

allowing us to estimate host specificity and habitat overlap of hispine beetles. The first quantitative index we used is the Weighted Generality (G_q). It represents the mean number of host species per hispine species (Bersier *et al.*, 2002; Dormann *et al*., 2009). The second index is called Niche Overlap (NO). Niche Overlap indicates the "mean similarity in interaction pattern between species of the same trophic level" (Dormann *et al*., 2009) and ranges from 0 to 1 (1 = perfect niche overlap), reflecting habitat boundaries. The index Interaction Evenness characterizing specialization at the network level was not calculated in this study, as the minimum network size (number of involved species) of 50 species suggested by Dormann et al. (2009) was not reached for our food web.

Results

Qualitative approach

Our food web was based on 5,077 individual associations of hispine beetles and food plants (Table 1, 2). Thirty hispine species interacted with 17 plant species and shared 99 interactions (Table 3), less than 20% (connectance value = 0.194) of the total potential number of interactions. Figure 1 illustrates quantitatively the heterogeneity and complexity of trophic interactions between hispine beetle and plant species, but also the disparity of hispine species abundances. Nestedness analysis recovered a food web temperature (T) of 37.22° and a nestedness value (N) of 0.63 (Table 3). Our observed food web was not significantly more nested than randomly generated food webs (null model CE; 1,000 replicates; mean ± SE: T = 41.27° ± 5.8°, NS).

Table 2. List of hispine species observed and their characteristics in Panama

				Larvae			Adult
Hispine tribes and species	**Code**	**No.**	**Plant family**	**Habitat**	**Shape**	**Dorsal shield**	**Habitat**
Arescini							
Chelobasis bicolor Gray	Ch.bic	16	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
Chelobasis preplexa Baly	Ch.per	24	Hel.	Rolled Leaf	Onisciform	None	Rolled Leaf
Cephaloleiini							
Aslamidium semicirculare Olivier	As.sem	507	Hel., Mar.	Open Leaf	Onisciform	None	Open Leaf - Upper Side
C. belti Baly	Ce.bel	258	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. costaricensis Uhmann	Ce.cos	12	Poa.	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
C. dilaticollis Baly	Ce.dil	93	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. dorsalis Baly	Ce.dor	7	Cos.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. erichsonii Baly	Ce.eri	253	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. instabilis Baly	Ce.ins	42	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. lata Baly	Ce.lat	195	Are.	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
C. metalescens Baly	Ce.met	219	Are.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. partita Weise	Ce.par	5	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. pretiosa Baly	Ce.pre	83	Cos., Hel.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. sallei Baly	Ce.sal	190	Hel., Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. stevensi Baly	Ce.ste	4	Mar.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. suturalis Baly	Ce.sut	1	Cos.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. trimaculata Baly	Ce.tri	2	Cos.	Rolled Leaf	Onisciform	None	Rolled Leaf
C. sp61 (Meskens C. – Panama)	Ce.sp61	378	Are.	Rolled or Open Leaf	Onisciform	None	Rolled Leaf
Imatidium thoracicum Fabr.	Im.tho	110	Hel., Mar.	Open Leaf	Onisciform	None	Open Leaf - Lower Side
Homalispa nevermanni Uhmann	Ho.ner	659	Are.	Rolled Leaf	Onisciform	None	Rolled or Open Leaf
Stilpnaspis panamensis Borowiec	St.pan	263	Are.	Rolled or Open Leaf	Onisciform	None	Open Leaf
Chalepini							
Platocthispa emorsitans Baly	Pl.emo	258	Hel., Mar.	Leaf Miner	Eruciform	None	Open Leaf
Delocraniini							
Delocrania panamensis Champion	De.pan	21	Are.	Open Leaf	Eruciform	Detritus	Open Leaf - Lower Side
Hemisphaerotini							
Spaethiella circumdata Boheman	Sp.cir	40	Hel.	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. marginata Champion	Sp.mar	204	Hel., Mar.	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. tristis Boheman	Sp.tri	239	Are.	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
S. sp1 (Meskens C. – Panama)	Sp.sp1	20	Poa.	Open Leaf	Eruciform	Exuvial-fecal Shield	Open Leaf
Prosopodontini							
Prosopodonta dorsata Baly	Pr.dor	481	Are.	Open Leaf	Eruciform	Urogomphus	Open Leaf
Spilophorini							
Calyptocephala antennata Spaeth	Ca.ant	296	Are.	Open Leaf	Eruciform	Exuvial Shield	Open Leaf
Calyptocephala brevicornis Boheman	Ca.bre	197	Are.	Open Leaf	Eruciform	Exuvial Shield	Open Leaf

No.: sampling result in Panama, number of individuals collected or observed; Code: species code used in qualitative association matrices; Plant family: plant family on which hispine species was observed; Are.: Arecaceae; Cos.: Costaceae; Hel.: Heliconiaceae; Mar.: Marantaceae; Poa.: Poaceae

Modularity analysis recovered a value of M = 0.469 (Table 3) indicating significantly higher modularity than expected by chance (100 random networks; mean ± SE: M = 0.399 ± 0.015, *P* < 0.01). NETCARTO identified five modules in the food web (Fig. 2), further confirming a compartmented structure.

Table 3. Summary of characteristics, descriptors and statistics for plant-hispine food web in Panama (Meskens *et al*., 2008)

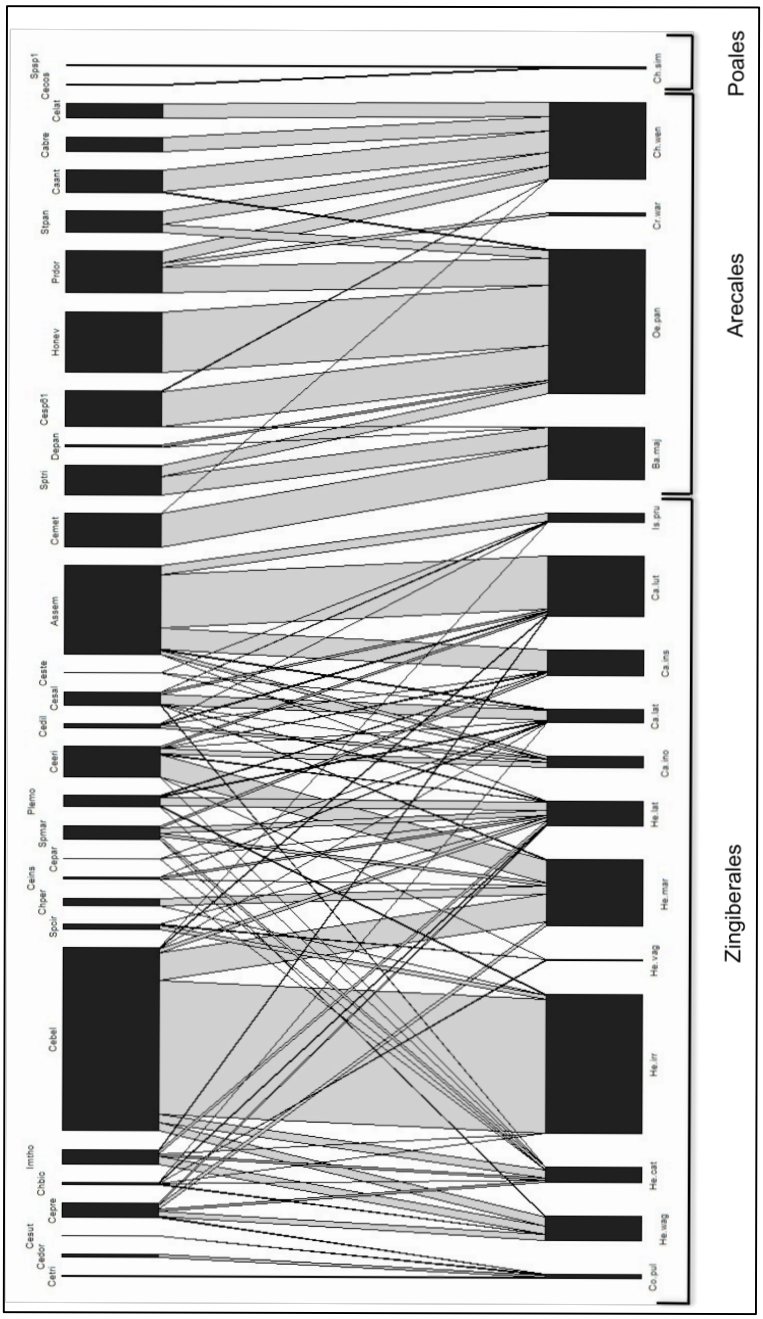
Insect family	Chrysomelidae
Insect sub-family	Cassidinae (hispine beetles)
Location	Lowland - Panama
Number of larval feeding guilds	3
Food web characteristics	
Number of plant species (H)	17
Number of plant individuals	4,671
Number of plant orders	3
Number of plant families	5
Number of hispine species (B)	30
Number of hispine individuals	5,077
Total number of species	47
Number of interactions (L)	99
Qualitative analysis	
Connectance	
C	0.194
Modularity	
M	0.469
#	5
Randomizations	0.399 ± 0.015
Nestedness analysis	
T	37.22°
T (CE)	41.27 ± 5.8°
P	NS
N	0.63
Quantitative analysis	
G_q	2.16
NO	0.09

C: food web connectance value = L/(B x H), ranging from 0 to 1; M: network modularity calculated by NETCARTO (Guimerà & Amaral, 2005); #: number of modules identified by NETCARTO (Guimerà & Amaral, 2005); Randomizations: mean M (± SE) for 100 similar-sized random networks in NETCARTO; T: matrix temperature calculated by ANINHADO (Guimarães & Guimarães, 2006); T (CE): mean T for null model CE (1,000 replicates) ± SE in ANINHADO (Guimarães & Guimarães, 2006); *P*: level of significance tested against null model CE; NS = not significant (*P* > 0.01); CE: null model used in Nielsen & Bascompte (2007); N: nestedness value = (100 – T)/100, ranging from 0 to 1 (1 = perfect nestedness; Nielsen & Bascompte, 2007); G_q: weighted generality (Bersier *et al*., 2002; Tylianakis *et al*., 2007); NO: niche overlap (Dormann *et al*., 2009)

Quantitative approach

R-package *bipartite* identified three completely isolated modules (or compartments) according to the plant order (Fig. 1). Weighted Generality showed that each hispine species fed on average 2.16 host species (G_q = 2.16; Table 3), indicating a high degree of host specificity. The index Niche Overlap (NO = 0.09; Table 3) revealed weak niche overlap, reflecting clear habitat boundaries.

Figure 1. Quantitative plant-hispine food web (function *plotweb* in R-package *bipartite*); lower and upper blocks represent respectively plant and hispine species; linkage width indicates frequency of each plant-beetle trophic interaction; lower limits represent modules boundaries according to plant order; see Table 1 for host plant species codes, and Table 2 for hispine beetle species codes.



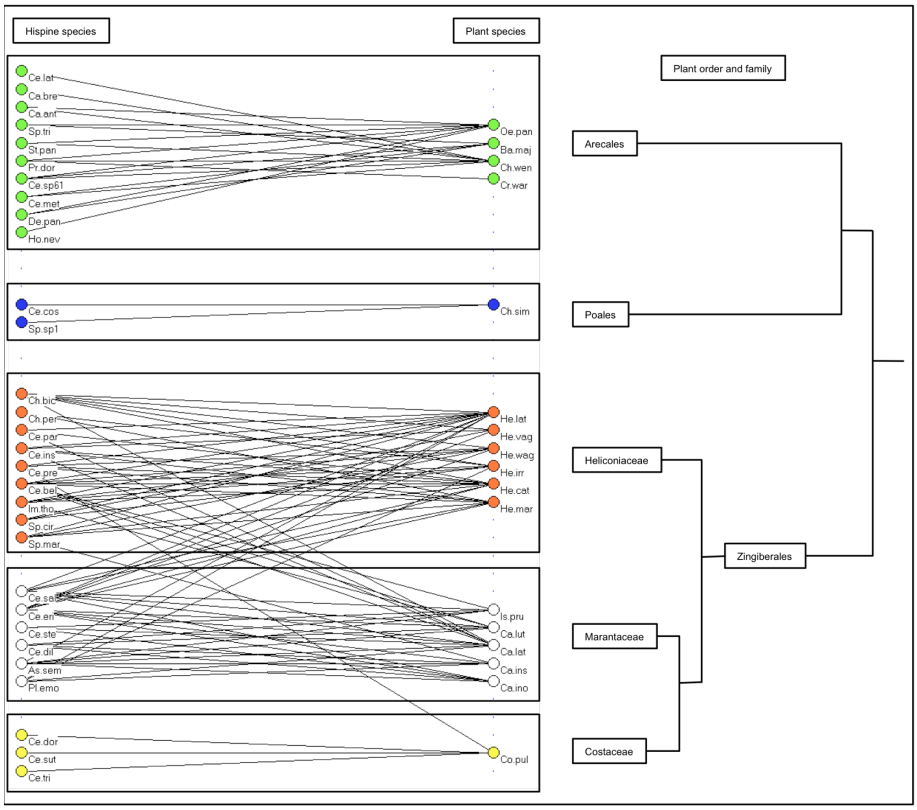
Discussion

Results from nestedness analysis show that the plant-hispine food web (N = 0.63; Table 3) is significantly less nested than expected by chance (Bascompte *et al.*, 2003) (Table 3; null model CE, $P > 0.01$, NS). Non-nested mutualistic networks are thought to be less robust to environmental perturbations (Almeida-Neto *et al.*, 2007).

The modularity analysis results (Table 3; Fig. 2) are consistent with a modular food web structure (Lewinsohn *et al.*, 2006; Thébault & Fontaine, 2010). NETCARTO identified in this food web, five well-defined modules corresponding to host plant taxonomy (Heliconiaceae, Marantaceae, Costaceae, Arecales and Poales, each constituting one module; Fig. 2). With a high connectance value (Table 3), our structure analysis supports the view that high connectance reduces: i) the likelihood that ecological networks are simultaneously nested and modular (Fortuna *et al.* 2010), and ii) the stability of food webs (Thébault & Fontaine, 2010), which confirms the contention of Strong (1983) that the specialized life histories and long reproductive cycles of hispine beetles may make them sensitive to environmental change. The modularity results and the Weighted Generality (G_q value = 2.16) indicate that these hispine species are feeding specialists at the plant familial or ordinal level (oligophagy; Jolivet, 1998) but generalists on genera and species within these taxonomic compartments (Prado & Lewinsohn, 2004; McKenna & Farrell, 2005, 2006). Specificity to higher taxonomic groupings of host plants may be largely responsible for the instances in which modularity is observed in plant-herbivore food webs (Fig. 2). Broad phenotypic traits observable at higher plant taxonomic levels may be particularly important in restricting the diets of hispine beetles. Such traits might include leaf secondary chemistry (e.g. flavonoid content and allelochemical protection; Williams & Herborne, 1977; Strong, 1982) and nutrient content (e.g., low nitrogen content of Heliconia species; Strong, 1982), but leaf toughness, abrasiveness and the presence of a leaf architecture which facilitates both feeding, and refuge from desiccation and

natural enemies is likely of equal or greater importance in this group of insects. These physical attributes of immature leaves differ highly among the host plant species in the clades Zingiberales, Arecales and Poales. Standing pools of water are present in the rolled leaves of many species of Zingiberales, but largely absent in Arecales and Poales. We can speculate that the largest two food web modules (Arecales/Poales and Zingiberales) may reflect two adaptive zones, which opened for hispine beetles at different points in their evolutionary history. The radiation of Cephaloleiini associated with Zingiberales, for example, is likely to have been more recent than that of Cephaloleiini species associated with Poaceae or Arecaceae (McKenna & Farrell, 2006). Different herbivore-plant food webs thus are expected to vary to the extent that they reflect the interplay of distribution and habitat boundaries (Montoya *et al*., 2006), plant attributes (Prado & Lewinsohn, 2004) and historical contingency.

Figure 2. Bipartite graph by modularity analysis for plant-hispine food web in Panama (Meskens *et al*., 2008); left circles represent hispine species; right circles represent plant species; lines joining two species represent recorded interactions; black boxes represent modules identified by NETCARTO; dashed black box represents potential module for Poales by removing uncertain records; black lines (right side) represent the respective phylogenetic relationships of the monocot families and orders that are presented as hypothesized by Stevens (2001) and Soltis *et al*. (2005); see Table 1 for host plant species codes, and Table 2 for hispine beetle species codes.



The modular structure of the hispine food web and the degree of host specificity (G_q = 2.16) illustrate the well-known feeding specialization within the subfamily of Cassidinae at the plant order and/or family-level (Strong *et al*., 1984; Jolivet, 1997; McKenna & Farrell, 2005; García-Robledo & Horvitz, 2009). Rolled leaf hispine beetles are known to have maintained a highly specialized feeding association with the plant order Zingiberales for ca. 60 million years (Wilf *et al*., 2000; McKenna & Farrell, 2006; García-Robledo & Horvitz, 2009), and therefore have figured prominently in the development of community ecological theory, especially in the context of tropical insects and

their host plants (Strong, 1982; McKenna & Farrell, 2005). With few interactions occurring across plant ordinal boundaries, also reflected by almost no common use of niches by beetle species (Niche Overlap = 0.09; Dormann *et al*., 2009), rolled leaf hispines appear to be an example of ecological-functional specialists (Ferry-Graham *et al.*, 2002) on plants that offer the same habitat conditions (humidity, physical protection, etc.) within young rolled leaf scrolls (Strong, 1983). Leaf scrolls are important feeding and mating sites for most rolled leaf hispine beetles (García-Robledo & Horvitz, 2009). Modularity in food web structure and low value of Niche Overlap can be defined as clear habitat boundaries (Montoya *et al.*, 2006) and strong structural trait matching, such as similar phenotypes and niche requirements of rolled leaf hispine beetle tribes, Cephaloleiini and Arescini on young rolled leaves of Zingiberales (Blüthgen *et al.*, 2008; Vázquez *et al*., 2009; Gómez *et al*., 2010). The ecological specialization we document may be consistent with the notion that specialization reduces interspecific competition and facilitates species coexistence by partitioning niche space (Dyer *et al*., 2007). The consequences of a modular structure may thus translate to the observed near absence of interspecific competition in rolled leaf hispines and related taxa, such as *Heliconia* species (Strong, 1982).

The modular structure we uncovered is also consistent with the known phylogenetic relationships of rolled leaf hispine beetles (McKenna & Farrell, 2005, 2006). *Cephaloleia* species in particular have been sampled with sufficient phylogenetic density to recover many of the interactions among members of the sampled lineage (McKenna & Farrell, 2006). When interpreted in a phylogenetic context most of the species belonging to the same feeding guild and tribe (e.g., Cephaloleiini) within a module share a common ancestor, and interact with phenotypically similar host plants (Gómez *et al*., 2010). Moreover, food web structure is consistent with the different feeding clades of *Cephaloleia* species (Zingiberales vs. Arecales; Fig. 1) identified by McKenna and Farrell (2005).

The host specificity of hispine beetles at the ordinal level may also be explained by host plant characteristics. Host plant species in this study are mainly either gap-dependent or understory plants (Zingiberales; Table 1). These plants have low population density in most tropical forests, and are poorly defended (Strong, 1983; Coley & Barone, 1996; Wright, 2002). Considering these two characteristics, small insects with poor dispersal abilities, such as hispine beetles might be expected to show a generalist habit within a plant order or family (and thus "create" a module) to reduce host locating time and consequent exposure to predators (Coley & Barone, 1996).

Some species complexes, such as *C. belti* or *C. erichsonii* Baly (D. Windsor and D. McKenna, pers. obs.), may complicate analysis by indicating that certain species are more generalist feeders than they actually are, thus affecting food web structure (Vázquez *et al.*, 2009). Further, our sampling of non-rolled leaf feeding taxa was small and furnishes a limited view on their habits. Studies investigating food web structure separately for each hispine tribe encountered in this study, and studies investigating food web structure at higher trophic levels, e.g., between hispine beetles and their parasitoids, may clarify factors influencing the evolution of host tissue use and host taxon specialization in Neotropical hispine beetles.

Finally, our food web was actually a relatively incomplete sampling of hispine beetle-plant associations existing in lowland central Panama because many plant species are rare or difficult to find. The binary matrix and resulting network could be expanded by sampling of beetle and plant species within a single geographic area and at replicate sites. Feeding trials could reduce uncertain plant-beetle associations (Futuyma & Moreno, 1988; Descampe *et al.*, 2008), and though a formidable task, "DNA barcoding" of both adults and larvae, and gut contents could reduce or eliminate taxonomic aggregations (species complexes) (Novotny & Basset, 2005; Dyer *et al.*, 2007; Jurado-Rivera *et al.*, 2008).

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Chapter 4.

« *Structure and specificity in plant-beetle food webs in Central America* »

Context

High richness Tropical communities are based on long-term evolutionary processes, such as the coevolution. A fundamental objective in ecology concerns how this Tropical biodiversity is maintained. A key to understand these maintaining processes is to define the extent to which Tropical phytophagous insects are specialized.

Because phytophagous beetles feed on their hosts as both immatures and adults they can be expected to have a significant impact on plant growth and survival, and depending on host specificity, to be among density dependent factors promoting the higher levels of plant diversity observed in Tropical forests (Janzen 1970; Connell 1971). We examined seven data sets of plant-beetle interactions including four different families (Chrysomelidae, Cerambycidae, Buprestidae and Curculionidae) and four feeding guilds within the Chrysomeloidea, in lower Central America (Panama and Costa Rica) to examine qualitatively food web structure and levels of host specificity.

In the last part of this thesis, we only performed the nestedness analysis to investigate food web structure because at the time of writing this paper, we were not in possession of NETCARTO allowing us to perform the modularity analysis. Nevertheless, preliminary results suggest a modular structure for these food webs (Meskens *et al., unpublished data*).

Structure and specificity in plant-beetle food webs in Central America

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Abstract. Recent qualitative studies in both temperate and tropical forests reveal the existence of variation in structure of food webs and the host specificity of phytophagous insects and their host plants. We examine four new and three combined (published and new) host association data sets to better characterize food web structure and levels of host specificity for phytophagous Coleoptera (beetles) in Neotropical forests. The data sets we examine come from a single region, lower Central America, and include representatives of the species-rich beetle superfamilies: Buprestoidea

(metallic wood boring beetles), Chrysomeloidea (leaf beetles and longhorned beetles), and Curculionoidea ("weevils"). A "nested", asymmetric structure was identified in each of the seven food webs accompanied by values of connectance considerably lower than is typical in mutualistic networks and some previously reported herbivore food webs. Host specificity values were high in all beetle taxa and did not vary significantly among feeding guilds. Findings from each of the superfamilies support assumptions of uniformly high levels of host specificity in phytophagous beetles, in contrast to results obtained in other tropical regions and in some other tropical insect taxa.

Keywords: *food web, nestedness, host specificity, Neotropics, feeding guild, Chrysomeloidea, Buprestiodea, Curculionoidea*

Introduction

Neotropical forests are among the most biodiverse habitats on earth (Wright 2002, Rull 2008), however the ecological factors generating and maintaining this diversity remain the subject of considerable debate (e.g., Wright 2002, Okuyama and Holland 2008). Some authors stress the importance of the Cretaceous rise in flowering plant (angiosperm) diversity and dominance as the major factor underlying the diversification of the angiosperm-associated insect taxa (Farrell 1998, McKenna and Farrell 2006, McKenna et al. 2009), hummingbirds (Bruna and Kress 2002), bees (Danforth et al. 2006) and other life forms. Insects and pathogens acting as negative density dependent mortality factors are thought to play a major role in maintaining high levels of tropical plant diversity by lowering the survival of contagiously distributed individuals (Janzen 1970, Connell 1971, Jones and Comita 2010). Food web analysis provides a useful tool for understanding how insects and plants interact in such complex and threatened ecosystems (Thompson 2009) and for interpreting variation in diversity measures among geographic regions.

Recent qualitative studies of networks involving mutualistic species, such as plants and their seed dispersers, pollinators, and ant guards (Bascompte et al. 2003, Guimarães et al. 2007), reveal a nested structure and species interactions with asymmetric specialization, that is, specialists interacting with generalists (Blüthgen et al. 2008; Fig. 1). Networks involving antagonistic species, such as plants and herbivores, often exhibit a compartmented structure and symmetric specialization, a possible result of persistent evolution of defenses and counter-defenses (Thébault and Fontaine 2008). Yet other studies have found similarities in the food web structures of both antagonists and mutualists (Lewinsohn et al. 2006, Sugiura et al. 2007, Thébault and Fontaine 2008). Because more than 40% of global terrestrial diversity is thought to be contained within plant-phytophage food webs (Price 2002), their analysis may be particularly useful in understanding the interactions of coevolved species (Thompson 2009)

and the consequences of disturbance on ecological communities (Bascompte et al. 2003).

Figure 1a. Packed binary matrix (reorganised) illustrating perfect nestedness (N = 0.997); columns represent insect species (A_i); rows represent plant species (P_j).

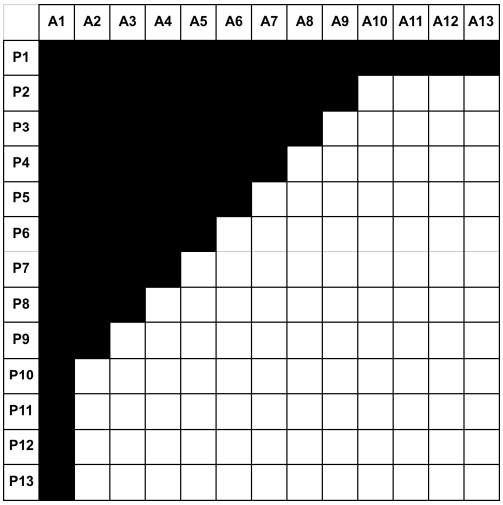
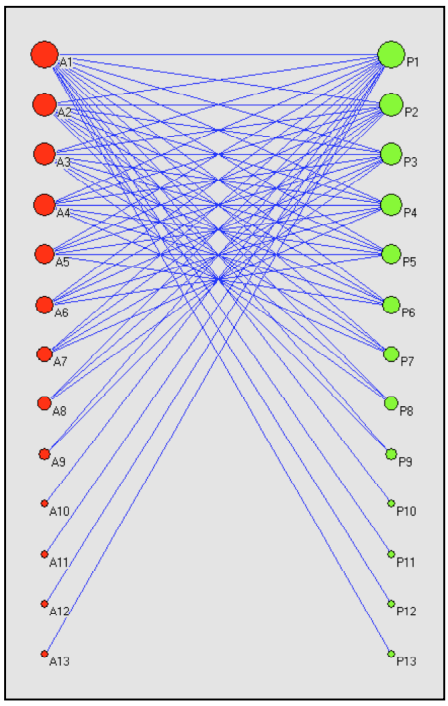


Figure 1b. Unweighted bipartite graph illustrating perfect nestedness in a plant-insect food web (N = 0.997); green circles represent plant species (P_j); red circles represent insect species (A_i); circle sizes are proportional to the number of interactions for each species; black lines represent species interactions. The bipartite graph was computed using PAJEK (Batagelj and Mrvar 2003; http://pajek.imfm.si/doku.php).



For the majority of phytophagous beetle species which attack plants both as immatures and adults, the consensus has been that they rarely feed at random, preferring instead hosts within a single plant family or genus, or more rarely, a single species (Strong et al. 1984, Barone 1996, Novotny et al. 2002). Recent studies suggest a strong latitudinal gradient in host specificity of phytophagous insects (Dyer et al. 2007, Schemske et al. 2009), and narrower host ranges in the Tropics (Coley and Barone 1996). Nevertheless, some recent large-scale studies report relatively low levels of host specificity for insect herbivores, including beetles, feeding on trees in both temperate and tropical forests (Novotny et al. 2002, Novotny and Basset 2005). Moreover, host specificity has been reported to vary depending on guild membership with specificity decreasing along a series from endophagy, exophagy, xylophagy to rhizophagy (Novotny and Basset 2005; McKenna and Farrell 2009).

Host specificity may affect the structure of ecological communities by increasing asymmetry within mutualistic networks (Bascompte et al. 2003, Fontaine et al. 2009), or by reducing interspecific competition in antagonistic networks (Dyer et al. 2007). Here, we test whether the network structure of Neotropical phytophagous beetles resembles that typical in mutualistic (e.g., ant-plant, pollinator-plant; Blüthgen et al. 2007) or antagonistic (plant-herbivore) networks. We draw on seven data sets including both published and new data for six families and three major superfamilies of herbivorous beetles (order Coleoptera) in lower Central America. We then use these large data sets to determine whether attributes of host specificity and food web structure vary among data sets despite differences in sampling methods and spatial scales. For each data set, we calculated “effective specialization” (Ødegaard 2000a, 2000b, Novotny et al. 2002) to compare levels of host specificity among beetle taxa. We then constructed food webs (also known as resource-consumer networks) using presence-absence data for plant and beetle species (Lewinsohn et al. 2006, Tylianakis et al. 2007) from which we obtained “connectance”, a food web descriptor (Olesen et al. 2007, Thébault and Fontaine 2008), and an index of “nestedness”, a food web structural pattern (Bascompte et al. 2003). These metrics provided a common "currency", which allowed us to describe the food web "architecture” (May 1990, Lewinsohn et al. 2006) of major beetle taxa (Chrysomeloidea, Curculionoidea and Buprestoidea) and feeding guilds within the Chrysomeloidea (“leaf-mining”, “leaf-chewing”, “seed-eating”, and “subcortical-mining”), and to compare our results to published surveys of ecological networks.

Methods

We analyzed seven beetle-plant interaction data sets obtained from studies performed on the Central American Isthmus (Costa Rica and Panama). We included only data sets containing at least 50 interacting species (plant and beetle species); a minimum size recommended by Bascompte et al. (2003) to ensure reasonable estimates of food web structure. Further, we accepted

only data sets based on direct feeding observations or adult emergence data. Therefore, we excluded several published beetle plant studies based on indirect feeding observations (e.g., Ødegaard 2000a and 2006, Meskens et al. 2008) because the collection methods employed in those studies underestimate host specificity (Meskens et al., *unpublished data*). Finally, while links to host plants identified only to genus were few (see below) we examined the effects they had on food web structure and host specificity by performing analyses both on entire data sets and on the same data sets from which all non species-level determinations had been removed (see Table 1).

Chrysomeloidea

Chrysomelidae

Cassidinae ("leaf-mining" hispine beetles in Costa Rica; network analysis code: HISP). We combined the adult emergence records of "leaf mining" hispine beetles from three published data sets; Memmott et al. (1994), Flowers and Janzen (1997) in the highly seasonal province of Guanacaste (dry forests), and Hespenheide and Dang (1999) in the nearly non-seasonal province of Heredia (OTS La Selva Biological Station; tropical premontane wet forests). The combined data set included 60 hispine species directly associated with 54 plant species in 23 plant families. Seven hispine species (8.9%) were linked to hosts identified only to genus (5 *Lasciasis* spp. and 2 *Desmodium* spp.).

Cassidinae ("leaf-chewing" tortoise beetles; Cassidinae (ss); network analysis code: CAS). We analyzed the adult feeding records of 142 Cassidinae species associated with 79 plant species in eight plant families, by combining the records of Windsor et al. (1992) plus more recent records from both lowland and highland Panamanian forests (L. Sekerka and D. Windsor, *unpublished data*), and Flowers and Janzen (1997) in Costa Rican dry forest (highly seasonal province of Guanacaste). Twenty-nine

Cassidinae species (20.4%) were linked to hosts identified only to genus (4 *Cordia* spp., 23 *Ipomoea* spp. and 2 *Tabebuia* spp.).

Bruchinae ("seed-eating" beetles; network analysis code: BRU1 and BRU2). We analyzed separately the adult emergence records for 32 species of adult Bruchinae emerging from 33 plant species in 14 plant families (including data by J. Wright, *unpublished data*) in Panama (BRU1; lowland and highland tropical wet forests), and 95 species of adult Bruchinae emerging from 90 plant species in 18 plant families (Janzen 1980; analyzed by Thébault and Fontaine 2008) (BRU2; highly seasonal dry forest of Guanacaste, Costa Rica). The Costa Rican and Panamanian data sets had five beetle species and three plant species in common. All Bruchinae species in these two studies were linked to hosts identified to species or unique morphospecies.

Cerambycidae ("subcortical-mining or xylophagous", long-horn beetles in Panama; network analysis code: CER). We analyzed adult emergence records for 80 species of Cerambycidae reared from 55 plant species in 28 plant families in Panama (lowland and highland tropical wet forests). All Cerambycidae were linked to hosts identified to species or unique morphospecies.

Curculionoidea

Curculionidae ("weevils"; network analysis code: CUR). We analyzed adult emergence records for 214 species of adult weevils (3 tribes: Baridinae, Cryptorhynchinae, Zygopinae) emerging from 135 plant species in 69 plant families in Panama (lowland and highland tropical wet forests). Thirty-six weevil species (13.8%) were linked to hosts identified only to genus (8 *Cecropia* spp., 2 *Eschweilera* spp., 3 *Inga* spp., 5 *Ipomoea* spp., 18 *Piper* spp.).

Buprestoidea

Buprestidae ("jewel" beetles; network analysis code: BUP). We analyzed data collected from 55 species of Buprestidae that had emerged from 47 plant species in 25 plant families in Panama (lowland and highland tropical wet forests). Five buprestid species (7.4%) were linked to hosts identified only to genus (3 *Inga* spp. and 2 *Cecropia* spp.).

The plant-beetle association data for Panamanian Bruchinae (BRU1), Cerambycidae (CER), Buprestidae (BUP) and Curculionidae (CUR) were recorded between 1969 and 2009. Sampling was performed in an unstructured manner; therefore sampling effort is reflected only by the total number of species reared. The entire data set is available on request at the STRI web site (http://biogeodb.stri.edu/bioinformatics/PAPB/). Species determinations were based on published keys, plates in the Biologia Centrali-Americani (Baly 1885) and by comparison to identified specimens in the STRI insect collection (Tupper Research and Conference Center, Panama).

Qualitative food web analysis

Binary interaction matrices (plant species by beetle species; presence or absence of interaction recorded within each cell) were constructed for each of the seven empirical data sets. Food webs were then analyzed (following Bascompte et al. 2003, Lewinsohn et al. 2006) to obtain numerical descriptors of their attributes (see Bersier et al. 2002).

The structural organization of food web networks was determined by computing matrix nestedness (Atmar and Patterson 1993). In our study, a nested structure is obtained when specialist beetles interact with plant species to form perfect subsets of the same plant species with which generalist beetles interact (Fig.1; Bascompte et al. 2003, Bascompte and Jordano 2007,). The **index of nestedness**, N (Table 1), is defined as: (100

– T)/100, and ranges from 0 to 1 (1 = maximum nestedness), where the factor "T" (temperature) of a binary matrix is a measure of the symmetrical distribution of unexpected presences and absences on both sides of the perfect nestedness boundary line, and ranges from 0° to 100° (Almeida-Neto et al. 2007). We used ANINHADO 3.0 (Guimarães and Guimarães 2006) to calculate T for our empirical networks. The null model CE in ANINHADO was chosen because it has the smallest type-I errors (Nielsen and Bascompte, 2007). To test if the observed value of N for the different networks was higher than expected by purely random association of beetle and plant species, each network was compared with 1,000 replicates generated by null model CE (*P* = the fraction of replicates that have a value of nestedness higher or equal to the observed value).

Host specificity within the different beetle families and subfamilies was evaluated using the index, **effective specialization** (F_{tS}, Table 1; Ødegaard 2000a, Novotny et al. 2002). This index is the ratio of the total number of beetle species feeding on all host plant species, divided by the number of host plant association records (Ødegaard 2000b, Novotny et al. 2002). The index has been used widely in the recent literature and it allowed us to quantitatively compare our results to previous studies. The same formula was used to calculate an index of "genus level" effective specialization (F_{tG}, Table 1) by removing specific epithets from the same identified plants. Moreover, we calculated four sample characteristics: the number of beetle species (B), the number of plant species (H), the number of plant-beetle interaction records (L or interaction richness), that is, the total number of "links" between beetles and plants within the network) and network size (S, the sum of plant and beetle species). **Connectance** (C) (Olesen et al. 2007, Thébault and Fontaine 2008) provides a descriptor of network structure (Table 1), the proportion of possible links actually observed in the network excluding any cannibalistic interactions or interactions within the same trophic level.

Table 1. Summary of characteristics, descriptors (formula and range), nestedness analysis results and statistics for each of the seven food webs

Beetle superfamily	Chrysomeloidea					Buprestoidea	Curculionoidea
Beetle family	Chrysomelidae				Cerambycidae	Buprestidae	Curculionidae
Beetle subfamily	Cassidinae		Bruchinae		(various)	(various)	(various)
Network analysis code	HISP	CAS	BRU1	BRU2	CER	BUP	CUR
Larval feeding guild	Leaf-miners	Leaf-chewers and scrappers	Seed-eaters		Subcortical-miners	Leaf and stem-miners	Root, stem and leaf-miners, and chewers
Network characteristics							
Number of host species (H; variable)	54	79 (75)	33	90	55	47	135 (132)
Number of beetle species (B; variable)	60	142 (118)	32	95	80	55	214 (198)
Number of host genera (G; variable)	48	47	29	45	48	42	100
Number of species (S; variable)	114	221 (193)	65	185	135	102	349 (330)
Interaction richness (L; variable; species level)	79	205 (176)	39	142	108	68	260 (239)
Interaction richness (A; variable; genus level)	74	175	36	111	106	42	248
Network descriptor (formula – range)							
Connectance C = L/(B x H); 0 to 1	0.024	0.018 (0.019)	0.037	0.017	0.024	0.026	0.009 (0.008)
Host specificity							
Effective specialization (species level) F_{IS} = (B/L); 0 to 1	0.76	0.69 (0.67)	0.82	0.67	0.74	0.81	0.82 (0.82)
Effective specialization (genus level) F_{IG} = (B/A); 0 to 1	0.81	0.81	0.89	0.86	0.75	0.92	0.86
Nestedness analysis							
Temperature T; 0° to 100°	9.73	3.46 (4.88)	15.01	7.22	8.00	12.50	3.47 (4.03)
Significance (p)	**	** (**)	**	**	**	**	** (**)
Nestedness							
N = (100 – T)/100 ; 0 to 1	0.90	0.96 (0.95)	0.85	0.93	0.92	0.87	0.96 (0.96)

Temperature (T): matrix temperature calculated by ANINHADO (Guimarães and Guimarães 2006); Significance (p): level of significance tested against null model CE (** = $p < 0.01$); Nestedness (N): value of index N calculated with ANINHADO; values in parentheses (CAS and CUR) were computed by removing all associations with unidentified plant species.

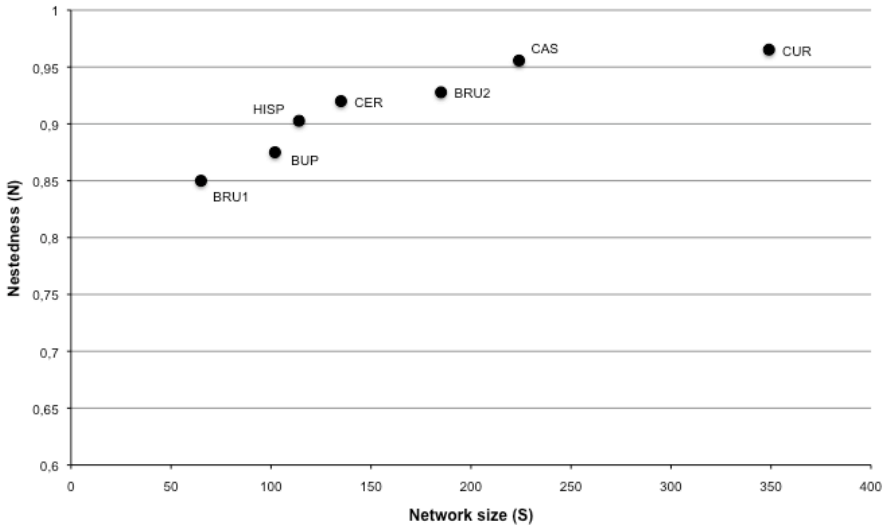
Results

Food web analyses

All nestedness values (N) obtained by ANINHADO (Guimarães and Guimarães 2006) were significantly higher than expected by chance (null model CE, 1000 replicates, $p < 0.01$; Table 1, Fig. 2). A nested structure was present in all seven food webs, with N ranging from 0.85 in the smaller of the two bruchid data sets (BRU1) to 0.96 in Curculionidae (CUR) (mean ± SD:

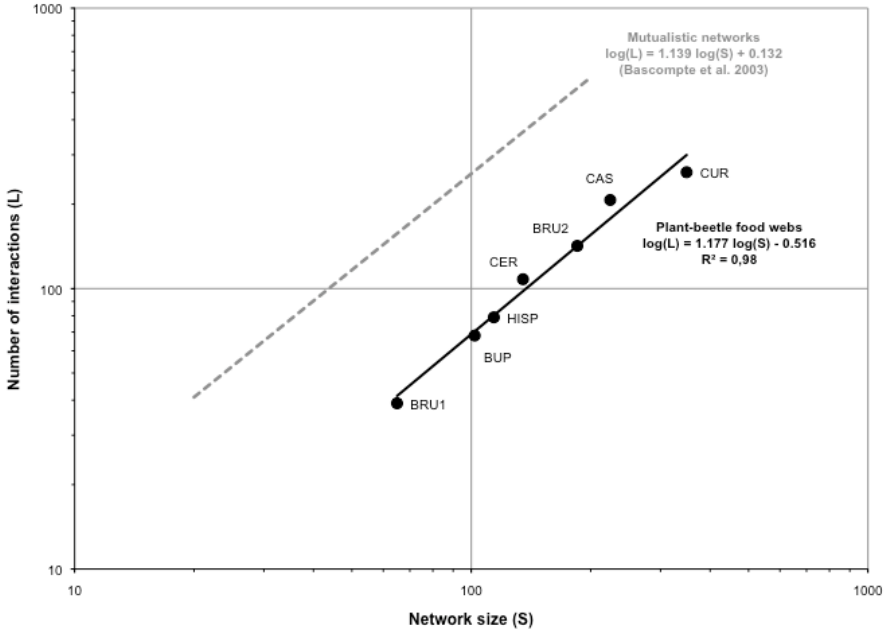
0.91 ± 0.04, n = 7). Nestedness reaches an asymptote at 0.95-0.97 for network sizes of approximately 200 or more species (power-law relationship, R^2 = 0.93; Fig. 2). Removal of ambiguously determined host plant species from the data matrix shifted nestedness in Cassidinae from 0.96 to 0.95 and had no effect on nestedness in Curculionidae (0.96) (Table 1).

Figure 2. Comparison of nestedness value (N) vs. network size (number of plant and beetle species; S = (B+H)) obtained by XY analysis of seven empirical plant-beetle food webs form Central America; grey dashed line represents the power-law relationship for mutualistic networks defined by Bascompte et al. (2003); N is the nestedness value given by ANINHADO (Guimarães and Guimarães 2006) for each community matrix; B and H are respectively the number of beetle and plant species involved in each community. See "Methods" for network analysis codes.



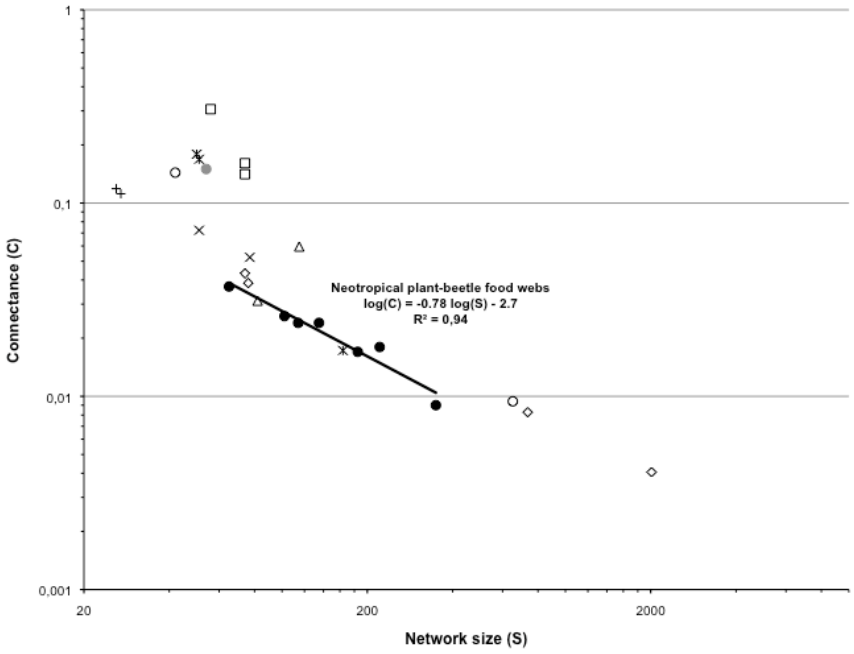
Interaction richness (L), the number of links between beetle and host plant species displayed a highly significant power-law relationship with network size (S) where log(L) = 1.177 log(S) - 0.516 (R^2 = 0.98, p < 0.01; Fig. 3).

Figure 3. Logarithmic plot of the number of interactions vs. network size (number of plant and beetle species; S = (B+H)) for all studied food webs (black circles); black line represents the best-fit power-law relationship for all studied food webs; dashed grey line represents the power-law relationship for mutualistic networks (Bascompte et al. 2003). See "Methods" for network analysis codes.



All seven food webs exhibited low values of connectance (Table 1, Fig. 4) ranging from 0.009 in Curculionidae (CUR) to 0.037 for the smaller of the two Bruchinae data sets (BRU1). The connectance values displayed a highly significant power-law relationship with network size (S) where log(C) = -0.78 log(S) – 2.7 (R^2 = 0.94, p < 0.01; Fig. 4). Removal of ambiguously determined host plants from the data matrices shifted connectance from 0.018 to 0.019 in Cassidinae and from 0.009 to 0.008 in Curculionidae (Table 1).

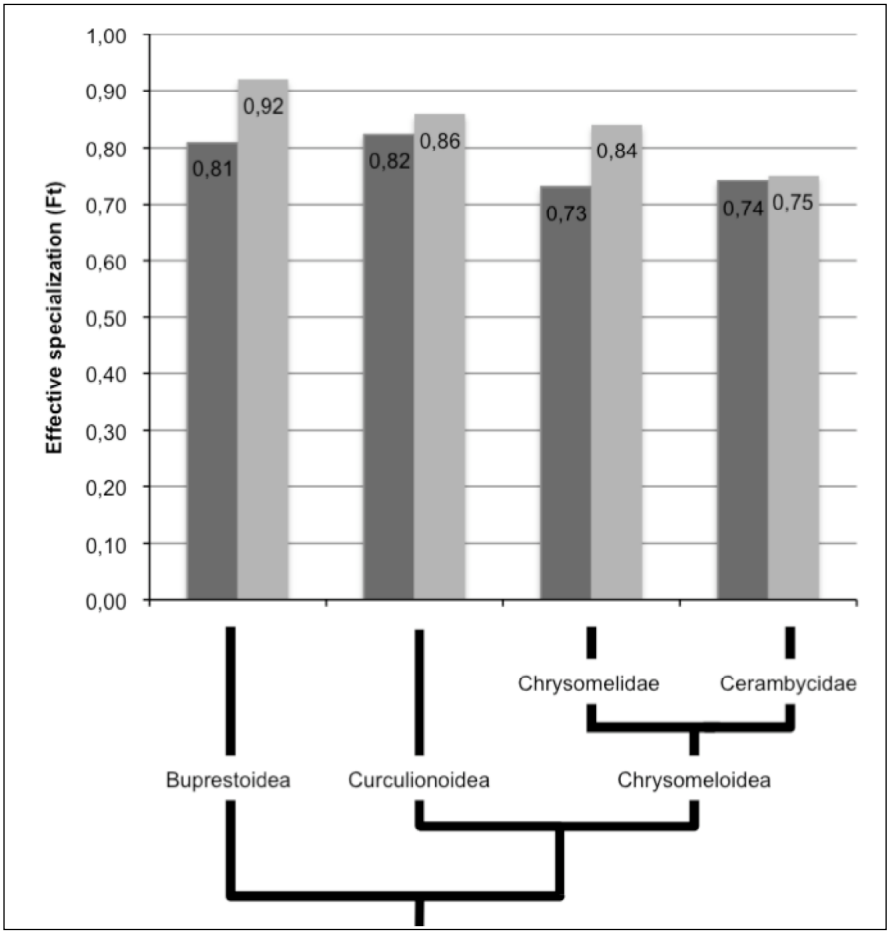
Figure 4. Logarithmic plot of connectance (C) vs. network size (S) for all known plant herbivore food webs. The black line represents the best-fit power-law relationship for our seven Central American Coleoptera food webs (black circles) which is contrasted with other taxonomic groups and locations; Lepidoptera[1] (open diamonds); Cerambycidae[2] (grey circle); Coleoptera[1] (open circles); Diptera[1] (open triangles) Orthoptera[1] (open squares); Hymenoptera[1] (straight crosses); Hemiptera[1] (crosses), and various insect orders[1] (asterisks); [1]: Thebault and Fontaine (2008); [2]: Sugiura et al. (2007).



Host specificity

Average host specificity values (effective specialization at the plant species level; F_{tS}) ranged from 0.67 to 0.83 (mean ± SD: 0.76 ± 0.06, n = 7; Table 1). Among the four subfamilies of Chrysomelidae, host specificity ranged from 0.67 to 0.83 (0.73 ± 0.07, n = 4) (Fig. 5). Although the host specificity of internal-feeding leaf miners, seed eaters and subcortical miners (0.76, 0.75, 0.74, respectively) was slightly higher than for external leaf chewers and scrapers (0.69), the difference was not statistically significant (Kruskal-Wallis One-Way ANOVA: F = 2.141, df = 3, ns), nor were these values significantly correlated with the decreasing sequence of host specificity for the same guilds reported by Novotny and Basset (2005) (Spearman Correlation: r_s = 0.31, df = 3, ns).

Figure 5. Comparison of effective specialization at both plant species and genus levels (F_{ts} and F_{tG}) vs. beetle superfamilies: Buprestoidea, Curculionoidea and Chrysomeloidea (includes Chrysomelidae and Cerambycidae); dark grey columns represent average effective specialization at plant species level (F_{ts}); light grey columns represent average effective specialization at plant genus level (F_{tG}); black lines (below) represent the phylogenetic relationships adapted from Hunt et al. (2007).



Variations in average effective host specialization among the three superfamilies were not significant, ranging from 0.74 in the Chrysomeloidea to 0.82 in the Curculionoidea (two Student *t*-tests: *t* = -0.087, -0.098; ns). Similarly, within the Chrysomeloidea, average host specificity did not vary significantly among the four families ranging from 0.73 in the Chrysomelidae to 0.82 in the Cerambycidae (0.77 ± 0.05, n = 4). While host specificity was slightly higher in the two basal superfamilies, Buprestidoidea and Curculionidae (0.81 and 0.82) than in the two more derived families,

Chrysomelidae (0.73) and Cerambycidae (0.74) these differences were not significant (Fig. 5; Kruskal-Wallis One-Way ANOVA: F = 0.8, df = 3, ns).

Computed "genus level" effective specialization (F_{tG}) ranged from 0.75 to 0.92 (Table 1, Fig. 5) (mean ± SD: 0.84 ± 0.06, n = 7; Table 1), higher by from 0.01 to 0.19 than the corresponding "species level" effective specialization (F_{tS}) observed in the seven food webs. Removal of host plant species from the two data matrices with the greatest number of ambiguous plant determinations changed Cassidinae effective specialization (F_{tS}) from 0.69 to 0.67 and had no effect in Curculionidae (0.82) (Table 1).

Discussion

Network analysis provides a means to describe and detect through structural differences the nature of interactions among suites of interacting species. A nested structure, with specialists predominantly interacting with species most often selected by generalists has been found commonly not only in mutualistic networks (Bascompte et al. 2003) but also in some plant-herbivore food webs (Thébault and Fontaine 2008). Other plant-herbivore food web structures have been identified in nature, including a complex web with multiple compartments each having a nested structure (flowerhead feeders, Tephritidae, Diptera; Lewinsohn et al. 2006). How herbivore phylogeny, geographic history, and associated factors affect web structure and diversity remain little explored despite a wealth of analytic tools. Variation in plant-insect food web structure potentially bears directly on the degree and nature of host specificity of herbivorous insects in general and by extrapolation the magnitude of global insect species richness (Erwin 1982, Odegaard 2002a, 2006, Novotny and Basset 2005) and the potential for herbivorous insects (particularly beetles) to affect forest diversity through negative effects on survival and fecundity of propagules and seedlings (Janzen 1970, Connell 1971, Jones and Comita, 2010).

A significantly nested food web structure was recovered in each of the seven Central American plant-beetle food webs studied. Average levels of nestedness recovered were higher than those reported by Bascompte et al. (2003) for both resource-consumer networks and mutualistic networks. As found in other types of networks, nestedness values increased smoothly with increasing network size until an asymptote was reached at values (0.95-0.97) close to the upper limit of nestedness (1.0) for networks having approximately 200 or more interacting species. The steady increase in nestedness with network size suggests similar processes determine the food web structure of each of these six taxa, and by extension, the networks of mutualists.

The food webs of Central American beetles and their host plants are similar to mutualistic networks in that the number of interactions increases according to a strict power-law relationship with network size (Fig. 3). Indeed, the slope of the power-law relationship for plant-beetle food webs (1.177) is strikingly similar to that obtained for mutualistic networks (1.139), further indication that the same forces are shaping the architecture of interactions in both types of networks. However, while the absolute number of interactions per species increases nonlinearly with increasing network size (log-log slopes exceeding one), the y-axis intercepts of these relationships in these two types of networks are substantially different. At a network size of 100 species (Fig. 3), for example, beetle-plant food webs have nearly 230 fewer interactions than mutualistic networks. Thus, while mutualistic and beetle-plant networks have similar nested structures, the number of links between species is vastly smaller in the latter.

Connectance, the proportion of possible links in a network that are realized, is known to be smaller in plant-herbivore than mutualistic networks (Thébault and Fontaine 2008). Connectance values reported here for Central American plant-beetle food webs decline at a steeper trajectory than was found in a survey of a wide array of herbivore taxa and study localities (Fig. 4) (Sugiura et al. 2007, Thébault and Fontaine 2008). In summary, both

network indices, the number of interactions and connectance, are informative in comparisons of mutualistic and plant-herbivore food webs which otherwise display similar values of nestedness.

The smaller numbers of interactions and lower connectance values for plant herbivore networks are likely the product of higher host specificity. Using effective specialization (F_t), a widely accepted index of specificity based on some of the same measures used in the food web construction, we find that Central American beetles display exceptionally high levels of host specificity, with an average value across all seven studies of 0.76 (Table 1; plant species level; F_{tS}), markedly higher than the average F_{tS} of 0.24 reported by Novotny et al. (2002) for Coleoptera feeding on woody plants (mainly species of Moraceae and Rubiaceae) in Papua New Guinea, and the F_{tS} of 0.21 reported by Prado and Lewinsohn (2004) for flowerhead-feeding Tephritidae (Diptera) on herbaceous Asteraceae in Brazil. Still other studies including those in temperate latitudes have reported herbivore F_{tS} values ranging from 0.08 to 0.89 (Table 2).

Table 2. Effective specialization at plant species level (F_{tS}), numbers of herbivore species (B), numbers of plant species (H), and number of interactions (L) reported in recent studies of plant-herbivore food webs. Portions of the Janzen (1980) and Memmott et al. (1994) data sets (reviewed in Thébault and Fontaine 2008) were included in the analyses presented in this paper and hence are not included in the table.

Herbivore order (family)	Location	H	B	L	F_{tS}	Reference
Coleoptera (Cerambycidae)	French Guiana	300	352	995	0.35	Tavakilian et al. 1997
Coleoptera (Cerambycidae)	Japan	28	26	109	0.24	Sugiura et al. 2007
Coleoptera (Chrysomelidae)	New Guinea	10	32	46	0.69	Basset and Samuelson 1996
Coleoptera (diverse)	Borneo	15	36	91	0.39	Nakagawa et al. 2003
Coleoptera (diverse)	New Guinea	36	NA	NA	0.24	Novotny et al. 2002
Diptera - Coleoptera - Lepidoptera (diverse)	Central America	71	93	114	0.82	Lewis et al. 2002
Diptera (Tephritidae)	New Guinea	53	29	48	0.60	Novotny et al. 2005
Diptera (Tephritidae)	Brazil	81	34	163	0.21	Prado and Lewinsohn 2004
Hemiptera (Aphididae)	Europe	26	25	47	0.53	Muller et al. 1999
Hemiptera (Membracidae - Nicomiidae)	Central America	42	35	77	0.45	Loye 1992
Hymenoptera (Chalcidae)	Europe	10	17	19	0.89	Dawah et al. 1995
Hymenoptera (Chalcidae)	Europe	10	16	19	0.84	Tscharntke et al. 2001
Lepidoptera (diverse)	Central America	293	443	1074	0.41	Dyer and Gentry 2002
Lepidoptera (diverse)	Hawaii (USA)	53	23	47	0.49	Henneman and Memmott 2001
Lepidoptera (diverse)	Hawaii (USA)	49	25	53	0.47	Henneman and Memmott 2001
Lepidoptera (diverse)	Borneo	21	29	109	0.27	Nakagawa et al. 2003
Lepidoptera (diverse)	New Guinea	36	NA	NA	0.51	Novotny et al. 2002
Lepidoptera (Sphingidae - Saturniidae)	Central America	1371	641	3568	0.18	Janzen and Hallwachs 2005
Orthoptera (Acrididae)	USA	53	22	184	0.12	Joern 1979
Orthoptera (Acrididae)	USA	53	21	157	0.13	Joern 1979
Orthoptera (Acrididae)	USA	42	14	180	0.08	Ueckert and Hansen 1971

NA: not available

The large discrepancy between the host specificity values found among Central American and Papuan forest Coleoptera (Novotny et al. 2002) is intriguing and perhaps indicative of real differences between herbivore communities on these distant landmasses with distinctly different biogeographic histories. However, because the research efforts reported here are separated widely in both space and time, we cannot be entirely certain that sampling protocols have not contributed to the differences observed. However, the tight relationships observed between the different metrics of network structure and network size in the data sets from Central America argue against the effects of sampling biases within the region,

suggesting tighter relationships of herbivores and their host plants than was previously realized. Until we have studies employing identical methodologies at distant sites, comparisons must be treated with caution.

Host specificity of Central American beetles calculated using only the host plant genus (F_{tG}), when averaged across the four beetle families was 0.84 (± 0.07), only slightly higher than at the species level (F_{tS}; 0.73 ± 0.07). That is, Central American beetles are almost as discriminating in selecting their host species as they are in selecting their host genera. This finding contrasts with the view that herbivores are genus specialists, and by implication not species specialists (Novotny et al. 2002, Novotny and Basset 2005). It now seems fairly clear that Central American Coleoptera, at least, should be considered specialists at both taxonomic levels. Further, we obtained little support for the idea that beetle host specificity varies significantly among feeding guilds or that phylogenetic relationships at the family and superfamily level have a significant effect on host specialization, whether calculated using plants determined to the genus or species level (Fig. 5). This clearly does not rule out the possibility that sampling of other orders of insects would not reveal such differences.

Future studies using comparable methodologies to uncover network structure, connectance, host specificity and the phylogenies of insects and plants stand to substantially improve our understanding of the factors, which maintain and generate diverse tropical communities.

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VI. Discussion

6.1. The plant-hispine community

The first part of this thesis (chapter 1, 2 and 3, Core section) presented the results of one of the largest and broadest surveys of hispine beetles (Cassidinae) conducted in the Neotropics. The objective was to identify hispine species assemblages and their associated host plants. According to the cluster analysis results (quantitative analysis), assemblages are defined by two main factors. The most important factor explaining beetle assemblages was linked to plant phylogeny at the order and/or family level. The second factor defining species assemblages was host plant habitat, such as understory, forest and tree-fall gap. Moreover, we concluded that a combination of different ecological factors (e.g., host range, size) and chemical defense (Strong, 1982) could explain the hispine species richness on particular host plants.

However, field observations of plant-hispine associations (mainly *occurrence* data) are subject to biases due to uncertain feeding records (e.g., "tourist individuals", mating sites, resting; Ødegaard, 2006). Our aims in the second part were thus to define the potential and realized feeding niches of hispine beetles, and to improve our field observations by removing uncertain feeding records. Feeding trial results have shown that hispine species fed on plant species within a single family (first degree of oligophagy; Jolivet, 1997) or at least, within a single order. These results confirmed earlier conclusions regarding hispine species assemblages.

Next we investigated the "architecture" of interactions among host plants and hispine beetles, and their host specificity, using a systematic approach, the food web approach. To characterize the structure of plant-hispine food webs in lowland Panama, based on *occurrence* data, we used three recent qualitative approaches: nestedness analysis, correspondence analysis and modularity analysis. Nestedness analysis results indicated that plant-hispine

food web structure was not significantly nested. Further, both modularity and correspondence analysis results identified different modules/compartments within the food web. This type of structure (modular or compartmented) has been encountered previously in plant-phytophagous insect food webs (Lewinsohn *et al*., 2006). Another characteristic of the plant-hispine food web was its high connectance value (C = 0.194). Such an extreme value of connectance is unusual and may be due to taxonomic aggregation, inadequate sampling or uncertain feeding records leading to "matrix noise" (Prado and Lewinsohn, 2004; Dunne *et al*., 2007). Moreover, high values of connectance eliminate the possibilities of simultaneously nested and modular structures (Fortuna *et al*., 2010). This was additionally confirmed by the nestedness analysis results. Nevertheless, high connectance could reflect the high host specificity exhibited by the hispine beetles, as each plant family represented one food web module densely connected, with almost no interaction between modules. By taking into account only one phylogenetic unit, such as the tribe Cephaloleiini, module limits could define those species sharing a common ancestor (see feeding clades in McKenna and Farrell, 2005), and indicate the evolutionary conservatism present in ecological interactions (Wilf *et al*., 2000; McKenna and Farrell, 2006; Gómez *et al*., 2010). Among modules defined by plant order levels, these limits could indicate habitat boundaries and differentiation in host use (Gómez *et al*., 2010), such as the different *Cephaloleia* species feeding on Zingiberales and Poales/Arecales (McKenna and Farrell, 2006).

As suggested by the two main factors (plant order and/or family, and habitats types) structuring their communities, hispine beetles can be considered as ecological-functional specialists (Ferry-Graham *et al*., 2002). The host specificity primarily responsible for the modular structure encountered in plant-hispine food webs reduces interspecific competition among hispines (Strong, 1982; Dyer *et al*., 2007). Both feeding trials and food web structure demonstrated that hispine host specificity is mainly defined at the plant family level. But high connectance values indicate non-overlapping modules are densely connected (Fortuna *et al*., 2010), showing

that hispine beetles are generalists in feeding on plants at lower taxonomic levels. Hispine host plants are mainly either gap or understory dwelling plant species, and thus occur at low population densities in tropical forests (Wright, 2002). The low specificity at the plant family level likely allows hispine beetles with poor dispersal abilities to reduce host locating time and consequent exposure to predators (Coley and Barone, 1996).

6.2. Neotropical plant-beetle food webs

The final part of this thesis (chapter 4, Core section) examines seven Neotropical plant-beetle food webs in Costa Rica and Panama (with a minimum network size of 50 interacting species), each based on *direct feeding* observations. These food webs include four major phytophagous beetle families. We used the nestedness index to characterize their structure, and the effective specialization metric to estimate their host specificity. Our results have demonstrated a significant nested structure (highly asymmetric; Thébault and Fontaine, 2008) similar to that reported by Bascompte *et al.* (2003) for mutualistic networks. Moreover, we show that Central American food webs (or antagonistic networks) and mutualistic networks seemed to follow the same structuring rules, such as increasing nestedness value with increasing network size (Bascompte *et al.*, 2003). Nevertheless, Neotropical food webs tended to possess a smaller number of associations per species (Thébault and Fontaine, 2008), and thus decreasing their connectance values ranging from 0.009 to 0.037. Additionally, results of effective specialization reveal high host specificity with little variance due to beetle families or feeding guilds. By showing a high level of host specificity at both genus and species levels, Neotropical phytophagous beetles can be considered as a highly selective pressure on plant species. Specialized beetles can dramatically reduce recruitment near reproductive plant adults, which tend to support high level of pests. Further, specialized beetles with poor dispersal abilities tend to concentrate their attack on highly dense patches of their host, such as leaf miner hispines on *Heliconia* (C. Meskens and D. M. Windsor, *personal observations*). These

specialized beetles may thus play a major role in maintaining high levels of tropical plant diversity ("negative density dependent"; Janzen, 1970; Connell, 1971; Wright, 2002). Nevertheless, the rolled-leaf hispines can not be considered as a highly selective pressure because their impact on host plants is limited due to their tiny damages to young leaves, and their host specificity at the family and/or order level.

Our results have showed a high level of host specificity for phytophagous beetles in Central America. Nevertheless, we have demonstrated that this high host specificity can be exhibited at different plant taxonomic levels (order/family for mainly rolled-leaf hispines vs. genus/species for Chrysomeloidea, Buprestoidea and Curculionoidea), and that host specificity does not vary significantly among beetles feeding guilds. These results tended to confirm recent studies indicating a strong latitudinal gradient in host specificity of phytophagous insects, e.g., larval Lepidoptera are more specialized (narrower diet range) in the Tropics (Dyer *et al*., 2007).

6.3. About the type of data collected

There are different sampling methods to study plant-herbivore insect interactions, e.g., canopy fogging, light and Malaise trapping, mass collecting, rearing (Novotny and Basset, 2005). The resulting data sets include two types of data: *direct feeding* observations or indirect observations (*occurrence* data). Indirect observations may lead to obtain a plant-insect matrix with a large number of "rare" or uncertain plant-beetle associations (Novotny and Basset, 2005; Blüthgen *et al*., 2008). Even though analyses of the hispine food webs based in Panama, and the Central America beetle food webs have revealed similar results of high level of beetle host specificity (at different taxonomic levels), the sampling procedures on which they are based highlight a major difference in the type of data sets (*occurrence* data vs. *direct feeding* data).

Four main points may explain and moderate this high host specificity. First, the hispine food web in Panama (*occurrence* data) possessed the highest connectance value, mainly due to matrix noise. High connectance does not allow a simultaneously nested and modular food web structure (Fortuna *et al*., 2010). Matrix noise dramatically increases interaction richness and similarly for other descriptor values, in comparison to a matrix constructed with *direct feeding* observations. All food webs resulting from this procedure showed lower connectance. Thus, the use of *occurrence* data in food webs is likely to lead to underestimates of host specificity. To decrease matrix noise and to evaluate beetle host specificity more reliably in food webs based on *occurrence* data, we removed erroneous associations and beating sheet data, which are well known to include tourists (Ødegaard, 2006). For example, we originally scored *Spaethiella tristis* Boheman on *Chusquea simpliciflora* Munro (Poales, Poaceae), but closer taxonomic scrutiny revealed that this was smaller undescribed species of *Spaethiella* known only from Poaceae (D. Windsor and L. Sekerka, *personal observations*). Moreover, to estimate the effect of matrix noise (uncertain records or erroneous host affiliations are common; Novotny *et al*., 2002) and rare species (Barone, 1998) on host specificity estimation, we computed networks by removing all records where less than one, five, 10, 20 and 30 individuals were observed feeding on a single plant species (record rarefaction). The computed networks using the limit of one and 10 individuals are indicated respectively with the suffixes (-1) and (-10) (Fig. 16). Indeed, as we removed matrix noise from *occurrence* food webs, values converged on those from *feeding* data food webs (Fig. 16; Meskens *et al., unpublished data*). As the minimum number of individuals was increased, host specificity significantly increased for *occurrence* food webs (Meskens *et al., unpublished data*). If the minimum acceptable number of observations was not appropriate for the sample size, the number of species remaining in the analysis necessarily decreased. As the plant-beetle species ratio gets closer to 1:1, we therefore begin to overestimate host specificity. In *feeding* studies, these uncertain records are almost absent, as these matrices are based on direct observations, thus the results for computed food webs are

similar for empirical *feeding* food webs (Meskens *et al., unpublished data*). Removing unsupported species runs the risk of removing accurate observations on rare species. Rare species represent an important part of rainforest biodiversity (Novotny and Basset, 2000) and their removal may decrease the estimation of real host specificity. To investigate host specificity of rare species, studies must be long-term surveys with large sample sizes (Novotny and Basset, 2000; Novotny *et al*., 2006).

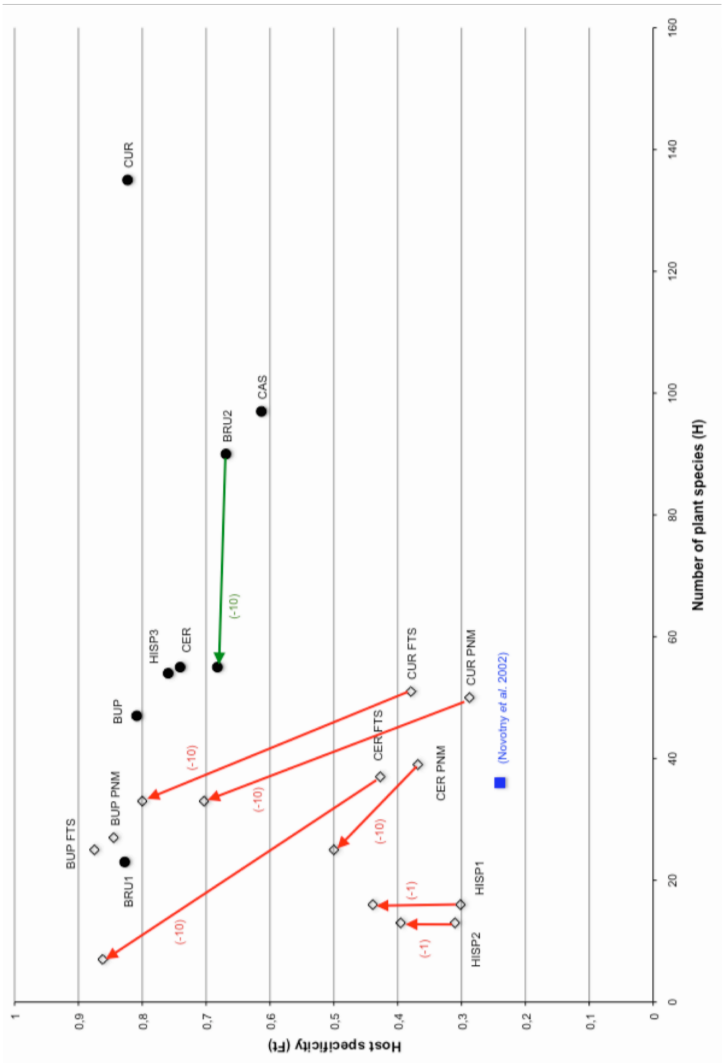

Fig. 16. Comparison of host specificity (F_t = B/L) vs. number of plant species (H) in *feeding* data networks (black circles), and in *occurrence* data networks (diamonds) obtained by XY analysis. Red arrows represent the effect of removing "matrix noise" from *occurrence* data networks; green arrow represents the effect of removing "matrix noise" from *feeding* data networks; blue square indicates host specificity result for Coleoptera feeding on trees in New Guinea (F_t = 0.24; Novotny et al. 2002); rolled-leaf hispine beetles, Panama (HISP1) = crosses; rolled-leaf hispine beetles, Costa Rica (HISP2) = diamonds; Curculionidae, Parque Natural Metropolitano, Panama (CUR PNM) = circles; Curculionidae, Fort Sherman, Panama (CUR FTS) = squares; Cerambycidae, Parque Natural Metropolitano, Panama (CER PNM) = empty circles; Cerambycidae, Fort Sherman, Panama (CER FTS) = triangles; Buprestidae Fort Sherman, Panama (BUP FTS) = empty squares; Bruchinae, Costa Rica (BRU2) = empty triangles.

Second, our study included a wide and disparate range of plant species. As observed by Barone (1998), the proportion of generalists in a study should decline as the number of plant species increases, which can also modify the estimation of host specificity.

Third, the present study integrates both larval and adult *feeding* records (emergences, direct feeding observations). Novotny and Basset (2005) pointed out that larval host plant ranges are more restricted than those of adults. For beetle taxa studied here, both larvae and adults feed on the same host plant species (only two exceptions in more than 100 species of Neotropical Cassidinae are known; D. M. Windsor, *personal observations*). Of the three superfamilies of phytophagous beetles included in our study, only Buprestoidea gave nearly identical results for both *direct feeding* and *occurrence* data (Meskens *et al., unpublished data*). The two *occurrence* food webs seem to be free of matrix noise, perhaps because adults rarely are found on plants other than those used for oviposition and larval development (D. M. Windsor, *personal observations*).

Fourth, the sampling of several *direct feeding* data sets in Central America took place over a wide area inducing some spatial separation of insects and plants. Therefore, some potential plant-beetle interactions cannot occur because of spatial incompatibility, decreasing connectance and overestimating host specificity. Nevertheless, at least for rolled-leaf hispine beetle species, Strong (1977a, b and 1982) concluded that considerable geographical replacement on host plants may occur in Central America.

VII. Conclusion

In 2002, more than 190 countries have decided that "achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional and national level as a contribution to poverty alleviation and to the benefit of all life on Earth" (UNEP, 2009; UNEP.org). The main drivers of global environment change (GEC) inducing this biodiversity loss are CO_2 enrichment, nitrogen deposition, climate, biotic invasions and land use. Their effects have been demonstrated on population abundances, community compositions and “less obvious alterations to the networks of interactions among species” (Tylianakis *et al.*, 2008). In order to quantify biodiversity loss, it is necessary to possess good data on species richness estimation and on “the background rate of change in ecological communities, particularly tropical ones” (Magurran and Querioz, 2010). Studying these ecological networks (or food webs) and communities may help us to understand the origin and maintenance of biodiversity, and how they might respond to and recover from disturbance, e.g., some particular network structure are known to more (or less) robust to environmental perturbations (Bascompte *et al*., 2003; Blüthgen *et al*., 2008).

In order to understand and estimate biodiversity, only focusing on individual interactions can be a dead-end approach. During the two last decades different broad research approaches (social science, ecology, biology…) are beginning to receive attention and recent analytical tools have been developed to investigate entire “communities” (social, ecological or metabolic networks) using a systemic approach. The food web approach includes different descriptors (e.g., connectance, effective specialization) and indexes (e.g., nestedness, modularity) to describe and to characterize structural properties of ecological networks, and to estimate host specificity. Even though food web analysis can be quantitative (weighted descriptors) or qualitative, the present study is focused on the second one, mainly due to the data sets analyzed (*direct feeding* observations; binary matrices). Defining network structure may help us to explore and to understand

ecological and evolutionary processes shaping biodiversity, and to compare it to other types of networks (e.g., mutualistic vs. antagonistic). Measuring host specificity of Tropical phytophagous insects allows us to estimate global arthropod diversity (Novotny *et al*., 2002). Moreover, this systemic approach may help us to make significant progress toward understanding how, and why, Tropical habitats are species-richer than Temperate ones (Dyer *et al*., 2007), and the underlying mechanisms responsible for it, such as the Janzen-Connell hypothesis ("negative density dependent"; Janzen, 1970; Connell, 1971; Jones and Comita, 2010).

As Tropical ecosystems are the richer ones on Earth (Wright, 2002), Tropical forests represent extraordinary opportunities to study and understand biodiversity. More than 40% of total terrestrial biodiversity encompass plant-herbivore food webs (or antagonistic ecological networks), mainly located in the Tropics (Price, 2002). This study is focused on plant-phytophagous beetle interactions in the Neotropics (lower Central America). The first step has targeted the interactions among hispine beetles (Coleoptera, Cassidinae; mainly rolled-leaf hispines) and their host plants in lowland Panama (*occurrence* data). To investigate more globally the food web structure and the host specificity of Central America phytophagous beetles (Costa Rica and Panama), the last part of this study is focused on three major Neotropical superfamilies of Coleoptera (Chrysomeloidea, Buprestoidea and Curculionoidea; four families), including seven new and published *direct feeding* data sets. This plant-phytophagous beetle system has been chosen for four main reasons. First, these beetle families include different feeding guilds (e.g., leaf-mining, leaf-chewing, seed-eating and subcortical-mining). Second, Neotropical phytophagous beetles are "suspected" to possess high host specificity. Third, a large number of plant-beetle species association records are available and well identified. Fourth, phylogenetic trees are starting to be available for most of these beetle clades.

Recent studies have documented low host specificity for phytophagous insects in tropical forests (Novotny *et al*., 2002; Ødegaard, 2006) and differences in host specificity among feeding guilds (Novotny and Basset, 2005). Other recent studies have shown a strong latitudinal gradient in host specificity of phytophagous insects, e.g., larval Lepidoptera are more specialized in the Tropics than in temperate zones (Dyer *et al*., 2007). Our results reveal little difference in specialization among feeding guilds (of lower Central America beetles) and higher overall values for host specificity (effective specialization), compared to other studies (Novotny *et al*., 2002; Novotny and Basset, 2005; Novotny *et al*., 2010). In addition, based on the seven long-term surveys of *direct feeding* data presented here, which are more reliable (Novotny *et al*., 2002), average Central American coleopteran effective specialization at the taxonomic species level ± SE was: F_{tS} = 0.76 ± 0.06, significantly higher than 0.24 given by Novotny *et al*. (2002) for phytophagous insects *feeding* records on trees. Beetle phylogeny (Hunt *et al*., 2007) appears to have a small or negligible affect on the levels of host specificity in the major phytophagous beetle superfamilies. These four beetle families may play a major role in generating and maintaining the exceptional biodiversity of Tropical forests ("negative density dependent"; Janzen, 1970; Connell, 1971; Jones and Comita, 2010). Nevertheless, the controversy and the debate concerning the difference of host specificity levels among tropical and temperate phytophagous insects, and the effects on plant populations are still in discussed (Novotny *et al*., 2002; Dyer *et al*., 2007; Novotny *et al*., 2010).

Moreover, our results show a difference of the taxonomic level of host specificity among hispine beetles (mainly rolled-leaf hispines, Cassidinae) and the other families and superfamilies. Rolled-leaf hispine beetles, which are known as ecological-habitat specialist, have maintained a highly specialized feeding association with the plant order and/or family. The modular structure of the plant-hispine food web in Panama (*occurrence* data) reflects the feeding clades identified by McKenna and Farrell (2005, 2006). This structure highlights the ecological and evolutionary scenarios

responsible for present-day plant-hispine interactions. This modular structure based on different densely connected modules dramatically increases the food web connectance (C = 0.194). At such high level of connectance, simultaneous nested and modular structures are rarely possible within one food web (Fortuna *et al*., 2010). On the other hand, the common nested structure identified for the seven Central America food webs (*direct feeding* data) reflects the beetle host specificity at the species level. Both structures (nested and modular) identified in this study "are thought to provide benefits to ecological communities" (Fortuna *et al*., 2010).

Regarding the analysis of plant-hispine food web in lowland Panama, a major improvement would be to target only well identified beetle phylogenetic units (e.g., Cephaloleiini and Arescini tribes separately) as feeding guilds, such as rolled-leaf hispines, are largely a mixture of phylogenetic and ecological aspects. This might "facilitate and ecological interpretation of a network, where the absence of a link may indicate a meaningful mismatch in species traits, which prevent its realization" (Blüthgen et al., 2010). Moreover, plant-hispine interaction sampling should take place in homogenous areas and during similar seasons to avoid non-overlapping host ranges, habitats and/or activity periods, which can lead to erroneous estimation of host specificity (Blüthgen *et al*., 2010).

In terms of perspectives of this study, further research direction using this innovative network approach would be to increase the number of host plant species surveyed, and to compare plant-hispine food webs in different homogenous areas. For example, in comparison to lower altitudes, higher altitudes tend to receive a larger amount of rain, to comprise relict species, and to possess examples indicative of *in situ* speciation (Escobar *et al*., 2006). Further, increasing the number of understory plant species, such as Arecales and Poales (shade-tolerant species investing more in defenses; Coley and Barone, 1996) in comparison to gap-dependent species (growing in high light) could allow us to better define and understand the modular structure of plant-hispine food web. Another research direction would to be

to link food web structure, host specificity and phylogenetic data for the entire Cassidinae subfamily, as basal hispine beetles are mainly endophagous feeding on monocotyledonous (family specialists) and true cassidines are exophagous and feeding mainly on dicotyledonous (species specialists). This study would allow us to better understand the evolutionary and ecological processes shaping contemporary plant-Cassidinae interactions, and present-day community structures.

Ecological network approach and its recent analytical tools may provide further broad research priorities to compare food web structures among Tropical and Temperate zones, in which phytophagous insects are suspected to possess different levels of host specificity. Several recent studies investigated the controversy of ecological network structure and stability (Tylianakis *et al*., 2008). The study of ecological network stability seems to be another broad research priority as the recent meta-analysis by Thébault and Fontaine (2010) demonstrated strong variations in the stability among mutualistic and tropich networks (or food webs) due to mainly indirect interactions, such as apparent competition in trophic networks. Moreover, this approach can be applied on different systems, such as crop plants and pests and/or pests and biological control agents (e.g., Aphididae and their Hymenoptera parasitoids). Identifying modular or nested structure in food webs combined with phylogenetic and ecological approaches may help us to better understand why some ecosystems are richer than others, to predict responses of entire communities to future effects of GEC in order to choose and prioritize conservation strategies.

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