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University of South Bohemia in České Budějovice
Faculty of Science

A cross-continental comparison of fruit-and seed-feeding insects in the rainforests of Panama, Thailand and Papua New Guinea

Ph.D. Thesis

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České Budějovice 2019

This thesis can be cited as:

Dahl, Chris. 2019: A cross-continental comparison of fruit-and seed-feeding insects in the rainforests of Panama, Thailand and Papua New Guinea. Ph.D. Thesis Series, No. 17. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, (158 pp).

Annotation

The aim of this thesis is to investigate community assemblages of fruit and seed feeding insects across three distinct biogeographic ForestGEO plots of Baro Colorado in Panama, Khao Chong in Thailand and Wanang in Papua New Guinea. It studies fruit and seed syndromes from which insects were reared per plant species across this rain forest plots, and describe host specialization in fruit and seed feeding insects. More specially, to compare seed feeding insects and their rate of seed attack among different insect groups are consistent across rainforest sites. Further it use plant phylogeny to explore plant floristic diversity, and explain ecological role of seed insect specialization in regulating plant species dynamic in maintaining high plant diversity in tropical rainforest regions.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 4 October 2019

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Chris Nazipa Dahl

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Entomology, Biology Centre CAS, supporting doctoral studies in the Entomology study programme.

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Financial support

The completion of projects presented in this thesis were funded through Czech Science Foundation (GAČR) Grant 13-09979S and 16-20825S; the Grant Agency of the University of South Bohemia (Grant GA JU 152/2016/P); the Centrum of Excellence for Tropical Biology (GAČR grant 14-36098G); the Center for Tropical Forest Science (small grant); and European Research Council Advanced Grant 669609. Field work on BCI was funded by a postdoctoral grant from the Academy of Finland to Sofia Gripenberg, Grants from the Smithsonian Institution Barcoding Opportunity FY013 and FY014 to Yves Basset.

Acknowledgements

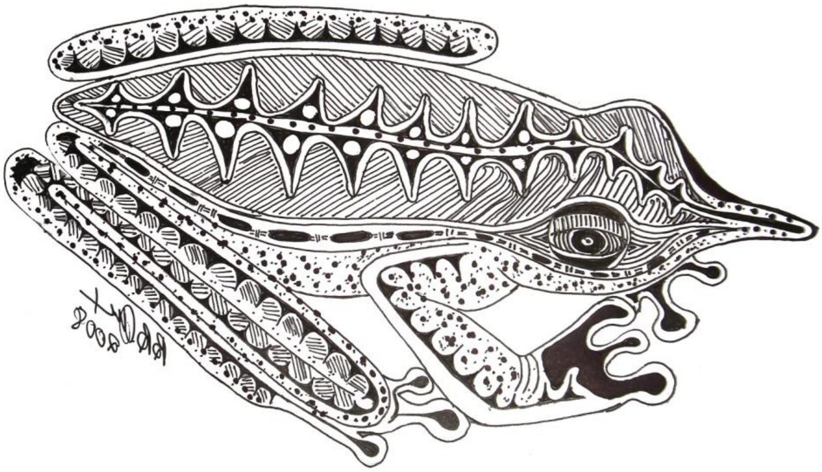
I would like to thank my supervisors, Yves Basset and Vojtěch Novotný for their significant contribution to this thesis. I am grateful to have been given the opportunity to study rainforests and their fruit-feeding insects across biogeographic distinct Forest Global Earth Observatory (ForestGEO) plots at Barro Colorado Island Barro (BCI) in Panama, Khao Chong (KHC) in Thailand and Wanang (WAN, PNG) in Papua New Guinea. I would like to extend my sincere thanks to ForestGEO; main scientists; Salomón Aguilar, Richard Condit, Robin Foster, Stephen Hubbell, and Rolando Pérez at the BCI ForestGEO plot; Sarayudh Bunyavejchewin, and Stuart J. Davies at the Thai KHC ForestGEO plot, and Vojtěch Novotný, and George Weiblen at the WAN ForestGEO plot. These scientists established the plots for the long term monitoring of forests so that ecologists can utilize the available data. Without the wealth of plant data from ForestGEO plots, this work would have not been possible.

I am indebted to numerous colleagues who assisted in various aspect of this project. In particular, Richard Čtvrtečka, Sofia Gripenberg, Héctor Barrios, Owen T. Lewis, Simon T. Segar, Petr Klimeš, Kateřina Sam, Joseph S. Wright and Philip T. Butterill made substantial contributions to data analyses. Scott E. Miller helped with identification of reared Lepidotera and DNA barcording. In-kind help was received from the Canadian Centre for DNA Barcoding and Southern China DNA for Barcoding Center that allowed to sequence insect specimens. I thank ForestGEO and the Smithsonian Tropical Research Institute (Panama), Khao Chong Botanical Garden (Thailand) and Binatang Research Centre and Wanang Conservation Area (BRC, PNG) for logistical support. Marjorie Cedeño, Indira Simon Chaves, Catalina D. Fernandez, Marleny Rivera (Panama); Pitoon Kongnoo, Montarika Panmeng, Sutipun Putnaul (Thailand), Jerome Doboria, Roll Lilip, Jonah Filip, Dominic Rinan and Ruma Umari (PNG) collected most of the insect material. My huge gratitude belongs paraecologists at the New Guinea Binatang Research Center, Madang. Furthermore, I am thankful to the Smithsonian Tropical Research Institute and the National Research Council of Thailand for the collecting permits; to the Madang Provincial Government and the Conservation and Environment Protection Authority of Papua New Guinea for research support and export permits.

I am thankful for sharing ideas and experience over the years with the team members of the Department of Eology at the Biology Centre of the Czech Academy of Sciences, namely: Čížek Lukáš, Antonin Krasa, Pavel Matos, Jimmy Moses, Conor Redmond, Legi Sam, Nichola Plowman, Pagi S. Toko, Carlo Lutz Seifert, Piotr Szefer, Daniel Souto-Vilarós and others, too numerous to mention. Many thanks to the miracle laddies: Jarmila Bajzeková, Michaela Borovanská, Eva Chocholová, Klárá Aurová and Hana Konvičková for logistical support.

Further, I would like to thank the Dahl and Kinibel families at Mazidaben (Buai roots), the Mambuan Family in Bogia and the Melemal Family in Marangis Village, Karkar Island, Madang Province. Most important, I am deeply grateful for the unlimited love and support of wife Liza Bardonado Dahl, daughter Christina E. Dahl and son Christian B. Dahl. Last, but not the least I would like to dedicate this thesis to the following important people who are sorely missed. In memory of Cathy Melemal Dahl (mama), Freddy Kinibel Dahl (papa) and Larry J. Orsak (a friend and mentor). Each passed on while I was abroad undertaking this Ph.D. study at the University of South Bohemia and Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic.

‘Wantaim dispela, mi tok bikpela *tenkyu’* (Pidgin), *khopkhun* (Thai) and *gracias* (Spanish): with this, a very many thank you, much love.



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18 June 2019

Dr OldrichNedved
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Dear Dr Nedved,

In anticipation of the submission of the PhD thesis of Mr Chris Dahl, I am detailing below the chapters that Chris will include in his thesis, as well as his contribution to each of these chapters.

Should you need more information on this matter, please do not hesitate to write to me.

Yours sincerely,

Yves Basset
ForestGEO Entomologist

Senior Editor, *Insect Conservation and Diversity*

Web pages: https://stri.si.edu/scientist/yves-basset

List of papers, manuscript, and author's contribution

The thesis is based on the following papers (listed chronologically):

I. **Dahl, Chris**, Richard Ctvrtecka, Sofia Gripenberg, Owen T. Lewis, Simon T. Segar, Petr Klimes, Katerina Sam, Dominic Rinan, Jonah Filip, Roll Lilip, Pitoon Kongnoo, Montarika Panmeng, Sutipun Putnau, Manat Reungaew, Marleny Rivera, Héctor Barrios, Stuart J. Davies, Sarayudh Bunyavejchewin, Joseph S. Wright, George D. Weiblen, Vojtech Novotny, Yves Basset. 2019. "The insect-focused classification of fruit syndromes in tropical rain forests: An inter-continental comparison". Biotropica 51 (1). pp. 39–49. (IF = 2.989). *[Chris Dahl conceived the study, participated in the field work and data collection, extraction of DNA samples, and was responsible for analysing the data and writing the manuscript]*.

II. Basset, Yves, **Chris Dahl**, Richard Ctvrtecka, Sofia Gripenberg, Owen T. Lewis, Simon T. Segar, Petr Klimes, Hector Barrios, John W. Brown, Sarayudh Bunyavejchewin, Buntika A. Butcher, Anthony I. Cognato, Stuart Davies, Ondrej Kaman, Milos Knizek, Scott E. Miller, Geoffrey E. Morse, Vojtech Novotny, Nantachai Pongpattananurak, Pairot Pramual, Donald L. J. Quicke, Robert K. Robbins, Watana Sakchoowong, Mark Schutze, Eero J. Vesterinen, Wen-zhi Wang, Yun-yu Wang, George Weiblen, Joseph S. Wright. 2018. "A cross-continental comparison of assemblages of seed-and fruit-feeding insects in tropical rainforests: faunal composition and rates of attack". Journal of Biogeoraphy 45 (6). pp. 1395–1407. (IF = 4.154). *[Chris Dahl helped to conceive the study, participated in the field work and data collection, extraction of DNA samples, participated to the analyses of data and contributed significantly to the writing of the manuscript]*.

III. **Dahl**, **Chris,** Richard Čtvrtečka, Sofia Gripenberg, Owen T. Lewis, Scott E. Miller, Philip T. Butterill, Simon T. Segar, Petr Klimeš, Dominic Rinan, Jonah Filip, Roll Lilip, Pitoon Kongnoo, Montarika Panmeng, Sutipun Putnaul, Manat Reungaew, Marleny Rivera, Héctor Barrios, Stuart J. Davies, Sarayudh Bunyavejchewin, Joseph S. Wright, George D. Weiblen, Vojtěch Novotný, and Yves Basset. "Plant - insect frugivore interaction networks in tropical forests: the effects of seasonality" (Manuscript). *[Chris Dahl conceived the study, participated in the field work and data collection, extraction of DNA samples, and was responsible for analysing the data and writing the manuscript].*

IV. Basset, Yves, Richard Ctvrteck, **Chris Dahl**, Scott E. Miller, Donald L. J. Quicke, Simon T. Segar, Héctor Barrios, Roger A. Beaver, John W. Brown, Sarayudh Bunyavejchewin, Sofia Gripenberg, Miloš Knížek, Pitoon Kongnoo, Owen T. Lewis, Nantachai Pongpattananurak, Pairot Pramual, Watana Sakchoowong and Mark Schutz. 2019. "Insect assemblages attacking seeds and fruits in a rainforest in Thailand". Entomological Science 22 (2). pp. 137-150. (IF = 1.073). *[Chris Dahl helped to conceive the study, participated in the field work and data collection, extraction of DNA samples, participated to the analyses of data and contributed to the writing of the manuscript].*

Co-author's agreement:

Yves Basset, and Vojtěch Novotný the supervisors of this Ph.D. thesis and co-author of chapters presented, fully acknowledges the major contribution of Chris Dahl.

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Yves Basset, Ph.D.

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Prof. RNDr. Vojtěch Novotný, CSc

Yves Basset, the corresponding author of ‘A cross-continental comparison of assemblages of seed-and fruit-feeding insects in tropical rainforests: faunal composition and rates of attack’ presented in Chapter II, supervisor of Ph.D. thesis and co-author of all presented papers, fully acknowledges the contribution of Chris Dahl in papers presented here.

Yves Basset, the corresponding author of ‘Insect assemblages attacking seeds and fruits in a rainforest in Thailand’ presented in Chapter IV, supervisor of Ph.D. thesis and co-author of all presented papers, fully acknowledges the contribution of Chris Dahl in papers presented here.

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Yves Basset, Ph.D.

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Introduction

Introduction

History and distribution of tropical rainforests

Where are the tropical rainforests of the world? I have always considered it for granted that they start right behind the fence of our garden in Papua New Guinea, only later to realize that this is not a generally shared perspective. Global distribution of rainforests is limited to areas with warm and moist climates that in our present climate form a single belt around the equator (Figure 1). The present composition of tropical rainforests is linked to the past of continental drift. Differences among regions have interested biogeographers, at least since the founder of the discipline of biogeography Alfred R. Wallace (who, in his explorations, nearly made it as far as the fence of my ancestor's garden in Papua New Guinea). Alfred R.Wallace, using his experience from sea voyages to S. America and SE Asia, demarcated distinct zoogeoraphic flora and fauna in the tropics (Wallace, 1876). His biogeographic regions have survived 150 years of scientific progress and have been only slightly adjusted by modern analyses (Holt et al., 2013). Wallace also identified the greatest discontinuity in animal distribution, referred to as 'the Wallace's line'. The species turnover along altitudinal gradient, one of key ecological gradients, was described from the tropics by Alexander Von Humboldt as a result of his 1799 expedition to the Andes in South America. Since then, modern remote sensing mapping techniques and databasing of museum collections have advanced our knowledge on broad scale patterns of distribution of plants and animals across tropical regions of the world (Barthlott et al., 2007). On the theoretical level, plate tectonics represent the major progress in our understanding of the forest distribution since the times of Wallace and Humboldt. The present distribution of rainforests is also closely related to rainfall gradients. For instance, high rainfall gradient may varies in relation with distance from equator, with seemingly change in floristic diversity and vegetation structure (Coley and Barone, 1996; Givnish, 1999). For example, Neotropical forests are characterized by a high abundance of understory fruiting shrubs and lianas (Corlett and Primack, 2006), in contrast, Southeast Asia forests are dominated by dipterocarps with seeds dispersed by wind during mass-fruiting events (Corlett and Primack, 2006; Baltzer and Davies, 2012), while Australasian forests have a high diversity of plant species that produce large, fleshy fruits (Chen et al., 2017; Dahl et al., 2019).

Figure 1. Map of the current potential tropical rainforest distribution (excluding anthropogenic deforestation, adapted from Michael Evans, Earthtimes.org; 2011).

Modern studies, even those focused on contemporary, community level ecological processes, should be replicated in different biogeographic areas, thus testing the observed patterns and mechanisms on multiple, partly independent continental pools of species. In my study, I am using three such biogeographic areas, with sites in the Neotropics, continental Oriental region and Australian region (which is most closely related to Australia).

Insect diversity in the tropics

Tropical forests cover approximately 19.6 million km^2 of the earth surface (Pimm and Sugden, 1994) and they host a majority of about 1.1 million insect species presently known to science (e.g., Stork, 2018). The highest insect diversity in tropical rainforest areas are explained by four general hypotheses (Turner et al., 1987). These include: (1) the historic hypothesis suggesting that a consistent and steady climate is conducive to high diversity in the tropic as a result of high speciation and low extinction rates; (2) the structural hypothesis, suggesting that there are many more ecological niches available for organisms in structurally complex rainforests (and coral reefs) in the tropics than in other ecosystems; (3) the dynamic hypothesis, suggesting that the intensity of predation and competition prevents any single species from ecosystem dominance, thus promoting high diversity; and (4) the energetic hypothesis, postulating that higher available energy translates into more resources and thus allows the coexistence of more species. These hypotheses call for either phylogenetic or ecological methods of study. Ecological studies study should have two stages: mapping the diversity patterns of plant and animal taxa, followed by the analysis of their interactions. My study focuses on the ecological, rather than phylogenetic, aspects of diversity. It incorporates community level assemblages to the study of interaction webs, specifically between plants and frugivorous insects.

The diversity and distribution of insect fauna varies considerably along latitudinal gradients (Novotny et al., 2006; Poisot et al., 2012; Novotny and Miller, 2014). Even after more than two centuries of studies of tropical insects, insect diversity remains poorly documented (e.g., Erwin, 1982; Basset et al., 2012; Novotny and Miller, 2014). For instance, global diversity estimates are often extrapolated from data obtained by small-scale insect community sampling (Erwin, 1982; Novotny et al., 2002; Basset et al., 2012). In plants, the latitudinal gradient in diversity is better documented (Barthlott et al., 2007; Anderson-Teixeira et al., 2014) than for insects (Basset et al., 2012). For example, there are 5 to 10 times more species of plants within 10,000 km^2 in the tropics compared to the temperate regions (e.g., Novotny et al., 2006; Basset et al., 2012), while locally, tropical lowland forests have on average 6 times more woody plant species in one hectare than the temperate forests (Novotny et al., 2006).

Estimating rainforest arthropod diversity remains difficult even on a local scale (May, 1988; Novotny et al., 2002). Among several entomological studies, Basset et al., (2012) provides the best data so far, as their study recorded a total of 130,000 individual arthropods including 6,144 species. They used these data to extrapolate to estimate that about 25,000 arthropod species may occur within 6,000 hectares of lowland forest in Panama. Perhaps the most discussed insect diversity estimate on a global scale was Erwin, (1982) based on the data he collected from the forest canopy with the fogging method. Canopy arthropod diversity was then extrapolated to 30 million species of arthropods on the Earth. But later, several authors disputed the 30 million global diversity estimate and reduced it to a range between 4.8 and 6.6 million (Basset et al., 1996; Ødegaard, 2000; Novotny et al., 2002; Stork, 2007; Hamilton et al., 2010). These estimates were based on studies of insects feeding on particular host plants and their specificity, in combination with counts of higher plant taxa in the tropics. These estimates may be improved by incorporating phylogeny to further estimates herbivore specificity (Novotny et al., 2002; Kitching, 2006; Basset et al., 2012).

Global insect diversity estimates may inflate in the near future because a range of small-bodied taxa, including hymenopteran parasitoids and dipterans, have not been properly documented from tropical regions in the past, but could be so in the future with the help of molecular methods (Novotny and Miller, 2014). These molecular taxonomic tools, including DNA barcoding (Novotny and Miller, 2014), have provided molecular information on an increasing number of insect species, to further advance morphological taxonomy. The advancement in molecular tools has increased the rate of species identification and description of new species. Molecular data can be also used to identify cryptic species, and reveal phylogenetic relationships among them (Miller, 2007). Most recently, DNA data have also revealed trophic relationship between species, including herbivores and their host plants (Miller, 2007). My research greatly relies on barcoding as an important methods of species identification, and a next step should be using such data to determine insect-plant trophic interactions in frugivorous insects and their hosts.

Knowledge of rainforest food web dynamics between plants and insects is central to our understanding of the mechanisms maintaining the high diversity of tropical forest vegetation (e.g., Paine, 1966; Novotny, 2009; Novotny et al., 2010). In particular, plant-herbivore interactions, together with plant-pathogen interactions, are key for maintaining the extraordinary diversity of tropical forests (Janzen, 1980; Condon et al., 2014). At the same time, plant-herbivore networks are difficult to study due to their extreme complexity. For instance, there are only few studies investigating plant-herbivore food web from a tropical forest (Gripenberg et al., 2019). There is only one, extrapolation of the dimension of the food web, for a New Guinean lowland forest, suggesting that 200 species of trees may support about 9,600 herbivorous insects that comprised 50,000 plant-herbivore interactions (Novotny et al., 2010). These webs include various guilds of herbivores, studied to a very different degree (Novotny and Basset, 2005; Basset et al., 2018).

In this study, I focused on a potentially important guild of herbivores that can directly affect host plant survival, but is also poorly studied. Fruit and seed-feeding insects can significantly contribute to seed mortality and therefore influence plant population dynamics (Janzen, 1980; Lewis and Gripenberg, 2008). Despite their importance, however, few local, let alone cross continental studies, have described in satisfactory detail the assemblages of fruit and seed-feeding insects in tropical rainforests (e.g., Ctvrtecka et al., 2014; Ctvrtecka et al., 2016; Basset et al., 2018; Dahl et al., 2019; Gripenberg et al., 2019). This is because these endophytic herbivores are difficult to locate and sample by targeted sampling protocols. In most cases, “blind” sampling of all fruits and subsequent rearing of insects is necessary, and hundreds of kilograms of fruit are required in order to obtain well resolved plant-frugivore webs (Ctvrtecka et al., 2014; Sam et al., 2017). Such massive sampling was indeed necessary in our target studies of the three rainforest sites.

Plant fruit syndromes and dispersal

Herbivore ecology, host specialization and diversity can greatly differ among individual herbivore guilds. A guild is defined as groups of species using the same resource in the same way (Basset and Arthington, 1992; Novotny et al., 2010). There is no well established classification system for guilds, and the definition of plant ‘resource’ is also vague. Novotny et al., (2010) provides one refined guild classification system, with fruits and seeds as one of the resources exploited in different ways (e.g., chewing, sucking) by individual guilds. For the purpose of our study, we sampled fruits per plant species and classified fruits into each finer categories, fruit syndromes (Dahl et al., 2019), attacked by specific insect guilds and taxa (Basset et al., 2018).

In tropical forests, fruit-bearing plants often rely on dispersers, such as frugivorous birds and mammals, to disperse fruits and seeds away from the parent trees in much larger degree than in temperate vegetation, where wind dispersal is much more common (Janson, 1983; Gautier-Hion et al., 1985; Florchinger et al., 2010). A large majority, about 70% of plant species, produce fleshy fruits in tropical forests (Florchinger et al., 2010; Grimbacher et al., 2013). The interaction between a plant species and its disperser is fraught with difficulties since the plant has to protect its seeds from predators and as well attract dispersers to its fruits. This involves various strategies that can morphologically manifest in a number of fruit and seed syndromes (i.e., sets of life history traits), evolved in response to pressure by dispersers as well as seed predators (Gautier-Hion et al., 1985). In this regard, dispersal syndromes are used to describe plants with certain fruit morphology adapted to specific dispersal modes by particular species of birds or mammals (Gautier-Hion et al., 1985). For instance, there is a ‘bird-monkey syndrome’ or a ‘ruminant-rodent-elephant’ dispersal syndrome. The ‘bird-monkey syndromes’ include fruits that are brightly coloured and seeds arilated with fleshy mesocarp (such as berries and drupes), while the ‘ruminant-rodent-elephant’ syndromes include fruits that are dull coloured with a distinct odour, with fibrous flesh, hard outer exocarp and seeds being well protected (Janson, 1983; Gautier-Hion et al., 1985).

There is extensive literature describing fruit and seed traits that considers fruit morphologies, size, weight, colour of the fruits and seeds for plants dispersed by frugivorous and granivorous animals (e.g., Howe and Smallwood, 1982; Janson, 1983; Gautier-Hion et al., 1985; Florchinger et al., 2010; Dahl et al., 2019). Dispersers may also have different preferences in different ecosystems. For instance, temperate forests birds prefer fruits that have red, black or blue colour, while birds in the tropics prefer more coloured fruits, such as red, black, white, blue and purple fruits (Janson, 1983; Gautier-Hion et al., 1985). However, the impact of insect predators on the fruit and seed morphology, and insect response to fruit traits possibly evolved in response to pressures by dispersers, are known to a much smaller degree. Our study contributes to this important issue.

Plant fruit-seed syndromes and insect seed predators

Tropical forests are known for high number of tree species and low densities of adult trees per species, compared to temperate forests that are dominated by a single or a few tree species (Janzen, 1970). Spatial distribution of individual mature tree species as well as their seedlings provide clear indirect evidence for negative density dependent factors regulating the population dynamics of tropical trees (LaManna et al., 2017). These processes can impact the seed, seedling or sapling stages, and less likely the mature stage of trees (Nathan and Muller-Landau, 2000; Lewis and Gripenberg, 2008).

The Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971), postulates that host-specific enemies, such as herbivorous insects and fungal pathogens, are the main factors important in regulating plant abundance at high densities, either by density-dependent or distance-dependent mortality. Herbivores and pathogens reach higher densities on locally abundant plant species (so called resource-concentration effect, Grez and Gonzalez, 1995), as high density patches are more easy to find and colonize. Further, parent trees may serve as sources of infection to their seeds and seedlings nearby, thus generating distance-dependent mortality (Janzen, 1970; Lewis and Gripenberg, 2008). Evidently, insect predators feeding on fruits and seeds may be important to the forest dynamics as they can kill individual plants – when still in the seed stage. For this effect to take place, insect seed predators must diversify to a larger extent to be host-specific because this will enhance their ability to lower recruitment of locally abundance species (Lewis and Gripenberg, 2008), an important ecological prerequisite for the Janzen-Connell hypothesis (Janzen, 1980; Connell, 1971). However, the species richness, host specialization and abundance of insects attacking seeds and fruits vary greatly among plant species and across rainforest regions (Stephenson, 1980; Martin and Lee, 1993; Basset et al., 2018). A strong distance-dependent mortality was documented in some systems (Wright, 1983) but other studies showed low levels of insect attacks on seeds and fruits, inconsistent with the Janzen-Connell hypothesis (Sam et al., 2017). On the other hand, some effects may be subtle and difficult to study. For instance, certain plant species respond to predispersal attack by insect predators by aborting developing seeds and fruits, thus minimizing energy loss while leading to under-estimation of the immediate impact that insects have on fruits and seeds in rainforests (Ehrlen, 1991).

There are two dominant approaches to assess the impact of seed predators on forest dynamics: experimental exclusion of insects by insecticides, and quantitative mapping of fruit-frugivore trophic webs, thus provides broader description of patterns of host use by seed predators, and specificity of plant enemies important for predicting communit-level effects (Gripenberg et al., 2019), thought to maintaining the high diversity of woody plant species (Janzen, 1970; Connell, 1971). The experiments can demonstrate the overall effects of insects but at the same time they are “black box” experiments, as we do not usually know which insects species have been excluded (Bagchi et al., 2014; Basset et al., 2019). The mapping of food webs can be accomplished by insect fruit and seed rearing programmes (e.g., Basset et al., 2018; Dah et al., 2019). Manipulative experiments may represent another promising approach. For instance, Auld, (1986) conducted manipulative experiment with weevils by offering them seeds of two potential host species, *Acacia elongate* and *A. myrtifolia*. Not surprisingly, he found that the weevil species had impacted significantly seed survival and growth near the trees, and away from parent trees. Similarly, Brancalion et al., (2010) studied insect seed predation on a palm species and observed that palm seeds were heavily infected during both post-dispersal and pre-dispersal phase, exhibiting distance-dependent effect as postulated by Janzen, (1970). It appears that bruchids, scolytids and possibly other beetle seed predators may be important in density-dependent effects on rainforest trees (Dracxler et al., 2010). These beetles attack seeds over seedlings, of palm and leguminous trees. Bruchine beetles are the main species responsible for Janzen–Connell in neotropical forests (Janzen, 1975). In Costa Rica, two species of bruchine beetles are host specific to *Guazuma ulmifolia*. One species attack fruits and seeds while still attach on the tree and the other attack mature seeds after they have fallen to the ground (Janzen, 1975).

The understanding of plant-herbivore dynamics requires detailed understanding of plant-herbivore interaction webs across plant and herbivore species, as well as rainforest ecosystems. Unfortunately, seed predation across a variety of tropical rainforests has been little studied in comparison with decomposition, folivory or pollination (Lewis and Gripenberg, 2008). In our study, we have conducted large, intense sampling of such interaction webs, focusing on inter-continental comparisons among lowland rainforests. This study should help to answer one of the pressing questions of tropical forest ecology, viz. how they maintain their exceptional plant diversity.

Aims and scope of the study

Frugivorous insects feeding on seeds and fruits represent an excellent system for studying ecological interactions that potentially maintain high tropical diversity. The aim of this dissertation is to quantitatively map plant-frugivore insect webs in three highly diverse forests representing three biogeographical regions of the tropics. This should allow us to assess the role of frugivores in forest dynamics, and to understand better the assembly rules for plant-insect food webs in tropical forests. The study is based on a large-scale rearing of insects within three well-documented forest sites, each with a Forest Global Earth Observatory vegetation plots (ForestGEO; Anderson-Teixeira et al., 2014): Barro Colorado Island in Panama; Khao Chong in Thailand and Wanang in Papua New Guinea.

Chapter I develops a new classification of rain forest plants into eight fruit syndromes based on fruit morphology and traits relevant to fruit-feeding insects. This is a novel classification taking into account the perspective of insect frugivores, rather than plants or vertebrate dispersers, as was the case in previous classification systems. We show that plant species bearing fleshy, indehiscent fruits with multiple seeds are important at all three sites. We also show that the Panamanian site has a high proportion of plant species bearing dry fruits but not the Thai and New Guinean sites, where fleshy drupes with thin mesocarps are mostly dominant. Dry winged seeds (that do not develop in capsules) reflected the occurrence of Dipterocarpaceae in KHC, Thailand. These differences may also determine the distribution of frugivorous insect communities. We show that fruit syndromes and colours are phylogenetically flexible traits with clustering at either plant genus or family levels. Despite such flexibility, plant phylogeny was the most important factor explaining the overall differences in the composition of fruit syndrome among the three study sites. We expect that the plant syndrome system may become an effective explanatory framework for the structure and composition of plant-frugivore food webs, in addition to plant phylogeny.

Chapter II investigates community convergence among entire insect assemblages attacking seeds and fruits at the three ForestGEO plots. We observe large differences in insect faunal composition, species richness and guild structure among the study plots. High species richness at the Panama site is due to high availability of dry fruits. We observe that insect assemblages were weakly influenced by seed predation syndromes, and the effect of phylogeny also varies among sites. The probability of a fruit to be attacked at any one site depends more on seed availability than plant traits.

In **Chapter III** we examine 12 plant-frugivore interaction networks generated by two frugivore taxa (Coleoptera and Lepidoptera) using fruit syndromes for focal plant species from 10 plant families at the three ForestGEO sites. We show that the drier and more seasonal Panamanian site supports a higher species diversity of insects than the Thai and New Guinean sites. Both Coleoptera and Lepidoptera had a similarly high diversity per biomass of fruits across the surveyed sites. However, the Lepidoptera reached higher species diversity per standardized number of individuals than the Coleoptera. Only the abundance of Thai frugivore increases seasonally with increasing rainfall. However, we observe a lower diversity of frugivore insects attacked fruits during the dry season than the wet season. Large differences in insect abundance do not influence interaction web parameters (generality, vulnerability, H2’ diversity) across seasons. The potential of insect herbivores to cause Janzen-Connell effects varies across continental species pools of plants and insects, and among wet and dry seasons.

Finally, in **Chapter IV** we analyse three years of data from insects rearing from fruits and seeds at the Khao Chong site in southern Thailand. We observed that (1) 43% of insects pests, were mostly seed eaters of Dipterocarpaceae with seed syndromes C1 (dry winged seed) and A1.2 (fleshy drupe with thin mesocarp). (2) Most of individuals in insect families (Pyraloidea, Tortricidae, Curculionoidea, Tephritidae, and Scolytinae) were reared from dry fruits, compared to fleshy fruits and (3) only 8% of frugivore insects reared were parasitized. We observe a large variability in rate of attack on fruits among plant species at Khao Chong. We show that the most heavily attacked trees are locally common species with large basal area, in accordance with the resource concentration hypothesis. No strong effects of phylogeny on the rates of attack are observed. Hence, insects reared from fleshy fruits have more stable populations than those reared from dry fruits.

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Chapter I

The insect-focused classification of fruit syndromes in tropical rain forests: An inter-continental comparison

in Biotropica 51 (2):39–49

Received: 9 September 2018 | Accepted: 5 December 2018
DOI: 10.1111/btp.12622

ORIGINAL ARTICLE

WILEY bioTROPICA ASSOCIATION FOR TROPICAL BIOLOGY AND CONSERVATION

The insect-focused classification of fruit syndromes in tropical rain forests: An inter-continental comparison

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Funding information
Czech Science Foundation, Grant/Award Number: 16-20825S; Center for Tropical Forest Science; University of South Bohemia, Grant/Award Number: GA JU 152/2016/P

Abstract

We propose a new classification of rain forest plants into eight fruit syndromes, based on fruit morphology and other traits relevant to fruit-feeding insects. This classification is compared with other systems based on plant morphology or traits relevant to vertebrate fruit dispersers. Our syndromes are based on fruits sampled from 1,192 plant species at three Forest Global Earth Observatory plots: Barro Colorado Island (Panama), Khao Chong (Thailand), and Wanang (Papua New Guinea). The three plots differed widely in fruit syndrome composition. Plant species with fleshy, indehiscent fruits containing multiple seeds were important at all three sites. However, in Panama, a high proportion of species had dry fruits, while in New Guinea and Thailand, species with fleshy drupes and thin mesocarps were dominant. Species with dry, winged seeds that do not develop as capsules were important in Thailand, reflecting the local importance of Dipterocarpaceae. These differences can also determine differences among frugivorous insect communities. Fruit syndromes and colors were phylogenetically flexible traits at the scale studied, as only three of the eight seed syndromes, and one of the 10 colors, showed significant phylogenetic clustering at either genus or family levels. Plant phylogeny was, however, the most important factor explaining differences in overall fruit syndrome composition among individual plant families or genera across the three study sites.

Abstract in Melanesian is available with online material.

KEYWORDS

ForestGEO, fruit color, plant traits, seed dispersal, seed predation, tropical insects

1 | INTRODUCTION

Tropical rain forests are known for their high number of tree species in comparison with temperate forests. Seed dispersal and survival represent potentially important but poorly documented processes maintaining the high tropical diversity of plants (Janzen, 1970; Nathan & Muller-Landau, 2000). Fruit-feeding insects may influence plant demography because they can kill individual trees while they are still at the embryo stage (Ehrlen, 1996). For example, seed predators in the beetle families Bruchinae and Scolytinae are responsible for high mortality of dry seeds of some rain forest trees (Janzen, 1980; Peguero & Espelta, 2013), while predation rates on seeds in fleshy fruits appears to be much lower (Basset et al., 2018; Ctvrtecka, Sam, Miller, Weiblen, & Novotny, 2016; Sam et al., 2017). Tropical forest trees rely mostly on frugivorous birds and mammals for seed dispersal (Florchinger, Braun, Bohning-Gaese, & Schaefer, 2010; Gautier-Hion et al., 1985; Janson, 1983). This leads to high variability of tropical fruits and seeds in their morphology, color, and size (Florchinger et al., 2010; Janson, 1983). Fruits with fleshy tissues surrounding seeds are a food resource for many animals such as ants (Altshuler, 1999; Borges, 2015), birds (Erard, Théry, & Sabatier, 2007; Gautier-Hion et al., 1985; Herrera, 1981; Mack, 2000; Pizo & Vieira, 2004), and mammals (Cáceres, Ditirich, & Monteiro-Filho, 1999; Janson, 1983), including bats (Kalka, Smith, & Kalko, 2008; Shanahan, So, Compton, & Corlett, 2001) and primates (Gautier-Hion et al., 1985). Mutualistic interactions between fruiting plants and frugivorous animals represent a significant component of interaction webs in tropical rain forests, with potential to influence rain forest ecosystem dynamics (Correa, Álvarez, & Stevenson, 2015; Janzen, 1980).

To help explain the diversity of fruits and seeds in an ecological context, both botanists and zoologists have proposed their own classification systems of fruit syndromes (Table 1). These systems focus on seed and fruit morphology from the perspective of either plants, or their vertebrate dispersers. For example, zoologists have based their classification on fruit morphology, size, mass, and color relevant to animal visitation to fruiting trees (Janson, 1983; Gautier-Hion et al., 1985, Table 1). However, current classification systems ignore seed predation and frugivory by insects. Furthermore, existing classification systems can allow individual plant species to be placed in multiple classes (Table 1), making comparative analyses among individual species and sites difficult. Here, we define a new classification system of fruit syndromes relevant to insect predation which accounts for different modes of oviposition and larval and adult feeding by insects and which allows individual plant taxa to be classified in a single class (see Table 1, Supporting Information Table S1). We relate and compare these fruit syndromes with those proposed on the basis of botanical and vertebrate studies.

Fruit and seed morphology can be described by multiple continuous (e.g., size), and categorical (e.g., color) variables. These can be used to organize plant species into relatively homogeneous groups, for instance using multivariate analysis methods, and then look for ecological or phylogenetic interpretations of these groups. Alternatively, we can define suites of traits, that is, syndromes, known to be relevant to a particular ecological process, such as dispersal or seed predation, and examine their importance in various ecosystems or geographic areas. Such syndromes can be useful as long as they are rigorously defined (Table 1) and combine traits that are functionally relevant. For instance, fruit fleshiness, number and size of seeds, and physical protection of seeds by a mesocarp all relate to vulnerability to seed predation by insects so that the study of particular combinations of these traits can provide insights into the insect predation pressure on plants. As for any categorical classification of continuous variability involving multiple traits, syndromes represent a simplification, but can help generate ecological hypotheses. For instance, the definition of discrete life-history syndromes has contributed to the development of ecological theory in the context of succession (Turner, 2008) and plant responses to herbivory (Herms & Mattson, 1992).

Tropical forest trees produce a wider variety of fruits ranging from fleshy to dry (e.g., achenes, Armesto, Díaz, Papic, & Willson, 2001). Most fleshy fruits are dispersed by animals while dry fruits are usually dispersed through other means (Du, Mi, Liu, Chen, & Ma, 2009; Florchinger et al., 2010; Gautier-Hion et al., 1985; Howe & Smallwood, 1982; Janson, 1983; Mack, 1993; Valido, Schaefer, & Jordano, 2011). Multiple factors have contributed to the evolution of the wide range of fruit and seed types observed in tropical forests. To assess the role of different factors in shaping the diversity of fruit traits, a helpful approach is to compare the relative frequencies of fruit syndromes across multiple forest sites. Inter-continental comparisons of ecological patterns are highly instructive, as they show the variance of these patterns in evolutionarily distinct species pools (Primack & Corlett, 2005), but data for such comparisons are rarely available. Inter-continental comparisons can shed light on different patterns of seed distribution and mortality, shaped mostly by the evolution of flowering plants, and the selection of dispersal agents or seed predators (Bolmgren & Eriksson, 2010; Janzen, 1971; Lewis & Gripenberg, 2008). Tropical rain forests vary in plant species composition and vegetation structure. These forests may also differ in

TABLE 1 Three classification systems of fruits used in previous studies (botany and zoology systems) and in this study (a novel entomology system). Consistent shading across systems denotes similar or equivalent categories

Botany system	Zoology system	Entomology system
Related to plant morphology	**Related to frugivory and seed dispersal**	**Related to seed predation**
Hickey & King, 1981; Zomlefer, 1994	Janson, 1983; Gautier-Hion et al.,1985	This study
Categories mutually exclusive	Categories not mutually exclusive	Categories in most cases mutually exclusive
Code (B-), Category	Code (Z-), Category	Code (E-), Category
B-A. Succulent, fleshy fruit	**Z-A. Color**	**E-A. Drupe (one seed per fruit)**
B-A1 Drupe—a single seed***	Z-A1 Color either red, white, black, or mixed (mostly dry fruits)	A1. Fleshy drupe
B-A2 Berry—a single fruit with several seeds†††	Z-A2 Color either orange, brown, yellow, green, purple (mostly fleshy fruits)	E-A1.1 Fleshy drupe with thick mesocarp (>5 mm)***
B-A3 Multiple fruit with several seeds †††	**Z-B. Type of flesh**	E-A1.2 Fleshy drupe with thin mesocarp (<5 mm)***
B-B. Dry fruit	Z-B1 Juicy soft	E-A2. Non-fleshy drupe***
B-B1. Dehiscent fruit	Z-B2 Juicy fibrous	E-A2.1 Non-fleshy with thick mesocarp (>5 mm)***
B-B1.1 Legume***	**Z-C. Protective coat**	E-A2.2 Non-fleshy with thin mesocarp (<5 mm)***
B-B1.2 Follicle***	Z-C1 Dehiscent coat***	**E-B. Fruit with multiple seeds**
B-B1.3 Capsule***	Z-C2 With aril***	E-B1 Fleshy indehiscent fruit with multiple seeds†††
B-B1.4 Others (silique, silicula, lomentum, etc.)***	Z-C3 Indehiscent coat—thin husk***	E-B2 Non-fleshy dehiscent fruit with multiple seeds, (dehiscence typically across multiple axes)***
B-B2. Indehiscent fruit	Z-C4 Indehiscent coat—thick husk***	**E-C. Dry fruit/seed**
B-B2.1 Samara***	**Z-D. Seed size**	E-C1 Dry winged seed that do not develop in capsule***
B-B2.2 Nut***	**Z-E. Number of seeds per fruit**	E-C2 Multiple dry seeds (with or without wings) that do develop in capsule (dehiscence typically across one single axis)***
B-B2.3 Achene***	Z-E1 Fruits with multiple seeds***	
B-B2.4 Others (caryopsis, utricle, etc.)***		
B-B3. Schizocarpic fruit		
B-B3.1 Cremocarp***		
B-B3.2 Double samara***		

Main fruit classification categories of botany, zoology and entomology study system.

Each symbol denotes similar/equivalent fruit category used by botany, zoology and entomology system. Also each symbol identify and set apart each cell when printed.

seasonality, climate, and fruiting periods, as well as the composition of frugivore faunas (Corlett & Primack, 2006). For example, forests in the Neotropics are characterized by a high abundance of understory fruiting shrubs. In contrast, many forests in Southeast Asia are dominated by dipterocarps with seeds dispersed by wind during mass-fruiting events (Corlett & Primack, 2006). Australasian rain forests have a high diversity of plant species that produce large, fleshy fruits (Chen, Cornwell, Zhang, & Moles, 2017). These differences in the production of fruits in rain forests may impact the way fruits, and seeds are attacked by insects (Supporting Information Table S1). Therefore, it is important to document the distribution of fruit syndromes relevant to insects across rain forest locations in distinct biogeographical regions. Our insect-oriented classification of fruit syndromes is based on 1,192 plant species collected at three tropical forest sites in Panama, Thailand, and Papua New Guinea. We quantified plant diversity and abundance represented by each syndrome in a phylogenetic context and across the three continents. We use this information to explore the resource base for fruit and seed eating insects in tropical rain forests.

2 | METHODS

2.1 | Study sites

We sampled three Forest Global Earth Observatories (ForestGEO) plots in biogeographically distinct rain forest regions: Neotropical: Panama: Barro Colorado Island (BCI, 50 ha plot); Oriental: Thailand: Khao Chong (KHC, 24 ha plot); and Australasian: Papua New Guinea: Wanang (WAN, 50 ha plot). ForestGEO (http://www.forestgeo.si.edu/) is a global network of permanent forest plots established to study long term forest ecosystem dynamics (Anderson-Teixeira et al., 2014). All three of our study sites are located in undisturbed lowland forests, either wet (KHC, WAN) or with a moderate dry season (BCI). Important characteristics of their vegetation are summarized in Supporting Information Table S2; see also Anderson-Teixeira et al. (2014) for details. We have obtained data on seed and fruit-feeding insects at all three sites through extensive rearing programs (Basset et al., 2018; Ctvrtecka et al., 2016) that became the basis for our fruit classification systems (Table 1).

2.2 | Plant surveys

We sampled available fruits from all plant species within or near permanent forest plots. This protocol was initiated in 2010 at BCI and introduced at KHC and WAN in 2013 (Basset et al., 2018). Sampling took place over 3 or 4 years at each site. During the first survey year at each site, we searched and sampled fruits and seeds haphazardly from all locally available trees, shrubs, lianas and (more rarely) epiphytes and herbs. In subsequent years, we restricted our sampling to plant species found in 10 families that are commonly distributed in these forest regions. Eight of these families are well represented across three sites, and two other families are only important locally, at a single site (Supporting Information Table S3). Data on plant abundance were taken from the most recent ForestGEO plot survey at each plot; these surveys record all stems with DBH >1 cm every 5 years (Anderson-Teixeira et al., 2014).

2.3 | Fruit classification systems

Each plant species sampled was assigned to a category using our new entomocentric classification and compared to the previous botany and zoology systems (Table 1). The botany system is based on plant morphology, while the zoology system was motivated largely with respect to plant dispersal by vertebrates. Our entomology system is concerned primarily with seed predation by insects. The first dichotomy in the botany system is whether the fruit is fleshy or dry. The former includes drupes, berries, and other fleshy fruits with multiple seeds. The dry fruits are classified as dehiscent, indehiscent, and schizocarps (Hickey & King, 1981; Zomlefer, 1994, Table 1). The zoology system uses fruit traits such as size, color, number of seeds, and seed protection (Janson, 1983; Gautier-Hion et al., 1985, Table 1). For our new entomology system, we selected 2–4 individual fruits per tree species, classified fruits by morphology, estimated their size (length and width to the nearest millimeter) and weight (to the nearest gram), and photographed them. We identified fruit color using a color scheme developed for vertebrate dispersal assessment by Janson (1983) and Gautier-Hion et al. (1985). To control for color choice biases, the Munsell color index system (Sturges & Whitfield, 1995) was used to match colors to black, blue, brown, green, orange, purple, red, violet, white, and yellow on the basis of pictures of ripened fruits.

Our previous analyses identified fleshiness as a critical trait for insect frugivores and seed predators (Ctvrtecka, Sam, Brus, Weiblen, & Novotny, 2014). The proposed entomology fruit classification system recognizes fleshiness as an important criterion, as does the botany system. Further, the number of seeds per fruit is included as an important variable for ovipositing insects (Table 1, Supporting Information Table S1). Finally, it takes into consideration the thickness and toughness of the mesocarp protecting seeds from insects (Table 1, Supporting Information Table S1). The individual categories correspond to "syndromes," each used by a different suite of insect taxa (Supporting Information Table S1). These fruit syndromes could be used to assess the diversity of food resources for insects that attack seeds in rain forests (Armesto & Rozzi, 1989; Corlett & Primack, 2006).

2.4 | Data analysis

Our analyses were based on all species sampled for fruits, both inside and outside the ForestGEO plots (1,192 species, Figures 1A, 2A, 3 and 4), using the number of species per category as a response variable. For tree species present within the ForestGEO plots (689 species), individual abundance and stem size data were available, and

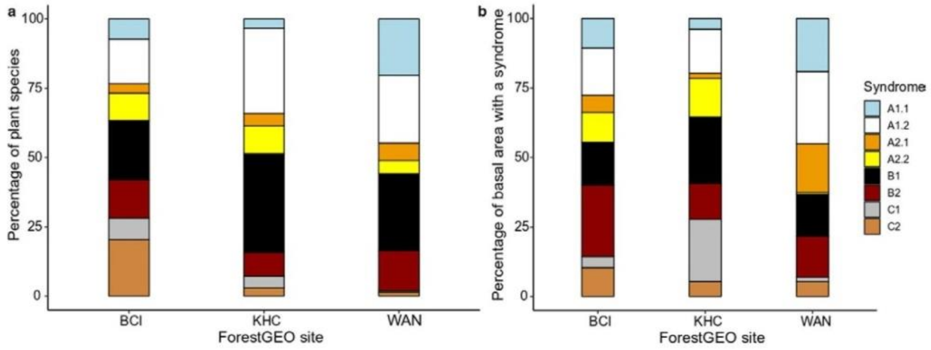

FIGURE 1 Percentage of plant species (a) and basal area (b) represented by individual fruit syndromes at each of the three ForestGEO sites. BCI: Barro Colorado Island, Panama; KHC: Khao Chong, Thailand; WAN: Wanang, Papua New Guinea

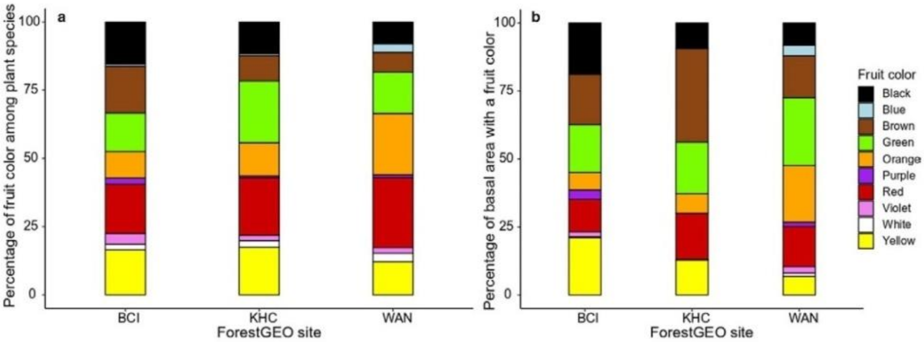

FIGURE 2 Percentage of fruit color represented by plant species (a) and basal area (b) at each of the three ForestGEO sites. BCI: Barro Colorado Island, Panama; KHC: Khao Chong, Thailand; WAN: Wanang, Papua New Guinea

we used basal area and density of stems per species in combination with the "species" fruit syndrome to quantify the ecological significance of fruit syndromes (including life form) as resource for insects and to make quantitative comparisons across sites (Figures 1B and 2B, Supporting Information Table S1, Basset et al., 2018). We compared the proportion of species, basal area, and stems represented by each fruit syndrome, life form, and fruit color among the study plots. At KHC, 14% of plant species (mostly unidentified lianas) were excluded from plant phylogeny analyses.

Since there was little species-level overlap between study sites, differences between plant communities were assessed by comparing composition at the plant genus level using the phylogenetic Chao-Sorensen index, which calculates the proportion of shared branch lengths between sites. We estimated the phylogenetic relationships between genera and families using the online interface of Phylomatic v3 (Webb, Ackerly, & Kembel, 2008) and the APG III (Angiosperm Phylogeny Group 2009) phylogeny. We built ultrametric trees using the BladJ function in Phylocom (Webb et al., 2008) and dated nodes using the calibration points from Wickstrom et al. (2001).

To test for phylogenetic clustering or over-dispersion of fruit syndromes and colors (coded as categorical traits) across the global generic and familial phylogenies of plants from all three sites, we calculated the mean phylogenetic distance (MPD) occupied by taxa that belonged to each of the eight syndromes and 10 colors. All analyses were abundance-weighted using the number of species within each genus/family (columns) with a given syndrome or color (rows). A genus or family could have multiple states. The significance of observed MPD was compared to null models generated through shuffling tip labels across 999 permutations (we tested for both clustering and over-dispersion and therefore use a two-tailed α of 0.025).

Often genera or families had multiple states (e.g., several syndromes), and we used the number of species within each genus or family to conduct abundance-weighted analyses using the R package "Picante" (Kembel et al., 2010). To evaluate simultaneous and separate effects of sites, fruit colors, and plant phylogeny on the variance in fruit syndromes, we performed multivariate analysis with variation partitioning among three sets of these explanatory variables, using canonical correspondence analysis (CCA) in Canoco ver. 5.10 (ter Braak & Smilauer, 2012). The analysis was performed at two levels of taxonomic resolution of the plant communities: (a) plant families; and (b) plant genera. We used the full datasets of all plant species, where fruit syndromes were measured, and retained all genera and families with available phylogenetic information. Each plant genus (or family) was regarded as a "sample" (i.e., individual rows in matrices), syndromes as a "species" (i.e., columns), and numeric values in the matrix were numbers of plant species (as dependent variable). The effect of phylogeny (at the genus or family level) was tested by including the phylogenetic principle co-ordinate axes (PCO axes) as co-variates. These axes were obtained from principle co-ordinates analysis of a distance matrix derived from the ultrametric phylogeny. We then used a forward selection (999 randomizations, variability adj., *p*-adj. <0.05) and selected the first 30 PCO axes as surrogates of the phylogenetic gradient.

To assess the robustness of the PCO axes, we also ran a similar analysis with 100 axes, which generated similar results. To avoid overestimating phylogenetic effects, the final number of retained significant PCO axes was adjusted considering also the number of degrees of freedom and mean squares for the three sets of the variables compared (Supporting Information Tables S4 and S5). We then calculated the percentage variance explained either by sites, colors, or phylogenetic axes, and by the three groups together. The results were visualized with biplot, using species-explanatory variables in the first two CCA axes. In addition, Venn diagrams indicating the amount of variance in syndromes explained by each of the two analyses were drawn using the R package "vennerable" (Chen, 2018). The efficiency of the two axes was calculated compared to unconstrained multivariate space (i.e., % of explanatory variance, Smilauer & Leps, 2014). Our analyses were computed with the R package (R Core Team, 2014).

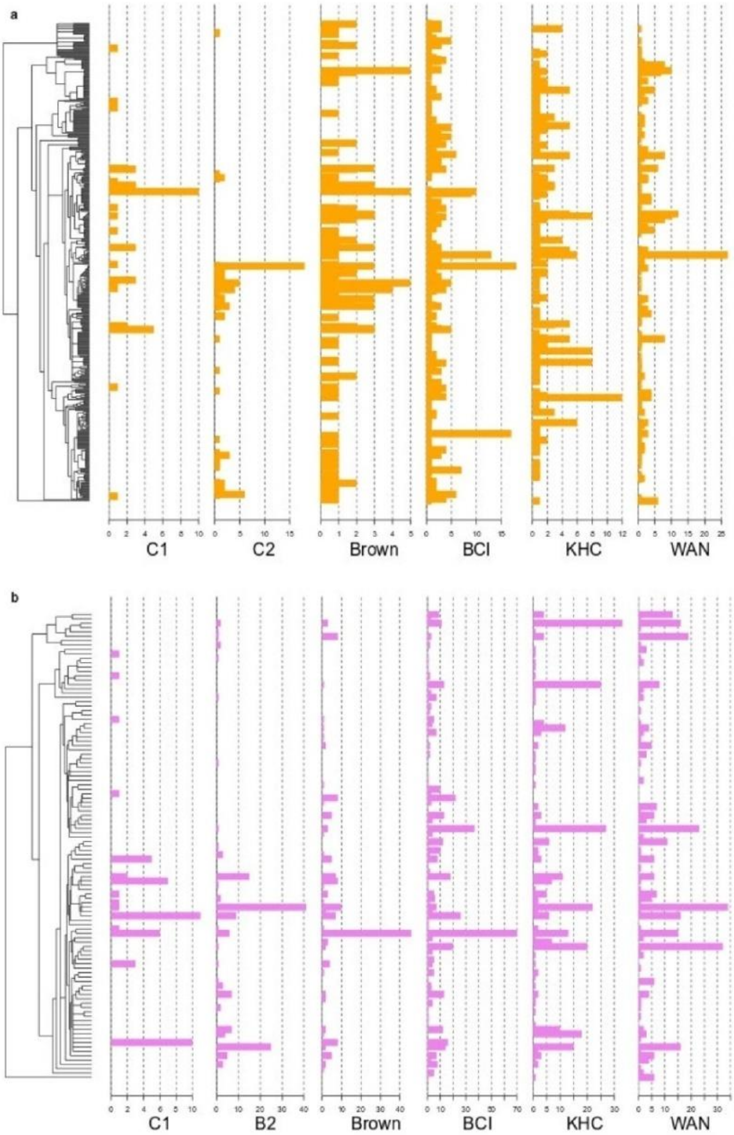

FIGURE 3 The number of species in phylogenetically ordered plant genera (a) and families (b) possessing a particular fruit syndrome (C1, C2, B2) or fruit color (brown), and the total number of species at each site. Only syndromes and colors showing significant phylogenetic clustering are shown

3 | RESULTS

3.1 | Plant diversity, composition, and fruit syndromes

A total of 1,192 plant species from 548 genera and 107 families were scored for fruit morphology and color, including 497 species from BCI, 360 from KHC, and 335 from WAN (Supporting Information Table S3). We obtained fruit syndrome data for 99% of species representing almost 100% of stems at BCI, 45% of species and 85% of stems in WAN and 45% of species and 66% of stems in KHC. Stem density representing particular fruit syndromes varied across study plots ($\chi^2 = 137020$, $df = 14$, $p < 0.001$, Supporting Information Figure S1).

The floristic similarity of the three plots at genus level was expressed using the phylogenetic Chao-Sorensen index. The similarity values ranged from 0.52 for KHC-WAN through 0.34 for BCI-KHC to 0.39 for BCI-WAN comparisons. The distribution of plant species among life forms differed significantly between study plots ($\chi^2 = 432.31$, $df = 14$, $p < 0.001$, Supporting Information Figure S2). Both KHC (87%), and WAN (80%) have a high proportion of trees, while only 40% of all plant species sampled at BCI were trees. In contrast, lianas (23%) and shrubs (28%) were relatively abundant at BCI in comparison with KHC (lianas 11.3%, shrubs 1.4%) and WAN (lianas 1.5%, shrubs 1.8%) plots. Less than 5% of plant species represented other plant life forms across the three study plots (Supporting Information Figure S2).

Every fruit syndrome was represented at each study site. Approximately half of all species at each site had one-seeded drupe fruits (A and B syndromes). The flora was dominated by fleshy fruits (A1 and B1 syndromes) in WAN (72% of species) and KHC (68%), but only 44% species had fleshy fruits at BCI. The distribution of individual syndromes differed among individual plots (plant species: $\chi^2 = 229$, $df = 14$, $p < 0.001$, basal area: $\chi^2 = 754.09$, $df = 14$, $p < 0.001$, Figure 1). The fleshy indehiscent fruits with multiple seeds (B1 syndrome) were important at all three sites. BCI had a higher proportion of dry fruits (C2 and C1) while at WAN and KHC, fleshy drupe with thin mesocarp fruits (A1.2) were important (Figure 1).

The proportion of plant species and basal area representing each fruit color differed significantly among plots (plant species: $\chi^2 = 108.44$, $df = 18$, $p < 0.001$; basal area: $\chi^2 = 595.73$, $df = 18$, $p < 0.001$, Figure 2). Blue, purple, violet, and white colors were always rare, together not exceeding 3.92% of species and 3.97% of basal area in any forest. The remaining colors (brown, black, red, green, orange, and yellow) each represented from 7.2% to 25.6% of species in each of the forest communities (Figure 2). Overall, there were more plant species with brown fruits on BCI and more species with orange fruits in WAN, but no color dominated any of the studied communities.

3.2 | Fruit syndromes and color in phylogenetic context

The number of genera represented by each syndrome ranged from 25 (C1) to 150 (B1) while the number of families ranged from 11 (C2) to 58 (B1). All syndromes were broadly phylogenetically distributed. We tested all eight fruit syndromes for phylogenetic clustering in their distribution among both genera and families and found only syndromes C1 ($n = 25$, $Z = -2.655$, $p = 0.002$) and C2 ($n = 67$, $Z = -3.778$, $p = 0.001$) significantly clustered at the genus level, and syndromes B2 ($n = 28$, $Z = -1.717$, $p = 0.009$) and C1 ($n = 15$, $Z = -1.731$, $p = 0.009$) clustered at the family level (Figure 3).

The number of genera represented by each color ranged from 14 (purple) to 153 (green) while the number of families ranged from 11 (blue) to 60 (green). We tested phylogenetic clustering for all 10 fruit colors and found only the color brown to be significantly clustered at genus level ($n = 107$, $Z = -2.609$, $p = 0.005$) and marginally significant at the family level ($n = 38$, $Z = -1.326$, $p = 0.035$).

The CCA analysis explained 16.6% of variability in fruit syndromes at the genus level and 35.7% at the family level by the effects of sites, colors and plant phylogeny (Figure 4, Supporting Information Figure S3 and Tables S4 and S5). The analysis separated fleshy from non-fleshy syndromes along the CCA1 axis, with red, orange, and black colors in fleshy fruits, and green and brown colors in non-fleshy fruits. WAN and KHC were associated with fleshy syndromes and BCI to non-fleshy syndromes. However, the largest proportion of the overall variability across canonical axes was explained by plant phylogeny, both at the genus and family level, while the effect of forest site was low (Figure 4, Supporting Information Figure S3).

4 | DISCUSSION

4.1 | Plant diversity, composition, and fruit syndromes

Our study provides an entomocentric assessment of fruit classification systems based on fruit morphology, particularly fleshiness, mesocarp thickness, and the number of seeds. As we expected, the three ForestGEO sites surveyed were distinct in their floral diversity as well as fruit syndromes and colors. The Neotropical BCI site was the most distinct in terms of plant species composition and fruit traits (fruit syndromes and colors), with KHC and WAN sites sharing both more phylogenetic- and trait-based similarity (Corlett & Primack, 2006). Corlett and Primack (2006) stated that Southeast Asian forest plots are dominated mostly by canopy tree species whereas Neotropical plots are rich in understory shrub species. These differences in the representation of life form categories (e.g., lianas, shrubs or trees) may explain much of the observed dissimilarity in fruit syndromes and fruit colors, and the overall pattern of fruit-feeding insect assemblages observed at the three rain forest regions (see Basset et al., 2018). For instance, BCI vegetation comprises a high proportion of shrub and liana species and has a high production of dry fruits. Lianas have a high proportion of dry fruits that were also often attacked by seed eaters, while fruits of shrubs are smaller and are rarely attacked by insects. In general, dry fruits are exposed to high insect damage compared to fleshy fruits at our study sites (Basset et al., 2018). Other studies from other tropical

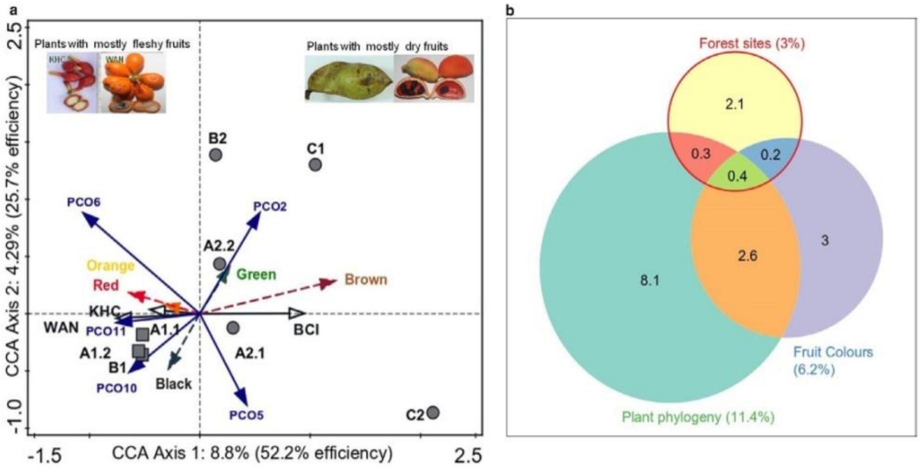

FIGURE 4 Canonical correspondence analysis (CCA) ordination of fruit syndromes based on their distribution in plant genera, with fruit color, forest site (BCI, KHC, WAN), and plant phylogeny (represented by PCO vectors) as explanatory variables (a) and Venn diagram visualizing the proportions of overall adjusted variability explained by each set of variables and their combinations (b). Centroids of individual seed syndromes in (a) are represented by circles for dry fruits and squares for fleshy fruits. CCA used forward selection of the individual predictors (999 randomizations, *p*-adj < 0.05) and variation partitioning among the three sets of variables (see Supporting Information Table S4 for details)

regions also found similar distinctions among plant life forms, fruit syndromes, and fruit colors (see Bolmgren & Eriksson, 2010; Chen, Fleming, Zhang, Wang, & Liu, 2004; Jara-Guerrero, De la Cruz, & Mendez, 2011).

Our fruit syndrome system represents a simple classification that emphasizes fruit traits relevant for insects (e.g., mesocarp thickness) rather than those important for vertebrates (e.g., fruit color). The present system offers a broad qualitative classification of fruits that could be further refined. For instance, Ctvrtecka et al. (2016) defined fleshiness as percentage of fruit volume represented by mesocarp and used a conditional inference tree to identify critical values of fleshiness and seed size of predictive value for frugivory by weevils. Basset et al. (2018) documented guild composition of frugivorous insects associated with individual syndromes in different geographic regions in the tropics.

The largest resource in the forests studied here is represented by fruits falling within the A1.2 and B1 syndromes. Interestingly, dry fruits are generally prevalent and are attacked by true seed feeders at BCI, while pulp feeders are common on fleshy fruits in KHC and WAN (Basset et al., 2018). The fruit syndromes therefore do not show inter-continental convergence in their frugivorous insect assemblages. The distribution of fruit syndromes reflected similarity in plant phylogenetic composition among the sites studied, with WAN and KHC being more similar to each other than to BCI.

We used stems per species abundance (as measured by basal area) to quantify the ecological dominance of each fruit syndrome, as overall resource availability is likely to be an important factor for predicting insect occurrence (Basset et al., 2018; Ctvrtecka et al., 2016). Basset et al. (2018) observed that seed eaters accumulate at a higher rate on plants with dry fruit syndromes relative to fleshy syndromes (BCI > KHC > WAN) across study plots.

Dry fruits tend to be abundant in dry tropical sites where fleshy fruits are less common (Ramirez & Traveset, 2010; Willson & Whelan, 1990). Most plant species producing black, orange, red, yellow, or brown fruits are reported as being vertebrate dispersed (Gautier-Hion et al., 1985). These fruit colors were prevalent in the fleshy fruit syndromes common at KHC and WAN but not at BCI. BCI retained mostly black/brown colored fruits (>21% of basal area), largely associated with small trees and shrubs and lianas. Black fruits were common among understory shrubs/herbs and are more likely to be visible to frugivorous birds than insect seed predators in Neotropical rain forests (Wheelwright & Janson, 1985). Furthermore, this may partly explain the low number of seed-feeding insects observed from fruit samples in BCI (Basset et al., 2018) and other dry forests (Janzen, 1980).

4.2 | Fruit syndromes and color in Aphylogenetic context

Both floristic and fruit syndrome similarities can be explained by a more pronounced dry season at BCI compared to the other two sites, promoting the dominance of Fabaceae (Chust et al., 2006; Condit, 1998). Fruit morphology can be shaped by mutualistic relationships with dispersers as well as antagonistic interactions with seed predators (Chen et al., 2004). Broadly speaking, BCI is the most phylogenetically distinctive site, yet many plant families and some genera have a pantropical distribution. The only syndromes aggregated on the plant phylogeny proved to be non-fleshy syndromes. Less surprisingly, fruit color also proved generally unconstrained by phylogeny. Clearly, the dry-fleshy continuum is at least partly explained by phylogeny, with color retaining a smaller degree of phylogenetic predictability. The fruit syndromes as well as colors thus retain phylogenetic flexibility to respond to local species pools of insect pests and vertebrate dispersers, irrespective of taxonomic composition of the regional floras. However, our multivariate analyses revealed a subtler correlation between phylogeny and plant traits, with plant phylogeny explaining much of the variance in the overall "community" of syndromes across all sites.

Even though our seed syndrome system has entomocentric interest, our results generally confirm those of others (Chen et al., 2017; Forget et al., 2007; Willson & Irvine, 1989). For example, fruiting trees bearing fleshy fruits coupled with an endozoochory relationship reliant on high local bird density are more prominent in tropical forest regions with high precipitation (Almeida-Neto, Campassi, Galetti, Jordano, & Oliveira-Filho, 2008). The high abundance of fruit flies reared from fleshy fruits from Papua New Guinean (Ctvrtecka et al., 2016) and Thai forest contrasts with lower numbers from Panamanian forest, with fewer fleshy fruits (Basset et al.,2018), suggesting our insect seed syndrome results reflect the endozoochory dichotomy pattern of fleshy versus dry fruits present across rain forest regions (Chen et al., 2017). Further, birds and mammals that consume fleshy fruits have played a role in the evolutionary diversification of fruit morphology (Valido et al., 2011; Whitney, 2009). Typically, a given color of fleshy fruits has a wide distribution among tropical plant communities (Willson & Whelan, 1990). We observed higher frequencies of preferred vertebrate colors (black, orange, red and green, or brown; Janson, 1983; Gautier-Hion et al., 1985; Willson & Whelan, 1990; Duan, Eben, & Rui-chang, 2005).

5 | CONCLUSION

There are many studies on fruit and seed syndromes by botanists and vertebrate zoologists. However, studies on insect fruit syndromes across inter-continental rain forest regions are few (Basset et al., 2018). We have shown large inter-continental variability in the representation of fruit syndromes and colors, with likely consequences for seed predators and dispersers. Plant species with fleshy and non-fleshy (dry) fruit syndromes may prefer different forest types and be attacked by different insect feeders (Basset et al., 2018), and fruits with different colors preferred by different vertebrate dispersers. The individual insect fruit syndromes and colors showed low levels of phylogenetic signal with only limited evidence of clustering across the plant phylogeny. However, in a multivariate context, plant phylogeny is clearly an important driver of overall syndrome composition. Both fruit syndromes and colors are, to some extent, evolutionarily flexible traits at higher taxonomic levels and capable of responding to local species pools of seed predators and dispersers. We consider our insect fruit syndromes to be ecologically useful. They can be further refined when additional information on the mode of attack by various frugivorous taxa becomes available.

ACKNOWLEDGMENTS

We are grateful to our colleagues of the Smithsonian Tropical Research Institute, (BCI, Panama), Khao Chong Botanical Garden (Thailand), and Binatang Research Centre and Wanang Conservation area (Papua New Guinea) for helping with this project which was funded by the Czech Science Foundation (GACR grant 16-20825S), the Center for Tropical Forest Science small grant scheme, the University of South Bohemia (grant GA JU 152/2016/P). The Canadian Centre for DNA Barcoding (Biodiversity Institute of Ontario) and Southern China DNA Barcoding Center (Kunming Institute of Zoology, Chinese Academy of Sciences) assisted with DNA sequencing of insect specimens.

DATA AVAILABILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.55nk147 (Dahl et al., 2018).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Dahl C, Ctvrtecka R, Gripenberg S, et al. The insect-focused classification of fruit syndromes in tropical rain forests: An inter-continental comparison. *Biotropica*. 2019;51:39–49. https://doi.org/10.1111/btp.12622

Supporting Information

Supplementary materials for Chapter I. The insect-focused classification of fruit syndromes in tropical rain forests: An inter-continental comparison - Biotropica.

Appendix I - Supporting Tables and Figures

Table S1. Syndrome categories for the entomology system. Codes refer to Table 1. Data are based on the rearing of ca 56,000 insects from seeds originating from Panama, Thailand and Papua New Guinea.

Syndrome	Insect taxa typically reared	Life history interpretation
E-A1.1	Lycaenidae	Female oviposits a single egg in fleshy fruit; large pulp-feeding larva
E-A1.2	Scolytinae, Tephritidae, Blastobasidae, Cosmopterigidae, Tortricidae	Female oviposits multiple eggs in fleshy fruit; high numbers of pulp-feeders and seed-eaters reared
E-A2.1	Baridinae	Female oviposits one or a few eggs; specialized pulp-feeders/seed-eaters often reared
E-A2.2	Sesiidae, Curculionidae	Female oviposits one or a few eggs; specialized seed-eaters often reared
E-B1	Nitidulidae, Drosophilidae, Stratiomyidae, Crambidae	Multiple oviposition events probable in fast-rotting fruits; scavengers and fungal-feeders often reared
E-B2	Anobiidae, Bruchinae, Curculionidae, Drosophiliade, Stratiomyidae,	Multiple oviposition events probable in high-resource fruits attacked before dispersal;
	Blastobasidae, Gelechiidae, Pyralidae, Tineidae	High numbers of insects reared, particularly Lepidoptera
E-C1	None specifically	Few insects reared as resources are limited
E-C2	Anthribidae	Larvae may feed on seeds and capsule before dispersal

Table S2. Salient characteristics of study sites, and plant, seed and insect variables measured across sites. Means are reported with se in brackets and p values refer to Kruskal-Wallis tests. Plot data are from Anderson-Teixeira *et al*. (2014) and Basset *et al*. (2018).

Variable	BCI	KHC	WAN	p
Site physiognomy and structure:				
Coordinates	9.15°N, 79.85°W	7.54°N, 99.80°E	5.24°S, 45.08°E	-
Elevation (m)	120-160	120-330	90-180	-
Annual average rainfall (mm)	2551	2665	3366	-
Annual average daily maximum air temperature (°C)	26.3	27.1	26.5	-
Average length of the dry season (days)	136	120	141	-
Total plant species in plot/% of spp. with fruits collected	499/99	802/45	748/45	-
% of plant spp. as trees/shrub/palm/liana/herb species	40/31/2/25/2	80/1/7/11/1	92/2/4/2/0	-
Plant samples:				
Years of collection	2010-2013	2013-2015	2013-2015	-
Total number of seeds or fruits collected	208,508	39,252	122,976	-
Total weight of samples (kg)	380.2	343.2	439.9	-
Mean sample fresh weight (g)	40.6 (0.5)	174.3 (5.9)	116.2 (1.9)	<0.001
Average fruit fresh weight (g)	25.6 (5.0)	18.3 (3.1)	11.4 (1.3)	<0.001
Average seed length (mm)	39.7 (2.4)	28.0 (1.1)	22.3 (0.9)	<0.01

Table S3. Plant families surveyed at the three study sites. **Denotes eight focal plant families with wide distributions and ***indicates two plant families present only at a single site.

Plant Family	Study plot / number of plant species				Comments
	BCI	KHC	WAN	Total	
Acanthaceae	3			3	
Achariaceae		1	1	2	
Anacardiaceae	5	5	7	17	
Annonaceae	11	33	16	60	BCI, KHC, WAN **
Apiaceae		1		1	
Apocynaceae	13	3	6	22	
Araceae	7	1	2	10	
Araliaceae	2	1	3	6	
Arecaceae	13	25	8	46	BCI, KHC, WAN **
Aristolochiaceae	2		1	3	
Asparagaceae		1	2	3	
Asteraceae	1			1	
Bignoniaceae	22			22	BCI***
Bixaceae	1			1	
Boraginaceae	4			4	
Brassicaceae			2	2	
Bromeliaceae	1			1	
Burseraceae	6	2	5	13	
Cactaceae	1			1	
Cannabaceae		1	2	3	
Capparaceae	1			1	
Caricaceae	2			2	
Celastraceae	7	3	6	16	
Chrysobalanaceae	4	1	1	6	
Clusiaceae	12	10	2	24	BCI***
Combretaceae	8	3	6	17	
Connaraceae	5	2		7	
Convolvulaceae	1	1		2	
Cucurbitaceae	4	1	2	7	
Dichapetalaceae	1		1	2	
Dilleniaceae	8	2		10	
Dioscoreaceae		1		1	

Dipterocarpaceae		7	1	8	
Ebenaceae	1	12	4	17	KHC***
Elaeocarpaceae	1		6	7	
Erythroxylaceae		1		1	
Escalloniaceae		1		1	
Euphorbiaceae	14	15	16	45	BCI, KHC, WAN **
Eupomatiaceae			1	1	
Fabaceae	70	13	15	98	BCI, KHC, WAN **
Fagaceae		7		7	
Gentianaceae		1		1	
Gnetaceae	1	1	6	8	
Heliconiaceae	1			1	
Icacinaceae			2	2	
Irvingiaceae		1		1	
Lamiaceae		2	7	9	
Lauraceae	9	4	13	26	BCI, KHC, WAN **
Lecythidaceae	2	2	5	9	
Loganiaceae	4	1	3	8	
Lythraceae	2	2	1	5	
Magnoliaceae		1		1	
Malpighiaceae	16			16	
Malvaceae	18	11	6	35	
Marantaceae	1	1	2	4	
Marcgraviaceae	1			1	
Melastomataceae	10	2	1	13	
Meliaceae	7	22	33	62	BCI, KHC, WAN **
Menispermaceae	5		2	7	
Monimiaceae	3		1	4	
Moraceae	20	20	32	72	
Musaceae		1		1	
Myristicaceae	3	4	19	26	WAN***
Myrtaceae	12	6	11	29	WAN***
Nyctaginaceae	2		1	3	
Ochnaceae	1			1	
Olacaceae	3			3	
Oleaceae			1	1	
Opiliaceae		1		1	
Oxalidaceae		1		1	

Pandaceae			1	1	
Pandanaceae		1	3	4	
Passifloraceae	3			3	
Pentaphylacaceae			1	1	
Phyllanthaceae		18	3	21	KHC***
Picramniaceae	1			1	
Piperaceae	1			1	
Pittosporaceae			1	1	
Poaceae	2	1		3	
Polygalaceae	1	2	1	4	
Polygonaceae	5			5	
Primulaceae	4	4	1	9	
Proteaceae			1	1	
Putranjivaceae		2	1	3	
Rhamnaceae	4	1		5	
Rhizophoraceae	1		1	2	
Rosaceae			1	1	
Rubiaceae	37	27	23	87	BCI, KHC, WAN **
Rutaceae	4	2	1	7	
Salicaceae	13	2	4	19	
Sapindaceae	26	6	16	48	BCI, KHC, WAN **
Sapotaceae	7	3	2	12	
Simaroubaceae	2	2	1	5	
Smilacaceae	3	1	1	5	
Solanaceae	10			10	
Staphyleaceae	1			1	
Stemonuraceae			2	2	
Theaceae			1	1	
Thymelaeaceae		1	1	2	
Torricelliaceae		1		1	
Ulmaceae	5			5	
Unidentified		46		46	
Urticaceae	4		1	5	
Verbenaceae	3			3	
Violaceae	3	1		4	
Vitaceae	3	1	4	8	
Vochysiaceae	1			1	
Zingiberaceae	1		1	2	

Table S4. Test of significance of the predictors in CCA affecting the seed syndromes, using plant genera as samples and forward selection of variables. For a diagram showing the first two canonical axes see Fig. 4. P(adj) was used with alpha < 0.05 for tests of significance. In the case of PCO phylogenetic axes, only the five most significant axes were retained (of 18 that were significant) to balance the variation partitioning analysis and avoid overestimating the effects of phylogeny and deep nodes (i.e. we retained a number of PCO that generated a similar DF and mean square to that for significant colours).

Forward Selection Results (matrix: 514 genera times 8 syndromes)				
a) effect of sites				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
BCI	2.2	64.8	11.6	0.001
KHC	1.2	35.2	6.4	0.001
b) effect of colors				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
Brown	3.97	46.41	21.7	0.002
Green	1.25	14.66	6.9	0.00333
Black	0.65	7.62	3.6	0.0125
Red	0.56	6.54	3.1	0.0125
Orange	0.6	6.98	3.3	0.01
c) effect of plant genera phylogeny				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
PCO.6	3.75	16.4	20.4	0.00375
PCO.11	2.62	11.47	14.7	0.005
PCO.2	2.21	9.68	12.6	0.00375
PCO.5	1.9	8.32	11.1	0.00429
PCO.10	1.75	7.65	10.4	0.00375

Table S5. Test of significance of the predictors in CCA affecting the seed syndromes using plant families as samples and forward selection. For a diagram of the first two canonical axes see Fig. S3. P(adj) was used with alpha < 0.05 for tests of significance.

Forward Selection Results: (matrix: 107 families times 8 syndromes)				
a) effect of sites				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
BCI	6.8	75.5	7.6	0.0015
KHC	2.2	24.5	2.5	0.028
b) effect of colors				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
Brown	14.4	52.9	17.7	0.005
Green	4.2	15.3	5.3	0.005
c) effect of plant families phylogeny				
Name	% explained (not adj.)	Contribution %	pseudo-F	P(adj)
PCO.30	14.6	31.3	18	0.015
PCO.10	5.3	11.2	6.8	0.01
PCO.3	4.2	9.1	5.8	0.01
PCO.1	2.8	6.1	4	0.03

Supporting Figures

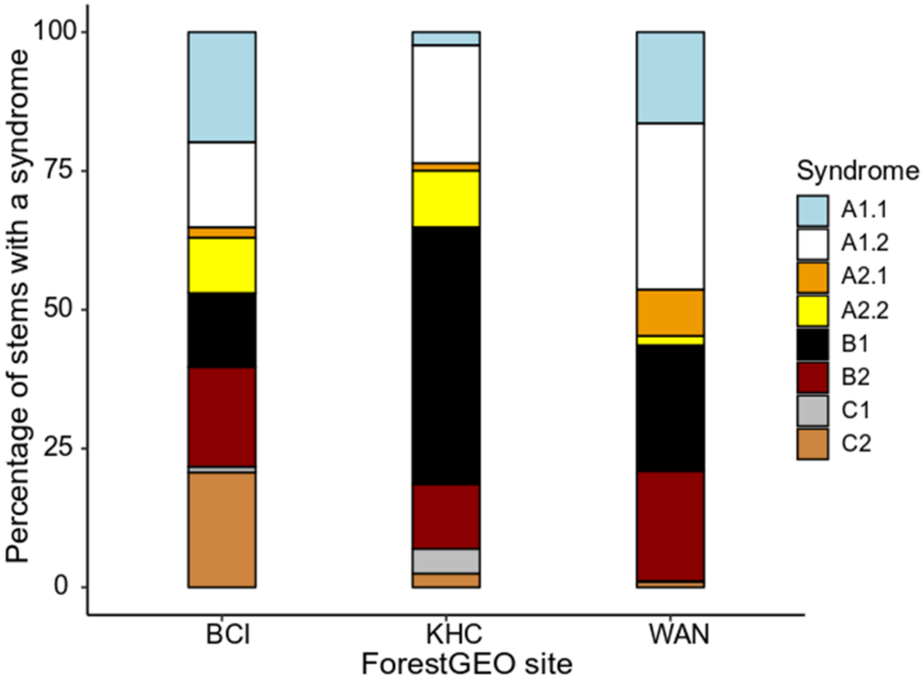

Figure S1. Percentage of stems represented by individual fruit syndromes at each of the three ForestGEO sites. BCI=Barro Colorado Island, Panama; KHC=Khao Chong, Thailand; WAN=Wanang, Papua New Guinea.

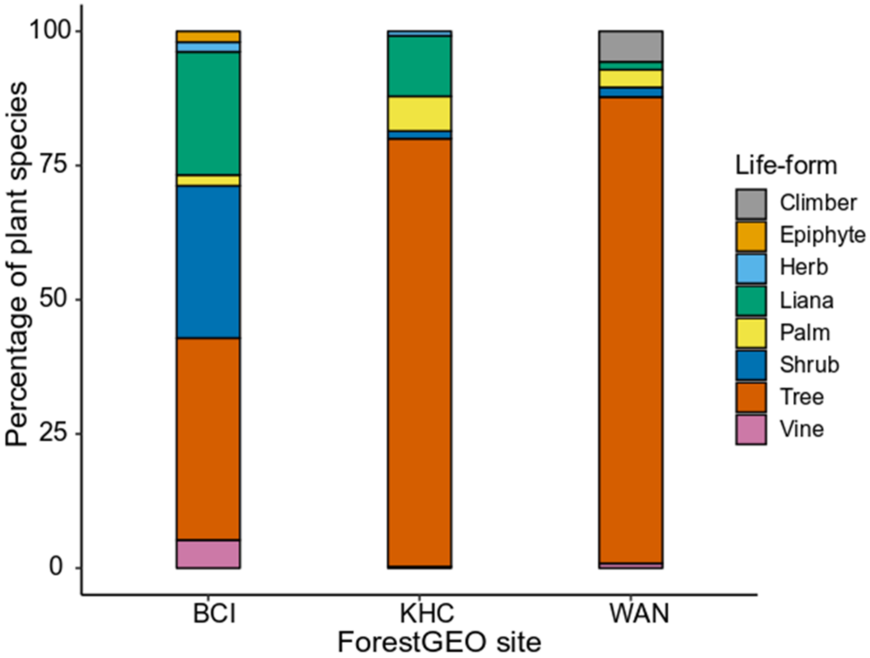

Figure S2. Percentage of plant species from each plant life form at the three ForestGEO study sites. BCI=Barro Colorado Island, Panama; KHC=Khao Chong, Thailand; WAN=Wanang, Papua New Guinea.

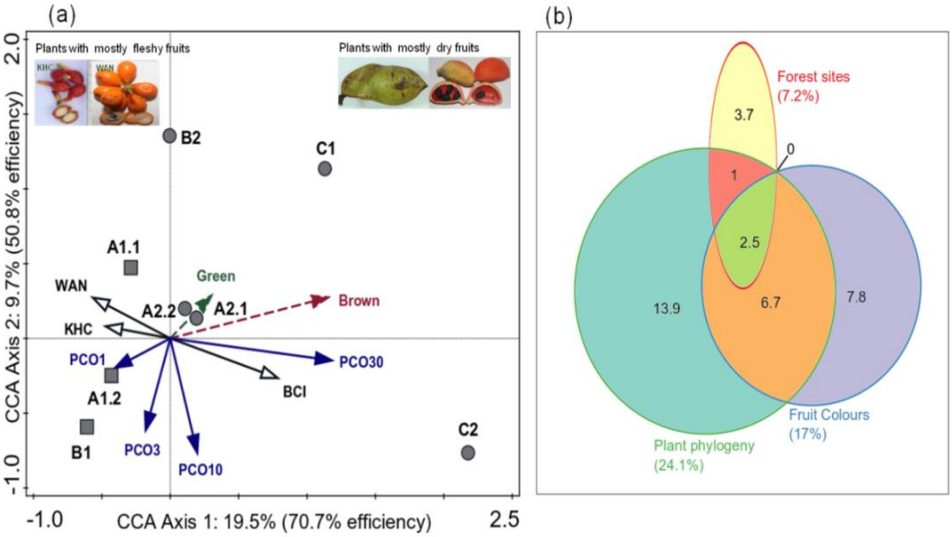

Figure S3. CCA ordination of fruit syndrome distribution based on plant families, fruit colour, forest site (BCI, KHC, WAN) and plant phylogeny (represented by PCO vectors) as explanatory variables (a), and Venn diagram visualizing the proportions of overall adjusted variability explained by each set of variables and their combinations (b). Centroids of individual seed syndromes (a) are represented by circles for the dry fruits and squares for the fleshy fruits. CCA used forward selection of the individual predictors (999 randomizations, p-adj< 0.05) and variation partitioning among the three sets of variables (see Table S5 for details).

Chapter II

A cross-continental comparison of assemblages of seed-and fruit-feeding insects in tropical rain forests: Faunal composition and rates of attack

in Journal of Biogeography 45 (6):1395–1407

DOI: 10.1111/jbi.13211

ORIGINAL ARTICLE

WILEY Journal of Biogeography

A cross-continental comparison of assemblages of seed- and fruit-feeding insects in tropical rain forests: Faunal composition and rates of attack

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Abstract

Aim: Insects feeding on seeds and fruits represent interesting study systems, potentially able to lower the fitness of their host plants. In addition to true seed eaters, a suite of insects feed on the fleshy parts of fruits. We examined the likelihood of

Funding information
Ministerstvo Zemědělství, Grant/Award Number: RO0117; 6779/2017-MZE-14151; Secretaria Nacional de Ciencia y Tecnología, Grant/Award Number: Sistema Nacional de Investigación; Suomen Akatemia; Grantová Agentura České Republiky, Grant/Award Number: 13-09979S; Royal Society, Grant/Award Number: Royal Society University Research Fellowship; Smithsonian Institution Barcoding Opportunity, Grant/Award Number: FY013 and FY014; Chulalongkorn University, Grant/Award Number: Ratchadaphiseksomphot Fund; ForestGEO Research Grant Program

Editor: Werner Ulrich

community convergence in whole insect assemblages attacking seeds/fruits in three tropical rain forests.

Location: Three ForestGEO permanent forest plots within different biogeographical regions: Barro Colorado Island (Panama), Khao Chong (Thailand) and Wanang (Papua New Guinea).

Methods: We surveyed 1,186 plant species and reared 1.1 ton of seeds/fruits that yielded 80,600 insects representing at least 1,678 species. We assigned seeds/fruits to predation syndromes on the basis of plant traits relevant to insects, seed/fruit appearance and mesocarp thickness.

Results: We observed large differences in insect faunal composition, species richness and guild structure between our three study sites. We hypothesize that the high species richness of insect feeding on seeds/fruits in Panama may result from a conjunction of low plant species richness and high availability of dry fruits. Insect assemblages were weakly influenced by seed predation syndromes, both at the local and regional scale, and the effect of host phylogeny varied also among sites. At the driest site (Panama), the probability of seeds of a plant species being attacked depended more on seed availability than on the measured seed traits of that plant species. However, when seeds were attacked, plant traits shaping insect assemblages were difficult to identify and not related to seed availability.

Main conclusions: We observed only weak evidence of community convergence at the intercontinental scale among these assemblages. Our study suggests that seed eaters may be most commonly associated with dry fruits at relatively dry tropical sites where fleshy fruits may be less prevalent.

KEYWORDS
convergence, guild structure, pulp eater, seed predator, seed rain, seed syndrome, species richness

1 | INTRODUCTION

Convergent evolution (or convergent phenotypic evolution: Mahler, Weber, Wagner, & Ingram, 2017) refers to the independent evolution of similar traits in different lineages resulting from strong selective pressures. Convergence is also recognized in ecological assemblages, and community convergence may be defined as the similarity in community structure or physiognomy of assemblages of co-occurring plants or animals resulting from comparable physical and biotic selective pressures (Bittleston, Pierce, Ellison, & Pringle, 2016; Samuels & Drake, 1997; Schluter, 1986; Smith & Wilson, 2002). Hence, community (or ecological) convergence can be sought in terms of (1) search for similarities in patterns of biodiversity and community structure, including species richness, relative abundance distributions, occurrence of trophic guilds, or (2) convergence in the characters of the species present (Korňan, Holmes, Recher, Adamík, & Kropil, 2013; Segar, Pereira, Compton, & Cook, 2013; Smith & Wilson, 2002). In this contribution, we focus on the former aspect of community convergence.

Several mechanisms have been proposed to explain community convergence. First, the likelihood of convergence may be driven by ecological opportunity, which may be based on what resources are in excess and can be easily employed (Agrawal, 2017). Second, biotic filtering, mostly based on species interactions, can result in community-level convergence (Smith & Wilson, 2002). Eventually, communities may reach similar ecological structure through different phylogenetic structures. In this case, empty niches are filled through a combination of colonization by pre-adapted species and/or niche shifts by resident lineages (Gillespie, 2004; Segar et al., 2013). Segar et al. (2013) demonstrated this mechanism for intercontinental fig wasps assemblages.

Of particular interest are examples of community convergence involving assemblages developing on different continents but under similar environmental conditions (Samuels & Drake, 1997). Community convergence on different continents has been reported for a variety of plant communities (Samuels & Drake, 1997) and vertebrate taxa. For the latter, convergence was observed with regard to morphology (fishes: Winemiller, 1991; birds: Ricklefs & Travis, 1980; mammals: Mares, 1993) or species richness (fishes: Irz et al., 2007; birds: Schluter, 1986). Yet, convergence is often evidenced at rather coarse levels, such as guilds (a group of species that exploit the same class of resources in a similar way: Root, 1973), as opposed to finer levels of scale such as species composition (Korňan et al., 2013; Samuels & Drake, 1997). One explanation may be that environmental conditions may determine the types of available niches and, therefore, the functional groups that can fill them, while species compositions within functional groups are influenced stochastically by the history of species arrivals (Fukami, Bezemer, Mortimer, & Putten, 2005).

It has been repeatedly demonstrated that assemblages of invertebrates, particularly arthropods, are rather different from those of vertebrates or plants, as including finer-grained patch sizes and geographical distributions, more complex seasonal and successional sequences, and more rapid generation turnover (Kremen et al., 1993). As a consequence, community convergence is more likely in vertebrates than invertebrates, because the latter are generally more closely tied to the specifics of their resources (Samuels & Drake, 1997). Nevertheless, convergence in arthropod communities has been observed in series reflecting island colonization (Gillespie, 2004), secondary succession (Hendrix, Brown, & Dingle, 1988), habitat restoration (Watts, Clarkson, & Didham, 2008), or in assemblages submitted to intense interspecific competition for patchy and ephemeral food resource, such as dung beetles (Inward, Davies, Pergande, Denham, & Vogler, 2011).

Convergent evolution of phytophagous insects is indicated, for example, by resistance to plant toxins, such as cardiac glycosides (Petschenka, Wagschal, Tschirnhaus, Donath, & Dobler, 2017). In contrast, lack of community convergence appears to be common in many phytophagous insect communities. This may result from host plants being sufficiently biochemically and structurally different to prevent certain modes of feeding or from interspecific competition being too weak to induce convergence (Lawton, 1984). One notable exception includes assemblages of highly specific fig wasps and their parasitoids enclosed in fig syconia. Segar et al. (2013) confirmed intercontinental community convergence in guild proportionality for these assemblages and predicted that similar examples could be observed for bounded communities with well-defined resource units, such as insect herbivores feeding on fruits.

Insects feeding on seeds and fruits represent tractable study systems for studying community convergence. In addition to true seed predators, a suite of insects (notably Diptera and Lepidoptera) feed on the fleshy parts of fruits (Ctvrtecka, Sam, Miller, Weiblen, & Novotny, 2016). The study of these insect assemblages can provide important information on how insect assemblages are structured in hyperdiverse tropical communities. Yet, we know very little about the basic biology and ecology of insect herbivores in tropical rain forests, with, specifically, only a handful of studies documenting whole assemblages of seed/fruit feeders (Ctvrtecka, Sam, Brus, Weiblen, & Novotny, 2014; Ctvrtecka et al., 2016; Nakagawa et al., 2003; Sam et al., 2017).

Following the results of Segar et al. (2013), we ask whether intercontinental convergence in discrete and specialist communities could be generalized to insects feeding on seeds and fruits in different tropical rain forests. While the barriers to community membership are weaker for seed- and fruit-feeding insects, these communities are still bounded. Because of the high diversity and phylogenetic extent of these assemblages (see Results), we took a simplistic approach in testing for differences in insect variables most likely to reflect community convergence, such as species richness and guild proportionality. Our general objectives were to test whether assemblages of insects feeding on seeds/fruits in three representative rain forests within different biogeographical regions (Neotropical, Oriental and Australian) converged towards predictable patterns of community structure influenced by plant phylogeny and/or plant functional traits ("seed predation syndromes," see methods).

We specifically aim at answering three key questions, derived from mechanistic hypotheses from the literature on plant–insect interactions (reviews in Strong, Lawton, & Southwood, 1984; Lewinsohn, Novotny, & Basset, 2005):

1. Are the community attributes of insect assemblages feeding on seeds/fruits (abundance, higher taxonomic composition, guild structure and species richness) similar, both at local and intercontinental scales, and when controlling for plant phylogeny? Further, can plant and seed traits predict the characteristics of these insect assemblages? The classic defence and plant apparency theory postulates that the breadth of an herbivore's diet depends on the apparency and mode of defence of its food–plant (Feeny, 1976). Under this hypothesis, the species richness of seed/fruit predator assemblages is expected to be similar for sets of host plants with particular functional traits, irrespective of plant family, provided that host traits are reasonably independent of plant phylogeny. The defence theory has been modified several times and is now part of a framework of three syndromes of plant defence, including (1) tolerance/escape, (2) low nutritional quality and (3) high nutritional quality and defence (Agrawal & Fishbein, 2006). Considering the antagonism between seeds and seed predators, fleshy fruits may represent an analogy with the tolerance/escape syndrome, as they are quickly dispersed by frugivores, while dry fruits (achenes) may be more likely to be nutritious but well defended against seed predators. If community convergence exists in assemblages of insects feeding on seeds/fruits, then we would expect community attributes to be influenced by seed predation syndromes (see Methods), including fruit fleshiness, and to be similar across locations.
2. Do rare tree species support less abundant, less diverse or functionally distinct seed/fruit-feeding insect assemblages than common tree species? The encounter frequency and resource concentration hypotheses state that more widespread or more locally abundant tree species may support a more abundant and richer herbivore fauna (Kelly & Southwood, 1999; Root, 1973). These hypotheses likewise predict differences in the species richness of seed/fruit feeder assemblages supported by rare and common host trees. Further, if community convergence exists at continental scale, we would at least expect similarity in some of the community attributes between insect assemblages feeding on

seeds/fruits of common tree species, as resources may be easily exploited in this case (Agrawal, 2017).

3. Are seed predation rates lower in forests of higher floristical diversity, do these rates vary among plant families, and can they be predicted from plant and seed traits? Plant resource in floristically diverse tropical forests may be difficult to track for insect herbivores and result in relatively low host specificity in these forests (Novotny et al., 2002). As abundant and specialized insect feeders are responsible for most of plant damage (Coley & Barone, 1996), we predict lower seed predation rates in floristically more diverse forests and/or within more diverse plant families. If the likelihood of community convergence depends to some extent on the ease of tracking resources (Agrawal, 2017), then we would also expect plant species richness to be one of the key factors shaping similarities in insect assemblages feeding on fruits/seeds.

2 | MATERIALS AND METHODS

2.1 | Study sites

Our study sites are three ForestGEO lowland rain forest plots (Anderson-Teixeira et al., 2014), located in different biogeographical regions (Table S1). These sites are detailed in Anderson-Teixeira et al. (2014), salient characteristics of the vegetation plots are also summarized in Table S1 and in Appendix S1, and maps can be consulted at http://www.forestgeo.si.edu/. Neotropical: Barro Colorado Island (BCI) is a 1,500 ha island created by the opening of the Panama Canal in 1914. The 50 ha plot is located in the centre of the island, which is a biological reserve. Oriental: the 24-ha plot at Khao Chong (KHC) is located in the protected forest of the Khao Ban Thad Wildlife Sanctuary in Southern Thailand. Australian: the 50-ha plot is located within the 10,000 ha Wanang Conservation Area in Papua New Guinea (WAN).

2.2 | Plant surveys

Field methods were similar for all study sites. Plant surveys spanned 3–4 years at each site (Table S1). During the first study year at each site, we surveyed seeds and fruits of locally abundant tree, shrub and liana (more rarely herb) species. During subsequent study years, we restricted our sampling effort to 10 plant families, which represented the most common families at each plot (eight families were common to all sites, two other families were well represented locally). We refer to these families as focal families and they included at all plots: Annonaceae, Arecaceae, Euphorbiaceae, Fabaceae, Lauraceae, Meliaceae, Rubiaceae, Sapindaceae; at BCI: Bignoniaceae, Clusiaceae; at KHC: Ebenaceae, Phyllanthaceae; at WAN: Myristicaceae, Myrtaceae. Unless specified, results are detailed for all host plant species. Seeds and fruits collected on plants or freshly fallen (without apparent decomposition) were surveyed within and/or near permanent plots (from an area <1,500 ha corresponding to the smallest study area, BCI). Rearing sample units included clusters of conspecific seeds/fruits of similar size collected from the same trees. We targeted as many individuals as possible for each plant species, typically >5. These sample units were weighted (fresh weight) and stored in individual plastic pots (details in Table 1 and Appendix S1).

2.3 | Insect rearing and processing

Rearing pots were stored under semi-natural conditions in covered but ventilated sheds under the forest canopy. They were checked twice weekly, and any emerging insect was collected, preserved, mounted and then identified with the assistance of taxonomists (see Table S2) and/or with molecular techniques. We obtained DNA Cytochrome c oxidase subunit I (COI, "DNA barcode") sequences from legs of representative insect specimens, and we used Barcode Index Numbers (BINs) derived from insect sequences to delineate species (Ratnasingham & Hebert, 2013). Data were deposited in different Barcode of Life projects (details in Appendix S1).

Insects reared from seeds/fruits were assigned to the following guild categories, inspired from Moran and Southwood (1982): seed eaters (coded as SE: larva feeding mostly on seed tissue), pulp eaters (PU: larva feeding mostly on mesocarp tissue), scavengers (SC: larva feeding mostly on decaying matter), fungal feeders (FU: larva feeding mostly on fungi) and parasitoids (PA: larva feeding on insect hosts). Guild assignment was done mostly at the familial or subfamilial level, but in some case at the generic or specific level, as indicated in Table S2 and Appendix S1.

2.4 | Measurement of plant traits and rates of seed attack

Host plants were identified and a suite of plant traits was determined as far as possible for each plant species, including plant life-form (tree, shrub, liana, herb, palm), seed syndrome, species abundance (no. of stems or basal area in vegetation plots), seed rain, seed fresh mean weight and mean length, and rates of seed attack (see below). Seeds and fruits exhibit a diversity of morphological and ecological features, which may represent important determinants of host use in seed- and fruit-feeding insect taxa. We selected eight putative seed predation syndromes (in analogy with "flower syndromes": Barth, 1981) on the basis of (1) plant traits particularly relevant to insects (Janzen, 1969; Ramírez & Traveset, 2010); and (2) previous comparisons of the distribution of seed/fruit categories at our three study sites (C. Dahl et al., unpubl. data). Each plant species was assigned a seed syndrome on the basis of seed/fruit appearance (fleshiness and dehiscence), number of seeds per fruit and measurement of mesocarp thickness (Table 2). Beside host chemistry and odour, these traits represent important variables for ovipositing female insects and the developing larvae (Díaz-Fleischer, Papaj, Prokopy, Norrbom, & Aluja, 2000; Messina, 1984). This classification of seed syndromes is compared elsewhere with other systems used in botany (based on morphology and mode of dispersal) and in vertebrate zoology (frugivory) (C. Dahl et al., unpubl. data). Seed rain was estimated using weekly censuses of litter traps (Wright, Calderón,

TABLE 1 Salient characteristics of plant traits and insect variables measured across sites (BCI: Barro Colorado Island, KHC: Khao Chong, WAN: Wanang). Means are reported with standard errors in brackets. Differences between means are tested by Kruskal–Wallis tests (significantly different groups indicated by different letters, Dwass-Steel post hoc tests), differences between proportions by chi-square tests. Details about study sites and plant samples are reported in Table S1

Variable	BCI	KHC	WAN	*p*
Plant traits (average per plant species)				
Average no. of stems per tree species per ha	13.02 (2.80)	7.97 (1.06)	9.17 (1.02)	.065
Average basal area per tree species per ha ($m^{-2} \times ha^{-1}$)	0.133 (0.02)^{a}	0.087 (0.01)^{b}	0.052 (0.01)^{c}	<.001
Average seed rain per tree species (dry $g \times m^{-2} \times yr^{-1}$)	0.53 (0.139)^{a}	0.009 (0.005)^{b}	0.015 (0.004)^{b}	<.001
Average seed fresh weight (g)	25.6 (5.0)^{a}	18.3 (3.1)^{a}	11.4 (1.3)^{b}	<.001
Average seed length (mm)	39.7 (2.4)^{a}	28.0 (1.1)^{b}	22.3 (0.9)^{c}	<.01
Average no. of seed eaters reared per seed	0.12 (0.03)^{a}	0.23 (0.14)^{b}	0.10 (0.04)^{a}	<.001
Apparent rate of seed attack (% seed attacked)	5.4 (0.43)^{a}	9.4 (0.89)^{b}	12.5 (0.98)^{b}	<.001
Apparent rate of seed attack (%)—10 focal plant families	6.1 (0.77)^{a}	10.1 (1.33)^{b}	12.2 (1.33)^{b}	.013
Insect samples:				
Total number of insects reared	27,610	17,555	35,434	—
Total number of insects reared—10 focal plant families	12,736	8,851	24,033	—
Proportion of samples with insects reared (%)	29.4	59.4	51.2	<.001
Proportion of samples with seed eaters reared (%)	12.6	10.9	12.7	.141
Mean insects reared per sample	2.6 (0.15)^{a}	7.6 (0.55)^{b}	7.5 (0.39)^{b}	<.001
Mean seed eaters reared per sample	1.17 (0.11)	1.08 (0.19)	1.07 (0.13)	.15
Mean seed eaters reared per attacked sample	9.3 (0.82)	9.8 (1.62)	8.5 (0.95)	.15
Mean insects reared per seed	0.51 (0.04)^{a}	1.71 (0.16)^{b}	0.53 (0.04)^{a}	<.001
Mean insects reared per g weight	0.23 (0.09)^{a}	0.11 (0.01)^{b}	0.08 (0.01)^{c}	<.001

TABLE 2 Number of individual insects reared from different seed syndromes, detailed for each study site (BCI: Barro Colorado Island, KHC: Khao Chong, WAN: Wanang). Sum refer to the total of insect reared, SE % to percentage of seed eaters and AT% to mean percent apparent rate of attack (standard error in bracket)

	BCI			KHC			WAN		
Seed syndrome[a]	**Sum**	***SE %***	**AT%**	**Sum**	***SE %***	**AT%**	**Sum**	***SE %***	**AT%**
A. Drupe (one seed per fruit)									
A1. Fleshy drupe									
A1.1 Fleshy drupe with thick mesocarp (>5 mm)	1125	32.2	12.5 (0.96)	1293	4.0	11.8 (1.7)	7676	12.4	11.5 (1.06)
A1.2 Fleshy drupe with thin mesocarp (<5 mm)	5655	62.1	10.5 (0.63)	3388	8.6	9.2 (0.96)	7092	10.0	11.1 (0.82)
A2. Non-fleshy drupe									
A2.1 Non-fleshy drupe with thick mesocarp (>5 mm)	1424	30.1	13.1 (1.23)	275	5.5	12.1 (3.19)	2844	24.1	17 (2.07)
A2.2 Non-fleshy drupe with thin mesocarp (<5 mm)	2748	51.6	11.6 (0.84)	1735	12.4	18.4 (2.59)	1116	31.1	17 (2.72)
B. Fleshy or non-fleshy fruit with multiple seeds									
B1 Fleshy indehiscent fruit with multiple seeds	3834	48.0	7.1 (0.69)	6388	7.2	14.6 (1.18)	5930	18.5	14.3 (1.22)
B2 Non-fleshy dehiscent fruit with multiple seeds (dehiscence typically across multiple axes)	7239	21.7	8.6 (0.55)	593	3.9	9.3 (2.2)	9665	2.5	8.4 (0.89)
C. Dry fruit/seed, often winged									
C1 Dry winged seed that do not develop in capsule	521	49.7	7.3 (0.86)	436	40.4	16 (2.11)	29	0	1.3 (1.25)
C2 Multiple dry seeds (with or without wings) that do develop in capsule/pod (opening across one axis)	4766	33.4	7.6 (0.51)	1654	22.2	28.7 (4.34)	462	3.7	32.2 (6.16)

[a] Recombined categories for some analyses: drupes = A1.1, A1.2, A2.1, A2.2; "fleshy fruits" = A1.1, A1.2, B1; "dry fruits" (achenes) = A2.1, A2.2, B2, C1, C2.

Hernandéz, & Paton, 2004; Appendix S1). Rates of seed attack may be derived with different approaches. Here, we consider an entomocentric view including, for each plant species, either the average number of seed eaters reared per seed or the percentage of seed attacked. The former has been identified as a critical factor for seed germination (Nakagawa et al., 2003), and we refer to the latter as the "apparent rate of seed attack." Average values for most plant traits at the community level (i.e. average for all plant species surveyed) are detailed in Table 1.

2.5 | Statistics: question (1)

For answering this question, our analyses aimed at evaluating differences in insect community structure between our study sites. Differences in insect variables suggest lack of community convergence, while a high similarity does not necessarily indicate community convergence. Sampling effort, either expressed as the number of seeds collected or the weight of samples, was significantly different between sites (Table S1). To account for this situation, our analyses considered average proportions within samples, rarefaction or expressed insect variables per unit seed or unit g fresh weight, for comparisons among study sites (see Appendix S1 for details). To compare insect faunal composition and species richness, we considered the following taxa, which were well studied, represented 48% of the total material collected and were also observed as important seed/fruit feeders in other tropical community studies (Ctvrtecka et al., 2016; Ramírez & Traveset, 2010): Bruchinae, Scolytinae, Curculionidae others than Scolytinae (Coleoptera), Tortricidae, Pyralidae (Lepidoptera), Stratiomyidae, Tephritidae (Diptera) and Braconidae (Hymenoptera). We evaluated differences across sites with Kruskal–Wallis tests, after a logit transformation (Warton & Hui, 2011). Faunal similarity was estimated with the Morisita-Horn index calculated with the "vegan" library of the R-language (Oksanen et al., 2011), separately for each main guild on the basis of the abundance of insect families at each site. We tested for differences in the distribution of insect guilds across seed syndromes and sites with contingency analysis.

To evaluate the possible existence of clusters formed by insect guilds and seed syndromes, we performed correspondence analyses (CA) for each site on the matrices of the abundance of the main insect guilds (seed eaters, pulp eaters, fungal feeders, scavengers, parasitoids) ordered by plant species (all plant species surveyed, 264, 237 and 257 species for BCI, KHC and WAN, respectively). These and other multivariate analyses (see below) were performed with Canoco 5.04 (ter Braak & Smilauer, 2012). We compared species richness (measurements of species diversity or evenness are less relevant in this context) for these same insect groups among study sites by computing rarefaction and extrapolation sampling curves of species richness with the R package "iNEXT" (Hsieh, Ma, & Chao, 2016). We considered the following datasets for comparing insect groups: all plant data available, data restricted to the 10 plant focal families and data restricted to BINs. We also compared total species richness separately for each study site and seed syndrome, with all plant data. With the same software, we calculated an estimated asymptotic species richness (Hsieh et al., 2016).

We tested the influence of plant and seed traits on insect assemblages as follows. First, we estimated the phylogenetic relationships between our focal host species using the software package Phylomatic (Webb & Donoghue, 2005; details in Appendix S1). We used a variance partitioning approach (Dray, Legendre, & Peres-Neto, 2006) to quantify the contribution of our explanatory variables (plant traits) and host phylogeny to structuring the composition of insect communities across plant hosts at each site, using Canonical Correspondence Analysis (CCA; details in Appendix S1). We used the following plant traits for each plant species: life-form, seed syndrome, seed type (as binary trait dry or fleshy), weight and length, and number of seeds per fruit. To obtain a balanced design, the analyses were restricted to the 10 focal plant families and to host species for which all measured variables were available. The representation of phylogeny through eigenvectors does not always capture phylogenetic structure fully (Freckleton, Cooper, & Jetz, 2011). Hence, we tested the influence of seed traits on insect community structure (i.e. presence of species) using a more explicit phylogenetic comparative framework: binomial Phylogenetic Linear Mixed Models (PGLMMs) (Ives & Helmus, 2011). Insect presence (response variable) was modelled using separate models for the traits: fruit length, number of seeds, fresh fruit weight and seed type (fixed explanatory variables), while including insect and host species identities as random effects. We included phylogenetic covariation as an additional random effect. We used the R package "pez" (Pearse et al., 2015) to construct PGLMMs (fitting models using restricted maximum likelihood; see Appendix S1).

2.6 | Statistics: question (2)

Common and rare tree species were defined as belonging to the first and last quartiles of abundance, respectively (Gaston, 1994), within each ForestGEO plot (vegetation data, Appendix S1). We compared the average abundance (all insects and seed eaters), species richness (rarefaction method as previously) and guild structure of all insects (proportion of individuals) reared from tree species belonging to the first and last quartile of abundance in vegetation plots. We tested for differences in guild structure in common tree species between different sites with contingency analysis.

2.7 | Statistics: question (3)

To evaluate differences in seed attack rates, we used the logit transformation (cf. above) to transform the apparent rate of seed attack and tested the significance of factors site and seed syndrome in a two-way ANOVA. We performed a similar analysis with log transformed abundance of seed eaters reared per unit seed. For each site, we analysed the statistical relationship between all continuous independent variables included in the multivariate analyses and the dependent variable presence/absence of seed eaters reared from all plant species surveyed (1/0), using quasibinomial generalized linear models (GLMs). We controlled for the phylogenetically non-independent data points as explained in Appendix S1. Further when seeds were attacked, we quantified the influence of continuous independent variables on three dependent variables (average number of seed eaters per unit seed, apparent rate of seed attack and number of species of seed eaters reared), using phylogenetic path analysis (PPA; Gonzalez-Voyer & von Hardenberg, 2014). The procedure and the assumptions of the models, which were calculated with the R

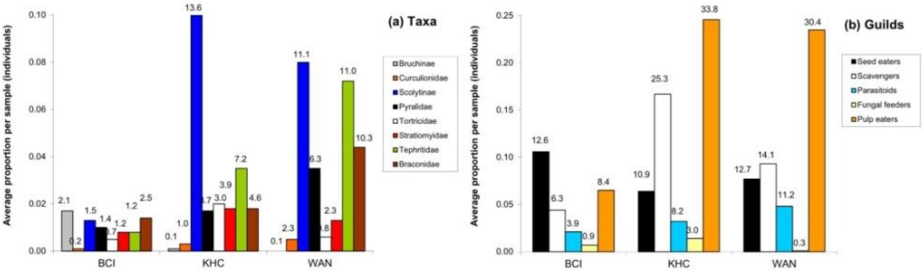

FIGURE 1 Plot of the average proportion of individuals of insect (a) taxa and (b) guilds reared per sample (all samples considered) for each study site. For taxa and guilds, proportions of particular taxa/guilds across sites are all significantly different (Kruskal–Wallis tests, all with $p < .05$). Figures above bars indicate, for each site, the percentage of samples in which a taxon or guild was present. Note that because values are averaged across all samples, proportions are rather small [Colour figure can be viewed at wileyonlinelibrary.com]

package "phylopath" (van der Bijl, 2017), are detailed in Appendix S1 and Figure S1. Eventually, to evaluate the possible effect of plant species richness on seed attack, we considered (1) the results of the GLMs described above, with the number of confamilial species for each plant species included as independent variable; and (2) the relationship between the number of confamilial plant species and the average percentage of seed attack per plant genus at each study site. In this case, considering plant genera instead of plant species reduced the possible effects of host phylogeny on the independence of data points.

3 | RESULTS

3.1 | Differences in the composition, guild structure and species richness of insect assemblages

At the three sites, 1,163 kg of seeds/fruits were reared, which yielded 80,600 insects representing at least 1,678 species reared from 1,186 plant species (Tables 1 and S1). Details about the salient differences between study sites in terms of plant and insect variables are further reported in Appendix S2. Average proportions per sample of particular taxa or guilds were all significantly different across sites (Figures 1, S2), with sometimes higher taxa absent at particular sites (Table S2). Generally, the highest faunal similarity was recorded between KHC and WAN (Table S3). Most insect reared were assigned to pulp eaters (present in 8%–34% of samples), scavengers (6%–25%) and seed eaters (11%–13%), and this general pattern was similar across sites and when restricting the data to the 10 focal plant families (Figures 1 and S2). Seed eaters overall represented 44.3% of all insects reared at BCI, whereas this percentage was only 14.2% and 14.3% at KHC and WAN, respectively. These general patterns were broadly similar for data restricted to 10 plant families, but with notable exceptions for the Curculionidae (Figure S2).

When all three study sites were considered together, it was apparent that a high relative proportion of Bruchinae and of seed eaters were reared from dry fruits (categories B2, C1 and C2, see Ramírez & Traveset, 2010), whereas a high relative proportion of Tephritidae and scavengers were reared from fleshy fruits (Figure S3; see Copeland, Luke, & Wharton, 2009). The highest and lowest relative proportion of seed eaters reared originated from categories C1 and A1.1-B2, respectively. The distribution of insect guilds across seed syndrome categories was significantly non-uniform within each site (BCI, $\chi^2 = 5{,}589.6$; KHC, $\chi^2 = 1{,}695.3$; WAN, $\chi^2 = 3{,}935.8$; all with $p < .001$), but appeared more resembling when comparing KHC and WAN data than when comparing BCI data with other sites (Figure 2). The proportion of plant species with dry fruits was higher at BCI (Table S1), but seed eaters were reared from a variety of syndrome categories, not just dry fruits (Table 2). This was confirmed by the large spread of insect guilds across seed syndrome categories, with no obvious clusters around guilds (CA, Figure S4). The distributions of insect guilds within syndrome categories were significantly non-uniform across sites, even when only drupes were considered ($\chi^2 = 7{,}639.2$, $p < .001$), fleshy fruits ($\chi^2 = 9{,}308.4$, $p < .001$) or dry fruits ($\chi^2 = 3{,}781.5$, $p < .001$; Figure 2, Table 2).

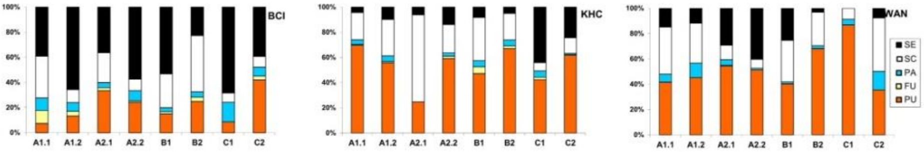

FIGURE 2 Proportion of the number of individuals reared from each seed syndrome category, detailed for each guild and each study site [Colour figure can be viewed at wileyonlinelibrary.com]

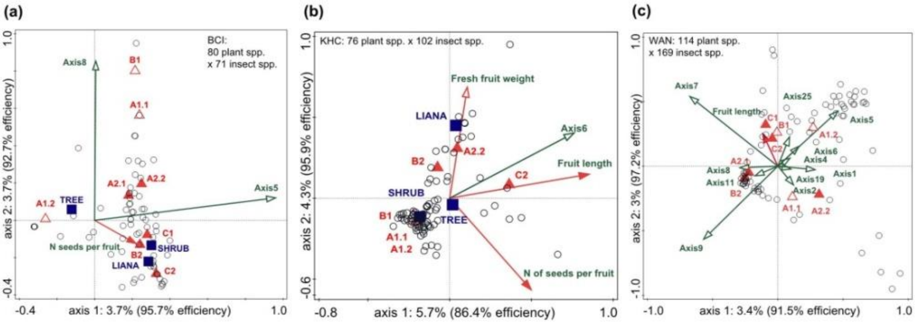

FIGURE 3 Plot of the variance in composition of insect species (circles; singletons excluded from analyses) explained by significant ecological variables (plant traits) and plant phylogeny in the first and second canonical axes of the CCA for (a) BCI, (b) KHC and (c) WAN. Continuous variables are coded as red vectors with closed arrows, factorial predictors as symbols. For the later, plant life-form is coded as blue squares, centroids of seed syndromes as red triangles (empty = fleshy fruits, solid = dry fruits). Plant phylogenetic axes are coded as green vectors with open arrows. The matrix sizes (plant species × insect species) and % of variability explained by each axis and their efficiency are indicated in the plots [Colour figure can be viewed at wileyonlinelibrary.com]

Overall insect species richness reared from samples followed the series BCI (total number of species observed = 1,178/number of species of seed eaters = 311) >WAN (462/77 spp.) > KHC (378/60 spp.). Rarefaction and asymptotic estimators indicated that BCI was the most species-rich site when considering all data and BINs. For the 10 focal species, species richness at BCI and WAN appeared to be similar (Figure S5). For seed syndromes surveyed with relatively high sample size, insect species accumulated faster on dry seeds/fruits at BCI (C2 > B2 > A1.2), whereas this pattern was opposite at KHC (B1 > A1.2 > C2) and WAN (A1.2 > A1.1 > B2; Figure S6).

3.2 | Influence of plant traits and phylogeny

The proportion of overall variance in insect faunal composition that was explained by all the explanatory variables in the CCAs was significant and relatively consistent (12.6%–18.7% after excluding singletons; Table S4), with seed syndrome, seed length and number of seeds, as best explanatory variables (Figure 3). However, the effects of host phylogeny (both overall and exclusive after controlling for plant traits) were much more variable among sites, ranging from 3.2% (BCI) to 72% (WAN) (Table S4). The results using binary occurrence of insect species (PGLMM) mirrored the multivariate CCAs but also confirmed the difficulty to predict insect faunal composition with plant traits. Seed type (dry/fleshy) did not influence faunal composition for any site. Still, fresh fruit weight had a significant positive relationship with the response variable for KHC (Table S5).

3.3 | Insect assemblages on rare and common tree species

Significantly more insects and seed eaters were reared from rare tree species than from common tree species at BCI (Mann–Whitney test, $U = 30.87$, $p > .001$ and $U = 6.26$, $p = .012$, respectively, n1 = 1044, n2 = 66; Figure 4). This pattern was opposite at WAN for seed eaters (U = 4.54, p = 0.033, n1 = 1036, n2 = 55) but not significant for all insects ($U = 0.67$, $p = .41$). At KHC, neither the abundance of all insects nor of seed eaters was significantly different between common and rare trees ($U = 0.08$, $p = .78$ and $U = 0.06$, $p = .81$, respectively, n1 = 392, n2 = 57; Figure 4). The rate of species accumulation was difficult to compare between common and rare trees, because of the relatively small sample of the latter, but extrapolations suggested that common tree species accumulated more species than rare tree species at all study sites (Figure S7a). The guild structure of common tree species was significantly non-uniform between study sites ($\chi^2 = 3721.6$, $p < .001$; Figure S7b). Further, the guild structure of insects reared from common and rare tree species was similar at KHC and WAN, but the proportion of seed eaters vs. other insects was significantly non-uniform across these tree categories at BCI (Fisher exact test, $p < .001$; Figure S7b).

3.4 | Rates of seed attack

Overall, apparent rates of seed attack at all study sites averaged 8.5 ± 0.7% per plant species (1,144 plant species considered). However, apparent rate of seed attack, both for all plants surveyed and for the focal 10 plant families, was significantly different and followed the series WAN>KHC>BCI (Table 1). The average number of seeds eaters reared per seed was also significantly different and followed the series KHC>BCI>WAN (Table 1). BCI had the lowest percentage of plant species attacked (by any insect guild) to plant species surveyed (64.1%), followed by KHC (71.3%) and WAN (77.4%). Apparent percent seed attack was high for C2 syndromes at KHC and WAN (Table 2). When seeds were attacked, the abundance of seed eaters per seed was significantly different both between sites and seed syndromes (two-way ANOVA, Table S6). This pattern

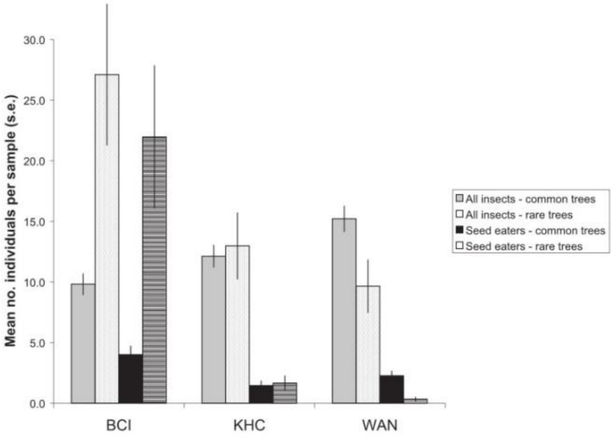

FIGURE 4 Abundance of all insects and seed eaters (means and *SE*) on common and rare tree species at each study site

was similar when considering the apparent rate of seed attack, with a stronger effect of seed syndrome than site (Table S6).

At BCI, 14% of the variance in the probability of rearing seed eaters (related to rates of seed attack, see Methods) from all plant species surveyed could be explained by the basal area of plant species and the seed rain (Table 3). At KHC, 17% of the variance could be explained by basal area, fruit length and the sum of seeds collected, whereas at WAN only 9% of the variance was explained by the number of confamilial species, the abundance in the plot and the sum of seeds collected (Table 3). The results were largely similar once the effect of phylogeny had been controlled for, but some variables no longer had explanatory power (such as basal area and number of confamilial species), suggesting a degree of phylogenetic conservatism (Table S7). When seeds were attacked, it was difficult to evidence direct paths in the PPAs to the dependent variables, as models were not significant for BCI and KHC (Table S8). For Wanang, the number of seeds per fruit had a direct positive path to the average number of seeds eaters reared per unit seed (Figure S8a), none of the independent variables influenced directly apparent rate of seed attack (Figure S8b), and only sampling effort had some direct and relatively large influence on the number of seed eaters reared (Figure S8c).

TABLE 3 Results of stepwise binary logistic regression describing the probability of rearing seed eaters for all plant species surveyed. Models are detailed separately for each study site (*SD*: standard error; *t*: *t*-value; Pr(>|t|): probability; R^2: coefficient of determination; *n*: number of plant species). For similar analyses controlling for the effect of host plant phylogeny, see Table S7

Model/ Parameter	Estimate	*SD*	*t*	Pr(>|t|)	R^2	*n*
BCI	—	—	—	—	.140	204
Constant	−0.434	0.185	−2.340	.020	—	—
Basal area	0.050	0.024	2.096	.037	—	—
Seed rain	1.313	0.653	2.011	.046	—	—
KHC	—	—	—	—	.167	156
Constant	−2.608	0.449	−5.810	.000	—	—
Basal area	0.058	0.030	1.917	.057	—	—
Fruit length	0.035	0.010	3.496	.001	—	—
Sum of seeds collected	0.003	0.001	2.879	.005	—	—
WAN	—	—	—	—	.086	240
Constant	−0.831	0.288	−2.889	0.004	—	—
No. of confamilial species	0.012	0.008	1.543	.124	—	—
Abundance in plot	0.000	0.000	1.453	.148	—	—
Sum of seeds collected	0.001	0.000	2.550	.011	—	—

As indicated previously, the influence of the number of confamilial species was non-existent or weak in explaining the probability of rearing seed eaters from plant species (Table 3). Further, when plant species were attacked, there was no significant linear relation (or any notable nonlinear relation) between the number of confamilial plant species and the average percentage of seed attack per plant genus at BCI ($F_{1,183} = 0.51$, $p = .48$), at KHC ($F_{1,109} = 0.23$, $p = .64$) or at WAN ($F_{1,104} = 2.53$, $p = .12$).

4 | DISCUSSION

4.1 | Synopsis of salient results

Most of observed differences in our study system contrast BCI to other sites. BCI has a relatively low plant species richness, with a high proportion of shrubs and lianas and a high proportion of dry fruits. Basal area per plant species is high, as is the average seed weight, seed length and seed rain per plant species. A high proportion of seed eaters was reared from BCI samples. However, a low proportion of seed samples yielded insects (this may be partly due to the small size of BCI samples), apparent rate of seed attack was low, and less plant species were attacked at BCI than at other sites. With reference to the questions formulated in the Introduction, our study indicated that (1) significant differences in insect assemblages exist at the study sites but it was difficult to predict the characteristics of these assemblages with plant and seed traits, including seed predation syndromes and fruit fleshiness. (2) Seeds of rare trees were more likely to be attacked than those of common trees only at BCI. Guild proportionality in insect assemblages on common tree species was not conserved between sites. (3) Rates of seed attack were not particularly low at the two floristically diverse sites and, within plant families, floristic richness had little apparent effects on rates of seed attack. As such, these results suggest only weak community convergence of these insect assemblages at the intercontinental scale.

4.2 | Intercontinental comparison of insect assemblages feeding on seeds/fruits

As far as we are aware our study represents the first intercontinental comparison with similar protocols of insect assemblages attacking seeds/fruits in tropical rain forests. Overall, it indicated large differences in insect faunal composition, species richness and guild structure between the three study sites (question 1). These patterns were similar when we restricted our dataset to the 10 focal plant families. Still, it can be argued that all insect guilds were represented at the three study sites and that the main guilds were also reared from all seed syndromes. This observation can be interpreted as partial convergence (Kořan et al., 2013) in guild structure of the phylogenetically distinct insect assemblages at the three study sites.

However, the predictive power of seed syndromes was relatively weak. At BCI, where the availability of dry fruits was high, nearly 45% of insects reared were seed eaters, whereas this proportion was <15% at KHC and WAN. In contrast, pulp eaters were proportionally better represented at KHC and WAN than at BCI. Further, at BCI dry seeds/fruits accumulated faster insect species than fleshy seed/fruits. In addition, the distribution of guilds appeared rather distinct for each category of seed syndromes. We conclude that for coarse functional comparison of insect assemblages between sites, the most distinctive dichotomy among our categories of seed syndromes is probably dry vs. fleshy fruits, although this variable cannot explain fine insect composition, which is best explained by seed syndromes. This suggests that insect community convergence, either on dry or fleshy fruits, must be rather weak, if it exists at all. Gripenberg et al. (2018) showed that seed polyphenols on BCI are more influenced by the host successional stage (pioneer vs. shade-tolerant tree species) than by plant apparency (height of tree). It is conceivable that insect communities feeding on seed/fruits may also be more likely to convergence according to their host successional status. However, data were lacking in this study to accurately score the successional status of many plant species.

Still, several studies reported higher damage on dry fruits than on fleshy fruits (Ctvrtecka et al., 2014; Janzen, 1969; Wright, 1990). Our results partially support the hypothesis that the pulp of fleshy fruits may, in addition to being a reward for vertebrates disseminating fruits (Gautier-Hion, 1990), also act as protection from the attack of specific seed eaters (Bolmgren & Eriksson, 2010). Specific adaptations may be required for insect ovipositing within or near the seed or for the insect larvae to reach the seed and start its development there in the presence of an abundant pulp (Wright, 1990). This certainly warrants further studies examining insect damage on different seed syndromes.

There were also overall differences between sites in the relative proportion of variance in insect faunal composition explained by plant traits and phylogeny. Our CCA and PGLMM results suggest that seed syndromes were important in shaping insect communities at BCI, while some seed traits were more important at KHC, and plant phylogeny represented the best predictor in this regard at WAN. This high influence of phylogeny at WAN was probably due to closely related plants hosting more similar insect communities, while at BCI and KHC plant traits were more important in shaping insect communities and independently of plant phylogeny. Apart from seed syndromes, seed length and number of seeds per fruit appeared to influence most significantly the composition of insect assemblages. Ctvrtecka et al. (2016) reported that fruits attacked by Diptera are significantly larger than fruits attacked by Coleoptera and Lepidoptera. Other studies likewise reported a positive effect of seed size on seed eaters (Janzen, 1969; Ramírez & Traveset, 2010; Sam et al., 2017). Overall, we conclude that the composition and guild structure of insect assemblages feeding on seeds/fruits in tropical rain forests are partly shaped by seed predation syndromes, both at the local and regional scale, but that the factors shaping these assemblages are hard to identify.

Further, assemblages of insects feeding on seeds/fruits were richer at BCI, than at other sites, when we considered rarefaction of either the number of species sorted or the number of BINs sequenced. This observation is not an artefact of different sample size. Different studies targeting weevils and Lepidoptera indicated, despite larger sample sizes in both the number of plant species surveyed and the number of insect individuals reared, much less species-rich insect assemblage feeding on fruits/seeds in Papua New Guinea or Kenya than at BCI (Copeland et al., 2009; Ctvrtecka et al., 2014, 2016). This high insect species richness at BCI is at odd when considering other insect assemblages that have been studied at our study sites. Butterflies are more diverse at KHC or WAN than at BCI (Basset et al., 2013), and Geometridae and litter ants are also more species-rich at KHC than at BCI (Y. Basset et al., unpubl. data). We hypothesize that the high species richness of insect feeding on seeds/fruits at BCI may result from a conjunction of low plant species richness and high seed availability of dry fruits (see below; it may also be partly related to the high proportion and productivity of shrubs and liana) per plant species, which may favour such rich insect assemblages.

4.3 | Insect assemblages attacking the seeds of rare and common trees

At BCI, more insects and seed eaters were reared from rare tree species than from common tree species, whereas these patterns did not exist at KHC and WAN. This appears to invalidate the resource concentration hypothesis (Root, 1973) and the higher likelihood of community convergence when abundant resources are available (Agrawal, 2017), in relation with question (2). If we assume that seed eaters are very host-specific (Janzen, 1980) and that few hosts can escape them, then we would expect a concentration of damage (and reared seed eaters) on rare tree species and a dilution of damage over common tree species. This mechanism would require a very high insect host specificity and ability to locate hosts, as well as a large resource base easily traceable over which seed eaters may be satiated (Wright, 1990). During the period of our study, these conditions were more like to exist at BCI than at KHC or WAN.

4.4 | Seed attack

Contrary to our predictions related to question (3), the number of confamilial plant species appeared to be unimportant in explaining the apparent rate of seed attack per plant species. Species-rich plant family did not sustain higher seed attack than less diverse plant families. Our logistic regressions indicated that at BCI the probability of seeds of a plant species being attacked depended more on seed availability than on the measured seed traits of that plant species. This issue was less clear at KHC (possible effect of sample size) or WAN (low variance explained). Other traits, such as seed chemistry, nutritional quality, fruiting frequency and host phylogeny may be substantial in this regard (Janzen, 1969; Nakagawa et al., 2003; Ramírez & Traveset, 2010). Resource availability may nevertheless represent a relatively important predictor of the probability of seed attack.

5 | CONCLUSIONS

Studies of insect herbivores in tropical rain forests are few, and often focus on leaf-feeding insects (Lewinsohn et al., 2005). The assemblages of seed-eating insects studied here did not conform to two out of three general predictions (see Introduction) that were coined more specifically for leaf-feeding insect herbivores. As such, seed-eating insects may represent a rather distinct guild from insect herbivores that may be difficult to study but may have great potential to lower the fitness of their hosts (Lewis & Gripenberg, 2008). As a further example, we note that in the tropics, increased damage or pathogens are often associated with increased rainfall (Coley & Barone, 1996). Our study suggests that seed eaters may be better reared from dry fruits and perhaps at relatively dry tropical sites where fleshy fruits may be less prevalent (Kissling, Böhning-Gaese, & Jetz, 2009). However, biogeographical and host phylogenetical factors may complicate this pattern.

ACKNOWLEDGEMENTS

We thank the Smithsonian Tropical Research Institute (Panama), Khao Chong Botanical Garden (Thailand) and Binatang Research Centre and Wanang Conservation Area (Papua New Guinea) for logistical support. Relevant collecting permits were obtained from MiAmbiente via the Smithsonian Tropical Research Institute, the National Research Council of Thailand and Papua New Guinea Research Institute, respectively. D. Catalina Fernandez, Indira Simon Chaves, Marjorie Cedeño, Marleny Rivera (Panama), Pitoon Kongnoo, Montarika Panmeng, Sutipun Putnaul (Thailand), Dominic Rinan, Jonah Philips, Roll Lilip and Ruma Umari (Papua New Guinea) collected most of the insect material. This study was supported by the Czech Science Foundation (16-20825S). Fieldwork on BCI was largely funded by a postdoctoral grant from the Academy of Finland to SG. Grants from the Smithsonian Institution Barcoding Opportunity FY013 and FY014 (to YB), from the ForestGEO Research Grant Program (to C.D.), and in-kind help from the Canadian Centre for DNA Barcoding and Southern China DNA Barcoding Center allowed to sequence insect specimens. Taxonomists listed in Table S2 helped with insect identification. Y.B. and H.B. are members of the Sistema Nacional de Investigación, SENACYT, Panama. S.G. holds a Royal Society University Research Fellowship. M.K. was partly supported by the Ministry of Agriculture of the Czech Republic (Resolution RO0117; 6779/2017-MZE-14151). D.L.J.Q. and B.A.B. were supported by Ratchadaphiseksomphot Fund, Chulalongkorn University (respectively Senior Postdoctoral Fellowship and Endowment Fund R/F_2559_019_05_23).

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BIOSKETCH

Yves Basset is interested in the community ecology of tropical arthropods.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Basset Y, Dahl C, Ctvrtecka R, et al. A cross-continental comparison of assemblages of seed- and fruit-feeding insects in tropical rain forests: Faunal composition and rates of attack. *J Biogeogr*. 2018;45:1395–1407. https://doi.org/10.1111/jbi.13211

Supporting Information

Supplementary materials for Chapter II. A cross-continental comparison of assemblages of seed-and fruit-feeding insects in tropical rain forests: Faunal composition and rates of attack - Journal of Biogeography.

Appendix II - Supporting Tables and Figures

Table S1. Salient characteristics of study sites and their plant samples. When available means are reported with se in brackets and p values refer to Kruskal-Wallis tests of differences between sites. Plot data are from Anderson-Teixeira *et al.* (2014) and Basset *et al.* (2013).

Variable	**BCI**	**KHC**	**WAN**	**p**
Site physiognomy and structure:				
WGS 1984 Coordinates	9.15°N, 79.85°W	7.54°N, 99.80°E	5.24°S, 145.08°E	-
Elevation (m)	120-160	120-330	90-180	-
Annual average rainfall (mm)	2551	2665	3366	-
Annual average daily maximum air temperature (°C)	26.3	27.1	26.5	-
Average length of the dry season (days)	136	120	141	-
Stems per ha in plot	4168	5062	5800	-
Number of tree species ⁄genera ⁄families recorded in plot	299/181/59	593/285/82	508/245/77	-
Mean ± s.e. canopy openness (%)	3.99 (0.194)	6.06 (0.445)	2.02 (0.205)	<0.001
Proportion of plant spp. as trees/shrub/palm/liana/herb species (%)	30/32/3/28/2	79/10/3/7/1	76/4/2/16/3	-
Proportion of plant spp. with drupes (%)	38.6	42.5	55.3	-
Proportion of plant spp. with dry	56.8	26.0	28.0	-

seeds/fruits (A2, B2, C1, C2) (%)				
Total seed rain (dry g x m-2 x yr-1)	108.0	7.0	10.8	
Plant samples:				
Years of collection	2010-2013	2013-2015	2013-2015	-
Number of plant species/plant families surveyed	497/82	357/66	332/67	-
Number of plant species surveyed within the 10 focal families	218	171	170	-
Number of samples collected	9,386	1,970	3,787	-
Total number of seeds or fruits collected	208,508	39,252	122,976	-
Ratio no. of seeds collected / no. of plant species surveyed	419.5	109.9	370.4	-
Mean number of seeds collected per sample	22.3 (1.3)	19.9 (0.7)	32.5 (0.9)	<0.001
Total weight of samples (kg)	380.2	343.2	439.9	-
Mean sample fresh weight (g)	40.6 (0.5)	174.3 (5.9)	116.2 (1.9)	<0.001
Total number of seeds or fruits collected - 10 focal plant families	89,800	21,040	92,755	-
Total weight of samples - 10 focal plant families (kg)	186.5	245.5	332.8	-

Table S2. Details of the taxonomic composition of the insect material (number of individuals and species) reared from seeds/fruits at the three study sites, along with guild assignment (see methods). A hyphen (-) in the column total of number of species indicates that the material was either not sorted, or not sorted consistently across sites (and not considered for analyses). For identifiers, "*" indicate the following persons who sorted the bulk of the insect material: Marleny Rivera, Indira Simon Chaves, D. Catalina Fernandez, Marjorie Cedeño (BCI), Pitoon Kongnoo, Montarika Panmeng, Sutipun Putnaul (KHC), Dominic Rinan, Ruma Umari, Jonah Philips, Roll Lilip, M. Mogia (WAN). See bottom of table for institutional affiliations of identifiers.

Family	Subfamily	Guild	Abundance				Number of species				Identifier(s)	Notes
			Total	BCI	KHC	WAN	Total	BCI	KHC	WAN		
COLEOPTERA												
Anthribidae		SE	796	11	396	389	22	3	8	11	R. Ctvrtecka	
Apionidae	Apioninae	SE	560	550	-	10	10	9	-	1	H. Barrios, R. Ctvrtecka	
Brentidae	Cyladinae	SE	9	9	-	-	2	2	-	-	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Byrrhidae		nc	1	1	-	-	-	-	-	-	*	
Callirhipidae		nc	1	-	1	-	-	-	-	-	*	
Carabidae		nc	1	1	-	-	-	-	-	-	*	No evidence of larvae eating seeds (Paarmann *et al.* 2002)
Cerambycidae		PU	48	45	3	-	11	8	3	-	*	
Chrysomelidae	Bruchinae	SE	1936	1931	2	3	43	42	1	-	G. Morse	
Chrysomelidae	Others	nc	4	1	-	3	-	-	-	-	*	
Coccinellidae		nc	8	-	-	8	-	-	-	-	*	
Colydiidae		nc	1	1	-	-	-	-	-	-	*	
Cucujidae		nc	130	130	-	-	-	-	-	-	*	
Cucujoidea		UN	41	-	41	-	-	-	-	-	*	

Curculionidae	Baridinae	SE	896	451	55	390		29	18	3	8	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Ceutorhynchinae	SE	14	14	-	-		1	1	-	-	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Conoderinae	SE	144	143	1	-		5	4	1	-	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Cossoninae	SE	9	-	-	9		2	-	-	2	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Cryptorhynchinae	SE	2052	1496	209	347		29	17	3	9	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Curculioninae	SE	1940	696	259	985		42	21	10	11	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Molytinae	SE	1895	663	762	470		58	47	7	4	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Orobitidinae	SE	5	-	5	-		2	-	2	-	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Curculionidae	Scolytinae	PU	15744	2688	5076	7980		104	39	44	21	A. Cognato, M. Knizek	
Curculionidae	Scolytinae	SE	2528	2528	-	-		1	1	-	-	A. Cognato, M. Knizek	*Pagiocerus frontalis* is SE
Curculionidae	Unknown	SE	345	147	90	108		20	6	6	8	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka	
Dermestidae		nc	2	2	-	-		-	-	-	-	*	
Dryophthoridae	Dryophthorinae	SE	73	-	73	-		1	1	-	-	H. Barrios, R. Ctvrtecka	
Dryophthoridae	Rhynchophorinae	SE	194	5	-	189		3	-	2	1	H. Barrios, R. Ctvrtecka	
Endomychidae		nc	1	-	1	-		-	-	-	-	*	

Erirrhinidae	Erirrhininae	SE	5	-	5	-		1	-	1	-	H. Barrios, H. Stockwell, M. Rowbotham, R. Ctvrtecka
Geotrupidae		nc	1	-	1	-		-	-	-	-	*
Histeridae		nc	10	3	1	6		-	-	-	-	*
Hydrophilidae		nc	2	2	-	-		-	-	-	-	*
Lathridiidae		nc	11	-	11	-		-	-	-	-	*
Leiodidae		nc	1	-	-	1		-	-	-	-	*
Limnichidae		nc	4	-	-	4		-	-	-	-	*
Lucanidae		nc	3	-	-	3		-	-	-	-	*
Nanophyidae		SE	44	-	44	-		5	-	5	-	R. Ctvrtecka
Nitidulidae		SC	1249	183	790	276		76	40	20	16	J. Jelinek
Ptinidae		SC	337	337	-	-		6	6	-	-	*
Scarabaeidae		nc	37	5	1	31		-	-	-	-	*
Silvanidae		SC	250	250	-	-		12	12	-	-	*
Staphylinidae		nc	407	45	277	85		-	-	-	-	*
Tenebrionidae		nc	12	10	-	2		-	-	-	-	*
Trogossitidae		nc	2	-	2	-		-	-	-	-	*
Unknown Coleoptera		UN	1527	70	125	1332		-	-	-	-	*
Total Coleoptera			33280	12418	8231	12631		485	277	116	92	
LEPIDOPTERA												
Arctiidae	Ctenuchiinae	PU	4	-	4	-		2	-	2	-	J. Heppner, S. Miller
Arctiidae	Lithosiinae	PU	2	-	1	1		2	-	1	1	*
Arctiidae	Unknown	PU	1	-	-	1		1	-	-	1	J. Heppner, S. Miller
Blastobasidae		SC	443	340	72	31		79	76	1	2	J. Heppner, S. Miller
Carposinidae		PU	130	-	-	130		1	-	-	1	J. Heppner, S. Miller
Cosmopterigidae		PU	242	137	11	94		20	12	3	5	J. Heppner, S. Miller

Cosmopterigidae		SE	257	257	-	-		1	1	-	-	J. Heppner, S. Miller	Some spp. SE
Crambidae	Pyraustinae	PU	312	-	312	-		11	-	11	-	J. Heppner, S. Miller	
Crambidae	Spilomelinae	PU	32	32	-	-		1	1	-	-	J. Heppner, S. Miller	
Crambidae	Unknown	PU	759	5	6	748		11	2	3	6	J. Heppner, S. Miller	
Depressariidae	Stenomatinae	PU	11	11	-	-		2	2	-	-	J. Heppner, S. Miller	
Depressaridae	Unknown	PU	1	-	1	-		1	-	1	-	J. Heppner, S. Miller	
Elachistidae		PU	1	1	-	-		1	1	-	-	J. Heppner, S. Miller	
Erebidae	Herminiinae	PU	4	-	4	-		1	-	1	-	J. Heppner, S. Miller	
Erebidae	Unknown	PU	1	-	-	1		1	-	-	1	J. Heppner, S. Miller	
Gelechiidae		PU	234	225	1	8		25	19	1	5	J. Heppner, S. Miller	
Geometridae		PU	2	-	2	-		2	-	2	-	J. Heppner, S. Miller	
Gracillariidae		PU	230	-	7	223		7	-	1	6	J. Heppner, S. Miller	
Heliodinidae		PU	35	35	-	-		3	3	-	-	J. Heppner, S. Miller	
Heliodinidae		SE	50	50	-	-		1	1	-	-	J. Heppner, S. Miller	One sp. SE
Lecithoceridae		PU	19	-	9	10		3	-	2	1	J. Heppner, S. Miller	
Limacodidae		PU	6	-	6	-		1	-	1	-	J. Heppner, S. Miller	
Lycaenidae	Polyommatinae	PU	48	-	1	47		2	-	1	1	R. Robbins, S. Miller	
Lycaenidae	Theclinae	PU	76	-	17	58		5	-	2	3	R. Robbins, S. Miller	
Lycaenidae	Theclinae	PU	20	7	-	13		7	5	-	2	R. Robbins, S. Miller	
Lycaenidae	Theclinae	SE	5	5	-	-		4	4	-	-	R. Robbins, S. Miller	Spp. of *Strephonota*, *Tmolus* and *Strymon* are SE
Noctuidae		PU	1	1	-	-		1	1	-	-	J. Heppner, S. Miller	
Nolidae		PU	44	-	11	33		4	-	2	2	J. Heppner, S. Miller	
Oecophoridae		SE	73	66	-	7		14	12	-	2	J. Heppner, S. Miller	
Psychidae		PU	1	-	1	-		1	-	1	-	J. Heppner, S. Miller	
Pterophoridae		PU	9	-	9	-		3	-	3	-	J. Heppner, S. Miller	

Pyralidae	Chrysauginae	SE	38	38	-	-		2	2	-	-	J. Heppner, S. Miller	*Clydonopteron pomponius* SE
Pyralidae	Galleriinae	SE	97	-	97	-		4	-	4	-	J. Heppner, S. Miller	
Pyralidae	Phycitinae	PU	300	14	286	-		4	0	4	-	J. Heppner, S. Miller	
Pyralidae	Unknown	SE	462	327	-	135		27	20	-	7	J. Heppner, S. Miller	
Sesiidae	Sesiinae	SE	257	257	-	-		8	8	-	-	J. Heppner, S. Miller	
Sesiidae	Unknown	SE	4	-	4	-		1	-	1	-	J. Heppner, S. Miller	
Stathmopodidae		PU	19	-	17	2		2	-	1	1	J. Heppner, S. Miller	
Tineidae		SC	1796	1013	642	141		159	149	6	4	J. Heppner, S. Miller	
Tortricidae	Chlidanotinae	PU	23	23	-	-		1	1	-	-	J. Brown, S. Miller	
Tortricidae	Olethreutinae	PU	412	412	-	-		12	12	-	-	J. Brown, S. Miller	
Tortricidae	Tortricinae	PU	12	12	-	-		3	3	-	-	J. Brown, S. Miller	
Tortricidae	Unknown	PU	999	5	314	680		38	1	11	26	J. Brown, S. Miller	
Unknown Lepidoptera		UN	883	123	346	414		116	8	40	68	*	
Total Lepidoptera			8355	3396	2181	2777		595	344	106	145		
DIPTERA													
Agromyzidae		nc	1	1	-	-		-	-	-	-	*	
Anisopodidae		nc	31	-	31	-		-	-	-	-	*	
Cecidomyiidae		nc	32	20	12	-		-	-	-	-	*	
Chloropidae		PU	37	37	-	-		3	3	-	-	*	
Dolichopodidae		nc	4	3	1	-		-	-	-	-	*	
Drosophilidae		SC	13501	3588	2109	7804		-	-	-	-	*	
Ephydridae		nc	4	4	-	-		-	-	-	-	*	
Hybotidae		nc	1	1	-	-		-	-	-	-	*	
Lauxaniidae		nc	1	1	-	-		-	-	-	-	*	
Limoniidae		PU	142	95	28	19		-	-	-	-	*	

Lonchaeidae		PU	338	257	81	-		-	-	-	-	C. Korytkowski	
Micropezidae		PU	126	123	-	3		-	-	-	-	*	
Muscidae		SC	183	11	172	-		-	-	-	-	*	
Mycetophilidae		FU	153	37	101	15		-	-	-	-	*	
Neriidae		PU	120	18	102	-		-	-	-	-	*	
Phoridae		nc	41	2	37	2		-	-	-	-	*	
Psychodidae		nc	325	1	324	-		-	-	-	-	*	
Richardiidae		PU	109	109	-	-		4	4	-	-	*	
Sarcophagidae		nc	1	1	-	-		-	-	-	-	*	
Sciaridae		FU	1026	729	290	7		-	-	-	-	*	
Sciomyzidae		nc	14	14	-	-		-	-	-	-	*	
Sphaeroceridae		nc	2	2	-	-		-	-	-	-	*	
Stratiomyidae	Sarginae	PU	598	598	-	-		8	8	-	-	R. Ctvrtecka	
Stratiomyidae	Unknown	PU	1116	25	483	608		22	7	8	7	R. Ctvrtecka	
Syrphidae		SC	134	15	87	32		6	3	2	1	R. Ctvrtecka	
Tachinidae		PA	149	62	87	-		-	-	-	-	*	
Tephritidae	Trypetinae	PU	844	844	-	-		12	12	-	-	M. Schutze, C. Korytkowski	
Tephritidae	Others	PU	4763	5	866	3892		132	2	29	101	M. Schutze	
Tipulidae		SC	319	46	273	-		-	-	-	-	*	
Unknown Diptera		UN	4934	176	1355	3403		-	-	-	-	*	
Total Diptera			29049	6825	6439	15785		187	39	39	109		
HYMENOPTERA													
Agaonidae		PA	146	-	-	146		-	-	-	-	*	Some spp. as parasitoids, inquilines
Agaonidae		SE	2089	1087	59	943		-	-	-	-	*	
Apidae		nc	1	-	-	1		-	-	-	-	*	

Bethylidae		PA	1	-	1	-		-	-	-	-	*
Braconidae	Acampsohelconinae	PA	2	2	-	-		2	2	-	-	D. Quicke
Braconidae	Agathidinae	PA	13	9	-	4		3	2	-	1	D. Quicke
Braconidae	Alysiinae	PA	194	10	132	52		19	3	8	8	D. Quicke
Braconidae	Brachistinae	PA	16	-	1	15		3	-	1	2	D. Quicke
Braconidae	Braconinae	PA	235	66	20	149		49	27	6	16	D. Quicke
Braconidae	Braconinae	SE	1	-	-	1		1	-	-	1	D. Quicke
Braconidae	Cenocoeliinae	PA	23	23	-	-		5	5	-	-	D. Quicke
Braconidae	Cheloninae	PA	228	65	31	132		39	21	6	12	D. Quicke
Braconidae	Doryctinae	PA	379	376	2	1		32	30	1	1	D. Quicke
Braconidae	Helconinae	PA	208	77	2	129		17	13	1	3	D. Quicke
Braconidae	Macrocentrinae	PA	4	4	-	-		3	3	-	-	D. Quicke
Braconidae	Microgastrinae	PA	86	63	7	16		42	30	4	8	D. Quicke
Braconidae	Opiinae	PA	496	56	123	317		56	11	19	26	D. Quicke
Braconidae	Orgilinae	PA	88	1	-	87		3	1	-	2	D. Quicke
Braconidae	Unknown	PA	375	1	32	342		9	1	8	-	*
Chalcididae		PA	100	-	-	100		-	-	-	-	*
Chalcidoidea		PA	181	181	-	-		-	-	-	-	*
Diapriidae		PA	39	8	31	-		-	-	-	-	*
Encyrtidae		PA	2	2	-	-		-	-	-	-	*
Eucharitidae		PA	9	9	-	-		-	-	-	-	*
Eucoilidae		PA	81	56	25	-		11	9	2	-	D. Quicke
Eulophidae		PA	270	252	5	13		20	18	1	1	D. Quicke
Eupelmidae		PA	1	1	-	-		-	-	-	-	D. Quicke
Eurytomidae	Eurytominae	SE	203	203	-	-		20	20	-	-	D. Quicke
Eurytomidae	Unknown	SE	168	50	50	68		29	15	3	11	D. Quicke
Figitidae		PA	20	20	-	-		-	-	-	-	*

Formicidae		nc	44	32	12	-	-	-	-	-	*
Ichneumonidae	Acaenitinae	PA	1	-	1	-	1	-	1	-	D. Quicke
Ichneumonidae	Banchinae	PA	7	-	3	4	3	-	1	2	D. Quicke
Ichneumonidae	Cremastinae	PA	14	-	3	11	8	-	3	5	D. Quicke
Ichneumonidae	Ichneumoninae	PA	1	-	1	-	1	-	1	-	D. Quicke
Ichneumonidae	Mesochorinae	PA	5	-	5	-	1	-	1	-	D. Quicke
Ichneumonidae	Orthocentrinae	PA	3	-	3	-	1	-	1	-	D. Quicke
Ichneumonidae	Pimplinae	PA	1	-	-	1	1	-	-	1	D. Quicke
Ichneumonidae		PA	25	22	-	3	12	12	-	-	*
Ormyridae		PA	2	-	2	-	-	-	-	-	*
Perilampidae		PA	1	1	-	-	-	-	-	-	*
Pteromalidae		PA	76	73	3	-	10	9	1	-	*
Tanaostigmatidae		PA	19	19	-	-	2	2	-	-	*
Torymidae		PA	34	16	7	11	8	5	1	2	*
Parasitica		PA	60	-	60	-	-	-	-	-	*
Unknown Hymenoptera		UN	1816	111	10	1695	-	-	-	-	*
Total Hymenoptera			7768	2896	631	4241	411	239	70	102	
				-	-	-		-	-	-	
UNKNOWN ARTHROPODA		UN	2148	2075	73	-	-	-	-	-	Mostly unidentified larvae
TOTAL			80600	27610	17555	35434	1678	899	331	448	

Table S3. Lower matrices of similarity (Morisita-Horn index) between study sites, calculated with the abundance of insect families, and detailed for each main guild. Grey cells denote the highest similarity for each guild matrix.

Guild/Site	**BCI**	**KHC**
Seed eaters		
KHC	0.897	-
WAN	0.916	0.932
Parasitoids		
KHC	0.926	-
WAN	0.910	0.983
Pulp eaters		
KHC	0.931	-
WAN	0.947	0.953
Scavengers		
KHC	0.939	-
WAN	0.897	0.821

Table S4. Summary results of CCAs performed with data sets (10 focal plant families) including all insect species or without singletons at each study site (* = summary plots of analyses in Fig. 3). Abbreviations of headers: Insect/Plant spp. = number of insect/plant species; Total Var %/ Total Var adj % = Percentage of total variance (raw and adjusted) explained; Var Ecol % = Percentage Variance explained by ecological variables (plant and seed traits); Var Phylo % = Percentage variance explained by plant phylogeny; Phylo Excl = Exclusive effect of phylogeny as % of total variability explained; Over % = Percentage of overlap of variance between ecological variables and plant phylogeny. Abbreviations of predictors: FL = fruit length; FW = fresh fruit weight; LF = life form; NS = Number of seeds per fruit; PA = phylogenetic axis (order); SS = seed syndrome.

Site	**Data set**	**Insect spp.**	**Plant spp.**	**Total Var %**	**Total Var adj %**	**Var Ecol %**	**Var Phylo %**	**Phylo Excl %**	**Over %**	**Significant predictors (p adj. < 0.05)** (ordered by forward selection of traits and phylogeny, respectively)
BCI	All insects	518	131	9.0	1.4	1.2	0.2	14.3	0.0	LF, SS; PA(15)
	w/o singletons *	71	80	27.5	12.6	12.2	2.3	3.2	1.9	SS, NS, LF; PA(5,8)
KHC	All insects	189	80	6.9	3.2	2.6	1.4	18.8	0.8	FL, NS; PA(6)
	w/o singletons *	102	76	28.1	15.8	14.2	2.9	10.1	1.3	FL, FW, SS, NS, LF; PA(6)
WAN	All insects	282	114	26.7	12.9	2.8	11.5	78.3	1.4	SS; PA(7,2,9,19,11,4,1,5,26,8,6)
	w/o singletons *	169	114	32.4	18.7	5.2	15.9	72.2	2.4	SS, FL; PA(7,9,2,25,11,4,8,1,6,19,5)

Table S5. Results of binomial Phylogenetic Linear Mixed Models (PGLMM) for each study site, including model coefficients and significance. Fixed effects are listed, while random effects are host plant species, herbivore species and phylogenetic covariance. Significant p-values of fixed effects are indicated in bold.

Site/Fixed Effect	Estimate	Standard Error	z-score	p-value
BCI				
Seed type (dry/fleshy)	0.188	0.210	0.897	0.370
No of Seeds	-0.001	0.002	-0.580	0.562
KHC				
Seed type (dry/fleshy)	0.260	0.341	0.762	0.446
Fruit Length	0.014	0.008	1.713	0.087
No of Seeds	-0.039	0.030	-1.280	0.201
Fresh Fruit Weight	0.011	0.005	2.219	**0.027**
WAN				
Seed type (dry/fleshy)	-0.160	0.249	-0.644	0.519

Table S6. Results of two way ANOVAs considering (a) the abundance of seed eaters per seed (when seeds are attacked) and (b) the apparent rate of seed attack, with fixed factors sites and seed syndromes.

Source	**Sum Squares**	**df**	**Mean Squares**	**F**	**p**
a) Abundance per seed					
Site	16.58	2	8.29	17.81	<0.001
Syndrome	16.55	6	2.76	5.92	<0.001
Site*Syndrome	42.33	12	3.53	7.58	<0.001
Error	818.5	1,758	0.47		
b) Apparent rate of attack					
Site	9.72	2	4.86	3.21	0.041
Syndrome	58.90	7	8.41	5.56	<0.001
Site*Syndrome	56.0	14	4.00	2.64	0.001
Error	4,995.8	3,301	1.51		

Table S7. Results of stepwise binary logistic regression (SBLR) describing the probability of rearing seed eaters for all plant species surveyed. Models are detailed separately for each study site (n indicates the number of plant species) and controlled for the effects of host plant phylogeny using the procedures outlined in Appendix S1.

Model/Parameter	**Estimate**	**sd**	**z-value**	**Pr(>|t|)**	**n**
SBLR BCI	-	-	-	-	204
Constant	-0.353	0.193	-1.822	0.068	-
Basal area	0.038	0.020	1.873	0.061	-
Seed rain	1.514	0.676	2.239	0.025	-
SBLR KHC	-	-	-	-	156
Constant	-2.608	0.822	-3.173	0.002	-
Basal area	0.058	0.030	1.944	0.052	-
Fruit length	0.049	0.011	4.616	0.000	-
Sum of seeds collected	0.004	0.001	3.819	0.000	-
SBLR WAN	-	-	-	-	240
Constant	-0.831	0.692	-1.201	0.230	-
No. of confamilial species	0.012	0.012	1.047	0.295	-
Abundance in plot	0.000	0.000	2.535	0.011	-
Sum of seeds collected	0.001	0.000	3.176	0.001	-

Table S8. Results of the phylogenetic path analyses, with details, for each site, dependent variable and model, of the number of conditional independencies (k), number of parameters (q), C statistic (C), its p-value (p), C-statistic information criterion (CICc), number of plant species included in the model, and the highest path coefficient if significant (p>0.05). Significant models are fully drawn in Fig. S8.

Site	**Dependent variable**	**model**	**k**	**q**	**C**	**p**	**CICc**	**No. plant species**	**Highest path coefficient**
BCI	Average no. seed eaters per unit seed	one	7	14	30.66	0.006	63.38	104	-
BCI	Average no. seed eaters per unit seed	two	8	13	38.57	0.001	68.61	104	-
KHC	Average no. seed eaters per unit seed	one	7	14	38.48	0	80.02	46	-
KHC	Average no. seed eaters per unit seed	two	8	13	55.21	0	92.58	46	-
WAN	Average no. seed eaters per unit seed	one	7	14	19.25	0.156	51.36	117	No. seeds per fruit
WAN	Average no. seed eaters per unit seed	two	8	13	59.82	0	89.36	117	-
BCI	Average apparent rate of seed attack	one	7	14	26.49	0.022	59.80	94	-
BCI	Average apparent rate of seed attack	two	8	13	39.96	0.001	70.51	94	-
KHC	Average apparent rate of seed attack	one	7	14	39.31	0	84.11	40	-
KHC	Average apparent rate of seed attack	two	8	13	47.66	0	87.66	40	-
WAN	Average apparent rate of seed attack	one	7	14	16.17	0.303	49.23	98	None large
WAN	Average apparent rate of seed attack	two	8	13	50.07	0	80.41	98	
BCI	No. of species of seed eaters collected	one	7	14	28.33	0.013	61.52	96	-

BCI	No. of species of seed eaters collected	two	8	13	30.19	0.017	60.63	96	-
KHC	No. of species of seed eaters collected	one	7	14	38.18	0	80.18	45	-
KHC	No. of species of seed eaters collected	two	8	13	67.88	0	105.62	45	-
WAN	No. of species of seed eaters collected	one	7	14	19.59	0.144	52.11	109	Sum seeds collected
WAN	No. of species of seed eaters collected	two	8	13	59.59	0	89.46	109	-

Supporting Figures

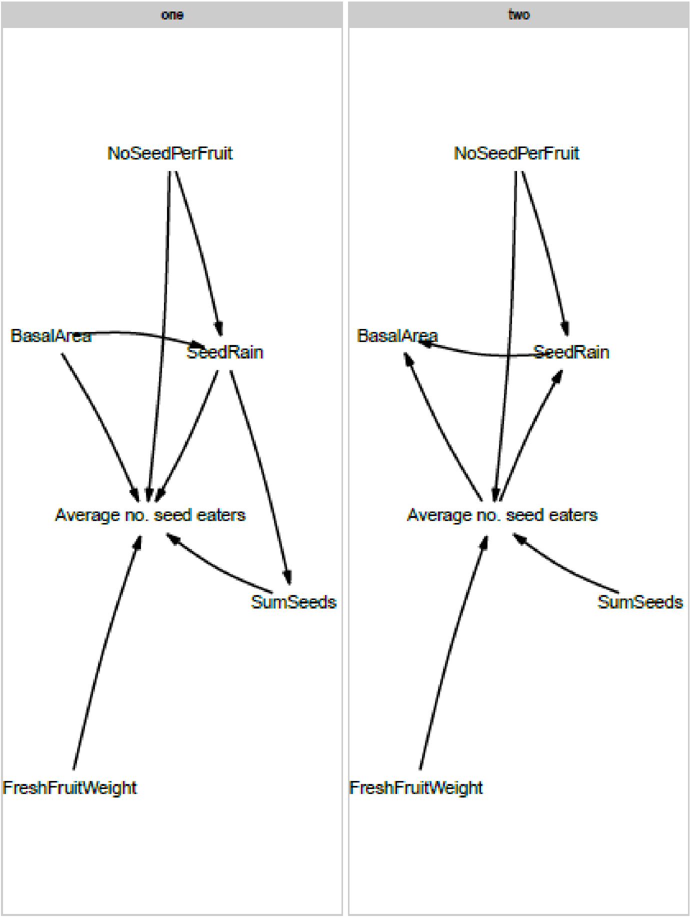

Fig. S1. Models used for the phylogenetic path analyses (assumptions detailed in Appendix S1). The second model is similar to the first one but has reverse paths from the dependent variable, here the average number of seed eaters per unit seed. The same models were used for the three dependent variables at each site (average number of seed eaters per unit seed, apparent rate of seed attack and number of species of seed eaters reared).

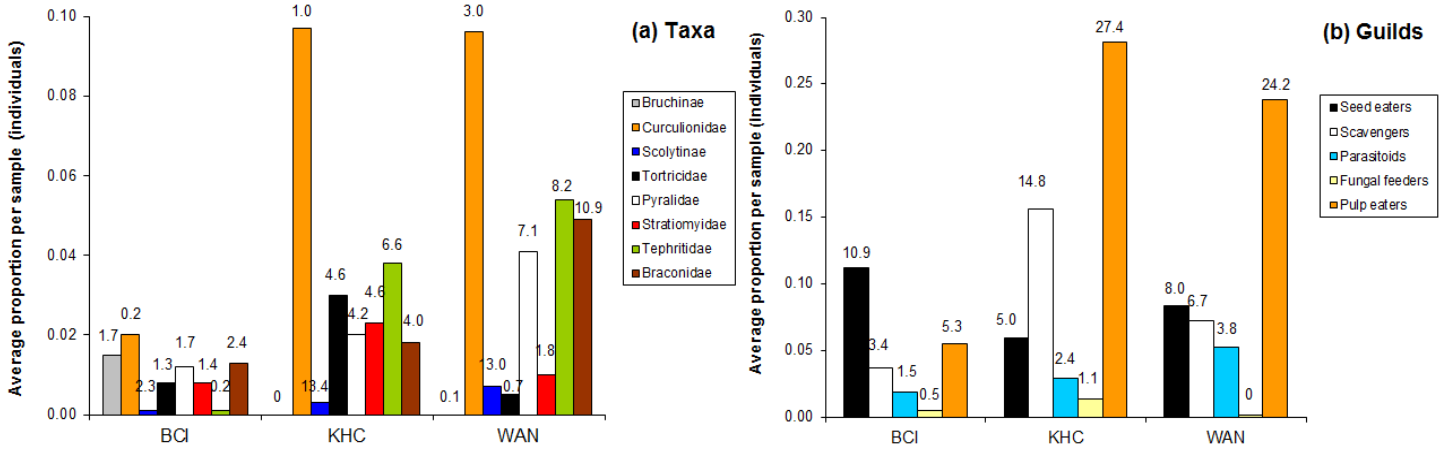

Fig. S2. Plot of the average proportion of individuals of (a) taxa and (b) guilds reared per sample at each study site. Data are restricted to the 10 focal plant families. For taxa and guilds, proportions of particular taxa/guilds across sites are all significantly different (Kruskal-Wallis tests, all with $p<0.05$). Figures above bars indicate, for each site, the percentage of samples in which a taxon or guild was present.

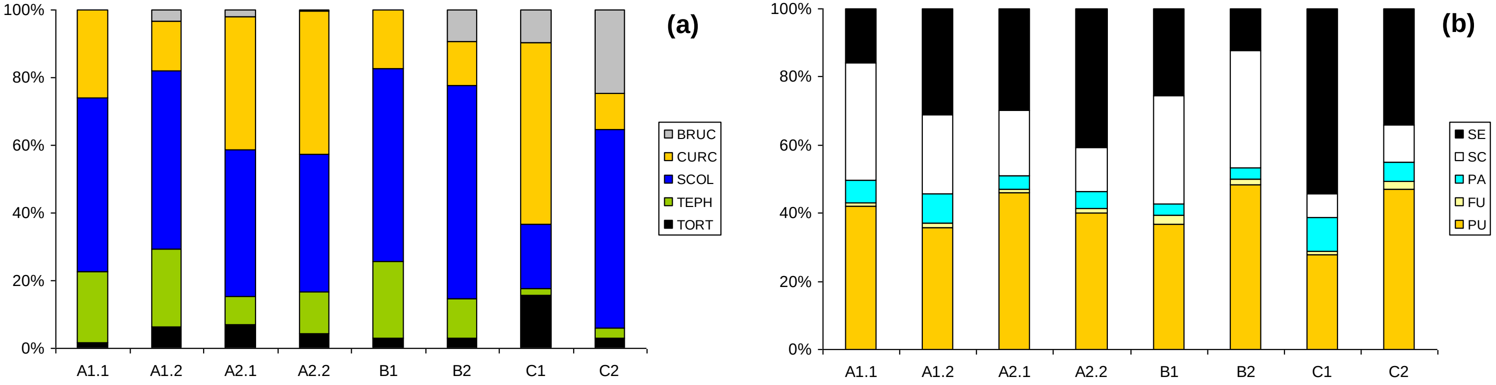

Fig. S3. Proportion of the number of individuals reared from each seed syndrome category at each study site, detailed for (a) the main taxa and (b) guilds. BRUC = Bruchinae, CURC = Curculionidae, SCOL = Scolytinae, TEPH = Tephritidae, TORT = Tortricidae, SE = seed eaters, SC = scavengers, PA = parasitoids, FU = fungal feeders, PU = pulp eaters.

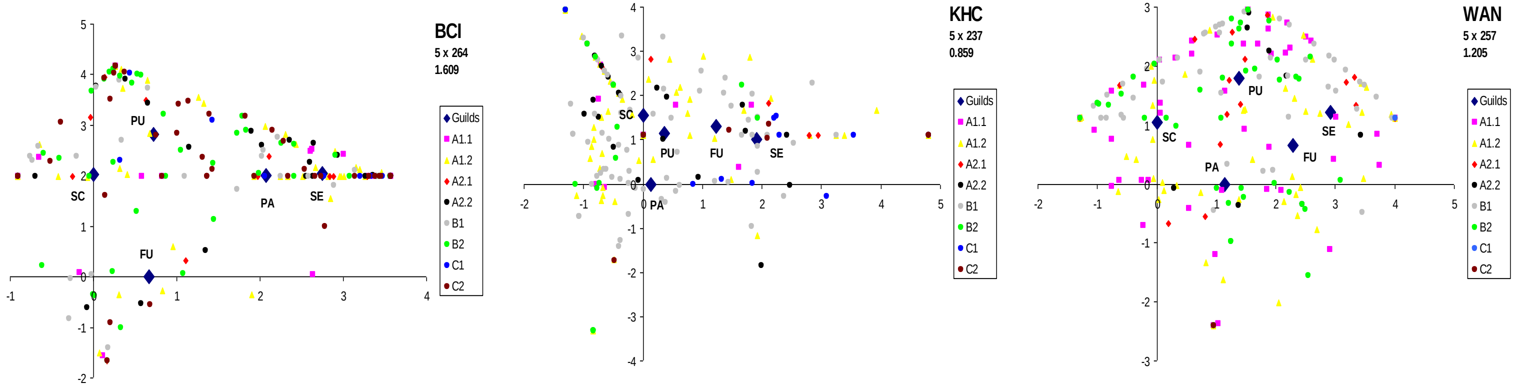

Fig. S4. Plots of plant species and guilds (large blue diamonds) in Axes 1,2 of the CA for BCI, KHC and WAN, respectively, with indication of the size of matrix (no. guild x no. plant species) and total eigenvalue. Different colors for plant species denote different seed syndromes.

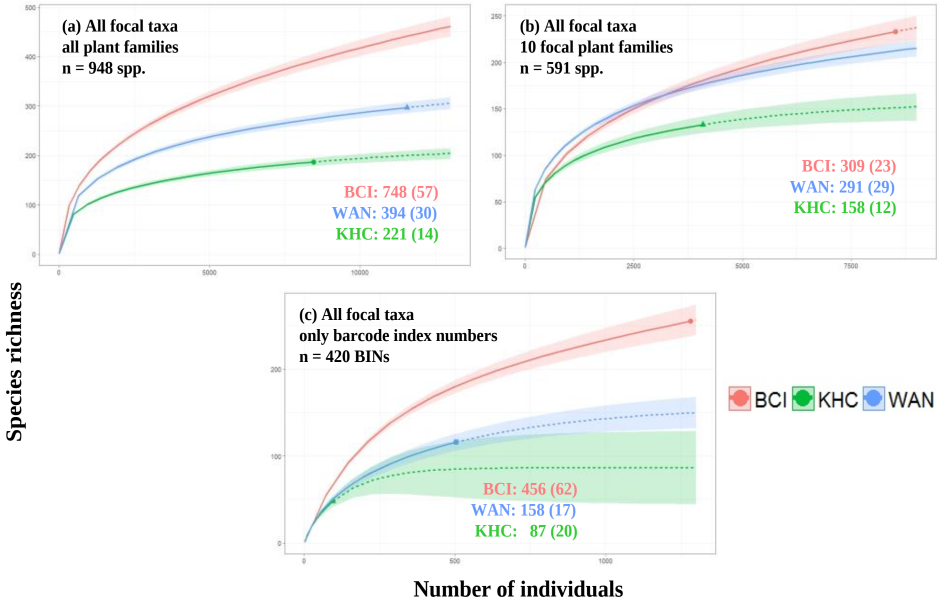

Fig. S5. Sample-size-based (solid line segment) and extrapolation (dotted line segments) of species richness with 95% confidence intervals (shaded areas) at each study site for (a) all data, (b) data restricted to the 10 focal plant families and (c) only Barcode Index Numbers. Estimates of asymptotic estimators (s.e.) are also indicated for each site.

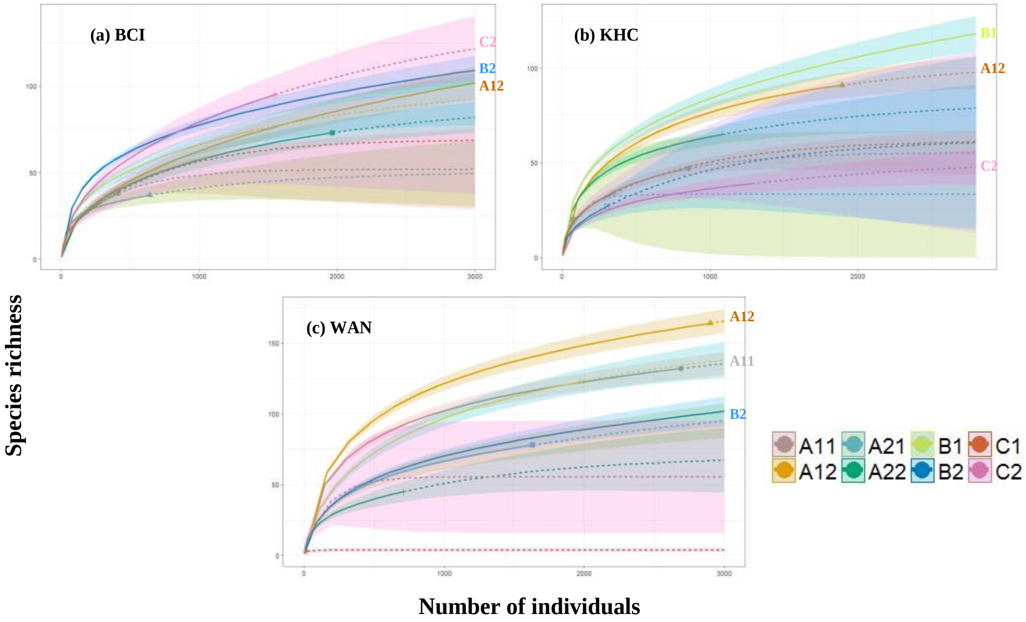

Fig. S6. Sample-size-based (solid line segment) and extrapolation (dotted line segments) of species richness with 95% confidence intervals (shaded areas) for focal insect taxa detailed by study site and seed syndrome. The endpoint of particular syndromes mentioned in the text are emphasized for clarity.

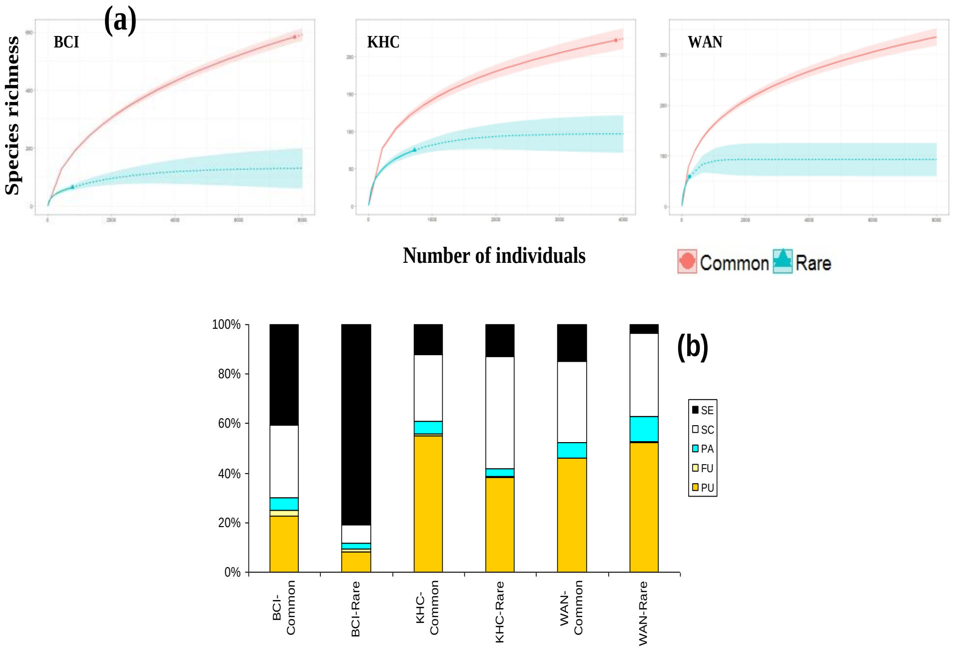

Fig. S7. Contrasts in common and rare tree species, detailed by study sites: (a) species accumulation curves (presentation as in Fig. S5) and (b) proportion of the sum of individuals within guilds.

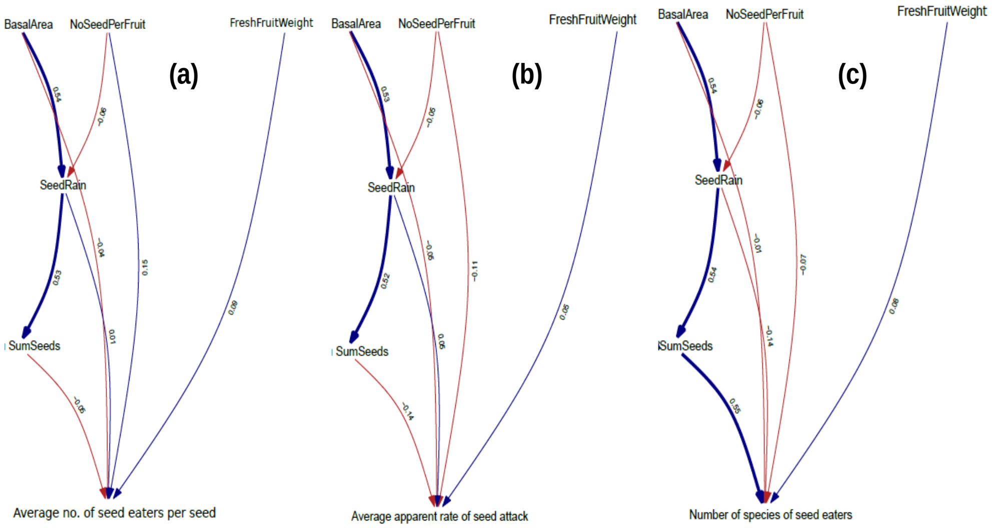

Fig. S8. Significant models of phylogenetic path analysis in Wanang for (a) the average number of seeds eaters reared per unit seed, (b) average apparent rate of seed attack and (c) number of species of seed eaters reared. Note that none of the models were significant at BCI and KHC (Table S8).

Chapter III

Plant-insect frugivore interaction networks in tropical forests: the effects of seasonality

Manuscript

Plant-insect frugivore interaction networks in tropical forests: the effects of seasonality

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Abstract

Plant-insect frugivore networks play important role in the ecology of tropical rainforests as tropical trees support a rich fauna of frugivorous insects whilst these insects have a potential to drive forest dynamics and maintain plant diversity via density-dependent plant mortality (Janzen-Connell hypothesis). These effects can differ across continents, with distinct continental species pools of plants and insects, as well as between wet and dry seasons. Here we examine 12 plant-frugivore interaction networks, comprising two major frugivore taxa (Coleoptera and Lepidoptera) in two seasons (wet and dry) at three sites on different continents (Panama, Thailand, New Guinea). We examine the composition and structure of plant-frugivore networks using fruit syndromes, defined with respect to fruit traits important for insects, thus facilitating inter-continental comparisons across distinct floras. Fruits were sampled and reared for frugivorous insects using plants from 10 focal families per site. A total of 2,149 kg of fruits yielded 18,183 frugivores (14,364 Coleoptera and 3,819 Lepidoptera) reared from 349 tree species representing 10,911 individual trees. Assemblages of frugivores varied with fruit syndromes, emphasizing the dichotomy between fleshy fruits and dry fruits (achenes). The species diversity of frugivores was highest in the drier and more seasonal site in Panama compared to the wet forests of Thailand and New Guinea. The species diversity of Coleoptera and Lepidoptera per biomass of fruit was high (>800 species for all three sites combined) and comparable among sites, but since Lepidoptera were less abundant, they included more species per number of individuals than Coleoptera. Dry season was characterized by lower abundance of attacked fruits, frugivorous insects and insect diversity, compared to wet season at all three sites. Interestingly, this large difference in abundance did not translate to differences in most interaction web parameters (generality, vulnerability, H_2’ diversity). Further, the abundance of frugivores increased seasonally with increasing rainfall only at the Thai site. Large differences in the structure of plant communities as represented by fruit syndromes existed between study sites. The syndromes represented by available fruits varied rapidly and unpredictably across months at each site. These results indicate, the effect of seasonality may be important in structuring composition of plant-frugivore interaction networks shaped by fruit morphological traits (syndromes) and the role frugivores may play towards driving forest dynamic.

Keywords

Fruit syndrome, insect predation, rainfall seasonality, Coleoptera, Lepidoptera

Introduction

Tropical rainforests support more than half of all plant and herbivorous insect species (Novotny et al., 2006; Hamilton et al., 2013). This strong latitudinal gradient in plants, insect herbivores and their interactions may be driven by a variety of ecological factors, including climate (Corely and Barone, 1996; Corlett and Primack, 2006). In tropical rainforests, the interaction between plants and frugivorous insects, including seed predators, is of particular importance as it has potential effects on forest dynamics. Seed dispersal away from parent trees and seed-seedling survival are crucial for the maintanance of high tropical tree species diversity (Janzen, 1970), whilst insect frugivores may be one of important mortality factors for seeds. Fruit-feeding insects range from innocuous to a serious mortality factor as they can destroy seeds while still at the embryo stage (Ehrlen, 1996), and may potentially destroy a large proportion of seed crops by either pre-dispersal or post-dispersal predation. Insect frugivores recruit predominately from Coleoptera, Lepidoptera, Diptera and Hymenoptera (Janzen, 1971; Ctvrtecka et al., 2016; Sam et al., 2017; Basset et al., 2018). They are known to feed on fleshy tissues of fruits and on seeds (Ctvrtecka et al., 2016; Basset et al., 2018).

Insect seed predators represent a highly specialized frugivore guild. Importantly, they may act as a negative density-dependent driver of plant populations, attacking disproportionately more common plant species and making it thus possible for less abundant plant species to establish themselves and coexist in the forest, escaping from specialized seed predators. Insect seed predators thus may be promoting high tropical plant diversity with numerous rare species, the characteristic pattern of tropical forests (Janzen, 1970; Downey et al., 2018). Fruit morphological traits, including fruit size, colour, and morphology, such as fleshiness, alongside with nutritional quality, seed chemistry, fruiting phenology and host phylogeny are all important determinants of attack rate by insects (Janzen, 1969; Wright, 1990; Nakagawa et al., 2003; Ramirez and Traveset, 2010; Segar et al., 2017). Thus, we proposed a classification system of fruit syndromes based on the fruit traits important to insect frugivores (Dahl et al., 2019).

Plant reproductive phenology is often driven by the seasonality of rainfall in tropical rainforests (Sakai, 2001; Kurten et al., 2017). Plant phenology can also be shaped by biotic interactions from herbivores, pollinators, and seed predators (Wright, 1990). Plant phenology can significantly affect insects feeding on young leaves, flowers, and fruits through temporal changes in plant resource availability (van Schaik et al., 1993). For example, during a fruiting event insects can time their oviposition of eggs on fruits so that developing larva may develop into adults during wet rather than during dry season when the food resources are available (Wolda, 1978; Coley and Barone, 1996; Givnish, 1999). On the other hand, rainfall seasonality may also constrain insect host specificity behaviour (Givnish, 1999). For instance, dry season and pest are proposed as selective filters acting on species distribution along rainfall gradient in tropical forests and may contribute importantly to species distributional limits and diversity (Baltzer and Davies, 2012). Similarly, conditions that reduce productivity such as poor soils, low rainfall, high seasonality may have reduce the rates of specificity of herbivory and, hence, have consequence for density dependent tree mortality (Givnish, 1999).

Despite their potential importance, ecological studies on the host specificity of fruit-insect interactions mediated through rainfall seasonality in rainforests are rare (e.g., Basset et al., 2019). Several studies showed wet tropical rainforest suffer higher herbivory pressure than dry forest areas (e.g., Brenes-Arguedas et al., 2009). The high level of species diversity in tropical forests may also drive seasonal differences in ecological interactions among species (Dyer et al., 2007). It allows for competition, predation and parasitism to be relatively more intense towards lower tropical latitudes (Dyer et al., 2007; Schleuning et al., 2012). Further, the high level of inter-specific interactions may have also cascading effects and drive tropical rainforest plants to increased chemical and mechanical defence against herbivores at lower latitudes (Dyer et al., 2007; Tylianakis et al., 2007; Schleuning et al., 2012). The ecological interactions hypothesis suggests that the interaction webs is more intense, than a sole focus on focal taxa or communities. In this study, we took the approach that focus on plant – frugivore webs and their seasonality.

Another serious gap in ecological studies is their geographic limitation to a single regional species pool. While many studies are replicated locally, across different sites, the studied communities are still drawn from the same evolutionarily determined regional species pool. This may influence also the strength of Janzen-Connell (JC) effects by herbivores. They may be weaker in drier, more seasonal rainforest habitats due to plant stress, lower survival and consequent attack by insects (Janzen, 1970; Connell, 1971; Givnish, 1999), and also vary due to differences in biogeographic history (Comita et al., 2014). For instance, dipterocarp-dominated forests in Malaysia exhibit supra mast fruiting that could lead to a weaker Janzen-Connell effect (Janzen, 1970; Bagchi et al., 2011) than in other rainforest localities.

In this study we used a different approach, selecting our sites for inter-continental comparisons, using three Forest Global Earth Observatory (ForestGEO) plots (see methods): Baro Colorado Island in Panama, Khao Chong in Thailand, and Wanang in Papua New Guinea. The study was partially standardized between continents by focusing on 10 plant families per site, eight of which were shared among all three sites, whilst two were not (Table S1).

Our study aims at answering two questions. (1) Is there a relationship between the abundance of Coleoptera and Lepidoptera frugivores and rainfall seasonality at the three rainforest plots? We also expect some inter-continental differences, can be influenced by rainfall gradient between wet humid and dry seasonal forests that may generate turnover of plant species across tropical rainforest regions (Brenes-Arguedas et al., 2009). Neotropical Panamanian rainforest site has a pronounced drier seaonality, with mostly plants producing dry fruits and but not the two Palaeotropic forest sites which contributes to the diversity of fleshy fruiting plants at the same time the Thai and New Guinean sites exhibit large floristic differences as the Thai site is dominated by dipterocarp trees (e.g., *Dipterocarpus costatus*), while the New Guinea site is not (Corlett and Primack, 2006). (2) Does the average host specificity in plant-frugivore food webs vary between dry and wet seasons? We expect that higher seasonality of rainfall in Panama may result in lower specialization in food webs as dry season limits the availability of resources (Dyer et al., 2007; Schleuning et al., 2012; Gentry, 1982). On the other hand, lower rainfall leads to higher proportion of plants with dry fruits, increasing the proportion of seed predators among frugivores at the expense of flesh eaters. In turn, this could increase overall host specificity in Panama as seed predators tend to be more specialized than frugivores eating fruit flesh (Willson and Whelan, 1990; Kissling et al., 2009).

Materials and Methods

Study sites

Our study sites are three Forest Global Earth Observatory (ForestGEO) plots situated in biogeographically distinct rainforest regions, each on a different continent: Neotropical - Panama: Barro Colorado Island (BCI has a 50 ha ForestGEO plot in a 1,500 ha island created by the opening of the Panama Canal in 1914); Oriental: Thailand - Khao Chong (KHC) has a 24 ha ForestGEO plot located in the protected forest of the Khao Ban Thad Wildlife Sanctuary in Southern Thailand) and Australasian: Papua New Guinea - Wanang (WAN has a 50 ha ForestGEO plot situated within the 10,000 ha of Wanang Conservation Area). ForestGEO (http://www.forestgeo.si.edu/) is a global network of permanent forest plots established to study long-term forest ecosystem dynamics (Anderson-Teixeira et al., 2014). Our three study plots were situated in diverse undisturbed lowland forests that spanned a rainfall gradient from wet low seasonal KHC, WAN to a moderate dry with stronger seasonality at BCI. Other characteristics of vegetation study plots are summarized in (Anderson-Teixeira et al., 2014; Basset et al., 2018).

Frugivore surveys

We used standardized field protocols and surveyed frugivores by sampling fruits for 3 to 4 years at each study site, followed by extensive rearing programs (Basset et al., 2018; Dahl et al., 2019). This survey protocol was initiated in 2010 at Barro Colorado Island and was replicated in 2013 at Khao Chong and Wanang ForestGEO plots. During the first year at each study site, we randomly searched for and sampled fruits from all locally available trees, shrubs, lianas and (more rarely) epiphytes and herbs within or near the permanent ForestGEO plots. In subsequent years, we restricted our sampling to plant species found in 10 focal families that are commonly distributed in each study forest. Eight of these families are well represented across all three sites while the two other families are only important locally each at a single site (Table S1). As much as possible we targeted > 5 individuals from each tree species and sampled fresh fruits whilst still attached, or abscised fruits, pods and seeds (only fresh fruits without any decomposition). Further, we selected 2-4 individual fruits per tree species, classified them with regard to morphology, and estimated their size (length and width to the nearest millimeter) and weight (to the nearest gram; Dahl et al., 2019).

Insect rearing and identification process

Fruits were placed within rearing plastic pots, covered with fine mesh lid and were kept in ventilated sheds under ambient environmental conditions. These rearing plastic pots were checked weekly for emerging adults for three months, allowing sufficient time for larvae to develop. Adult insects emerging during rearing were stored individually in vials with 95% ethanol or pinned and mounted (for most Coleoptera and Lepidoptera specimens). They were assigned to morpho-species with the assistance of taxonomists (e.g., Basset et al., 2018) and with the use of molecular techniques (Ratnasingham and Hebert, 2013). For molecular purposes, we extracted legs of representative insect specimens and obtained DNA Cytochrome c oxidase subunit I (COI, DNA barcode) sequences and used Barcode Index Numbers (BINs) for insect sequences to delineate species (Ratnasingham and Hebert, 2013). All DNA sequences were deposited in projects of the Barcode of Life Data System (www.boldsystems.org).

Furthermore, to assess host specificity of frugivore insect communities, we assigned frugivores to individual feeding guilds, and the fruits to syndromes, based on their morphology relevant to insects (Tables S2, S3). All reared insects were assigned to guilds: *seed eaters* (coded as SE: larva feeding mostly on seed tissue), *pulp eaters* (PU: larva feeding mostly on mesocarp tissue), *scavengers* (SC: larva feeding mostly on decaying matter), *fungal feeders* (FU: larva feeding mostly on fungi) and *parasitoids* (PA: larva feeding on insect hosts). Each reared species per fruit syndrome is assigned to a higher taxanomic level, mostly family or subfamily. To allow for food-web analysis (consult, Basset et al., 2018 for details).

Plant trait measurement and insect seed predation

We characterized the plants from which insects were reared by a suite of plant traits for each species. These included plant life form (tree, shrub, liana, herb, palm), fruit syndromes, species abundance (number of stems in vegetation plots), fresh fruit/seed mean weight and length, and rates of seed attack. We identified eight categories of insect fruit syndromes at each ForestGEO study plot (Dahl et al., 2019 for details) on the basis of plant fruit morphology traits (e.g., fleshiness, dehiscence, number of seeds per fruit and measurement of mesocarp thickness) mostly relevant for insect seed predators (Janzen, 1969; Ramırez and Traveset, 2010; Basset et al., 2018). These traits represent important variables for ovipositing female insects and the developing larvae (Basset et al., 2018; Dahl et al., 2019). These traits are used to assess host specificity by insect frugivores but also considered important for seed germination process (e.g., Nakagawa et al., 2003; Baltzer and Davies, 2012; Lewis and Gripenberg, 2008).

Rainfall and frugivores

At each ForestGEO site, we obtained daily rainfall measurement for 3 to 4 years during the study period (Figures S1, Table S4). BCI has 3 to 4 months of dry season, from mid-December/January to April (Wolda, 1983; Leigh et al., 2004; Beckman and Muller-Landau, 2011), while KHC has a milder 2 to 3 months of dry seasons from January to March (Baltzer and Davies, 2012). WAN has 2 to 3 months of mild dry season from July to September (McAlpine et al., 1983, Figure S1, Table S4). Monthly rainfall data (e.g., Figures S1, Table S4) and insect seed predators reared from fruit syndromes per study site that allowed us to assess the relationships with host specificity and insect seed predation (Dahl et al., 2019). Our continuous sampling and rearing allowed us to estimate the seasonality of frugivorous insects reared from the fruits (Figures S1). We used the data from the 10 focal tree families for inter-continental comparison, to ensure compatibility of data among different forest types (Table S1). Further, we focused on two key taxa, Coleoptera and Lepidoptera. These taxa are ecologically well studied and are known to feeds on various fleshy tissues and also seeds of many tropical rainforest fruits (e.g., Copeland et al., 2009; Brown et al., 2014; Ctvrtecka et al., 2014; Sam et al., 2017), that provide baseline for comparisons in this study.

Data analysis

Our analyses were based on all tree species (349 species) obtained within 10 focal families sampled for fruits at three ForestGEO plots. Individual fruits sampled per tree species assigned to a fruit syndrome were used to identify food source for insect frugivores in order to quantify host specificity and insect seed predation (Dahl et al., 2019). We used mean monthly rainfall as an explanatory factor for frugivore abundance, defining dry season by < 100 mm of rainfall per month (Baltzer and Davies, 2012; Figures S1, Table S4). We used linear model to test for correlation between rainfall and frugivore abundance for two taxa and three forest plots. The species diversity of frugivores was analysed using rarefaction and extrapolation sampling curves of species richness with the R package ‘iNEXT’ (Hsieh et al., 2016).

We used higher taxa (mostly subfamilies and familiesas units for analysis) for frugivores, separating (i) Coleoptera and Lepidoptera, (ii) dry and wet season, and (iii) study sites between fruit syndromes as units of analysis for plants, in the construction of 12 interaction webs (Figures 4, S1). We generated bipartite networks (Dormann et al., 2008) for each season and site for both Coleoptera and Lepidoptera, and calculated the following quantitative network metrics: 1) generality, as the mean abundance weighted number of hosts used by each herbivore species, 2) vulnerability, the mean abundance weighted number of herbivores using each host species (Tylianakis et al., 2007), 3) connectance, the proportion of the actually observed interactions per species relative to the total possible interactions divided by the number of species in the network, weighted by interaction frequency (Dunne et al., 2002) and 4) network specialization (H_2') as a measure of specialization at the network level that is based on the deviation between the number of interactions of a species and the expected total of interactions for each network, assuming that all species interact with their partners in proportion to their observed frequency total (Blüthgen et al., 2006). We used the function 'network level' in the R package bipartite (Dormann et al., 2008). All analyses were computed with the R package (R Core Team, 2017).

Results

A total of 2,149 kg of fruits yielded 18,183 seed predators of which Coleopterans represented about 79% (14,364 insects reared) and Lepidopterans 21% (3,819 insects reared). The fruits were sampled from 349 plant species representing 10,911 trees at the three ForestGEO sites (see Tables S1, S2, S3). The relative importance of different fruit syndromes as food source for Coleopteran and Lepidopteran frugivores varied significantly during the year, and among the study sites (Figure 1). In Coleoptera, each site had unique or almost unique syndrome(s) contributing to the frugivorous community: A1.1 and A2.1 (fleshy and non-fleshy drupe with a thick mesocarp) at BCI, C2 syndrome at KHC (multiple dry seeds that develop in capsule) and C1 (dry winged seed that does not develop in capsule) at WAN. In contrast, A1.2 (fleshy drupe with thin mesocarp) was the only syndrome important at all three sites. The patterns recorded for Lepidoptera were similar; the differences between sites were larger than between Coleoptera and Lepidoptera within a single site. The only marked difference was higher importance of the B1 syndrome (fleshy indehiscent fruit with multiple seeds) for Coleoptera than Lepidoptera at KHC and WAN. Interestingly, this syndrome, although important at these two sites, was almost absent from BCI.

The differences among months in seed syndrome composition were significant (χ^2 test, $p < 0.05$) for both taxa at each of the three sites. However, seasonality was not extreme as most of the syndromes were present throughout the year. The individual syndromes did not exhibit distinct seasonal peaks, except for B2 (non-fleshy dehiscent fruits with multiple seeds) which were concentrated during the dry season at BCI.

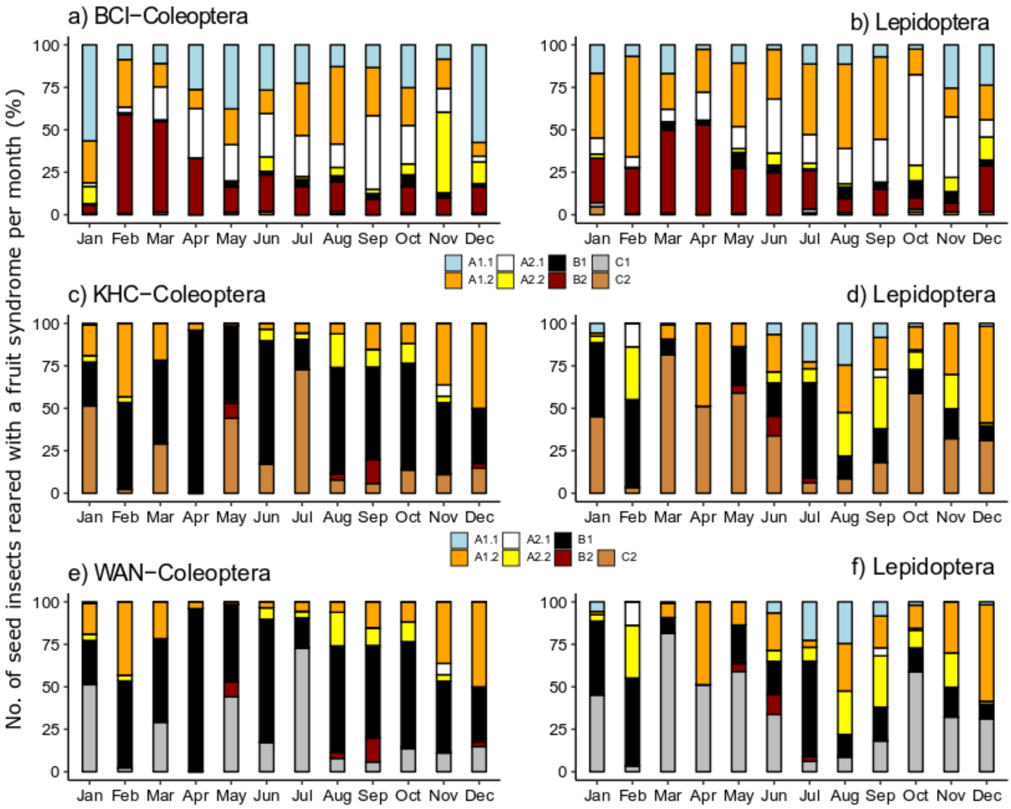


Figure 1. Seasonal variation of Coleoptera and Lepidoptera seed predators reared from individual fruit syndromes (A1.1 – C2) at three forests. (BCI, KHC and WAN). See Methods for fruit syndromes.

Species diversity of frugivores was compared using species accumulation curves based on the number of insect individuals (Figures 2). These curves showed that for Coleoptera, BCI was more than twice more diverse than WAN and KHC, both exhibited almost identical diversity. While the KHC and WAN curves approached an asymptote at ~ 100 species per site and required only 3,000 individuals to capture most of the local species diversity at BCI our surveys were still adding new species after rearing 8,000 individuals when it exceeded 200 species (Figure 2a). Sampling effort in Lepidoptera was less complete, with only the least diverse site, KHC, reaching asymptote at ~ 80 species. The species diversity was intermediate at WAN and highest again at BCI, with > 250 species (Figure 2b). When comparing the two taxa across all three sites, Lepidoptera proved to be more species diverse per a given number of individuals than Coleoptera (Figure 2c). However, Lepidoptera had lower number of individuals per fruit biomass than Coleoptera so that the numbers of species reared from the entire survey was comparable between the two taxa, at ~ 400 species shared (Figure 2c).

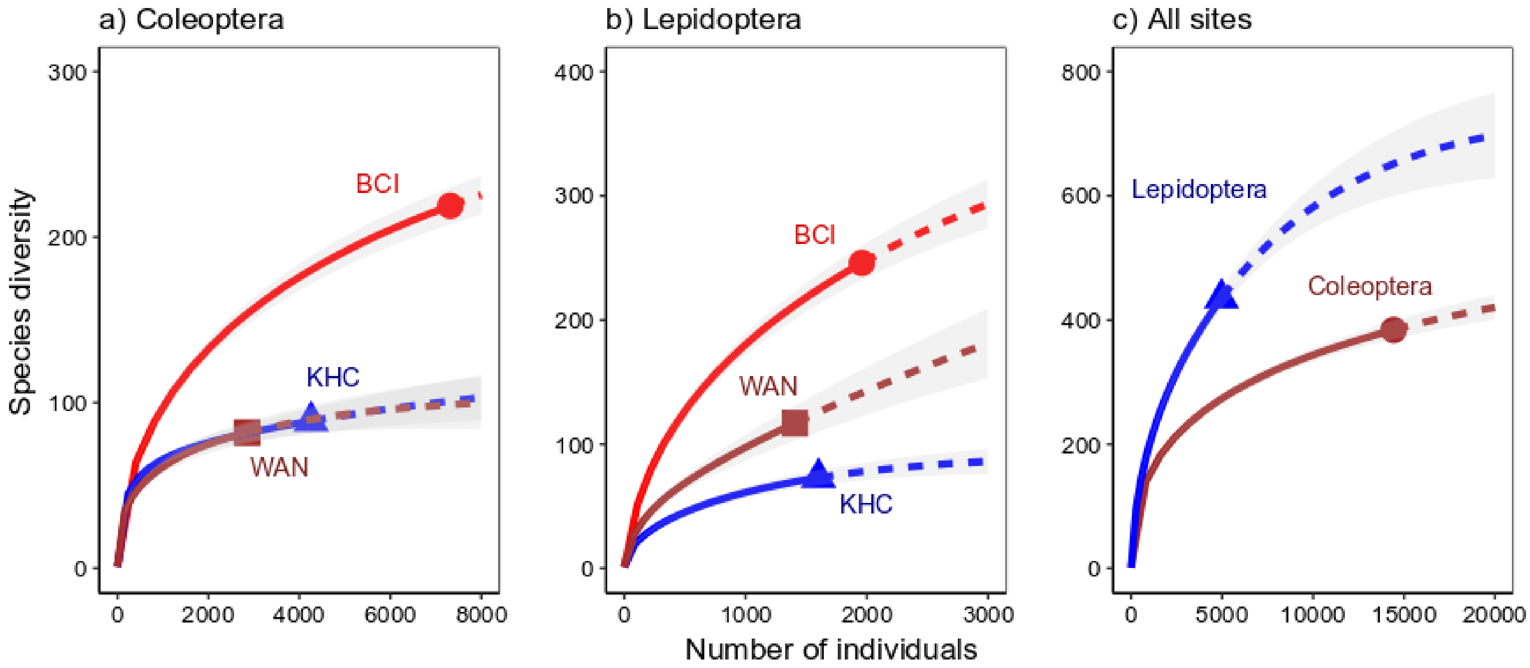

a) Coleoptera
b) Lepidoptera
c) All sites
Species diversity
Number of individuals
BCI
KHC
WAN
BCI
WAN
KHC
Lepidoptera
Coleoptera
300
200
100
0
0
2000
4000
6000
8000
400
300
200
100
0
0
1000
2000
3000
800
600
400
200
0
0
5000
10000
15000
20000

Figure 2. Rarefaction curves showing the diversity of Coleoptera (a) and Lepidoptera (b) frugivores at individual sites, and all sites combined (c). The frugivores were reared from the 10 focal families per site. Extrapolated curves are dashed, 95% confidence intervals are shaded in grey.

We found no significant correlation among Coleopterans (R^2 = 0.0063, $F_{1,46}$ = 0.291, p = 0.87, n = 48 months) and Lepidopterans (R^2 = 0.0056, $F_{1,46}$ = 0.260, p = 0.61, n = 48 months) frugivore abundance with increasing rainfall at BCI (Figures 3, S1). Hence, there was a significant correlation among Coleopterans (R^2 = 0.199, $F_{1,34}$ = 8.432, p = 0.0064, n = 36 months) and Lepidopterans (R^2 = 0.223, $F_{1,34}$ = 9.75, p = 0.0036, n = 36 months) frugivore abundance increased with increasing rainfall at KHC (Figures 3, S1). While, no significant correlation was observed among Coleopterans (R^2 = 0.0185, $F_{1,34}$ = 0.639, p = 0.43, n = 36 months) and Lepidopterans (R^2 = 0.1061, $F_{1,34}$ = 0.365, p = 0.55, n = 36 months) frugivore abundance with increasing rainfall at WAN (Figures 3, S1).

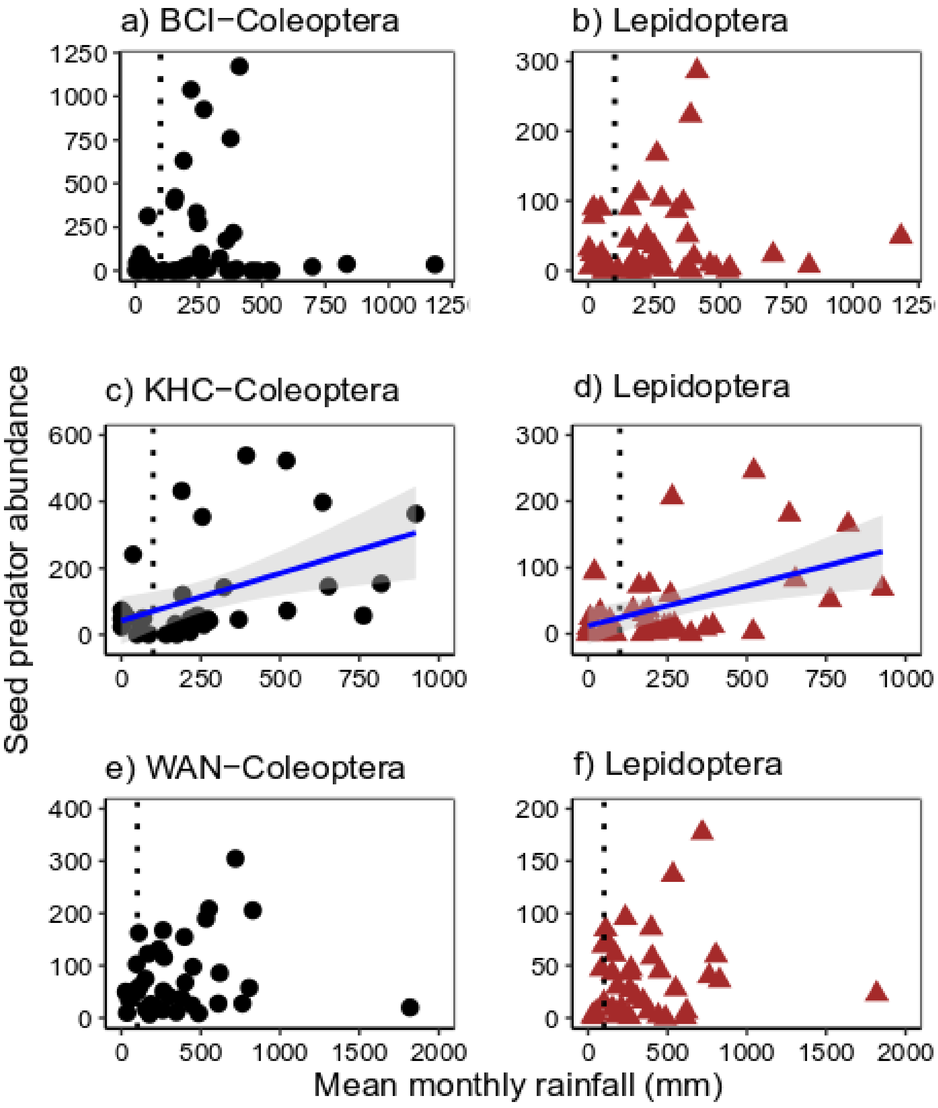
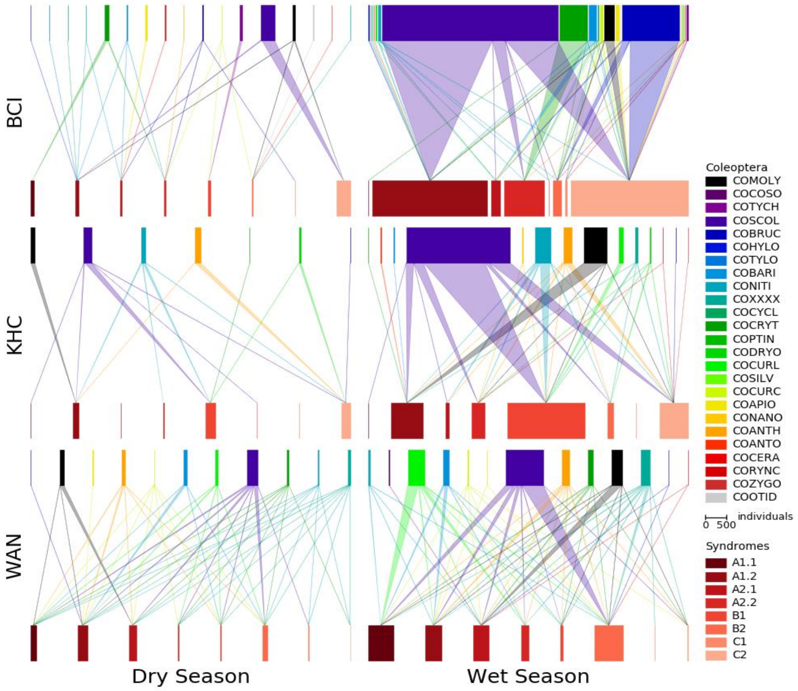


Figure 3. Correlation between frugivore abundance with rainfall, analyse comprise individual months of 3-4 years of sampling per site. The vertical dotted line indicates average 100 mm rainfall used to separate dry and wet seasons.

There was a clear difference between sparse, low abundance networks from dry season as compared to those in wet season, for both Coleoptera and Lepidoptera at all three sites (Figures 4). These visual differences translated to significantly higher number of frugivore taxa in wet than dry season (Table 1). However, differences in network metrics remain statistically non-significant between the seasons (Table 1). The differences between sites were significant for connectance (high in WAN) and H_2' diversity (Table 1). Coleoptera networks in WAN were characterized by higher generality and vulnerability values than those at the other two sites (Figures 5). However, no metrics differed significantly between Coleoptera and Lepidoptera (Table 1). In addition to differences between sites in plant syndromes, described above, there were also important taxonomic differences in frugivores among the sites (Figures 4). In Coleoptera, Scolytinae numerically dominates all three sites. The new two top frugivore taxa per site are, Bruchinae and Cryptorhynchinae at BCI, Molytinae and Nitidulidae at KHC and Curculioninae and Molytinae at WAN (Figures 4, Table S2). In Lepidoptera, the top frugivore taxon was Tineidae at BCI and KHC, and this taxon was the third most common at WAN (sharing this rank with Polyommatinae). Further, the next two top frugivore taxa were Cosmopterigidae and Olethreutinae at BCI, Phyticinae and Pyraustinae at KHC and Carposinidae at WAN (Figures 4, Table S3).

a)


BCI
KHC
WAN
Dry Season
Wet Season
Coleoptera
COMOLY
COCOSO
COTYCH
COSCOL
COBRUC
COHYLO
COTYLO
COBARI
CONITI
COXXXX
COCYCL
COCRYT
COPTIN
CODRYO
COCURL
COSILV
COCURC
COAPIO
CONANO
COANTH
COANTO
COCERA
CORYNC
COZYGO
COOTID
0 500 individuals
Syndromes
A1.1
A1.2
A2.1
A2.2
B1
B2
C1
C2

b)

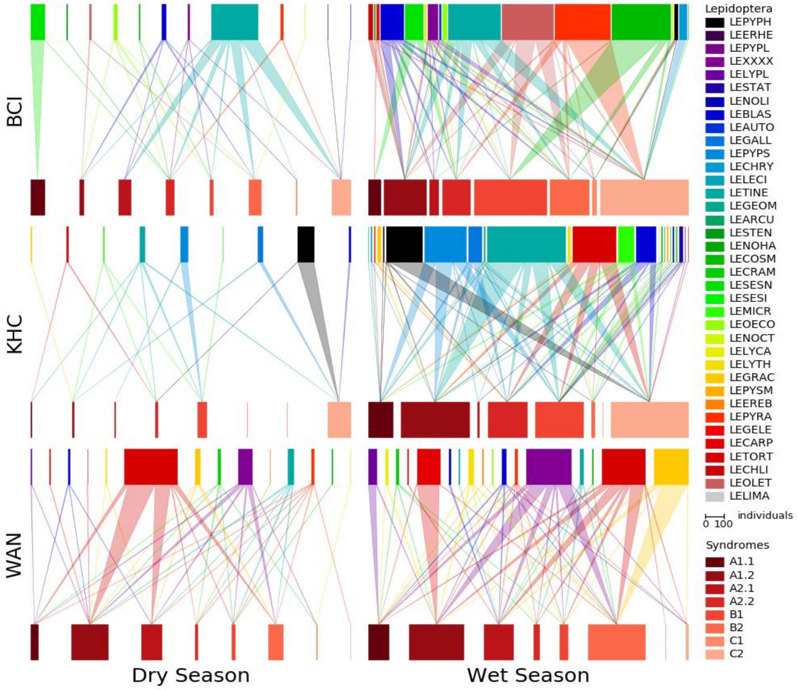

BCI
KHC
WAN
Dry Season
Wet Season
Lepidoptera
LEPYPH
LEERHE
LEPYPL
LEXXXX
LELYPL
LESTAT
LENOLI
LEBLAS
LEAUTO
LEGALL
LEPYPS
LECHRY
LELECI
LETINE
LEGEOM
LEARCU
LESTEN
LENOHA
LECOSM
LECRAM
LESESN
LESESI
LEMICR
LEOECO
LENOCT
LELYCA
LELYTH
LEGRAC
LEPYSM
LEEREB
LEPYRA
LEGELE
LECARP
LETORT
LECHLI
LEOLET
LELIMA
0 100 individuals
Syndromes
A1.1
A1.2
A2.1
A2.2
B1
B2
C1
C2

Figure 4. Bipartite networks showing a) Coleopteran and b) Lepidopteran frugivores in dry and wet season at three study sites. Bars at the bottom indicate relative abundance of individual fruit syndromes, upper bars indicate frugivorous taxa (families or subfamilies, see Tables S2, S3), and width of the links indicate their frequency.

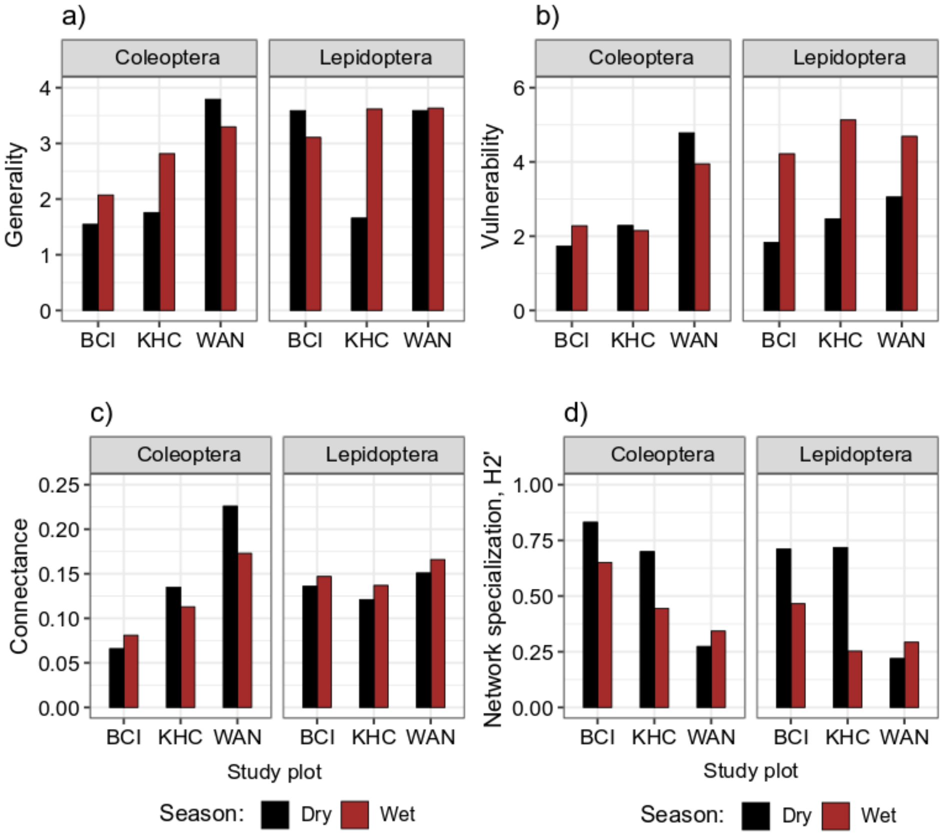


Figure 5. Network metrics for frugivore insectsin dry and wet seasons across tropical latitudes: a) generality, b) vulnerability, c) connectance, and d) network specialization (H_2’).

Table 1. Effect of rainfall season (dry vs wet season), taxon (Coleoptera vs Lepidoptera) and site (BCI, KHC, WAN) on network metrics for interaction networks between fruit syndromes and insect families. Significant (*) results at $P \leq 0.05$.

	Season		Taxon		Study site	
Network Metric	F	P	F	P	F	P
No. of insect families	6.121	0.043*	0.762	0.412	0.588	0.581
Generality	1.137	0.322	2.551	0.154	3.010	0.114
Vulnerability	3.141	0.120	1.423	0.272	2.597	0.143
Connectance	0.024	0.882	0.298	0.602	4.788	0.049*
Specialization H_2'	5.189	0.057	1.756	0.227	9.289	0.011*

Discussion

Few studies have investigated quantitative food-web interaction networks across three distinct ForestGEO rainforest plots (Y. Basset et al., unpubl. data) and none, to our knowledge, has compared the effect of seasonality on the assemblages of the plant-frugivore networks in this context. We found that the assemblages between frugivorous Coleoptera and Lepidoptera reared during wet and dry seasons were quiet dissimilar at three ForestGEO study plots, each from a different continent. Large differences in species pools among the regions are evident, represented by differences in the representation of fruit between fleshy vs. dry syndromes that contribute to frugivore assemblages at each site. For example, BCI in Panama has a relatively high percentage of frugivore insects reared from dry fruit syndromes, but not Khao Chong (KHC), Thailand and Wanang (WAN), New Guinea sites. Both Thai and New Guinean frugivorous insects were reared mostly from fleshy and non-fleshy drupe syndromes (Basset et al., 2018; Dahl et al., 2019).

The lack of strong seasonal trends in frugivorous insect assemblages in our study is consistent with other rainforest studies (e.g., Moles et al., 2003; Chen et al., 2017; Jeffs et al., 2018). Our sites mostly experience from medium to low rainfall seasonality. That they proved to be highly variable from month to month with fruit syndromes contributing to frugivorous communities. Thus, this result likely suggests asynchronous contributions by numerous tree species with their fruit crops to the overall resource supply during the year (Boivin, 2019), lacking a predictable turnover in fruit syndrome composition of fruits that would generate seasonal trends in frugivore abundance, diversity and composition. Our result showed partial positive relationship among frugivore insects’ abundance and rainfall at three rainforest plots, reflecting, and local seasonality of rainfall pattern (e.g., Corely and Barone 1996). The varying intensity of rainfall and predispersal seed predation by tropical frugivorous insects highlight that the high degree of mortality by seed predators may not be always a significant mortality factor. Host plant mortality during seed dispersal, seed to seedling transition, and sapling phase may be more important for plant species dynamics (Crawley 1992; Lewis and Gripendberg 2008; Boivin et al., 2019).

The generality values could in theory indicate potential of frugivores, particularly seed eaters, to maintain plant diversity by Janzen-Connell effects. Here the low specialization of beetles in WAN and Lepidoptera in general indicates low potential for such herbivore-plant dynamics, as also indicated by low abundance of frugivorous beetles reported from Papua New Guinea by another similar study (Ctvrtecka et al., 2014; 2016). In contrast, beetles in KHC and BCI may be effective Janzen-Connell agents, and one such case of density-dependent regulation by seed predators was indeed previously reported from Panama (Wright, 1983). The vulnerability values were higher for beetles in WAN than KHC and BCI. While KHC and especially BCI networks were heavily dominated by one or a few interactions than, the network in WAN comprised higher number of less frequent interactions. In Lepidoptera, dry season networks had consistently lower vulnerability than wet season ones. This demonstrate clearly a consequence of lower fruits and insect abundance in dry season, leading to many rare interactions being absent in dry season. Interestingly, this was not the case for Lepidoptera where lower abundance did not translate in a decrease in vulnerability values in dry seasons. This may also typically reflect life history stage of predispersal by insect seed predators across the rainforest sites. For example, most frugivore Lepidoptera require relatively humid condition and fleshy syndromes with thick mesocarp of fruits that are necessary for the adult female insects ovipositing, and for larva development, thus limiting further larval desiccation in dry harsh conditions (Jeff et al., 2018). Moreover, our result may also indicate that Lepidoptera seed predators in forests of Panama and Thailand are relatively more specialized and better adapted to pronounced seasonal dry forests than the New Guinean forest (Basset et al., 2018).

Seasonal differences in interaction webs have been documented in other studies (e.g., Lewis et al., 2002). However, such differences do not necessarily affect network parameters. For instance, Tylianakis et al., (2007) found a distinct variation in interaction webs along of a gradient from natural to modified habitats but the web metrics did not reveal differences in quantitative webs. However, it is surprising that a marked decline in species diversity and abundance of frugivores in dry season caused a relatively small change in network parameters, compared to wet season. In support, our quantiative webs provide an example of the extend of seasonality in frugivore abundance thus reflects the rainfall seasonality at difference rainforest localities. For example, we found at the three-rainforest plots, a decrease in frugivore abundance networks in the dry seasons, which may perhaps mark transition from the beginning of the wet season with increased frugivore abundance (see Wolda, 1978). In addition, frugivore abundance observed in this study may mirror this pattern being lowest in the dry seasons and highest towards the wet seasons (Wolda, 1978; Lewis et al., 2002). Thus seasonality of rainfall between dry and wet seasons may also correlate to species turnover (see Brenes-Arguedas et al., 2009), such as it may provide partial control for defensive adaptation of plants that may influence rate of frugivore seed attack (e.g., Basset et al., 2018). For instance, it has been shown pathogen and insect herbivory damage is higher in wet seasons compare to dry seasons (Brenes-Arguedas et al., 2009; Bagchi et al., 2014). Finally, the differences between seasonality of rainfall pattern per study rainforest plots may also explain the outcome of frugivore seed predation network parameters. For example, seasonal semi-deciduous tree species may require specialized frugivore insects with their ability to search and locate its host tree species in Panamanian forest compared to Thai and New Guinean forests (Basset et al., 2018).

Conclusion

We showed that the assemblages of frugivores varied largely with fruit syndromes across rainforest study sites. The frugivore species diversity was highest in the drier and more seasonal site in Panama compared to the wet forests of Thailand and New Guinea (Basset et al., 2018). Additionally, despite lower frugivore abundance, we find that Lepidoptera constitute high species richness community compared to Coleoptera. We find that the effect of seasonality was important in determining frugivore abundance at all study sites. The frugivores attacking fruits and seeds was rather low at dry seasons compared to wet seasons across three studied rainforest localities. Moreover, our web networks indicate that frugivore host specificity per site may be influence by the dominance of plants with fleshy vs. dry fruit syndromes. This finding was similar to that described previously for assemblages of fruit and seed feeding insects across studied rainforest plots (Basset et al., 2018). This may support the conclusion the frugivore web interactions were less stronger during dry seasons.(Basset et al., 2018). Finally, this study may provide guide to the methods and sample size necessary to document plant frugivore networks in tropical rainforests, and demonstrate the approach to inter-continental analysis relying on fruit syndromes defined as food resources for insects in tropical rainforests.

Acknowledgements

We are grateful to our colleagues of the Smithsonian Tropical Research Institute, (BCI, Panama), Khao Chong Botanical Garden (Thailand) and Binatang Research Centre and Wanang Conservation area (Papua New Guinea) for helping with this project which was funded by the Czech Science Foundation (GACR grant 16-20825S), the Center for Tropical Forest Science small grant scheme, the University of South Bohemia (grant GA JU 152/2016/P). The Canadian Centre for DNA Barcoding (Biodiversity Institute of Ontario) and Southern China DNA Barcoding Center (Kunming Institute of Zoology, Chinese Academy of Sciences) assisted with DNA sequencing of insect specimens.

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Supporting Information

Supplementary materials for Chapter III. Plant-insect frugivore interaction networks in tropical forests: the effects of seasonality - Manuscript

Appendix III - Supporting Tables and Figures

Table S1. The number of individual trees /tree species sampled from the 10 focal plant families per study plot, including eight families sampled at all three sites, and two site-specific families at each site.

	Study plot			
Plant family	BCI	KHC	WAN	Focal
Annonaceae	117 /7	582 /18	172/12	All sites
Arecaceae	235/8	383/15	39/6	All sites
Bignoniaceae	172/11	-	-	BCI
Clusiaceae	255/4	-	-	BCI
Ebenaceae	-	208/10	-	KHC
Euphorbiaceae	119/6	62/8	707/10	All sites
Fabaceae	1,533/46	1,259/10	912/12	All sites
Lauraceae	478/8	173/3	264/10	All sites
Meliaceae	83/5	226/10	400/27	All sites
Myristicaceae	-	-	389/14	WAN
Myrtaceae	-	-	533/9	WAN
Phyllanthaceae	-	31/8	-	KHC
Rubiaceae	192/14	119/8	683/18	All sites
Sapindaceae	77/17	345/5	163/10	All sites
Total	3,261/126	3,388/95	4,262/128	10,911/349

Table S2. The number of frugivorous Coleoptera from individual taxa (subfamilies or families) with their guild classification, reared at each study site. Guilds: UN=feeding guild not known, PU=Pulp eater, SC=Scavenger, SE=Seed eater (see Basset et al., 2018). The top three taxa at each site in bold.

Taxon Code	Family	Subfamily	Guild	BCI	KHC	WAN	Total
COANTH	Anthribidae		SE	10	335	240	585
COAPIO	Apionidae	Apioninae	SE	-	-	3	3
COAPIO	Brentidae	Apioninae	SE	126	-	-	126
COOTID	Brentidae	Cyladinae	SE	2	-	-	2
COCERA	Cerambycidae		PU	16	3	-	19
COBRUC	Chrysomelidae	Bruchinae	SE	**1,349**	2	3	1,354
COSCOL	Curculionidae	Scolytinae	PU	**4,384**	**2,591**	**1,120**	8,095
COBARI	Curculionidae	Baridinae	SE	174	1	198	373
COZYGO	Curculionidae	Conoderinae	SE	-	1	-	1
COCOSO	Curculionidae	Cossoninae	SE	-	-	1	1
COCRYT	Curculionidae	Cryptorhynchinae	SE	**744**	3	162	909
COCURC	Curculionidae	Curculioninae	SE	5	107	**430**	542
COCYCL	Curculionidae	Cyclominae	SE	1	-	-	1
COMOLY	Curculionidae	Molytinae	SE	292	**615**	**334**	1,241
COTYCH	Curculionidae	Tychiinae	SE	81	-	-	81
CODRYO	Dryophthoridae	Rhynchophorinae	SE	5	75	3	83
CONANO	Nanophyidae		SE	-	12	-	12
CONITI	Nitidulidae		SC	70	**469**	60	599
COPTIN	Ptinidae		SC	1	-	-	1
COSILV	Silvanidae		SC	4	-	-	4
COXXXX	Unknown		UN	20	45	267	332
			Total	7,284	4,259	2,821	14,364

Table S3. The number of frugivorous Lepidoptera from individual taxa (subfamilies or families) with their guild classification, reared at each study site. See Table S3 for guild classification.The top three taxa at each site in bold.

TaxonCode	Family	Subfamily	Guild	BCI	KHC	WAN	Total
LEMICR	Microlepidoptera		UN	-	85	-	85
LEARCU	Arctiidae		PU	-	4	-	4
LEARCU	Ctenuchiinae		PU	-	4	-	4
LEAUTO	Autostichidae		PU	8	-	-	8
LEBLAS	Blastobasidae		SC	140	115	29	284
LECARP	Carposinidae		PU	-	-	**125**	125
LECOSM	Cosmopterigidae		PU	**316**	2	3	321
LEPYPS	Crambidae	Pyraustinae	PU	-	**260**	-	260
LEPYSM	Crambidae	Spilomelinae	PU	-	1	-	1
LESTEN	Depressariidae	Stenomatinae	PU	2	-	-	2
LEERHE	Erebidae	Herminiinae	PU	-	3	-	3
LEGELE	Gelechiidae		PU	15	1	5	21
LEGEOM	Geometridae		PU	-	1	-	1
LEGRAC	Gracillariidae		PU	-	19	**206**	225
LELECI	Lecithoceridae		PU	-	3	1	4
LELIMA	Limacodidae		PU	-	1	-	1
LELYPL	Lycaenidae	Polyommatinae	PU	-	-	**47**	47
LELYTH	Lycaenidae	Theclinae	PU	-	11	34	45
LENOHA	Noctuidae	Hadeninae	PU	-	1	-	1
LENOLI	Nolidae		PU	-	2	8	10
LEOECO	Oecophoridae		SE	40	-	5	45
LECHRY	Pyralidae	Chrysauginae	SE	39	-	-	39
LEGALL	Pyralidae	Galleriinae	SE	-	93	-	93
LEPYPH	Pyralidae	Phycitinae	SE	19	**278**	-	297
LEPYPL	Pyralidae	Pyralinae	SE	-	2	-	2
LESESN	Sesiidae	Sesiinae	SE	170	-	-	170
LESTAT	Stathmopodidae		PU	-	18	-	18
LETINE	Tineidae		SC	**527**	**449**	**47**	1,023
LECHLI	Tortricidae	Chlidanotinae	PU	23	-	-	23
LEOLET	Tortricidae	Olethreutinae	PU	**284**	-	-	284
LEXXXX	Unknown		UN	60	-	313	373
			Total	1,643	1,353	823	3,819

Table S4. Summary of rainfall seasonality patterns described in the literature and this study.

Study site	Rainforest Region	Study Year	Dry season:	Wet season:	Mean rainfall (mm)/yr	Reference
BCI, Panama	Neotropic	2010-2013	mid Dec to Apr	May to Nov	238.3	
			mid Dec to Apr/May	Aug to Nov.		Leigh 1999, this study
			Jan to Apr	May to Nov		Wolda, 1983
KHC, Thailand	Southeast Asia	2013-2015	Jan to Mar	Apr to Dec	268	Baltzer and Davies 2012, this study
WAN, PNG	New Guinea	2013-2015	Jul to Sept	Oct to Jun	372.1	MacAlpine et al. 1983, this study

Table S5. Summary of network metrics for Coleoptera and Lepidoptera frugivores in wet and dry season at each study site. H_2’ is network specialization.

Study plot	Season	Insect taxon	No.of taxa	Generality	Vulnerability	Connectance	H_2’
BCI	Dry	Coleoptera	17	1.547	1.735	0.066	0.832
-	Wet	Coleoptera	19	2.074	2.281	0.081	0.651
-	Dry	Lepidoptera	12	3.588	1.834	0.136	0.711
-	Wet	Lepidoptera	17	3.108	4.221	0.147	0.466
KHC	Dry	Coleoptera	7	1.758	2.291	0.135	0.7
-	Wet	Coleoptera	14	2.816	2.152	0.113	0.445
-	Dry	Lepidoptera	9	1.66	2.467	0.121	0.718
-	Wet	Lepidoptera	24	3.617	5.133	0.137	0.253
WAN	Dry	Coleoptera	11	3.792	4.784	0.226	0.274
-	Wet	Coleoptera	13	3.298	3.95	0.173	0.343
-	Dry	Lepidoptera	14	3.59	3.059	0.151	0.22
-	Wet	Lepidoptera	17	3.634	4.691	0.166	0.293

Supporting Figures

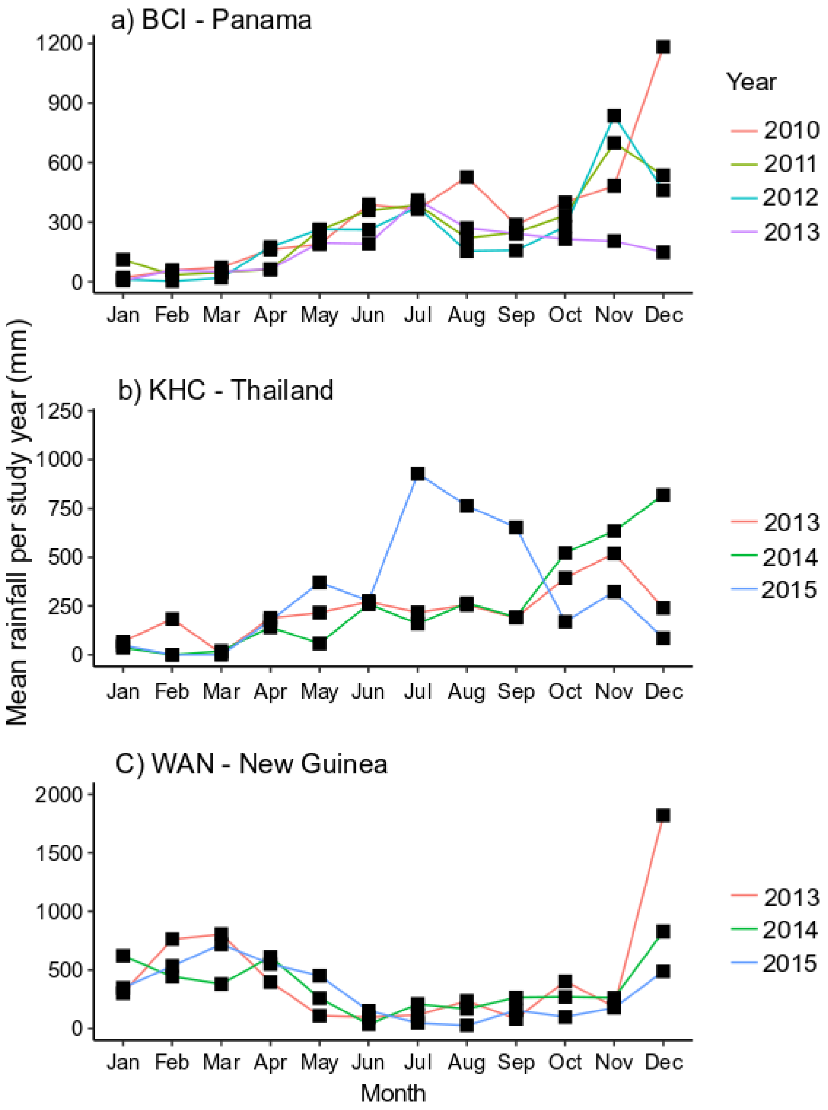

Figure S1. Monthly rainfall (mm) pattern during the years of sampling, on average our sampling years corresponded to typical rainfall seasonality patterns reported in the literature (see Table S4).

Chapter IV

Insect assemblages attacking seeds and fruits in a rainforest in Thailand

in Entomological Science 22 (2):137-150

Entomological Science (2019) **22**, 137–150 doi: 10.1111/ens.12346

ORIGINAL ARTICLE

Insect assemblages attacking seeds and fruits in a rainforest in Thailand

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Abstract

Insect seed predators are important agents of mortality for tropical trees, but little is known about the impact of these herbivores in rainforests. During 3 years at Khao Chong (KHC) in southern Thailand we reared 17,555 insects from 343.2 kg or 39,252 seeds/fruits representing 357 liana and tree species. A commented list of the 243 insect species identified is provided, with details about their host plants. We observed the following. (i) Approximately 43% of identified species can be considered pests. Most were seed eaters, particularly on dry fruits. (ii) Approximately 19% of parasitoid species (all Opiinae) for which we could determine whether their primary insect host was a pest or not (all *Bactrocera* spp. breeding in fruits) can be considered beneficials. (iii) The seeds/fruits of approximately 28% of the plant species in this forest were free of attack. Phyllanthaceae, Rubiaceae and Meliaceae were attacked relatively infrequently; in contrast, Annonaceae, Fabaceae, Sapindaceae and Myristicaceae were more heavily attacked. There was no apparent effect of plant phylogeny on rates of attack but heavily attacked tree species had larger basal area in the KHC plot than rarely attacked tree species. (iv) Insects reared from fleshy fruits were more likely to show relatively stable populations compared to insects reared from dry fruits, but this was not true of insects reared from dipterocarps, which appeared to have relatively stable populations throughout the study period. We tentatively conclude that insects feeding on seeds and fruits have little effect on observed levels of host abundance in this forest.

Key words: dipterocarp, parasitoid, pest, seed predation, Tephritidae.

INTRODUCTION

Insect seed predators represent important agents of mortality for tropical rainforest trees because they often

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Received 24 September 2018; accepted 16 December 2018.

kill the plant embryo, or make the fruit unsuitable for seed dispersers (Janzen 1970; Lewis & Gripenberg 2008). Insects feeding internally on fleshy fruits can also cause significant loss of plant fitness and economic damage by, notably, fruit abortion (Stephenson 1981). There is abundant work published on seed predators as pests of economic plants (e.g. Zehnder *et al.* 2007) or on seed- and fruit-feeding insects in temperate areas (e.g. Turgeon *et al.* 1994) but in comparison little is known about these in tropical rainforests where community-level studies of insects feeding on seeds (dry fruits, achenes) and fleshy fruits are extremely rare. This is because it is difficult to survey the extremely diverse range of potential host plants with adequate spatial and temporal sampling effort, particularly with regard to predispersal seed predation (Ctvrtecka *et al.* 2014). To the best of our knowledge, there are currently only six such examples that are relatively comprehensive: (i) Janzen's studies of insect seed predation in Guanacaste (targeting beetles and summarized in Janzen 1971) led to the formulation of the Janzen–Connell hypothesis, explaining the coexistence of tree species in tropical forests as resulting from negative density-dependence processes (Janzen 1970); (ii) Nakagawa, Hosaka and their colleagues have studied insect seed predation in dipterocarp forests at two locations in Malaysia (Nakagawa *et al.* 2003, 2005; Hosaka *et al.* 2009, 2011; Iku *et al.* 2017); (iii) Copeland *et al.* (2009) made a broad survey of insects feeding on wild fruits in Kenya, targeting tephritids; (iv) Ramírez and Traveset (2010) published a comprehensive survey of insect seed predators in different habitats in Venezuela, including discontinuous patches of forest; (v) Ctvrtecka and colleagues studied insects feeding on both seeds and fruits with high sampling effort in a lowland forest of Papua New Guinea (Ctvrtecka *et al.* 2014, 2016; Sam *et al.* 2017); and (vi) more recently, Gripenberg *et al.* (2018, unpubl. data) undertook a similar survey on Barro Colorado Island in Panama.

The present contribution adds the first study in Thailand. We have summarized the higher faunal composition of the insects reared from seeds and fruits at this location (Basset *et al.* 2018) and intend to discuss interaction networks in detail elsewhere. In this contribution, we attempt to answer various questions related to three general hypotheses that are particularly relevant to the identity per se of the plants surveyed and insect species reared.

First, forests could act as reservoirs of both fruit/-seed-feeding pests and their parasitoids. For example, most research on frugivorous insects from wild fruit is specifically concerned with discovering the range of reservoir hosts of fruit flies (Tephritidae), which are major pests of commercial fruit crops (Allwood *et al.* 1999; Copeland *et al.* 2009). Given that most insect herbivores in tropical rainforests are reasonably host-specific (Novotny *et al.* 2002), it is not immediately clear whether a relatively pristine forest could contribute significantly as a reservoir of pests of cultivated plants, or of potential parasitoids of such pests. Furthermore, forest pests attacking the seeds of ecologically and economically important species of timber trees, such as many species of Dipterocarpaceae (Lyal & Curran 2000), could spread into plantations of these species. The forest might also potentially act as a reservoir of pests of stored products because these insects usually feed on a resource low in water (Subramanyam 1995), similar to that of seed predators of dry fruits (achenes; Janzen 1980). Rainforests might also act as reservoirs of beneficial insects, such as parasitoids of pest species (Aluja *et al.* 2014). The enemy hypothesis states that predatory insects and parasitoids are more effective at controlling populations of herbivores in diverse systems of vegetation than in simple ones (Russell 1989). For example, there is evidence that diverse wet and dry forests in Mexico and Central America act as reservoirs of parasitoids attacking fruit flies in fruit orchards. This mechanism contributes to the value of tropical tree conservation in Mexico (Aluja *et al.* 2014).

Second, the identity of the plants and insects involved in interactions is crucial for two reasons. The identity of plants whose levels of seed/fruit attack stand out from the rest of the local vegetation (i.e. rarely or heavily attacked) is important because it can shed light on patterns of insect host shifts and use (Janzen 1985) and, ultimately, to practical measures of crop protection. The identity (or absence) of the enemies of seed eaters, such as insect parasitoids, is also important because some granivores and frugivores might be relatively free of enemies, perhaps suggesting effective defenses. The nasty host hypothesis proposes that insect herbivores feeding on plant hosts with strong and/or distinctive chemical defenses might support a reduced load of parasitoids because their tissues may be more toxic to parasitoids (Gauld *et al.* 1992). Given the potential importance of insect seed predators in tropical tree mortality (Lewis & Gripenberg 2008), this hypothesis could have consequences for the local distribution of tree species and the dynamics of their populations.

Finally, seed predators are thought to be satiated by mass production of seeds, which promotes escape from predation. The satiation hypothesis has been well-studied in dipterocarp forests of Malaysia (Curran & Webb 2000). The whereabouts of seed-predators of mast-fruiting trees, such as dipterocarps in many forests, in between periods of masting, which can be as long as several years, is crucial for these specialized insects (Hosaka *et al.* 2011). The extent of annual fluctuations of seed predators in tropical rainforests has not been well-studied, with the exception of dipterocarp seed predators, which might maintain populations by prolonged dormancy and/or alternative hosts (Hosaka *et al.* 2011). This issue could help understanding patterns of insect attack on particular plant species, and their local distribution and abundance. Here again the identity of both plants and insects are crucial to evaluate potential patterns.

The general aims of this paper are to document (as far as possible) the identity of insects attacking seeds and fruits, as well as their main parasitoids, in a lowland rainforest in Thailand. Our specific questions are as follows:

1. Does this forest represent a potential reservoir of pests for seed and fruit crops or seeds of valuable timber trees, such as dipterocarps, in Thailand?
2. Does this forest represent a reservoir of parasitoids potentially able to control pests of seeds and fruits in Thailand?
3. Which taxa of seed/fruit-feeding insects are relatively free of parasitoids?
4. Which tree species suffer unusual rates of seed/-fruit attack in this forest? Are these tree species particularly rare or abundant in this forest?
5. Which insect species maintain relatively high and stable populations during the study years?

MATERIALS AND METHODS

Study site

Our study site included the 24 ha ForestGEO permanent vegetation plot (https://forestgeo.si.edu/; see below) at Khao Chong (KHC; 7°32′N, 99°47′E, altitude 120–330 m) and the surrounding forest (i.e. an area of ca. 1,500 ha). This permanent plot is located in the protected lowland seasonal evergreen forest of the Khao Ban Thad Wildlife Sanctuary in southern Thailand and is described in detail by Anderson-Teixeira *et al.* (2014). Mean annual rainfall is 2,665 mm and mean daily maximum air temperature is 27.1°C. KHC experiences a 2–3 months of seasonal drought from January to March (drought defined as any month receiving <100 mm of rainfall: Baltzer & Davies 2012). In the ForestGEO plot, all trees with a diameter at breast height of 1 cm or greater have been mapped and identified to species (Anderson-Teixeira *et al.* 2014). There are 593 tree species, representing 285 tree genera and 82 plant families in the plot, with approximately 300 species per hectare (Baltzer & Davies 2012). The proportion of plant species with dry fruits (achenes) is 26.0% and total seed rain is 7.0 dry $g \times m^{-2} \times year^{-1}$ (Basset *et al.* 2018). Although 13 dipterocarp species grow at KHC (representing 11.8% of stems and 23% of the basal area in the ForestGEO plot; Bunyavejchewin *et al.* 2011), phenological studies reported that the reproductive phenology of the KHC forest was more similar to tropical forests with similar rainfall seasonality in other parts of the world than it was to dipterocarp-dominated forests in ever wet regions of Southeast Asia (Kurten *et al.* 2017).

Survey of plants and rearing of insects

Plant surveying and the rearing of insects from seeds and fruits are detailed in Basset *et al.* (2018). Briefly, in 2013 we surveyed seeds and fruits of locally abundant tree, shrub and liana (more rarely herb) species. During 2014 and 2015, we restricted our sampling effort to 10 plant families, which represented the most common families at KHC. We refer to these families as focal families and they included: Annonaceae, Arecaceae, Ebenaceae, Euphorbiaceae, Fabaceae, Lauraceae, Meliaceae, Phyllanthaceae, Rubiaceae and Sapindaceae. Unless specified, results are detailed for all host plant species. Seeds and fruits collected on plants or freshly fallen (without apparent decomposition) were targeted, thus focusing on predispersal attack (i.e. on insects attacking developing or mature seeds in the canopy of trees). Host plants were identified and their seeds/fruits assigned to the following seed and fruit "syndromes" (hereafter seed syndromes for brevity; see Basset *et al.* 2018 for more details): A1.1, fleshy drupe with thick mesocarp (>5 mm); A1.2, fleshy drupe with thin mesocarp (<5 mm); A2.1, non-fleshy drupe with thick mesocarp (>5 mm); A2.2, non-fleshy drupe with thin mesocarp (<5 mm); B1, fleshy indehiscent fruit with multiple seeds; B2, non-fleshy dehiscent fruit with multiple seeds; C1, dry winged seed that does not develop in capsule; and C2, multiple dry seeds (with or without wings) that develop in a capsule/pod (opening across one axis). These categories were recombined in some analyses as just "fleshy fruits" (= A1.1, A1.2, B1) or "dry fruits" (achenes = A2.1, A2.2, B2, C1, C2).

Rearing sample units included clusters of conspecific seeds/fruits of similar size collected from the same trees. We targeted as many individuals as possible for each plant species, typically >5. These sample units were weighed (fresh weight) and stored in individual plastic pots. Pots were lined with tissue paper and covered with very fine netting for ventilation and to avoid subsequent colonization/contamination of fruits by, notably, drosophilid flies (Copeland *et al.* 2009). Rearing pots were stored under seminatural conditions in covered but ventilated sheds under the forest canopy. They were checked twice weekly, and any emerging insects were collected, preserved, mounted and then identified (see below). Seeds/fruits were stored for 3 months and then dissected to ensure that there were no developing larvae inside. Seed/fruits with live larvae were reared for longer, whereas other seeds/fruits were discarded.

Insect identification

The level of identification was unequal among insect orders owing to the availability of specialists on particular insect groups. In general, beetle and moth families were identified mostly to species level, whereas for Diptera and Hymenoptera only Stratiomyidae, Tephritidae and Ichneumonoidea were sorted to species level. We obtained DNA cytochrome c oxidase subunit I ("DNA barcode") sequences from legs of representative specimens, and we used Barcode Index Numbers derived from insect sequences to delineate species (Ratnasingham & Hebert 2013). Unfortunately, most of the original high-quality DNA samples were spoiled in the sequencing laboratory of the Smithsonian Tropical Research Institute (Panama City, Republic of Panama), and in the meantime the remaining specimens had been exposed to high humidity, so we were unable to obtain DNA sequences from all species. Data were deposited in the Barcode of Life projects KHCSP and KHCTE (398 sequences). Full specimen data for specimens sequenced (including those that failed), including images and host plants, are available on BOLD (www.boldsystems.org), accessible from the dataset KHCFRUIT using a DOI (dx.doi.org/10.5883/DS-KHCFRUIT). Morphological identification of specimens, when possible, was carried out by RT, SEM, JWB, DLJQ, MK, PP, MS, and by colleagues cited in the Acknowledgments. For Lepidoptera, nomenclature follows Holloway (2011) and Holloway *et al.* (2001). Insect vouchers are deposited at the Thai Department of National Parks, Wildlife and Plant Conservation (Bangkok, Thailand) and the National Museum of Natural History, Smithsonian Institution (Washington, D.C., USA).

Insects reared from seeds/fruits were assigned to a guild system at the family, subfamily, or in some cases at the generic or specific level (details in Basset *et al.* 2018). Here we only consider three guilds: seed eaters (coded as SE, larva feeding mostly on seed tissue), pulp eaters (PU, larva feeding mostly on mesocarp tissue), and parasitoids (PA, larva feeding on insect hosts). Members of the moth families Blastobasidae and Tineidae, which are predominantly scavengers, were not included in the analyses; however, when available, we provided basic information about them.

Assessing the pest status of insect species identified is not an easy task. For Lepidoptera, we examined the list of species of economic importance compiled by Zhang (1994). We further considered for pest species the number of citations occurring in the *Review of Applied Entomology* (up to 1994) as an indication of the severity of the pest (Zhang 1994). Additionally, we considered the host records of Kuroko and Lewvanich (1993) for Thailand. For Tephritidae we followed the nomenclature and pest status as indicated in Doorenweerd *et al.* (2018). The pest status of Scolytinae was inferred from Browne (1961) and other sources indicated in Appendix S1, as for the rest of the beetles. Finally, we also considered the species listed as pests and beneficial insects in Thailand (Hutacharern & Tubtim 1995).

Regarding the parasitoids, we considered interactions between members of the Braconidae and Icheumonidae (both Ichneumonoidea) and their insect hosts; these two families represented most of the parasitoids that we reared. Unlike with the host plants, our interpretations of the hosts of the reared parasitoids only reflect "high expectations of interactions", not documented interactions. This is because parasitized hosts were not isolated and reared individually, the parasitoids instead being reared from samples including relatively high numbers of seeds and fruits. To assign putative hosts to each parasitoid species, we applied three simple rules in decreasing level of importance: (i) as many ichneumonoid lineages are rather conservative in host use, we followed Quicke (2015) to select the most likely host order or family; (ii) we then examined for each parasitoid species, the co-occurrence of primary consumers in each sample from which this parasitoid species was reared; and finally (iii) we considered the highest abundance of putative host reared in samples in which the parasitoid species was also reared. We emphasize that our host assignments must not be taken as definite records (Shaw 1994).

Statistical analyses

A main host plant/insect was defined if 80% of reared individuals originated from this host. Sampling effort for a particular plant species could be assessed as the number of samples obtained, or the sum of seeds collected, or the total weight of seeds. To examine which plant species were rarely attacked by insects, we considered species with a high number of seeds collected but none attacked (i.e. no insect reared from the seeds), as this variable is more directly relevant to the regeneration of the plant species. We considered the distribution of the number of seeds free of attack for each tree species, ranked in decreasing number. Host species "rarely attacked" were defined as species belonging to the first quartile of this distribution. It was more challenging to define host species "heavily attacked" and for this we considered insect load on their hosts both in terms of species richness and abundance. With regard to insect species richness, we considered for each host species the number of insect species reared from a main host, excluding insect singletons. With regard to insect abundance, we considered the number of insects reared per seed (per unit seed), to reduce the effect of sampling effort, and calculated these values for hosts relatively well sampled (for which >75 seeds were collected). We compared the abundance in the KHC permanent plot of rarely versus heavily attacked tree species (abundance not defined for liana species) with Mann–Whitney tests for the variables "number of stems" (i.e. number of individuals per tree species) and "basal area" (i.e. total cross-sectional area of all stems in the plot measured at breast height).

Our analyses about insect interannual variation in abundance are limited by only 3 years of data, but motivated by the lack of data for tropical species other than those attacking dipterocarp seeds (i.e. Nakagawa *et al.* 2003). We used the stability index of Wolda (1983) to estimate the magnitude of change in insect abundance between study years (2013–2015). The index is calculated as the natural logarithm of the variance in the natural logarithms of the abundances (+1) of the individual species. We included insect species reared from the 10 focal families plus the Dipterocarpaceae for these analyses and considered the average number of insects reared per seed among samples obtained each year as a measure of insect abundance. We tested for differences in the average stability index of species: (i) of pulp versus seed eaters; (ii) reared from dipterocarps versus non-dipterocarps; and (iii) reared from fleshy versus dry fruits with Mann–Whitney tests. For (ii) and (iii) we considered only insects reared from a main host, in order to relate unequivocally insect species to either plant family or seed syndrome. Raw data (abundance per year) for insect species are indicated in Appendix S1.

We evaluated the influence of host plant phylogeny on our results as follows. First, we estimated the phylogenetic relationships between host species present at KHC using the software package Phylomatic (Webb & Donoghue 2005; details in Basset *et al.* 2018). Second, we tested for phylogenetic signal for all tree species attacked, for trees rarely or heavily attacked and for host trees from which Ichneumonoidea were reared. We calculated the D statistic for phylogenetic signal in a binary trait (Fritz & Purvis 2010). The value of the D statistic is based on the sum of changes between sister clades across the phylogeny. Highly clumped traits tend to have lower D values, closer to 0. We compared the scaled value of the observed D statistic to values generated under a simulated Brownian model of phylogenetic structure and one resulting from no phylogenetic structure (each with 10,000 permutations) using the R package "Caper" (Orme 2013). We used a complementary significance-based approach to provide further support for these results, by testing for phylogenetic signal according to the mean phylogenetic distance (MPD) between tree species. We used standardized effect sizes of MPD generated under null models of tip label randomization (999 runs) as implemented in the R package "Picante" (Kembel *et al.* 2010).

RESULTS

Faunal composition and occurrence of pests and beneficial insects

During the 3-year study, we collected 1,970 samples comprising 343.2 kg or 39,252 seeds/fruits from 357 liana and tree species (and a few herbs) representing 66 plant families. From these samples we reared 17,555 insects (8,851 individuals from the 10 focal plant families). There was a relatively high incidence of Alysiinae (Hymenoptera: Braconidae) and a relatively low incidence of Bruchinae (Coleoptera: Chrysomelidae), Baridinae (Coleoptera: Curculionidae), Cosmopterigidae (Lepidoptera) and Sesiidae (Lepidoptera) in comparison with sites in Panama and Papua New Guinea (Basset *et al.* 2018). Appendix I details the 243 species (totaling 8,949 individuals) in the guilds of seed/pulp eaters and parasitoids that we were able to identify or morphotype. Approximately 71% of the morphospecies could be identified to genus and 28% of them to species. This material included mostly beetles, with Curculionidae (53 spp. and 5,644 individuals; including 22 spp. and 4,262 individuals of Scolytinae) and Anthribidae (8 spp. and 396 individuals) predominating. Tephritidae and Stratiomyidae represented 26 and 8 species, and 814 and 464 individuals, respectively. Moths were dominated by Tortricidae (16 spp., 337 individuals), Crambidae (15 spp., 321 individuals) and Pyralidae (14 spp., 390 individuals), while Braconidae were represented by 54 species and 344 individuals (Appendix I). Most of the insects reared were pulp eaters (127 spp., 73.7% of individuals), followed by seed eaters (55 spp., 22.5%) and parasitoids (62 spp., 4%; Appendix I). Among pulp eaters, two species of *Coccotrypes* were the most abundant and reared from numerous hosts, whereas the most abundant seed eater was an unidentified species of *Aclees* reared mostly from

Mucuna phaseoleae (Fabaceae). Note that the scolytines *Coccotrypes carpophagus*, *C. dactyliperda* and *C. gedeanus* could be considered seed eaters rather than pulp eaters (Appendix I). In addition, 796 specimens of Tineidae and Blastobasidae were reared from 56 host species, but the larvae of these families are more likely to be scavengers. We reared at least one species of *Lateantenna* (Blastobasidae, *L. inana* (Butler, 1881)), one of *Opogona* (Tineidae), three of *Phaeoses* (Tineidae) and one of *Tineovertex* (Tineidae).

Of the 69 taxa identified to species-level, 30 (43%) could be considered pests (Appendix I). This includes two ambrosia beetles that usually do not breed in seeds. The insect taxa in which the proportions of reported pest species to species identified were highest included: Nanophyidae (100%), Crambidae (67%), Tortricidae (55%), Scolytinae (36%) and Tephritidae (26%). The origin of these pest species is summarized in Figure 1. Most pests were seed eaters, and were reared mostly from Dipterocarpaceae and from hosts with seed syndromes C1 (dry winged seed) and A1.2 (fleshy drupe with thin mesocarp). Most pest species and individuals were reared from dry fruits as opposed to fleshy fruits (Fig. 1). Only one pest of stored products, *Pyralis pictalis* (Curtis, 1834), was reared from the seeds and fruits collected in the Khao Chong forest.

We obtained 57 samples from seven of 13 dipterocarp species growing at KHC, totaling 1,240 seeds (10.3 kg; 3.1% of total seeds reared), which yielded 425 insects (14 samples lacked insects). Out of these, we obtained 236 weevils and moths whose individual larvae likely feed on and kill a single seed (Hosaka *et al.* 2009). This suggests that approximately 19% of dipterocarp seeds were lost by weevils and moths. Insects reared from dipterocarp seeds included at least 26 species of seed and pulp eaters (Appendix II), mostly belonging to the Curculionidae, Nanophyidae and Tortricidae. The most abundant species were an unidentified species of *Alcidode*s (Curculionidae) reared from *Parashorea stellata*, and *Andrioplecta shoreae* Komai, 1992 reared from four dipterocarp hosts. In comparison, Nakagawa *et al.* (2003) reared 1,419 insects representing 51 species from 20,215 seeds of 24 dipterocarp species in Sarawak. Only four species were in common between their study and ours (Appendix II). In Pasoh, Malaysia, Hosaka *et al.* (2009) recorded at least 32 insect species from two consecutive mast-fruiting events of 15 species of dipterocarps (3,779 insects reared from 27,483 seeds). Senthilkumar *et al.* (2009) studied seed predation in *Dipterocarpus retusa* Blume in Assam, India, and recorded nine species of seed predators. In Thailand, at least 12 species of seed predators have been recorded from dipterocarps (Hutacharern & Tubtim 1995; DNP 2018). Because of incomplete identifications, different taxonomists studying the insect material and inconsistent use of DNA barcoding, it is difficult to compare the lists of taxa provided by these dipterocarp studies. Nonetheless, they suggest a relatively low overlap with the fauna feeding on dipterocarp seeds at KHC. The densities of reared insect individuals per dipterocarp seed appears to be higher at Khao Chong during the study period (0.34 insect per seed) compared with Lambir Hills (0.07 insect per seed; Nakagawa *et al.* 2003) or Pasoh (0.14 insect per seed; Hosaka *et al.* 2009), during periods of mast fruiting. One species of Blastobasidae and two species of Tineidae were reared from Dipterocarpaceae at KHC.

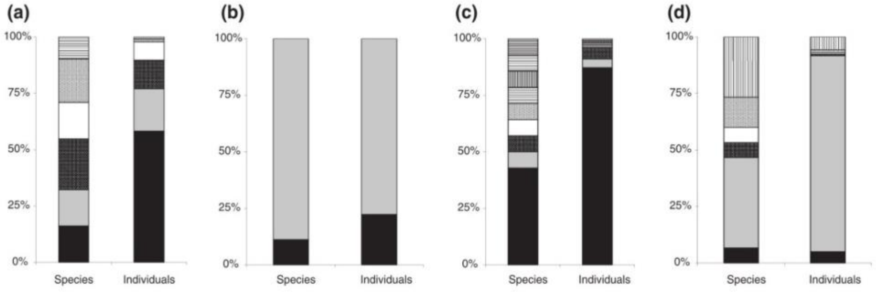

Figure 1 Source of pest species recorded at Khao Chong, Thailand, detailed for species and individuals and by (a) insect families, ▤, other lepidoptera, ⬚, Pyraloidea, □, Tortricidae, ▩, Curculionoidea, ▒, Tephritidae, ■, Scolytinae, (b) insect guilds, ▒, seed eaters, ■, pulp eaters, (c) main host family, ▤, Meliaceae, ▤, Fabaceae, ▥, Myrtaceae, ▤, Moraceae, ⬚, Lauraceae, □, Dilleniaceae, ▩, Chrysobalanaceae, ▒, Sapindaceae, ■, Dipterocarpaceae, and (d) main host seed syndromes, ▥, A1.2 - Fleshy, ⬚, B1 – Fleshy, □, C2 – Dry, ▩, B2 – Dry, ▒, C1 – Dry, ■, A2.2 - Dry. Curculionoidea do not include Scolytinae, which are indicated separately.

Of 27 parasitoid species for which we could identify the main insect hosts and verify whether the host was considered a pest of fruits or seeds, five species (18.5%) could be considered beneficial (Appendix I). All these species were Opiinae attacking *Bactrocera* pests (Tephritidae) breeding in the fruits of many host plant species. In addition, the larvae of *Hermetia illucens* (Linnaeus, 1758) recycle manure, so this species can also be considered beneficial (Appendix I).

Levels of parasitism of insects attacking seeds and fruits

Our data allowed us to present only crude estimates of the level of parasitism due to Ichneumonoidea (mostly Braconidae; Appendix I, Table 1). Overall, approximately 8.2% and 2.9% of insect species and individuals were parasitized, respectively. The level of parasitism was not notably different between pulp and seed eaters (Fisher's exact test, $P = 0.483$). Tephritidae was the most commonly attacked family by Braconidae, followed by Curculionidae (not including Scolytinae). *Bactrocera irvingiae* Drew & Hancock, 1994 and *Andrioplecta shoreae* Komai, 1992 reared from several host plant species, appeared to be the species most commonly parasitized by braconids. Insect taxa that appeared to be infrequently parasitized (Stratiomyidae, Pyralidae, Crambidae and Scolytinae; Table 1) could be under attack by parasitoids other than braconids. For example, *Coccotrypes* spp. (Scolytinae) are known to be attacked by the braconid genera *Spathius*, *Bracon* and *Diospilus* (Quicke 2015). These genera were infrequently reared at Khao Chong and obtained from other putative hosts. We also note that there was no obvious correlation between the number of species of parasitoids and prey reared from particular plant families (only main hosts considered: Spearman rank correlation, $r_s = 0.112$, $P > 0.25$, $n = 31$ plant families). Finally, most species of parasitoids were reared from main host plant species with syndrome A1.2 (40.9% of species), B1 (25.0%) and A2.2 (18.2%).

Rates of seed attack

Of 357 plant species surveyed, seeds/fruits of 101 were free of attack (28.3%). The first quartile of the distribution of these species represented 71% of the total number of seeds not attacked. *Antidesma neurocarpum* (Phyllanthaceae) was the most avoided plant species, with 344 seeds not attacked (Fig. 2). Other tree species rarely attacked (first quartile of the distribution in Fig. 2) included 11 Rubiaceae, 9 Annonaceae, 9 Arecaceae, 7 Meliaceae, and 6 Euphorbiaceae, Fabaceae and Phyllanthaceae each. Plant families with a high proportion of seeds not attacked (>15%) included Apocynaceae, Clusiaceae, Meliaceae, Anacardiaceae, Rubiaceae, Celastraceae, Phyllanthaceae and Sapotaceae (Fig. 2). Of those, Phyllanthaceae, Rubiaceae and Meliaceae were species-rich and collected with a high sampling effort, and hence, could be considered families relatively infrequently attacked by insects. Seed syndrome B2 (non-fleshy) also had a relatively high proportion of seeds free of attack (Fig. 2).

The main hosts of insects at KHC (as defined in Methods and Materials) belonged to 40 species and 16 plant families. Only *Parashorea stellata* (Dipterocarpaceae) and *Lepisanthes rubiginosa* (Sapindaceae) supported more than two insect species. Dipterocarpaceae, Annonaceae and Fabaceae had a relatively high load of insect species, as well as seed syndromes B1, A1.2 and C1, a mixture of dry and fleshy fruits (Fig. 3a). The 25 most heavily attacked host species (in terms of insect abundance) often belonged to Annonaceae, Fabaceae, Sapindaceae and Myristicaceae (Fig. 3b). The highest

Table 1 Levels of parasitism due to Ichneumonoidea for the main higher insect taxa reared from seeds and fruits at Khao Chong, Thailand, presented in decreasing percentages of species parasitized

Taxa	No. spp. reared	No. spp. parasitized	% species parasitized	% individuals parasitized
Tephritidae	26	7	26.9	7.0
Anthribidae	8	1	12.5	0.3
Curculionidae[†]	26	3	11.5	0.8
Tortricidae	13	1	7.7	4.6
Stratiomyidae	8	0	0.0	0.0
Pyralidae	8	0	0.0	0.0
Crambidae	15	0	0.0	0.0
Scolytinae	22	0	0.0	0.0
All pulp eaters	113	8	7.1	6.3
All seed eaters	34	4	11.8	0.7
All	147	12	8.2	2.9

[†] Without Scolytinae.

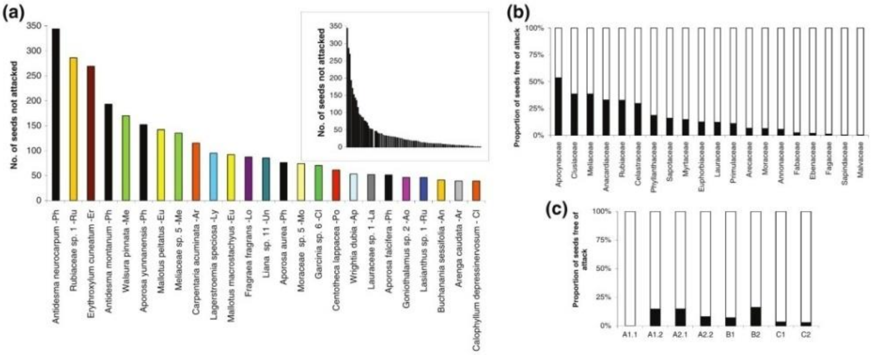

Figure 2 Plant species free of seed attack at Khao Chong, Thailand. (a) Main figure: first quartile of the distribution with name of species detailed and plant families abbreviated and colored similarly. Inset: full distribution of the number of seeds free of attack for each species not attacked. An, Anacardiaceae; Ao, Annonaceae; Ap, Apocynaceae; Ar, Arecaceae; Cl, Clusiaceae; Er, Erythroxylaceae; Eu, Euphorbiaceae; Ge, Gentianaceae; La, Lauraceae; Lo, Loganiaceae; Ly, Lythraceae; Me, Meliaceae; Mo, Moraceae; Ph, Phyllanthaceae; Po, Poaceae; Ru, Rubiaceae; Un, Unknown. (b) Proportion of seeds free of attack (black) detailed by plant family (when number of plant species surveyed ≥3). (c) Same, detailed by seed syndrome. A1.1, fleshy drupe with thick mesocarp (>5 mm); A1.2, fleshy drupe with thin mesocarp (<5 mm); A2.1, non-fleshy drupe with thick mesocarp (>5 mm); A2.2, non-fleshy drupe with thin mesocarp (<5 mm); B1, fleshy indehiscent fruit with multiple seeds; B2, non-fleshy dehiscent fruit with multiple seeds; C1, dry winged seed that does not develop in capsule; C2, multiple dry seeds (with or without wings) that develop in a capsule/pod (opening across one axis).

numbers of insect reared were obtained from *Mezzettia parviflora* (Annonaceae). Overall densities of insects were also relatively high on Meliaceae and Anacardiaceae (Fig. 3c). On average the highest densities of insects reared per seed and plant species were obtained from hosts with syndrome C2 (multiple dry seeds). There was no significant difference between the number of stems in the plot of tree species rarely and heavily attacked (Mann–Whitney $U = 192.5$, $P = 0.808$). However, heavily attacked tree species had significantly larger basal areas in the plot than rarely attacked tree species ($U = 309.0$, $P < 0.001$; mean ± S.E. = 6.08 ± 1.145 m^{-2} and 1.28 ± 0.439 m^{-2}, respectively).

Insect fluctuation during study years

Overall the highest densities per unit seed over the 3-year study were attained by several species of Scolytinae (Appendix I). There was no significant difference between the average stability index of pulp-eating species and that of seed-eating species (Mann–Whitney $U = 1481.5$, $P = 0.927$). However, the average stability index of species reared from dipterocarp hosts was significantly smaller (more stable) than that of species reared from non-dipterocarp hosts ($U = 710.0$, $P = 0.027$; Fig. 4a). Furthermore, the average stability index of species reared from fleshy fruits was significantly smaller (more stable) than that of species reared from dry fruits ($U = 313.0$, $P = 0.010$; Fig. 4b).

Host plant phylogenetic signals

Figure 5 provides a visual interpretation of how all/rarely/heavily attacked plant species, and from which Ichneumonoidea were reared, clustered across the whole plant phylogeny at KHC. The first three categories showed a limited phylogenetic signal with the D statistic relatively high (all plant species attacked: $D = 0.862$, $P(D > 0) = 0.0001$, $P(D < 1) = 0.0001$; species rarely attacked: $D = 0.781$, $P(D > 0) = 0.005$, $P(D < 1) = 0.0023$; species heavily attacked: $D = 0.855$, $P(D > 0) = 0.025$, $P(D < 1) = 0.0001$). For plant species hosting Ichneumonoidea, there was clearly no phylogenetic signal ($D = 0.994$, $P(D > 0) = 0.418$, $P(D < 1) = 0.0001$). Significance tests of phylogenetic signal according to MPD indicated that all categories were not clumped across plant phylogeny (all species: MPD observed = 358.9, MPD random mean = 342.5, $P = 0.92$; species rarely attacked: MPD observed = 305.3, MPD random mean = 328.1, $P = 0.23$; species heavily attacked: MPD observed = 364.8, MPD random mean = 329.6, $P = 0.88$; species

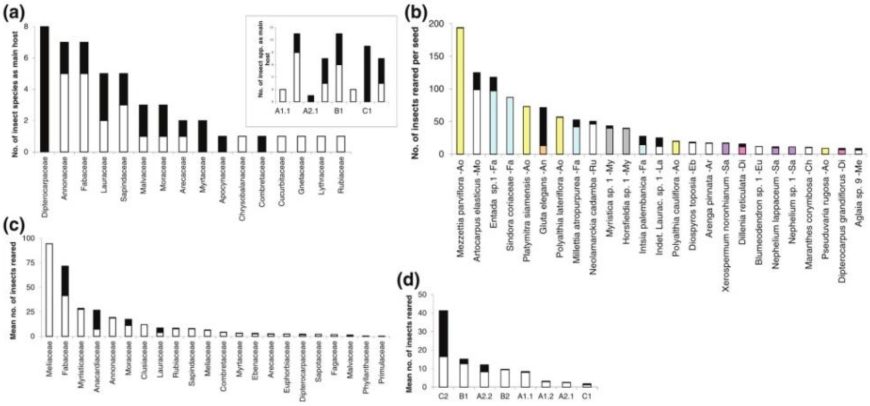

Figure 3 Heavily attacked plant species at Khao Chong, Thailand. (a) Number of insect species (black, seed eaters; white, pulp eaters) reared from main hosts, detailed by plant families. Inset: same presentation, detailed by seed syndrome. (b) Number of insects reared per seed for the 25 most attacked plant species. Black, seed eaters; white or different color, pulp eaters (same color denotes same plant family). An, Anacardiaceae; Ao, Annonaceae; Ar, Arecaceae; Ch, Chrysobalanaceae; Di, Dilleniaceae; Di, Dipterocarpaceae; Eb, Ebenaceae; Eu, Euphorbiaceae; Fa, Fabaceae; La, Lauraceae; Me, Meliaceae; Mo, Moraceae; My, Myristicaceae; Ru, Rubiaceae; Sa, Sapindaceae. (c) Average number of insects reared per seed and plant species, detailed by plant family (black, seed eaters; white, pulp eaters). (d) Same presentation, detailed by seed syndrome. A1.1, fleshy drupe with thick mesocarp (>5 mm); A1.2, fleshy drupe with thin mesocarp (<5 mm); A2.1, non-fleshy drupe with thick mesocarp (>5 mm); A2.2, non-fleshy drupe with thin mesocarp (<5 mm); B1, fleshy indehiscent fruit with multiple seeds; B2, non-fleshy dehiscent fruit with multiple seeds; C1, dry winged seed that does not develop in capsule; C2, multiple dry seeds (with or without wings) that develop in a capsule/pod (opening across one axis).

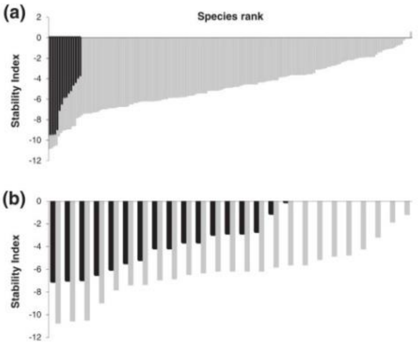

Figure 4 Insect species at Khao Chong, Thailand, ranked by their stability index. (a) Species reared from non-dipterocarp hosts (gray bars) versus species reared from dipterocarp hosts (black bars). (b) Species reared from fleshy fruits (gray bars) versus dry fruits (black bars).

hosting Icheumonoidea: MPD observed = 355.9, MPD random mean = 330.3, *P* = 0.78).

DISCUSSION

Insect assemblages feeding on seeds and fruits in tropical rainforests are challenging to study, primarily because of low rates of attack, high plant diversity and the high sampling effort required to rear sufficient numbers of insect specimens to provide meaningful statistics (Ctvrtecka *et al.* 2014). Furthermore, the taxonomic knowledge of insects reared from native seeds and fruits of tropical countries is often limited (Nakagawa *et al.* 2003; Miller *et al.* 2014). Regarding the questions asked in this study, we observed the following.

1. Approximately 43% of species identified could be considered pests. Most were seed eaters, particularly on dry fruits (but only a single pest of stored products was recorded), belonging to Nanophyidae, Tortricidae, Crambidae, Scolytinae and Tephritidae.

2. Approximately 19% of parasitoid species for which we could assess whether the main insect host is a pest could be considered beneficial. All these species were Opiinae with Bactrocera pests breeding in fruits as main hosts.

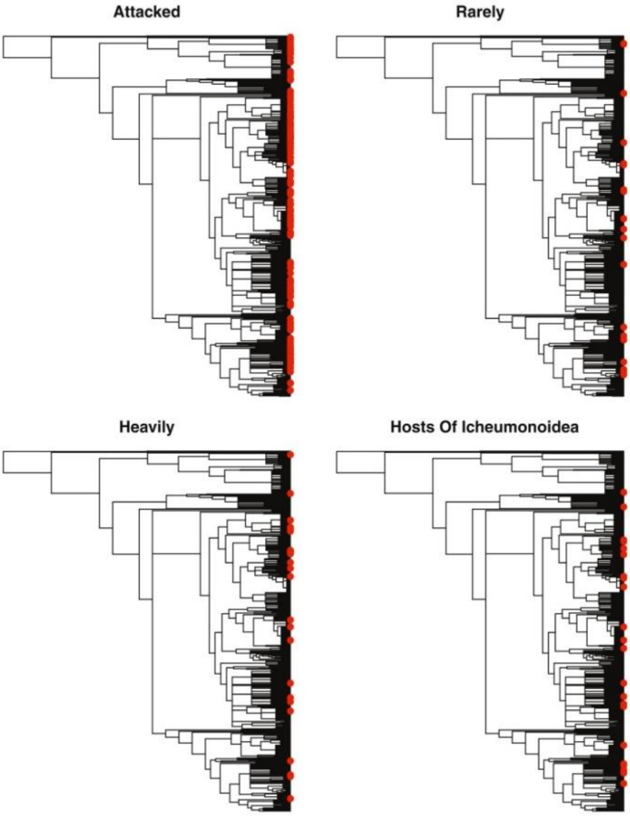

Figure 5 Maximum clade credibility consensus trees depicting the phylogenetic relationships between 622 host plant species at Khao Chong, Thailand, for each consensus tree. Taxa marked in red indicate (a) all species attacked, (b) species rarely attacked, (c) species heavily attacked, and (d) species from which Ichneumonoidea were reared.

3. Overall, approximately 8% of insect species reared from seeds/fruits were parasitized by Ichneumonoidea, with Tephritidae being the family most commonly attacked.

4. The seeds/fruits of approximately 28% of plant species in the KHC forest were free of attack. The seeds/fruits of Phyllanthaceae, Rubiaceae and Meliaceae were attacked relatively infrequently by insects. In contrast, fruits and seeds of species of Annonaceae, Fabaceae, Sapindaceae and Myristicaceae were more likely to be heavily attacked, with multiple dry seeds (syndrome C2) often well attacked. There was no apparent effect of plant phylogeny on rates of attack but heavily attacked tree species had larger basal area in the KHC plot than rarely attacked tree species.

5. The highest densities per unit seed over the three study years were attained by several species of Scolytinae, as these beetles can produce large broods inside fruits. Insects reared from fleshy fruits were more likely to show relatively stable populations compared to insects reared from dry fruits, except for insects reared from dipterocarps, which appeared to have relatively stable populations during the study years at KHC.

The proportion of pest species recorded in our study is probably inflated because in the tropics insect pests are far better known than native forest insects, especially those reared from native seeds and fruits (Miller *et al.* 2014). We encountered two general categories of pests: (i) various beetles species breeding in the dry seeds of dipterocarps that appear to be rather specific (Nakagawa *et al.* 2003); and (ii) polyphagous species of Tephritidae breeding in fleshy fruits. The former species could be of concern because modest dipterocarp plantations have been established in Thailand since the 1980s (Weinland 1998). However, densities of the most common pest feeding on dipterocarps, *Alcidodes* sp. 15, were rather low, reaching 0.16 insect per seed on average during the 3-year study. *Bactrocera irvingiae* was the most commonly reared tephritid from fleshy fruits, but this species is not considered a pest. *Dacus longicornis* Wiedemann, 1830, a pest of Cucurbitaceae, reached densities of 0.44 flies per fruit on our focal hosts, but was not very abundant when all plant species surveyed were considered. We conclude that, during our study years, the KHC forest did not support insect pests in densities that could cause concern to timber species (dipterocarps) or fruit crops. Less than 20% of parasitoid species appeared to have insect pests as hosts. As we have little evidence that the KHC forest acts as a reservoir of insect seed/fruit pests, it is difficult to argue that the same forest acts as a reservoir of beneficial insect species. A better test of this issue would be to compare parasitoid and seed insect assemblages in commercial crops contiguous with natural forests, such as in Mexico (Aluja *et al.* 2014). However, in Thailand such situations are rare, with habitats contiguous to natural forests represented primarily by buffalo fields, maize plantations or holiday resorts (D.J. Quicke, pers. obs., 2018).

A more interesting question related to parasitoids is whether some seed insects could be relatively free of ichneumonoid parasitoids. In Costa Rica, Janzen (1980) observed that Bruchinae seed predators are rarely attacked by parasitoids. At KHC, Bruchinae are replaced by Anthribidae and Curculionidae (Basset *et al.* 2018), whose species frequently were attacked (except for Scolytinae, Table 1). Furthermore, many of the Tephritidae species were attacked by braconids. We reared approximately 50% fewer individuals of Stratiomyidae (Appendix I) but did not record any braconid attacks on these flies. There are very few Ichneumonoidea parasitoids of Stratiomyidae (Quicke 2015), which are attacked only as eggs by various Chalcididae and Trichogrammatidae (Robertson 1987). We also note that there was no obvious correlation (negative or positive) between the number of prey and parasitoids reared from particular plant families, and that there was no phylogenetic signal relating host plant species from which Ichneumonoidea were reared. Although these represent weak tests of the nasty host hypothesis (Gauld *et al.* 1992), these observations do not appear to support it (and see Quicke 2012 for other considerations). Our rearing scheme, albeit imperfect to obtain reliable data about the identity of parasitoid hosts and level of parasitism, nevertheless suggests that the action of parasitoids at KHC might be too infrequent to induce strong differences in seed/fruit crops, with possible consequences on local tree abundance.

There are certainly different reasons for seeds of particular plant species to be attacked less frequently by insects. First, plant chemistry might be an important determinant; because seeds represent the most valuable part of the plant, they are usually well protected (Janzen 1969; Ramírez & Traveset 2010). At present we lack data for most KHC plant species to provide a context for discussing plant chemistry (see Gripenberg *et al.* 2018 for such a discussion). Our phylogenetic tests indicated only limited phylogenetic signal for the categories of plant species attacked by seed and pulp eaters, as well as for plant species rarely heavily attacked. This suggests that insects overall might not be very selective regarding attacking or avoiding particular clades of plant species, even if they may be reasonably host-specific. Second, sample size is certainly important (Ctvrtecka *et al.* 2014), but among our focal plant families, we could nevertheless crudely assign species to the categories rarely and heavily attacked. The next important variable is probably local host abundance. We found that host species heavily attacked have on average a higher basal area (but not number of stems) in the KHC plot than rarely attacked host species. This suggests that seed and pulp eaters are influenced primarily by seed/fruit production, which is probably more dependent on basal area than on number of stems. It seems less likely that seed and pulp eaters are directly limiting the local abundance of heavily attacked tree species.

The observations that dipterocarp mast fruiting does not occur at KHC (Kurten *et al.* 2017), and insect densities in dipterocarp seeds during the study years were higher than in Malaysian dipterocarp forests experiencing mast fruiting (Nakagawa *et al.* 2003; Hosaka *et al.* 2009), support the hypothesis of satiation of seed predators by mast fruiting (Curran & Webb 2000). However, it is not clear why insects reared from dipterocarp seeds at KHC should have more stable populations than insects reared from non-dipterocarp hosts. This might be related to easy host-switching and alternative hosts for insects feeding on dipterocarp seeds (Nakagawa *et al.* 2003). The low faunal turnover between dipterocarp insects at KHC and in Malaysia is also of interest, suggesting that different insect assemblages could be well adapted to either mast-fruiting events or the lack of these events. We also strongly suspect that low host specificity in insects breeding in fleshy fruits could explain the more stable populations of these species as opposed to those breeding in dry fruits. This issue will be explored elsewhere with more adequate data.

In conclusion, most of the evidence (often indirect) suggests that insects feeding on seeds and fruits at KHC have a limited impact on host abundance in this forest. Insect densities were low, as was the number of confirmed insect pests, and heavily attacked tree species were not notably less abundant than other species. This situation appears similar to that described for a lowland rainforest in Papua New Guinea (Ctvrtecka *et al.* 2014; Sam *et al.* 2017). This could be a consequence of the high plant diversity at these two locations, but it also might be related to the relative occurrence of fleshy versus dry fruits (Basset *et al.* 2018). It is obvious that more surveys of insects feeding on seeds and fruits are required at different rainforest locations to adequately discuss this issue.

ACKNOWLEDGMENTS

We thank the Khao Chong Botanical Garden (Thailand) for logistical support. Collecting permits were obtained from the National Research Council of Thailand. Montarika Panmeng and Sutipun Putnaul collected most of the insect material, which was identified as indicated in the main text and with the further help of Jeremy Holloway, Steve Lingafelter, Chris Lyal, Alma Solis, Don Davis and Nicholas Silverson. This study was supported by the Czech Science Foundation (16-20825S). Grants from the Smithsonian Institution Barcoding Opportunity FY013 and FY014 (to YB) and in-kind help from the Canadian Centre for DNA Barcoding and Southern China DNA Barcoding Center allowed the sequencing of insect specimens. YB and HB are members of the Sistema Nacional de Investigación, SENACYT, Panama. SG holds a Royal Society University Research Fellowship. MK was partly supported by the Ministry of Agriculture of the Czech Republic (Resolution RO0117; 6779/2017-MZE-14151). DLJQ was supported by the Ratchadaphiseksomphot Fund, Chulalongkorn University.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Appendix I. Details of insects reared from seeds and fruits and Khao Chong, Thailand.

Appendix II. Seed and pulp eaters reared from Dipterocarpaceae at Khao Chong, Thailand.

Supporting Information

Supplementary materials for Chapter IV. Insect assemblages attacking seeds and fruits in a rainforest in Thailand - Entomological Science.

Additional Supporting Information may be found on the Supporting Information section at the end of the article via https://onlinelibrary.wiley.com/doi/full/10.1111/ens.12346.

Appendix SI. Details of the insects reared from seeds and fruits and Khao Chong, Thailand.

Appendix SII. Seed and pulp eaters reared from Dipterocapaceae at Khao Chong, Thailand.

Summary

Thesis Summary

This thesis evaluates cross-continental assemblages of fruit-and seed-feeding insects among three biogeographical regions including each a Forest Global Earth Observatory (ForestGEO) plots located in major tropical regions: Neotropical: Panama: Barro Cororado Island, BCI: Oriental: Thailand: Khao Chong, KHC: and Australian: Papua New Guinea: Wanang, WAN. Wanang is the first ForestGEO plot established south of the Wallace line in the Australian region. ForestGEO is a global network of permanent forest plots where researchers can study of tropical and (temperate) forest ecosystems (http://www.forestgeo.si.edu/). These forest locations are known for their high level of local biodiversity (Anderson-Teixeira et al., 2014). The present study adds insect herbivore data to the existing vegetation data, creating thus one of the few cross-continental studies comparing assemblages of tropical forest insects on their host plants (e.g., Ctvrtecka et al., 2014; Basset et al., 2018; Dahl et al., 2019).

We took advantage of the rich data and facilities provided by the network of the ForestGEO (Anderson-Teixeira et al., 2014) and our own extensive insect sampling analyse global patterns in plant-frugivore food webs (e.g., Basset et al., 2018). The results may improve our understanding of forest ecosystem dynamics (Lewis and Gripenberg, 2008), plant and insect phenology (Wright, 1990), rainforest food webs (Dahl et al., 2019) and their possible response to climate change (Pimm and Sugden, 1994).

Specifically, we tested whether interaction within networks composed of plants and frugivores display cross-continental similarity in the patterns of host use with respect to phylogenetic relatedness of plants /or with respect to plant functional traits (i.e., fruit and seed predation syndromes). We have assessed the differences between insect predation syndromes and host plant specialization among continents by constructing food webs across three ForestGEO forest plots. The results are based on standardized sampling protocol applied to a 24 hectare plot at KHC and 50 hectare plot at BCI, and WAN (Basset et al., 2018; Dahl et al., 2019). At the end of 3 years of extensive field sampling and data processing, we obtained approximately 1,163 kg fruits from 1,186 species which yielded 80,600 frugivore insect specimens representing at least 1,678 (Basset et al., 2018). The taxonomy of insects reared

from tropical seeds has been always challenging because most of these insects are poorly known and studied in the tropics. We therefore delineated insect morphospecies with DNA barcoding (DNA Cytochrome c oxidase subunit I sequences). The molecular data were used to confirm identifications based on morphology and to test for morphologically cryptic species.

In Chapter I we propose a new classification of rainforest tree species into eight fruit syndromes based on fruit morphology and other traits relevant to fruit-feeding insects designed a used insect fruit syndrome classification system and compared it to the existing systems of botanists (Hickey and King, 1981; Zomlefer, 1994) and vertebrate zoologists (Gautier-Hion et al., 1985). We observed large inter-continental variability among fruit syndromes and colors, with likely consequences for seed predators and dispersers. We argue that plant species with fleshy and non-fleshy (dry) fruit syndromes may be preferred in different forest types and are attacked by different insect predators (Basset et al., 2018). Likewise, fruits with different colors are preferred by different vertebrate dispersers (Gautier-Hion et al., 1985). We found that insect fruit syndromes and colors show a weak phylogenetic signal with only limited evidence of clustering across the plant phylogeny. With multivariate analysis, we observed plant phylogeny as an important driver of overall syndrome composition. We found that fruit syndromes and colors were evolutionarily flexible traits at higher taxonomic levels and capable of influencing seed predator and disperser assemblages. Plant phylogeny was important when explaining overall fruit syndrome composition across plant families or genera. This study highlights that the Panamanian forest has a distinct distribution of fruit syndromes from the two more humid forests in Thailand and New Guinea.

In Chapter II we utilize the insect fruit classification described in Chapter I (Dahl et al., 2019), and tested ecological hypotheses that high seed damage by insect predators and pathogens may correlate with increase rainfall in the tropics (Coley and Barone, 1996; C. Dahl et al., unpublished data). We revealed that true insect seed feeders prefer dry fruits at dry seasonal tropical forests where fleshy fruits are less prevalent (Kissling et al., 2009). Overall, our results indicate that insects feeding on fruits and seeds play a potentially important role in maintaining diversity of vegetation by predispersal seed predation (Lewis and Gripenberg, 2008).

In Chapter III we investigate the effects of seasonality on insect seed predator abundance and plant-frugivore interaction networks. We show that frugivore insect diversity was high at the drier and more seasonal Panamanian site compared to the Thai and New Guinean sites (Basset et al., 2018; Basset et al., 2019). We observed a community with high species richness community for frugivorous Lepidopterans, despite their low abundance. Effects of seasonality were important in determining frugivore abundance at all study sites. Likewise, the abundance of frugivores attacking fruits were lower during the dry seasons than during the wet seasons at all surveyed sites. We argue that insect host specificity and the structure of food webs may be influenced by relative dominance of plants with fleshy vs. dry fruit syndromes (Basset et al., 2018; Dahl et al., 2019). Since frugivore web interactions are weaker during the dry seasons, this supports the hypothesis that high seed predation rate is more intense during the wet seasons.

Finally, Chapter IV data analyses examine the insect rearing data for food web dynamics over time for a single site (Khao Chong) during three years of insect rearing. Our results suggest that insect seed predators identified as major pests do not have much impact on abundant hosts. We argue that low insect densities and diversity prevent the occurrence of major pests. Overal, this study showed that insect feeding on fruits and seeds of many tropical tree species may not be of economic significance (Zehnder et al., 2007) but indicated that forests may act as reservoirs of fruit and seed feeding pests, but also their parasitoids (Copeland et al., 2006).

In summary, the present study indicates that predispersal insect-seed predators are capable to potentially reduce fitness of their host tree species, but this may be limited to a subset of plant species only Thai and New Guinean rainforests host similar fruit syndrome distributions dominated by fleshy fruits, therefore also host similar assemblages of insects' communities feeding on them. In contrast Panamanian forest is dominated by dry fruits and specific insects communities feeding on them. This study also provides a guide to the methods and require sample sizes that are necessary to document plant frugivore networks in complex ecosystems, and demonstrates the comparative approach to inter-continental analysis of such networks. However, this may require a more efficient collection methods that may be used to rear only insect attacking fruits and seeds and their interactions could be studied with metabarcoding. Moreover, eventually, has potential importance for the tests of the Janzen-Connell mechanisms of maintaining plant diversity in rainforests.

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Curriculum Vitae

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Supervisors: Yves Basset, Ph.D. and prof. RNDr. Vojtěch Novotný, CSc

A cross-continental comparison of fruit-and seed-feeding insects in the rainforests of Panama, Thailand and Papua New Guinea.

2008-2010: M.Sc. in Zoology, University of Papua New Guinea, Port Moresby.

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Species richness, community composition and distribution of frogs along an altitudinal gradient in Papua New Guinea.

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Employment history

2012-2013: Research Supervisor at the New Guinea Binatang Research Center (NGBRC), Madang, Papua New Guinea.

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International Conference

2019: Plant-insect frugivore interaction networks in tropical forests: the effects of seasonality [Talk]. 7th Conference of the Czech Society for Ecology, Olomouc, Czech Republic.
2018: Cross-continental comparison of plants and a novel classification of syndromes in the tropical rainforests of Panama, Thailand and Papua New Guinea [Talk]. European Conference of Tropical Ecology, Paris, France.
2014: Where is the peak of the altitudinal diversity in New Guinea frogs [Talk]. 51st Annual Meeting of the Association for Tropical Biology and Conservation, Cairns, Australia.
2014: Frog diversity in a tropical rich biodiversity region **-** the eastern New Guinea. "The land of the unexpected". University of South Bohemia, Ceske Budejovice, Czech Republic.
2009: The distribution and conservation status of the montane frog fauna of Mt Michael, Eastern Highlands Province, Papua New Guinea [Poster]. Student Conference on Conservation Science, University of Cambridge, UK.
2007: The distribution, abundance and conservation status of the montane frog fauna of Mt Michael, Papua New Guinea [Talk]. Biodiversity, Extinction Crisis Conference - A Pacific Response, Sydney.
2005: Conservations status and diversity of Melanesian frogs [Talk]. San Gabriel Herpetology Society in Los Angeles, Museum of Vertebrate Zoology at Berkeley, USA, and University of South Bohemia, Ceske Budejovice, Czech Republic.
2004: Montane frog fauna of Mt Michael, Eastern Highlands Province [Talk]. The 6th New Guinea Biological Conference, Manokwari, Indonesia.

Grant awards

2019: Dahl, C. University of South Bohemia, Ceske Budejovice, CZ. Book printing support, 16,051 CZK. A pocket guide to frogs of lowland forest around Kau, Morox, Ohu and Wanang Villages of Madang Province, Papua New Guinea.
2013: Dahl, C., et al. Centre for Tropical Forest Science Research Grants Program, USA to New Guinea Research Center, Madang; US $10,000 (PI).
2013-2016: Dahl. C., and Novotny, V. Australia and Pacific Science Foundation to NG BRC, Madang, AU $26,000 (PI).
2008: Dahl, C., and Bronikowski, E. Association of Zoos and Aquariums Conservation Endowment Fund, US $19,130 (PI).
2000-2010: Dahl, C., et al., PNG MAMAGRAUN Trust Fund to NG BRC, Madang, PG K48,000 (Co PI).
2004: First prize for the best student presenter at the 6th Biological Conference, Manokwari, Indonesia.

2009: Runner-up for the Miriam Rothschild Scholarship Program in Conservation Leadership, Cambridge University.

Trainnings and workshops

2014: Use of DNA Barcoding to identify insect specimens to species. Smithsonian Tropical Research Institute, Panama.
2012: Experimental design and statistics. Griffith University, Australia
2010: Tropical ecology field course, study design and data analysis. Tropical Biology Association UK at Danum Valley, Malaysia.
June-July 2010: Emerging amphibian disease workshop, James Cook University, Australia.
2008: IBISCA Arthropod survey in temperate forest, France. France Museum, IBISCA-AUVERGNE Pronatura International.
2008: Tropical ecology field course, study design and data analyses, (combine with 10 students from University of South Bohemia, České Budějovice. NGBRC, Madang.
2005: DNA Sequencing, PCR. University of Minnesota, St. Paul, USA.
2003: Field techniques in ecology. Wildlife Conservation Society (WCS), Goroka.
2002: Field techniques in ecology. WCS, Goroka.
2001: Museum biological specimen preparation, conservation in Melanesia. Smithsonian Institute, USA and University of Papua New Guinea.
1996: Reefs and impact of pollution monitoring methods. Australia Institute of Marine Studies and Motupore Island Research Center, Port Moresby.

Biodiversity surveys

2016: Herpetofauna survey, Gulf province. Papua LNG with Environmental and Social Impact Assessment and Management Specialists (ERIAS).
2013: Elevala Herpetofauna survey. Horizon Oil LTD Papua. Consultant to John Douglas Environment Service and Environment Resource Management (ERM).
2012: Arthropods Mt Wilhelm altitudinal survey. IBISCA, PNG Pronatura International.
2011: Altitudinal amphibian survey. Solomon Islands.
2009: BBC Scientific Expedition. Hepertofauna, Mt Bosavi, Southern Highlands Province.
2009: Rapid Assessment Biodiversity surveys, Muller Range, Southern Highlands Province.
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2006: Northern and Southern regions Herpetofauna surveys, PNG. Louisiana State University, USA (C. Austin).
2005: Herpetofauna survey, Managlas WMA, Oro Province. Partners with Melanesia

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2004 - 2007: Herpetofauan surveys to Eastern Highlands Prov., Southern Highlands Prov. and Simbu Prov. Conservation International (S. Richards).
1995: Kikori insect survey, Gulf Province and Southern Highlands Prov. World Wide Fund for Nature, Papua New Guinea.

Professional travel

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Popular media publication

2019: A pocket guide to frogs of lowland forests around Kau, Morox, Ohu and Wanang Villages of Madang Province, Papua New Guinea (Chris Dahl, Stephen J. Richards).
2019: An island haven for frogs (by Michael Lucy, UNE Media Team, Australia)
2009: The frogs and moths of Mt Wilhelm National Park in Post Courier newspaper, (by Chris Dahl).
2007: Frogs hit the limelight in Post Courier newspaper (by Chris Dahl)
2006: interviewed on NBC Radio, frog research in Papua New Guinea.
2005: Conservation man in The National newspaper, (by Damaris Minikula)
1997: Driman bilong Chris kisim em raun long ovasis in Wantok newspaper. (by Larry Orsak).
1995: The Binatang Boys in Post Courier newspaper (by Larry Orsak).

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A cross-continental comparions of fruit-and seed-feeding insect in the rainforests of Panama, Thailand and Papua New Guinea
Ph.D. Thesis Series, 2019, No. 17

Printed in the Czech Republic by Typodesign
Edition of 20 copies

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