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Host specificity and interaction networks of insects feeding on seeds and fruits in tropical rainforests

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Abstract

In the tropics, antagonistic seed predation networks may have different properties than mutualistic pollination and seed dispersal networks, but the former have been considerably less studied. We tested whether the structure of antagonistic tripartite networks composed of host plants, insects developing within seeds and fruits, and their insect parasitoids could be predicted from plant phylogenetic distance and plant traits. We considered subsets of the networks ("subnetworks") at three rainforest locations (Panama, Thailand, Papua New Guinea), based on insect families, plant families or plant functional groups. We recorded 3,197 interactions and observed a low percentage of realized interactions, especially in Panama, where insect host specificity was higher than in Thailand or New Guinea. Several factors may explain this, including insect faunal composition, incidence of dry fruits, high fruit production and high occurrence of Fabaceae at the Panamanian site. Host specificity was greater among seed-eaters than pulp-eaters and for insects feeding on dry fruits as opposed to insects feeding on fleshy fruits. Plant species richness within plant families did not influence insect host specificity, but site characteristics may be important in this regard. Most subnetworks were extremely specialized, such as those including Tortricidae and Bruchinae in Panama. Plant phylogenetic distance, plant basal area and plant traits (fruit length, number of seeds per fruit) had important effects on several network statistics in regressions weighted by sampling effort. A path analysis revealed a weak direct influence of plant phylogenetic distance on parasitoid richness, indicating limited support for the "nasty host hypothesis". Our study emphasizes the duality between seed dispersal and seed predation networks in the tropics, as key plant species differ and host specificity tends to be low in the former and higher in the latter. This underlines the need to study both types of networks for sound practices of forest regeneration and conservation.

Key words: Barro Colorado Island; functional group; nasty host hypothesis; plant phylogeny; quantitative food web; seed predation.

113   *Author contributions* – YB, SG and OL conceived and designed the study with input from HB, CD and RC. JWB, SB, BAB, AIC,

114   SJD, OK, MK, GEM, NP, PP, DLJQ, WS, RU, EJV and GW contributed plant and insect data. YB, LRJ, PTB, GPAL, PK and

115   STS analyzed the data. All authors contributed to the writing of the manuscript, with notable input from YB, LRJ, STS, PTB,

116   SEM, VN, SJW, SG and OL.

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Host specificity and interaction networks of insects feeding on seeds and fruits in tropical rainforests

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124 **Introduction**

125

126 Community assembly and the relationships among interacting species are frequently studied using ecological interaction networks

127 (Jordano et al. 2003, Blüthgen et al. 2006, Poisot et al. 2015, Dáttilo & Rico-Gray 2018), as the structure of these networks may

128 be critically linked to the dynamics and stability of interacting species within the community (Paniagua et al. 2009). Mutualistic

129 networks involving the processes of pollination and seed dispersal have been relatively well studied in tropical rainforests, often

130 emphasizing vertebrates (e.g., Olesen & Jordano 2002, Schleuning et al. 2012, Escribano-Avila et al. 2018). To date, antagonistic

131 networks, incorporating information on the frequency of each trophic interaction and emphasizing invertebrates in rainforests

132 have been assembled for leaf miners, gallers, leaf-chewers, their hosts and their parasitoids (review in Morris et al., 2014), and,

133 more rarely, for seed predators (Gripenberg et al. 2019).

134

135 In tropical rainforests, insects are the main seed predators, especially before seed dispersal (Janzen 1971). Insects that kill seeds

136 either before or after dispersal influence the population dynamics of individual plant species, and ultimately, plant diversity and

137 assemblage composition (Lewis & Gripenberg 2008). In addition to true seed predators, other functional groups of insects,

138 notably in Diptera and Lepidoptera, feed on the fleshy parts of fruits (Ctvrtecka et al. 2016). This guild of "pulp eaters" (as

139 opposed to "seed eaters") can cause fruit abortion and fall, with consequences for plant population dynamics (Stephenson 1981).

140 In the tropics data regarding insect assemblages feeding on seeds/fruits are infrequent (Gripenberg 2018). So far, interaction

141 networks have been built for tephritid flies breeding in tropical flower heads (Prado & Lewinsohn 2004) or in tropical fruits

142 (Novotny et al. 2010), and for the whole assemblage of seed predators in one Panamanian rainforest (Gripenberg et al. 2019).

143

144 Concealed insect herbivores, such as seed/fruit predators, are more specialized than insect herbivores that feed externally

145 (Novotny & Basset 2005). Studies in tropical rainforests have often confirmed the high host specificity of seed/fruit predators

146 (Janzen 1980, Hopkins 1983, Nakagawa *et al.* 2003, Copeland et al. 2009, Ctvrtecka et al. 2014, Sam et al. 2017, Gripenberg et

147 al. 2019). Since foliar chemistry and plant phylogeny predict patterns of host use by caterpillars in tropical rainforests with high

148 concordance (Segar et al. 2017, Volf et al. 2017), we expect that plant phylogeny may also influence assemblages of seed/fruit

149 insects in tropical rainforests.

150

151   Resource availability, such as the production of young leaves, is key to understanding the local distribution of insect folivores in

152   tropical rainforests (e.g., Basset 2001). Likewise, it may be crucial to explain the structure and species interactions in assemblages

153   of insects feeding on seeds/fruits in tropical rainforests. In mutualistic networks, biotic specialization decreases with increasing

154   plant diversity, because high plant diversity may reduce relative plant abundance and related plant resources (Schleuning et al.

155   2012). However, antagonistic and mutualistic networks may be structured differently (Morris et al. 2014).

156

157   We expect that plant phylogeny (Segar et al. 2020), local plant diversity and abundance, seed availability and functional plant

158   traits (Basset et al. 2018) may influence interaction networks involving insects breeding in seeds and fruits in tropical rainforests.

159   In this contribution we test whether the structure of antagonistic tripartite networks composed of host plants, insects breeding in

160   seeds and fruits and their insect parasitoids at three representative rainforest locations within different biogeographical regions

161   can be predicted from different plant variables. Because of the high diversity of our study systems (see results), we consider

162   subsets of the overall networks ("subnetworks") at each location, either based on insect families, plant families or plant traits. We

163   answer the following questions.

164

165   1. Accounting for host-plant phylogenetic relatedness, does herbivore host specificity vary among (a) insect families or feeding

166   guilds? (b) plant families or functional groups? and (c) three tropical forests?

167

168   One important variable accounting for network structure is specialization (Blüthgen et al. 2006), which is positively related to

169   host phylogenetic isolation (Jorge et al., 2017). Host specificity is likely to differ among insect guilds associated with fruit pulp

170   versus seeds, as seeds are better chemically protected than pulp (Janzen 1971). Plant traits may also influence seed predator load

171   and host specificity (Janzen 1971, 1980, Basset et al. 2018, Dahl et al. 2019). Low plant richness may favor high insect host

172   specificity, as suggested by comparisons of insect herbivores in temperate and tropical forests (Novotny et al. 2002).

173

174   **2.** Does plant phylogenetic relatedness or plant functional similarity explain the structure of interaction networks between seeds or

175   fruits and their insect predators across study sites or across different local subnetworks?

176

177   Related insect herbivores tend to feed on related host plants (Ehrlich & Raven 1964), because related plants may often

178   (Berenbaum 2001, Rønsted et al. 2012) but not always (Sedio et al. 2018) share similar chemical defences. In turn, specialized

179   herbivores, particularly seed predators, may be adapted to detoxify chemical defences (Kergoat et al. 2005). Hence, the structure

180   of plant-herbivore interaction networks may have a strong phylogenetic signal (Weiblen et al. 2006, Segar et al. 2020).

181   Alternatively, plant apparency theory (Feeny 1976) has been incorporated into a framework of three syndromes of plant defence,

182   including (1) tolerance/escape, (2) low nutritional quality and (3) high nutritional quality and defence (Agrawal & Fishbein

183   2006). Under this framework, plant functional traits may predict the structure of seed predator networks as well as, or better than,

184   plant phylogenetic relatedness.

185

186   **3.** Does the species richness of parasitoid assemblages feeding on seed/fruit predators reflect the traits of host plants, i.e. do the

187   effects of plants on herbivores cascade upwards to affect the next trophic level?

188

189   The nasty host hypothesis proposes that insect herbivores feeding on plant hosts with strong and/or distinctive chemical defenses

190   may support reduced loads of parasitoids because herbivore tissues may be more toxic to parasitoids (Gauld et al. 1992). Thus,

191   this hypothesis predicts that plant phylogenetic relatedness, as a surrogate for plant chemistry (Berenbaum, 2001), (a) should have

192   a significant effect on the species richness of parasitoids, (b) that this effect should be strong, and (c) that this effect should also

193   be positive (i.e., mean phylogenetic distance is predicted to be negatively correlated with parasitoid species richness).

194

Material and methods

196

Study sites

198

199   Our study sites are three ForestGEO lowland rainforest plots (Anderson-Teixeira et al. 2014) located in different biogeographical

200   regions. Salient characteristics of the plots and seed/fruit samples are detailed in Basset et al. (2018) and in Table 1. Neotropical:

201   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 in the

202   heart of the island, which is near the center of more than 700 km^2 of protected forests. Oriental: the 24-ha plot at Khao Chong

203   (KHC) is in the protected forest of the Khao Ban Thad Wildlife Sanctuary in southern Thailand. Australasian: the 50-ha plot is

204   located in the 10,000 ha Wanang (WAN) Conservation Area in Papua New Guinea. Marked differences between BCI and the two

205   other sites include lower plant richness, higher percentage of species with dry seeds, higher percentage of Fabaceae species,

206   higher average seed rain per plant species and lower ratio of realized to potential interactions (Table 1).

207

Plant surveys, phylogeny and functional traits

209

210   Field methods were similar for all study sites (details in Basset et al. 2018, Gripenberg et al. 2019). Plant surveys spanned several

211   years at each site (Table 1; Appendix S1A). During the first study year at each site, we indiscriminately surveyed seeds and fruits

212 of locally abundant tree, shrub and liana (more rarely herb) species, to obtain an overview of the local community. During

213 subsequent study years at KHC and WAN, we restricted our sampling effort to the 10 plant families that were most common at

214 each plot. Eight of these focal families were common to all sites: Annonaceae, Arecaceae, Euphorbiaceae, Fabaceae, Lauraceae,

215 Meliaceae, Rubiaceae and Sapindaceae. Unless specified, results are detailed for all host plant species. Seeds and fruits collected

216 on plants or freshly fallen (without apparent decomposition) were opportunistically surveyed within and/or near permanent plots

217 (from an area < 1,500-ha corresponding to the smallest study area, BCI). Rearing sample units consisted of 1 to 200 seeds and/or

218 fruits collected from a single plant. We targeted as many individual plants as possible for each species, typically > 5. To evaluate

219 the phylogenetic relationships between sampled host plant species at each site, we estimated the relationships between our focal

220 species using the R package S.PhyloMaker (Qian & Yi 2016). We used the updated phylogeny and node ages derived from a

221 sequence-based study by Zanne et al. (2014) as a Megatree. Our focal species were placed within the Megatree where possible

222 and placed to family where not possible. This procedure generates three alternative topologies which differ with respect to the

223 resolution of unplaced taxa. We selected the phylogeny derived from "Scenario 3" as this has been shown to be robust to

224 uncertainty at the higher taxonomic level (Qian & Yi 2016). Note that polytomies in the phylogeny underestimated DSI* slightly

225 for herbivores feeding on a few species within a family. However, we expect this effect to be quite small, especially because it

226 does not apply to monophages that would still have maximum specialization.

227

228 To obtain similarity matrices of plant functional traits for each site, we first compiled a matrix of functional traits relevant to

229 seeds and fruits for each plant species, including numerical and categorical variables (Appendix S1B). We then used hierarchical

230 daisy clustering methods to identify functional groups. Finally, we used a mixed Principal Component Analysis (PCA) for

231 numerical and categorical variables to interpret the functional groups. Scores of each plant species on the PCA axes were used to

232 build similarity matrices of functional traits that were used in subsequent analyses. In sum, 1,186 plant species could be assigned

233 to one of five functional groups, coded A, B, C, D or E (A: often fleshy green fruits; B; often dry dehiscent fruits; C: often fleshy

234 orange fruits; D: often red fruits; E: often small fleshly black-green fruits). Appendix S1B provides details about the composition

235 of the matrix of functional traits, computational steps and relevant references, as well as the results obtained.

236

237 **Insect rearing and processing**

238

239 Methods for rearing seed/fruits insects are detailed in Basset et al. (2018) and Appendix S1C. Insects were identified with the

240 assistance of taxonomists (see Basset et al. 2018) and/or by molecular techniques (Appendix S1C). Insects reared from

241 seeds/fruits were assigned to the following guild categories (Basset et al. 2018): seed eaters (coded as SE: larva feeding mostly on

242 seed tissue), pulp eaters (PU: larva feeding mostly on mesocarp tissue), and parasitoids (PA: larva feeding on insect hosts). Seed

243 and pulp eaters consisted mainly of seven taxa that represented most of the material reared and are considered in analyses

244 restricted to insect taxa: Bruchinae, Scolytinae, Curculionidae others than Scolytinae (Coleoptera), Tortricidae, Pyralidae

245 (Lepidoptera), Stratiomyidae and Tephritidae (Diptera). Bruchinae were well represented only at BCI. Hereafter for sake of

246 simplicity, we refer to these seven taxa as "insect families". For parasitoids, analyses were restricted to Braconidae and

247 Ichneumonidae because they represented most (69%) of the parasitoids reared from samples and their taxonomy was supported by

248 molecular data.

249

250 **Interactions, topologies and subnetworks**

251

252 Trophic relationships were inferred from the number of primary consumers reared from samples of seeds/fruits of host plant

253 species at the first trophic level (coded as level 1-2). For the third trophic level, we considered interactions between Braconidae

254 and Ichneumonidae and their insect hosts (coded as level 2-3). Contrary to interactions between the first and second trophic

255 levels, third level data only reflected expected interactions, not documented interactions, because parasitized hosts were not

256 isolated and reared individually, the parasitoids instead being reared from samples including relatively high numbers of seeds and

257 fruits. To assign putative hosts to each parasitoid species, we applied three simple rules, as detailed in Appendix S1D: (1) since

258 many parasitoid lineages are rather conservative in host use, we followed Quicke (2015) to select the most likely host order or

259 family. (2) In case of conflicts, we examined for each parasitoid species the consistency of co-occurrence with the putative host

260 species in all samples from which the parasitoid species was reared. (3) Eventually, we considered the highest abundance of

261 putative host reared in samples in which the parasitoid species was also reared. We considered expected interactions between

262 hosts plants and parasitoids (coded as level 1-3) to answer Question 3.

263

264 We constructed tripartite and quantitative interaction networks for the three full networks at BCI, KHC and WAN and for

265 meaningful subsets of the data. This approach was selected because of the complexity of the full networks, which involved an

266 order of magnitude more interacting species than most published networks (see Results; Schleuning et al. 2012, de Aguiar et al.

267 2019). Breaking a complex network into smaller sub-networks can reveal interesting patterns (Lewinsohn et al. 2006, de Aguiar et

268 al. 2019) and has been performed with networks including insects (Quinto et al. 2012), which are far more diverse than those

269 based on vertebrates (Schleuning et al. 2012). Each of our three full networks can be viewed as a collection of empirical

270 subnetworks built by sampling interactions of a particular taxonomic/functional group within a locality, a general approach which

271 is consistent with most published networks (de Aguiar et al. 2019). In addition, subnetworks were relatively independent from

272 each other, thus motivating analyses at the level of subnetworks (see "subnetwork structure" below). The meaningful subsets

273 (hereafter "topologies" for sake of brevity and in reference to how subnetworks are arranged) were based on (A) insect taxa: the

274 distribution of particular insect families on plant species (n=7 taxa, resulting in 19 subnetworks); (B) plant family: the

275 distribution of insect species within particular plant families (n=8, 24 subnetworks); or (C) plant functional groups: the

276 distribution of insect species on particular plant functional groups (n=5, 15 subnetworks). Topology (A) is more relevant to

277 Questions 1 and 3 of the Introduction, whereas topologies (B) and (C) are more relevant to Question 2. Topologies (A) and (C)

278 included insects reared from all host plants at each site, whereas for topology (B) we restricted the data to focal plant families.

279 Further, for topologies (B) and (C) we also included in subnetwork illustrations plant species that were surveyed but yielded no

280 reared insects (these "empty hosts" were not considered in the calculation of subnetwork statistics, see below). Variables as

281 surrogates of either resource availability or sampling effort are discussed in Basset et al. (2018). Here, we consider that resource

282 availability is most accurately tracked as the square root of the number of seeds collected for each plant species.

283

284 **Data analyses**

285

286 *Question 1: herbivore host specificity*

287

288 Quantitative metrics accounting for network-wide specialization (Blüthgen et al. 2006, Dormann et al. 2009) may be biased by

289 sample size (Morris et al. 2014) and by non-random sampling of the plant phylogeny (Redmond et al. 2018). To overcome these

290 challenges, we calculated herbivore specificity with the rescaled distance-based specialization index (DSI* - Jorge et al. 2014,

291 2017), which measures trophic specialization by accounting for host phylogenetic relatedness and resource availability. This

292 quantitative metric accounts for differences in abundance and sampling effort of consumers and is largely independent of sample

293 size. Briefly, DSI* measures specialization as a deviation from a random expectation involving the mean pairwise phylogenetic

294 distance between hosts and is rescaled to enable the comparison of consumers that differ in their recorded sample sizes. DSI*

295 varies between −1 (maximum achievable generalization) and 1 (monophages or maximum achievable specialization; Jorge et al.

296 2017). At each site we calculated DSI* for all seed- and pulp-eating insect species considering all host plant data available. DSI*

297 was not calculated for parasitoid species, as, due to many missing data, we could not build reliably a phylogeny for insect

298 herbivores.

299

300 To answer Questions 1a-1c, we first tested for differences in DSI* among herbivore taxa and guilds (pulp and seed eaters), plant

301 families and functional groups, and study sites. We used non-parametric Kruskal-Wallis tests because DSI* values were skewed

302 towards high specialization. Then, we used DSI* as the response variable in a model including taxa, guilds, sites and taxa:site and

303 guild:site interactions as independent variables. To control for variation among insect families on DSI* (excluding Bruchinae,

304 collected only at BCI), we performed a linear mixed model with site and insect guild as fixed effects and insect family as a

305 random effect. To control for variation among plant families, we performed a linear mixed model with mean DSI* of the

306 herbivores feeding on each plant species as the dependent variable, site and plant functional groups as fixed factors, and plant

307 family as a random factor. To evaluate the effect of local plant richness, we calculated the correlation between the average DSI*

308 of the insect assemblage feeding on each focal plant family at each study site (n=24 site-family combinations) and the species

309 richness of these plant families at each site.

310

311 *Question 2: subnetwork structure*

312

313 We addressed Question 2 by modeling indices for network properties at the level of the full network or subnetwork (hereafter

314 "network statistics"). Rather than focusing on any single metric, we calculated the following standard network statistics reflecting

315 network structure (Morris et al. 2014): degree of compartmentalization (number of compartments); weighted quantitative network

316 specialization index H2'; weighted quantitative generality (effective number of host species per consumer species); nestedness

317 (specialization asymmetry); weighted quantitative vulnerability (effective number of consumer species per host plant species);

318 and connectance (degree of redundancy in the study system). Appendix S1E describes these network statistics in greater detail.

319 All network statistics were calculated with the R package Bipartite (Dormann et al. 2018). We also reported the number of species

320 in both trophic levels and the sum of links for each subnetwork, as well as sampling intensity sensu Schleuning et al. (2012).

321 Models were estimated separately for topologies A-C (insect families, plant families and plant functional groups) and trophic

322 levels 1-2. We considered network statistics as independent data points in models, because of (a) no overlap between insect and

323 plant species across study sites; and (b) for a particular site and topology, the average pairwise species overlap between

324 subnetworks was 4.3%, 2.2% and 4.1% for herbivore families, plant families and functional groups, respectively. Network

325 statistics were also calculated for trophic levels 2-3 and 1-3 (see Question 3, below). We did not calculate network statistics when

326 subnetworks were too small (number of species in the lower level < 5).

327

328 We used null models to assess how network metrics deviated from those expected from a random distribution of interactions. Null

329 models were implemented for three full networks and 58 subnetworks using Patefield's algorithm ("r2dtable" in Bipartite's

330 nullmodel function, Dormann et al. 2018), in which the marginal species totals are constrained as per the respective observed

331 networks. We ran 1,000 randomisations for subnetworks and 200 for full networks (due to the time and CPU demands of running

332 analyses on large sparse networks). We evaluated whether network statistics differed significantly between study sites by

333 performing simple Kruskal-Wallis and Dwass-Steel tests. We refined this analysis by using three types of regression models. To

334 account for the effect of sampling effort, the number of observed interaction events (i.e., the number of links; Schleuning et al.,

335 2012) was used as a weighting factor in each regression. The first type of regression (hereafter "model type I") included mixed

336 models with network statistics as dependent variables, sites and insect guilds as fixed factors and topologies (insect and plant

337 families, plant functional groups) as random factors.

338

339 Following Chamberlain et al. (2014), we modeled network statistics with beta regression (H2' and Connectance), generalized

340 linear models with Poisson (Number of compartments) or Gaussian (all other statistics) error distribution, separately for each

341 topology A-C. Models type II parsed the effects of plant phylogeny and of plant ecological variables (resource and functional

342 traits), and were calculated as:

343

344 Network statistic = MPD + FDis,

345

346 where MPD is the average plant relatedness (mean phylogenetic distance between plant species included in subnetwork,

347 calculated with the function mpd of the R package Picante, Kembel et al. 2010), and FDis is the functional dispersion within the

348 subnetwork, calculated with the function fdisp of the R package FD (Laliberté et al. 2014). FDis quantifies trait diversity as the

349 mean distance in multidimensional trait space of individual plant species to the centroid of all species (Laliberté & Legendre

350 2010). FDis was calculated with variables accounting for (a) plant resource (no. of stems and basal area in ForestGEO plots, seed

351 rain (g dry weight x m^{-2}), equivalent to total fruit biomass and estimated from litterfall traps, Basset et al. 2018); and (b) plant

352 traits (fruit length and weight (partly related to seed size and biomass), number of seeds per fruit). We used the function betareg

353 of the R package betareg to perform beta regressions (Gruen et al. 2012). For other regressions, we performed model

354 simplification to extract the variables with significant predictive power with the built-in functions glm and step (backward

355 selection of variables) of the R package (R Core Team 2018). We eventually tested the significance of estimators by an ANOVA

356 (type 2 test) with the function anova in the R package 'car' (Fox and Weisburg 2019).

357

358 A last series of models considered more specifically the effects of plant variables (hereafter "models type III"):

359

360 Network statistic = Plant species richness + MPD + CWM_1 + CWM_2 + … + CWM_n,

361

362 where Plant species richness was the number of confamilial species in ForestGEO plots (for topology B) or the number of plant

363 species in functional groups in ForestGEO plots (for topology C), and CWM_n was the community weighted mean of trait n,

364 weighted by the number of samples collected and calculated with function dbFD of R package FD (Laliberté et al. 2014; plant

365 species richness could not be included as an independent variable for the topology based on herbivore families). Independent

366 variables accounted for sampling effort, plant species richness, mean phylogenetic distance, plant resource and plant traits (as

367 defined in models type II; only continuous variables). Before analyses, highly correlated variables (r>0.7) were removed from

368 models. Regressions were calculated and the significance of estimators tested as described previously.

369

370 *Question 3: parasitoid species richness*

371

372 To approach question 3, we computed a path analysis with a bottom-up flow of correlations implying direct and indirect

373 correlations between herbivore species richness, parasitoid species richness (dependent variables) and selected independent

374 variables. This analysis was performed at the level of the plant species, considering all plant species (n= 618) at the three sites

375 from which seed predators were reared. Independent variables included mean phylogenetic distance and variables related to plant

376 resource or plant traits (Appendix S1F). They were selected based on (a) a rationale for each path explained in Appendix S1F; and

377 (b) the best predictors in the regressions performed previously (see previous section and results). The mean phylogenetic distance

378 of a plant species to all other plant species was calculated with the function cophenetic of the R package Picante (Kembel et al.

379 2010). The model was calculated with the Ωnyx software (von Oertzen et al. 2015).

380

381 **Data deposition**

382

383 Interaction data were deposited in figshare, https://doi.org/10.25573/data.11444571.v1. Molecular insect data were deposited in

384 the following Barcode of Life projects (BOLD, www.boldsystems.org): BCI: 2,310 sequences in projects BCISP and PSPLP;

385 KHC: 398 sequences in KHCSP and KHCTE; WAN: 1,646 sequences in WANSP, FRUT and CURCU. Full data for specimens

386 sequenced (including those that failed), including images and host plants, are available on BOLD, accessible by DOI for the

387 datasets dx.doi.org/10.5883/DS-BCISP (BCI), dx.doi.org/10.5883/DS-KHCFRUIT (KHC) and dx.doi.org/10.5883/DS-

388 PNGFRUIT (WAN).

389

390 **Results**

391

392 We collected 1,163 kg of seeds and fruits, which produced 80,600 insects across the three sites (Table 1). The composition and

393 species richness of the insect material is discussed elsewhere (Basset et al. 2018). This contribution analyzes the 3,197

394 interactions across a total of 1,176 plant, 1,015 herbivore and 318 parasitoid species at the three study sites (Table 1). Only 0.58%

395 of the potential 553,160 interactions were realized (Table 1). Since most properties of subnetworks do not represent properties of

396 whole networks (Jordano 2016), we detail network statistics for the full networks (level 1-2) of BCI, KHC and WAN in Table 1,

397   for comparison with other studies. These results emphasize differences between BCI and the other two sites, which we analyze

398   in more depth by considering subnetwork data.

399

400   **Question 1: insect host specificity**

401

402   Differences in the median value of DSI* across insect families were significant (W=114.0, p < 0.001, d.f.=6; Fig. 1b). Bruchinae

403   were by far the most specialized taxa, followed by Pyralidae, Curculionidae, Scolytinae, Tortricidae, Tephritidae and

404   Stratiomyidae (Fig. 1b). Seed eaters were significantly more specialized than pulp eaters (Mann-Whitney test, U=91.8, p< 0.001,

405   d.f.=1; Fig. 1c). There were also significant differences between the median DSI* of insect faunas feeding across plant families

406   (W= 50.9, p > 0.001, d.f.=7). For example, on average, insects feeding on Fabaceae were rather specialized whereas those feeding

407   on Meliaceae were less so (Fig. 1d). Differences in median of DSI* for insects feeding across plant functional groups were also

408   significantly different (W= 53.1, p < 0.001, d.f.=4). In particular, insects feeding on plants belonging to functional group B (dry

409   dehiscent fruits) were far more specialized than those feeding on group C plants (fleshy orange fruits; Fig. 1e). Overall, insects

410   feeding on dry fruits were significantly more specialized than those feeding on fleshy fruits (Mann-Whitney test, U=39.9, p<

411   0.001, d.f.=1; Fig. 1f). The percentage of true monophagous species (DSI*=1) was higher at BCI (69.5%) than at KHC (25.3%)

412   and WAN (18.9%) and the median of DSI* was significantly different across sites (Kruskal-Wallis test, W=201.8, p < 0.0001,

413   d.f.=2; Fig. 1a).

414

415   The mixed linear model with DSI* as dependent variable and insect family as random factor indicated that the effect of site was

416   significant, but not that of insect guild (seed eater versus pulp eater; Table S1). When considering the mean DSI* of insect species

417   feeding on plant families, a similar model indicated that the effects of both site and plant functional group (coded A to E, see

418   Appendix S1B) were significant, but not their interaction (Table S1). There was no correlation between the average DSI* of

419   insects feeding on the eight focal plant families at the three study sites and the local species richness of these plant families (r = -

420   0.16, p = 0.45; Fig. S1).

421

422   **Question 2: variables affecting the structure of subnetworks**

423

424   We illustrate nine of the 58 subnetworks studied (Fig. 2) and detail characteristics of all subnetworks in Appendix S2. In null

425   models, most (95.6%) observed network statistics deviated significantly from those expected from a random distribution of

426   interactions (Table S2), with nestednes involved in nearly all cases where the deviation was not significant (Table S2). The

427   distribution of the six main network statistics is summarized in Fig. 3 for the topology based on insect herbivore families and

428 trophic levels 1-2. BCI subnetworks had significantly more compartments, significantly higher degree of specialization (H2'),

429 and significantly lower effective number of host species per consumer species (generality) than KHC and WAN. In particular, for

430 all herbivore families, H2' was also higher at BCI than at other sites (Appendix S2). Subnetworks based on Bruchinae,

431 Tortricidae, Curculionidae and Pyralidae were in general more specialized than those based on Tephritidae, Scolytinae and

432 Stratiomyidae (Appendix S2). Food webs based on stratiomyid flies were rather unspecialized, rarely parasitized by braconids and

433 their subnetwork at WAN represented the most unspecialized subnetwork of all subnetworks analyzed. Conversely, the most

434 specialized subnetwork was based on Tortricidae at BCI, followed closely by Bruchinae at BCI (Appendix S2). Other network

435 variables were not significantly different between sites (Fig. 3). Mixed models weighting the effect of sampling effort (models

436 type I), for the topology based on insect families, confirmed the strong effect of sites on the number of compartments, H2' and

437 generality (Table S3).

438

439 For the topology based on plant families, BCI had significantly more compartments than KHC and WAN, and KHC had

440 significantly larger effective number of consumer species per host plant species (vulnerability) than BCI and WAN (Fig. S1).

441 Nestedness and Connectance were not significantly different between sites for any of the topologies considered (Fig. S1). For the

442 topology based on plant functional groups, the number of compartments and H2' were significantly higher at BCI than at the

443 other sites, whereas generality and vulnerability were significantly lower at BCI (Fig. S2). Mixed models weighting the effect of

444 sampling effort (models type I) for both topologies based on plant families and functional groups confirmed the significant effect

445 of site on all network statistics (Table S3).

446

447 When parsing the effects of plant phylogeny and ecological variables (models type II, Table S4), the significance of effects could

448 be ranked overall as mean phylogenetic distance (MPD) > functional dispersion (FDis). MPD was a significant predictor of

449 network statistics calculated for plant functional groups (topology C), while FDis was more important to predict network statistics

450 calculated for plant families (topology B). Note that for models based on plant families, the effect of MPD may be low due to the

451 limited range of MPD within plant families. This also applies for FDIs in models based on plant functional groups.

452

453 Not surprisingly, models best explained by CWM (models type III) were related to plant functional groups (65-98% of variance

454 explained, Table 2). Over the different topologies, the variation explained by the type III models was greatest for number of

455 compartments and least for generality. Several variables were reasonably good predictors of subnetwork structure, in order of

456 importance seed rain, mean phylogenetic distance and number of plant species, as well as number of seeds per fruit (Table 2).

457 Plant species richness was a good predictor of network statistics (connectance, nestedness, generality), only for topology B based

458 on plant families. In models describing network statistics for plant functional groups, variables related to plant traits were

459 important to predict H2', while variables related to plant resources were important for number of compartments, vulnerability,

460 connectance and nestedness (Table 2).

461

462 **Question 3: species richness of herbivores and parasitoids**

463

464 Our path analysis model explained 19% of the variance in the number of parasitoid species supported by each plant species (Fig.

465 4). As expected, significant paths existed between plant traits and herbivore species richness, and between plant resources and

466 herbivore species richness. Mean plant phylogenetic distance influenced plant traits but not directly herbivore species richness.

467 The strongest direct paths (as judged from standardized path coefficients) influencing parasitoid species richness originated from

468 herbivore species richness (positive), mean phylogenetic distance (negative) and basal area (positive). Thus, although the effect of

469 mean plant phylogenetic distance was significant and negative on parasitoid species richness (as predicted by the nasty host

470 hypothesis of Gauld et al. 1992), its direct path was about five times smaller than the corresponding direct path originating from

471 herbivore species richness, pointing to other explanations.

472

473 **Discussion**

474

475 In this contribution we examined the interaction networks involving seeds and fruits, the insects feeding on them and their

476 parasitoids, at three tropical sites. To analyze the 3,197 interactions reported, we considered three "topologies" (how subnetworks

477 are arranged) resulting in 58 different subnetworks, which were largely independent from each other. This strategy was possible

478 because of the very low overlap of interacting species between subnetworks but may not be applicable to other types of networks,

479 such as mutualistic networks. Topology A, based on families of seed predators, may be useful to entomologists, whereas

480 topologies B and C (based on plant families and functional groups) may be more interesting to botanists. Some interactions may

481 not have been documented in our study system, since attack rates were rather low (8.5% of seeds/fruits attacked, Basset et al.

482 2018) and substantial sampling effort may be required to rear insects attacking seeds and fruits, For example, Ctvrtecka et al.

483 (2014) consider a minimum sample size of 5kg of fruits/seeds per plant species adequate to rear weevils feeding on fruits/seeds.

484 This condition was achieved for only 3% of our plant species. Low sampling effort may result in inflated insect host specificity

485 and network specialization (Blüthgen et al. 2006). Sampling effort in the field (collecting seeds/fruits, rearing insects) was higher

486 at BCI than at the other sites (Table 1). Hence, we believe that the high host specificity documented at BCI is not an artefact.

487 Another obvious limitation in our study was the indirect documentation of linkages between insect herbivores and parasitoids (see

488   Methods). Some of the linkages reported here will need confirmation but given the limited data on tropical seed predators they

489   are nevertheless valuable.

490

491   **Insect host specificity**

492

493   Insect host specificity varied significantly between insect families. While some seed- or fruit-feeding taxa are known to be

494   extremely specialized in rainforests (Bruchinae: Janzen 1980, Curculionidae: Ctvrtecka et al. 2014), others are less so

495   (Tephritidae: Novotny et al. 2010). These trends were confirmed in our study, which also indicated that Stratiomyidae, a taxon

496   rarely considered in studies of frugivorous insects, are less specialized pulp eaters than Tephritidae. Seed-eaters were more host-

497   specific than pulp-eaters, confirming that insect host specificity for tropical herbivore guilds in both temperate and tropical forests

498   decreases in the sequence: seed-eaters > leaf-miners > pulp-feeders > leaf-chewers > sap-suckers > xylophages > root-feeders

499   (Novotny & Basset 2005). This partially reflects the plant's allocation of nitrogen and chemical defences to the tissues consumed

500   by these guilds, as young leaves are sometimes better defended than seeds (Janzen 1971, 1980, Bazzaz et al. 1987, Zangerl &

501   Bazzaz 1992, Kergoat et al. 2005). Insects feeding on dry fruits were also more host specific than those feeding on fleshy fruits.

502   Insect host specificity varied significantly among plant families and functional groups, and the effect of site was important in

503   most of our analyses. Overall, we observed the lowest percentage of realized interactions and highest insect host specificity at

504   BCI. This trend was apparent when considering both entire networks (Table 1) and subnetworks (Figs 1,3). Similar levels of host

505   specificity for entire networks and across subnetworks may be explained by the preponderance of highly specialized fruit/seed

506   consumers, with very few generalist consumers present in more than one subnetwork.

507

508   This high insect host specificity at BCI is likely to result from the following factors. (1) Insect faunal composition: highly host

509   specific and diverse Bruchinae are prevalent at BCI and absent from KHC and WAN. (2) Fruit fleshiness: BCI has the highest

510   proportion of dry fruits (supporting highly host specific insects), possibly because of lower rainfall at BCI as compared to our

511   other sites (Kissling et al. 2009). (3) Fruit production: BCI has on average four times higher seed rain per plant species than KHC

512   or WAN (i.e., high fruit production and low plant species richness: Table 1). Our regressions confirmed the importance of

513   variables such as basal area or seed rain to predict insect specialization. (4) Fabaceae: there is a high percentage of Fabaceae

514   species at BCI (Table 1), which support many host-specific insect species.

515

516   In mutualistic networks, such as pollination and seed dispersal networks, specialization decreases with increasing plant diversity.

517   An explanation may be that high plant diversity reduces relative plant abundance and related plant resources, resulting in hard

518   evolutionary constraints on specialists (Schleuning et al. 2012, Escribano-Avila et al. 2018). Antagonistic networks may be

519   different (Morris et al. 2014). Lewinsohn and Roslin (2008) discuss the species richness and host specificity of folivorous

520   insects in tropical rainforests, and our BCI data appear to follow their contention that high species richness may be promoted by

521   more insect species per plant species (Table 1) or higher herbivore host specificity (Fig. 1a). In sum, low plant richness and high

522   insect host specificity at BCI suggests that antagonistic networks based on seed predation may follow the same rules as

523   mutualistic networks, with low plant richness strengthening interactions and favoring high insect host specificity (Novotny et al.

524   2002).

525

526   **Subnetwork structure**

527

528   Our analyses emphasized the strong effects of site on the different network statistics. Plant assemblages at different rainforests

529   may be phylogenetically different or may possess different traits, or both. Tree assemblages are phylogenetically distinct in many

530   rainforests (Webb 2000) and there are important differences in seed functional traits between our rainforest sites (Appendix S1B:

531   Table App2-S1; Dahl et al., 2019). Plant phylogenetic distance had an important effect on subnetwork structure (e.g.,

532   specialization H2' and generality in subnetworks based on insect families), but this effect was not overwhelming, as variables

533   related to plant traits or plant resource were also important in this regard. A more explicit inclusion of the hierarchical structure of

534   phylogenies in predicting interaction identities might provide increased explanatory power. Ideally, further analyses would

535   include phylogenies for hosts, herbivores and parasitoids (Ives and Godfray 2006), but herbivore and parasitoid phylogenies are

536   not currently available. Plant traits such as fruit length and number of seeds per fruit, were important predictors of network

537   statistics (Table 2). Other variables related to host phenology, such as the duration of fruiting season and its synchronization

538   within/among years, may well be important in this regard (Janzen 1976), but they could not be tested in this study, for lack of

539   reliable data at all sites. Variables accounting for plant resource (basal area, seed rain) were also important whereas the effect of

540   mean phylogenetic distance was not excessive. This would lend support to the modified plant defence theory (Agrawal &

541   Fishbein 2006). Both plant resource and plant traits were reasonably good predictors of subnetwork structure, particularly for

542   models based on plant functional groups, emphasizing the interest in this topology as a predictive framework for subnetwork

543   structure.

544

545   **Upward cascades in the subnetworks**

546

547   The nasty host hypothesis (Gauld et al. 1992) argues that tropical plants often possess highly active chemical defenses, which may

548   lead to greater host specialization and sequestration of secondary compounds in insect herbivores, and reduced loads of

549   parasitoids on particularly well-defended host plants. However, to date, evidence in favor of this hypothesis is mixed (Quicke

550 2012, Morris et al. 2014). Alternatively, Smilanich et al. (2009) observed that secondary metabolites sequestered by herbivores

551 may compromise their immune response, making them more vulnerable to successful parasitism (the "vulnerable host

552 hypothesis"). In our study system, we used plant phylogenic distance as a surrogate for plant chemistry (Berembaum 2001), as

553 chemical data for tropical fruits and seeds are limited (Gripenberg et al. 2018). Our path model indicated that most of the

554 explained variance in parasitoid species richness on host plants could be attributed to a direct path originating from herbivore

555 species richness, whereas the corresponding path originating from mean plant phylogenetic distance was less important.

556

557 The nasty host hypothesis (Gauld et al. 1992) explains parasitoid loads on plants principally with regard to plant chemistry. Were

558 this hypothesis correct, we would have expected a large direct path from mean plant phylogenetic distance to parasitoid species

559 richness. The larger direct path observed from herbivore species richness to parasitoid species richness seems rather consistent

560 with both the resource concentration and resource base hypotheses (Root 1973, Price 1992), predicting that local assemblages of

561 parasitoids may be more diverse when their herbivore hosts are diverse (Hawkins & Lawton 1987) and vulnerable (Smilanich et

562 al. 2009).

563

564 **Conclusions**

565

566 The stability of mutualistic networks is promoted by a highly connected and nested architecture, whereas stability in antagonistic

567 networks is promoted by a compartmentalized and weakly connected structure (Morris et al. 2014). The subnetworks with the

568 highest number of compartments were those based on Curculionidae, Fabaceae and functional group B (large dry fruits, protected

569 and dehiscent) at BCI. Webs that are strongly compartmentalised (i.e. have high modularity) might be expected to be stable

570 (across modules) because changes in abundance (or extinction) of individual species (within modules) are less likely to cascade to

571 affect nodes in other parts of the network beyond the affected compartment or module (Thébault and Fontaine 2010). In

572 antagonistic insect-plant networks where the host is immobile (a property that distinguishes them from many other food webs),

573 modularity will often result from trait matching and phylogenetic conservatism in plant traits. High levels of trait matching in

574 most cases will make insect herbivores particularly prone to co-extinction following loss of their host plants. Interactions such as

575 those for Stratiomyidae at Wanang which display lower trait matching may be more robust to random plant species loss but the

576 subnetwork overall will be less resilient to the loss of key nodes rich in fleshy fruit (e.g. well-connected plant genera).

577

578 Seed dispersal networks have on average a low specialization (H2') compared to our seed predation subnetworks (Blüthgen et al.

579 2007: average 0.28; average 0.79 for all our subnetworks). Low H2' promotes high redundancy and increased seed dispersal

580 (Blüthgen et al. 2007). Everything else being equal, plant species supporting generalist dispersers but specialized seed predators

581 with low attack rates may be able to produce large number of viable seeds and may be at an advantage over other plant

582 competitors. This is in line with the plant defence syndrome of high nutritional quality and defence (Agrawal & Fishbein 2006).

583 Further, reviewing seed dispersal networks in the tropics, Escribano-Avila et al. (2018) indicated that woody plants bearing small

584 juicy berries containing many tiny seeds often represent keystone species. From the viewpoint of conserving insects feeding on

585 fruits/seeds, the plants most important in seed predation networks are those which support many insect species (i.e., with high

586 number of consumer species per host plant species, vulnerability). Although we did not study many of the plant families

587 considered by Escribano-Avila et al. (2018), we note that our plant families with high vulnerability (Appendix S2) usually do not

588 bear berries with tiny seeds. This indicates that, from a conservation viewpoint, key plant species in the tropics may differ

589 between networks of seed dispersal and seed predation.

590

591 In summary, our study emphasizes the duality between seed dispersal and seed predation networks in the tropics as the former are

592 not very specific whereas the latter are far more specialized and may include different key plant species. From the viewpoint of

593 forest regeneration and conservation, this underlines the need to study both types of network including a variety of potential key

594 plant species.

595

607

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609

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732 Zanne A. E. et al. 2014. Three keys to the radiation of angiosperms into freezing environments. – Nature 506: 89–92.

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734   Supplementary material at xxx.

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737 **Table 1.** Salient characteristics of the study sites and their plant, insect and network interactions. Plot data are from Anderson-

738 Teixeira *et al.* (2014).

739

Variable	BCI	KHC	WAN
Site physiognomy and structure:			
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
Number of tree species∕genera∕families recorded in plot	299/181/59	593/285/82	508/245/77
Percentage of plant spp. with dry seeds/fruits	56.8	26.0	28.0
Percentage of Fabaceae species to total spp. richness in plot	14.0	3.1	4.8
Total fruit production (seed rain; dry g x m-2 x yr-1)	108.0	7.0	10.8
Average fruit production per species (dry g x m-2 x yr-1)*	0.596	0.141	0.157
Plant samples:			
Years of collection	2010-2013	2013-2015	2013-2015
Number of plant species/plant families surveyed	497/82	357/66	332/67
No. plant species surveyed within the 10 focal families	218	171	170
Total number of seeds or fruits collected	208,508	39,252	122,976
Total weight of samples (kg)	380.2	343.2	439.9
Insect samples:			
Total number of insects reared	27,610	17,555	35,434
Number of individuals / species of seed eaters**	11,059/311	2,100/59	3,935/77
Number of individuals / species of pulp eaters**	5,670/214	7,265/161	9,403/193
Number of individuals / species of parasitoids***	775/161	359/61	961/96
Interactions:			
Number of interactions realized / % realized-potential****	892/0.26	917/1.01	1,388/1.20
Plant species with most seeds/fruit reared	*Mikania leiostachya*	*Caryota mitis*	*Mastixiodendron pachyclados*
Most abundant herbivore species	*Pagiocerus frontalis*	*Coccotrypes myristicaceae*	*Coccotrypes* sp.n.3
Most abundant parasitoid species	Dorylinae sp. 156	Alysiinae sp. 13	*Diospilus* sp. 2
Network statistics for full network (level 1-2):			
Average DSI* ± s.e.	0.906±0.013	0.577±0.029	0.503±0.028
Number of compartments	85	20	9
H2'	0.914	0.664	0.657
Generality	1.85	6.84	5.15
Nestedness	0.96	1.61	2.20
Vulnerability	2.23	4.69	6.05
Connectance	0.006	0.021	0.022

740 * Plant species recorded in litterfall traps
741 ** Seven focal taxa only, see methods
742 *** Braconidae and Ichneumonidae only
743 **** No. of interactions between levels 1-2 and 2-3; percentage of realized to potential interactions
744

745 **Table 2**. Summary of the best regression models (type III) with network statistics as dependent variables, for each topology considered (A-C). Independent variables include

746 Mean Phylogenetic Distance (MPD) and the community weighted mean (CWM) of functional traits (see text for details). *** p< 0.001, ** p<0.01, * p<0.05, independent

747 variables listed according to decreasing standardized coefficients, best estimators indicated in bold.

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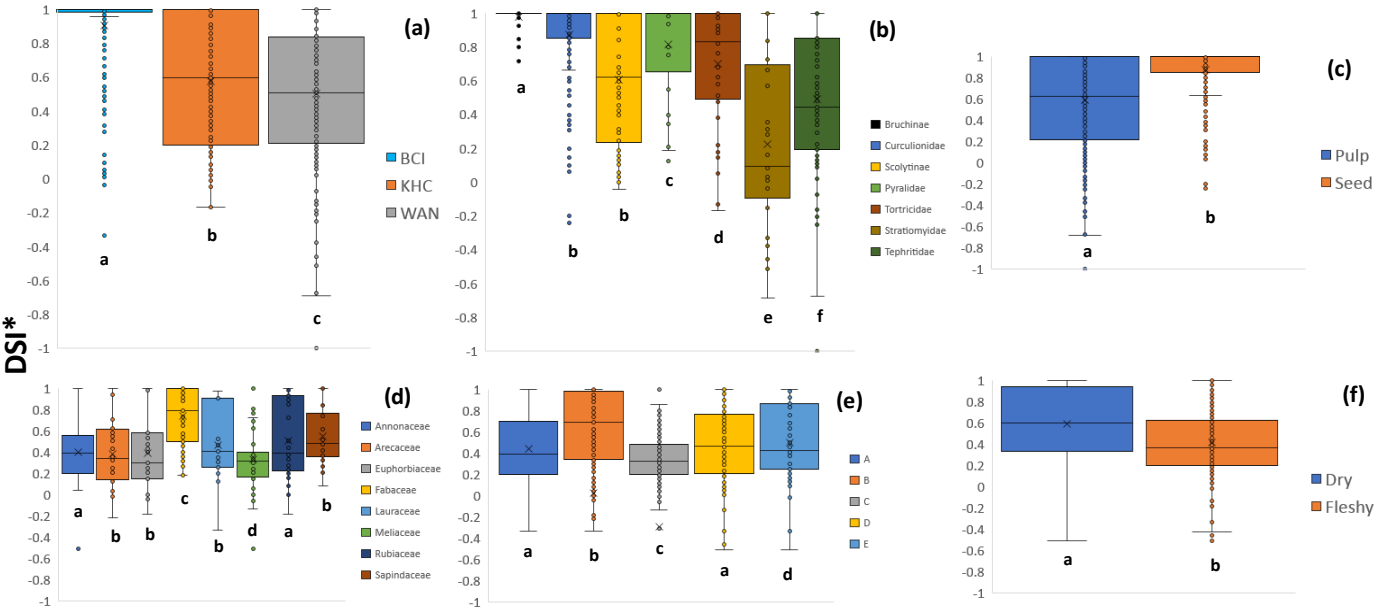| Topology/ Subnetwork variable	Significant variables	R^2	F	p	AIC
A. Insect families					
Specialization H2'	**MPD*****, Fruit length***, Basal area***, No. seed per fruit***, Seed Rain***	0.400	2457.1	<0.001	-3670.1
Connectance	n.s.	-	-	-	-
No. compartments	**Seed Rain*****, Fruit length***, MPD***, Basal area***, No. seed per fruit***	0.581	2637.9	<0.001	26903.0
Generality	**MPD****	0.500	15.9	<0.001	104.1
Nestedness	n.s.	-	-	-	-
Vulnerability	**Fruit length***	0.342	5.2	0.022	94.5
B. Plant families					
Specialization H2'	**No. seed per fruit*****, Basal area***, MPD***, Fruit length***, Seed Rain***	0.107	446.1	<0.001	-1999.3
Connectance	**No. plant species*****, MPD***, Basal area***, No. seed per fruit***, Fruit length***	0.618	1948.5	<0.001	-7143.4
No. compartments	**Basal area*****, Seed Rain***, Fruit length***, No. plant species***, MPD**	0.925	18.5	<0.001	5481.6
Generality	**No. plant species***, Fruit length*	0.351	3.4	0.037	44.6
Nestedness	**No. plant species*****	0.467	8.8	0.002	150.4
Vulnerability	n.s.	-	-	-	-
C. Plant functional groups					
Specialization H2'	**No. seeds per fruit*****, Basal area***, Seed rain***, MPD***, Fruit length***	0.696	4169.4	<0.001	-10018.1
Connectance	**Seed Rain*****, MPD***, Fruit length***, No. seed per fruit***, Basal area***	0.749	5125.6	<0.001	-20987.9
No. compartments	**Seed Rain*****, Basal area***, Fruit length***, No. seed per fruit***, No. plant species***	0.987	56.9	<0.001	13211.0
Generality	n.s.	-	-	-	-
Nestedness	**MPD*****, Basal area*	0.650	11.2	0.002	60.7
Vulnerability	**Seed rain*****, Basal area**, MPD*	0.835	9.1	0.002	52.3

750

752 **FIGURE CAPTIONS**

753

754 **Fig. 1.** Summary distribution of the specialization index DSI*. Box and whisker plots across (a) sites, (b) insect families, (c)

755 insect guilds, (d) plant families, (e) seed functional groups and (f) categories of fruit fleshiness. Groups with different letters are

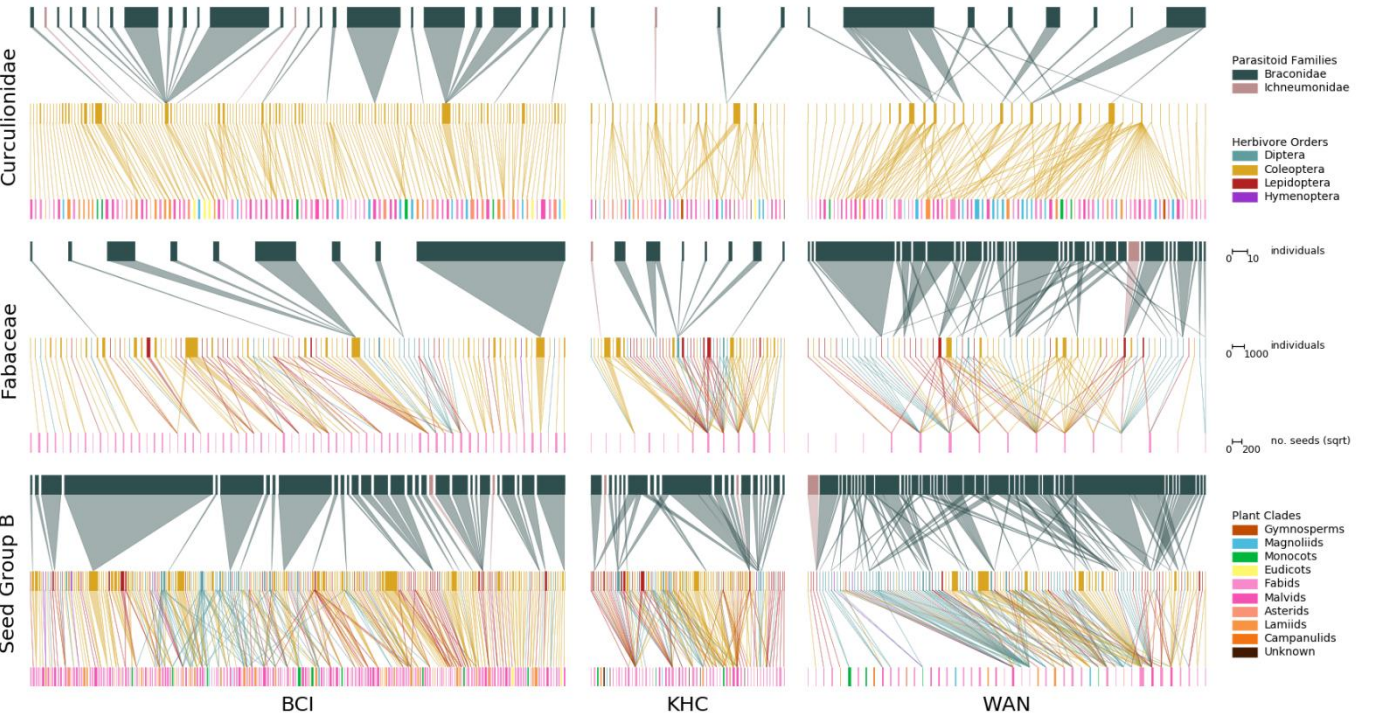756 significantly different (Dwass-Steel tests, $p < 0.05$).

757

758 **Fig. 2.** Examples of interaction subnetworks at BCI (left), KHC (middle) and WAN (right). Top: topology based on insect family,

759 here the Curculionidae (without Scolytinae). Middle: topology based on plant family, here the Fabaceae (including plant species

760 lacking insects attacking seeds or fruits). Bottom: topology based on plant functional group, here Group B (dry fruits, protected,

761 dehiscent and relatively large, see Appendix S1B). For each subnetwork, the abundance of parasitoid species (top series of

762 rectangles) and herbivore species (middle series of rectangles) are represented by the number of individual reared, whereas the

763 abundance of plant species (bottom series of rectangles, coloured by plant clades following APG IV: Chase *et al.*, 2016) are

764 represented by the square root of the number of seeds collected. Parasitoid families, herbivore orders and plant clades are

765 identified by distinct colours as coded on the right. The scale for each level is also indicated on the right. All subnetwork nodes

766 are ordered as to minimize the number of crossed interactions. From left to right and top to bottom these subnetworks are coded

767 as HB-CURC-BCI, HB-CURC-KHC, HB-CURC-WAN, PL-FABA-BCI, PL-FABA-KHC, PL-FABA-WAN, FG-B-BCI, FG-B-

768 KHC and FG-B-WAN in Appendix S2.

769

770 **Fig. 3.** Summary distribution of the six main network level statistics across study sites (BCI, KHC, WAN) for subnetworks based

771 on insect herbivore families and trophic levels 1-2 (plants-insect herbivores; n=18). The Bruchinae subnetwork for BCI was not

772 included as it has no equivalent at other study sites. Groups with different letters are significantly different (Dwass-Steel tests, $p <$

773 0.05).

774

775 **Fig. 4.** Results of path analysis testing direct and indirect correlations beween the species richness of seed- and fruit-eating insects

776 (HerbSpp), parasitoid species richness (ParaSpp), mean phylogenetic distance (MPD), plant traits (fruit length, Length and

777 ordination scores delineating plant functional groups, PCA1) and plant resource (basal area, BA and seed rain, SeedRain), for 618

778 host plant species. Standardized path coefficients are in parentheses. Significant ($p<0.05$) and insignificant paths are indicated by

779 solid and dashed lines, respectively. The rationale of the model is detailed in Appendix S1F.

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Fig. 1. Summary distribution of the specialization index DSI*. Box and whisker plots across (a) sites, (b) insect families, (c) insect guilds, (d) plant families, (e) seed functional groups and (f) categories of fruit fleshiness. Groups with different letters are significantly different (Dwass-Steel tests, p< 0.05).


Fig. 2. Examples of interaction networks at BCI (left), KHC (middle) and WAN (right). Top: topology based on insect family, here the Curculionidae (without Scolytinae). Middle: topology based on plant family, here the Fabaceae (including plant species lacking insects attacking seeds or fruits). Bottom: topology based on plant functional group, here Group B (dry fruits, protected, dehiscent and relatively large, see Appendix S1B). For each subnetwork, the abundance of parasitoid species (top series of rectangles) and herbivore species (middle series of rectangles) are represented by the number of individual reared, whereas the abundance of plant species (bottom series of rectangles, coloured by plant clades following APG IV: Chase *et al.*, 2016) are represented by the square root of the number of seeds collected. Parasitoid families, herbivore orders and plant clades are identified by distinct colours as coded on the right. The scale for each level is also indicated on the right. All subnetwork nodes are ordered as to minimize the number of crossed interactions. From left to right and top to bottom these subnetworks are coded as HB-CURC-BCI, HB-CURC-KHC, HB-CURC-WAN, PL-FABA-BCI, PL-FABA-KHC, PL-FABA-WAN, FG-B-BCI, FG-B-KHC and FG-B-WAN in Appendix S2.
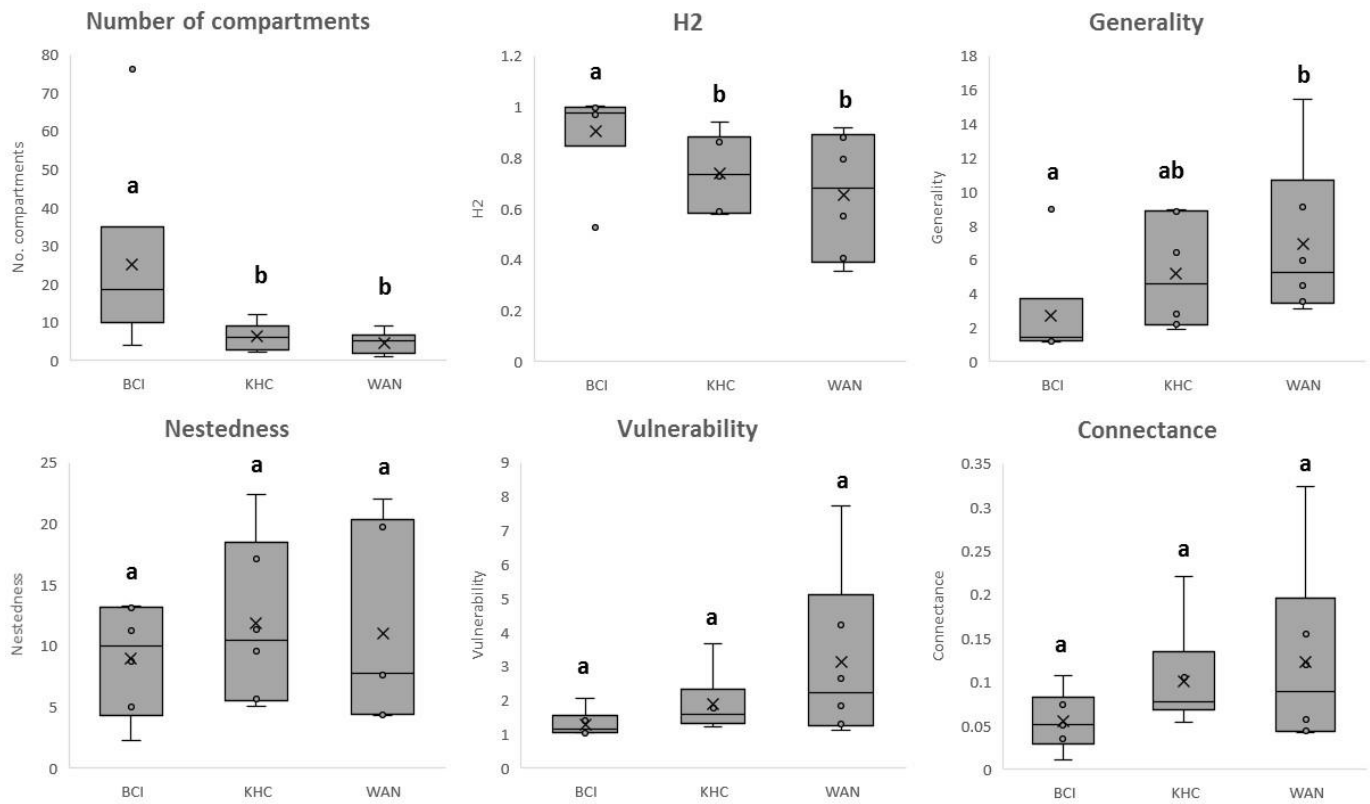
Fig. 3. Summary distribution of the six main network level statistics across study sites (BCI, KHC, WAN) for subnetworks based on insect herbivore families and trophic levels 1-2 (plants-insect herbivores; n=18). The Bruchinae subnetwork for BCI was not included as it has no equivalent at other study sites. Groups with different letters are significantly different (Dwass-Steel tests, p< 0.05).
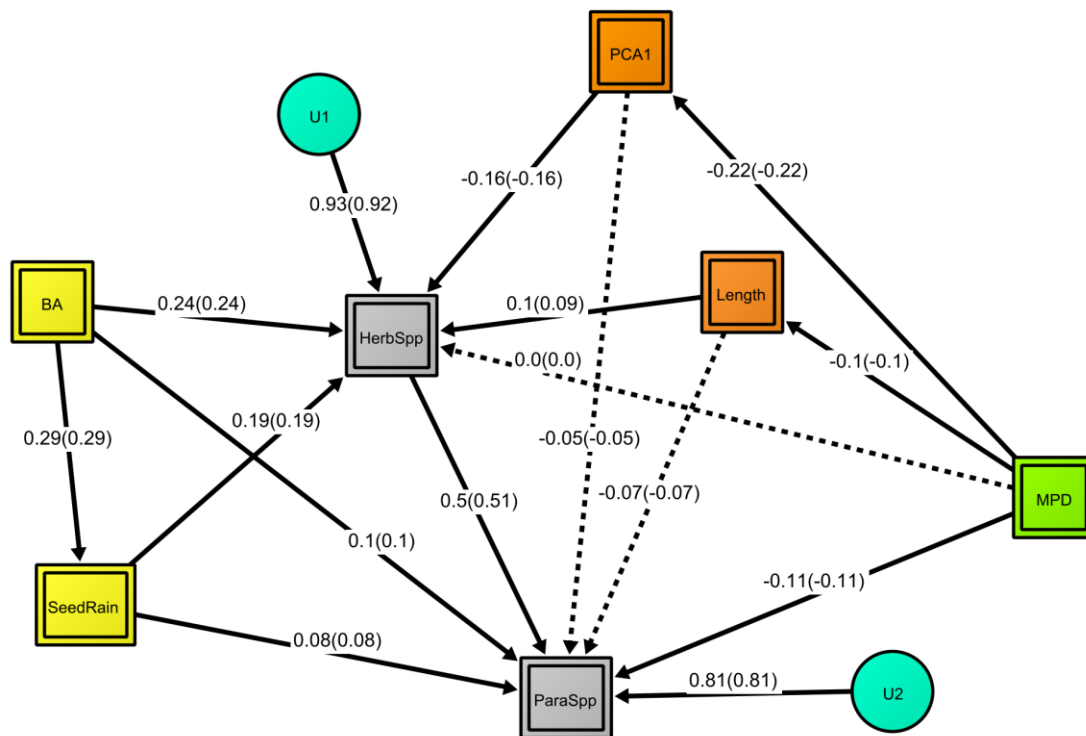
Fig. 4. Results of path analysis testing direct and indirect correlations beween the species richness of seed- and fruit-eating insects (HerbSpp), parasitoid species richness (ParaSpp), mean phylogenetic distance (MPD), plant traits (fruit length, Length and ordination scores delineating plant functional groups, PCA1) and plant resource (basal area, BA and seed rain, SeedRain), for 618

809   host plant species. Standardized path coefficients are in parentheses. Significant (p<0.05) and insignificant paths are indicated
810   by solid and dashed lines, respectively. The rationale of the model is detailed in Appendix S1F.