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Rodrigo Augusto Santinelo Pereira, Finn Kjellberg

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2 Chapter 13

3 Mutualism as a source of evolutionary innovation: insights from insect-plant interactions

4 Rodrigo Augusto Santinelo Pereira<sup>1\*</sup> and Finn Kjellberg<sup>2\*</sup>

5 <sup>1</sup> Depto de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade  
6 de São Paulo, Brasil.

7 <sup>2</sup> CEFÉ, CNRS, Université Montpellier, Université Paul Valéry, Montpellier, EPHE, IRD,  
8 Montpellier Cédex 5, France

9 \* Corresponding author: [raspereira@yahoo.com.br](mailto:raspereira@yahoo.com.br); [finn.kjellberg@cefe.cnrs.fr](mailto:finn.kjellberg@cefe.cnrs.fr)

10

11 **Abstract:**

12 Mutualism is ubiquitous in nature and probably all species in the world are involved in some  
13 form of such ecological interaction. The evolution of mutualisms is often envisioned in a  
14 framework where individual selection leads to reciprocal exploitation that results in net  
15 benefits to all the interacting parties, *i.e.* in a framework in which individual selection  
16 intrinsically destabilises the mutualistic association. However, mutualisms are stable, and  
17 rarely turn into parasitism. At a macroevolutionary scale, mutualisms have been a source of  
18 major evolutionary innovations and have benefited all the species involved. Indeed,  
19 complementary traits of the partners enable them to colonise new ecological niches. In this  
20 chapter we discuss mutualism as a source of evolutionary innovation, using some insect-plant  
21 associations as examples. Firstly, we dedicate a topic to theoretical aspects and hypotheses on  
22 evolutionary forces stabilizing mutualisms (and their pitfalls). We present some examples of  
23 mutualisms that allowed insects and/or plants to expand their ecological niches. Finally, we  
24 focus on fig tree – animal interactions that, putatively, allowed this plant lineage to diversify in  
25 subtropical and tropical ecosystems and to form a group of keystone species for the  
26 functioning of many forest ecosystems.

27

28 **Learning objectives:**

29 After completing this chapter, you should understand the following:

- 30 • To understand the evolution of mutualisms we need to look at natural selection  
31 operating at different levels
- 32 • The general architecture of mutualistic associations
- 33 • Mutualisms are ecologically and evolutionarily stable
- 34 • Mutualistic associations may boost species diversification by creating new ecological  
35 opportunities (innovations) for the interacting species

36

37 **Key-words:** co-diversification, co-evolution, ecological interactions, mutualism conflicts, plant  
38 defences, pollination

39

## 40 1. Introduction

41 Mutualisms, defined as interspecific interactions that are beneficial to all the involved  
42 partners, are ubiquitous in nature. Probably all species in the world are involved in some form  
43 of mutualistic interaction (Bronstein et al. 2006). In this chapter we will address direct  
44 mutualisms in which the partners are in physical contact. Direct mutualisms may be further  
45 divided into symbiotic and non-symbiotic mutualisms (Boucher et al. 1982). In symbiotic  
46 mutualisms, individuals are physiologically integrated, whereas in non-symbiotic mutualism,  
47 despite some physiological co-adaptation, individuals of the interacting species are at least at  
48 some stage of their life cycle physiologically independent (Boucher et al. 1982). Many  
49 symbiotic mutualisms involve the exchange of nutritional and energetic services (*e.g.*  
50 photosynthesis). Mutualisms, and particularly non-symbiotic ones, can also involve protection  
51 and dispersal of gametes or propagules.

52 Often, individuals benefit directly from their mutualistic behaviour. Many definitions  
53 restrict mutualism to these cases. However, in a number of interactions that constitute  
54 undisputed examples of mutualisms, the individuals performing the act that is beneficial to the  
55 other species do not benefit directly from this act. This is typically the case in gynodioecious  
56 *Ficus* species (Box 1). Pollinator individuals that ensure pollination by entering functionally  
57 female inflorescences (figs) die without reproducing: their reproductive value is equal to zero  
58 (Kjellberg et al. 2005; Pereira 2014). A definition of mutualism that would exclude half of *Ficus*  
59 species and many other undisputed cases of mutualisms would be useless. In the case of the  
60 fig pollinating wasp mutualism, the wasps maximise their fitness by trying to reach receptive  
61 figs as fast as possible, *i.e.* without engaging in the difficult task of avoiding female figs, and  
62 this is beneficial for the fig trees as it results in pollination. In this situation, individual selection  
63 on the fig wasp is beneficial for the host *Ficus* species, not to a particular individual of that  
64 species. Reciprocally we expect the interaction to be stable because individual selection on fig

65 trees results in phenotypes that allow the survival of populations of its associated wasps.  
66 Hence, in many situations, mutualism benefits cannot be defined at the level of individual  
67 interactions. Defining mutualism at the population level could in a number of cases be  
68 operational as the an interaction may vary from mutualistic to parasitic across populations  
69 (Addicott 1986; Thompson 1999; Friberg et al. 2019). However, the structuring of mutualists  
70 into populations and species may be so different that a population level approach is not  
71 operational. For instance, *Ficus hirta* present gradual genetic variation among populations  
72 while its pollinating wasp in South-East China constitutes a single population (Yu et al. 2019). In  
73 such cases, selective processes do not occur at the same geographic scales for the partners.  
74 Different geographic population structure between mutualists is probably the rule rather than  
75 the exception in non-symbiotic mutualisms (Alvarez et al. 2010). Therefore, in this chapter, we  
76 define mutualisms as interspecific interactions that are beneficial for the species involved and  
77 that result in adaptive innovation. Obligate interactions resulting from infection of a host and  
78 subsequent adaptation of the host to its pathogen do not enter within the limits of this  
79 definition if they do not result in biological innovation. Such interactions can become obligate  
80 when curing the host from its pathogen results in self-poisoning (Dedine et al. 2001).

81       Generally, organisms that associate in a mutualism differ radically in biological traits and  
82 life habits. The combination of these traits confers them new biological capacities, allowing  
83 them to colonise new ecological niches. At a macro-evolutionary scale, mutualisms have been  
84 at the source of major biological innovation (Margulis and Neelson 1989; Wheat et al. 2007;  
85 Leigh Jr 2010). A classic example is the endosymbiotic origin of mitochondria and plastids  
86 through the inclusion of prokaryotic organisms into the pre-eukaryotic cell (Gray 2017). This  
87 association improved the energetic machinery of eukaryotes and allowed some of them to  
88 become primary producers (Margulis 1996). Several other symbiotic mutualisms have been the  
89 source of key innovations at the origin of major new modes of life. For instance,  
90 representatives of at least seven phyla (Annelida, Arthropoda, Ciliophora, Mollusca,

91 Nematoda, Platyhelminthes and Porifera) are known to associate with chemosynthetic  
92 bacteria (Dubilier et al. 2008). This type of mutualism allows representatives from these  
93 diverse lineages of animals to colonize habitats presenting high concentrations of reduced  
94 energy sources, such as sulphide and methane. These habitats are generally transient but at  
95 different time scales ranging from whale carcasses and sunken wood on the deep-sea floor to  
96 somewhat more stable habitats, such as hydrothermal vents, cold seeps, shallow-water coastal  
97 sediments and continental margins (*e.g.* mangrove areas) (Dubilier et al. 2008). Herbivores,  
98 including insects of different orders and vertebrates derive their digestive capacities from  
99 bacteria, fungi, protozoa and other microorganisms. The fine mechanisms involved have been  
100 investigated for cockroaches, termites, attine ants, sap-feeding insects and ruminant  
101 vertebrates (Mueller et al. 2001; Nalepa et al. 2001; Aanen et al. 2002; Kamra 2005; Baumann  
102 2005; Koike and Kobayashi 2009; Douglas 2009; Caldera et al. 2009). While taxonomically and  
103 biologically highly heterogeneous, these digestive mutualisms are all based on the capacity of  
104 the animal to collect carbon rich resources that they cannot digest by themselves. The  
105 associated microorganisms provide the metabolic capacities to degrade these carbon rich  
106 resources, such as cellulose, hemicelluloses and lignin, to detoxify secondary plant compounds  
107 and to convert nitrogen into available amino acids. The association of plants with mycorrhizal  
108 fungi (*e.g.* fungi belonging to the Phylum Glomeromycota and forming arbuscular mycorrhiza)  
109 is most often mutualistic, improving water and nutrient uptake by the plant, especially the  
110 uptake of phosphate and nitrogen (Brundrett 2004). Plant fine roots are constrained in how  
111 thin they can become because of the size of their genome which results in large nuclei. Fungi  
112 have much smaller genomes allowing smaller nuclei and hence allowing fungal filaments to be  
113 much thinner than plant fine roots. Therefore, mycelia explore the soil at a much finer scale  
114 and at lower constitutive costs than plant roots. Mycorrhizal fungi are associated with roots of  
115 70 – 90% of land plant species, representing one of the most extensive terrestrial symbioses  
116 (Parniske 2008). It may have played a central role in the colonisation of terrestrial habitats by

117 enabling plants to exploit mineral soils. The association between plants and nitrogen-fixing  
118 bacteria is another example of mutualism allowing ecological innovation. The bacteria  
119 metabolise inert N<sub>2</sub> present in the atmosphere into the utilizable form ammonia (NH<sub>3</sub>) through  
120 the action of nitrogenases. This mutualism is present in a diversity of plant lineages including  
121 ferns, gymnosperms, mono- and eudicots. The plants host the endophytic bacteria in their  
122 roots. Among them, the root nodule symbiosis is particularly sophisticated and complex. The  
123 root nodules house high bacteria densities in a structure that provides an anaerobic  
124 microenvironment favourable for nitrogenase activity (Markmann and Parniske 2009; Soto et  
125 al. 2009). The mutualisms exemplified above are just a few examples of known mutualisms  
126 that represent biological innovations central to the colonisation of earth and the functioning of  
127 extant ecosystems. There are many other examples, as for instance lichens associating fungi  
128 and algae/cyanobacteria (Nascimbene and Nimis 2006), or sessile/slow-moving marine animals  
129 and algae/cyanobacteria (Venn et al. 2008).

130 In the following, we first present some theoretical considerations on mutualisms.  
131 Indeed, developing a comprehensive theory of mutualisms is still an open endeavour. We  
132 propose a framework within which a theory may emerge. Then we develop some examples of  
133 insect-plant and insect-fungi mutualisms that are at the origin of major evolutionary  
134 innovation. Finally, we focus on the fig tree – associated animal interactions that allowed this  
135 plant lineage to diversify in subtropical and tropical ecosystems and to become keystone  
136 species for the functioning of some forest ecosystems.

137

138 **Box 1.** Pollination by deceit in fig trees.

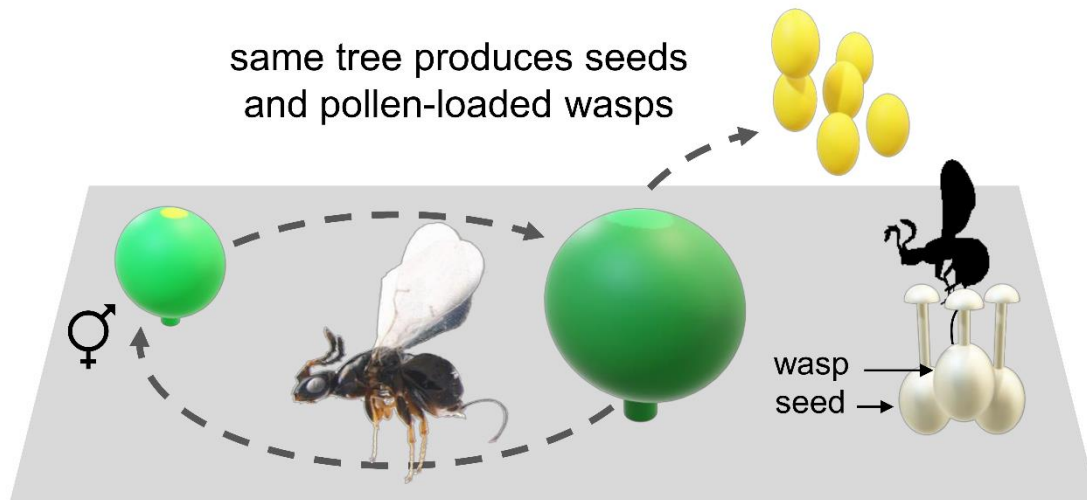
139 An intriguing example is pollination by deceit in gynodioecious *Ficus* species (Kjellberg et  
140 al. 1987; Figure 1). Functionally “male” trees produce pollinating wasps (pollen vectors) and  
141 pollen, and female trees produce seeds but neither pollen nor wasps. Female trees bear figs in

142 which all the styles of pistillate flowers are too long to allow wasp oviposition. Wasps entering  
143 figs of female trees pollinate but die without reproducing. Functionally male trees bear figs in  
144 which all pistillate flowers have short styles. These flowers may receive an egg and produce a  
145 wasp, but they rarely produce seeds. As a result, although male figs present both male and  
146 female flowers, they are functionally male. This is a case of pollination by deceit and figs on  
147 female trees constitute a lethal trap for the wasps. Considering the difference in generation  
148 time between fig trees (counted in years) and fig wasps (counted in months) and the huge  
149 difference in population sizes (ranging from 10<sup>th</sup> of thousands to millions of times larger insect  
150 population sizes), a simple prediction is that the capacity to recognise female trees and avoid  
151 them will evolve rapidly. Nevertheless, approximately half of the 800 *Ficus* species are  
152 functionally dioecious and they correspond to a limited set of monophyletic lineages. Why is it  
153 so? The pollinating fig wasps are attracted to the fig tree by volatile compounds released by  
154 the receptive inflorescences. For the compounds that are perceived by the pollinators, the  
155 inflorescences of female and “male” trees emit exactly the same relative proportions  
156 (Hossaert-McKey et al. 2016; Proffit et al. 2020). Hence, a first answer is male-female mimicry.  
157 However, a better formulation is probably, male-female mimicry makes distinguishing  
158 between male and female trees complicated. Then the question becomes, could it be  
159 worthwhile to take the time and efforts to distinguish male and female trees, can being choosy  
160 be selected? In some *Ficus* species, there is hardly any selection for avoiding female trees  
161 because figs on female and “male” trees are not receptive at the same time of the year, so that  
162 the wasps never get a chance to choose between sexes. Most pollinators emerge from figs on  
163 “male” trees at a time when there are no receptive male figs. They will fail to reproduce  
164 irrespective of whether they avoid female trees or not (Kjellberg et al. 1987). In *Ficus* species in  
165 which “male” and female trees are receptive at the same time, the race to enter receptive figs  
166 (Conchou et al. 2014) selects against taking the time required to distinguish highly similar  
167 phenotypes.

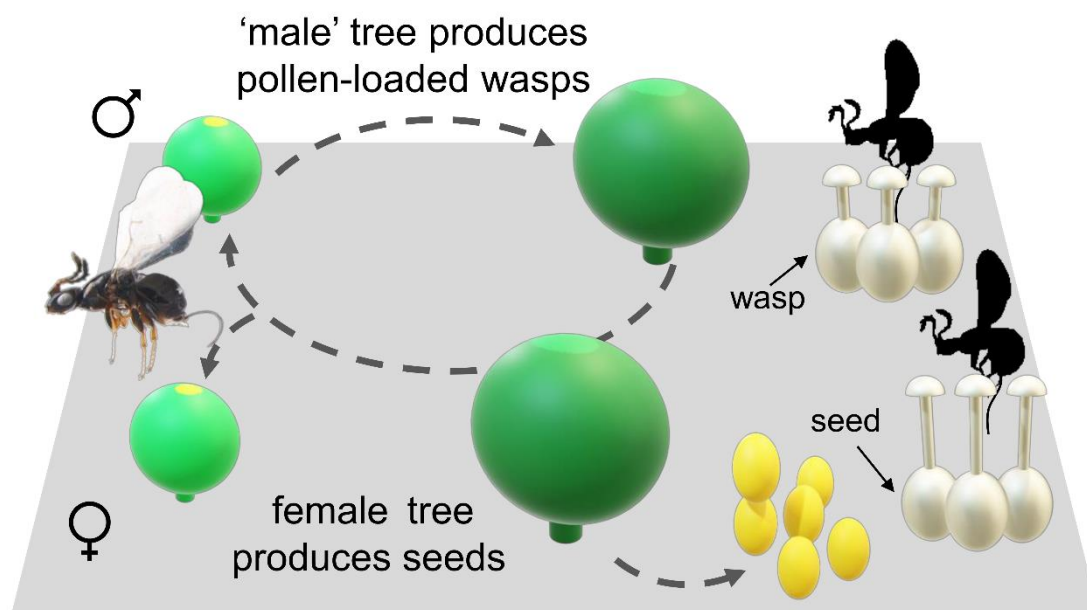
168



## A Monoecious species



## B Gynodioecious species



169

170 **Figure 1.** Reproductive systems in fig trees. A – in monoecious species seeds and pollen-loaded  
171 wasps are produced in the same fig. B – in gynodioecious (functionally dioecious) species,  
172 functionally male trees produce pollen-loaded wasps and female trees produce seeds.

173

174

175 **2. Theoretical remarks**

176 The main pitfall of a vision in which adaptive innovation drives the evolution of mutualistic  
177 associations is formulations suggesting that a species acts for the sole benefit of another one  
178 and reciprocally. Such formulations cannot be defended. There is no way escaping that  
179 individuals are selected to maximize their fitness. In a mutualism this may, or may not, be at  
180 the expense of the partner species. In this context, the evolution of mutualisms is often  
181 envisioned in a framework where individual selection leads to reciprocal exploitation that  
182 results in net benefits to all the interacting parties, *i.e.* in a framework in which individual  
183 selection intrinsically destabilises the mutualistic association (Axelrod and Hamilton 1981;  
184 Herre and West 1997). Therefore, it has been proposed that host sanction against non-  
185 cooperative partners may be important for mutualism stability. Host sanctions would be any  
186 traits evolved specifically to reduce the fitness of a partner that becomes non-cooperative.

187 Lists of mechanisms limiting non-cooperative behaviour or partner overexploitation and  
188 derived from intraspecific cooperation theory have been proposed (Herre et al. 1999; Yu 2001;  
189 Sachs et al. 2004; Doebeli and Hauert 2005; West et al. 2007; Bergmüller et al. 2007). While  
190 cooperation models have stimulated theoretical studies on mutualism, they do not find  
191 consistent empirical support to explain the stability of a range of mutualistic associations. For  
192 example, models based on retaliation (sanctions or refusing future interactions) are probably  
193 only of importance for animals with developed cognition capacity, as they require the ability to  
194 remember past interactions and behave accordingly. Sanction has been invoked to explain  
195 cooperation reinforcement in some mutualistic association, such as legume plants – N<sub>2</sub>-fixing  
196 bacteria, fig trees– pollinating fig wasps and yucca – pollinating yucca moths (Pellmyr and Huth  
197 1994; Kiers et al. 2006; Kiers and Denison 2008; Jandér and Herre 2010; Leigh Jr 2010; Jandér  
198 et al. 2012). In these examples, when the *Rhizobium* bacteria do not provide nitrogen, or when  
199 the fig wasps/yucca moths do not pollinate, the non-cooperating partner pays a cost, as the O<sub>2</sub>

200 flux to the radicular modules where bacteria are housed stops or the flower ovaries or  
201 inflorescences where fig/yucca pollinators laid their eggs provide less nutrients or abort.  
202 However, there is no demonstration that these host traits that have been selected to respond  
203 to non-cooperative partners, *i.e.* that they qualify as sanctions (Frederickson 2013). Further no  
204 theoretical framework has been proposed within which sanction could evolve. Indeed, there is  
205 an intrinsic problem with the strong differences in generation time and populations sizes  
206 between the interacting species (see Box 1). Individuals of the slow-reproduction small  
207 population size species interact with many individuals and many generations of their  
208 associated species. Such asymmetry is constitutive of most mutualistic associations. As a  
209 consequence, in visions of mutualism stability based on an evolutionary race between species,  
210 the partner with larger population sizes and the shorter generation time should win the race  
211 and reap the highest profits possible from the association. In the process, it would overcome  
212 any adaptation of the other species to limit overexploitation.

213         Contrary to the predictions based on ideas stemming from within species cooperation  
214 theory, mutualistic associations are robust to the presence of non-cooperative partners.  
215 Indeed, phylogenetic evidences from a range of biological systems evidences that mutualisms  
216 rarely evolve into parasitic interactions in nature (Frederickson 2017). A striking example of  
217 this robustness is provided by the diffuse mutualism involving seed dispersion by vertebrates.  
218 Seed dispersion networks are structured into complex mosaics of frugivorous guilds associated  
219 with plant guilds and involve plant traits such as seed size, fruit colour, fruit flesh nutrient  
220 content, etc, with few specialist species (Shanahan et al. 2001; Albert et al. 2013; Sarmiento et  
221 al. 2014). Poor dispersers and seed predators are frequent among members of the frugivorous  
222 guilds (Howe 1986; Compton et al. 1996; Shanahan et al. 2001). Nevertheless, even with this  
223 widespread occurrence of non-cooperating partners, about 70-90% of tropical trees present  
224 adaptations favouring animal seed dispersion (Howe and Smallwood 1982). Thinking about the  
225 stability of mutualisms and their role in biological innovation obliges to envision their evolution

226 from two different perspectives. The individual selection perspective is a truism: only genes  
227 that increase in frequency in a population become fixed. Genes that decrease in frequency will  
228 disappear. Therefore, individual selection may lead to population size decrease and to species  
229 extinction by increasing the relative frequency of a gene that reduces population size. On the  
230 other hand, one of the defining characters of life is that living organisms have a history.  
231 Indeed, life on earth has survived for over 3 billion years. Species that have survived and given  
232 rise to new ones are those in which short-term individual selection was compatible with, or  
233 even favored, long-term species survival. Consequently, extant species derive from species in  
234 which some intrinsic biological traits resulted in lack of short-term selection favoring genes  
235 that would lead to species extinction. Only lineages in which there was no such destructive  
236 short-term selection have survived. If a lineage loses these biological traits, it will go extinct.  
237 This intrinsic property of life is illustrated by sexual reproduction. Most species on earth  
238 engage in some form of sex. Nevertheless, asexual lineages regularly appear. In the  
239 phylogenies, there are no asexual lineages but only isolated asexual species at the end of  
240 phylogenetic branches. This means that asexual species are evolutionary dead ends (Maynard-  
241 Smith 1978). Why is loss of sex so rare that it does not drive life on earth to extinction? Simply  
242 because extant lineages derive from species in which some intrinsic trait resulted in short term  
243 selection for sex and most often species inherit this trait from their ancestors (Gouyon 1999).  
244 As a result, loss of sex remains exceptional. We can apply the same line of thinking to  
245 mutualisms. Only mutualisms in which there is no short-term selection against the mutualism,  
246 or mutualisms in which no mutation that destabilizes the mutualism can arise, survive over  
247 evolutionary times. This explains why mutualisms are intrinsically stable (Frederickson 2017)  
248 and, hence, why we do not expect to find adaptations specifically evolved to punish cheating  
249 mutualists (i.e. that would take the benefits from the mutualism without reciprocating).  
250 Therefore, sanctions do not explain mutualism stability (Frederickson 2013).

251           The mutualism between *Yucca* and *Yucca*-moths provides an example of the pitfalls  
252 associated with thinking in terms of sanctions. *Yuccas* are pollinated by *Tegeticula* moths that  
253 lay eggs in the flowers. The moth collects pollen with its modified labial palps and deposits  
254 some of it on the flower stigma after oviposition. The moth larva feeds on the developing  
255 young seeds. If the larva eats too many developing seeds in the ovary, the flower aborts  
256 leading to the death of the larva. From these observations, it is tempting to suggest that *Yucca*  
257 plants have developed a mechanism to limit oviposition by its mutualistic pollinator. If the  
258 pollinator turns into a parasite eating too many seeds, it is punished. However, *Tegeticula*  
259 belong to a group of moths that do limited damages in the ovaries. Lack of host  
260 overexploitation predates the mutualism (Yoder et al. 2010). Similarly, *Yucca* belong to a  
261 lineage of plants in which there is abundant early abortion of developing fruits. Abortion rates  
262 of fruits containing few developing seeds and of damaged flowers are high. Hence, the  
263 mechanisms limiting over-exploitation predated the evolution of the mutualism and do not  
264 qualify as sanctions. We propose that the evolution of the mutualism was made possible by  
265 pre-existing traits of the associates (Frederickson 2013). This is further demonstrated that one  
266 *Tegeticula* pollinated species, *Hesperoyucca whipplei*, is not a *Yucca*. The moth has jumped  
267 host, and has become the active pollinator of a new lineage (Pellmyr 2003). This lineage did  
268 not have a history of co-adaptation with the moth and therefore could not have developed  
269 adaptations against overexploitation by mutualist *Tegeticula* moths. Despite this, the  
270 association has thrived demonstrating that the adaptations limiting exploitation pre-existed to  
271 the mutualism. Successful host switching has occurred in several active pollination mutualisms.  
272 It has also been shown that *Epicephala* moths have colonised and pollinate actively four  
273 different lineages of plants within Phyllanthaceae converting pollinated lineages pollinated by  
274 generalist insects into lineages engaged in active pollination mutualisms (Kawakita 2010).  
275 Further, some chalcid fig-wasp lineages that do not belong to the Agaonidae have become fig-  
276 pollinators despite 70 Ma year co-diversification of the mutualistic association between *Ficus*

277 and Agaonidae (Jousselin et al. 2001). Hence, we have numerous examples of new associations  
278 that became mutualisms because of pre-existing traits of one or both partners involved in the  
279 new mutualism.

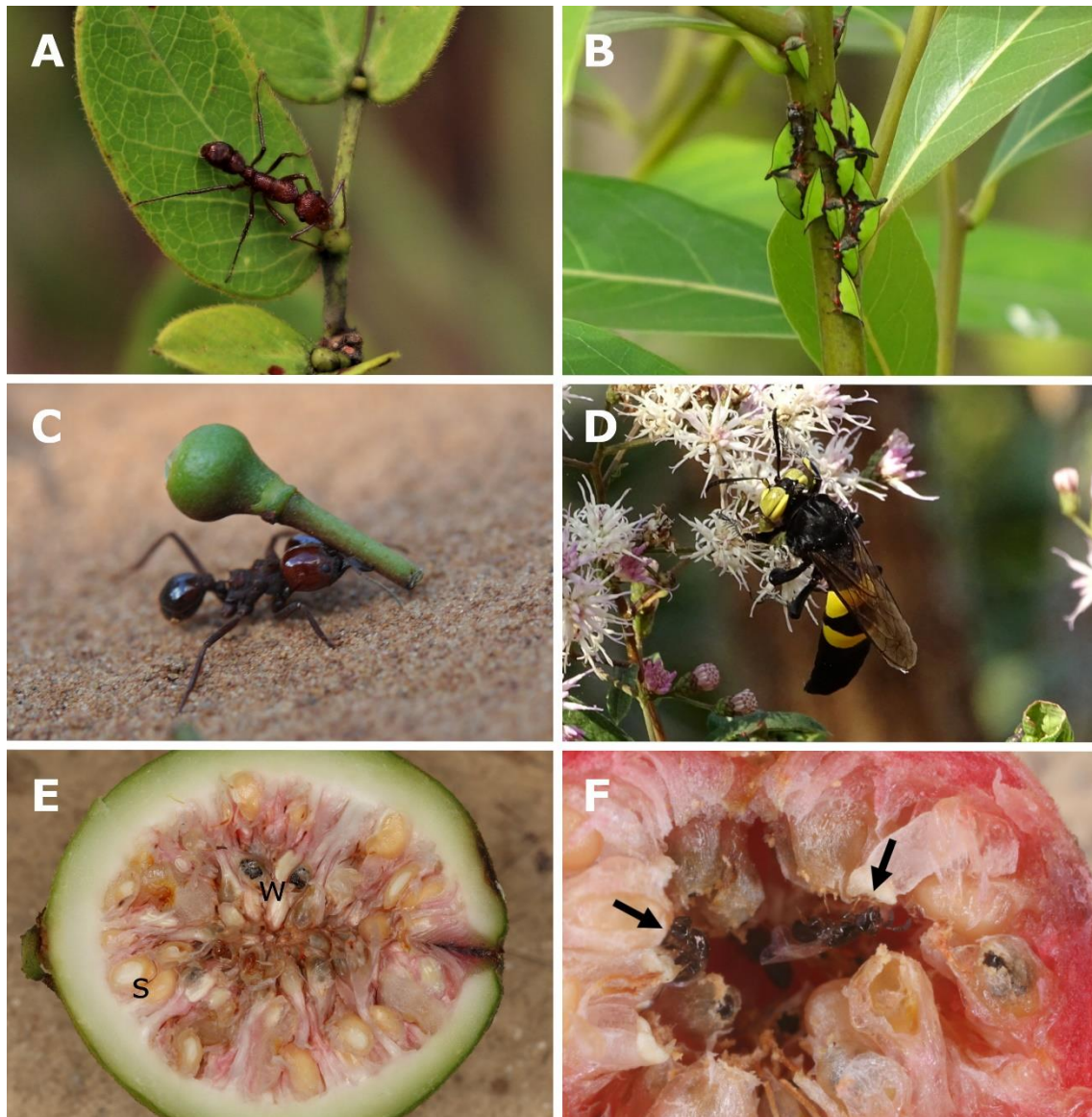
280         The complementarity of species traits in mutualisms are generally striking. In both  
281 symbiotic and non-symbiotic mutualism, in general, there is a combination of a structural  
282 component, representing goods provided by a long-lived partner (*e.g.*, 3D structure, sheltering  
283 or food), and a service supplied by a short-lived partner (*e.g.*, gamete transport, resource  
284 transport from the surroundings, protection against natural enemies or biosynthetic  
285 capacities) (Leigh Jr 2010). In addition, mutualisms involve members of distant taxonomic  
286 groups, as exemplified by marine invertebrates – bacteria, mammals/insects – gut  
287 microorganisms, plant – root bacteria/fungi, fungi – algae/cyanobacteria, and plant – insect  
288 associations. Large phylogenetic distance between the partners is probably important for  
289 mutualism stability, as it enhances the combination of contrasting abilities, and decreases  
290 niche overlap. As a consequence, the potential for selective conflicts between partners is  
291 reduced, and the potential for overcoming them is enhanced. Indeed, the larger organism  
292 generally controls the arena in which the life of the smaller, shorter-lived organism is played out.  
293 Controlling the arena may canalise selective forces acting on the smaller partner. In this topic  
294 we argue that mechanisms to reinforce cooperation, acting exclusively at individual (or gene)  
295 level are not sufficient to stabilize mutualistic associations, although these mechanisms play a  
296 role in reducing conflicts between partners. One example of such mechanism is the vertical  
297 transmission of the symbiont that occurs in sap-sucking insects – gut bacteria (Baumann 2005)  
298 and ant/beetle – cultivated fungi (Aanen et al. 2002; Mueller et al. 2005), which increases the  
299 symbiont endogamy, limiting the selection of non-cooperative traits via kin selection. In other  
300 types of mutualism, specially the non-symbiotic ones, it is necessary to consider spatio-  
301 temporal dynamics at community level and the multilevel selection to accommodate an  
302 evolutionary framework of mutualisms (Gomulkiewicz et al. 2003; Wilson and Wilson 2008;

303 O’Gorman et al. 2008; Nowak et al. 2010). Mutualism as source of evolutionary innovation that  
304 expands the partners capacities appears as a general aspect of + / + interactions. Such  
305 emergent attribute can sometimes transcend pure genetic determinism if one considers  
306 cultural evolution in animals with developed cognitive capacities, as for example corvids and  
307 primates, that have allowed them to better explore the ecosystem, and eventually use new  
308 niches (Wimsatt 1999; Castro and Toro 2004; Marzluff and Angell 2005; Vale et al. 2017).

309

### 310 **3. Some examples of mutualisms involving insects and plants**

311 Most seed plants are sessile during their sporophytic phase, *i.e.* during most of their life cycle.  
312 Hence, many of their responses to ecological challenges, such as defence against herbivores,  
313 gamete transfer and seed dispersal, may rely on mutualistic associations with mobile animals  
314 (see previous topics). On the other hand, animals may expand their ability to use plant  
315 resources thanks to mutualistic interactions with microorganisms (Douglas 2009) or by tending  
316 phytophagous insects (Heil and McKey 2003). Those mutualisms can be classified according to  
317 the services provided by one of the partners, usually the one smaller in size and with shorter  
318 generation time (Table 1). In the following, we present some examples of insect-plant  
319 mutualisms involving defence against herbivores, digestion of plant products, and pollination  
320 by insects (Figure 2).



321

322 **Figure 2.** Examples of mutualisms involving insects and plants. Protection mutualism: A –  
 323 *Ectatomma tuberculatum* ant probing an extrafloral nectary in Leguminosae. Digestive  
 324 mutualisms: B – *Aconophora* sp. (Auchenorrhyncha: Membracidae), associated with  
 325 *Baumannia* ( $\gamma$ -proteobacteria) and *Sulcia* (Bacteroidetes); C – *Atta laevigatta* carrying a floral  
 326 bud. Pollination mutualisms: D - *Editha magnifica* wasp visiting *Vernonia* flowers; non-  
 327 specialized insects may eventually act as pollinators. E – immature seeds and fig wasps in the  
 328 monoecious *F. maxima*; note that seeds (s) are produced in flowers closer to the fig wall, while  
 329 wasps (w) are produced closer to the fig cavity. F – fig wasps collecting pollen (arrows) in the  
 330 monoecious *F. albert-smithii*. Photo credits: A – D (Kleber Del-Claro), E – F (Finn Kjellberg).

331



332 Table 1. Examples of mutualisms involving insects and plants

Mutualism Classes	Provided services	Systems	Partner integrations	Sub-divisions	Some examples	References
Protection mutualism	Protection against herbivores	Predatory insects – plants	Non-symbiotic	Extra-floral nectar, domatia and food bodies	Myrmecophilic plants (several of flowering plants and ferns)	Heil & Mckey (2003)
				Honey dew from phloem-feeding hemipterans (trophobionts)	Hemiptera – Formicidae mainly, but also Anthribidae, Coccinellidae, Apoidea, Tachinidae, Syrphidae and Neuroptera	Delabie (2001)
Digestive mutualism	Degrade carbon rich sources ( <i>i.e.</i> cellulose, hemicelluloses and lignin), detoxify secondary plant compounds and convert nitrogen in available amino acids	Insects – microorganisms	Symbiotic	General feeders	Blattodea, Coleoptera and Psocoptera	Nalepa et al. (2001), Douglas (2009)
				Plant sap feeders	Hemiptera – <i>Baumannia</i> , <i>Buchnera</i> , <i>Carsonella</i> , <i>Portiera</i> , <i>Sulcia</i> and <i>Tremblaya</i> bacteria, and clavicipitacean fungi	Douglas (2009)
				Fungus – growing insects	Attine ants, Macrotermitinae termites and Scolytinae (ambrosia beetles) – several fungi	Mueller et al. (2005)
Pollination mutualism	Gamete transport	Several insect orders – seed plants	Non-symbiotic	Nectar, pollen and sheltering as reward	bees, beetles, dipterans, lepidopterans, thrips and wasps	Rech et al. (2014)
			Symbiotic	Brood-site pollination	Obligate: fig trees – fig wasps, leafflowers – leafflower moths and yucca – yucca moths senita cactus – senita moths, Silene – <i>Hadena</i> / <i>Perizoma</i> , Lithophragma – <i>Greya</i> and globeflower – globeflower flies	Sakai (2002), Weiblen (2002) Kephart et al. (2006), Hembry & Althoff (2016)

333

334

335 *3.1. Protection mutualisms*

336 Animals and plants can obtain protection against natural enemies from mutualistic  
337 associations. Particularly, two interrelated systems have been extensively studied: ant-plant-  
338 herbivore and ant-hemipteran interactions. Insect herbivory is a key factor in plant  
339 communities that effects plant productivity, survival and reproduction (Showalter 2000).  
340 Plants can derive protection against herbivores by associating with predatory ants. As ants are  
341 among the most important predators of arthropods, they can constitute an effective plant  
342 defence. Indeed, while many herbivores have overcome plant chemical defences, few have  
343 evolved efficient adaptations against predatory ants (Heil and McKey 2003).

344         Plants can attract facultative mutualist ants by providing direct rewards (energy-rich  
345 extrafloral nectar and food bodies), as well as indirect rewards such as carbohydrate-rich  
346 excretions (honeydew) of phloem-feeding hemipterans (trophobionts). In this later tripartite  
347 mutualism, ants defend their trophobionts from predatory and parasitoid insects, resulting in  
348 another nested defensive interaction (Delabie 2001; Heil and McKey 2003; McKey et al. 2005).  
349 The direct and indirect rewards provided by the plants for the ants are poor in nitrogen and  
350 present highly unbalanced amino acid compositions. Ants may complement this unbalanced  
351 diet by hunting herbivorous insects or by harvesting some of their hemipteran trophobionts  
352 (Del-Claro et al. 2016; Calixto et al. 2018). Some ants, especially species adapted for living in  
353 the canopy, are associated with endosymbiotic microbes that help them to cope with such  
354 nutritional imbalance. The need of supplementation of nitrogen from external sources, seems  
355 to have driven the selection of prey-foraging strategies and physiological adaptations (Heil and  
356 McKey 2003; McKey et al. 2005).

357         In over 100 genera of tropical angiosperms, plants are involved in more permanent, in  
358 some cases obligatory, mutualisms with protective ants. These plants have specialised

359 structures, called domatia, and used as nest by the ants. Domatia can occur in hollow stems  
360 (e.g., *Cecropia*, *Leonardoxa* and *Macaranga*), thorns (*Acacia*), petioles (*Piper*), or leaf pouches  
361 (*Hirtella*, *Maieta*, *Scaphopetalum* and *Tococa*). In addition to domatia, plants may offer food  
362 rewards, such as extrafloral nectar, food bodies or both. Plants benefit from protection  
363 services and, additionally, from nutrients mobilised by the ants. It is estimated that 80% of the  
364 carbon in *Azteca* ant's bodies can be derived from their *Cecropia* host tree, whereas about 90%  
365 of the plant's nitrogen comes from ants debris (i.e., exuviae, dead larvae, workers, and remains  
366 of arthropod prey) (Heil and McKey 2003).

367         Defensive mutualisms have evolved many times between insect species. Ants show  
368 trophobiotic relationships with lepidopteran larvae of the families Lycaenidae, Riodinidae, and  
369 Tortricidae, and heteropteran species of the families Coreidae, Pentatomidae and Plataspidae.  
370 In addition to ants, hemipteran species have trophobiotic relationships with a range of other  
371 insect groups, such as Anthribidae, Coccinellidae, Apoidea (and other aculeate Hymenoptera),  
372 Tachinidae, Syrphidae and Neuroptera (Delabie 2001). However, protection mutualism is more  
373 frequent and better studied in ant-hemipteran interactions. Sap-feeding hemipterans are  
374 particularly vulnerable to predation. Some species are sessile at some life-stages. Even in more  
375 mobile species, to access the phloem, hemipteran insects introduce their stylets deep into  
376 plant tissues; a process that takes minutes to hours. While they are attached to the host plant,  
377 they are not able to remove the mouthparts quickly to escape predators. Moreover, because  
378 of their feeding mode, hemipteran insects excrete large quantities of honeydew. These insects  
379 have one additional problem; they have to get rid of such excretion that can accumulate on  
380 their bodies and serve as substrate for fungus growing. Thus, trophobiosis has this "cleaning"  
381 benefit for sap feeding insects (Delabie 2001).

382

383 *3.2. Digestive mutualism*

384 Mutualism with microorganisms has allowed several groups of animals to feed on plants.  
385 Indeed, plant tissues constitute a source of energy and nutrients rich in cellulose and  
386 allelochemicals, which are primarily inaccessible to animal digestion. Plant feeding insects and  
387 vertebrates derive their digestive capabilities from bacteria, fungi, protozoa and other  
388 microorganisms. The most studied groups include cockroaches, termites, attine ants, plant  
389 sap-feeding insects and ruminants (Mueller et al. 2001; Nalepa et al. 2001; Aanen et al. 2002;  
390 Kamra 2005; Baumann 2005; Koike and Kobayashi 2009; Douglas 2009; Caldera et al. 2009).  
391 Despite their remarkable taxonomic and ecological diversity, digestive mutualisms all share the  
392 attribute of making use of the large metabolic capacities of microorganisms to degrade carbon  
393 rich sources (i.e. cellulose, hemicelluloses and lignin), detoxify secondary plant compounds and  
394 convert nitrogen in available amino acids.

395 Detritivorous termites and cockroaches are associated with bacteria, fungi and protozoa  
396 that degrade cellulosic material and recycle nitrogen from insect waste (Nalepa et al. 2001;  
397 Douglas 2009). Analogous interactions occur in ruminant vertebrates, allowing them to  
398 assimilate carbon present in cellulosic compounds and metabolise anti-nutritional and toxic  
399 substances of plants. Cattle support an impressively diverse and complex microorganism  
400 community. One millilitre of rumen liquor can enclose as many as  $10^{10}$ – $10^{11}$  cells of 50  
401 bacterium genera,  $10^4$ – $10^6$  ciliate protozoa from 25 genera,  $10^3$ – $10^5$  zoospores of five  
402 anaerobic fungus genera and  $10^8$ – $10^9$  bacteriophages (Kamra 2005). Nevertheless, the  
403 bacterial diversity is largely underestimated, as many rumen bacteria cannot be cultivated in  
404 the laboratory. Species survey based on DNA sequences suggest that 300-400 species of  
405 bacteria are present in the rumen (Koike and Kobayashi 2009).

406 Despite of the large metabolic capacity of microorganisms, the amount of vegetal  
407 material that insects can process is ultimately constrained by their gut volume. This limitation  
408 is overcome by insects that cultivate fungi in their nests (Douglas 2009). Fungus cultivation is

409 well known in the Neotropical Attini ant tribe and in the Old-World termite subfamily  
410 Macrotermitinae (Mueller et al. 2001; Aanen et al. 2002; Caldera et al. 2009). However, fungus  
411 cultivation is much more widespread and is carried out by siricid woodwasps, cerambycid  
412 beetles and plant-ants (Douglas 2009; Defosse et al. 2009). All the 210 plus attine ant species  
413 rely on cultivation of fungi of the tribe Leucocoprineae as their main food. The majority of  
414 cultivated fungi belong to two genera, *Leucoagaricus* and *Leucocoprinus*. Basal attine species  
415 cultivate fungi on dead-plant matter and caterpillar frass, while derived lineages collect fresh  
416 leaves and flowers as substrate for fungi, suggesting that the development of a new mode of  
417 fungi cultivation is an innovation that has allowed ant species diversification. The fungi  
418 cultivated by derived attine ants are also highly specialized on the mutualism. They have  
419 evolved a specialised structure rich in nutrients, the gongylidia, that serves as ant food. This  
420 structure is formed by densely packed clusters of hyphae that are easily harvested by the ants  
421 (Mueller et al. 2001). The mutualism involving fungus-growing termites is analogous with the  
422 attine-fungus association, as it allows termites to make use of a diversity of vegetal food  
423 sources such as wood, dry grass, and leaf litter. This mutualism is restricted to a single termite  
424 subfamily, Macrotermitinae, which is associated with fungi of the genus *Termitomyces*. In  
425 contrast with fungal symbionts of the attine ants that are usually propagated clonally and  
426 vertically by dispersing queens, *Termitomyces* reproduce sexually and are most often  
427 horizontally transmitted (Aanen et al. 2002).

428         A particular mutualism involves sap-feeding insects and intracellular bacteria. This  
429 system is one of the most specialized digestive mutualisms in terms of partner integration.  
430 Intracellular bacteria reside in specialized host cells (bacteriocytes) that constitute an organ  
431 called the bacteriome and are vertically transmitted - the symbionts migrate to the ovaries and  
432 enter the germ cells. This specialised mutualism is restricted to the monophyletic clade  
433 constituted by members of the two hemipteran suborders, Sternorrhyncha (psyllids,  
434 whiteflies, aphids, and mealybugs) and Auchenorrhyncha (sharpshooters)]. This clade

435 comprises over 10,000 representatives. Within this clade different insect lineages are  
436 associated with different bacterium genera: *Buchnera* (aphids), *Carsonella* (psyllids), *Portiera*  
437 (whiteflies), *Tremblaya* (Mealybugs) and *Baumannia* (sharpshooters). Plant sap is an  
438 unbalanced diet for insects as it is rich in carbohydrates relative to free amino acids and it is  
439 deficient in essential amino acids. The symbiotic bacteria convert non-essential amino acids  
440 into essential ones, allowing sap-feeding insects to circumvent their intrinsic nutritional  
441 limitation. In contrast to other sap-feeders that feed on sap circulating in the phloem,  
442 sharpshooters feed on xylem. The bacteriome in *Homalodisca* sharpshooters is bilobed, with  
443 one portion hosting *Baumannia* bacteria that can synthesize vitamins and cofactors, and the  
444 other portion hosting *Sulcia* bacteria that can synthesize essential amino acids (Baumann 2005;  
445 Moya et al. 2008; Douglas 2009).

446

### 447 3.3. Pollination mutualism

448 Gamete transfer in seed plants is strongly constrained by the sessile nature of their  
449 predominant life stage, the sporophyte. Therefore, seed plants must rely on animal and abiotic  
450 pollen vectors (wind and sometimes water) to achieve cross fertilisation. Several groups of  
451 insects including bees, wasps, beetles, dipterans, lepidopterans and thrips, are involved in non-  
452 symbiotic and symbiotic pollination mutualisms. Non-symbiotic mutualisms include cases of  
453 diffuse reciprocal adaptation, where independent plant lineages share convergent floral  
454 attributes called pollination syndromes associated with pollinator guilds that present particular  
455 sensory biases and particular pollen transfer attributes (Rech et al. 2014; Dellinger 2020). In  
456 those mutualisms, insects benefit from food (nectar, pollen, oil), shelter (mainly for mate  
457 encounter) as well as other resources used for nest construction or their reproductive  
458 behaviour (oil, resin and floral scent) (Agostini et al. 2014).

459 Symbiotic pollination mutualisms are associations in which the plant provide breeding  
460 sites for their pollinators. Most of them qualify as nursery pollination mutualism. Larvae of  
461 pollinators feed on ovules/seeds or other floral parts (Sakai 2002; Dufaÿ and Anstett 2003;  
462 Hembry and Althoff 2016). Among these, brood-site pollination mutualism (*sensu* Hembry and  
463 Althoff 2016, *i.e.* association where pollinators feed on developing plant ovules) has evolved  
464 independently in several groups of plants and insects (Dufaÿ and Anstett 2003). It is often  
465 obligate (*e.g.*, fig trees – fig wasps, yucca – yucca moths and leafflowers – leafflower moths,  
466 senita cactus – senita moths), but can also be facultative when the plant is pollinated both by  
467 insects whose offspring develop feeding on seeds and by generalist insect pollinators that visit  
468 flowers to feed on pollen and/or nectar; *e.g.* *Silene* – *Hadena/Perizoma*, *Lithophragma* – *Greya*  
469 and globeflower – globeflower flies (Kephart et al. 2006; Hembry and Althoff 2016). In these  
470 cases, the relationship with the seed eating taxa varies locally from mutualism to antagonism  
471 depending on the local pollination efficiency of generalist pollinators.

472 In brood-site pollination mutualisms the plants are selected to attract pollinators that  
473 will feed on developing seeds. We propose that the pollinating insects are selected to be host  
474 specialists so that they collect pollen from plants of and oviposit in plants of the same species,  
475 thus ensuring successful seed to feed their offspring. In most cases, the plants are selected to  
476 try to kill the developing pollinator larvae as the pollinator offspring will not carry pollen from  
477 their natal plant, and the system remains stable because this selection has not succeeded  
478 (Addicott et al. 1990). For instance, in the fig tree – fig wasp system, about 50% of the plant  
479 ovules are consumed by the pollinator larvae (Kjellberg et al. 2005). However, breeding  
480 pollinators does not come at a cost in *Ficus* as most consumed ovules produce female  
481 pollinating wasps, that will carry pollen from their natal fig (Jousselin and Kjellberg 2001;  
482 Kjellberg et al. 2001; Jousselin et al. 2003a).

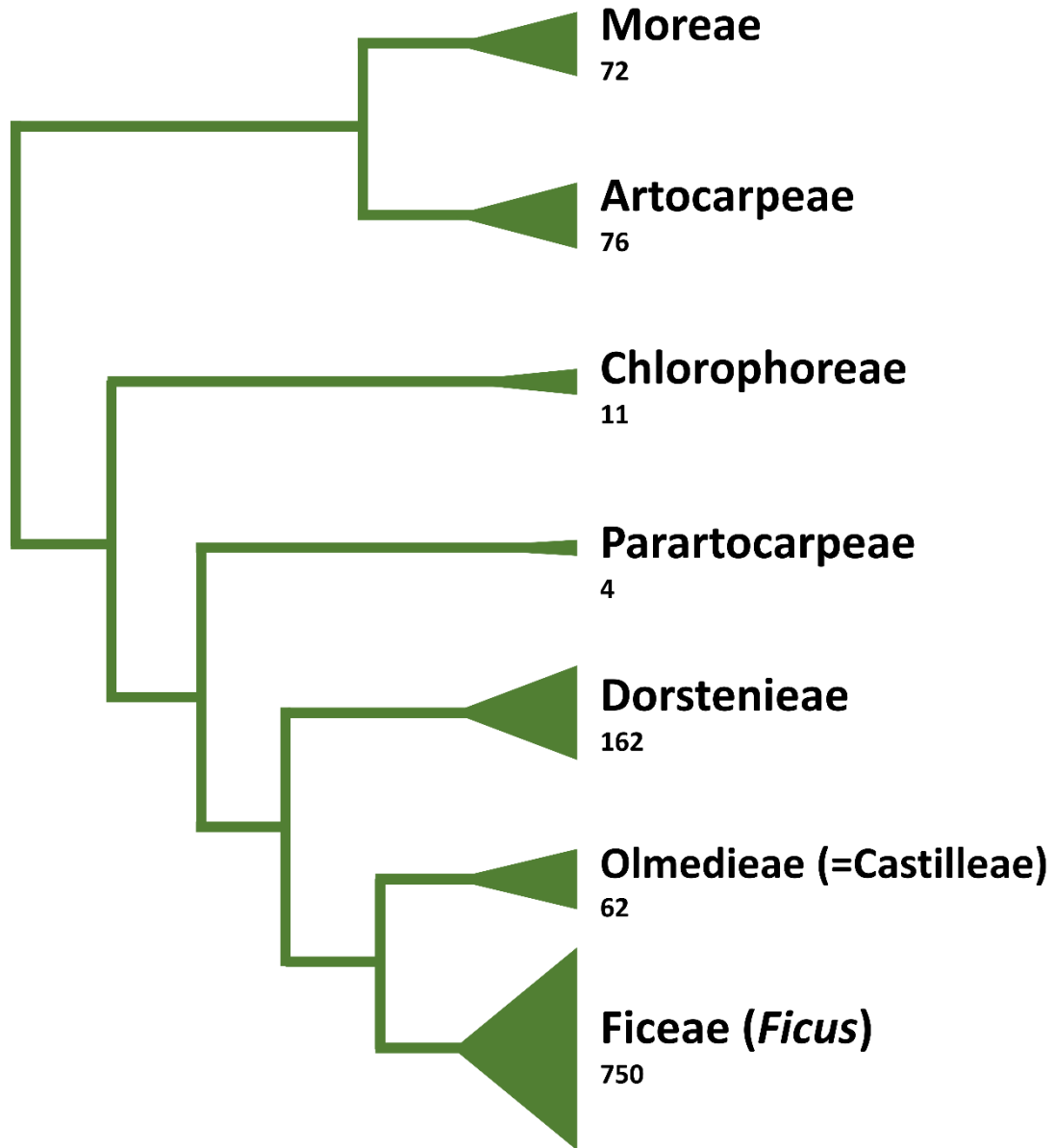
483

484 **4. Brood-site pollination mutualism in fig trees: did it boost species and lifeform**  
485 **diversifications?**

486 Fifty years ago, William Ramirez and Jacob Galil/Dan Eisikowitch independently discovered  
487 active pollination by some fig wasps (Hymenoptera: Agaonidae). Female actively pollinating  
488 wasps collect pollen into specialised pockets before leaving their natal figs, and later remove  
489 pollen from the pockets and deposit it on the stigmas in another fig containing receptive  
490 pistillate flowers (Galil and Eisikowitch 1969; Ramírez 1969). These observations marked the  
491 modern evolutionary studies of fig trees (*Ficus* spp) and their associated animals, making it a  
492 model system to investigate the evolution of mutualisms (Borges et al. 2018). The fig trees  
493 belong to Moraceae family, which includes ca. 39 genera. With a pantropical/subtropical  
494 distribution, *Ficus* encompasses approximately 70% of all 1,100 described Moraceae species  
495 (Zerega et al. 2005; Gardner et al. 2017). Wind pollination is ancestral in Moraceae (Gardner et  
496 al. *Taxon, accepted*). Nevertheless, representatives of a series of genera are involved in  
497 nursery pollination mutualisms. They include representatives of genera *Artocarpus* (gall  
498 midges), *Antiaropsis/Castilla* (thrips), *Dorstenia* (flies) and *Mesogyne* (bees), and *Ficus* (chalcid  
499 wasps) (Sakai et al. 2000; Sakai 2001; Zerega et al. 2004; Olotu et al. 2011; Araújo et al. 2017).  
500 Among these, only in *Ficus* do the wasp larvae feed on developing plant ovules. This is  
501 probably the sole Moraceae genus in which fertilisation of female flowers generally directly  
502 benefits the wasp's offspring. Fig trees stand out for their generalised obligate-specialized  
503 pollination mutualism and their taxonomic diversity. *Ficus* is about seven times more speciose  
504 than the second moracean genus in number of species (*i.e.*, *Dorstenia* with 113 spp, which is  
505 followed by *Artocarpus* with ca. 70 spp) (Figure 3). *Ficus* is, in addition, functionally diversified.  
506 The genus presents diverse life-forms (freestanding and hemi-epiphytic trees, shrubs and  
507 climbers), breeding systems (monoecious and gynodioecious), pollination mode (active and  
508 passive), seed dispersion syndromes (birds and volant and non-volant mammals, as well as



509 reptiles and fishes)] that allow them to occupy a range of (micro)habitats (Shanahan et al.  
510 2001; Joussein et al. 2003b; Harrison 2005; Coelho et al. 2014).



511

512 **Figure 3.** Phylogeny of Moraceae tribes according to Gardner et al. (*Taxon, accepted*). Terminal  
513 widths are proportional to the squared root (number of species).

514

515 Each *Ficus* species is pollinated by one or some species of host species-specific agaonid

516 wasp (Ramírez 1970; Kjellberg et al. 2005). Approximately half of *Ficus* species are

517 monoecious, bearing urn shaped inflorescences (also called syconium or fig) that enclose both  
518 pistillate and staminate flowers. The other species are structurally gynodioecious (see next  
519 paragraph), but functionally dioecious (Berg 1989). Fertilised pollen-loaded pollinating female  
520 wasps are attracted by a blend of volatile substances released by the receptive figs (Grison-  
521 Pigé et al. 2002; Souza et al. 2015). The pollinating wasp enters the fig through the ostiole, a  
522 pore closed by floral involucral bracts and pollinates (actively or passively) the pistillate  
523 flowers. In some of them, the wasp inserts its ovipositor through the style to lay one egg  
524 precisely between the nucellus and the inner integument (Jansen-Gonzalez et al. 2012).  
525 Ovaries that receive a wasp egg turn into galls where the pollinator larvae will develop feeding  
526 on endosperm, and those that have been pollinated and have escaped wasp oviposition will  
527 produce seeds. Approximately four to eight weeks later (up to 9 months later for the pollinator  
528 of the common fig!), depending on the temperature (Pereira et al. 2007), the offspring  
529 complete their development. The males emerge first, locate and copulate with the females still  
530 enclosed in their natal galls. Then, the fertilised females emerge from their galls, actively  
531 collect pollen from staminate flowers at anthesis (in passively pollinated species the pollen  
532 from dehiscing anthers adheres spontaneously to the wasp's body) and leave the natal fig to  
533 search for another tree bearing receptive figs (Frank 1984). Subsequently the figs ripen and  
534 become attractive to a diversity of vertebrate frugivores that can act as seed dispersers  
535 (Shanahan et al. 2001). The figs that are not consumed by frugivores fall to the ground and  
536 turn into a resource for a diverse range of more or less specialised animals, mainly insects  
537 (Palmieri and Pereira 2018).

538 Gynodioecious *Ficus* species present two types of plants – one that produces figs  
539 containing only pistillate flower (*i.e.*, 'female' tree). The pollinator attracted to a fig in a female  
540 tree pollinates but cannot lay eggs, as the styles are too long for the wasp ovipositor to reach  
541 the ovary of these flowers (Figure 1B). Thus, the pistillate flowers of female trees are  
542 pollinated by deceit, as the pollinator is attracted by a deceptive resource signalling. Pollen

543 production, on the other hand, occurs in the functionally male trees, which have figs that  
544 enclose both pistillate and staminate flowers (Figure 1B). Wasp can oviposit in ‘male’ trees as  
545 the pistillate flowers have short styles. ‘Male’ trees do not produce seeds because either the  
546 wasps deposit pollen precisely on the stigmas of the flowers into which they oviposit (active  
547 pollination) or because of poor germination of pollen in ‘male’ trees (Jousselin and Kjellberg  
548 2001). However, pollen is dispersed by the pollinator offspring in the same way of in  
549 monoecious *Ficus* species (Figure 1A; Weiblen 2002).

550         The ancestral mode of pollination of Moraceae and their closest relatives Cannabaceae  
551 and Urticaceae is wind pollination involving an explosive mechanism of pollen dispersal with  
552 stamens inflexed in the flower bud that, when they distend cause a rapid anther movement,  
553 releasing large amounts of pollen (Pedersoli et al. 2019). This pollen release mechanism can  
554 achieve an exceptional initial velocity of Mach 0.7 (232 m.s<sup>-1</sup>) in mulberry plants (Taylor et al.  
555 2006). This mechanism may be an adaptation to circumvent the wind limitation in the tropical  
556 forest understorey (Bawa and Crisp 1980). In this context, the fig – fig wasp mutualism brings  
557 out a singular innovation in pollen dispersion, by combining initial wind dispersal of the wasps  
558 followed by wasp chemotaxis allowing cross-pollination at amazingly low population densities  
559 (Ware and Compton 1994). This is achieved despite the dispersing wasps only surviving 24-48  
560 hours outside figs (Kjellberg et al. 1988; Jevanandam et al. 2013). Despite this temporal  
561 constraint, agaonid wasps can regularly disperse pollen over remarkably long distances  
562 comparatively to usual insect pollinated plants. It is demonstrated that average pollination  
563 distances by agaonid wasps within a fig population may reach several tens of kilometres  
564 (Compton et al. 1988; Nason et al. 1996; Nazareno and Carvalho 2009; Ahmed et al. 2009).  
565 Long-distance pollen dispersal, at least in monoecious *Ficus* species, is mediated by wind.  
566 Collection of aerial plankton shows that wasps pollinating monoecious *Ficus* species are  
567 dispersed by the wind above the forest canopy over long distances (Compton et al. 2000;  
568 Harrison and Rasplus 2006). When a wasp detects the plume of receptive fig scent released

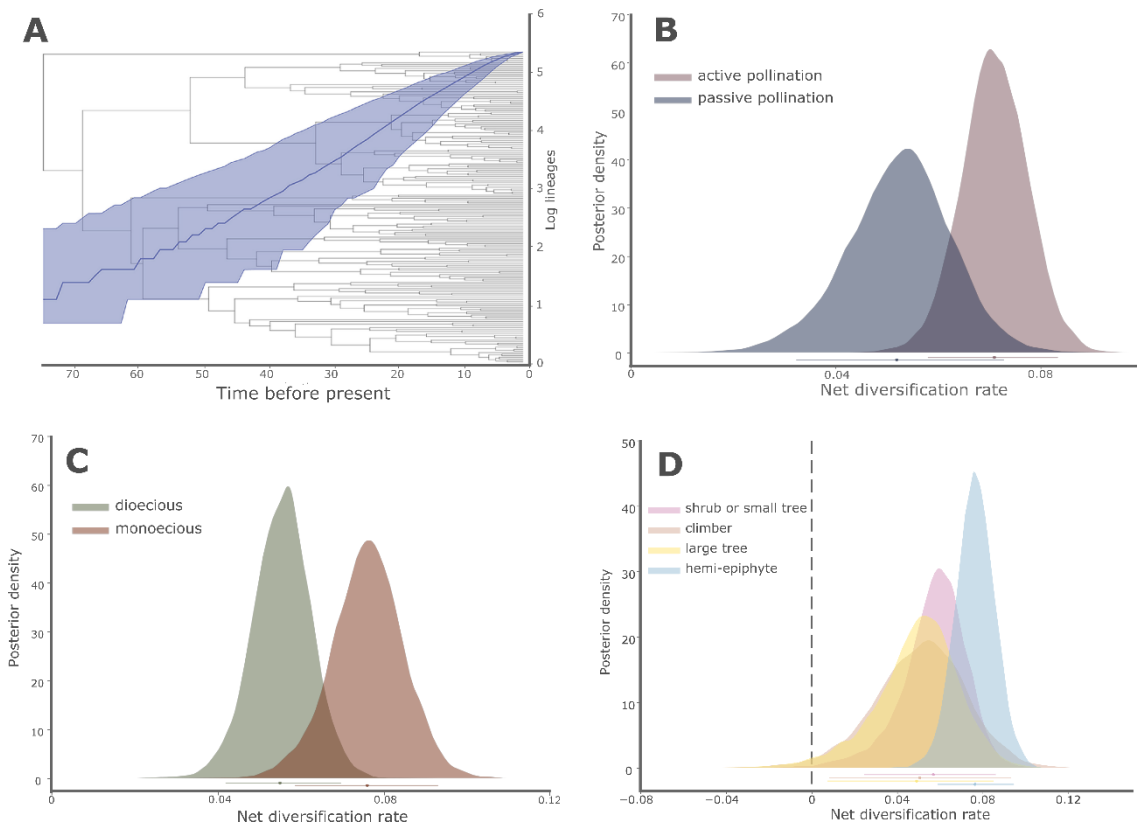
569 from a receptive tree, it moves down, out of the main wind current and then flies upwind to  
570 reach the tree (Ware and Compton 1994).

571 The pollination mutualism seems to have opened other evolutionary opportunities in  
572 *Ficus*, such as the active pollination that increases the efficiency of pollen transfer from the  
573 plant to the insect. As a consequence, the plants can invest in other aspects of male function  
574 such as producing more male inflorescences or in the case of *Ficus*, breeding more pollinator  
575 offspring (Sakai 2002; Pellmyr et al. 2020). Active pollination is present in two thirds of the  
576 *Ficus* species. Agaonid wasps that actively pollinate fig trees transport the pollen clumped into  
577 body containers (*i.e.*, pollen pockets), which apparently shares functional analogies with the  
578 cohesive pollen dispersion in pollinia observed in Asclepiadaceae and Orchidaceae (Ramírez  
579 2007). It is postulated that pollen clumping can improve the pollination success as it decreases  
580 the pollen waste during transport, and increases its probability of being deposited on a  
581 conspecific stigma (Johnson and Edwards 2000). However, this is not ever true in *Ficus* as  
582 actively pollinating wasps may carry limiting quantities of pollen (Kjellberg et al. 2014). Indeed,  
583 in the fig system as in other actively pollinated systems, the wasps, not the tree decide how  
584 much pollen they load into their pockets. In general, canopy fig tree species produce  
585 synchronous crops within trees but asynchronous among trees, making fig crops available at  
586 population level year-round (Milton et al. 1982; Windsor et al. 1989; Figueiredo and Sazima  
587 1997; Pereira et al. 2007). Trees are selected to produce synchronous crops for wasp attraction  
588 as in some *Ficus* species producing small figs consumed by local animals (*e.g.* *F. guianensis*, *F.*  
589 *caulocarpa*, *F. subpisocarpa*), fig receptivity is synchronised within crop, wasp emergence is  
590 synchronised within crop, but fig ripening is scattered over a longer period of time (Chiang et  
591 al. 2018). Synchronous fig crops lead to year-round fruiting because of strong within fig  
592 protogyny associated with the production of synchronised crops. If receptive figs are  
593 particularly abundant at one period of the year, then there is selection on trees to produce  
594 crops that will pollinate these figs, *i.e.* that will release wasps at that time. These pollinating

595 figs were receptive several weeks earlier, leading to the selection of figs producing crops that  
596 would pollinate them. Hence, there is frequency dependent selection favouring year-round  
597 production of crops due to the strong protogyny of figs and long wasp development time.  
598 Year-round fig production makes figs keystone resources for the year round survival of highly  
599 diversified frugivorous vertebrates, allowing different seed-dispersion mutualism to arise  
600 (Shanahan et al. 2001). Moreover, monoecious fig trees, which are adapted to long-distance  
601 pollen dispersion (Compton et al. 2000; Harrison 2003), usually produce huge fig crops that  
602 consequently result in a massive production of small seeds. Thus, capacity to be pollinated at  
603 very low densities coupled with the massive seed dispersion by canopy frugivores allow fig  
604 trees to colonise highly transient habitats and unlikely (in terms of frequency) habitats.

605 We postulate that brood-site pollination mutualism has boosted species diversification  
606 in *Ficus*, by opening new adaptive opportunities. As a matter of comparison the sister group of  
607 *Ficus* (*i.e.*, tribe Castilleae, represented by 10 genera and approximately 60 species) is nearly 13  
608 times less speciose than *Ficus* (Gardner et al. 2017). Pollination biology in Castilleae is not well  
609 known, but pollination by thrips is reported for *Antiaropsis decipiens* and *Castilla elastica*, and  
610 potential bee/vespid pollination for *Mesogyne insignis* (Sakai 2001; Zerega et al. 2004; Olotu et  
611 al. 2011). These three genera have 1-3 species each, and there is no evidence that those insect  
612 pollinations parallel the fig – fig wasp mutualism in terms of pollination efficiency. Patterns of  
613 diversification/extinctions in *Ficus* lineages support a diversification hypothesis based on new  
614 ecological opportunities. Bruun-Lund et al. (2018), based on a dated and comprehensive  
615 phylogenetic hypothesis, demonstrated that fig trees follow the evolutionary model of  
616 ‘museum of diversity’, with gradual accumulation of species over time coupled with very low  
617 extinction rates (Figure 4A). They showed that key innovations directly or indirectly associated  
618 with the reproductive biology of fig trees correlated with higher diversification rates in clades  
619 where those features were present. For instance, actively pollinated *Ficus* species diversify  
620 faster and present lower extinction rates than passively pollinated ones (Figure 4B). Similarly,

621 monoecious and hemi-epiphytic species diversified faster than gynodioecious and other life  
622 forms (Figures 4C-D).



623

624 **Figure 4.** Diversification rate in *Ficus* (Bruun-Lund et al. 2018). A: lineage-through-time plot  
625 depicted on the dated phylogenetic tree from Cruaud et al. (2012). B-D: net diversification rate  
626 in *Ficus* lineages, according to pollination modes, reproductive systems and lifeforms. Bruun-  
627 Lund et al. (2018) was published under the terms of the Creative Commons Attribution-  
628 NonCommercial-No Derivatives License (CC BY NC ND).

629

630 Bruun-Lund et al. (2018) hypothesized the following scenario to describe the success of  
631 fig trees. As the fig – fig wasp mutualism emerged by the late Cretaceous (75-90 Ma) (Cruaud  
632 et al. 2012), fig trees expanded into vacant niches left by the putative massive plant extinctions  
633 at the Cretaceous–Paleogene boundary (~65 Ma) (Wilf and Johnson 2004), thanks to their  
634 capacity to colonize unlikely suitable habitats, and to cross-pollinate at low population density.  
635 Then, *Ficus* would have diversified at a constant rate, as fig trees have several attributes of  
636 pioneer plants, such as fast growth, small seeds, high fecundity and flexible rooting habits.

637 Those features make the fig – fig wasp association very robust and successful. Indeed, fossil  
638 evidences support that the fig – fig wasp mutualism is stable along its evolutionary history.  
639 Fossil specimens of a pollinating fig wasp from a limestone bed in England (~34 Ma), and  
640 pollinating and non-pollinating fig wasps from Dominican amber (10-20 Ma), as well as pollen  
641 morphology, display the same set of associated anatomical characters as modern species  
642 (Peñalver et al. 2006; Compton et al. 2010; Farache et al. 2016).

643 A major challenge in the study of mutualisms is how to go beyond particular examples  
644 and biological models and draw generalisations. We may list some challenges:  
645 “Most fig papers start with the statement that the fig-fig wasp mutualism is a model system. Is  
646 it a model system of anything else than figs?” Richard T. Corlett  
647 “What will matter a lot to whether the paper is accepted (...) is how you frame the results. (...)”  
648 They absolutely have to convincingly inform broader concepts in ecology, evolution and/or  
649 behavior that aren't system specific. That's always a little hard with figs.” Judith L. Bronstein  
650 “Fig wasps are wonderful” E. Allen Herre

651 What we hope to have shown in this chapter is that figs and fig wasps and more  
652 generally brood-site pollination mutualisms, and case studies of mutualisms have something to  
653 tell us, beyond fascinating stories. We have tried to approach generalisation. This is a first  
654 sketch, and as such, we have made a number of provocative statements. The aim was to  
655 suggest alternative perspectives, to stir reflexion. A very important point is that as long as we  
656 do not know enough about a biological model it is very easy to make false inferences. To  
657 understand mutualisms we need strong biological data on the different systems.

658

659

660 **Conclusion**

661 The view that mutualism represents reciprocal exploitation leads to (1) a false assumption of  
662 an inherent conflict of interest between interacting parties, and (2) that the evolution of  
663 strategies that limit non-cooperative behaviours, such as sanctions, is condition for mutualism  
664 stability. However, phylogenetic evidences from a range of mutualistic systems point out that  
665 traits claimed as sanctioning acts were preexisting adaptations. Indeed, mutualisms are stable  
666 at macroevolutionary timescales, and rarely evolve to parasitic associations. In general,  
667 mutualisms involve members of distant taxonomic groups that combines a structural (goods  
668 provided by a long-lived partner) and a service component supplied by a short-lived partner.  
669 Thus, mutualism is a source evolutionary innovations that expand the partners ecological  
670 capacities. The brood-site pollination in the fig tree – fig wasp mutualism resulted in a set of  
671 innovations, such as efficient long-distance pollination and year-round massive seed rain, that  
672 opened new ecological niches and allowed a remarkable net diversification at a  
673 macroevolutionary timescale.

674

675 **Key points:**

- 676 • Cooperation theory
- 677 • Mechanisms to limit non-cooperative behaviour
- 678 • Mutualism as source of evolutionary innovations

679

680 **Questions**

- 681 • Do the architecture “goods vs. services” occur in other examples of mutualisms?



- 682       • In addition to *Ficus*, are lineages involved in mutualistic associations in general more  
683           diverse than close-related lineages not engaged in mutualisms?  
684       • What should be the insights resulted from expanding mutualism theory to non-pure  
685           biological interactions (*i.e.* culture vs. animals)?

686

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