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Chapter 13

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

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Abstract:

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

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

1. Introduction

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

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

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

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

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

enabling plants to exploit mineral soils. The association between plants and nitrogen-fixing bacteria is another example of mutualism allowing ecological innovation. The bacteria metabolise inert N₂ present in the atmosphere into the utilizable form ammonia (NH₃) through the action of nitrogenases. This mutualism is present in a diversity of plant lineages including ferns, gymnosperms, mono- and eudicots. The plants host the endophytic bacteria in their roots. Among them, the root nodule symbiosis is particularly sophisticated and complex. The root nodules house high bacteria densities in a structure that provides an anaerobic microenvironment favourable for nitrogenase activity (Markmann and Parniske 2009; Soto et al. 2009). The mutualisms exemplified above are just a few examples of known mutualisms that represent biological innovations central to the colonisation of earth and the functioning of extant ecosystems. There are many other examples, as for instance lichens associating fungi and algae/cyanobacteria (Nascimbene and Nimis 2006), or sessile/slow-moving marine animals and algae/cyanobacteria (Venn et al. 2008).

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

Box 1. Pollination by deceit in fig trees.

An intriguing example is pollination by deceit in gynodioecious *Ficus* species (Kjellberg et al. 1987; Figure 1). Functionally “male” trees produce pollinating wasps (pollen vectors) and 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 10th 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.

2. Theoretical remarks

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

Lists of mechanisms limiting non-cooperative behaviour or partner overexploitation and derived from intraspecific cooperation theory have been proposed (Herre et al. 1999; Yu 2001; Sachs et al. 2004; Doebeli and Hauert 2005; West et al. 2007; Bergmüller et al. 2007). While cooperation models have stimulated theoretical studies on mutualism, they do not find consistent empirical support to explain the stability of a range of mutualistic associations. For example, models based on retaliation (sanctions or refusing future interactions) are probably only of importance for animals with developed cognition capacity, as they require the ability to remember past interactions and behave accordingly. Sanction has been invoked to explain cooperation reinforcement in some mutualistic association, such as legume plants – N₂-fixing bacteria, fig trees– pollinating fig wasps and yucca – pollinating yucca moths (Pellmyr and Huth 1994; Kiers et al. 2006; Kiers and Denison 2008; Jandér and Herre 2010; Leigh Jr 2010; Jandér et al. 2012). In these examples, when the *Rhizobium* bacteria do not provide nitrogen, or when the fig wasps/yucca moths do not pollinate, the non-cooperating partner pays a cost, as the O₂

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

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

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

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

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

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

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

3. Some examples of mutualisms involving insects and plants

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

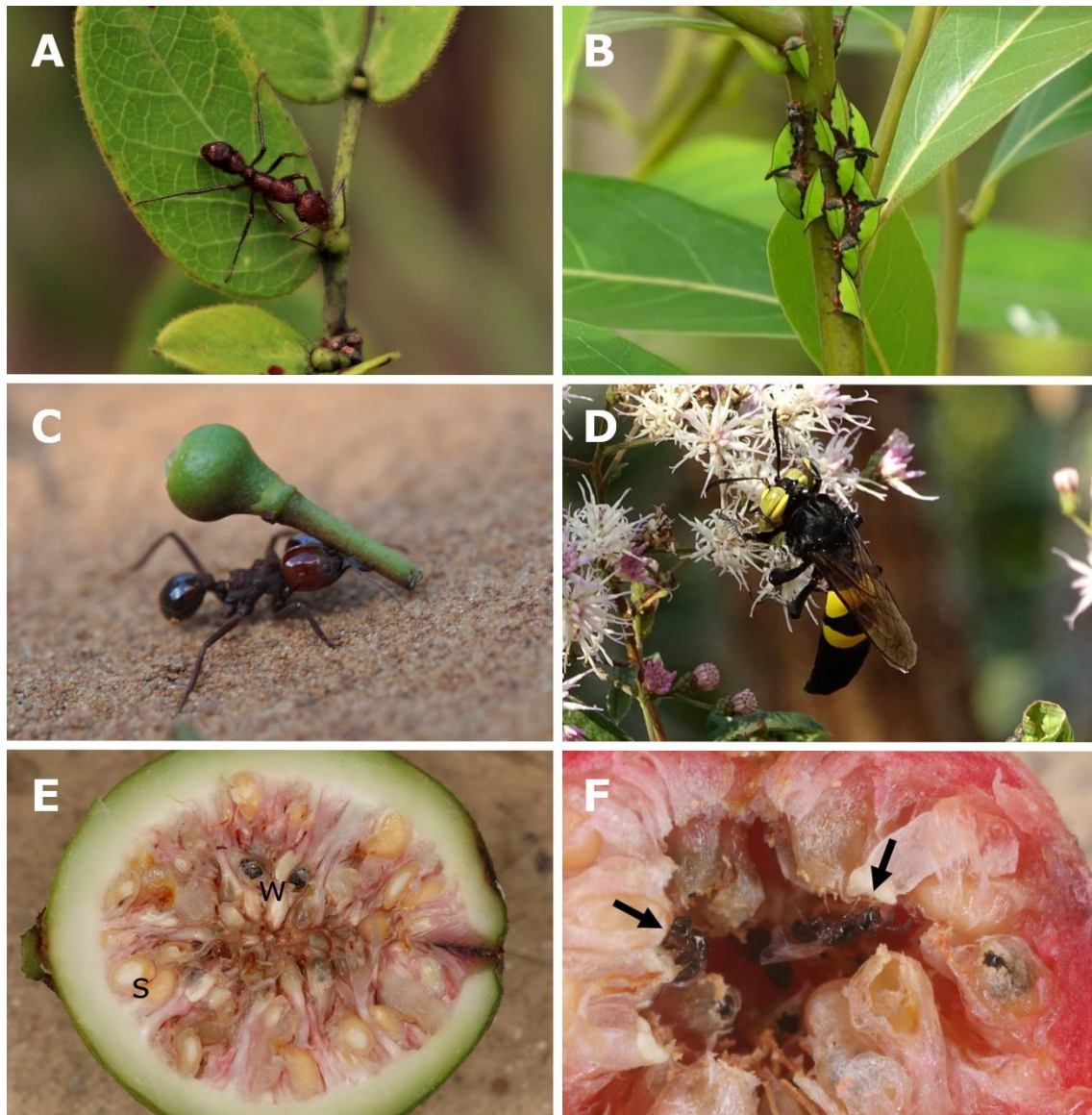


Figure 2. Examples of mutualisms involving insects and plants. Protection mutualism: A – *Ectatomma tuberculatum* ant probing an extrafloral nectary in Leguminosae. Digestive mutualisms: B – *Aconophora* sp. (Auchenorrhyncha: Membracidae), associated with *Baumannia* (γ -proteobacteria) and *Sulcia*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

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 – Hadenia/Perizoma, Lithophragma – Greya 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

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

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

3.2. Digestive mutualism

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

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

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

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

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

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

3.3. Pollination mutualism

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

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

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

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

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

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

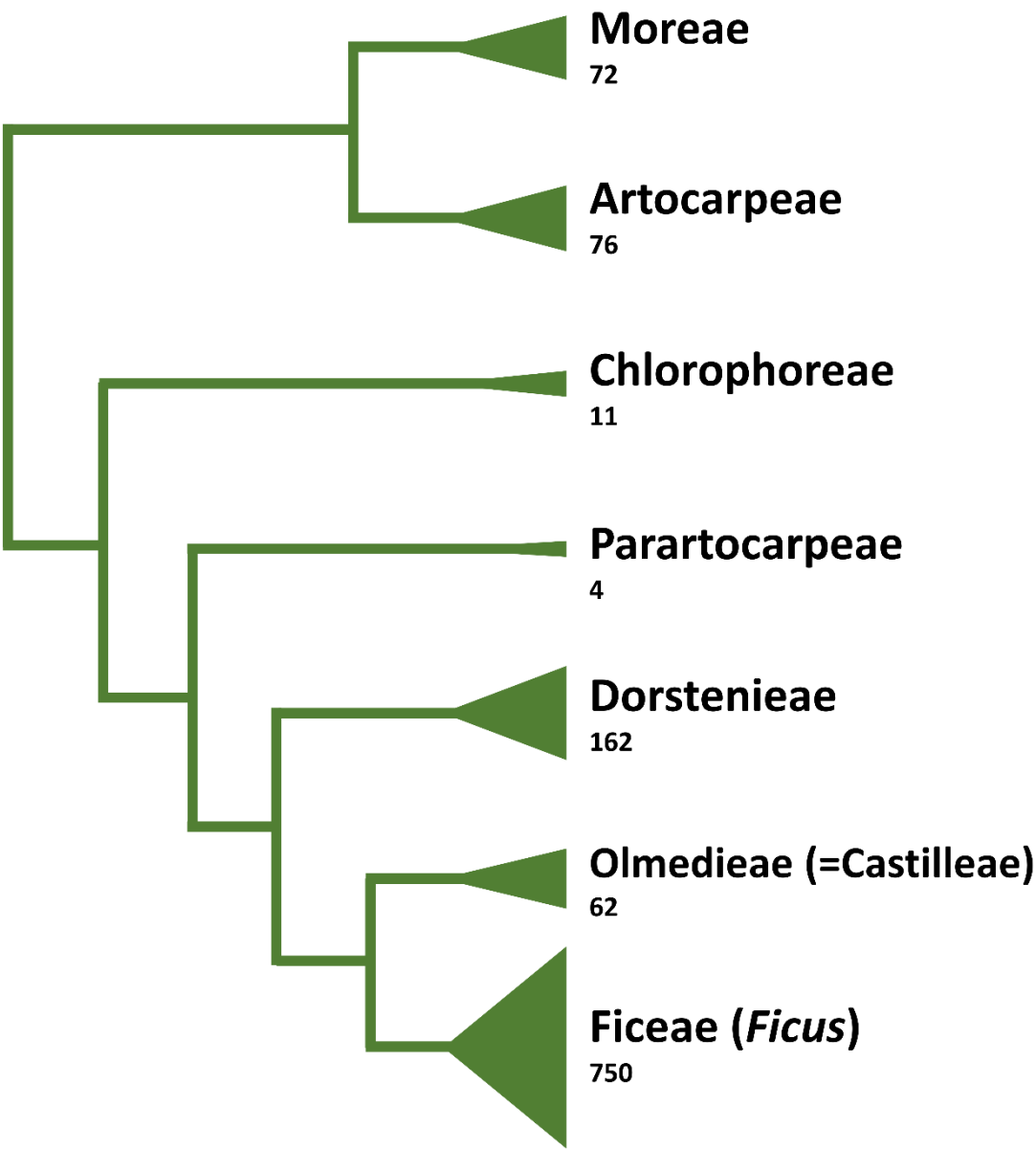


Figure 3. Phylogeny of Moraceae tribes according to Gardner et al. (*Taxon, accepted*). Terminal widths are proportional to the squared root (number of species).

Each *Ficus* species is pollinated by one or some species of host species-specific agaonid wasp (Ramírez 1970; Kjellberg et al. 2005). Approximately half of *Ficus* species are

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

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

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

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

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

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

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

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

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

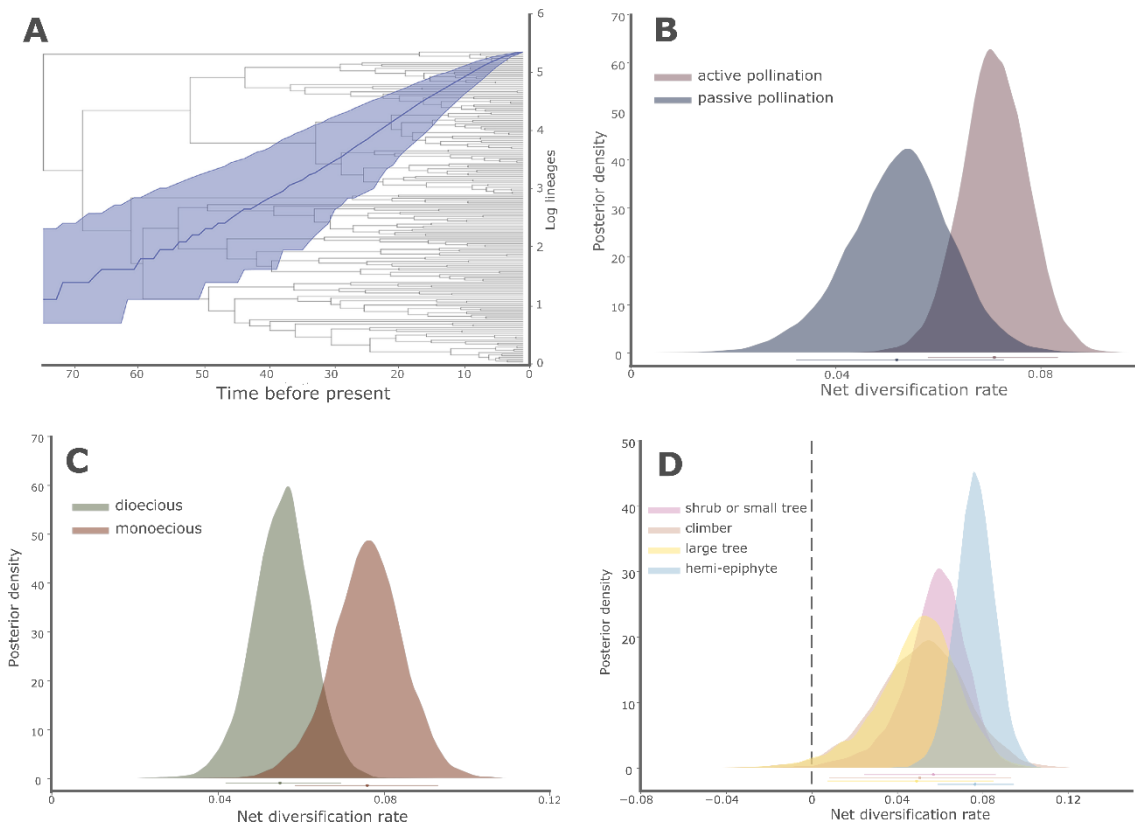


Figure 4. Diversification rate in *Ficus* (Bruun-Lund et al. 2018). A: lineage-through-time plot depicted on the dated phylogenetic tree from Cruaud et al. (2012). B-D: net diversification rate in *Ficus* lineages, according to pollination modes, reproductive systems and lifeforms. Bruun-Lund et al.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

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

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

A major challenge in the study of mutualisms is how to go beyond particular examples and biological models and draw generalisations. We may list some challenges:

“Most fig papers start with the statement that the fig-fig wasp mutualism is a model system. Is it a model system of anything else than figs?” Richard T. Corlett

“What will matter a lot to whether the paper is accepted (...) is how you frame the results. (...) They absolutely have to convincingly inform broader concepts in ecology, evolution and/or behavior that aren't system specific. That's always a little hard with figs.” Judith L. Bronstein

“Fig wasps are wonderful” E. Allen Herre

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

Conclusion

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

Key points:

- Cooperation theory
- Mechanisms to limit non-cooperative behaviour
- Mutualism as source of evolutionary innovations

Questions

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

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

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