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Biology of Fungi and Their Bacterial Endosymbionts

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Running title: Endosymbiotic bacteria of fungi

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Mycoavidus cysteinexigens; *Rhizopus microsporus*

36 **Abstract**

37 Heritable symbioses, in which endosymbiotic bacteria (EB) are transmitted vertically between
38 host generations, are an important source of evolutionary novelties. A primary example of such
39 symbioses is the eukaryotic cell with its EB-derived organelles. Recent discoveries suggest that
40 endosymbiosis-related innovations can be also found in associations formed by early divergent
41 fungi in the phylum Mucoromycota with heritable EB from two classes, Betaproteobacteria and
42 Mollicutes. These symbioses exemplify novel types of host-symbiont interactions. Studies of
43 these partnerships fuel theoretical models describing mechanisms that stabilize heritable
44 symbioses, control the rate of molecular evolution, and enable the establishment of mutualisms.
45 Lastly, by altering host phenotypes and metabolism, these associations represent an important
46 instrument for probing the basic biology of the Mucoromycota hosts, which remain one of the
47 least explored filamentous fungi.

49 **1. Introduction**

50 Fungi are increasingly appreciated for their ability to form intimate associations with bacteria
51 (31, 89). Among them, the symbioses of early divergent fungi in the phylum Mucoromycota
52 with an array of heritable endosymbiotic bacteria (EB) from two classes, Betaproteobacteria and
53 Mollicutes, stand out as the most highly co-evolved and ancient. The clade of Mucoromycota
54 includes three subphyla, Mucoromycotina, Mortierellomycotina, and Glomeromycotina (115).
55 Most Mucoromycota engage in plant-related lifestyles of decomposers of plant debris, plant
56 mutualists, and plant pathogens (115). Interactions with animals are uncommon in this group of
57 fungi.

58 Partnerships with bacteria formed by Mucoromycota have diverse fitness outcomes,
59 involve transfer of various goods and services, and represent a range of degrees of co-evolution.
60 In this review, we will focus on four very distinct symbioses partnering arbuscular mycorrhizal
61 fungi (AMF, subphylum Glomeromycotina) with ‘*Candidatus Glomeribacter gigasporarum*’
62 (*CaGg*, Betaproteobacteria, **Fig 1**) and ‘*Candidatus Moeniiplasma glomeromycotorum*’ (*CaMg*,
63 Mollicutes, **Fig 2**) as well as on associations of *Rhizopus microsporus* (*Rm*, subphylum
64 Mucoromycotina) with *Burkholderia* EB (Betaproteobacteria, **Fig 1**), and *Mortierella elongata*
65 (*Me*, subphylum Mortierellomycotina) with *Mycoavidus cysteinexigens* (*Mc*, Betaproteobacteria,
66 **Fig 1**). Despite their marked differences, these Mucoromycota-EB associations provide

67 important insights into the host-symbiont biology. Studies of these symbioses inform
68 evolutionary models describing the mechanisms that stabilize heritable symbioses, control the
69 rate of molecular evolution, and lead to the establishment of mutualisms. In addition, by altering
70 host phenotypes and metabolism, these partnerships are a valuable source of information about
71 the biology of Mucoromycota, which remain one of the least explored groups of filamentous
72 fungi.

73 Heritable symbioses in which EB are transmitted from one host generation to the next can
74 range from antagonisms to mutualisms. Importantly, strictly vertically transmitted symbionts
75 that lower host fitness are unlikely to persist in a host population (28, 60). Evolutionary stability
76 of such antagonistic symbioses requires that, in addition to passaging from parents to offspring,
77 symbionts engage in horizontal transmission between hosts (28, 60). Alternatively, harmful
78 symbionts can be maintained stably if they deliver occasional benefits to the host, forming a
79 conditional mutualism (40, 61, 62, 105).

80 Mutualisms are reciprocal exploitations that nonetheless provide net benefits to each
81 partner (42). This definition emphasizes an inherent vulnerability of mutualisms to instabilities
82 and breakdowns, which stem from conflicting interests of the interacting partners. Vertical
83 transmission is a powerful mechanism that stabilizes mutualisms over evolutionary time (1, 4,
84 18, 21, 27, 104, 130). This stabilizing role is related to the fact that heritability of symbionts
85 aligns partner reproductive interests and facilitates reciprocal selection. While coupling of
86 reproductive efforts maximizes fitness of the partners, it does not eliminate conflicts among the
87 symbionts. Such conflicts are a potential source of instabilities in heritable mutualisms. They
88 intensify when symbiont populations are genetically diverse due to symbiont mixing, which can
89 lead to the emergence of rivaling strategies for the utilization of host resources (30).

90 In established mutualisms, several tactics are possible to control symbiont mixing,
91 including uniparental inheritance of symbionts (13), transmission of only a fraction of parental
92 symbionts to each offspring (29), and separation of an intrahost symbiont population into a
93 reproductive germline and a non-reproductive somatic lineage (29). Host control over symbiont
94 mixing evolved independently multiple times in various symbiotic systems, including eukaryotic
95 cells and their organelles (13) as well as nutritional symbioses of insects that rely on EB for
96 essential metabolites, such amino acids and vitamins (67, 74). While beneficial to the host, long-
97 term evolutionary consequences of suppressed symbiont mixing can be detrimental to the

98 symbionts and the symbiosis as a whole. Symbiont population subdivisions, transmission
99 bottlenecks, and clonality reduce the effective size of a symbiont population and magnify the
100 impact of genetic drift relative to natural selection (90). As a consequence, symbiont populations
101 become vulnerable to accumulation of slightly deleterious (88) and eventual extinction (78). In
102 heritable EB, this process is associated with genomic decay and reduction of the genome size (7,
103 67, 74, 84). Such degenerative genome evolution has been observed empirically in free-living
104 bacteria evolving under conditions of a small effective population size (84), and inferred from
105 molecular evolution patterns in multiple heritable EB that provision insects with essential
106 metabolites (7, 67, 74). Another important consequence of degenerative evolution in heritable
107 EB is acceleration of the molecular evolution rate compared to free-living relatives (73, 87).

108 Remarkably, most of the Mucoromycota-EB symbioses are ancient (15, 72, 121, 124).
109 Two are mutualisms (AMF-*CaGg* and *Rm-Burkholderia*), one is an antagonism (*Me-Mc*), and
110 one remains unresolved in terms of partner fitness outcomes (AMF-*CaMg*). As a consequence,
111 Mucoromycota-EB associations exemplify diverse mechanisms that control evolutionary
112 stability and longevity in symbioses with vertically transmitted EB. Moreover, with the
113 exception of *Burkholderia* EB, symbionts of Mucoromycota appear to evolve faster than their
114 free-living relatives (20, 81), and thus offer insights into how molecular rate acceleration is
115 achieved in EB with different lifestyles. In addition, these symbioses allow for exploring
116 theoretical predictions that specify conditions necessary for mutualisms to arise. Many such
117 predictions have not been tested rigorously because very few heritable partnerships outside
118 Mucoromycota are amenable to experimental manipulation.

119 In this review, we summarize key features of Mucoromycota-EB partnerships, use
120 molecular evolution patterns apparent in these symbioses to speculate about uncertainties
121 surrounding some of their aspects, describe how studies of the Mucoromycota-EB associations
122 inform and validate theoretical models of symbiosis evolution, and detail how they can be used
123 to generate specific insights into the facets of host biology that historically have been recalcitrant
124 to investigation. In the process, we highlight future research directions.

125

126 **2. Host-symbiont biology and symbiosis stability**

127 **2.1. AMF-*CaGg* mutualism**

128 *CaGg* is a betaproteobacterium (**Fig 1**) and a mutualist of AMF from the family Gigasporaceae
129 (9, 64, 72). AMF are obligate biotrophs that colonize roots of most terrestrial plants and
130 facilitate plant uptake of mineral nutrients from the soil (114) in exchange for photosynthesis-
131 derived monosaccharides (41) and fatty acids (17, 44, 48, 63) coming from the plant. The
132 association that AMF form with plants, arbuscular mycorrhiza, dates back to the Early Devonian,
133 400 MYA (97), and is one of the oldest mutualisms on the planet. AMF are increasingly
134 recognized in agronomy as sustainable biofertilizers of the future (127).

135 *CaGg* is vertically transmitted through AMF generations (10) and shows variable
136 distribution across host populations, with some AMF individuals harboring the EB and some
137 being *CaGg*-free (12, 72). This pattern suggests that *CaGg* is a nonessential partner of AMF.
138 Serial sub-culturing of AMF can lead to elimination of *CaGg* under laboratory conditions (64).
139 For AMF, phenotypic consequences of *CaGg* loss include reduced elongation and branching of
140 pre-symbiotic hyphae that emerge from spores in the presence of plant roots (64) (**Fig 3**). At the
141 subcellular level, the absence of *CaGg* from pre-symbiotic hosts is accompanied by a decline in
142 the volume of lipid droplets present in fungal cells (64). Without *CaGg*, spore fatty acids
143 become less abundant, with particular depletion of palmitic acid (106). Pre-symbiotic AMF are
144 unable to synthesize palmitate (123) because they lack genes encoding the fatty acid synthase
145 enzyme complex (118, 129). Consequently, the efficiency of how spore energy reserves are
146 utilized is important for the AMF ability to associate with a plant host. In fungi cured of *CaGg*,
147 reductions in lipid droplet volume and fatty acid abundance are accompanied by elevated
148 expression of genes and proteins involved in beta-oxidation of fatty acids and the pentose
149 phosphate pathway, suggesting a shift towards pathways that provide reducing power (126). In
150 contrast, pre-symbiotic fungi harboring *CaGg* acquire their reducing power due to elevated
151 mitochondrial oxidative phosphorylation and ATP biosynthesis (107, 126). These increases are
152 associated with respiration rates 50% higher than in the cured fungi (126). Overall, *CaGg*
153 appears to interact with AMF energy metabolism in ways that mobilize ATP and fuel pre-
154 symbiotic growth. Interestingly, similar effects are caused by strigolactones, plant hormones that
155 AMF perceive and respond to by enhancing hyphal branching, proliferation of mitochondria and
156 increasing respiration (8, 54). Remarkably, the strigolactone treatment also induces a
157 proliferation of *CaGg* cells (3), which suggests that the fungal mitochondrion might be the

158 primary target of both *CaGg* and plant strigolactones. However, the proximate mechanism of
159 how *CaGg* regulates pre-symbiotic activities of AMF remains elusive.

160 As we discussed earlier, in heritable EB that provision insects with essential metabolites,
161 genes in all functional categories are vulnerable to accumulation of slightly deleterious mutations
162 and decay (7, 67, 74). However, the symbiont genes responsible for essential services to the
163 host, such as those needed for the biosynthesis of amino acids (112) or vitamins (2), maintain
164 their functionality due to host-level selection (19). These observations suggest that clues
165 concerning *CaGg* factors that interact with AMF metabolism might be gleaned from the *CaGg*
166 genomic data. With sizes ranging from 1.34 Mb to 2.36 Mb (36, 71), the genomes of *CaGg* are
167 substantially streamlined compared to their free-living *Burkholderia* relatives (131). However,
168 there are reasons to suspect that the mechanisms of genome contraction in *CaGg* are different
169 from those that govern degenerative genome reduction in heritable EB of insects. In particular,
170 *CaGg* rate of mutation accumulation of 2.03×10^{-9} substitutions per site per year (71) is
171 comparable to that of free-living bacteria, and much lower than 2.2×10^{-7} substitutions per site
172 per year estimated in *Buchnera aphidicola*, *Ba* (76). *Ba* is an essential mutualist that provisions
173 phloem-feeding aphids with amino acids missing from their sugar-rich diet, and a model for
174 understanding degenerative genome evolution in heritable EB (77, 112, 117, 128). Importantly,
175 unlike heritable essential mutualists of insects, *CaGg* shows evidence of rare recombination and
176 host switching/horizontal transmission (71, 72). This pattern is consistent with a relatively large
177 effective size of the *CaGg* population estimated at 1.44×10^8 (71) and larger than 1.0×10^7 in *Ba*
178 (34). Accordingly, forces of natural selection are expected to operate in the *CaGg* population,
179 and in fact, *CaGg* appears to be as effective at purging slightly deleterious mutations as free-
180 living bacteria (71). As a consequence, only the genes encoding biosynthesis of costly
181 metabolites available to *CaGg* from the host are expected to be lost from *CaGg* genomes.
182 Consistent with this prediction, *CaGg* appears to rely on host-derived arginine as its energy
183 source (36). Conversely, EB retains the capacity for the energetically expensive and complex
184 biosynthesis of vitamin B₁₂ (36), which is a cofactor essential to some bacteria and humans but
185 has no apparent role in the metabolism of fungi (99, 120). Consequently, the vitamin B₁₂
186 biosynthetic pathway must be preserved by *CaGg* for its own benefit. These patterns suggest
187 that identifying genomic clues to how *CaGg* reprograms the energy metabolism of its fungal host
188 may not be as simple as in heritable EB with degenerate genomes.

189 *CaGg* is transmitted uniparentally, along clonal lineages of its AMF hosts. AMF show
190 no direct evidence of sexual mating and rely on large multinucleate spores for asexual
191 proliferation. Intrahost populations of *CaGg* are genetically uniform (72). Such genetic
192 homogeneity could be attributed to a rate of mutation accumulation in *CaGg* that is comparable
193 to that of free-living bacteria (71). This low mutation rate (71) and a relatively large effective
194 population size in *CaGg* (71) are also likely to be responsible for the extraordinary evolutionary
195 longevity of the AMF-*CaGg* symbiosis, which dates back to the Early Devonian (72).

196 What remains uncertain are the forces that allow *CaGg* to maintain a relatively large
197 population size. It is possible that the ultimate cause is related to the nature of *CaGg* association
198 with AMF. *CaGg* services are not essential to AMF, or, in other words, AMF are only
199 facultatively reliant on *CaGg* (64, 72). Such reliance suggests that fitness benefits of carrying
200 EB vary depending on specific conditions, with certain environments favoring EB presence and
201 others selecting against it (101, 102). A variable selective landscape is expected to support
202 retention of genetic competence for horizontal transmission and recombination (85), which are
203 present in *CaGg* (71, 72). However, the specific environmental factors responsible for AMF
204 facultative rather than obligate dependence on *CaGg* are unknown. It could be speculated that
205 these factors are related to conditions affecting pre-symbiotic activities of obligately biotrophic
206 AMF, such as the number of spore germination attempts and the extent of hyphal proliferation.
207

208 **2.2. *Rm-Burkholderia* mutualism**

209 *Rm*, like most other Mucoromycotina, is a saprotroph that also can act as an opportunistic
210 pathogen of plants and humans (93, 108). While multiple *Burkholderia* EB species have been
211 found in different isolates of this fungus, such as *Burkholderia rhizoxinica*, *Br* (51, 68, 95, 96,
212 113, 125), *Burkholderia endofungorum* (94) and *Burkholderia* sp. (55, 70) (**Fig 1**), no
213 *Burkholderia* EB have been found in other Mucoromycotina (111). Moreover, even within *Rm*
214 some strains do not harbor these EB (55, 93).

215 The *Rm-Burkholderia* mutualism has become a model for understanding fungal-bacterial
216 symbioses because it can be manipulated experimentally, hosts can be cured of symbionts, and
217 partners separated and reassembled back into a functional symbiosis (51, 55, 68, 70, 95). This
218 versatility is related to the genomic makeup of *Burkholderia* EB. The 3.75 Mb genome of *Br*
219 (52) supports functional capabilities important for *Burkholderia* EB persistence outside the host

220 cellular environment and host recolonization as well as endosymbiotic lifestyle and vertical
221 transmission (51, 68, 95). Recolonization of the fungal mycelium is possible due to the activity
222 of *Burkholderia* secretion systems. These systems include the Type II Secretion System, which
223 translocates fungal cell wall-degrading enzymes chitinase and chitosinase (68) as well as the
224 Type III Secretion System (51), which delivers effectors for host manipulation directly into the
225 host cytoplasm (22). The establishment of symbiosis is associated with alterations of the *Rm*
226 lipid metabolism (55). Host lipids are also important for the maintenance of the symbiosis, as
227 they likely provide substrates for *Burkholderia* energy metabolism (52, 53) (**Fig 3**).

228 Nearly 10% of the *Br* genome is comprised of secondary metabolite gene clusters (52,
229 53). Secondary metabolites are low molecular weight compounds with potent physiological and
230 antimicrobial activities often deployed in interspecific interactions (47). In the *Rm-Burkholderia*
231 symbiosis, an antimitotic polyketide rhizoxin is synthesized cooperatively by both partners (95,
232 108). In contrast to essential metabolites provisioned by EB to insect hosts (6, 75), rhizoxin is
233 not essential to *Rm* survival. However, it allows *Rm* to engage in pathogenesis of plants (108).
234 Such reliance of *Rm* on its EB for secondary metabolites is an important and lifestyle altering
235 evolutionary innovation, as Mucoromycota, including *Rm*, contain only a limited repertoire of
236 secondary metabolite gene clusters (55, 70, 124).

237 For vertical transmission, *Burkholderia* exploits asexual sporangiospores and sexual
238 zygosporangia of *Rm*, exerting different degrees of control over formation of these two types of
239 propagules (70, 96) (**Fig 3**). EB transmission via asexual sporangiospores allows for co-
240 dispersal of partner lineages. However, the extreme bottleneck size, varying from one to four
241 *Burkholderia* cells per *Rm* sporangiospore (70, 96), suggests that additional mechanisms must be
242 in place to prevent rapid genomic degeneration of EB genomes. Like other Mucoromycotina, in
243 addition to asexual proliferation via sporangiospores, the *Rm* hosts can mate and form sexual
244 zygosporangia (70). Consequently, it would not be unexpected for the zygosporangia to provide an
245 arena for mixing of symbionts associated with host parental lineages. While this hypothesis
246 remains to be tested, such mixing would be important for the retention by *Burkholderia* EB of
247 molecular evolution patterns resembling those of free-living *Burkholderia* rather than those of
248 heritable EB of insects, such as ‘*Candidatus* Tremblaya princeps’, a closely related nutritional
249 mutualist of mealybugs (20) (**Fig 1**).

250

251 **2.3. Me-Mc symbiosis**

252 *Mc* is a betaproteobacterium (**Fig 1**) auxotrophic for cysteine, which is provisioned by its *Me*
253 host (86). Like other Mortierellomycotina, *Me* can be isolated from the soil and roots of trees
254 (16, 124). Importantly, not all strains of *Me* harbor *Mc* (124). The *Mc* genome of 2.6 Mb
255 represents an intermediate level of contraction compared to the genomes of its close relatives
256 *CaGg* and *Burkholderia* EB of *Rm* (33, 124). Elimination of *Mc* from the *Me* hyphae results in
257 improved mycelial growth (59, 124) (**Fig 3**). Changes in the colony morphology are
258 accompanied by accumulation of fatty acids that otherwise fuel *Mc* energy metabolism (124).
259 Collectively, the phenotypic effects of *Mc* elimination suggest that it is a parasite of *Me*.

260 Interestingly, the *Me-Mc* symbiosis is believed to have originated 350 MYA (124), which
261 raises questions concerning the exact nature of this association and factors that control its
262 evolutionary stability. As mentioned before, it is unlikely for strictly vertically inherited
263 parasites to persist in a host population (28, 60) unless they engage in horizontal transmission
264 (28, 60), or in a conditional mutualism (40, 61, 62, 105). As the population structure of *Mc* is
265 unknown, it is not clear whether this heritable EB undergoes horizontal transmission. However,
266 as *Me* is a heterothallic fungus in which sexual reproduction requires two compatible mates (35),
267 host mating interactions could facilitate horizontal transmission of *Mc*. It is also possible that *Mc*
268 offers some conditional services to *Me*. For example, it could protect its host against more
269 virulent horizontally transmitted parasites (61, 62). Alternatively, costs and benefits of the *Mc*
270 infection may vary spatially and temporally, and be related to the biosynthesis of secondary
271 metabolites (40, 105). Mucoromycota genomes, as we mentioned earlier, contain only a limited
272 repertoire of secondary metabolite gene clusters (55, 70, 124). In contrast, the *Mc* genome
273 harbors several of them, including one cluster encoding an insecticidal toxin, which potentially
274 could be expressed under specific environmental conditions to aid the fungal host (33, 124).
275 Such secondary metabolite complementation would resemble provision of rhizoxin by
276 *Burkholderia* EB to *Rm* (95, 108). As long as metabolic benefits provisioned by *Mc* occasionally
277 outweigh its cost to *Me*, the symbiosis could be evolutionarily stable (40, 105).

278

279 **2.4. AMF-CaMg symbiosis**

280 Like *CaGg*, *CaMg* is a heritable EB of AMF (79). In fact, both *CaGg* and *CaMg* can coexist in
281 a single AMF host (26, 121). *CaMg* is an uncultivable mollicute in the *Mycoplasma pneumoniae*

282 group of the family Mycoplasmataceae (79, 80) (**Fig 2**). Even though the *CaMg* host range
283 extends to all major lineages of Glomeromycotina (79, 83, 121) as well as to other
284 Mucoromycota, including *Endogone* (25), not all host populations harbor this EB. The role of
285 *CaMg* in the biology of AMF is unknown. The *CaMg* genomes are highly reduced in size,
286 ranging from 0.66 to 1.23 Mb (80, 122). Consequently, *CaMg* is metabolically dependent on the
287 host, with the major source of energy remaining undiscovered (80, 122). Presence of the genes
288 encoding host-interactive proteins as well as genes acquired horizontally from fungi, including
289 Glomeromycotina and Mortierellomycotina (80, 122), suggests that *CaMg* is able to manipulate
290 its host biology.

291 While the metabolic capacity of the *CaMg* genomes does not offer obvious clues as to
292 whether it is a mutualist or antagonist, inferences can be made from the genome architecture (80,
293 81) and the population structure of *CaMg* (121). In contrast to heritable EB that act as
294 mutualists, *CaMg* displays uncommon genome plasticity (80, 81), remarkably high levels of
295 intrahost genetic diversity (83, 121), and population-level recombination (81, 121). These
296 patterns could be interpreted as an indication of an antagonistic arms race with the host (80, 81,
297 121). Genome plasticity in *CaMg* could be also viewed as a countermeasure to genomic
298 degeneration experienced by *CaMg* (81). *CaMg*, while being heritable in AMF, is derived from
299 horizontally transmitted animal-infecting mycoplasmas (80). Like its mycoplasma ancestors,
300 *CaMg* is missing DNA repair mechanisms, a deficiency that contributes to rapid accumulation of
301 mutations, resulting in one of the fastest rates of evolution among bacteria (81). As recombination
302 and mobile genetic element (MGE) activity underlying *CaMg* genomic plasticity are common in
303 other mycoplasmas, *CaMg* must have retained these mechanisms after the host switch to fungi
304 and the transition from horizontal to vertical transmission (81). Importantly, the two
305 explanations of *CaMg* genomic plasticity, as an adaptation that facilitates exploitation of AMF
306 versus a countermeasure to genomic degeneration, are not mutually exclusive. Conversely, it
307 cannot be dismissed that, with genomic plasticity representing a vestige of its mycoplasma
308 ancestry, *CaMg* is a conventional mutualist providing yet unknown benefits to AMF. It is also
309 possible that it is a conditional mutualist that aids the host only under specific conditions (40, 61,
310 62, 105).

311 The age of the AMF-*CaMg* symbiosis likely pre-dates the diversification of the
312 Mucoromycota (121), attesting to considerable evolutionary stability of this heritable association.

313 Such stability could be attributed to an apparent balance between the forces contributing to
314 genomic degeneration versus plasticity experienced by *CaMg* (81). In particular, reconstructing
315 the patterns of accumulation of slightly deleterious mutations during *CaMg* evolution revealed a
316 significant acceleration of this process after ancestral *CaMg* had switched from horizontal to
317 vertical transmission (81). In contrast, the evolution rates along terminal phylogenetic branches
318 leading to present day *CaMg* (**Fig 2**) do not appear to be elevated, which suggests that, over
319 time, *CaMg* has refined the mechanisms responsible for purging of slightly deleterious mutations
320 (81).

321

322 **2.5. Why are heritable EB common in Mucoromycota?**

323 Fungal-bacterial symbioses are not unique to the phylum Mucoromycota (89). However, the
324 associations formed with EB by these early divergent fungi are distinct due to a high degree of
325 co-evolution between the partners. It has been proposed that the propensity of Mucoromycota to
326 host EB is related to the aseptate nature of their hyphae, which allow free migration of EB across
327 the host mycelium (26). Another tantalizing explanation is related to the recent discovery that,
328 unlike Dikarya, early divergent fungi share with bacteria the use of 6-methyladenine (m6A)
329 DNA modification (69). 6mA is by far the most common type of DNA modifications in
330 bacteria, important for bacterial cell defense relying on restriction-modification systems (14). In
331 contrast to prokaryotes, the role of 6mA in eukaryotes has not been understood until recently (32,
332 39, 65, 66, 69, 133, 135). Recent studies revealed that 6mA is not only present in eukaryotes,
333 but plays an important role in gene expression (39, 49, 133, 135). Remarkably, the genomes of
334 early-divergent fungi contain up to 3% of 6mA, a level substantially higher than that in other
335 eukaryotes (69). Moreover, 6mA modifications appear to concentrate at the transcriptional starts
336 of expressed genes, a pattern consistent with gene activation (69). Consequently, it is attractive
337 to speculate that the shared use of 6mA DNA modification is a condition predisposing
338 Mucoromycota to bacterial manipulation, a hypothesis that remains to be tested.

339

340 **3. Exploring evolutionary models**

341 **3.1. Molecular evolution rate acceleration**

342 The rate of molecular evolution is expected to be higher in a population of a small effective size
343 that rapidly accumulates slightly deleterious mutations due to genetic drift compared to a

344 population of a larger size where such mutations are eliminated by natural selection (87).
345 Importantly, molecular evolution rate acceleration relative to free-living taxa is one of the
346 hallmarks of heritable EB (75), including *CaGg* (20). However, as we discussed earlier, with its
347 low mutation rate and a relatively large effective population size (71), *CaGg* appears to defy
348 predictions concerning the causes that underlie evolution rate acceleration. In fact, modeling of
349 the rates of evolution under various parameters of mutation and recombination suggested that the
350 evolution rate acceleration in *CaGg* is a consequence of the long-term maintenance of a largely
351 clonal population coupled with infrequent recombination (71).

352 Even though *Mc* is evolving significantly slower than *CaGg*, its evolution rate is
353 accelerated relative to free-living *Burkholderia* and *Burkholderia* EB of *Rm* (**Fig 1, Table 1**).
354 The genome of *Mc* contains multiple genes involved in DNA repair, including *polA*, *dnaQ*, *mutS*,
355 and *mutL* (33), which encode DNA polymerase I with proofreading activity, ϵ subunit of DNA
356 polymerase III with 3'→5' DNA-directed proofreading exonuclease activity, the MMR5
357 mismatch repair protein that recognizes and binds mismatched nucleotides, and MMR3
358 mismatch repair protein with endonuclease activity, respectively. While retention of these DNA
359 repair mechanisms suggests that the evolution rate acceleration in *Mc* is not caused by an
360 increased supply of mutations, the specific cause has yet to be found.

361 Unlike *CaGg* and *Mc*, *Burkholderia* EB of *Rm* evolve at a rate comparable to that of their
362 free-living relatives (20), which is somewhat surprising in a heritable EB. In the absence of
363 specific data, two hypotheses can be formulated that explain such a low evolutionary rate. First,
364 the *Rm-Burkholderia* mutualism is still at an early stage of co-evolution between the partners,
365 before the population of *Burkholderia* EB had a chance to decline in effective size and start
366 accumulating slightly deleterious mutations that disable DNA repair mechanisms. Alternatively,
367 the *Rm-Burkholderia* symbiosis is already ancient. Yet the genomes of EB are arrested at the
368 present state of evolution due to the nature of the symbiosis in which EB control host
369 reproductive biology, are free to mix, and thereby retain a large effective population size that
370 allows for symbiont-level selection. A moderate size of the *Br* genome and its retention of DNA
371 repair genes *polA*, *dnaQ*, *mutS*, and *mutL* (52) support both hypotheses. Accordingly, additional
372 work is needed to explain the low rate of molecular evolution in *Burkholderia* EB.

373 *CaMg* evolves at a rate that exceeds the rates observed in rapidly evolving animal-
374 associated mycoplasmas and is one of the fastest among bacteria (81). As we indicated earlier, the

375 genomes of *CaMg* are missing genes responsible for DNA repair, which contributes to a rampant
376 accumulation of mutations (80). This mutational decay is countered by genome plasticity (80,
377 82). In turn, a dynamic equilibrium between the forces that drive the ongoing genome decay and
378 its restoration contributes to evolutionary antiquity of the AMF-*CaMg* symbiosis (81). The same
379 forces are also likely responsible for the ultra-rapid evolution in *CaMg*. Importantly, this
380 mechanism is distinct from the one governing the rapid evolution of heritable EB with
381 populations of a small effective size (73, 87). It also differs from the mechanism operating in
382 *CaGg* in which molecular evolution rate acceleration can be attributed to rare recombination
383 events in a predominantly clonal population with a relatively large effective size (71).

384

385 **3.2. Mutualism origins**

386 **3.2.1. Antagonism-to-mutualism transition in heritable symbioses.** In the *Rm-Burkholderia*
387 symbiosis, elimination of EB from the host mycelium abolishes asexual proliferation of the
388 fungus (96) and affects its ability to mate, either impeding sex completely or reducing the rate of
389 zygospore formation (70) (**Fig 3**). These two patterns suggest that symbionts interact with host
390 reproduction and, by doing so, they control their own transmission (70). According to one of the
391 theoretical models describing conditions required for mutualism establishment, the symbiont's
392 ability to achieve control of its own transmission is the key prerequisite for the antagonism-to-
393 mutualism transition in heritable symbioses (134). While the evolutionary history of the *Rm-*
394 *Burkholderia* mutualism is uncertain, present-day antagonistic interactions between naturally
395 EB-free (non-host) *Rm* and *Burkholderia* isolated from the host suggest that it originated as an
396 antagonism (55). The symbiont's control over own transmission is expected to facilitate
397 reciprocal selection between the partners, leading to utilization of symbiont services by the host
398 (134). In the *Rm-Burkholderia* symbiosis, these services include EB-mediated synthesis of
399 rhizoxin, which, as we discussed earlier, enables pathogenesis of plants by *Rm* (95, 108).
400 Overall, the *Rm-Burkholderia* mutualism supports the evolutionary model suggesting that a
401 heritable mutualism could evolve from an antagonism (134).

402

403 **3.2.2. Host addiction to an antagonistic symbiont.** Another theoretical model describing the
404 antagonism-to-mutualism transition, which gained support from the patterns displayed by the *Rm*
405 and *Burkholderia* partners, is the addiction model (1). According to this model, a host

406 antagonized by a parasitic symbiont will develop mechanisms that counterbalance parasite's
407 negative effects. These mechanisms may make the host addicted to the symbiont's continued
408 presence (92). In the *Rm-Burkholderia* symbiosis, the non-hosts exhibiting growth inhibition
409 when confronted by EB isolated from host fungi represent a pre-addiction stage of the fungus
410 (55). Mutualism establishment between the cured host and *Burkholderia* EB as well as bacterial
411 presence inside the host hyphae in the established symbiosis are associated with elevated
412 expression of fungal genes involved in lipid metabolism (55, 70). Activities of these enzymes
413 result in accumulation of triacylglycerol (TAG) and phosphatidylethanolamine (PE) at a ratio of
414 about 1:1 (55) (**Fig 3**). Perturbation of this ratio in favor of TAG over PE shifts the *Rm-*
415 *Burkholderia* interaction into antagonism, suggesting that the accumulation of TAG and PE at a
416 specific ratio is part of the fungal addiction syndrome to EB.

417 In addition to EB impact on host lipid metabolism, *Rm* is addicted to *Burkholderia* for
418 reproduction (70). Bacteria hijacked a component of the host's reproductive machinery by
419 gaining control over the expression of *ras2-1* (70), a gene encoding a G-protein involved in
420 asexual and sexual reproduction in other fungi (45, 46, 58). The exact mechanism of bacterial
421 control over *ras2-1* expression and the evolutionary trajectory that lead to it are unknown.
422 However, a tantalizing clue comes from observations made in yeast *Saccharomyces cerevisiae* in
423 which hyper-activation of Ras signaling induces programmed cell death (38). Accordingly, it is
424 attractive to speculate that in the ancestrally antagonistic relationship between *Rm* and
425 *Burkholderia* (55), establishing control over *ras2-1* expression by EB was an important
426 component of co-evolution between the partners, leading to adaptive changes in host regulation
427 of its Ras2-1 signaling (70).

428

429 **4. Fungal-bacterial symbioses: a window into the fungal biology**

430 The phylum Mucoromycota is one of the least understood lineages of filamentous fungi because
431 its representatives have been remarkably recalcitrant to genetic analysis and manipulation.
432 However, recent studies of fungal-bacterial symbioses involving Mucoromycota suggest that
433 novel insights into various aspects of the Mucoromycota biology can be gleaned from a
434 systematic dissection of these associations.

435

436 **4.1. Lipid metabolism of Mucoromycota**

437 Most Mucoromycota are oleaginous fungi that accumulate lipids to at least 20% of their biomass
438 (119). In all Mucoromycota symbioses that can be manipulated experimentally (AMF-*CaGg*,
439 *Me-Mc*, *Rm-Burkholderia*), symbiont elimination results in alterations of host lipid metabolism
440 (55, 59, 106, 107, 124, 126) (**Fig 3**). While the significance of these perturbations is different in
441 each of the systems, they all speak to the central role of lipid metabolism in host-EB interactions
442 involving Mucoromycota. Importantly, the examination of host responses to EB contributed to a
443 refined understanding of lipid metabolic pathways in Mucoromycota (55, 59). It also revealed
444 that some of the Mucoromycota lipid metabolic enzymes affected by EB are unique to the early
445 divergent fungi and not found in Dikarya (55).

446

447 **4.2. Reproductive biology of Mucoromycotina**

448 Reproductive dependence of *Rm* on *Burkholderia* EB established this symbiosis as a model for
449 understanding how asexual and sexual reproduction is regulated in Mucoromycotina (70, 96).
450 Several important insights have been already generated in this system. These findings include a
451 discovery that only one of the multiple paralogs of Ras2, a small GTPase central to the
452 reproductive development of other fungi, plays a role during both mating and asexual
453 proliferation of Mucoromycotina (70). In addition, a negative impact of cyclic AMP on
454 Mucoromycotina mating has been confirmed in this system (70). Lastly, candidate receptors of
455 mating pheromones unique to Mucoromycotina have been identified (70). Unlike Dikarya,
456 Mucoromycotina rely on trisporic acids and their precursors for communication between sexual
457 partners (132). While the biosynthesis of these molecules is fairly well understood (132),
458 mechanisms of their perception have been elusive.

459

460 **4.3. Reproductive biology of AMF**

461 Glomeromycotina are one of oldest and most common symbionts of plants (114). Despite their
462 close phylogenetic relationship with Mucoromycotina and Mortierellomycotina (115), they
463 display several phenotypic features that superficially set them apart from these other
464 Mucoromycota. First, unlike other predominantly saprotrophic Mucoromycota,
465 Glomeromycotina are obligate biotrophs. They have lost the fatty acid synthase, which is the
466 key enzyme complex responsible for the biosynthesis of fatty acids (118, 129). As a
467 consequence, AMF rely on their plant hosts for energy metabolism substrates. Second, although

468 cryptic recombination appears to occur in AMF (23, 24, 98), there is no direct evidence that
469 these fungi engage in a sexual process in which the union of gametangia leads to the formation
470 of zygospores typical for Mucoromycotina and Mortierellomycotina. Third, AMF do not form
471 asexual sporangiospores that are used for dispersal by most other Mucoromycota, with the
472 exception of *Endogone*. Instead, they generate large multinucleate resting spores that
473 phenotypically resemble azygospores formed by many Mucoromycotina under several specific
474 conditions (5, 37, 109, 110).

475 The apparent loss of sexual mating and sporangiospore-mediated dispersal in
476 Glomeromycotina may be attributed to selective pressures exerted by their obligate mutualism
477 with plants. In particular, genetic recombination is expected to be disfavored in mutualistic
478 microbes because new recombinant genotypes are less likely to be co-adapted to common host
479 genotypes (56, 57, 103). However, once recombination is lost, accumulation of slightly
480 deleterious mutations becomes a threat to evolutionary longevity of an asexual population (78).
481 Under such circumstances, asexual propagation becomes a key modulator of the population load
482 of deleterious mutations. Specifically, multinucleate propagules, such as those formed by AMF,
483 are more effective in purging of slightly deleterious mutations compared to uninuclear
484 propagules, like sporangiospores (43, 91, 100). Consequently, they are expected to be favored.

485 Theoretical considerations suggest that the reproductive biology of extant
486 Glomeromycotina could be solely a product of their interactions with plants. However, given the
487 role of *Burkholderia* EB in the reproductive biology of *Rm* (70, 96) and the propensity of AMF
488 for hosting diverse EB (11, 79), it is tempting to speculate that the loss of mating and
489 sporangiospore formation might have been facilitated by interactions of ancestral
490 Glomeromycotina with EB capable of modulating host reproductive biology.

491

492 **4.4. Innate immunity in Mucoromycotina**

493 The utility of the *Rm-Burkholderia* symbiosis as a model for fungal-bacterial interactions is
494 enhanced by the existence of non-host strains of *Rm* that do not harbor EB and interact
495 antagonistically with EB isolated from the host (55). Specifically, co-cultivation of cured *Rm*
496 with its own *Burkholderia* EB or *Burkholderia* isolated from other *Rm* hosts re-establishes a
497 functional symbiosis whereby bacteria populate fungal hyphae and spores (55, 70). In contrast,
498 non-host *Rm* strains do not become colonized by EB isolated from host *Rm* strains (55). A

499 similar absence of colonization was observed in other non-host Mucoromycotina such as
500 *Rhizopus oryzae* and *Mucor circinelloides* during co-cultivation with EB of *Rm* (55). Moreover,
501 the non-host fungi are antagonized by these bacteria and change their growth pattern by reducing
502 hyphal extension around bacterial colonies (55). These observations indicate that *Burkholderia*
503 isolated from *Rm* offers an excellent probe for exploring innate immunity of Mucoromycotina,
504 which, as we mentioned earlier, possess a limited repertoire of secondary metabolites that could
505 be deployed as a defense against bacterial invasions.

506

507 **5. Conclusions**

508 Heritable symbioses formed with bacteria by the members of the phylum Mucoromycota stand
509 out among other fungal-bacterial relationships. Despite their ecological and metabolic diversity,
510 these associations are all highly co-evolved and most are ancient. They have been a source of
511 important insights into the mechanisms that stabilize heritable symbioses, control the rate of
512 molecular evolution, and enable the establishment of mutualisms. They revealed novel aspects
513 of host-microbe biology and provided a unique framework for exploring genetically intractable
514 Mucoromycota. These advances establish heritable symbioses between Mucoromycota and EB
515 as convenient and versatile research targets. Importantly, it is highly likely that many
516 Mucoromycota-EB associations with unique biological properties will soon be discovered.
517 Consequently, we expect that the current explosion of studies conducted on fungal-bacterial
518 symbioses is a good prognostic for the future expansion of this research area.

519

520 **Summary Points**

- 521 1. The associations of Mucoromycota with EB exemplify novel host-microbe interactions and
522 mechanisms that stabilize heritable symbioses over long evolutionary periods.
- 523 2. Some EB of Mucoromycota display molecular evolution rate acceleration relative to free-
524 living bacteria that cannot be attributed to accumulation of slightly deleterious mutations in a
525 population of a small effective size.
- 526 3. Studies of the Mucoromycota-EB symbioses allow for testing predictions of theoretical
527 models describing the origins of mutualisms.
- 528 4. Examination of the Mucoromycota-EB symbioses provides insights into the biology of
529 genetically intractable fungal hosts.

530 5. Novel Mucoromycota-EB symbioses are expected to be discovered.

531

532 **Future Issues**

533 1. What is the proximate mechanism that allows *CaGg* for manipulation of pre-symbiotic
534 AMF?

535 2. What is the evolutionary age of the *Rm-Burkholderia* symbiosis?

536 3. Is the *Me-Mc* symbiosis a conditional mutualism?

537 4. What is the nature of the AMF-*CaMg* symbiosis?

538 5. Is the shared use of m6A DNA modification predisposing Mucoromycota to harboring EB?

539

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542

543 **Literature Cited**

544 1. Aanen DK, Hoekstra RF. 2007. The evolution of obligate mutualism: if you can't beat
545 'em, join 'em. *Trends in Ecology & Evolution* 22: 506-09

546 2. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, et al. 2002. Genome sequence
547 of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature*
548 *Genetics* 32: 402-07

549 3. Anca IA, Lumini E, Ghignone S, Salvioli A, Bianciotto V, Bonfante P. 2009. The *ftsZ*
550 gene of the endocellular bacterium '*Candidatus Glomeribacter gigasporarum*' is
551 preferentially expressed during the symbiotic phases of its host mycorrhizal fungus.
552 *Molecular Plant-Microbe Interactions* 22: 302-10

553 4. Axelrod R, Hamilton WD. 1981. The evolution of cooperation. *Science* 211: 1390-96

554 5. Benjamin RK, Mehrotra BS. 1963. Obligate azygospore formation in two species of
555 *Mucor* (Mucorales). *Aliso* 5: 235-45

556 6. Bennett GM, Moran NA. 2013. Small, smaller, smallest: the origins and evolution of
557 ancient dual symbioses in a phloem-feeding insect. *Genome Biology and Evolution* 5:
558 1675-88

- 559 7. Bennett GM, Moran NA. 2015. Heritable symbiosis: The advantages and perils of an
560 evolutionary rabbit hole. *Proceedings of the National Academy of Sciences of the United*
561 *States of America* 112: 10169-76
- 562 8. Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, et al. 2006.
563 Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLOS*
564 *Biology* 4: e226
- 565 9. Bianciotto V, Bandi C, Minerdi D, Sironi M, Tichy HV, Bonfante P. 1996. An obligately
566 endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Applied*
567 *and Environmental Microbiology* 62: 3005-10
- 568 10. Bianciotto V, Genre A, Jargeat P, Lumini E, Becard G, Bonfante P. 2004. Vertical
569 transmission of endobacteria in the arbuscular mycorrhizal fungus *Gigaspora margarita*
570 through generation of vegetative spores. *Applied and Environmental Microbiology* 70:
571 3600-08
- 572 11. Bianciotto V, Lumini E, Bonfante P, Vandamme P. 2003. '*Candidatus* Glomeribacter
573 gigasporarum' gen. nov., sp nov., an endosymbiont of arbuscular mycorrhizal fungi.
574 *International Journal of Systematic and Evolutionary Microbiology* 53: 121-24
- 575 12. Bianciotto V, Lumini E, Lanfranco L, Minerdi D, Bonfante P, Perotto S. 2000. Detection
576 and identification of bacterial endosymbionts in arbuscular mycorrhizal fungi belonging
577 to the family Gigasporaceae. *Applied and Environmental Microbiology* 66: 4503-09
- 578 13. Birky CW, Jr. 1995. Uniparental inheritance of mitochondrial and chloroplast genes:
579 Mechanisms and evolution. *Proceedings of the National Academy of Sciences of the*
580 *United States of America* 92: 11331-38
- 581 14. Blow MJ, Clark TA, Daum CG, Deutschbauer AM, Fomenkov A, et al. 2016. The
582 epigenomic landscape of prokaryotes. *PLoS Genetics* 12: e1005854
- 583 15. Bonfante P, Desirò A. 2017. Who lives in a fungus? The diversity, origins and functions
584 of fungal endobacteria living in Mucoromycota. *ISME Journal* 11: 1727
- 585 16. Bonito G, Hameed K, Ventura R, Krishnan J, Schadt CW, Vilgalys R. 2016. Isolating a
586 functionally relevant guild of fungi from the root microbiome of *Populus*. *Fungal*
587 *Ecology* 22: 35-42

- 588 17. Bravo A, Brands M, Wewer V, Dormann P, Harrison MJ. 2017. Arbuscular mycorrhiza-
589 specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development
590 of arbuscular mycorrhiza. *New Phytologist* 214: 1631-45
- 591 18. Bull JJ, Rice WR. 1991. Distinguishing mechanisms for the evolution of cooperation.
592 *Journal of Theoretical Biology* 149: 63-74
- 593 19. Canbäck B, Tamas I, Andersson SGE. 2004. A phylogenomic study of endosymbiotic
594 bacteria. *Molecular Biology and Evolution* 21: 1110-22
- 595 20. Castillo DM, Pawlowska TE. 2010. Molecular evolution in bacterial endosymbionts of
596 fungi. *Molecular Biology and Evolution* 27: 622-36
- 597 21. Connor RC. 1986. Pseudo-reciprocity: investing in mutualism. *Animal Behaviour* 34:
598 1562-84
- 599 22. Costa TR, Felisberto-Rodrigues C, Meir A, Prevost MS, Redzej A, et al. 2015. Secretion
600 systems in Gram-negative bacteria: structural and mechanistic insights. *Nature Reviews*
601 *Microbiology* 13: 343-59
- 602 23. Croll D, Sanders IR. 2009. Recombination in *Glomus intraradices*, a supposed ancient
603 asexual arbuscular mycorrhizal fungus. *BMC Evolutionary Biology* 9
- 604 24. den Bakker HC, VanKuren NW, Morton JB, Pawlowska TE. 2010. Clonality and
605 recombination in the life history of an asexual arbuscular mycorrhizal fungus. *Molecular*
606 *Biology and Evolution* 27: 2474-86
- 607 25. Desirò A, Faccio A, Kaech A, Bidartondo MI, Bonfante P. 2015. *Endogone*, one of the
608 oldest plant-associated fungi, host unique Mollicutes-related endobacteria. *New*
609 *Phytologist* 205: 1464-72
- 610 26. Desirò A, Salvioli A, Ngonkeu EL, Mondo SJ, Epis S, et al. 2014. Detection of a novel
611 intracellular microbiome hosted in arbuscular mycorrhizal fungi. *ISME Journal* 8: 257-
612 70
- 613 27. Doebeli M, Knowlton N. 1998. The evolution of interspecific mutualisms. *Proceedings*
614 *of the National Academy of Sciences of the United States of America* 95: 8676-80
- 615 28. Fine PEM. 1975. Vectors and vertical transmission: An epidemiologic perspective.
616 *Annals of the New York Academy of Sciences* 266: 173-94
- 617 29. Frank SA. 1996. Host control of symbiont transmission: The separation of symbionts into
618 germ and soma. *American Naturalist* 148: 1113-24

- 619 30. Frank SA. 1996. Host-symbiont conflict over the mixing of symbiotic lineages.
620 *Proceedings of the Royal Society of London Series B-Biological Sciences* 263: 339-44
- 621 31. Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A. 2011. Bacterial-
622 fungal interactions: hyphens between agricultural, clinical, environmental, and food
623 microbiologists. *Microbiology and Molecular Biology Reviews* 75: 583-609
- 624 32. Fu Y, Luo GZ, Chen K, Deng X, Yu M, et al. 2015. N6-methyldeoxyadenosine marks
625 active transcription start sites in *Chlamydomonas*. *Cell* 161: 879-92
- 626 33. Fujimura R, Nishimura A, Ohshima S, Sato Y, Nishizawa T, et al. 2014. Draft genome
627 sequence of the betaproteobacterial endosymbiont associated with the fungus *Mortierella*
628 *elongata* FMR23-6. *Genome Announcements* 2: e01272-14
- 629 34. Funk DJ, Wernegreen JJ, Moran NA. 2001. Intraspecific variation in symbiont genomes:
630 Bottlenecks and the aphid-Buchnera association. *Genetics* 157: 477-89
- 631 35. Gams W, Chien C-Y, Domsch KH. 1972. Zygospore formation by the heterothallic
632 *Mortierella elongata* and a related homothallic species, *M. epigama* sp.nov. *Transactions*
633 *of the British Mycological Society* 58: 5-IN2
- 634 36. Ghignone S, Salvioli A, Anca I, Lumini E, Ortu G, et al. 2012. The genome of the
635 obligate endobacterium of an AM fungus reveals an interphylum network of nutritional
636 interactions. *ISME Journal* 6: 136-45
- 637 37. Ginman A, Young TWK. 1989. Azygospore morphology in *Mucor azygospora* and *M.*
638 *bainieri*. *Mycological Research* 93: 314-20
- 639 38. Gourlay CW, Ayscough KR. 2006. Actin-induced hyperactivation of the Ras signaling
640 pathway leads to apoptosis in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*
641 26: 6487-501
- 642 39. Greer EL, Blanco MA, Gu L, Sendinc E, Liu J, et al. 2015. DNA methylation on N6-
643 adenine in *C. elegans*. *Cell* 161: 868-78
- 644 40. Gyllenberg M, Preoteasa D, Saikkonen K. 2002. Vertically transmitted symbionts in
645 structured host metapopulations. *Bulletin of Mathematical Biology* 64: 959-78
- 646 41. Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, Requena N. 2011. A versatile
647 monosaccharide transporter that operates in the arbuscular mycorrhizal fungus *Glomus* sp
648 is crucial for the symbiotic relationship with plants. *Plant Cell* 23: 3812-23

- 649 42. Herre EA, Knowlton N, Mueller UG, Rehner SA. 1999. The evolution of mutualisms:
650 exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution* 14:
651 49-53
- 652 43. Jany JL, Pawlowska TE. 2010. Multinucleate spores contribute to evolutionary longevity
653 of asexual Glomeromycota. *American Naturalist* 175: 424-35
- 654 44. Jiang Y, Wang W, Xie Q, Liu N, Liu L, et al. 2017. Plants transfer lipids to sustain
655 colonization by mutualistic mycorrhizal and parasitic fungi. *Science* 356: 1172-75
- 656 45. Kalleda N, Naorem A, Manchikarla RV. 2013. Targeting fungal genes by diced siRNAs:
657 a rapid tool to decipher gene function in *Aspergillus nidulans*. *PLoS One* 8: e75443
- 658 46. Kana-uchi A, Yamashiro CT, Tanabe S, Murayama T. 1997. A *ras* homologue of
659 *Neurospora crassa* regulates morphology. *Molecular & General Genetics* 254: 427-32
- 660 47. Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism — From
661 biochemistry to genomics. *Nature Reviews Microbiology* 3: 937-47
- 662 48. Keymer A, Pimprakar P, Wewer V, Huber C, Brands M, et al. 2017. Lipid transfer from
663 plants to arbuscular mycorrhiza fungi. *eLife* 6
- 664 49. Koziol MJ, Bradshaw CR, Allen GE, Costa ASH, Frezza C, Gurdon JB. 2016.
665 Identification of methylated deoxyadenosines in vertebrates reveals diversity in DNA
666 modifications. *Nature Structural & Molecular Biology* 23: 24-30
- 667 50. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics
668 Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-4
- 669 51. Lackner G, Moebius N, Hertweck C. 2011. Endofungal bacterium controls its host by an
670 *hrp* type III secretion system. *ISME Journal* 5: 252-61
- 671 52. Lackner G, Moebius N, Partida-Martinez L, Hertweck C. 2011. Complete genome
672 sequence of *Burkholderia rhizoxinica*, an endosymbiont of *Rhizopus microsporus*.
673 *Journal of Bacteriology* 193: 783-84
- 674 53. Lackner G, Moebius N, Partida-Martinez LP, Boland S, Hertweck C. 2011. Evolution of
675 an endofungal lifestyle: Deductions from the *Burkholderia rhizoxinica* genome. *BMC*
676 *Genomics* 12: 210
- 677 54. Lanfranco L, Fiorilli V, Venice F, Bonfante P. 2017. Strigolactones cross the kingdoms:
678 plants, fungi, and bacteria in the arbuscular mycorrhizal symbiosis. *Journal of*
679 *Experimental Botany*: erx432

- 680 55. Lastovetsky OA, Gaspar ML, Mondo SJ, LaButti KM, Sandor L, et al. 2016. Lipid
681 metabolic changes in an early divergent fungus govern the establishment of a mutualistic
682 symbiosis with endobacteria. *Proceedings of the National Academy of Sciences of the*
683 *United States of America* 113: 15102-07
- 684 56. Law R. 1985. Evolution in a mutualistic environment. In *The Biology of Mutualism.*
685 *Ecology and Evolution*, ed. DH Boucher, pp. 145-70. New York: Oxford University Press
- 686 57. Law R, Lewis DH. 1983. Biotic environments and the maintenance of sex—some
687 evidence from mutualistic symbioses. *Biological Journal of the Linnean Society* 20: 249-
688 76
- 689 58. Lee N, Kronstad JW. 2002. *ras2* controls morphogenesis, pheromone response, and
690 pathogenicity in the fungal pathogen *Ustilago maydis*. *Eukaryotic Cell* 1: 954-66
- 691 59. Li Z, Yao Q, Dearth SP, Entler MR, Castro Gonzalez HF, et al. 2017. Integrated
692 proteomics and metabolomics suggests symbiotic metabolism and multimodal regulation
693 in a fungal-endobacterial system. *Environmental Microbiology* 19: 1041-53
- 694 60. Lipsitch M, Nowak MA, Ebert D, May RM. 1995. The population dynamics of vertically
695 and horizontally transmitted parasites. *Proceedings of the Royal Society of London Series*
696 *B-Biological Sciences* 260: 321-27
- 697 61. Lipsitch M, Siller S, Nowak MA. 1996. The evolution of virulence in pathogens with
698 vertical and horizontal transmission. *Evolution* 50: 1729-41
- 699 62. Lively CM, Clay K, Wade MJ, Fuqua C. 2005. Competitive co-existence of vertically
700 and horizontally transmitted parasites. *Evolutionary Ecology Research* 7: 1183-90
- 701 63. Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, et al. 2017. Fatty
702 acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* 356:
703 1175-78
- 704 64. Lumini E, Bianciotto V, Jargeat P, Novero M, Salvioli A, et al. 2007. Presymbiotic
705 growth and sporal morphology are affected in the arbuscular mycorrhizal fungus
706 *Gigaspora margarita* cured of its endobacteria. *Cellular Microbiology* 9: 1716-29
- 707 65. Luo G-Z, Blanco MA, Greer EL, He C, Shi Y. 2015. DNA N6-methyladenine: a new
708 epigenetic mark in eukaryotes? *Nature Reviews Molecular Cell Biology* 16: 705-10

- 709 66. Luo GZ, Wang F, Weng X, Chen K, Hao Z, et al. 2016. Characterization of eukaryotic
710 DNA N6-methyladenine by a highly sensitive restriction enzyme-assisted sequencing.
711 *Nature Communications* 7: 11301
- 712 67. McCutcheon JP, Moran NA. 2012. Extreme genome reduction in symbiotic bacteria.
713 *Nature Reviews Microbiology* 10: 13-26
- 714 68. Moebius N, Üzümlü Z, Dijksterhuis J, Lackner G, Hertweck C. 2014. Active invasion of
715 bacteria into living fungal cells. *eLife* 3: e03007
- 716 69. Mondo SJ, Dannebaum RO, Kuo RC, Louie KB, Bewick AJ, et al. 2017. Widespread
717 adenine N6-methylation of active genes in fungi. *Nature Genetics* 49: 964-68
- 718 70. Mondo SJ, Lastovetsky OA, Gaspar ML, Schwardt NH, Barber CC, et al. 2017. Bacterial
719 endosymbionts influence host sexuality and reveal reproductive genes of early divergent
720 fungi. *Nature Communications* 8: 1843
- 721 71. Mondo SJ, Salvioli A, Bonfante P, Morton JB, Pawlowska TE. 2016. Nondegenerative
722 evolution in ancient heritable bacterial endosymbionts of fungi. *Molecular Biology &*
723 *Evolution* 33: 2216–31
- 724 72. Mondo SJ, Toomer KH, Morton JB, Lekberg Y, Pawlowska TE. 2012. Evolutionary
725 stability in a 400-million-year-old heritable facultative mutualism. *Evolution* 66: 2564-76
- 726 73. Moran NA. 1996. Accelerated evolution and Muller's ratchet in endosymbiotic bacteria.
727 *Proceedings of the National Academy of Sciences of the United States of America* 93:
728 2873-78
- 729 74. Moran NA, Bennett GM. 2014. The tiniest tiny genomes. *Annual Review of Microbiology*
730 68: 195-215
- 731 75. Moran NA, McCutcheon JP, Nakabachi A. 2008. Genomics and evolution of heritable
732 bacterial symbionts. *Annual Review of Genetics* 42: 165-90
- 733 76. Moran NA, McLaughlin HJ, Sorek R. 2009. The dynamics and time scale of ongoing
734 genomic erosion in symbiotic bacteria. *Science* 323: 379-82
- 735 77. Moran NA, Mira A. 2001. The process of genome shrinkage in the obligate symbiont
736 *Buchnera aphidicola*. *Genome Biology* 2: Research0054.1–54.12
- 737 78. Muller HJ. 1964. The relation of recombination to mutational advance. *Mutation*
738 *Research* 1: 2-9

- 739 79. Naito M, Desirò A, Gonzalez JB, Tao G, Bonfante P, et al. 2017. ‘*Candidatus*
740 *Moeniiplasma glomeromycotorum*’, an endobacterium of arbuscular mycorrhizal fungi.
741 *International Journal of Systematic and Evolutionary Microbiology* 67: 1177–84
- 742 80. Naito M, Morton JB, Pawlowska TE. 2015. Minimal genomes of mycoplasma-related
743 endobacteria are plastic and contain host-derived genes for sustained life within
744 Glomeromycota. *Proceedings of the National Academy of Sciences of the United States*
745 *of America* 112: 7791-96
- 746 81. Naito M, Pawlowska TE. 2016. Defying Muller’s ratchet: Heritable endobacteria escape
747 extinction through recombination and genome plasticity. *mBio* 7: e02057-15
- 748 82. Naito M, Pawlowska TE. 2016. The role of mobile genetic elements in evolutionary
749 longevity of heritable endobacteria. *Mobile Genetic Elements* 6: e1136375
- 750 83. Naumann M, Schüßler A, Bonfante P. 2010. The obligate endobacteria of arbuscular
751 mycorrhizal fungi are ancient heritable components related to the Mollicutes. *ISME*
752 *Journal* 4: 862-71
- 753 84. Nilsson AI, Koskiniemi S, Eriksson S, Kugelberg E, Hinton JCD, Andersson DI. 2005.
754 Bacterial genome size reduction by experimental evolution. *Proceedings of the National*
755 *Academy of Sciences of the United States of America* 102: 12112-16
- 756 85. O’Fallon B. 2008. Population structure, levels of selection, and the evolution of
757 intracellular symbionts. *Evolution* 62: 361-73
- 758 86. Ohshima S, Sato Y, Fujimura R, Takashima Y, Hamada M, et al. 2016. *Mycoavidus*
759 *cysteinexigens* gen. nov., sp. nov., an endohyphal bacterium isolated from a soil isolate of
760 the fungus *Mortierella elongata*. *International Journal of Systematic and Evolutionary*
761 *Microbiology* 66: 2052-7
- 762 87. Ohta T. 1972. Population size and rate of evolution. *Journal of Molecular Evolution* 1:
763 305-14
- 764 88. Ohta T. 1973. Slightly deleterious mutant substitutions in evolution. *Nature* 246: 96-98
- 765 89. Olsson S, Bonfante P, Pawlowska TE. 2017. Ecology and evolution of fungal-bacterial
766 interactions. In *The Fungal Community: its Organization and Role in the Ecosystem*, ed. J
767 Dighton, JF White, pp. 563-83. Boca Raton: Taylor & Francis
- 768 90. Orive ME. 1993. Effective population size in organisms with complex life-histories.
769 *Theoretical Population Biology* 44: 316-40

- 770 91. Otto SP, Orive ME. 1995. Evolutionary consequences of mutation and selection within
771 an individual. *Genetics* 141: 1173-87
- 772 92. Pannebakker BA, Loppin B, Elemans CP, Humblot L, Vavre F. 2007. Parasitic inhibition
773 of cell death facilitates symbiosis. *Proceedings of the National Academy of Sciences of*
774 *the United States of America* 104: 213-5
- 775 93. Partida-Martinez LP, Bandemer S, Ruchel R, Dannaoui E, Hertweck C. 2008. Lack of
776 evidence of endosymbiotic toxin-producing bacteria in clinical *Rhizopus* isolates.
777 *Mycoses* 51: 266-69
- 778 94. Partida-Martinez LP, Groth I, Schmitt I, Richter W, Roth M, Hertweck C. 2007.
779 *Burkholderia rhizoxinica* sp. nov. and *Burkholderia endofungorum* sp. nov., bacterial
780 endosymbionts of the plant-pathogenic fungus *Rhizopus microsporus*. *International*
781 *Journal of Systematic and Evolutionary Microbiology* 57: 2583-90
- 782 95. Partida-Martinez LP, Hertweck C. 2005. Pathogenic fungus harbours endosymbiotic
783 bacteria for toxin production. *Nature* 437: 884-88
- 784 96. Partida-Martinez LP, Monajembashi S, Greulich KO, Hertweck C. 2007. Endosymbiont-
785 dependent host reproduction maintains bacterial-fungal mutualism. *Current Biology* 17:
786 773-77
- 787 97. Remy W, Taylor TN, Hass H, Kerp H. 1994. Four hundred-million-year-old vesicular
788 arbuscular mycorrhizae. *Proceedings of the National Academy of Sciences of the United*
789 *States of America* 91: 11841-43
- 790 98. Ropars J, Toro KS, Noel J, Pelin A, Charron P, et al. 2016. Evidence for the sexual origin
791 of heterokaryosis in arbuscular mycorrhizal fungi. *Nature Microbiology* 1: 16033
- 792 99. Roth JR, Lawrence JG, Bobik TA. 1996. Cobalamin (coenzyme B12): Synthesis and
793 biological significance. *Annual Review of Microbiology* 50: 137-81
- 794 100. Roze D, Michod RE. 2001. Mutation, multilevel selection, and the evolution of propagule
795 size during the origin of multicellularity. *American Naturalist* 158: 638-54
- 796 101. Russell JA, Moran NA. 2005. Horizontal transfer of bacterial symbionts: Heritability and
797 fitness effects in a novel aphid host. *Applied and Environmental Microbiology* 71: 7987-
798 94

- 799 102. Russell JA, Moran NA. 2006. Costs and benefits of symbiont infection in aphids:
800 variation among symbionts and across temperatures. *Proceedings of the Royal Society B-*
801 *Biological Sciences* 273: 603-10
- 802 103. Sachs JL, Essenberg CJ, Turcotte MM. 2011. New paradigms for the evolution of
803 beneficial infections. *Trends in Ecology & Evolution* 26: 202-09
- 804 104. Sachs JL, Mueller UG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation.
805 *Quarterly Review of Biology* 79: 135-60
- 806 105. Saikkonen K, Ion D, Gyllenberg M. 2002. The persistence of vertically transmitted fungi
807 in grass metapopulations. *Proceedings of the Royal Society of London Series B-*
808 *Biological Sciences* 269: 1397-403
- 809 106. Salvioli A, Chiapello M, Fontaine J, Hadj-Sahraoui AL, Grandmougin-Ferjani A, et al.
810 2010. Endobacteria affect the metabolic profile of their host *Gigaspora margarita*, an
811 arbuscular mycorrhizal fungus. *Environmental Microbiology* 12: 2083-95
- 812 107. Salvioli A, Ghignone S, Novero M, Navazio L, Venice F, et al. 2016. Symbiosis with an
813 endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic
814 potential. *ISME Journal* 10: 130-44
- 815 108. Scherlach K, Busch B, Lackner G, Paszkowski U, Hertweck C. 2012. Symbiotic
816 cooperation in the biosynthesis of a phytotoxin. *Angewandte Chemie* 51: 9615-18
- 817 109. Schipper MA. 1976. Induced azygospore formation in *Mucor (Rhizomucor) pusillus* by
818 *Absidia corymbifera*. *Antonie Van Leeuwenhoek* 42: 141-4
- 819 110. Schipper MAA, Gauger W, Van Den Ende H. 1985. Hybridization of *Rhizopus* species.
820 *Microbiology* 131: 2359-65
- 821 111. Schmitt I, Partida-Martinez LP, Winkler R, Voigt K, Einax E, et al. 2008. Evolution of
822 host resistance in a toxin-producing bacterial-fungal alliance. *ISME Journal* 2: 632-41
- 823 112. Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H. 2000. Genome sequence of
824 the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* 407: 81-6
- 825 113. Silipo A, Leone MR, Lanzetta R, Parrilli M, Lackner G, et al. 2012. Structural
826 characterization of two lipopolysaccharide O-antigens produced by the endofungal
827 bacterium *Burkholderia* sp HKI-402 (B4). *Carbohydrate Research* 347: 95-98
- 828 114. Smith SE, Read DJ. 2008. *Mycorrhizal Symbiosis*. New York: Academic Press

- 829 115. Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, et al. 2016. A phylum-level
830 phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*
831 108: 1028-46
- 832 116. Tajima F. 1993. Simple methods for testing the molecular evolutionary clock hypothesis.
833 *Genetics* 135: 599-607
- 834 117. Tamas I, Klasson L, Canbäck B, Naslund AK, Eriksson AS, et al. 2002. 50 million years
835 of genomic stasis in endosymbiotic bacteria. *Science* 296: 2376-79
- 836 118. Tang N, San Clemente H, Roy S, Bécard G, Zhao B, Roux C. 2016. A survey of the gene
837 repertoire of *Gigaspora rosea* unravels conserved features among Glomeromycota for
838 obligate biotrophy. *Frontiers in Microbiology* 7: 10.3389/fmicb.2016.00233
- 839 119. Thevenieau F, Nicaud J-M. 2013. Microorganisms as sources of oils. *OCL* 20: D603
- 840 120. Tisserant E, Malbreil M, Kuo A, Kohler A, Symeonidi A, et al. 2013. Genome of an
841 arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis.
842 *Proceedings of the National Academy of Sciences of the United States of America* 110:
843 20117-22
- 844 121. Toomer KH, Chen X, Naito M, Mondo SJ, den Bakker HC, et al. 2015. Molecular
845 evolution patterns reveal life history features of mycoplasma-related endobacteria
846 associated with arbuscular mycorrhizal fungi. *Molecular Ecology* 24: 3485-500
- 847 122. Torres-Cortés G, Ghignone S, Bonfante P, Schübler A. 2015. Mosaic genome of
848 endobacteria in arbuscular mycorrhizal fungi: Transkingdom gene transfer in an ancient
849 mycoplasma-fungus association. *Proceedings of the National Academy of Sciences of the*
850 *United States of America* 112: 7785-90
- 851 123. Trépanier M, Becard G, Moutoglis P, Willemot C, Gagné S, et al. 2005. Dependence of
852 arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. *Applied and*
853 *Environmental Microbiology* 71: 5341-7
- 854 124. Uehling J, Gryganskyi A, Hameed K, Tschaplinski T, Misztal PK, et al. 2017.
855 Comparative genomics of *Mortierella elongata* and its bacterial endosymbiont
856 *Mycosporium cysteinexigens*. *Environmental Microbiology* 19: 2964-83
- 857 125. Uzum Z, Silipo A, Lackner G, De Felice A, Molinaro A, Hertweck C. 2015. Structure,
858 genetics and function of an exopolysaccharide produced by a bacterium living within
859 fungal hyphae. *ChemBioChem* 16: 387-92

- 860 126. Vannini C, Carpentieri A, Salvioli A, Novero M, Marsoni M, et al. 2016. An interdomain
861 network: The endobacterium of a mycorrhizal fungus promotes antioxidative responses in
862 both fungal and plant hosts. *New Phytologist* 211: 265-75
- 863 127. Weber OB. 2014. Biofertilizers with arbuscular mycorrhizal fungi in agriculture. In
864 *Mycorrhizal Fungi: Use in Sustainable Agriculture and Land Restoration*, ed. ZM
865 Solaiman, LK Abbott, A Varma, pp. 45-66. Berlin, Heidelberg: Springer
- 866 128. Wernegreen JJ, Moran NA. 1999. Evidence for genetic drift in endosymbionts
867 (*Buchnera*): analyses of protein-coding genes. *Molecular Biology and Evolution* 16: 83-
868 97
- 869 129. Wewer V, Brands M, Dörmann P. 2014. Fatty acid synthesis and lipid metabolism in the
870 obligate biotrophic fungus *Rhizophagus irregularis* during mycorrhization of *Lotus*
871 *japonicus*. *Plant Journal* 79: 398-412
- 872 130. Weyl EG, Frederickson ME, Yu DW, Pierce NE. 2010. Economic contract theory tests
873 models of mutualism. *Proceedings of the National Academy of Sciences of the United*
874 *States of America* 107: 15712-16
- 875 131. Winsor GL, Khaira B, Van Rossum T, Lo R, Whiteside MD, Brinkman FS. 2008. The
876 *Burkholderia* Genome Database: facilitating flexible queries and comparative analyses.
877 *Bioinformatics* 24: 2803-4
- 878 132. Wöstemeyer J, Schimek C. 2007. Trisporic acid and mating in Zygomycetes. In *Sex in*
879 *Fungi: Molecular Determination and Evolutionary Implications*, ed. J Heitman, JW
880 Kronstad, JW Taylor, LA Casselton, pp. 431-43. Washington, D.C.: ASM Press
- 881 133. Wu TP, Wang T, Seetin MG, Lai Y, Zhu S, et al. 2016. DNA methylation on N6-adenine
882 in mammalian embryonic stem cells. *Nature* 532: 329-33
- 883 134. Yamamura N. 1993. Vertical transmission and evolution of mutualism from parasitism.
884 *Theoretical Population Biology* 44: 95-109
- 885 135. Zhang G, Huang H, Liu D, Cheng Y, Liu X, et al. 2015. N6-methyladenine DNA
886 modification in *Drosophila*. *Cell* 161: 893-906
- 887
- 888

889 **Terms and Definitions**

890 **AMF:** arbuscular mycorrhizal fungi, soil fungi that colonize roots of most terrestrial plants and
891 facilitate plant uptake of mineral nutrients from the soil in exchange for photosynthesis-
892 derived metabolites

893 **Burkholderia EB:** a heritable endosymbiotic bacterium of *Rhizopus microsporus*

894 **CaGg:** ‘*Candidatus Glomeribacter gigasporarum*’, a heritable endosymbiotic bacterium of
895 arbuscular mycorrhizal fungi

896 **CaMg:** ‘*Candidatus Moeniiplasma glomeromycotorum*’, a heritable endosymbiotic bacterium of
897 arbuscular mycorrhizal fungi

898 **EB:** endosymbiotic bacteria

899 **Effective population size:** a parameter that determines the rate of change in the composition of a
900 population caused by genetic drift

901 **Genetic drift:** the process of evolutionary change involving the random sampling of genes from
902 the parental generation to produce the offspring generation

903 **Mc:** *Mycoavidus cysteinexigens*, a heritable endosymbiotic bacterium of *Mortierella elongata*

904 **Me:** *Mortierella elongata*, a soil fungus in the subphylum Mortierellomycotina

905 **Rm:** *Rhizopus microsporus*, a soil fungus in the subphylum Mucoromycotina

906 **Horizontal transmission:** passage of symbionts between hosts of the same generation

907 **Mutualism:** a type of symbiosis in which reciprocal exploitation provides net benefits to each
908 partner

909 **Symbiosis:** the living together of dissimilar organisms

910 **Vertical transmission:** passage of symbionts from one host generation to the next

911 **Zygospor:** a resting spore formed by fusion of gametangia during sexual reproduction of
912 Mucoromycota

913

914 **Reference Annotations**

915 55. Lastovetsky OA, Gaspar ML, Mondo SJ, LaButti KM, Sandor L, et al. 2016. Lipid
916 metabolic changes in an early divergent fungus govern the establishment of a mutualistic
917 symbiosis with endobacteria. *Proceedings of the National Academy of Sciences of the*
918 *United States of America* 113: 15102-07

919 **Host lipid metabolism plays a role in the establishment of the *Rm-Burkholderia* mutualism.**
920 **Some lipid metabolic genes active in this process are only found in early divergent fungi.**

921
922 70. Mondo SJ, Lastovetsky OA, Gaspar ML, Schwardt NH, Barber CC, et al. 2017. Bacterial
923 endosymbionts influence host sexuality and reveal reproductive genes of early divergent
924 fungi. *Nature Communications* 8: 1843

925 ***Burkholderia* EB interact with sexual reproduction in *Rm*. This interaction revealed**
926 **candidate receptors of trisporic acids, mating pheromones unique to Mucoromycotina.**

927
928 69. Mondo SJ, Dannebaum RO, Kuo RC, Louie KB, Bewick AJ, et al. 2017. Widespread
929 adenine N6-methylation of active genes in fungi. *Nature Genetics* 49: 964-68

930 **The m6A DNA modification, which is common in bacteria, is also found in early divergent**
931 **fungi and plays a role in gene activation.**

932
933 71. Mondo SJ, Salvioli A, Bonfante P, Morton JB, Pawlowska TE. 2016. Nondegenerative
934 evolution in ancient heritable bacterial endosymbionts of fungi. *Molecular Biology &*
935 *Evolution* 33: 2216–31

936 **In contrast to degenerately evolving heritable essential EB of insects, genome evolution in**
937 ***CaGg* is non-degenerative.**

938
939 81. Naito M, Pawlowska TE. 2016. Defying Muller’s ratchet: Heritable endobacteria escape
940 extinction through recombination and genome plasticity. *mBio* 7: e02057-15

941 **Genome plasticity counters genomic degeneration in *CaMg*.**

942
943 115. Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, et al. 2016. A phylum-level
944 phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*
945 108: 1028-46

946 **This paper placed AMF into the phylum Mucoromycota and inspired our speculations**
947 **about the impact of EB on the reproductive biology of AMF.**

948
949 **Related Resources**

950 Charlesworth B. 2009. Effective population size and patterns of molecular evolution and
951 variation. *Nature Reviews Genetics* 10: 195-205
952
953

954 **Table 1.** The rate of evolution in *Mc* differs from the evolution rates in other EB and free-living
 955 relatives^a.

956

Ingroup (GenBank accession no.)	Outgroup (GenBank accession no.)	Relative rate statistic ^a
<i>Mycoavidus cysteinexigens</i> FMR23-6 (NZ_DF850521) ' <i>Ca. Glomeribacter gigasporarum</i> ' BEG34 (NZ_CAFB00000000)	<i>Burkholderia phytofirmans</i> PsJN (NC_010681)	22.88****
<i>Mycoavidus cysteinexigens</i> FMR23-6 (NZ_DF850521) ' <i>Ca. Glomeribacter gigasporarum</i> ' IN211 (PRJNA276133)	<i>Burkholderia phytofirmans</i> PsJN (NC_010681)	17.95****
<i>Mycoavidus cysteinexigens</i> FMR23-6 (NZ_DF850521) <i>Burkholderia rhizoxinica</i> HKI454 (NC_014722)	<i>Burkholderia phytofirmans</i> PsJN (NC_010681)	506.65****
<i>Mycoavidus cysteinexigens</i> FMR23-6 (NZ_DF850521) <i>Burkholderia phytofirmans</i> PsJN (NC_010681)	<i>Ralstonia pickettii</i> 12J (NC_010682)	773.73****
<i>Mycoavidus cysteinexigens</i> FMR23-6 (NZ_DF850521) <i>Burkholderia glumae</i> BGR1 (NC_012724)	<i>Ralstonia pickettii</i> 12J (NC_010682)	864.33****

957 ^aResults were obtained using Tajima's 1D relative rate test (116) implemented in MEGA7 (50)
 958 and conducted on DNA sequences at 27 loci listed in **Fig 1**.

959 ^bThe 1D relative rate statistic distribution is the same as the distribution of χ^2 .

960 ****, significant at $P \leq 0.0001$.

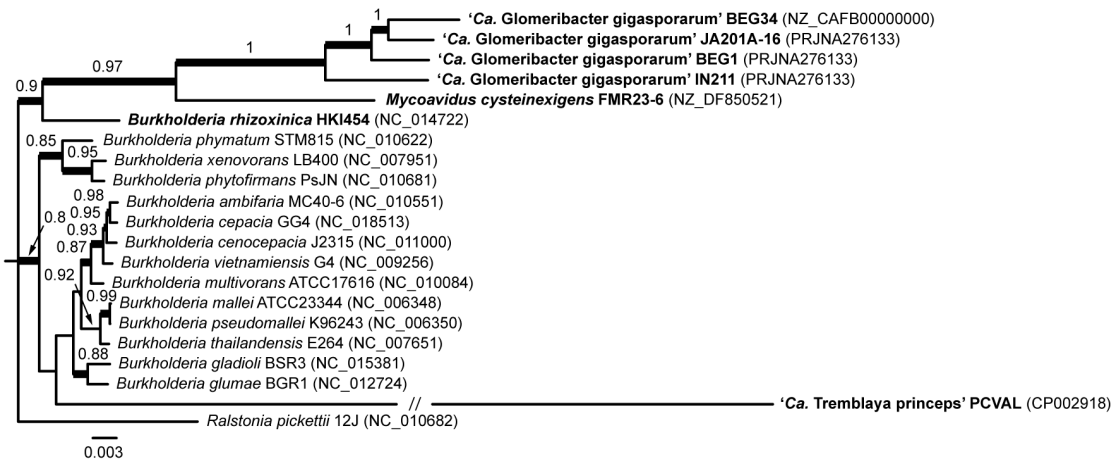
961 **Figure legends**

962 **Figure 1.** Evolutionary history of *CaGg*, *Mc* and *Burkholderia* EB reconstructed using
963 nucleotide sequences at 16S rRNA, 23S rRNA, and 25 protein-coding loci (*nusA*, *pyrG*, *rplA*,
964 *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*,
965 *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, and *rpsS*). Bayesian posterior probabilities over 0.80 are shown
966 above branches. Branches with maximum likelihood bootstrap support over 70% are thickened.
967 Sequences of EB are in bold: *CaGg* of *Gigaspora margarita* BEG34, *CaGg* of *Gigaspora*
968 *margarita* JA201A-16, *CaGg* of *Racocetra castanea* BEG1, *CaGg* of *Cetraspora pellucida*
969 IN211, *Mycoavidus cysteinexigens* of *Mortierella elongata* FMR23-6, *Burkholderia rhizoxinica*
970 of *Rhizopus microsporus*, ‘*Ca. Tremblaya princeps*’ of citrus mealybug *Planococcus citri*.
971 Figure modified from (71).

972
973 **Figure 2.** Phylogenetic placement of ‘*Ca. Moeniiplasma glomeromycotorum*’ based on amino
974 acid sequences at 19 protein-coding loci (*dnaG*, *infC*, *nusA*, *rplA*, *rplB*, *rplC*, *rplE*, *rplF*, *rplM*,
975 *rplN*, *rplP*, *rplT*, *rpmA*, *rpsB*, *rpsC*, *rpsE*, *rpsJ*, *rpsS* and *smpB*). Bayesian posterior probabilities
976 over 0.90 are indicated above branches. Branches with maximum-likelihood bootstrap support
977 over 70 % are thickened. Sequences of *CaMg* are in bold: *CaMg* of *Dentiscutata heterogama*
978 FL654, *CaMg* of *Rhizophagus clarus* NB112A, *CaMg* of *Racocetra verrucosa* VA103A. Figure
979 modified from (79).

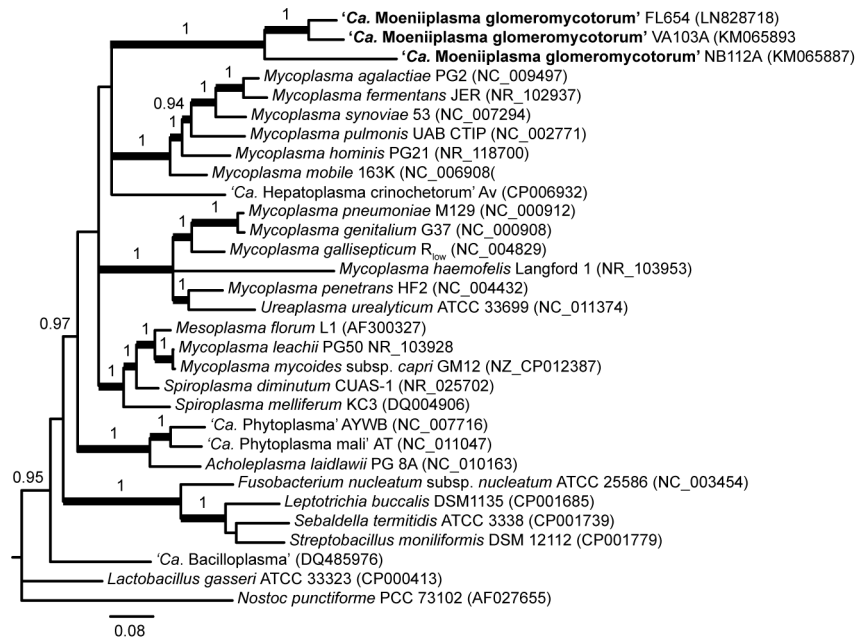
980
981 **Figure 3.** Cartoon representation of phenotypic effects that EB have on their Mucoromycota
982 hosts. *CaGg* improves germ tube extension and branching during pre-symbiotic growth of AMF
983 (left). *Burkholderia* EB interacts with *Rm* asexual sporulation and mating (center); images
984 modified from (70). *Mc* reduces colony expansion in *Me* (right). Red ovals represent EB; fungal
985 structures, including AMF spores and germ tubes, *Rm* zygospores and sporangia with
986 sporangiospores, and *Me* mycelia, are not drawn to scale. FA, fatty acids.

987 **Figure 1**



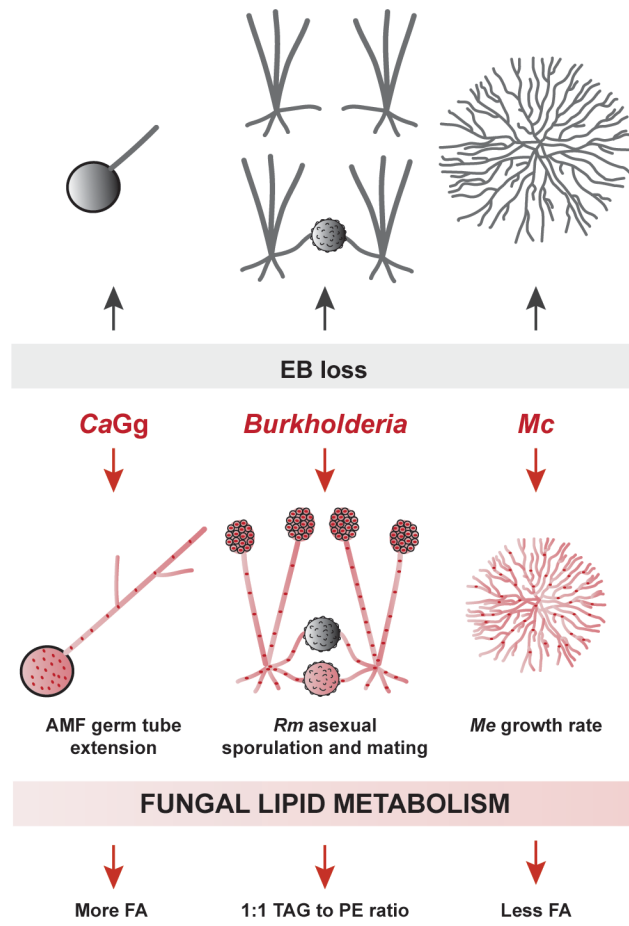
988

989 **Figure 2**



990

991 **Figure 3**



992