Complex behavioral manipulation drives mismatch between host and parasite diversity

Short title: Mismatch between host and parasite diversity

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25 **Abstract**

26 Parasites and hosts are intimately associated such that changes in the diversity of one
 27 partner are thought to lead to changes in the other. We investigated this linked
 28 diversity hypothesis in a specialized ant-*Ophiocordyceps* system in three forests
 29 across 750 km in Central Amazonia. All species belonging to the fungal genus
 30 *Ophiocordyceps* associated with ants have evolved some degree of behavioral control
 31 to increase their own transmission, but the leaf-biting behavior is the most complex
 32 form of host manipulation. Such a system requires control of the mandibular muscles
 33 and a distinct shift in behavior, from climbing vegetation to walking on leaves to
 34 rasping leaf veins in the seconds before death. The need to induce complex behavior
 35 may limit host availability and represent a constraint on parasite diversity. The
 36 consequence for community structure is that complex behavioral manipulation leads
 37 to a mismatch between ant hosts and the diversity of their fungal parasites.

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39 **Keywords:** ants, behavior manipulation, interaction, *Ophiocordyceps*, tropical forests.

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42 **Introduction**

43 Species diversity varies considerably between habitats and regions [1], and the
 44 factors driving such heterogeneity typically depend on the scale of the analysis [2]. At
 45 the local level, interactions among species are known to play an important role in
 46 structuring communities [3]. This is especially the case when the interacting species
 47 occupy different trophic levels, which leads to a stronger link between the diversity of
 48 consumers and the diversity of resources [4–6]. Parasite-host interactions are
 49 examples of such trophic effects. Parasites tend to be host specific with hosts serving
 50 as both the habitat and the dispersal agents for parasites [7]. This implies that changes
 51 in host abundance often lead to changes in parasite abundance. Such specificity is
 52 considered to lead to arms races that promote overall diversity at the community level
 53 [8].

54 Within the framework of linked diversity in host-parasite systems, the sub-set
 55 of parasites that manipulate behavior has not been considered. The effect of parasites
 56 on their hosts is not only to reduce host fitness but in some cases also involves a
 57 manipulation of host behavior that directly increases parasite fitness [9]. In these
 58 cases, other constraints acting on the parasite related to its need to control behavior as
 59 a life-history strategy may affect the coupling of diversity across scales. The
 60 interaction between ants and the ascomycete fungus *Ophiocordyceps* provides a
 61 convenient model for understanding the roles of behavioral manipulation on patterns
 62 of host and parasite diversity. Ants infected by *Ophiocordyceps* species die in specific
 63 locations outside the nest where the microenvironment is ideal for fungal sporulation
 64 and subsequent dispersal to new hosts [10–12]. Dying outside the nest is considered
 65 adaptive for the fungus, because it avoids the cleaning behavior of ant workers that
 66 may prevent the completion of the fungal lifecycle inside the colony [10]. Depending

on which fungal species is involved, infected ants may die attached to stems (Fig. 1C), buried in the leaf-litter, attached to tree bark (Fig. 1D) or biting leafs (Fig. 1E). Biting leaf veins or leaf tissue is the most complex form of manipulation and maintains the ant in situ after death giving the fungus the necessary 24-48 hours to grow adhesive mycelia that bind the ant to the plant [11]. This behavioral manipulation is ancient with vein biting occurring at least since the Eocene [12]. Recent evidence has shown that this host-parasite relationship is highly specific with each host species examined having its own specific parasite species [13].

To explore the linked diversity hypothesis between parasite and host we worked with a large dataset of more than 70,000 samples representing 340 ant species with knowledge on parasite diversity built up from 2,700 samples collected from three Amazonian sites across a 750km transect (Fig. 1A). We specifically compare the composition of infected and non-infected ant species among sites and how the complex behavioral manipulation by the fungus *Ophiocordyceps* can affect the parasite assemblage structure.

Materials and Methods

We sampled ants and their fungal parasites in three Amazonian forests. Two of them (Maracá Ecological Station, 3° 22'N, 6° 127'W and Viruá National Park, 1° 27'N, 61° 01'W) are situated in forest reserves in Roraima State (extreme North of Brazil). The third (Ducke Reserve, 2° 57'S, 59° 56' W) is situated 25 km North of Manaus, Central Amazonia (Fig 1A). The sites cover a latitudinal gradient (~ 750 km) in Amazonian forests and encompass wide environmental heterogeneity, including areas of open and dense forests, and areas subject to different degrees of seasonal flooding [14]. We sampled both, parasitized and non-parasitized ants in 9 plots per

92 site covering approximately an area of 9 km². In each plot, we carefully searched for
 93 infected ants buried in soil/litter, and attached to vegetation and tree trunks: habitats
 94 where the infected ants are most commonly found. The three-dimensional volume
 95 sampled per plot was ~ 500 m³: 250 m length, 1 m wide and 2 m in height, resulting
 96 in 13,500 m³ in total. Two persons sampled each plot for at least 1.5 hours (~ 40
 97 hours/person over the three sites); one focusing on all infected ants and the other on
 98 non-infected ants belonging to the genera infected by *Ophiocordyceps*. In the Upper
 99 Amazon, this and previous research [15] has shown that the following seven ant
 100 genera are infected: *Camponotus*, *Cephalotes*, *Daceton*, *Dolichoderus*, *Ectatomma*,
 101 *Pachycondyla* and *Paraponera* genera. To contrast the assemblage of infected ants
 102 with the whole ant community, we used a comprehensive ant survey of 30 plots per
 103 site, with over 70,000 samples collected. This survey included 900 1 m² litter samples
 104 (Winkler sacks), 900 pitfall-traps and 900 sardine baits regularly distributed among
 105 the three sites to describe the ant assemblage composition (see [14] for additional
 106 details).

107 The data were organized in three matrices: 1) all ant species collected at the
 108 three sites, 2) all species from the genera known to be suitable hosts (i.e. *Camponotus*,
 109 *Cephalotes*, *Daceton*, *Dolichoderus*, *Ectatomma*, *Pachycondyla* and *Paraponera*
 110 genera) and 3) only the species we discovered to be infected. This last category was
 111 created because not all species in a genus are infected. To provide further
 112 understanding of the role of complex behavioral manipulation on ant community-
 113 level patterns, we also constructed matrices of ant species according to the type of
 114 manipulation (Fig 1B). We reduced the dimensionality of all matrices using Principal
 115 Coordinate Analysis (PCoA) based on the Sørensen dissimilarity index.
 116 Presence/absence data were used to avoid overestimation of species with larger nests.

117 We compared assemblage composition between the three areas using non-parametric
118 multivariate analysis of variance [16]. The statistical significance of each analysis was
119 based on 9,999 Monte Carlo permutations.

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122 **Results**

123 We found that the ant assemblage composition was markedly different
124 between areas (npMANOVA, $p < 0.001$; Fig 1F). We recorded 343 species from 58
125 different genera in 11 sub-families. We found very little overlap of species between
126 areas; only 72 out of 343 ant species (~20%) were sampled in each of the three sites.
127 Although we recorded 58 genera of ants only 7 genera contain species that are
128 infected by *Ophiocordyceps* (these are *Camponotus*, *Cephalotes*, *Daceton*,
129 *Dolichoderus*, *Ectatomma*, *Pachycondyla* and *Paraponera*). The assemblage
130 composition of 68 species of ants belonging to these 7 genera also were different
131 between areas (npMANOVA, $p < 0.001$, Fig S1). However, the assemblage of
132 infected ants did not mirror the community structure; either of all ants from all genera
133 or all ants from the genera that we identified as containing infected species. The
134 assemblage composition of species of ants that are infected by *Ophiocordyceps* was
135 not different between sites (npMANOVA, $p = 0.109$; Fig 1G). Put another way,
136 despite the fact that ~20% of the ant species were shared among the three locations
137 the infected ant species were similar between these three very geographically and
138 ecologically different sites. We had expected that different areas would have different
139 infected species assemblages reflecting the general pattern of ant diversity across
140 three sites, i.e. linked diversity between hosts and parasites. The linked diversity in
141 the host-parasite system only matched when the infected ants that are manipulated to

bite into plant tissue are removed from the analysis (npMANOVA, $p < 0.001$, Fig. 1H).

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145 Discussion

146 To infect an ant worker, *Ophiocordyceps* fungus produces spores that are
147 released onto the forest floor or onto vegetation, over both of which foraging ants
148 move. The spores of this fungus are very large, with a relatively thin cell wall and
149 devoid of pigmentation [i.e. hyaline 17], making them sensitive to dehydration and
150 UV radiation. Therefore, for fungal life cycle completion, an ant must pass, at the
151 correct time of the day, over the spores scattered on the forest floor to be infected. In
152 addition, the parasite must overcome the host population structure to complete their
153 life cycle. Ants are very interactive organisms, and the competition between colonies
154 of the same species at local scale has been frequently demonstrated [18]. Colony-
155 colony competition implies an additional barrier for the transmission between
156 colonies of a specialized parasite. However, the complex behavioral manipulation by
157 *O. unilateralis* seems to circumvent these barriers by creating a relative large
158 minefield areas ($\sim 30\text{m}^2$, [11]) where the foraging ants can be infected by spores. This
159 is achieved by very high densities of manipulated/killed hosts in a small area known
160 as graveyards (Pontop et al). Virtually, in all the plots where we found species of ants
161 known to be a host to *O. unilateralis* complex we also found ants infected by fungi
162 within the *O. unilateralis* complex. And over the 750 km range the same group of
163 *Camponotus* species were infected despite those areas having limited overlapp in
164 *Camponotus* species assemblage (Fig 2). Other groups of *Ophiocordyceps* that infect
165 ant species with large colonies, such as the ant genera *Cephalotes* and *Dolichoderus*
166 (both included in this study) also create graveyards, but in smaller areas (personal

167 observations). In the latter case, the dead ants died attached in one tree trunk [17], and
168 sites without any infected ants were more common (Fig 2).

169 Biting behavior requires a control of the mandibular muscles that involves a
170 reduction in muscle organelle abundance [12]. It also requires a distinct shift in
171 behavior in the seconds before biting as infected ants shift from a wandering behavior
172 to rasping of either the major veins or leaf edges. Other complexes of *Ophiocordyceps*
173 cause ants to die on leaves (*O. lloydii*, 17) but they do not cause ants to bite into the
174 plant tissue. We suggest that the nature of complex manipulation and the necessary
175 additional control of the host's phenotype that is entailed limit the potential host range
176 of fungi investing in manipulation. Transmission requires this complex control of
177 behavior, which in turn requires multiple effects at the physiological and neuronal
178 level. The consequence for community structure is that even across large geographical
179 areas, complex behavioral manipulation results in a mismatch between host and
180 parasite diversity patterns. However, despite this apparent constraint the evolution of
181 behavioral manipulation seems to be a successful strategy and ant hosts of *O.*
182 *unilateralis* group were by far the most abundant hosts we discovered.

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193 **References**

- 194 1 Gaston, K. J. 2000 Global patterns in biodiversity. *Nature* 405, 220–227.
- 195 2 Ricklefs, R. E. 2004 A comprehensive framework for global patterns in
196 biodiversity. *Ecology Letters* 7, 1–15. (doi:10.1046/j.1461-0248.2003.00554.x)
- 197 3 Chase, J. M. & Leibold, M. A. 2003 Ecological niches: linking classical and
198 contemporary approaches. Chicago: The University of Chicago Press.
- 199 4 Polis, G. A. & Strong, D. R. 1996 Food Web Complexity and Community
200 Dynamics. *American Naturalist* 147, 813–846.
- 201 5 Gravel, D., Massol, F., Canard, E., Mouillot, D. & Mouquet, N. 2011 Trophic
202 theory of island biogeography. *Ecology letters* 14, 1010–6. (doi:10.1111/j.1461-
203 0248.2011.01667.x)
- 204 6 Janz, N., Nylin, S. & Wahlberg, N. 2006 Diversity begets diversity: host
205 expansions and the diversification of plant-feeding insects. *BMC evolutionary*
206 *biology* 6, 4. (doi:10.1186/1471-2148-6-4)
- 207 7 Combes, C. 2001 The ecology and evolution of intimate interactions. Chicago:
208 University of Chicago Press.
- 209 8 Hudson, P. J., Dobson, A. P. & Lafferty, K. D. 2006 Is a healthy ecosystem one
210 that is rich in parasites? *Trends in Ecology & Evolution* 21, 381–385.
- 211 9 Poulin, R. 2011 Parasite Manipulation of Host Behavior: An Update and
212 Frequently Asked Questions. In *Advances in the Study of Behavior* (ed H. J.
213 Brockmann), pp. 151–186. Burlington: Elsevier.
- 214 10 Andersen, S. B., Gerritsma, S., Yusa, K. M., Mayntz, D., Hywel-Jones, N. L.,
215 Billen, J., Boomsma, J. J. & Hughes, D. P. 2009 The life of a dead ant: the

- 216 expression of an adaptive extended phenotype. *American naturalist* 174, 424–33.
217 (doi:10.1086/603640)
- 218 11 Pontoppidan, M., Himaman, W., Hywel-Jones, N. L., Boomsma, J. J. & Hughes,
219 D. P. 2009 Graveyards on the move: the spatio-temporal distribution of dead
220 *Ophiocordyceps*-infected ants. *PloS one* 4, e4835.
221 (doi:10.1371/journal.pone.0004835)
- 222 12 Hughes, D. P., Wappler, T. & Labandeira, C. C. 2011 Ancient death-grip leaf
223 scars reveal ant-fungal parasitism. *Biology letters* 7, 67–70.
224 (doi:10.1098/rsbl.2010.0521)
- 225 13 Evans, H. C., Elliot, S. L. & Hughes, D. P. 2011 Hidden diversity behind the
226 zombie-ant fungus *Ophiocordyceps unilateralis*: four new species described from
227 carpenter ants in Minas Gerais, Brazil. *PloS one* 6, e17024.
228 (doi:10.1371/journal.pone.0017024)
- 229 14 Baccaro, F. B., De Souza, J. L. P., Franklin, E., Landeiro, V. L. & Magnusson,
230 W. E. 2012 Limited effects of dominant ants on assemblage species richness in
231 three Amazon forests. *Ecological Entomology* 37, 1–12. (doi:10.1111/j.1365-
232 2311.2011.01326.x)
- 233 15 Sanjuán, T., Henao, L. G. & Amat, G. 2001 Distribución espacial de *Cordyceps*
234 spp. (Ascomycotina: Clavicipitaceae) y su impacto sobre las hormigas en selvas
235 del piedemonte amazónico de Colombia. *Revista de Biología Tropical* 49, 945-
236 955.
- 237 16 Anderson, M. J. 2006 A new method for non-parametric multivariate analysis of
238 variance. *Austral Ecology* 26, 32–46.

- 239 17 Evans, H. C. 2002 Entomopathogenic fungi associated with ants (Formicidae): a
240 review. In *Trichomycetes and Other Fungal Groups* (eds M. JK & H. BW), pp.
241 119–144. Enfield: Science Publishers.
- 242 18 Parr, C. L. & Gibb, H. 2010 Competition and the role of dominant ants. In *Ant*
243 Ecology (eds Lach L C. L. Parr & K. L. Abbott), pp. 77–96. Oxford: Oxford
244 University Press.

Figure 1. (A) Map of study area. (B) Relative infection levels by ant genera showing where the ants died and the seven more specious non-infected genera sampled in 27 plots (note that *Pheidole* bar is at different scale). Black bar shows the number of infected species and gray bars the number of non-infected species. (C) *Pachycondyla inversa* infected by *Ophiocordyceps kniphofioides* var. *ponerinarum* attached to a stem. (D) *Cephalotes atratus* killed by *O. kniphofioides* var. *kniphofioides* buried in the mosses of a tree trunk. (E) *Camponotus atriceps* parasitized by *O. unilateralis* s.l. biting a leaf edge. PCoA ordination plots indicating (F) the differences in species composition among the three sites using all data, (G) congruence in species composition of all infected ant species found in 25 out of 27 plots and (H) different assemblage composition of non-biting infected ants. Some plots are stacked in the last two figures, because had the same infected ant species composition.

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Figure 2. Distribution of ant species ordered by the first axis of PCoA analysis in study plots at Maracá, Viruá and Ducke sites. Occurrence of uninfected ants are in gray. Plots where ant species were infected by *unilateralis* complex (biting plant tissue) are showed in red. Blue and green bars represent plots were ants infected by *australis* and *kniphofioides* complex were found, respectively. In the later case, the infected ants were found on litter and buried in tree trunks.