

1 **Ecological change and conflict reduction led to the evolution of a** 2 **transformative social behavior in ants**

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10 **Abstract**

11 Behavioral innovations can be ecologically transformative for lineages that perform them and for
12 their associated communities. Many ecologically dominant, superorganismal, and speciose ant
13 lineages use a mouth-to-mouth fluid exchange behavior – trophallaxis – to share both exogenously
14 sourced and endogenously produced materials across their colonies, while lineages that are less
15 abundant, less cooperative and less speciose tend not to perform this behavior. How and why this
16 behavior evolved and fixed in only some ant lineages remains unclear and whether this trait enables
17 ants' ecological dominance is not yet understood. Here we show that trophallaxis evolved in two
18 major events ~110 Ma in lineages that today encompass 36% of ants, and in numerous smaller and
19 more recent events. We found that trophallaxis evolved early only in ant lineages that had reduced
20 intra-colonial conflict by losing workers ability to reproduce. Our causal models indicate that this
21 signature behavior of superorganismal ants required social cooperation and ecological opportunism,
22 and likely contributed to the large colony sizes and speciation patterns of the ants that use it and
23 dominate our landscapes today. We hypothesize that the early evolution of trophallaxis was brought
24 about by a major shift in terrestrial ecosystems through the origin and diversification of flowering
25 plants and the consequent opportunistic inclusion of nectar and sap-sucker honeydew in the ant diet.

26

27 **Keywords:** behavior, trophallaxis, evolution, phylogenetic path analysis, comparative phylogenetic
28 methods, speciation, Bayesian neural network

29

30 Introduction

31 Changes in intra- and inter-species behavior have had dramatic impacts on the ecology of our
32 planet. Novel behaviors can impact not only a species' own evolutionary trajectory through selection,
33 speciation and extinction dynamics, but also the evolutionary trajectories of associated species, and
34 even entire ecosystems¹⁻³. The evolution from carnivorous wasps to nectar- and pollen-foraging
35 bees⁴ was transformational for plant reproduction with the evolution and co-evolution of countless
36 pollination syndromes and adaptations and likely promoted the diversification of many flowering plant
37 clades^{5,6}. Occasionally new behaviors and their associated morphology can be so crucial, that
38 through selection, speciation and extinction dynamics, entire clades exclusively display central
39 adaptations around the behavior. This occurred in the evolution of mammals when a novel social
40 transfer of material evolved – milk⁷⁻¹⁰. Lactation and nursing behaviors were so essential to the
41 mammalian lineage that these traits have been retained across the 300 Ma long evolutionary history
42 and among the >4000 living species⁷.

43 Behavioral traits and associated phenotypic adaptations were central to the evolution of one of the
44 most ecologically dominant invertebrate clades: the ants^{3,11,12}. The ant phylogeny holds extreme
45 diversity in ecology, morphology, life-history traits, but also in behavior. At one extreme are the living
46 descendants of early-branching lineages with small, often monomorphic colonies of predatory
47 specialists, and at the other extreme are the more derived ecosystem-dominating generalist species
48 with colossal colonies and high levels of queen-worker dimorphism. The latter show dramatic
49 reproductive division of labor, to the point where a single colony can be considered a superorganism,
50 where the sexuals are the germline and sterile workers are the soma¹³.

51 A majority of ecosystem-dominating generalist ants perform a behavior called stomodeal
52 trophallaxis, wherein adult workers regurgitate the contents of their crop to nestmates in a mouth-to-
53 mouth interaction¹⁴, though it is not clear why these traits appear to co-vary. It has been suggested
54 that trophallaxis behavior evolved to transport sugary liquids acquired through mutualisms with sap-
55 suckers or with plants^{15,16}. Species that use trophallaxis tend not to have a sting and tend to have
56 sterile workers or workers that cannot be mated, suggesting that these traits might prohibit the

57 evolution of trophallaxis¹⁴. In some ants where trophallactic fluid has been characterized
58 (*Camponotus*), it has been shown to contain not only food but also endogenously produced materials
59 including proteins, small molecules, RNA and even hormones that shift with colony maturation¹⁷⁻¹⁹.
60 This suggests that in such superorganismal species, trophallaxis behavior may enable a form of
61 social circulatory system or shared metabolism across the colony^{14,19}, and large colonies have been
62 proposed to require more coordination²⁰⁻²². At present, the evolutionary dynamics that led to the
63 single or repeated origins of this behavioral trait remain largely unexplored. Similarly unclear is the
64 link between trophallaxis and the emergence of superorganismality. Finally, it is not known whether
65 trophallaxis, like lactation and nursing in mammals, is an irreversible key innovation or a volatile trait
66 in ants.

67 In this study, we tested whether trophallaxis behavior enabled the shift from predaceous ant
68 ancestors to more modern generalists, assessed which pressures led trophallaxis to evolve and how
69 this behavior impacted speciation. We combine deep learning predictions, phylogenetic comparative
70 methods and causal models to infer the trophallaxis behavior across hundreds of ant species and
71 reconstruct its evolution and impact on the long diversification history of this clade.

72 **Results**

73 After compiling all records of trophallaxis from the literature (265 papers, 205 species, Supp Table1),
74 we used a Bayesian neural network to train a predictive model able to infer the presence or absence
75 of this behavior based on phylogenetic, ecological, and morphological data and life-history traits,
76 which could be collected for a larger number of species. These included the inclusion of sugary liquid
77 food in the diet, colony size, presence of workers with full reproductive potential, presence of sting,
78 and proxies for climate preferences (see Methods and Supplementary Discussion for more
79 information).

80 Our predictive model reached a cross-validation test accuracy of 84% (Supp Table 2). Since a BNN
81 inherently quantifies the uncertainty around predictions, we could identify a posterior probability
82 threshold above which the expected accuracy increases to 95%. Using this threshold, 24% of the
83 species in the test set were classified as uncertain. To further assess the accuracy of our model we

84 reached out to myrmecologists with expertise on certain species groups to have their determination
85 of trophallaxis behavior and compared their assessment with our predictions. Our model's
86 myrmecologist accuracy was 88% and included both false negatives and false positives (Supp Table
87 2)

88 Using feature permutation, we identified phylogeny (Δ accuracy of 4.5%), the inclusion of sugary
89 liquid in the diet (Δ accuracy of 4.5%), presence or absence of a sting (Δ accuracy of 2.3%) and
90 presence or absence of reproductive workers (Δ accuracy of 1.7%) as the most important features
91 for the prediction of trophallaxis behavior (Supplement Fig 1).

92 With our trained model we predicted trophallaxis behavior for 253 additional species, thus more than
93 doubling the taxonomic coverage for this trait and representing 15 of the 17 ant subfamilies.

94 **The ancestral ant**

95 To understand the evolutionary context around the emergence of trophallaxis, we used phylogenetic
96 comparative methods and included four additional traits in our analyses for each species: sugary
97 liquids in the diet, workers that can be mated (referred to hereafter as reproductive workers), sting,
98 and colony size. This allowed us to assess the concerted evolution of traits, rates of transition and
99 ancestral phenotypes.

100 We estimated that the ancestral ant had reproductive workers (posterior probability, $pp = 0.983$) and
101 a sting ($pp = 0.999$), while it was unlikely to rely on sugary liquids ($pp = 0.037$) and did not engage
102 in trophallaxis ($pp = 0.002$) (Figure 1, Supp Fig 2). The ancestral states of the maximum clade
103 credibility (MCC) tree for colony size were ambiguous, and thus, we cannot assess how the
104 evolutionary history of colony size interplays with the evolution of trophallaxis.

105 Our ancestral state reconstructions indicated that workers with full reproductive potential were lost
106 repeatedly in up to six large events between 125 and 90 Ma, and in up to seven events around 60-
107 50 Ma. Gamergates (a form of fully reproductive workers) were secondarily acquired once in the
108 lineage leading to *Metapone madagascariensis* (Myrmicinae) (Fig 1B, Fig 2). Sting was lost at least

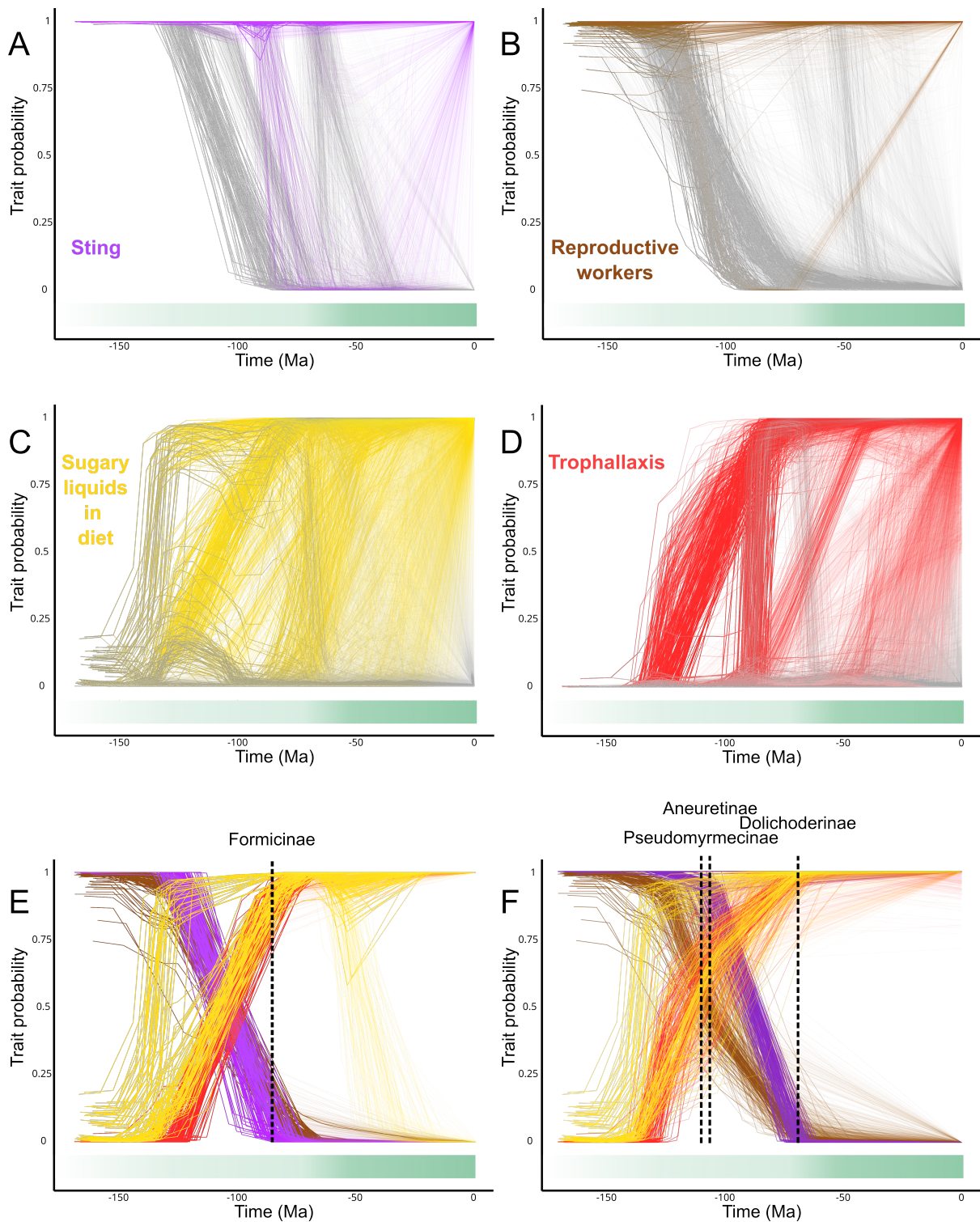
109 nine times starting between 115 and 90 Ma and was secondarily evolved at least four times, all within
110 the genus *Stenammina* (Myrmicinae) (Fig 1A).

111 The integration of sugary liquid in the ant diet likely evolved independently multiple times, in few
112 events occurring 140-100 Ma and many more sporadic events 75-10 Ma (Fig 1C, Fig 2). The first
113 event temporally overlapped with a phase of rapid diversification of early flowering plants (~110
114 Ma)²³, and the latter with the time when angiosperms became dominant following the End
115 Cretaceous mass extinction (66-50 Ma)²⁴. These ecological shifts led to the origination of new
116 biomes and ecosystems for ants to conquer, new food sources for sap-suckers to exploit, and thus
117 highly abundant low-risk food sources (nectar and honeydew) upon which ants could forage.

118 Mirroring the adoption of sugary liquid in the diet, we found that two of the five biggest ant subfamilies
119 evolved trophallaxis approximately 110 Ma (Formicinae and Dolichoderinae, Fig 1E and 1F, Fig 2).
120 This was followed by more sporadic losses and gains of trophallaxis in other subfamilies throughout
121 the Cenozoic (Fig 1D).

122 **Conditions for the evolution of trophallaxis**

123 Analyzing the coherent timing across the evolution of traits in different ant lineages, a pattern
124 emerges for the conditions that allowed this social transfer to evolve. The most frequent sequence
125 of evolved traits observed across all our ant species was the early (140-90 Ma) integration of sugary
126 liquid in the diet, the early loss of worker reproductive potential (100-110 Ma) and the early evolution
127 of trophallaxis (120-90 Ma). This sequence of events occurred in the two distinct early evolutions of
128 trophallaxis (Fig 1E and 1F, Fig 2). These two events make up four ant subfamilies
129 (Pseudomyrmeiinae, Dolichoderinae, Aneuretinae and Formicinae) that together account for ~36%
130 of all ant extant species. This leads us to the hypothesis that trophallaxis behavior, a novel social
131 transfer of material⁷, only evolves given the pressure to share liquid food and when intra-colonial
132 reproductive conflict is sufficiently reduced.



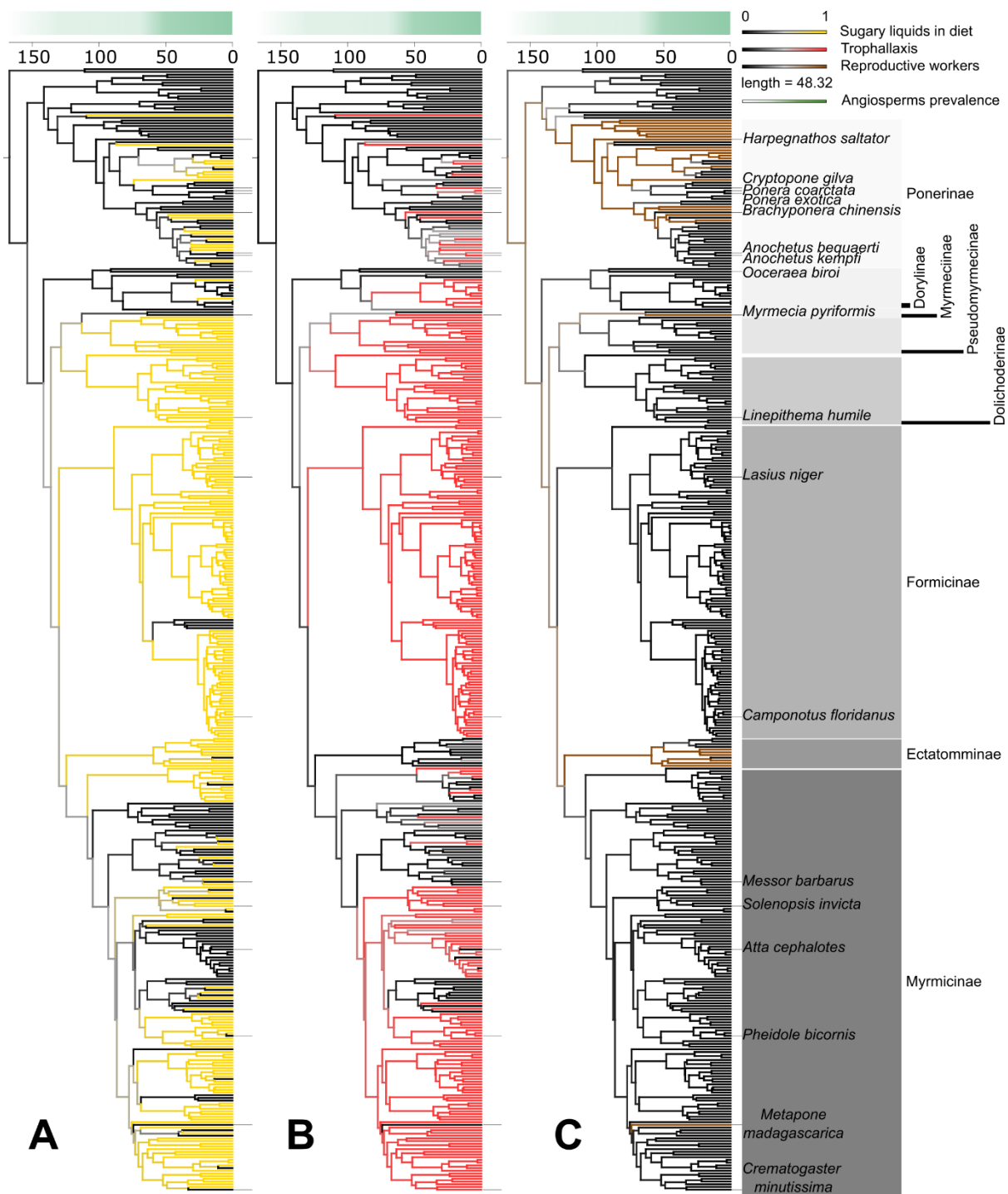
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134 **Figure 1:** Phenograms showing the probability of presence ($p = 1$) or absence ($p = 0$) of phenotypic
135 and behavioral traits in ants inferred through phylogenetic comparative methods. The inferred
136 probabilities are mapped along the branches of the ant phylogeny and plotted against time to show
137 the timing of gains and losses. The traits are A) sting, B) reproductive workers, C) sugary liquids
138 presence in the diet and D) trophallaxis behavior. All traits are shown for two large ant clades that
139 acquire trophallaxis early in their evolution, E) Formicinae and F) Aneuretinae, Pseudomyrmecinae

140 *and Dolichoderinae. The phenograms are based on a posterior sample of 100 trees. To account for*
141 *the uncertainty of predicted presence or absence of trophallaxis across living species we used their*
142 *posterior probability as inferred by the BNN model rather than its binary predictions (see Methods).*
143 *On each graph angiosperm prevalence is indicated by a gradient of opacity based on Benton 2022²⁴.*

144

145 Looking across the ant phylogeny, most ant lineages that did and did not evolve trophallaxis did so
146 in accordance with our hypothesis. In the two early gains of trophallaxis (Fig 1E and 1F, Fig 2), ants
147 first began opportunistically eating sugary liquids when these novel food sources became abundant
148 (Fig 2A). Shortly after or at the same time, trophallaxis evolved (Fig 2B), likely as a safe and efficient
149 way to transport and share these new liquid food sources, but trophallaxis only evolved early on in
150 lineages that had also reduced intra-colonial conflict by blocking worker reproductive potential (Fig
151 2C). The gain of liquid food in the diet alone was insufficient to gain trophallaxis behavior as
152 evidenced by Ectatomminae (Fig 2 & Supp Fig 3). Ectatomminae began drinking sugary liquids
153 during the early diversification of flowering plants, but none evolved trophallaxis. Some
154 Ectatomminae species lost worker reproductive potential rather late, about 40-25 Ma, and yet they
155 still do not perform true trophallaxis. This indicates that there was either some form of critical period
156 connected to ecological changes, or that these species may be transitioning to using trophallaxis,
157 perhaps through mandibular pseudotrophallaxis^{16,25}. In multiple other subfamilies, the species
158 present in our dataset never evolved trophallaxis in accordance with our hypothesis (Leptanillinae,
159 Apomyrminae, Proceratiinae, Amblyoponinae and Agroecomymecinae, Fig 2): sugary liquids were
160 never integrated into the diet, workers lost full reproductive potential, and trophallaxis never evolved.
161 This indicates that the reduction of intra-colonial conflict is insufficient to drive the evolution of
162 trophallaxis.



163

164 **Figure 2:** Ancestral state reconstruction integration of A) sugary liquid food in the diet, B)
 165 trophallaxis, and C) presence of reproductive workers on the MCC tree for 416 ant species. Colored
 166 branches indicate that the trait is likely present at a given time (in Ma) and black that it is absent.
 167 Major subfamilies of interest are labeled. The green gradient illustrates the prevalence of
 168 angiosperms as described in Benton & al. 2022²⁴.

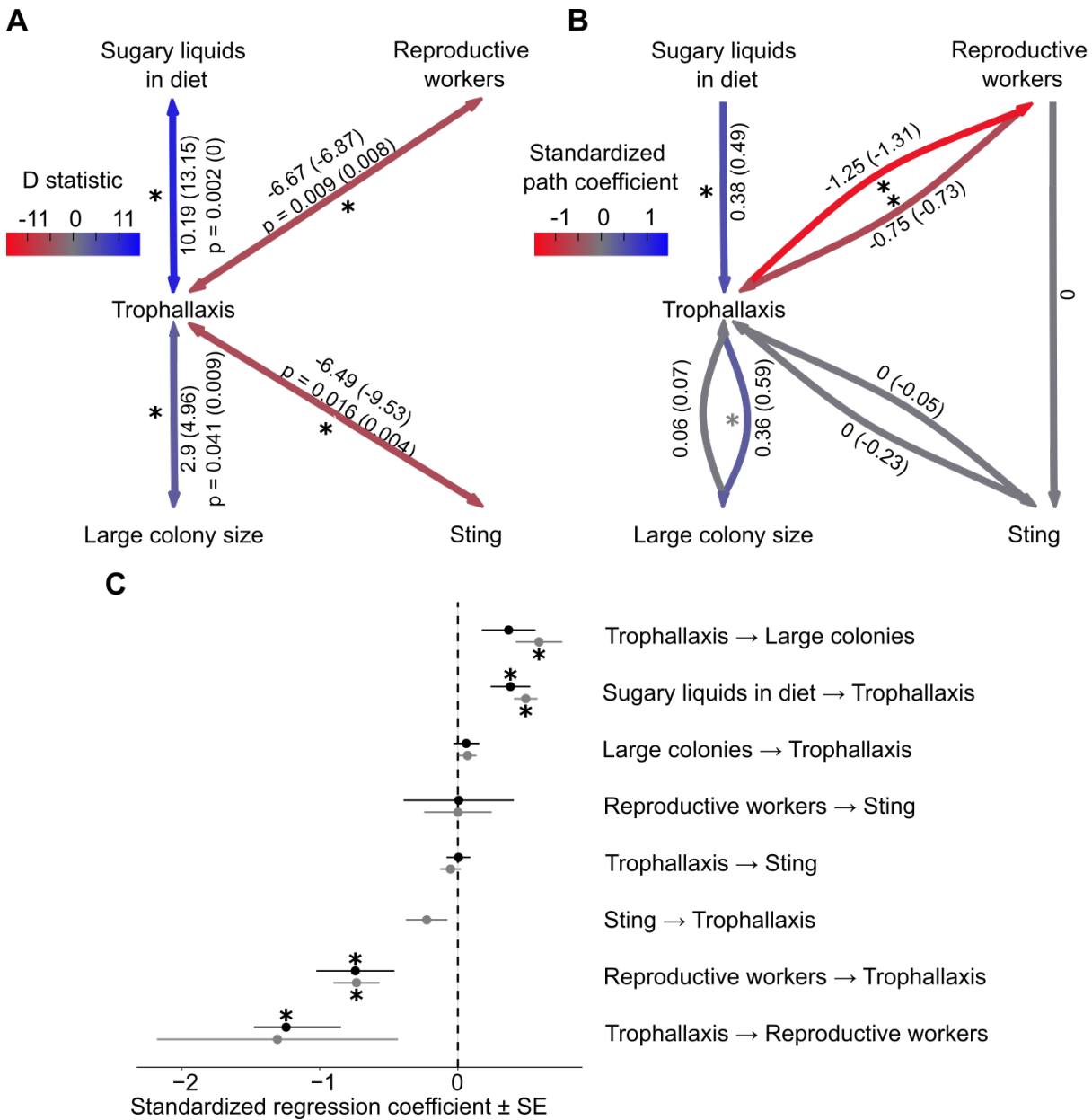
169 In Ponerinae and Myrmicinae, the evolutionary patterns of our four traits are complex, as almost all
170 traits evolved and/or were lost multiple times over a large range of dates. Our ancestral state
171 reconstructions are ambiguous about whether the most recent common ancestor of Myrmicines had
172 already begun eating sugary liquids (Fig 2, Supp Fig 3), and thus conclusions are difficult for this
173 particular subfamily. Ponerine ants rarely perform trophallaxis (16/56 species in our dataset), but
174 those that did evolve trophallaxis also lost worker reproductive potential somewhat consistent with
175 our hypothesis. In Myrmicines, there are many genera and species whose trait evolution is consistent
176 with our model (e.g. *Tetramorium*). However, we also have many Myrmicinae species not known to
177 drink sugary liquids but known or predicted to use trophallaxis, such as *Pheidole bicornis* or
178 *Crematogaster minutissima*. One species, from the subfamily Myrmeciinae, *Myrmecia pyriformis* is a
179 clear exception to our model, in that they have reproductive workers, do not drink sugary liquids and
180 use trophallaxis²⁶.

181 **Traits shaping evolution of trophallaxis**

182 To understand how these traits correlated with trophallaxis over the evolutionary history of ants, we
183 used a phylogenetic d-test²⁷ (Fig 3A). We found that diet including sugary liquid (p statistic = 0.002),
184 absence of reproductive workers (p statistic = 0.009), absence of sting (p statistic = 0.016) and large
185 colony size (p statistic = 0.041) all correlated with trophallaxis. Small (p statistic = 0.136) and medium
186 (p statistic = 0.182) colony sizes did not.

187 To disentangle causality in these correlations, we used phylogenetic path analysis using
188 phylogenetic generalized least squares and d-separation²⁸. We built models of the trophallaxis-
189 correlated traits based on the results of the d-test (Supp Fig 4 & 5), and the best models, according
190 to AIC, were averaged (Fig 3 B and C). We found that sugary liquids in the ant diet had a significant
191 and positive effect on trophallaxis behavior (effect size 0.38 on only known data, 95% CI [0.09; 0.66]),
192 consistent with our observations given the ancestral states. Trophallaxis, in turn, increased the
193 likelihood of large colony sizes (0.36, 95% CI [-0.02, 0.74]), but large colony sizes did not increase
194 the likelihood of trophallaxis (0.06, 95% CI [-0.13, 0.24]). This indicates that trophallaxis likely did not
195 evolve as a means to better coordinate large colonies, but rather may have improved coordination

196 once the behavior had already been adopted for food sharing. Trophallaxis behavior strongly
 197 promoted the loss of reproductive workers (-1.25, 95% CI [-2.02; -0.47]), and reproductive workers
 198 strongly inhibited the evolution of trophallaxis (-0.75, 95 % CI [-1.3; -0.19]), indicating that
 199 reproductive conflict is likely a much more potent inhibitor of social sharing than previously
 200 considered. The results of both the correlation and path analyses on the evolution of sting indicate
 201 that while lack of sting may correlate with trophallaxis, there is unlikely to be a causal relationship.



202

203 **Figure 3: Representation of correlation and causality of traits with trophallaxis.** A) Correlation
 204 of traits with trophallaxis according to d-test with the D statistic when analyzes are launched on the
 205 known and verified 211 species, and on all species including those with imputed data in parentheses

206 *and the corresponding p-values. B) Phylogenetic path analysis based on the PGLS model-averaging*
207 *with the standardized regression coefficient when analyzes are launched on the known and verified*
208 *211 species, and on all species including those with imputed data in parentheses. Nodes indicate*
209 *the presence of the trait. Red edges indicate a negative correlation while blue edges mark a positive*
210 *correlation. C) The standardized path coefficient between traits and standard error for the*
211 *phylogenetic path analysis only on the 211 species for which we know the trophallaxis behavior,*
212 *either from literature or experts (in black) or on the full dataset, including imputed data (in gray).*
213 *Stars indicate significance based on 95% confidence intervals for one (gray) or both datasets (black).*

214 **Speciation and extinction analyses**

215 Given that some of the most species-rich lineages of ants engage in trophallaxis, we tested whether
216 trophallaxis could have impacted speciation and extinction events over the ant phylogeny. Using a
217 Bayesian Analysis of Macroevolutionary Mixtures (BAMM)²⁹ with STructured Rate Permutations on
218 Phylogenies test (STRAPP)³⁰ we found that trophallaxis significantly lead to speciation events ($p =$
219 0.0423), with lineages engaging in trophallaxis showing 1.6x higher speciation rates (no trophallaxis
220 $= 0.07 \pm 0.004$; trophallaxis $= 0.11 \pm 0.007$) and 2.8x higher extinction rates (no trophallaxis $=$
221 0.007 ± 0.004 ; trophallaxis $= 0.02 \pm 0.007$). However, when accounting for the possibility of other,
222 unmeasured traits affecting diversification using a state-dependent model with hidden states
223 (HiSSE)³¹, we found that the preferred model implied character independent diversification with three
224 hidden states ($\Delta AIC > 8000$; Supp Table 3). Thus, the HiSSE model rejects a direct link between
225 trophallaxis and increased speciation rates. While this discrepancy between methods can derive
226 from model identifiability issues^{32,33}, it can also indicate that trophallaxis might have played an
227 indirect role in ant diversification or that speciation patterns cannot be ascribed solely to this trait.

228 **Discussion**

229 Our results suggest that trophallaxis behavior evolved to allow transport of sugary liquids that
230 became abundant first during the diversification of angiosperms and more so later when
231 angiosperms became dominant. This represents a key shift from a specialist diet (on insect prey) to

232 a generalist diet (including both prey and sugary liquids). This type of dietary shift, in other systems,
233 has been linked to faster diversification^{34,35}, range expansion^{36,36,37} and invasion^{38–41}. In spite of clear
234 advantages to becoming a generalist, not all ants did so in their early evolutionary history when
235 sugary liquids became abundant – ant lineages that retained the worker ability to reproduce did not
236 evolve the social transfer of trophallaxis. Only the ant lineages that became sugar-eating generalists
237 and also reduced intra-colonial conflict early in their evolutionary history, losing the worker capacity
238 to reproduce, evolved trophallaxis. Extrapolating from our dataset to the extant number of species
239 in these clades, this sequence of trait evolution likely occurred in the evolution of about 36% of all
240 ant species, the most consistent pattern of trait evolution over time across our dataset.

241 Behavior has been proposed to both drive evolution and to buffer it¹ and indeed there is evidence
242 for both in different contexts. Studying the impact of behavior on macroevolution is often difficult
243 because we cannot easily measure behavior from fossils and it can be difficult to access some extant
244 species. Here we have adapted new deep-learning tools to impute behavior, allowing us to bridge
245 this gap and study the evolution of a complex behavior over a large phylogeny. Bayesian neural
246 networks provided a flexible tool to integrate multiple traits along with phylogenetic structure in our
247 predictions, while embracing and explicitly quantifying the uncertainties around them. In our
248 analytical framework these uncertainties were then propagated to the comparative phylogenetic
249 analysis and ancestral state estimations to provide robust inferences.

250 Trophallaxis likely first evolved in ants as a mode of sharing exogenous food, yet in ants that perform
251 this behavior frequently (e.g. *Camponotus*), trophallactic fluid has become a rich and complex
252 socially transferred material similar to mammalian milk^{17,19}. Various proteins and small molecules
253 used elsewhere in ant physiology have been co-opted and neo- or sub-functionalized as they
254 became part of trophallactic fluid¹⁸. Juvenile hormone and juvenile hormone esterase provide an
255 example of developmental hormones and hormone regulators that are critical in insect development,
256 that once co-opted to trophallactic fluid, allow workers to manipulate larval development through
257 adult-to-larva trophallaxis^{17,18}. Once a social transfer, like milk-feeding or trophallaxis, evolves it
258 creates a private physiological channel between bodies that has far reaching implications for social
259 life and the evolutionary and ecological success of social organisms⁷. A potentially important

260 consequence of the evolution of trophallaxis is the possibility of an individually tailored diet for larvae,
261 which more easily allows body-size variation between otherwise genetically equivalent individuals.
262 Such asymmetries are hallmarks of committed cooperative systems after major evolutionary
263 transitions in individuality⁴², as they can entrench a cooperative system into rigid specialization.

264 Our scenario for the evolution of trophallaxis in ants differs from the evolutionary path inferred for
265 wasps, another eusocial hymenopteran lineage^{43,44}. In wasps, trophallaxis likely evolved from adults
266 pre-chewing prey and providing it to larval young, and generally, adult-adult trophallaxis only occurs
267 in highly eusocial wasps⁴³. In ants, because the data on larval-adult trophallaxis are so sparse¹⁴, we
268 were not able to explore the possibility that another social transfer may have played a role in the
269 evolution of trophallaxis. Adult-adult stomodeal trophallaxis alone is truly a continuous trait rather
270 than a binary one, where some species use the behavior only in the context of food (most Ponerines
271 that engage in trophallaxis), while others perform it routinely across contexts (*Camponotus*). Looking
272 at our model's predictions for the Myrmicinae or Dorylinae (our BNN predicted that New World army
273 ants use trophallaxis, even though, to our knowledge, trophallaxis behavior has never been
274 reported), it is possible that the use of some other form of social transfer has led to our model's
275 uncertainty or unexpected predictions. Further investigation of these clades would help verifying the
276 predictions and refining the model.

277 Our result, that ancestral ants likely had workers that could mate, brings significant doubt to the idea
278 that all ants are superorganismal. It has previously been postulated that all ants are
279 superorganismal¹³ because they have passed a 'point-of-no-return' of having morphologically fixed
280 reproductive division of labor. A main point of evidence for this comes from fossils of ants from a
281 sister clade to crown Formicidae where winged queens and unwinged workers were fossilized
282 together in the same group⁴⁵. However, the winged individuals may have been dispersal morphs
283 while workers may have retained reproductive potential in the form of a functional spermatheca⁴⁶.
284 These two conditions are met in the extant ant *Harpegnathos saltator* known to have both "true
285 queen" (winged) and gamergates⁴⁷ (reproductive workers). Others have also noted this potential
286 ambiguity in the fossil record⁴⁸. Investigations of fossil worker spermathecae could indicate whether
287 ancestral ant workers could be mated. Our result suggests that gamergates are not secondarily

288 evolved⁴⁹, but rather that reproductive workers were present in the common ancestor to all ants.
289 This, in turn, means that according to Boomsma's definition of superorganismality, the common
290 ancestor to all ants had not passed this point of no return and that fully reproductive workers may
291 have been retained as a plesiomorphic trait, and thus not all ants are superorganismal. Our results
292 indicate instead that superorganismality evolved multiple times within Formicidae, presenting a rich
293 playground to study major evolutionary transitions in individuality⁴².

294 Our study demonstrates a link between environmental changes brought about by the rise of flowering
295 plants, with its transformational impact on most terrestrial ecosystems, and a simple but important
296 ant behavior that had notable impact on the evolution of different ant lineages. This shift was enabled
297 by ant-plant and ant-sap-sucker interactions. These resulted in adaptations across multiple partners,
298 and thus significant rewiring of ecosystems^{3,50}. Ants, and in particular ants that rely on such
299 mutualisms, are known to be ecosystem engineers, moving their aphid cattle from plant to plant,
300 protecting trees from predators, cultivating fungus gardens and planting seeds. Understanding the
301 evolutionary underpinnings of behavioral traits is crucial to grasp their fundamental role in
302 determining the ecological success of the most dominant organisms in the animal kingdom, such as
303 *Homo sapiens* and the ants.

304 **Methods**

305 **Data collection**

306 We completed the reports of trophallaxis from Meurville & LeBoeuf¹⁴ by manually searching literature
307 up to early 2021 for reports of occurrence or absence of trophallaxis behavior, ending up with data
308 for trophallaxis behavior of 205 species from 265 sources (Supp Table 1). Because some subfamilies
309 were more represented than others, and in order to have more even taxon sampling, we imputed
310 trophallaxis behavior (Supp Fig 6). To do so, we used a Bayesian Neural Network (BNN) as
311 implemented in the npBNN library⁵¹ (<https://github.com/dsilvestro/npBNN>), that provided posterior
312 probabilities for a binary trait, based on life-history, morphological and ecological traits collected from
313 literature. These traits were either easy to collect for a large number of species (temperature and

314 humidity data, presence or absence of a sting) or were linked to some of our hypotheses (Supp
315 Table 4).

316 **Phylogeny**

317 We used the MCC tree produced by Economo et al. 2018⁵² as a reference for the ant phylogeny of
318 over 14,000 species, as it was the largest and most recent ant phylogeny at the time. We corrected
319 species names according to antwiki (https://www.antwiki.org/wiki/Ant_Names) valid species names
320 in 2021, and removed morphospecies. In order to include the phylogenetic signal in the BNN to
321 predict trophallaxis behavior, we transposed the tree into eigenvalues using the R packages ape
322 v5.4.1 and PVR v0.3, providing us with 11 eigenvalues (50% of the phylogenetic signal) per species
323 that we used as an input to the BNN.

324 **Temperature and humidity**

325 We retrieved temperature and humidity data from the GABI database (Release 18.01.2020) and
326 Antweb (October 2020). Temperature and precipitation data were obtained from the coordinates
327 referenced in the two aforementioned databases, using the R package raster v3.3.13. For each
328 specimen of each species, we collected the annual temperature and precipitation recorded at this
329 location. For each species, we then extracted the minimal and maximal values of temperatures and
330 precipitations, thus providing a range of temperatures and precipitations for each species' habitat,
331 ending in four continuous traits: minimal and maximal temperature and minimal and maximal
332 precipitation.

333 **Drinking sugary liquids**

334 Mention of a species drinking sugary liquids in the field, tending aphids, or having repletes was
335 reported as 1, and specialized predators or ants not interested in sugary foods were attributed a 0.
336 For four species we accepted information indicating species drink sugary liquids in the lab
337 (*Strumigenys lewisi*, *S. canina*, *Cephalotes varians* and *Streblognathus aethiopicus*). For 32 species
338 in 17 genera (*Acanthognathus*, *Anochetus*, *Apomyrma*, *Cerapachys*, *Cryptopone*, *Discothyrea*,
339 *Leptogenys*, *Myopias*, *Myrmecina*, *Neivamyrmex*, *Platythyrea*, *Ponera*, *Prionopelta*,

340 *Probolomyrmex*, *Proceratium*, *Typhlomyrmex* and *Tapinoma*), the species-level diet information was
341 impossible to retrieve, so it was inferred from the genus-level.

342 **Sting**

343 Whether species have a sting or not was retrieved from the supplementary material of Blanchard &
344 Moreau⁵³. As sting is not considered to be an evolutionarily labile trait, we extrapolated the data on
345 presence or absence of a sting from genus to species level. Missing values in the training set were
346 retrieved manually from scientific literature (Supp Table 4).

347 **Colony size**

348 Colony size data were retrieved manually from various sources. Because colony size is variably
349 reported in the literature (sometimes maximum, minimum, average or single observations), we
350 recorded what was available. To face this heterogeneity in data collection, we split colony size by
351 quartile (1st quartile: under 60 individuals, 2nd quartile: 60-268, 3rd quartile: 269-2000, 4th quartile:
352 2001-306000000). To binarize the data, each quartile was considered a trait.

353 **Reproductive workers**

354 Categorical data on presence or absence of gamergates (ants with worker fate but that can be mated
355 and produce diploid offspring) was retrieved from antwiki,
356 <https://www.antwiki.org/wiki/Category:Gamergate> (October 2021). Genera not listed were assumed
357 to have workers that could not be mated. Species from genera known to have gamergates but for
358 which no data was found were not included in the dataset, as we do not know how labile the presence
359 or absence of gamergates is within a genus.

360 **Data imputation**

361 We trained a BNN model on 161 species split as training (90%) and test sets (10%). To evaluate the
362 test accuracy of the model we performed a 10-fold cross-validation analysis. The BNN model

363 included a fully connected network with two hidden layers of 20 and 10 nodes, respectively, with
364 ReLU activation function⁵⁴.

365 The cross-validation test accuracy of our model was 84% (Supp Table 2). We had, on average over
366 the 10 test-samples, 11.6 True Positives, 1.8 True Negatives, 1.6 False Positives and 1 False
367 Negative.

368 To further evaluate the accuracy of our model, we sent the list of species for which we predicted
369 trophallaxis behavior (including both predictions that species would engage in trophallaxis and
370 predictions that a species would not engage in trophallaxis) to 10 expert myrmecologists (C. Lebas,
371 N. Idogawa, A. Yamada, P. Slingsby, R. Mizuno, J. Heinze, A. Lenoir, V. Nehring, F. Savarit and X.
372 Cerda, Supp Table 5) and asked them if they had observed or not observed stomodeal trophallaxis
373 between adults for any species on the list. We used their answers to estimate the accuracy of our
374 imputations. Experts provided responses for 48 species, 19% of our imputed species. We have 37
375 True Positives, 5 True Negatives, 3 False Positives and 3 False Negatives, leading to a
376 myrmecologist accuracy of 88% (Supp Table 2).

377 After running the trained model on 253 unlabeled species, we obtained a dataset of 416 species:
378 161 species with known trophallaxis behavior and all known traits, 2 species with known trophallaxis
379 behavior but missing temperature and precipitation data (*Myrmecaria natalensis eumenoides* and
380 *Myrmoteras jaitrongi*, Supp Table 4), 48 species with trophallaxis behavior confirmed or denied by
381 myrmecologists, and 205 species with trophallaxis behavior imputed by the BNN. In cases of
382 mismatch between our predictions and the experts' assessments, we gave priority to the trophallaxis
383 behavior indicated by the experts.

384 **Evolutionary analyses**

385 We used sMap v1.0.7⁵⁵ to infer ancestral states using phylogenetic comparative methods. For each
386 trait (presence or absence of reproductive workers, sting, trophallaxis and sugary liquid food in
387 species diet; discretized colony size) we tested different Markov models in a maximum likelihood
388 framework and ranked them based on Akaike weights (Supp Fig 7).

389 Analyses were launched with 1000 simulations using the 'sMap' command. We launched maximum
390 likelihood analyses on both the stem maximum clade credibility tree and 100 stem posterior trees in
391 order to take variability into account (Supp table 6). The rest of the analysis was done only on the
392 maximum clade credibility tree. We then built a Bayesian model with a log normal prior where μ is
393 the natural logarithm of the maximum likelihood model rates and σ is 1. We then computed the
394 posterior probability of each model and used it to average Bayesian models with the 'Blend-sMap'
395 command. This provided the ancestral state reconstruction for the stem maximum clade credibility
396 tree.

397 **Trait correlation and phylogenetic path analysis**

398 We used a d-test²⁷ as implemented in sMap⁵⁵ to estimate the co-occurrence of each trait with
399 trophallaxis. We performed this test on the known dataset and verified species (211 species) to avoid
400 circularity and on the full dataset (416 species). We performed the ML analysis and Bayesian
401 analyses as before, only adding the -pp 100 option to draw 100 posterior predictive samples. Then,
402 we average Bayesian models and pairwise merged the trophallaxis model with every trait using the
403 'Merge-sMap' command to finally use 'Stat-sMap' with default values to run the D-test.

404 To test how traits influenced trophallaxis behavior, we used phylogenetic path analysis as proposed
405 by von Hardenberg and Gonzalez-Voyer²⁸, on our dataset of 416 species, and on only the 211
406 species with known trophallaxis behavior (from literature and imputations verified by
407 myrmecologists). This method allows the comparison of possible causal relationships between traits
408 while testing for direct or indirect effects and considering the non-independence of the traits due to
409 phylogeny. We first built causal quantitative models as directed acyclic graphs (DAGs, Supp Fig 4)
410 and accounted for phylogeny through phylogenetic generalized least squares analysis (PGLS), using
411 the d-separation method^{28,56}. Building the models, running the phylogenetic path analysis and
412 performing model selection was done in R with the Phylopath v.1.1.3⁵⁷, in order to take both
413 continuous and discrete response variables into account, with 500 bootstrap.

414 The best models were models seven, one, three and five for the full dataset, and models one, three
415 and seven for the reduced dataset (Supp Fig 4 and 5). Because we cannot identify the best model,

416 we averaged the models using the `average()` function in the `phylopath` package. This provided a
417 model that reflects the results of the best models.

418 **Speciation and extinction analyses**

419 We used reconstructed birth-death processes to estimate the dynamics of speciation and extinction
420 rates across the ant phylogeny and assess whether the evolution of trophallaxis might have affected
421 species diversification. To this end we used the state-dependent models implemented in the R
422 package `HiSSE` (v 1.9.6)³¹ and the mixture models implemented in `BAMM` (v 2.5.0)⁵⁸.

423 For the `HiSSE` analysis, we used the tree from Economo et al 2018⁵² tree with 14,183 species, and
424 set the trophallaxis state to unknown for all species missing from our imputed dataset. We tested
425 four models (Supp table 3), starting with (1) the original `BiSSE` model⁵⁹, with turnover (speciation +
426 extinction) and extinction fraction (extinction / speciation) dependent on that trait state. Because the
427 `BiSSE` model is prone to false positives³¹, we also ran a character-dependent `HiSSE` model (2) with
428 two hidden states, where diversification rate variation can occur as function of both trophallaxis and
429 an unobserved (hidden) trait. We additionally tested character independent models with only hidden
430 traits (of two or three) affecting turnover and extinction fraction and with no effect of trophallaxis. We
431 compared the fit of these models through maximum likelihood using AIC.

432 For the `BAMM` analysis, we started estimating empirical priors using the `setBAMMpriors` function
433 from `BAMMtools` v2.1.9, and launched `BAMM` on a reduced phylogeny for the 416 species in our
434 dataset with species-specific sampling fractions determined at the genus level and default
435 parameters for other arguments (Supp Table 7). We then performed a `STRAPP` analysis³⁰ test with
436 the `BAMMtools` function `traitDependentBAMM`, to estimate if rates differ between species using and
437 not using trophallaxis. We used the resulting P-value to assess whether significant evidence of a
438 trait-dependent effect was detected and the mean diversification rates across species engaging or
439 not with trophallaxis to quantify the magnitude of that difference.

440

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452 **Author contributions**

453 M-P.M, D.S and A.C.L conceived the experiments. M-P.M and A.C.L collected the traits data from
454 literature. M-P.M conducted the analyses under the supervision of D.S and A.C.L. M-P.M, D.S and
455 A.C.L made the figures and wrote the manuscript with comments from all authors.

456 **Competing interests**

457 The authors declare that they have no competing interests.

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