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# Distinguishing cophylogenetic signal from phylogenetic congruence clarifies the interplay between evolutionary history and species interactions

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1 **Distinguishing cophylogenetic signal from phylogenetic congruence clarifies the**  
2 **interplay between evolutionary history and species interactions**

3

4 Running title: Cophylogenetic signal does not imply phylogenetic congruence

5

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7

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12

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14

15 **Abstract:**

16

17 Interspecific interactions, including host-symbiont associations, can profoundly  
18 affect the evolution of the interacting species. Given the phylogenies of host and  
19 symbiont clades and knowledge of which host species interact with which symbiont,  
20 two questions are often asked: "Do closely related hosts interact with closely related  
21 symbionts?" and "Do host and symbiont phylogenies mirror one another?". These  
22 questions are intertwined and can even collapse under specific situations, such that  
23 they are often confused one with the other. However, in most situations, a positive  
24 answer to the first question, hereafter referred to as "cophylogenetic signal", does not  
25 imply a close match between the host and symbiont phylogenies. It suggests only that  
26 past evolutionary history has contributed to shaping present-day interactions, which  
27 can arise, for example, through present-day trait matching, or from a single ancient  
28 vicariance event that increases the probability that closely related species overlap  
29 geographically. A positive answer to the second, referred to as "phylogenetic

30 congruence”, is more restrictive as it suggests a close match between the two  
31 phylogenies, which may happen, for example, if symbiont diversification tracks host  
32 diversification or if the diversifications of the two clades were subject to the same  
33 succession of vicariance events.

34 Here we apply a set of methods (ParaFit, PACo, and eMPress), which  
35 significance is often interpreted as evidence for phylogenetic congruence, to  
36 simulations under three biologically realistic scenarios of trait matching, a single  
37 ancient vicariance event, and phylogenetic tracking with frequent cospeciation events.  
38 The latter is the only scenario that generates phylogenetic congruence, whereas the  
39 first two generate a cophylogenetic signal in the absence of phylogenetic congruence.  
40 We find that tests of global-fit methods (ParaFit and PACo) are significant under the  
41 three scenarios, whereas tests of event-based methods (eMPress) are only significant  
42 under the scenario of phylogenetic tracking. Therefore, significant results from global-  
43 fit methods should be interpreted in terms of cophylogenetic signal and not  
44 phylogenetic congruence; such significant results can arise under scenarios when hosts  
45 and symbionts had independent evolutionary histories. Conversely, significant results  
46 from event-based methods suggest a strong form of dependency between hosts and  
47 symbionts evolutionary histories. Clarifying the patterns detected by different  
48 cophylogenetic methods is key to understanding how interspecific interactions shape  
49 and are shaped by evolution.

50

51 **Keywords:** cophylogeny, codiversification, phylogenetic methods, symbiosis,  
52 parasitism, coevolution.

53 **Introduction:**

54

55 Antagonistic or mutualistic interactions, such as parasitism, herbivory, seed  
56 dispersal, or pollination, are key components of ecological communities (Bascompte  
57 and Jordano 2013; Mittelbach and McGill 2019). Patterns of interactions (*i.e.* who  
58 interacts with whom) are shaped by evolutionary history through a variety of  
59 processes. For example, interspecific interactions may be constrained to species with  
60 matching traits, as frequently observed in pollination or host-parasite interaction  
61 networks (Muchhala and Thomson 2009; Morand et al. 2015), in which case species  
62 evolutionary history matters as soon as the traits involved in the interactions are  
63 evolutionarily conserved. Interspecific interactions may also be constrained by  
64 historical contingencies such as past dispersal and/or geographic events: for instance,  
65 if lineages have been geographically isolated following a vicariance event, these  
66 lineages do not interact simply because they do not co-occur (Althoff et al. 2014; Perez-  
67 Lamarque et al. 2022a). Interspecific interactions can also be transmitted from  
68 generation to generation on evolutionary time scales, as in the case of symbionts that  
69 are vertically transmitted from parental to descendant hosts (Bright and Bulgheresi  
70 2010). Reciprocally, interactions affect the evolution of the interacting species, for  
71 instance through disruptive selection, stabilizing selection, or coevolution. Over  
72 macroevolutionary scales, such effects can leave an imprint on the phylogenetic trees  
73 of the interacting clades (Harmon et al. 2019; Hembry and Weber 2020; Hayward et al.  
74 2021). An extreme case corresponds to phylogenetic tracking, which can happen, for  
75 example, when host speciation events lead to the subsequent speciation of symbionts  
76 that are closely associated with them (Fahrenholz 1912). Analyzing interacting species  
77 through the lens of their past evolutionary history is therefore fundamental for  
78 understanding how interspecific interactions shape and are shaped by evolution.

79

80 Given host-symbiont cophylogenetic data, *i.e.* a phylogenetic tree for both the  
81 host and the symbiont clades and knowledge of which host species interact with which

82 symbiont, two patterns are often investigated (Supplementary Box 1): (i) whether  
83 closely-related hosts interact with closely-related symbionts, hereafter referred to as  
84 “cophylogenetic signal”, and (ii) whether host and symbiont phylogenies mirror one  
85 another (with pairs of interacting hosts and symbionts that tend to occupy similar  
86 positions in the two trees), hereafter referred to as “phylogenetic congruence” (Blasco-  
87 Costa et al. 2021). By definition, phylogenetic congruence can occur only when the  
88 number of host and symbiont species is similar, with mostly ‘one-to-one’ interactions  
89 between one host and one symbiont species. In this case, patterns of cophylogenetic  
90 signal and phylogenetic congruence tend to collapse, such that they can be studied  
91 interchangeably. In this context, some cophylogenetic methods, referred to as global-  
92 fit methods, have been developed to test for phylogenetic congruence using linear  
93 algebra techniques, such as the fourth-corner statistics (ParaFit, Legendre et al. 2002)  
94 and procrustean superposition (PACo, Balbuena et al. 2013). Numerous  
95 cophylogenetic systems observed in nature, however, are characterized by ‘many-to-  
96 many’ interactions, as illustrated by densely-connected networks of interspecific  
97 interactions (Ronquist and Nylin 1990; Bascompte and Jordano 2013; Pichon et al.  
98 2023). The same global fit methods have been regularly applied to such systems, and  
99 interpreted as tests of phylogenetic congruence (see *e.g.* Fuzessy et al. 2022; Suzuki et  
100 al. 2022 for recent examples). If, under these situations, global-fit methods actually do  
101 not measure phylogenetic congruence, this can lead to a misinterpretation of the  
102 results.

103

104         Patterns of cophylogenetic signal and phylogenetic congruence can reflect  
105 different processes (Fig. 1). The occurrence of cophylogenetic signal simply suggests  
106 that past evolutionary history has contributed to shaping present-day interactions. For  
107 instance, a cophylogenetic signal can arise if interactions are shaped by evolutionarily  
108 conserved traits (Fig. 1a “trait matching”) or by past biogeographic events (Fig. 1b  
109 “vicariance”), even if the diversification of the host and symbiont clades was not  
110 influenced by the interactions between host and symbiont species. The occurrence of

111 phylogenetic congruence, on the other hand, suggests a potential (co-)dependency  
112 between the host and symbiont diversifications, causing both phylogenetic trees to  
113 look alike. For instance, phylogenetic congruence can emerge (i) if the diversification  
114 of vertically transmitted symbionts tracks host diversification, generating a pattern of  
115 concomitant diversification events happening in both host and symbiont clades,  
116 referred to as “codiversification” (Fig. 1c “phylogenetic tracking”), (ii) if the  
117 diversifications of the two clades were subject to the same succession of vicariance  
118 events, also resulting in a pattern of concomitant codiversification, or (iii) under  
119 preferential host switching, *i.e.* if symbionts diversify by preferentially transferring to  
120 closely-related host species, generating a pattern of phylogenetic congruence without  
121 concomitant divergence times, referred to as “pseudo-codiversification” (de Vienne et  
122 al. 2013; Althoff et al. 2014). Coevolution, *i.e.* the reciprocal evolutionary changes in  
123 interacting lineages induced by selective pressures exerted by one another, may also  
124 generate phylogenetic congruence in some cases (*e.g.* aphid-associated bacterial  
125 endosymbionts (Jousselin et al. 2009)), but is not a necessary nor sufficient condition  
126 for observing phylogenetic congruence (Poisot 2015). Phylogenetic congruence implies  
127 cophylogenetic signal, but the reverse is not true.

128

129         Here, in an effort to clarify the conclusions that can be drawn from various  
130 cophylogenetic methods, we analyze their outputs under various evolutionary  
131 scenarios, using simulations. Cophylogenetic methods can be divided into two main  
132 types of approaches (see de Vienne et al. (2013) and Dismukes et al. (2022) for reviews  
133 of these methods): the global-fit methods mentioned above, such as ParaFit and PACo,  
134 and the event-based methods, such as TreeMap (Page 1994a, 1995), TreeFitter  
135 (Ronquist 2003a), Jane (Conow et al. 2010), or eMPress (Santichaivekin et al. 2021).  
136 Event-based methods try to reconcile the host and symbiont phylogenies by fitting  
137 a set of reconciliation events (*e.g.* cospeciation, host transfer, intra-host duplication, or  
138 symbiont loss) to the symbiont phylogeny (Page 1994b; Ronquist 2003b). We have  
139 shown before, in a different context, that global-fit and event-based methods can

140 sometimes output contrasting results (Perez-Lamarque and Morlon 2023), suggesting  
141 that they measure different patterns. We evaluate here their outputs under three  
142 biologically realistic scenarios of host-symbiont evolution by trait matching,  
143 vicariance, and phylogenetic tracking. The latter is the only scenario that generates  
144 phylogenetic congruence when simulating frequent cospeciations, whereas the first  
145 two generate a cophylogenetic signal in the absence of phylogenetic congruence. We  
146 find that global-fit methods provide a test of cophylogenetic signal rather than  
147 phylogenetic congruence, whereas event-based methods provide a test of  
148 phylogenetic congruence, and discuss the implications of these results for the study of  
149 cophylogenetic systems.

150

### 151 **Simulations of three biologically realistic scenarios:**

152

153 In the first simulated scenario, we assumed that present-day host-symbiont  
154 interactions are more likely between species having complementary traits following a  
155 trait-matching expression with a unidimensional continuous trait (Fig. 1a – “trait  
156 matching”; Supplementary Methods 1). We independently simulated two  
157 phylogenetic trees for the host and symbiont clades using a birth-death model and on  
158 each tree, we then independently simulated the evolution of traits modulating present-  
159 day interactions. By using a Brownian motion for trait evolution, closely-related hosts  
160 and closely-related symbionts tend to have similar trait values (*i.e.* phylogenetic signal  
161 in species traits). Finally, we assumed that the degree of specialization of the symbionts  
162 (*i.e.* the number of hosts that a given symbiont interacts with) follows a Poisson  
163 distribution with parameter  $\lambda=1.5$  and attributed the present-day host-symbiont  
164 interacting pairs following a trait-matching expression. As a result, closely related host  
165 species interact with closely related symbiont species (*i.e.* cophylogenetic signal),  
166 although the phylogenetic trees were simulated independently and are therefore not  
167 congruent. We generated 1,000 simulations with varying clade sizes (from 10 to 200  
168 species per clade; Supplementary Methods 1) and replicated the simulations with

169 fewer host species associated with each symbiont species by using a Poisson  
170 distribution with parameter  $\lambda=1$ .

171

172 In the second simulated scenario, we assumed that host and symbiont species  
173 interact at random as long as they occupy the same biogeographic area. At first, all  
174 hosts and symbionts simultaneously occupy a single area and diversify  
175 independently, until a vicariance event splits the area into three separate areas (Fig. 1b  
176 – “vicariance”; Supplementary Methods 1). Half of the host and symbiont species are  
177 then isolated in separate areas; the other half experiences allopatric speciation as their  
178 population is split into two. Following the vicariance event, each host and symbiont  
179 lineage diversifies independently in its area, resulting in a phylogenetic signal in  
180 biogeographic repartition (*i.e.* closely related species tend to occupy the same area).  
181 Although the host and symbiont diversifications are not independent (they both  
182 undergo a burst of speciation events at the time of vicariance), the host and symbiont  
183 phylogenetic trees are not congruent as they experienced different events of  
184 diversification before and after the vicariance (Fig. 1b). Finally, we assumed that the  
185 degree of specialization of the symbionts follows a Poisson distribution with  
186 parameter  $\lambda=1.5$  and randomly attributed host-symbiont present-day interactions  
187 within each area. This scenario thus produces cophylogenetic signal but no  
188 phylogenetic congruence. We generated 1,000 simulations with varying clade sizes  
189 (Supplementary Methods 1) and replicated the simulations with fewer host species  
190 associated with each symbiont species ( $\lambda=1$ ).

191

192 In the third simulated scenario, we assumed that the symbiont diversification  
193 tracks the host diversification. Symbionts species are vertically transmitted over long-  
194 time scales from host generation to host generation (at the level of host individual or  
195 at the level of the whole host lineage) and cospeciate at host speciation events resulting  
196 in codiversification (Fig. 1c – “phylogenetic tracking”; Supplementary Methods 1). In  
197 addition, we assumed that symbiont lineages experience a given number of host



198 transfers from a donor host to a receiver host (with replacement of the previous  
199 symbiont lineage); this number was uniformly sampled between 0 and half the  
200 number of extant host species. Finally, intra-host duplication occurs at rate 0.001 event  
201 per million year per lineage and host lineages can lose their symbionts with a  
202 probability of 0.1 at present. The latter processes (transfer, duplication and loss)  
203 dampen but maintain the phylogenetic congruence between host and symbiont  
204 phylogenies, rendering it more realistic. This third scenario of phylogenetic tracking  
205 with frequent cospeciations produces both cophylogenetic signal and phylogenetic  
206 congruence. We generated 1,000 simulations with varying clade sizes (from 10 to 200  
207 host species; Supplementary Methods 1). We also replicated the simulations with less  
208 cospeciations and more host transfers (number uniformly sampled between 50% and  
209 75% of the number of extant host species) and intra-host duplications (rate of 0.0015  
210 event per million year per lineage). Under these simulations of phylogenetic tracking  
211 with infrequent cospeciations, the pattern of phylogenetic congruence is greatly erased  
212 as cospeciation events represent a minority of simulated events.

213

214 We therefore obtained a total of 6,000 simulations with different host and  
215 symbiont species richness values and ratios of one-to-one interactions (Supplementary  
216 Figs. S1 & S2). As expected, our simulations under phylogenetic tracking produced  
217 systems with a much higher proportion of one-to-one interactions than simulations  
218 under trait matching and vicariance, which were mainly constituted of many-to-many  
219 interactions, especially under low symbiont specialization ( $\lambda=1.5$ , Supplementary Fig.  
220 S2).

221

### 222 **Fitting cophylogenetic methods:**

223

224 For each simulated cophylogenetic data, we first applied the global-fit methods  
225 ParaFit and PACo using the functions *parafit* and *PACo* from the R-packages ape  
226 (Paradis et al. 2004) and *paco* (Hutchinson et al. 2017) respectively, amended to avoid

227 technical issues when the number of host or symbiont species is low (Perez-Lamarque  
228 and Morlon 2023). We measured the strength of the cophylogenetic signal using the  
229 “ParaFit global statistic” in ParaFit, whereas, in PACo, we ran the symmetric option  
230 for the procrustean superposition to obtain  $R^2$ , defined as  $R^2 = 1 - m^2$ , where  $m^2$  is the  
231 sum of the squared residuals of the symmetric procrustean superposition (Blasco-  
232 Costa et al. 2021).  $R^2$  is comprised between 0 and 1, and  $R^2$  close to 0 indicates low  
233 cophylogenetic signal, whereas  $R^2$  close to 1 indicates high cophylogenetic signal.  
234 Following Legendre et al. (2002) and Hutchinson et al. (2017), the significance of the  
235 ParaFit and PACo tests was first evaluated using 10,000 randomizations obtained by  
236 independently shuffling which host species are associated with each symbiont species,  
237 hereafter referred to as “null model 1”. Second, following Ronquist (1998) and  
238 Sanmartín and Ronquist (2004), we assessed their significance by randomly shuffling  
239 the host species labels, hereafter referred to as “null model 2”. We also investigated  
240 whether the effect size of the global-fit methods, *i.e.* values of the ParaFit global statistic  
241 and PACo’s  $R^2$ , can be used as indicators of phylogenetic congruence (Blasco-Costa et  
242 al. 2021). Finally, we tested whether global-fit approaches were more likely to be  
243 significant when the ratio of one-to-one interactions was low using generalized linear  
244 models.

245

246 Second, we applied the event-based method eMPress to each simulation  
247 (Santichaivekin et al. 2021). eMPress reconciles the host and symbiont tree topologies  
248 by using maximum parsimony to fit events of host transfers, duplications, and losses;  
249 each event being associated with a given cost. We chose eMPress over all the other  
250 existing event-based methods, as it can be automatically and rapidly run using the  
251 command line. As with most event-based methods, eMPress does not handle  
252 symbiont species that interact with multiple hosts (Dismukes et al. 2022). However,  
253 symbionts often interact with several hosts in nature, and also in our simulated  
254 scenarios of trait matching and vicariance. We thus tested two strategies for running  
255 eMPress following Sanmartín and Ronquist (2002): (i) subsampling one host at

256 random per symbiont species (Su et al. 2022) or (ii) randomly generating bifurcating  
257 sub-trees for symbionts with multiple hosts, such that each tip in the symbiont tree is  
258 associated with a single host (Satler et al. 2019). We used the command-line version of  
259 eMPress with the commands “python empres\_cli.py reconcile” to reconcile the trees  
260 and “python empres\_cli.py p-value” to assess the significance. We tested 5 different  
261 combinations of cost values for duplication (d), host transfer (t) or loss (l) events: (i)  
262 d=1, t=1, l=1; (ii) d=4, t=1, l=1; (iii) d=2, t=1, l=2; (iv) d=4, t=2, l=1; or (v) d=2, t=3, l=1. A  
263 reconciliation is considered significant if its total cost is lower than 95% of the costs of  
264 1,000 reconciliations obtained after randomly shuffling the host species labels (“null  
265 model 2”; Ronquist 1998; Sanmartín and Ronquist 2004). Phylogenetic trees are  
266 considered congruent if the reconciliation estimates less host transfer than  
267 cospeciation events (Groussin et al. 2017; Perez-Lamarque and Morlon 2023).

268

### 269 **Outputs of cophylogenetic methods:**

270

271 For ParaFit, we found significant tests in 34% of the trait-matching simulations,  
272 45% of the vicariance simulations, and 98% of the phylogenetic tracking (obtained with  
273 “null model 1” and  $\lambda=1$  or 1.5; Table 1). Significant tests for PACo were even more  
274 frequent, reaching 48%, 63%, and 99% of the simulations in the three scenarios,  
275 respectively (Table 1). Qualitatively similar results were obtained when evaluating the  
276 significance by shuffling the host species labels (“null model 2”; Supplementary Table  
277 S1). As expected, global-fit tests were more often significant in simulations with higher  
278 numbers of host and symbiont species (Supplementary Table S2, Supplementary Figs.  
279 S3 & S4). In trait matching and vicariance simulations, when increasing the ratio of  
280 one-to-one host-symbiont interactions (*i.e.* reducing the mean number of associated  
281 hosts per symbiont by using  $\lambda=1$ ), global-fit tests were less often significant (Table 1;  
282 Supplementary Table S3; Supplementary Figs. S3 & S4; generalized linear models: p-  
283 values<0.05). In other words, global-fit tests are actually more likely to be significant  
284 when there are frequent many-to-many host-symbiont interactions, while we would

285 expect the contrary if these tests measured phylogenetic congruence. Overall, these  
286 results indicate that global-fit methods tend to measure cophylogenetic signal in  
287 general rather than specifically phylogenetic congruence.

288

289 In terms of interpretation of the effect size of the global-fit methods, the ParaFit  
290 global statistic varies with the total number of species, and is thus difficult to interpret  
291 in itself: it cannot be used to distinguish phylogenetic congruence from cophylogenetic  
292 signal (Fig. 2; Supplementary Fig. S3). In contrast, for PACo, we found that  $R^2$  is  
293 generally higher than 0.25 when there is a pattern of phylogenetic congruence (in 99%  
294 of the scenario of phylogenetic tracking with frequent cospeciations), whereas it is  
295 lower than 0.5 when there is a pattern of cophylogenetic signal alone (in 99% of the  
296 scenarios of trait-matching or vicariance; Figs. 2 & 3). Hence, a significant test with  $R^2$   
297  $> 0.50$  is strong support for phylogenetic congruence (although not definite evidence,  
298 it may be possible to obtain higher  $R^2$  values under scenarios without congruence with  
299 different simulation choices), whereas a significant test with  $R^2 < 0.25$  suggests that the  
300 system presents a cophylogenetic signal without phylogenetic congruence (Fig. 3). A  
301 significant test with  $R^2$  values between 0.25 and 0.50 is harder to interpret, as  $R^2$  tends  
302 to correlate positively with the ratio of one-to-one interactions and  $R^2$  values above  
303 0.25 are sometimes reported in simulations without phylogenetic congruence  
304 (Supplementary Fig. S4). PACo alone is therefore often not sufficient to identify a  
305 pattern of phylogenetic congruence.

306

307 For eMPress, when subsampling one host per symbiont species, the  
308 reconciliation was significant in only 5% of the trait-matching simulations and 16% of  
309 the vicariance simulations (the two scenarios generating cophylogenetic signal  
310 without phylogenetic congruence), and congruent in none (Table 1). When generating  
311 random bifurcations in the symbiont tree, it was significant in 34% and 80% of the trait-  
312 matching and vicariance simulations, respectively, but congruent in none (Table 1).  
313 Indeed, eMPress estimated on average 5 times more host transfers than cospeciations

314 for both scenarios of trait matching and vicariance (Table 1; Fig. 2; Supplementary Fig.  
315 S5). In contrast, when simulating phylogenetic tracking with a majority of cospeciation  
316 events, eMPress gave significant reconciliations in 100% of the simulations, 92% of  
317 which were congruent (Table 1). Results were qualitatively similar when choosing  
318 alternative event costs (Supplementary Tables S4, S5, & S6). In all simulated scenarios,  
319 significant reconciliations were more frequent when the number of host and symbiont  
320 species were larger (Supplementary Tables S4, S5, & S6). When simulating  
321 phylogenetic tracking with infrequent cospeciations, eMPress reconciliations were  
322 still significant in 99% of the simulations, but congruent in only 34% of them; this is  
323 expected, given that the number of cospeciations is lower than the number of host  
324 transfers in these simulations (Fig. 2; Table 1; Supplementary Tables S7). Overall, our  
325 findings suggest that event-based methods can specifically detect patterns of  
326 phylogenetic congruence and distinguish them from a “simple” cophylogenetic signal.

327

328

## 329 **Discussion:**

330

331 Using simulations, we have assessed the ability of different cophylogenetic  
332 methods to distinguish phylogenetic congruence from cophylogenetic signal. The  
333 distinction is important, as these patterns are indicative of different processes,  
334 phylogenetic congruence revealing more intricate evolutionary histories between  
335 hosts and symbionts than cophylogenetic signal (Fig. 4). We have shown that global-  
336 fit methods typically return significant results as soon as there is cophylogenetic signal  
337 in species interactions, including in the absence of phylogenetic congruence, meaning  
338 that they cannot distinguish the two patterns. In contrast, event-based methods can be  
339 used to specifically detect phylogenetic congruence.

340

341 Given that global-fit methods detect cophylogenetic signal rather than  
342 phylogenetic congruence, Mantel tests measuring phylogenetic signal in species

343 interactions could be used in place of these methods (Perez-Lamarque et al. 2022b). It  
344 would be useful, in the future, to compare the behavior of Mantel tests to global-fit  
345 approaches. Regarding event-based methods other than eMPress, such as TreeMap  
346 (Page 1994a, 1995), TreeFitter (Ronquist 2003a), or Jane (Conow et al. 2010), we expect  
347 them to have similar behaviors given that they are also cost-based and use maximum  
348 parsimony. There are however notable differences between them; for example,  
349 eMPress (like TreeMap or Jane) sets a null cost to cospeciation events, therefore  
350 implicitly favoring cospeciation over other reconciliation events (host transfers, intra-  
351 host duplication, and symbiont loss), while TreeFitter (Ronquist 2003a) allows setting  
352 a positive cost to cospeciation events. Given that cospeciation events may not be that  
353 frequent in nature (Ronquist 1995), TreeFitter is probably less likely to overestimate  
354 cospeciation events in the reconciliation. More sophisticated probabilistic methods,  
355 such as the version of the amalgamated likelihood estimation (ALE) that considers  
356 branching orders in addition to tree topology (Szöllősi et al. 2013), may also perform  
357 better as it would provide only time-consistent reconciliations (Maestri et al. 2023).

358

359 Several recent studies have interpreted significant results of global-fit  
360 approaches as evidence for phylogenetic congruence and signs of codiversification  
361 (*e.g.* Fuzessy et al. 2022; Suzuki et al. 2022). Our findings suggest that there is evidence  
362 for cophylogenetic signal in the studied systems, which is already insightful in itself,  
363 but that little can be said about phylogenetic congruence before event-based methods  
364 are applied. Applying event-based methods could drastically change the biological  
365 conclusions that have been drawn.

366

367 Our results suggest that the complementary use of PACo (fast and easy to run)  
368 and eMPress (more computationally intensive but more informative) can be most  
369 useful for analyzing cophylogenetic data, with a careful interpretation of the results  
370 (Fig. 4). We recommend to begin by using PACo; if the test is not significant, there is  
371 neither cophylogenetic signal nor phylogenetic congruence in the data, suggesting

372 independent evolution. If the PACo test is significant but with a low  $R^2$  ( $R^2 < 0.25$ ), there  
373 is a cophylogenetic signal but no congruence, suggesting that evolutionary history  
374 played a role in shaping present-day interactions, but that there was not a strong co-  
375 dependency during the evolutionary history of the two clades. If the PACo test is  
376 significant with a higher  $R^2$  ( $R^2 > 0.25$ ), there is a cophylogenetic signal, and potentially  
377 also phylogenetic congruence, but the latter needs to be validated using eMPress, as  
378  $R^2 > 0.25$  also frequently occur in systems without phylogenetic congruence. Before  
379 running eMPress, if some symbiont species interact with several host species, we  
380 recommend randomly sampling one host species per symbiont (rather than, for  
381 example, generating random bifurcations in the symbiont tree, which seems to foster  
382 false positives). If the eMPress test supports phylogenetic congruence (significant  
383 reconciliation with a larger number of cospeciation events compared with the number  
384 of host transfers), this suggests a strong co-dependency during the evolutionary  
385 history of the two clades, such as phylogenetic tracking, successive vicariance events,  
386 or preferential host switching. As eMPress uses only the tree topologies and not the  
387 branch lengths, no conclusion can be drawn about the concomitance of divergence  
388 times in the host and symbionts trees. Additional analyses are thus needed to be able  
389 to distinguish phylogenetic congruence with concomitant divergence times (pattern of  
390 codiversification arising, *e.g.* from phylogenetic tracking or successive vicariance  
391 events) from phylogenetic congruence with non-concomitant divergence times  
392 (pattern of pseudo-codiversification arising, *e.g.* from preferential host switching;  
393 Ronquist 2003b; de Vienne et al. 2013). One possibility is to check whether eMPress  
394 reconciliations include time-inconsistent host transfers (*i.e.* “back-in-time” transfers  
395 between non-contemporary host lineages; Maestri et al. 2023), which would suggest  
396 pseudo-codiversification.

397

398 An advantage of global-fit methods is that their hypothesis testing is rather  
399 flexible. One can easily imagine designing more constrained randomization strategies,  
400 for example, to specifically test the influence of biogeography or trait matching on the

401 observed cophylogenetic signal (Perez-Lamarque and Morlon 2023). In parallel, data  
402 augmentation and other machine-learning techniques may allow overriding the  
403 computational bottleneck that limits the implementation of more complex process-  
404 based models. Altogether, such advancements will facilitate linking patterns to  
405 processes in cophylogenetic systems.

406

407 **Concluding remarks:**

408

409 To conclude, our results imply that using global-fit methods alone is not  
410 sufficient to robustly assess a pattern of phylogenetic congruence. In a given  
411 cophylogenetic system, finding both significant global-fit tests and significant  
412 reconciliations using event-based approaches suggests that there is a pattern of  
413 phylogenetic congruence that can be linked to various processes such as phylogenetic  
414 tracking, successive vicariance events, or pseudo-codiversification. In contrast, finding  
415 significant global-fit tests but no significant reconciliations with event-based  
416 approaches suggests that phylogenetic congruence is unlikely; the cophylogenetic  
417 signal in this system may rather emerge from processes such as trait matching or  
418 biogeographical contingency, but not from phylogenetic tracking or pseudo-  
419 codiversification. Clearly distinguishing patterns of cophylogenetic signal and  
420 phylogenetic congruence and carefully interpreting outputs of cophylogenetic  
421 methods are key if we are to understand the processes that shape present-day  
422 communities.



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424

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430

431 **Supplementary Information:**

432

433 Supplementary information can be found on DRYAD: 10.5061/dryad.7wm37pvzn  
434 (temporary link: [https://datadryad.org/stash/share/u1-stLek8-  
435 xsOgCKnCdGzPXl6KaQopUuY3h1NNOOfCeI](https://datadryad.org/stash/share/u1-stLek8-<br/>435 xsOgCKnCdGzPXl6KaQopUuY3h1NNOOfCeI))

436

437 **Code availability:**

438

439 All the codes for generating the simulations and performing the analyses are available  
440 through the following link:

441 [https://github.com/BPerezLamarque/Scripts/tree/master/Cophylogenetic\\_signal](https://github.com/BPerezLamarque/Scripts/tree/master/Cophylogenetic_signal).

442 Upon publication, all the codes for generating the simulations and performing the  
443 analyses will be available on DRYAD: <https://doi.org/10.5061/dryad.7wm37pvzn>

444 (temporary link: [https://datadryad.org/stash/share/u1-stLek8-  
445 xsOgCKnCdGzPXl6KaQopUuY3h1NNOOfCeI](https://datadryad.org/stash/share/u1-stLek8-<br/>445 xsOgCKnCdGzPXl6KaQopUuY3h1NNOOfCeI))

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448 **Conflicts of interests:**

449

450 The authors declare no conflict of interests.

451

452 **Author contributions:**

453

454 BPL and HM designed the study, BPL performed the analyses, and both authors wrote  
455 the manuscript.

456

457 **References:**

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568 **Table 1: ParaFit and PACo (global-fit methods) are often significant in all simulated**  
569 **scenarios, including scenarios of trait matching and vicariance that do not generate**  
570 **phylogenetic congruence, whereas eMPress (an event-based method) supports**  
571 **phylogenetic congruence only under scenarios of phylogenetic tracking with**  
572 **frequent cospeciations, which generate phylogenetic congruence:** This table  
573 indicates the percentages of simulations for which ParaFit, PACo, or eMPress output  
574 a significant test, under three scenarios (Fig. 1): (a) present-day interactions dictated  
575 by trait matching (with more ( $\lambda=1.5$ ) or less ( $\lambda=1$ ) host species associated with each  
576 symbiont species), (b) present-day interactions at random following a single vicariance  
577 event (with more ( $\lambda=1.5$ ) or less ( $\lambda=1$ ) host species associated with each symbiont  
578 species), or (c) present-day interactions resulting from phylogenetic tracking (with  
579 frequent or infrequent cospeciation events). For eMPress, we report the percentage of  
580 significant reconciliations based on permutations alone (P) or based on selecting  
581 among these the ones that have more cospeciation than host transfer events (P+C). We  
582 consider eMPress to support phylogenetic congruence when conditions P and C are  
583 met (in bold). Results by clade size are given in Supplementary Tables S1-S7. eMPress  
584 results correspond to host-symbiont reconciliations ran with relative costs  $d=4$ ,  $t=1$ ,  
585 and  $l=1$  for duplications, host transfers, and losses, respectively (other costs  
586 combinations provided qualitatively similar results – see Supplementary Tables S4-  
587 S6).

Methods		(a) Trait matching		(b) Vicariance		(c) Phylogenetic tracking	
		$\lambda=1.5$	$\lambda=1$	$\lambda=1.5$	$\lambda=1$	Frequent co-speciations	Infrequent co-speciations
Global-fit methods	Percentage of significant tests using ParaFit	39%	29%	52%	38%	99%	96%
	Percentage of significant tests using PACo	50%	45%	68%	59%	100%	99%
Event-based methods	Percentage of significant tests using eMPress with one host per symbiont	P: 5% P+C: 0%	P: 8% P+C: 0%	P: 16% P+C: 0%	P: 18% P+C: 0%	P: 100% P+C: 92%	P: 99% P+C: 34%
	Percentage of significant tests using eMPress with random bifurcations	P: 34% P+C: 0%	P: 23% P+C: 0%	P: 80% P+C: 0%	P: 58% P+C: 0%		

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595 **Figures legends:**

596

597 **Figure 1: Three mock examples of host-symbiont systems (here represented as**  
598 **gophers and lice) that generate a cophylogenetic signal. Only the third example also**  
599 **generates phylogenetic congruence.**

600 **(a) Trait matching.** Here host and symbiont phylogenetic trees evolve independently.  
601 Traits also evolve independently on each phylogeny following Brownian motion  
602 processes, which results in a phylogenetic signal in species traits (*i.e.* closely related  
603 species tend to have similar trait values; see the color gradient). Host-symbiont  
604 interactions at present are more likely between species having complementary traits,  
605 following a trait-matching expression. Because of trait matching, closely related host  
606 species interact with closely related symbiont species (*i.e.* cophylogenetic signal),  
607 although the phylogenetic trees are independent (*i.e.* no phylogenetic congruence).

608 **(b) Vicariance:** Here hosts and symbionts interact at random as long as they occupy  
609 the same biogeographic area. They occupy a single area until a vicariance event (*i.e.*  
610 the formation of a biogeographic barrier) splits this area into three separate areas,  
611 isolating populations and leading to speciation (a species occupying two areas at the  
612 time of vicariance immediately experiences allopatric speciation). Following the  
613 vicariance event, each host and symbiont lineage radiates independently on its area,  
614 without dispersal between areas, which results in a phylogenetic signal in  
615 biogeographic repartition (*i.e.* closely related species tend live on the same area).  
616 Because interactions happen at random within each area, this scenario generates  
617 cophylogenetic signal without phylogenetic congruence.

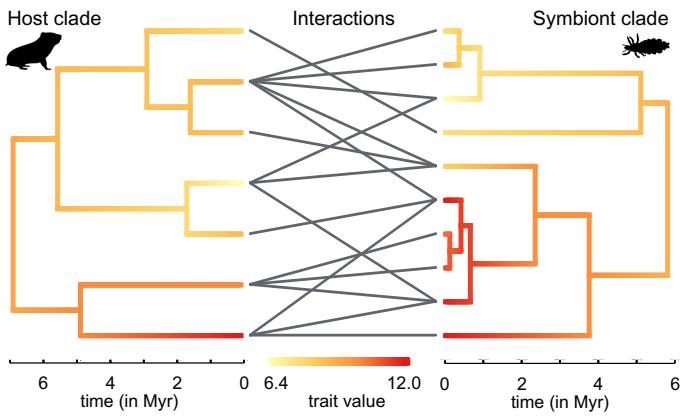
618 **(c) Phylogenetic tracking:** Here symbiont species are vertically transmitted over long-  
619 time scales along host lineages and hosts speciations concomitantly lead to symbiont  
620 speciations (“cospeciation”) resulting in a pattern of codiversification. In addition,  
621 symbiont lineages can experience horizontal host transfers from a donor host to a  
622 receiver host (with replacement of the previous symbiont lineage), intra-host  
623 duplication, and host lineages can lose their symbionts. This scenario generates both



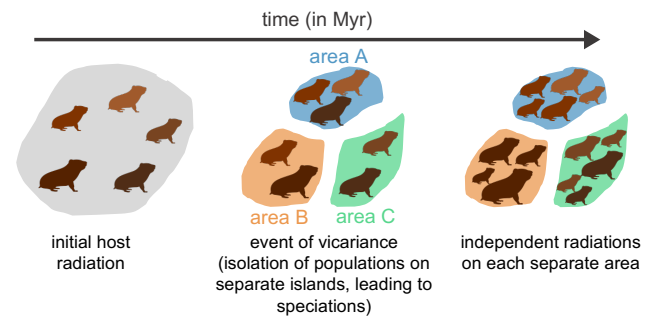
624 cophylogenetic signal and phylogenetic congruence. The greater the number of host  
 625 transfers, intra-host duplications, and symbiont loss, the lower the phylogenetic  
 626 congruence, as these events disrupt the symbiont phylogeny with regard to the host  
 627 phylogeny (Ronquist 2003a,b).

628  
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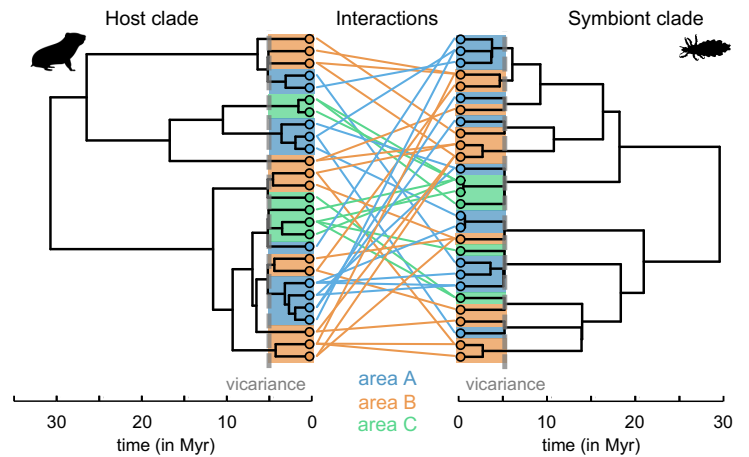
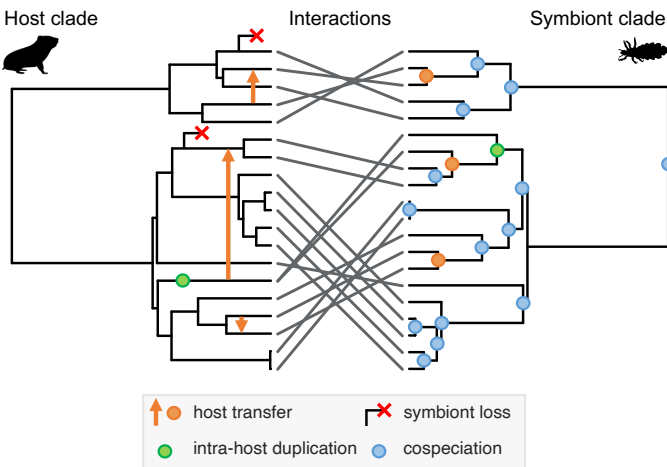
**(a) Trait matching**



**(b) Vicariance**

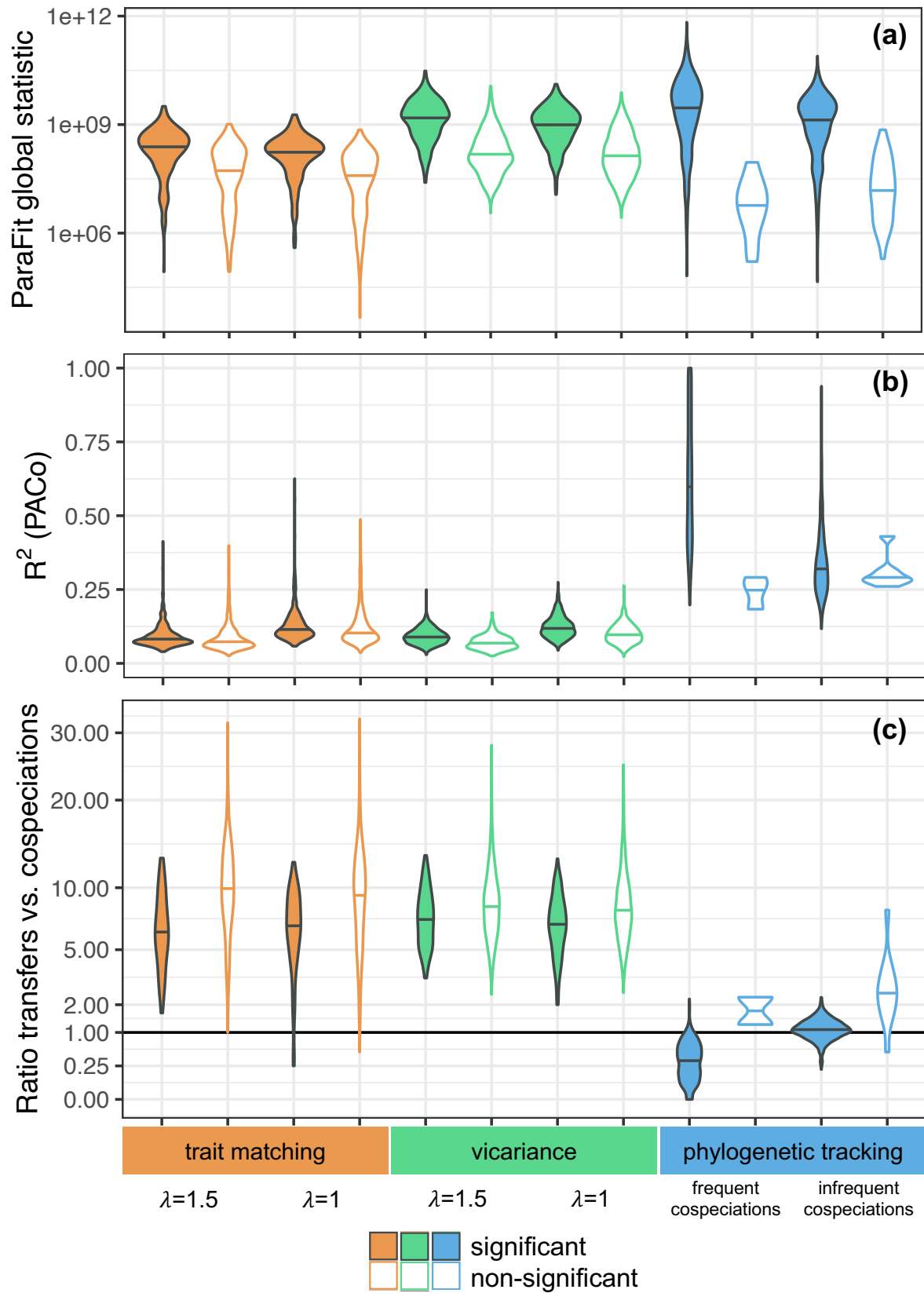


**(c) Phylogenetic tracking**



630 **Figure 2: eMPress correctly distinguishes patterns of phylogenetic congruence**  
631 **(scenario of phylogenetic tracking with frequent cospeciations) from**  
632 **cophylogenetic signal alone (scenarios of trait matching and vicariance), whereas**  
633 **ParaFit cannot and PACo can only in some cases:** Distribution of ParaFit global  
634 statistics (a), PACo's  $R^2$  (b), and the ratio between the number of host transfers and the  
635 number of cospeciations in eMPress reconciliations (c) as a function of the test  
636 significance and the simulation scenario of trait matching, vicariance, or phylogenetic  
637 tracking. Here, we only reported eMPress reconciliations obtained when subsampling  
638 one host per symbiont species (with relative costs  $d=4$ ,  $t=1$ , and  $l=1$ ), but similar results  
639 were observed when using random bifurcations (Supplementary Fig. S5).

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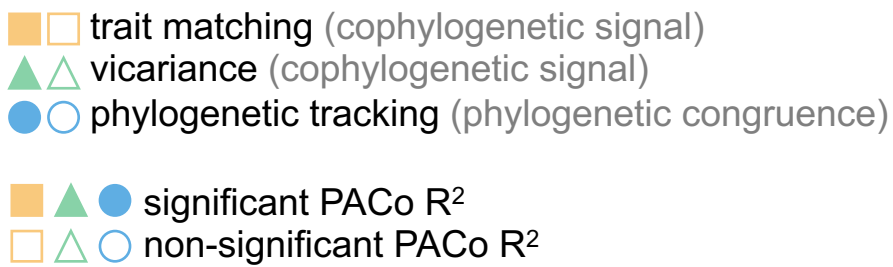
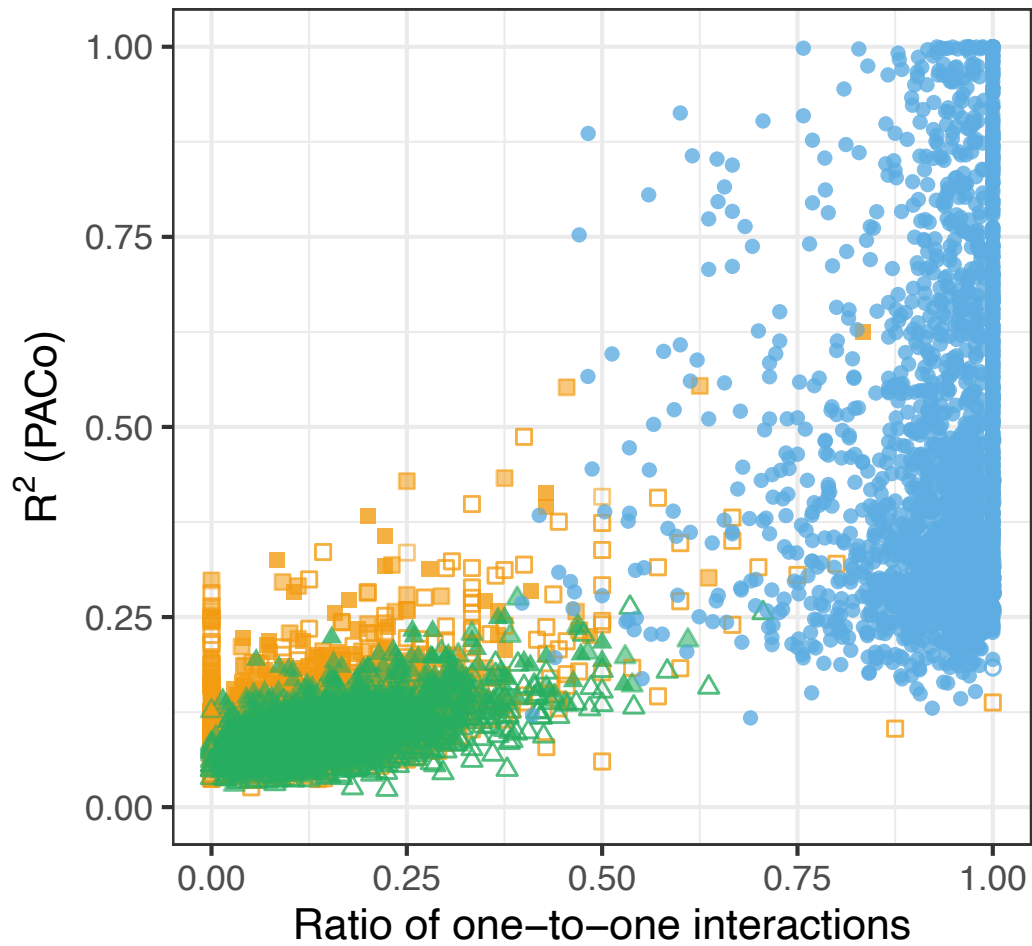


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643 **Figure 3: PACo's statistic ( $R^2$ ) tends to increase with the ratio of one-to-one**  
 644 **interactions; however, PACo tests tend to be more often significant when the ratio**  
 645 **of one-to-one interaction is low under scenarios of trait matching and vicariance.**

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649 **Figure 4: Patterns of cophylogenetic signal and phylogenetic congruence can be**  
 650 **generated by various processes:**

651 Event-based and global-fit methods differently measure these patterns: Event-based  
 652 methods can robustly identify phylogenetic congruence, whereas global-fit methods  
 653 measure cophylogenetic signal. Some of the statistics of global-fit methods (e.g. the  $R^2$   
 654 of PACo) can inform whether the cophylogenetic signal may be due to phylogenetic  
 655 congruence: a low  $R^2$  ( $R^2 < 0.25$ ) indicates that there is a cophylogenetic signal but no  
 656 phylogenetic congruence, whereas a higher  $R^2$  ( $R^2 > 0.25$ ) suggests there is a  
 657 cophylogenetic signal and potentially also phylogenetic congruence, but the latter  
 658 needs to be validated using eMPress, as  $R^2 > 0.25$  also frequently occur in systems  
 659 without phylogenetic congruence (Fig. 3).

660

