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<sup>6</sup> **IN FOCUS**  
<sup>7</sup> **Natural history matters: how biological**  
<sup>8</sup> **constraints shape diversified interactions in**  
<sup>9</sup> **pollination networks**

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15 IN FOCUS: Sazatornil, F.D., Moré, M., Benitez-Vieyra, S., Cocucci, A.A.,  
16 Kitching, I.J., Schlumpberger, B.O., Oliveira, P.E., Sazima, M. & Amorim,  
17 F.W. (2016) Beyond neutral and forbidden links: morphological matches  
18 and the assembly of mutualistic hawkmoth-plant networks. *Journal of An-*  
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21 **Species-specific traits and life-history characteristics constrain**  
22 **the ways organisms interact in nature. For example, gape-limited**  
23 **predators are constrained in the sizes of prey they can handle and**  
24 **efficiently consume. When we consider the ubiquity of such con-**  
25 **straints it is evident how hard it can be to be a generalist partner**  
26 **in ecological interactions: a free living animal or plant can't simply**  
27 **interact with every available partner it encounters. Some pairwise**  
28 **interactions among coexisting species simply do not occur; they**  
29 **are impossible to observe despite the fact that partners coexist in**  
30 **the same place. Sazatornil *et al.* (1) explore the nature of such**  
31 **constraints in the mutualisms among hawkmoths and the plants**  
32 **they pollinate. In this iconic interacion, used by Darwin and Wal-**  
33 **lace to vividly illustrate the power of natural selection in shaping**  
34 **evolutionary change, both pollinators and plants are sharply con-**  
35 **strained in their interaction modes and outcomes.**

36

37 **Keywords:** complex networks, forbidden links, long-tubed flowers, mutual-  
38 ism, pollination, Sphingidae

39        Size-limited foragers show clear restrictions on the size of prey items they  
40      can efficiently handle. In the case of plant-pollinator interactions, size un-  
41      coupling between pollinator bodies and flower sizes (i.e., length of spur or  
42      corolla tubes) or structure are specially relevant in filtering out a range of  
43      potential partners (2). As a general trend, also observed in frugivorous anim-  
44      als dispersing seeds, larger (long-tongued) species can exploit longer-tubed  
45      flowers and a wider range of flower sizes. The size of the feeding apparatus  
46      (e.g., tongue length, gape width) limits the maximum size of flower or fruit  
47      an animal mutualist can efficiently use. Phenotypic trait matching is thus  
48      a key influence in the effectiveness of plant-animal interactions shaped by  
49      these size effects, where the interaction outcomes depend on close matching.  
50      The idea, when applied to the bizarre flowers of some plants pollinated by  
51      sphingid moths (Lepidoptera: Sphingidae) (Fig. 1), was seminal in Dar-  
52      winian evolutionary theory to support the potential of natural selection in  
53      shaping adaptations (3). Wallace (4) in his book, *Creation by law*, vividly  
54      uses the famous example of the Malagasy orchid and its sphingid pollinator  
55      to refute the arguments of the Duke of Argyll against natural selection and  
56      Darwinism:

57            "There is a Madagascar Orchis—the *Angraecum sesquipedale*—  
58      with an immensely long and deep nectary. How did such an  
59      extraordinary organ come to be developed? Mr. Darwin's [[p.  
60      475]] explanation is this. The pollen of this flower can only be  
61      removed by the proboscis of some very large moths trying to get  
62      at the nectar at the bottom of the vessel. The moths with the  
63      longest proboscis would do this most effectually; they would be  
64      rewarded for their long noses by getting the most nectar; whilst  
65      on the other hand, the flowers with the deepest nectaries would  
66      be the best fertilized by the largest moths preferring them. Con-  
67      sequently, the deepest nectaried Orchids and the longest nosed

68        moths would each confer on the other a great advantage in the  
69        'battle of life.' This would tend to their respective perpetuation  
70        and to the constant lengthening of nectar and noses."

71        Phenotypic fitting of corolla length and shape and the pollinators' feeding  
72        apparatus and body sizes are important because the better the fit, the better  
73        the consequences in terms of fitness outcomes for the interaction partners  
74        (5). Yet the expectation of perfect trait matching across populations or com-  
75        munities is too simplistic (6): "arms races" as initially suggested by Darwin  
76        and Wallace are frequently asymmetric, originating pollinator shifts rather  
77        than tight phenotypic trait matching (Fig. 2)(7). Therefore, extensive local  
78        variation in phenotypic mismatch exists in different plant-pollinator systems  
79        (e.g., 2; 6; 8), with pollinator-mediated selection geographic mosaics of loc-  
80        ally coevolved partners where tight phenotypic matching is not necessarily  
81        the rule.

82        Recent work by Sazatornil *et al.* (1) provides evidences that the types  
83        of trait mismatching outlined in Fig. 2 limit the ranges of host plants for  
84        sphingid pollinators, and ultimately shape their complex plant-pollinator net-  
85        works. By using a comparative analysis of five different hawkmoth/flower  
86        assemblages across four South American biotas (Atlantic rainforest and Cer-  
87        rado in Brazil, Chaco montane dry woodland, and the ecotone between west-  
88        ern Chaco woodland and Yungas montane rain forest in Argentina) they  
89        tested the contributions of phenotypic matching to explain observed patterns  
90        of moth-flower interactions.

91        How are these moth-flower interactions assembled? Pairwise patterns of  
92        trait matching scale-up to conform complex webs of interaction where in  
93        some cases interaction topology is linked to phenotypic fit between partners

94 while in others the interaction pattern appears more determined by neut-  
95 ral processes (i.e., probability of interspecific encounter). Sazatornil *et al.*  
96 (1) use a comparative analysis of hawkmoth proboscis length ( $HPL$ ) and  
97 the effective length of the flower ( $EFL$ ) for every pairwise interaction re-  
98 corded.  $EFL$  is just the corolla tube length (as in Fig. 2 for long-tubed  
99 and salverform corollas) or the stamen protrusion length in brush-type and  
100 funnel-shape flowers (as in Fig. 1). The authors first tested a neutral model,  
101 where interactions are independent of trait-matching and driven solely by  
102 variation in local abundance. Under this hypothesis distribution paramet-  
103 ers (mean and standard deviation) must be the same for both the simulated  
104 and empirical distributions. They further tested a Forbidden links hypo-  
105 thesis, where interactions occurred only if  $HPL \geq EFL$ . Sazatornil *et al.*  
106 further tested the morphological match hypothesis, where the probability of  
107 occurrence of an interaction depends on the frequency of possible pairwise  
108 differences between  $HPL$  and  $EFL$ , i.e., all possible pairwise  $HPL - EFL$   
109 differences were weighted by their respective interaction frequency.

110 The trait matching between  $HPL$  and  $EFL$  is crucial in this type of  
111 interaction and determines its outcome in terms of fitness for both partners.  
112 Nilsson (5) demonstrated experimentally that shortening the nectary tube  
113 of long-spurred corollas decreased both seed set and pollinia removal for  
114 *Platanthera* orchids. Further experimental evidence has been provided for  
115 long-tongued nemestrinid flies pollinating long-tubed irises in South Africa,  
116 where increased mismatch decreases both plant fitness and the nectar ex-  
117 traction efficiency of the pollinators (9) (also see 6, and references therein).  
118 Sazatornil *et al.* extend those results to the scale of the whole moth-plant

119 assemblage and demonstrate that trait matching successfully predicts the  
120 diversity of interactions recorded. Interestingly enough, the interaction pat-  
121 terns in two local assemblages from ecotone areas of the argentinian Chaco  
122 woodland-Yungas montane rain forest transition are better fitted by a neutral  
123 model where pairwise interactions are driven by probability of interspecific  
124 encounter. Yet Sazatornil *et al.* did not include the morphological difference  
125 for parameter estimation when interactions were not recorded. Thus the test  
126 of the mismatch hypothesis implicitly includes forbidden links effects: a full  
127 mismatch of corolla tube/proboscis lengths actually means a forbidden link.  
128 Furthermore, a fraction of unobserved interactions was likely caused by phen-  
129 ological uncoupling between flowering and hawkmoth activity phenophases  
130 (14; 1). In any case the mismatch hypothesis somehow captures the fact  
131 that a fraction of the unobserved interactions in these hawkmoth/flower as-  
132 semblages is due to extreme phenotypic mismatch, i.e., size-related forbidden  
133 links (1); also see (10) for evidences with hummingbird-flower interactions.

134       Forbidden links represent a family of causes for not observing specific in-  
135 teractions when sampling diversified plant-animal interaction networks, and  
136 stem on biological causes deeply linked to the fascinating natural history de-  
137 tails of these interactions (14). The raw material for phenotypic mismatches  
138 in the specific case of hawkmoth-flower interactions is the extreme variability  
139 of the two pivotal traits determining their outcomes: proboscis length and  
140 corolla/spur or nectary depth (Fig. 2) (2; 3; 5; 11). This variation is extens-  
141 ive for both the plant and pollinator partners and observable at individual,  
142 population and species levels.

143       Sazatornil et al. approach would be most useful for proper tests of coe-

<sup>144</sup> volutionary hypotheses in hawkmoth/flower assemblages (and plant-animal  
<sup>145</sup> mutualisms in general): assessing match/mismatch patterns for every pos-  
<sup>146</sup> sible pairwise interaction among partners within complex webs of interac-  
<sup>147</sup> tion where multiple life-history attributes may contribute biological reasons  
<sup>148</sup> for forbidden links. The morphological match hypothesis is not the only  
<sup>149</sup> mechanism to explain patterns of hawkmoth–plant interactions, where other  
<sup>150</sup> life-history limitations may operate generating forbidden links, e.g., pheno-  
<sup>151</sup> logical mismatches (for example in the case of long-distance or elevational  
<sup>152</sup> migratory hawkmoths), constraints from foraging for oviposition sites (12),  
<sup>153</sup> energetic constraints due to balances of nectar availability/foraging costs  
<sup>154</sup> (13), etc. Most importantly, this paper nicely illustrates how the fascinating  
<sup>155</sup> natural history details of these interactions can be use to build meaningful  
<sup>156</sup> testable models to assess the mechanisms beyond structure and function of  
<sup>157</sup> megadiversified webs of interactions among free-living species.

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<sup>163</sup> tubed flowers. Comments and suggestions by Rodrigo Medel and Jeferson  
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<sup>212</sup> **Figures**

<sup>213</sup> **Figure 1.** Morphological mismatches set important biological constraints  
<sup>214</sup> for size-limited foragers, including e.g., predators, pollinators, and frugi-  
<sup>215</sup> vores. In plant-animal mutualisms (e.g., plant-pollinator interactions), a  
<sup>216</sup> morphological mismatch between partners sets size limits that filter out a  
<sup>217</sup> range of phenotypes that otherwise could eventually interact. Two main coe-  
<sup>218</sup> volutionary trends in hawkmoth-flower interactions involve arms-race trends  
<sup>219</sup> (with progressively longer spurs and proboscides) and pollinator shifts (where  
<sup>220</sup> short-tongued moths are replaced as legitimate pollinators by long-tongued  
<sup>221</sup> species when corolla tubes increase in length). In many cases, pollination is  
<sup>222</sup> impossible when the proboscis is longer than the spurs because the pollen  
<sup>223</sup> or pollinaria are attached further from the base of the proboscis. When this  
<sup>224</sup> happens the pollen or pollinaria may be scratched away by the forelegs  
<sup>225</sup> when the proboscis is rolled to a loose spiral; yet in other cases, actual pollen  
<sup>226</sup> transfer may occur when long-tongued moths visit short-tubed flowers. If  
<sup>227</sup> the proboscis is shorter than the spur, transfer of the pollen or pollinaria is  
<sup>228</sup> possible as long as the proboscis can get in contact with the sexual organs  
<sup>229</sup> of the flowers. Other reasons for forbidden links include, e.g., phenological  
<sup>230</sup> differences (14). Thus, a number of the potential interactions that could  
<sup>231</sup> take place in a given mutualistic assemblage simply cannot occur because  
<sup>232</sup> of biological reasons: these are forbidden interactions. Photo: A sphingid  
<sup>233</sup> moth, *Manduca sexta* visiting a flower of *Tocoyena formosa* (Rubiaceae) in  
<sup>234</sup> the Brazilian Cerrado; tongue and corolla tube lengths approximately 100  
<sup>235</sup> mm. Top, approaching and probing a flower; bottom, extracting nectar.

<sup>236</sup> Photo courtesy of Felipe Amorim.

<sup>237</sup>

<sup>238</sup> **Figure 2.** The mechanistic basis of morphological mismatches in hawkmoth-  
<sup>239</sup> flower interactions. For example, nectar accessibility in *Angraecum sesqui-*  
<sup>240</sup> *pedale* long-tubed flowers. A, only small volumes of nectar can be exploited  
<sup>241</sup> if the spur is 27 cm long. B, moths with an average tongue length of 22 cm  
<sup>242</sup> can obtain about 50  $\mu L$  nectar from a spur of 33.3 cm average length and  
<sup>243</sup> an average nectar volume of 165  $\mu L$ . C, a spur 43 cm long could offer nectar  
<sup>244</sup> to a moth with a 22-cm-long proboscis only if it contains more than 240  $\mu L$   
<sup>245</sup> nectar. Lack of fit prevents interactions, but also marginal fitting renders  
<sup>246</sup> interactions unlikely because of energetic constraints. Modified and redrawn  
<sup>247</sup> from (3).

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