

1 **Running title:** Forbidden interactions
2 **Number of words:** ~1949
3 **Number of tables:** 0
4 **Number of figures:** 2
5 **Number of references:** 14

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

10 **Pedro Jordano¹**

11 *1. Integrative Ecology Group, Estación Biológica de Doñana, Consejo Superior*
12 *de Investigaciones Científicas (EBD-CSIC), Avenida Americo Vespucio s/n,*
13 *E-41092 Sevilla, Spain*

14 *Correspondence author. jordano@ebd.csic.es

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-*
19 *imal Ecology*, 00, 000–000. doi:10.1111/1365-2656.12509

20
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 interaction, 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 *Angræcum 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-

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

158 **Acknowledgments**

159 My work was funded by a Severo-Ochoa Excellence Grant (SEV2012-0262)
160 from the Spanish Ministerio de Economía y Competitividad (MINECO),
161 and RNM-5731 from the Junta de Andalucía. Andrea Cocucci generously
162 provided material for Fig. 1 and insightful discussions on sphingids and long-
163 tubed flowers. Comments and suggestions by Rodrigo Medel and Jeferson
164 Vizentin-Bugoni helped to improve the manuscript.

References

- [1] Sazatornil, F.D., Moré, M., Benitez-Vieyra, S., Cocucci, A.A., Kitching, I.J., Schlumpberger, B.O., Oliveira, P.E., Sazima, M. & Amorim, F.W. (2016) Beyond neutral and forbidden links: morphological matches and the assembly of mutualistic hawkmoth-plant networks. *Journal of Animal Ecology*, 00, 000–000.
- [2] Cocucci, A.A., Moré, M. & Sérsic, A.N. (2009) Restricciones mecánicas en las interacciones planta-polinizador: estudio de casos en plantas polinizadas por esfíngidos. *Interacciones planta—animal y la conservación de la biodiversidad* (eds R. Medel, R. Zamora, M. Aizen & R. Dirzo), pp. 43–59. CYTED, Madrid.
- [3] Arditti, J., Elliott, J., Kitching, I.J. & Wasserthal, L.T. (2012) “Good Heavens what insect can suck it”—Charles Darwin, *Angraecum sesquipedale* and *Xanthopan morgani praedicta*. *Botanical Journal of the Linnean Society*, 169, 403–432.
- [4] Wallace, A.R. (1867) Creation by law. *The Quarterly Journal of Science*, 4, 471–488.
- [5] Nilsson, L.A. (1988) The evolution of flowers with deep corolla tubes. *Nature*, 334, 147–149.
- [6] Anderson, B., Terblanche, J.S. & Ellis, A.G. (2010) Predictable patterns of trait mismatches between interacting plants and insects. *BMC Evolutionary Biology*, 10, 204.
- [7] Wasserthal, L.T. (2014) The Pollinators of the Malagasy star orchids *Angraecum sesquipedale*, *A. sororium* and *A. compactum* and the evolution of extremely long spurs by pollinator shift. *Botanica Acta*, 110, 343–359.
- [8] Moré, M., Amorim, F.W., Benitez-Vieyra, S., Medina, A.M., Sazima, M. & Cocucci, A.A. (2012) Armament imbalances: match and mismatch in plant-pollinator traits of highly specialized long-spurred orchids. *PLoS ONE*, 7, e41878.
- [9] Pauw, A., Stofberg, J. & Waterman, R.J. (2009) Flies and flowers in Darwin’s race. *Evolution*, 63, 268–279.
- [10] Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird-plant network. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 281, 20132397.
- [11] Miller, W.E. (1997) Diversity and evolution of tongue length in hawkmoths (Sphingidae). *Journal of the Lepidopterists Society*, 51, 9–31.

- 201 [12] Alarcón, R., Davidowitz, G. & Bronstein, J.L. (2008) Nectar usage in a south-
202 ern Arizona hawkmoth community. *Ecological Entomology*, 33, 503–509.
- 203 [13] Borrell, B. (2005) Long tongues and loose niches: Evolution of euglossine bees
204 and their nectar flowers. *Biotropica*, 37, 664–669.
- 205 [14] Bascompte, J. & Jordano, P. (2014) *Mutualistic Networks*. Princeton Univer-
206 sity Press, Princeton, NJ.

207 **Pedro Jordano**

208 *Integrative Ecology Group, Estación Biológica de Doñana,*
209 *Consejo Superior de Investigaciones Científicas (EBD-CSIC),*
210 *Avenida Americo Vespucio s/n,*
211 *E-41092 Sevilla, Spain*

212 Figures

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

235

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

246



