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6 **IN FOCUS**  
7 **Natural history matters: how biological**  
8 **constraints shape diversified interactions in**  
9 **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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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. Pairwise patterns of trait matching scale-up to  
91       conform complex webs of interaction where in some cases interaction topology  
92       is linked to phenotypic fit between partners while in others the interaction  
93       pattern appears more determined by neutral processes (i.e., probability of

interspecific encounter).

How are these moth-flower interactions assembled? Sazatornil *et al.* (1) use a comparative analysis of hawkmoth proboscis length (*HPL*) and the effective length of the flower (*EFL*) for every pairwise interaction recorded. *EFL* is just the corolla tube length (as in Fig. 2 for long-tubed and salverform corollas) or the stamen protrusion length in brush-type and funnel-shape flowers (as in Fig. 1). The authors first tested a neutral model, where interactions are independent of trait-matching and driven solely by variation in local abundance. Under this hypothesis distribution parameters (mean and standard deviation) must be the same for both the simulated and empirical distributions. They further tested a Forbidden links hypothesis, where interactions occurred only if  $HPL \geq EFL$ . Sazatornil *et al.* further tested the morphological match hypothesis, where the probability of occurrence of an interaction depends on the frequency of possible pairwise differences between *HPL* and *EFL*, i.e., all possible pairwise  $HPL - EFL$  differences were weighted by their respective interaction frequency.

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

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

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## Figures

**Fig. 1** Morphological mismatches set important biological constraints for size-limited foragers, including e.g., predators, pollinators, and frugivores. In plant-animal mutualisms (e.g., plant-pollinator interactions), a morphological mismatch between partners sets size limits that filter out a range of phenotypes that otherwise could eventually interact. Two main coevolutionary trends in hawkmoth-flower interactions involve arms-race trends (with progressively longer spurs and proboscides) and pollinator shifts (where short-tongued moths are replaced as legitimate pollinators by long-tongued species when corolla tubes increase in length). Pollination is impossible when the proboscis is longer than the spurs because the pollen or pollinaria are attached further from the base of the proboscis. When this happens the pollen or pollinaria are scratched away by the forelegs when the proboscis is rolled to a loose spiral. If the proboscis is shorter than the spur, transfer of the pollen or pollinaria is possible as long as the proboscis can get in contact with the sexual organs of the orchid. Other reasons for forbidden links include, e.g., phenological differences (14). Thus, a number of the potential interactions that could take place in a given mutualistic assemblage simply cannot occur because of biological reasons: these are forbidden interactions. Photo: courtesy of Andrea Cocucci. A sphingid moth, *Agrius cingulata*, visiting a flower of *Bauhinia mollis* (Fabaceae), Las Yungas, Argentina.



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

