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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 or structure are specially  
42 relevant in filtering out a range of potential partners (2). The idea, when  
43 applied to the bizarre flowers of some plants pollinated by sphingid moths  
44 (Lepidoptera: Sphingidae) (Fig. 1), was seminal in Darwinian evolutionary  
45 theory to support the potential of natural selection in shaping adaptations  
46 (3). Wallace (4) in his book, *Creation by law*, vividly uses the famous example  
47 of the Malagasy orchid and its sphingid pollinator to refute the arguments  
48 of the Duke of Argyll against natural selection and Darwinism:

49       "There is a Madagascar Orchis—the *Angræcum sesquipedale*—  
50 with an immensely long and deep nectary. How did such an  
51 extraordinary organ come to be developed? Mr. Darwin's [[p.  
52 475]] explanation is this. The pollen of this flower can only be  
53 removed by the proboscis of some very large moths trying to get  
54 at the nectar at the bottom of the vessel. The moths with the  
55 longest proboscis would do this most effectually; they would be  
56 rewarded for their long noses by getting the most nectar; whilst  
57 on the other hand, the flowers with the deepest nectaries would  
58 be the best fertilized by the largest moths preferring them. Con-  
59 sequently, the deepest nectaried Orchids and the longest nosed  
60 moths would each confer on the other a great advantage in the  
61 'battle of life.' This would tend to their respective perpetuation  
62 and to the constant lengthening of nectar and noses."

63       Phenotypic fitting of corolla length and shape and the pollinators' feeding  
64 apparatus and body sizes are important because the better the fit, the better  
65 the consequences in terms of fitness outcomes for the interaction partners  
66 (5). Yet the expectation of perfect trait matching across populations or  
67 communities is too simplistic (6): "arms races" as initially suggested by  
68 Darwin and Wallace are frequently asymmetric, originating pollinator shifts

rather than tight phenotypic trait matching (Fig. 2)(7). Extensive local variation in phenotypic mismatch exists in different plant-pollinator systems (e.g., 2; 6; 8), with pollinator-mediated selection geographic mosaics of locally coevolved partners.

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

How are these moth-flower interactions assembled? Sazatornil *et al.* (1) first tested a neutral model, where interactions are independent of trait-matching. Under this hypothesis distribution parameters (mean and standard deviation) must be the same for both distributions. They further tested a Forbidden links hypothesis, where interactions occurred only if the hawkmoth proboscis length (HPL) is equal to or greater than the effective length of the flower (EFL). 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). 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

94 by their respective interaction frequency.  
 95 Th trait matching between HPL and EFL is crucial in this type of in-  
 96 teraction and determines its outcome in terms of fitness for both partners.  
 97 Nilsson (5) demonstrated experimentally that shortening the nectary tube  
 98 of long-spurred corollas decreased both seed set and pollinia removal for  
 99 *Platanthera* orchids. Further experimental evidence has been provided for  
 100 long-tongued nemestrinid flies pollinating long-tubed irises in South Africa,  
 101 where increased mismatch decreases both plant fitness and the nectar extrac-  
 102 tion efficiency of the pollinators (9). Sazatornil *et al.* extend those results  
 103 to the scale of the whole moth-plant assemblage and demonstrate that trait  
 104 matching successfully predicts the diversity of interactions recorded. In-  
 105 terestingly enough, the interaction patterns in two local assemblages from  
 106 ecotone areas of the Chaco woodland-Yungas montane rain forest transition  
 107 are better fitted by a neutral model where pairwise interactions are driven by  
 108 probability of interspecific encounter. Yet Sazatornil *et al.* did not include  
 109 the morphological difference for parameter estimation when interactions were  
 110 not recorded. Thus the test of the mismatch hypothesis implicitly includes  
 111 forbidden links effects: a full mismatch of corolla tube/proboscis lengths  
 112 actually means a forbidden link. Furthermore, a fraction of unobserved in-  
 113 teractions was likely caused by phenological uncoupling between flowering  
 114 and hawkmoth activity phenophases (14; 1). In any case the mismatch hy-  
 115 pothesis somehow captures the fact that a fraction of the unobserved inter-  
 116 actions in these hawkmoth/flower assemblages is due to extreme phenotypic  
 117 mismatch, i.e., size-related forbidden links (1); also see (10) for evidences  
 118 with hummingbird-flower interactions.

Forbidden links represent a family of causes for not observing specific interactions when sampling diversified plant-animal interaction networks, and stem on biological causes deeply linked to the fascinating natural history details of these interactions (14). The raw material for phenotypic mismatches in the specific case of hawkmoth-flower interactions is the extreme variability of the two pivotal traits determining their outcomes: proboscis length and corolla/spur or nectary depth (Fig. 2) (2; 3; 5; 11).

Sazatornil et al. approach would be most useful for proper tests of coevolutionary hypotheses in hawkmoth/flower assemblages (and plant-animal mutualisms in general): assessing match/mismatch patterns for every possible pairwise interaction among partners within complex webs of interaction where multiple life-history attributes may contribute biological reasons to expect forbidden links. The morphological match hypothesis is not the only mechanism to explain patterns of hawkmoth-plant interactions, where other life-history limitations may operate generating forbidden links, e.g., phenological mismatches (for example in the case of long-distance or elevational migratory hawkmoths), constraints from foraging for oviposition sites (12), energetic constraints due to balances of nectar availability/foraging costs (13), etc.

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<sup>143</sup> long-tubed flowers.

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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).

