

1 **Running title:** Forbidden interactions  
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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-*  
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 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 com-  
67 munities is too simplistic (7): "arms races" as initially suggested by Darwin  
68 and Wallace are frequently asymmetric, originating pollinator shifts rather

69 than tight phenotypic trait matching (Fig. 2). Extensive local variation  
70 in phenotypic mismatch exists in different plant-pollinator systems (e.g.,  
71 2; 7; 8), with pollinator-mediated selection geographic mosaics of locally  
72 coevolved partners.

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

82 How are these moth-flower interactions assembled? Sazatornil *et al.* (1)  
83 first tested a neutral model, where interactions are independent of trait-  
84 matching. Under this hypothesis distribution parameters (mean and stand-  
85 ard deviation) must be the same for both distributions. They further tested  
86 a Forbidden links hypothesis, where interactions occurred only if the hawk-  
87 moth proboscis length (HPL) is equal to or greater than the effective length  
88 of the flower (EFL). EFL is just the corolla tube length (as in Fig. 2 for long-  
89 tubed and salverform corollas) or the stamen protrusion length in brush-type  
90 and funnel-shape flowers (as in Fig. 1). Sazatornil *et al.* further tested the  
91 morphological match hypothesis, where the probability of occurrence of an  
92 interaction depends on the frequency of possible pairwise differences between  
93 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 of  
98 long-spurred corollas decreased both seed set and pollinia removal for *Plat-*  
99 *anthera* orchids. Further experimental evidence has been provided for long-  
100 tongued nemestrinid flies pollinating long-tubed irises in South Africa, where  
101 increased mismatch decreases both plant fitness and the nectar extraction ef-  
102 ficiency of the pollinators (6). Sazatornil *et al.* extend those results to the  
103 scale of the whole moth-plant assemblage and demonstrate that trait match-  
104 ing successfully predicts the diversity of interactions recorded. Interestingly  
105 enough, the interaction patterns in two local assemblages from ecotone areas  
106 of the Chaco woodland-Yungas montane rain forest transition are better fit-  
107 ted by a neutral model where pairwise interactions are driven by probability  
108 of interspecific encounter.

109 Yet Sazatornil *et al.* did not include the morphological difference for  
110 parameter estimation when interactions were not recorded. Thus the test  
111 of the mismatch hypothesis implicitly includes forbidden links effects: a full  
112 mismatch of corolla tube/proboscis lengths actually means a forbidden link.  
113 In any case the mismatch hypothesis somehow captures the fact that a frac-  
114 tion of the unobserved interactions in these hawkmoth/flower assemblages is  
115 due to extreme phenotypic mismatch (i.e., size-related forbidden links).

116 Sazatornil *et al.* approach would be most useful for proper tests of coe-  
117 evolutionary hypotheses in hawkmoth/flower assemblages (and plant-animal  
118 mutualisms in general): assessing match/mismatch patterns for every pos-

119 sible pairwise interaction among partners within complex webs of interaction  
120 where multiple life-history attributes may contribute biological reasons to  
121 expect forbidden links. The morphological match hypothesis is not the only  
122 mechanism to explain patterns of hawkmoth–plant interactions, where other  
123 life-history limitations may operate generating forbidden links, e.g., phenolo-  
124 gical mismatches (for example in the case of long-distance or elevational mi-  
125 gratory hawkmoths), energetic constraints due to nectar availability/foraging  
126 costs balances, etc.

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132 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, a morphological mismatch between partners sets size limits that filter out a range of phenotypes that otherwise could eventually interact. Other reasons for forbidden links include, e.g., phenological differences (9). 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: Andrea Cocucci. An sphingid moth, *Agrius cingulata*, visiting a flower of *Bauhinia mollis* (Fabaceae), Las Yungas, Argentina.

