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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-*
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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 reasons 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). They include phenological, size, microhabitat,
138 sensory, accessibility, or any other type of mismatches between partners that
139 explain why some pairwise interactions never occur. The raw material for
140 phenotypic mismatches in the specific case of hawkmoth-flower interactions is
141 the extreme variability of the two pivotal traits determining their outcomes:
142 proboscis length and corolla/spur or nectary depth (Fig. 2) (2; 3; 5; 11).
143 This variation is extensive for both the plant and pollinator partners and

¹⁴⁴ observable at individual, population and species levels.

¹⁴⁵ Sazatornil et al. approach would be most useful for proper tests of coe-
¹⁴⁶ volutionary hypotheses in hawkmoth/flower assemblages (and plant-animal
¹⁴⁷ mutualisms in general): assessing match/mismatch patterns for every pos-
¹⁴⁸ sible pairwise interaction among partners within complex webs of interac-
¹⁴⁹ tion where multiple life-history attributes may contribute biological reasons
¹⁵⁰ for forbidden links. The morphological match hypothesis is not the only
¹⁵¹ mechanism to explain patterns of hawkmoth–plant interactions, where other
¹⁵² life-history limitations may operate generating forbidden links, e.g., pheno-
¹⁵³ logical mismatches (for example in the case of long-distance or elevational
¹⁵⁴ migratory hawkmoths), constraints from foraging for oviposition sites (12),
¹⁵⁵ energetic constraints due to balances of nectar availability/foraging costs
¹⁵⁶ (13), etc. Match–mismatch patterns are expected when we consider these
¹⁵⁷ interactions across geographic mosaics at different spatial scales, with hot-
¹⁵⁸ and cold-spots of phenotypic matching generating variable outcomes of in-
¹⁵⁹ teractions (6; 8; 9; 14) . Most importantly, Sazatornil et al. nicely illustrate
¹⁶⁰ how the fascinating natural history details of these interactions can be used
¹⁶¹ to build meaningful testable models to assess the mechanisms beyond struc-
¹⁶² ture and function of megadiversified webs of interactions among free-living
¹⁶³ species.

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214 **Pedro Jordano**

215 *Integrative Ecology Group, Estación Biológica de Doñana,*
216 *Consejo Superior de Investigaciones Científicas (EBD-CSIC),*
217 *Avenida Americo Vespucio s/n,*
218 *E-41092 Sevilla, Spain*

²¹⁹ **Figures**

²²⁰ **Figure 1.** Morphological mismatches set important biological constraints
²²¹ for size-limited foragers, including e.g., predators, pollinators, and frugi-
²²² vores. In plant-animal mutualisms (e.g., plant-pollinator interactions), a
²²³ morphological mismatch between partners sets size limits that filter out a
²²⁴ range of phenotypes that otherwise could eventually interact. Two main coe-
²²⁵ volutionary trends in hawkmoth-flower interactions involve arms-race trends
²²⁶ (with progressively longer spurs and proboscides) and pollinator shifts (where
²²⁷ short-tongued moths are replaced as legitimate pollinators by long-tongued
²²⁸ species when corolla tubes increase in length). In many cases, pollination is
²²⁹ impossible when the proboscis is longer than the spurs because the pollen
²³⁰ or pollinaria are attached further from the base of the proboscis. When this
²³¹ happens the pollen or pollinaria may be scratched away by the forelegs
²³² when the proboscis is rolled to a loose spiral; yet in other cases, actual pollen
²³³ transfer may occur when long-tongued moths visit short-tubed flowers. If
²³⁴ the proboscis is shorter than the spur, transfer of the pollen or pollinaria is
²³⁵ possible as long as the proboscis can get in contact with the sexual organs
²³⁶ of the flowers. Other reasons for forbidden links include, e.g., phenological
²³⁷ differences (14). Thus, a number of the potential interactions that could
²³⁸ take place in a given mutualistic assemblage simply cannot occur because
²³⁹ of biological reasons: these are forbidden interactions. Photo: A sphingid
²⁴⁰ moth, *Manduca sexta* visiting a flower of *Tocoyena formosa* (Rubiaceae) in
²⁴¹ the Brazilian Cerrado; tongue and corolla tube lengths approximately 100
²⁴² mm. Top, approaching and probing a flower; bottom, extracting nectar.

²⁴³ Photo courtesy of Felipe Amorim.

²⁴⁴

²⁴⁵ **Figure 2.** The mechanistic basis of morphological mismatches in hawkmoth-
²⁴⁶ flower interactions. For example, nectar accessibility in *Angraecum sesqui-*
²⁴⁷ *pedale* long-tubed flowers. A, only small volumes of nectar can be exploited
²⁴⁸ if the spur is 27 cm long. B, moths with an average tongue length of 22 cm
²⁴⁹ can obtain about 50 μL nectar from a spur of 33.3 cm average length and
²⁵⁰ an average nectar volume of 165 μL . C, a spur 43 cm long could offer nectar
²⁵¹ to a moth with a 22-cm-long proboscis only if it contains more than 240 μL
²⁵² nectar. Lack of fit prevents interactions, but also marginal fitting renders
²⁵³ interactions unlikely because of energetic constraints. Modified and redrawn
²⁵⁴ from (3).

²⁵⁵



