IN FOCUS

Natural history matters: how biological constraints shape diversified interactions in pollination networks

Species-specific traits constrain the ways organisms interact in nature. Some pairwise interactions among coexisting species simply do not occur; they are impossible to observe despite the fact that partners coexist in the same place. I discuss these 'forbidden links' of species interaction networks. Photo: the sphingid moth *Agrius cingulata* visiting a *Bauhinia mollis* flower; Las Yungas, Argentina. Courtesy of Andrea Cocucci.

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Species-specific traits and life-history characteristics constrain the ways organisms interact in nature. For example, gape-limited predators are constrained in the sizes of prey they can handle and efficiently consume. When we consider the ubiquity of such constrains, it is evident how hard it can be to be a generalist partner in ecological interactions: a free-living animal or plant cannot simply interact with every available partner it encounters. Some pairwise interactions among coexisting species simply do not occur; they are impossible to observe despite the fact that partners coexist in the same place. Sazatornil *et al.* (2016) explore the nature of such constraints in the mutualisms among hawkmoths and the plants they pollinate. In this iconic interaction, used by Darwin and Wallace to vividly illustrate the power of natural selection in shaping evolutionary change, both pollinators and plants are sharply constrained in their interaction modes and outcomes.

Size-limited foragers show clear restrictions on the size of prey items they can efficiently handle. In the case of plant–pollinator interactions, size uncoupling between pollinator bodies and flower sizes (i.e. length of spur or corolla tubes) or structure is especially relevant in filtering out a range of potential partners (Cocucci, Moré & Sérsic 2009). As a general trend, also observed in frugivorous animals dispersing seeds, larger (long-tongued) species can exploit longer-tubed flowers and a wider range of flower sizes. The size of the feeding apparatus (e.g. tongue length, gape width) limits the maximum size of flower or fruit an animal mutualist can efficiently use. Phenotypic trait matching is thus a key influence in the effectiveness of plant–animal interactions shaped by these size effects, where the interaction outcomes depend on close matching. The idea, when applied to the bizarre flowers of some plants pollinated by sphingid moths (Lepidoptera: Sphingidae; Fig. 1), was seminal in Darwinian evolutionary theory to support the potential of natural selection in shaping adaptations (Arditti *et al.* 2012). Wallace (1867) in his book, *Creation by law*, vividly uses the famous example of the Malagasy orchid and its sphingid pollinator to refute the arguments of the Duke of Argyll against natural selection and Darwinism:

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Fig. 1. Morphological mismatches set important biological constraints for size-limited foragers, including 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 co-evolutionary 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). In many cases, 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 may be scratched away by the forelegs when the proboscis is rolled to a loose spiral; yet in other cases, actual pollen transfer may occur when long-tongued moths visit short-tubed flowers. 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 flowers. Other reasons for forbidden links include phenological differences (Bascompte & Jordano 2014). 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. Photograph: A sphingid moth, *Manduca sexta* visiting a flower of *Tocoyena formosa* (Rubiaceae) in the Brazilian Cerrado; tongue and corolla tube lengths approximately 100 mm. Top, approaching and probing a flower; bottom, extracting nectar. Photograph courtesy of Felipe Amorim.
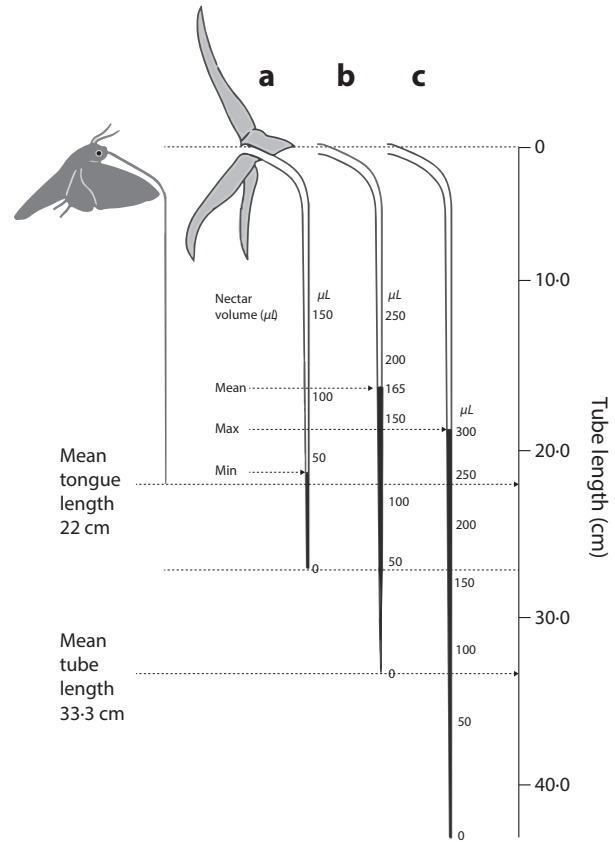

Fig. 2. The mechanistic basis of morphological mismatches in hawkmoth–flower interactions. For example, nectar accessibility in *Angraecum sesquipedale* long-tubed flowers. (a) only small volumes of nectar can be exploited if the spur is 27 cm long. (b) moths with an average tongue length of 22 cm can obtain about 50 μL nectar from a spur of 33·3 cm average length and an average nectar volume of 165 μL. (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 μL nectar. Lack of fit prevents interactions, but also marginal fitting renders interactions unlikely because of energetic constraints. Modified and redrawn from Arditti *et al.* (2012).

this. The pollen of this flower can only be removed by the proboscis of some very large moths trying to get at the nectar at the bottom of the vessel. The moths with the longest proboscis would do this most effectually; they would be rewarded for their long noses by getting the most nectar; whilst on the other hand, the flowers with the deepest nectaries would be the best fertilized by the largest moths preferring them. Consequently, the deepest nectaried Orchids and the longest nosed moths would each confer on the other a great advantage in the 'battle of life'. This would tend to their respective perpetuation and to the constant lengthening of nectar and noses.

Phenotypic fitting of corolla length and shape and the pollinators' feeding apparatus and body sizes are important because the better the fit, the better the consequences in terms of fitness outcomes for the interaction partners (Nilsson 1988). Yet the expectation of perfect trait

There is a Madagascar Orchis–the *Angræcum sesquipedale*–with an immensely long and deep nectary. How did such an extraordinary organ come to be developed? Mr. Darwin's [[p. 475]] explanation is

matching across populations or communities is too simplistic (Anderson, Terblanche & Ellis 2010): 'arms races' as initially suggested by Darwin and Wallace are frequently asymmetric, originating pollinator shifts rather than tight phenotypic trait matching (Fig. 2; Wasserthal 2014). Therefore, extensive local variation in phenotypic mismatch exists in different plant–pollinator systems (e.g. Cocucci, Moré & Sérsic 2009; Anderson, Terblanche & Ellis 2010; Moré *et al.* 2012), with pollinator-mediated selection geographic mosaics of locally co-evolved partners where tight phenotypic matching is not necessarily the rule.

Recent work by Sazatornil *et al.* (2016) 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 rain forest 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? Pairwise patterns of trait matching scale-up to conform complex webs of interaction where in some cases interaction topology is linked to phenotypic fit between partners whilst in others the interaction pattern appears more determined by neutral processes (i.e. probability of interspecific encounter). Sazatornil *et al.* (2016) 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 summary image). 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 ≥ 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; that is, 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 (1988) 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 (Pauw, Stofberg & Waterman 2009; also see Anderson, Terblanche & Ellis 2010; and references therein). Sazatornil *et al.* extend those results to the scale of the whole moth–plant assemblage and demonstrate that trait matching successfully predicts the diversity of interactions recorded. Interestingly enough, the interaction patterns in two local assemblages from ecotone areas of the Argentinian Chaco woodland–Yungas montane rain forest transition are better fitted by a neutral model where pairwise interactions are driven by probability of interspecific encounter. Yet Sazatornil *et al.* did not include the morphological difference for parameter estimation when interactions were not recorded. Thus, the test of the mismatch hypothesis implicitly includes forbidden links effects: a full mismatch of corolla tube/proboscis lengths actually means a forbidden link. Furthermore, a fraction of unobserved interactions was likely caused by phenological uncoupling between flowering and hawkmoth activity phenophases (Bascompte & Jordano 2014; Sazatornil *et al.* 2016). In any case, the mismatch hypothesis somehow captures the fact that a fraction of the unobserved interactions in these hawkmoth–flower assemblages is due to extreme phenotypic mismatch, that is size-related forbidden links (Sazatornil *et al.* 2016); also see Vizentin-Bugoni, Maruyama & Sazima (2014) for evidences with hummingbird–flower interactions.

Forbidden links represent a family of reasons 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 (Bascompte & Jordano 2014). They include phenological, size, microhabitat, sensory, accessibility or any other type of mismatches between partners that explain why some pairwise interactions never occur. 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; Nilsson 1988; Miller 1997; Cocucci, Moré & Sérsic 2009; Arditti *et al.* 2012). This variation is extensive for both the plant and pollinator partners and observable at individual, population and species levels.

Sazatornil *et al.* approach would be most useful for proper tests of co-evolutionary 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 for 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, for example phenological mismatches (e.g. in the case of long-distance or elevational migratory hawkmoths), constraints from foraging for

oviposition sites (Alarcón, Davidowitz & Bronstein 2008), energetic constraints due to balances of nectar availability/foraging costs (Borrell 2005), etc. Match–mismatch patterns are expected when we consider these interactions across geographic mosaics at different spatial scales, with hot and cold spots of phenotypic matching generating variable outcomes of interactions (Pauw, Stofberg & Waterman 2009; Anderson, Terblanche & Ellis 2010; Moré *et al.* 2012; Bascompte & Jordano 2014). Most importantly, Sazatornil *et al.* nicely illustrate how the fascinating natural history details of these interactions can be used to build meaningful testable models to assess the mechanisms beyond structure and function of megadiversified webs of interactions among free-living species.

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