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Abstract

One of the reasons why flowering plants became the most diverse group of land plants is their association with animals to reproduce. The earliest examples of this mutualism involved insects foraging for food from plants and, in the process, pollinating them. Vertebrates are latecomers to these mutualisms, but birds, in particular, present a wide variety of nectar-feeding clades that have adapted to solve similar challenges. Such challenges include surviving on small caloric rewards widely scattered across the landscape, matching their foraging strategy to nectar replenishment rate, and efficiently collecting this liquid food from well-protected chambers deep inside flowers. One particular set of convergent traits among plants and their bird pollinators has been especially well studied: the match between the shape and size of bird bills and ornithophilous flowers. Focusing on a highly specialized group, hummingbirds, we examine the expected benefits from bill-flower matching, with a strong focus on the benefits to the hummingbird and how to quantify them. Explanations for the coevolution of bill-flower matching include 1) that the evolution of traits by bird-pollinated plants, such as long and thin corollas, prevents less efficient pollinators (e.g., insects) from accessing the nectar, and 2) that increased matching, as a result of reciprocal adaptation, benefits both the bird (nectar extraction efficiency) and the plant (pollen transfer). In addition to nectar feeding, we discuss how interference and exploitative competition also play a significant role in the evolution and maintenance of trait matching. We present hummingbird-plant interactions as a model system to understand how trait matching evolves and how pollinator behavior can modify expectations based solely on morphological matching, and discuss the implications of this behavioral modulation for the maintenance of specialization. While this perspective piece directly concerns hummingbird-plant interactions, the implications are much broader. Functional trait matching is likely common in coevolutionary interactions (e.g., in predator-prey interactions), yet the physical mechanisms underlying trait matching are understudied and rarely quantified. We summarize existing methods and present novel approaches that can be used to quantify key benefits to interacting partners in a variety of ecological systems.

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Keywords: Bill-corolla fit, Hummingbird-pollinated plants, Mutualism, Nectarivory, Behavioral plasticity, Trait matching

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1) Introduction to plant-pollinator functional trait matching

A fundamental question in ecology is how specialization evolves and is maintained. Plant-pollinator mutualisms are a model system for the study of specialization and both plants and their animal pollinators can vary from being highly generalized to being strongly specialized. For example, plants with a generalist pollination strategy might receive pollination services from a wide range of pollinator taxa (e.g., Fig. 1A, Hernández-Conrique et al. 2007), while plants that are highly specialized often restrict access to a few pollinator taxa (e.g., Fig. 1B, Sargent and Vamosi 2008; Soteras et al. 2018). Specialization on the part of the plant is thought to arise due to costs of associating with less efficient visitors and selection by the most effective pollinator (Pauw et al. 2020). Animals vary in their effectiveness as pollinators and visits by ineffective pollinators can carry heavy fitness costs for a plant, including excessive pollen loss (the male plant gamete), increased heterospecific pollen transfer (Ashman and Arceo-Gómez 2013), consumption of nutritive food rewards (e.g., nectar) without pollinating (i.e. robbing), and even damage to the flower (Rojas-Nossa et al. 2021). These costs might drive the evolution of floral traits that filter less effective pollinators. Likewise, more effective pollinators (e.g., transporting more conspecific pollen) shape selection for floral traits that further enhance their effectiveness as pollinators. In some cases, highly specialized plants are pollinated by animals that have in turn specialized to feed on that plant's food rewards (Brown and Kodric-Brown 1979; Serrano-Serrano et al. 2017). Such interactions, where both plant and pollinator are reciprocally specialized, create a tighter coevolutionary association that frequently involves the evolution of specialized traits that further enhance specialization (Proctor et al. 1996).

Specialized traits that enhance reciprocal specialization are often matched between plant and pollinator (i.e. 'trait matching'). At broad taxonomic scales, selection favors similarity in traits across groups of pollinators and plants (Johnson and Anderson 2010; Medeiros et al. 2018), and trait matching is

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3 93 frequently studied in the context of pairwise interactions between plant and pollinator species. For
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5 94 example, the length of hawkmoth tongues may closely match the length of floral tubes (Johnson et al.
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7 95 2017) in a community of hawkmoths pollinating long-tubed flowers. A classic case of pairwise trait
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9 96 matching occurs between *Angraecum sesquipedalia*, a Madagascan orchid with an extremely long
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11 97 nectar spur, and its presumed coevolved specialist pollinator, the long-tongued sphinx moth, *Xanthopan*
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13 98 *morganii* (Darwin 1862; Johnson and Anderson 2010; Arditti et al., 2012). Trait matching is often
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15 99 inferred by means of comparing the morphology of plant and pollinator, and is assumed to be functional
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17 100 (Castellanos et al. 2007; Maglianesi et al. 2014; Weinstein and Graham, 2017). That is, trait matching is
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19 101 predicted to simultaneously improve the precision of pollen placement on the pollinator and subsequent
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21 102 deposition on the stigma and enhance the extraction of floral rewards by pollinators (Castellanos et al.
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23 103 2007). In addition, trait-matching may limit interspecific competition, both for the pollinator, by limiting
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25 104 which plant species a given pollinator may profitably visit, and simultaneously for the plant, by reducing
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27 105 heterospecific pollen transfer (Waser and Fugate 1986; Ashman and Arceo-Gómez 2013; Maglianesi et
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29 106 al. 2014; Fonseca et al., 2016). From the plant’s perspective, important factors to consider include:
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31 107 sequence of plants/flowers visited by the pollinator, the visitation timing and distance between those
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33 108 flowers, the number of pollinators and their morphologies, the adhesive properties, size, and amount of
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35 109 pollen grains deposited per floral visit, and the degree of reproductive incompatibility of the plant species
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37 110 involved. When a pollinator visits a flower, it can already be carrying and could deposit heterospecific
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39 111 pollen (which has the potential to reduce plant fitness, Fonseca et al., 2016; Waser and Fugate 1986).
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41 112 Furthermore, any heterospecific pollen left on the pollinator’s body can reduce space available for pollen
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43 113 from a conspecific plant, reducing pollination probability. Lastly, conspecific pollen can be lost and
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45 114 wasted by pollinator delivery to a heterospecific plant (Morales and Traveset, 2008).
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51 116 Avian adaptations to collect floral nectar have been summarized in the appropriately named ‘syndrome of
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53 117 anthophily’ (Stiles 1981). Features of this syndrome include a bill that is usually slender (e.g., Fig. 1),
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55 118 often long and/or curved, and a bifurcated tongue tip (grooved, fringed, and/or capable of rolling into a
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3 119 tube) that is extendable beyond the bill tip. In contrast with other nectar-feeding animals (like some bats
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5 120 that store their long tongues deeper inside their bodies; Muchhala 2006), specialized nectarivorous birds
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7 121 not only have evolved elongated tongues to reach the nectar, but also long bills to hold them and allow
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9 122 them to be inserted inside the nectar chamber with enough precision to collect the liquid reward
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11 123 efficiently (Stiles 1981). In fact, bill length and shape are considered key to functional trait matching,
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13 124 because while tongues may extend far beyond the bill tip, in most birds tongues have motion control only
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15 125 at their base and are too flimsy to traverse barriers within the flower on their own (e.g., Rico-Guevara et
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17 126 al. 2019). Here, we focus on hummingbirds, the most speciose group of anthophilous vertebrates.
18
19 127 Hummingbirds exhibit a wide array of traits associated with their dependence on nectar feeding (Stiles
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21 128 1981; Fleming and Muchhala 2008), that allow them to feed on flowers well enough to make their living
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23 129 out of small volumes of nectar. Presumed adaptations to nectarivory in hummingbirds range from the
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25 130 traditionally recognized ones (such as elongated bills) that evolved more than 30 million years ago (Mayr
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27 131 2004), to specialized tongue elastic properties described recently (Rico-Guevara et al. 2015).
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29 132 Hummingbird feeding apparatus present a variety of minute structures at their tips that are believed to be
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31 133 adaptations to improve collection and transport of nectar (Rico-Guevara and Rubega 2011, 2017). We
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33 134 discuss the evolution of presumed functional trait matching between hummingbird bills and flowers, and
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35 135 propose tools to study this interaction from biomechanical and behavioral perspectives.
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41 137 Just like their pollinators, hummingbird-pollinated plants have repeatedly evolved a suite of traits that
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43 138 characterizes them as thochiliphilous (plants that use hummingbirds as pollination vectors). Such
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45 139 convergent traits deviate to varying degrees from the ancestral states of entomophilous (insect-pollinated)
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47 140 plants and include larger flowers, increased correspondence with the bills of the hummingbirds that are
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49 141 pollinating those flowers, fine-tuned placement of reproductive organs to contact hummingbird surfaces
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51 142 (different areas in the bill, head, and throat), larger volumes of more dilute nectar, overall larger caloric
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53 143 rewards, reduced or absent nectar amino acids, reddish coloration, reduced scent cues, and in some cases,
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55 144 hanging (pendant) flowers that are reached via hovering, or inflorescences shaped to support the weight of
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3 145 clinging birds (Stiles 1978; Martínez del Río et al. 2001; Rocca and Sazima 2010; Pauw 2019). Some
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5 146 species may explore other food sources when there is intense competition, for instance, recent studies
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7 147 have shown that hummingbirds include non-throchilophilous plants in their diet (e.g., Bee hummingbirds,
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9 148 Dalsgaard et al. 2008, 2009; Waser et al. 2018; Wessinger et al. 2019), demonstrating the importance of
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11 149 studying behavioral plasticity and expanding studies to all the potential sources of nectar in a given
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13 150 environment. Throchilophilous plants (~7000 spp.) have likely independently evolved hundreds of times
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15 151 across 68 families (Thomson and Wilson 2008; Abrahamczyk and Renner 2015), highlighting the
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17 152 importance of hummingbirds as pollinators in a variety of ecosystems.
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22 154 Traits that are likely involved in functional trait matching, such as bill and floral morphologies are well
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24 155 characterized. Likewise, the interactions between hummingbird and ornithophilous plant species in
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26 156 pollination networks are also well known. The key gap in our knowledge is understanding how the
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28 157 expected benefits from functional trait matching are achieved. In this perspective piece, we discuss how
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30 158 hummingbird behavior and morphological matching between the feeding apparatus of hummingbirds and
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32 159 the floral structures of plants interact and modulate the benefits for both the plant and pollinator. From the
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34 160 hummingbird perspective, we discuss adaptations that enhance the extraction of floral rewards, focusing
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36 161 on nectar drinking efficiency, as this is the most prominent link between the evolution of nectarivory in
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38 162 birds and coevolution with ornithophilous plants. To understand the general benefits of trait matching we
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40 163 present novel methods that could allow researchers to quantify the benefits of trait matching. The tools we
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42 164 present provide a window into how the hummingbirds interact with the flowers at the moment of the
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44 165 floral visit, highlighting the importance of considering behavior as an integral part of the study of trait
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46 166 matching between hummingbirds and the plants they pollinate.
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52 168 **2) Evolution of trait matching in hummingbird-plant interactions**
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Determining whether trait matching is functional (i.e., benefits both hummingbird and plant) depends in part on understanding how mutualists may be driving coevolution. The close physical match between slender hummingbird bills and the elongated and narrow-entranced corollas of hummingbird pollinated plants is frequently interpreted as sufficient evidence of reciprocal adaptation between these two mutualist groups (see Cronk and Ojeda 2008). Indeed, there is strong evidence that hummingbird pollination drives the diversification of hummingbird-pollinated plants and the evolution of their floral morphology (Temeles et al. 2002; Pauw 2019; Wessinger et al. 2019).

However, floral traits that appear to be matched with hummingbird pollination may also be to some extent a consequence of historical contingency, rather than having evolved to match exclusively within the plant-hummingbird association. Hummingbird pollination is thought to be rarely ancestral and transitions from insect pollination to hummingbird pollination are the norm (Tripp and Manos 2008; Serrano-Serrano et al. 2017; Dellinger et al. 2019; Wessinger et al. 2019). In transitions to hummingbird pollination, morphological traits associated with hummingbird pollinated flowers might therefore evolve as a function of increasing exclusion of less efficient insect pollinators (and release from interspecific competition via heterospecific pollen transfer) when hummingbird pollination is assured (Arcos et al. 2019). Experimental studies provide substantial support for this interpretation (Mackin et al. 2021). Elongated and narrow-entranced corollas reduce the efficiency of insects as pollinators and also reduce insect pollinator preference for these flowers, making them less able to handle the flower and extract nectar (Castellanos et al. 2004; Gegear et al. 2017; Arcos et al. 2019). Likewise, transitions from hummingbird pollinated to insect pollinated, or to mixed pollination systems exhibit shifts toward shorter and wider-entranced corollas that enhance insect pollination, but have no effect on hummingbird pollination effectiveness (Tripp and Manos 2008; Arcos et al. 2019).

Altogether, the evidence suggests that the match between hummingbird bills and corollas may be driven in large part not by coevolution between hummingbirds and thochiliphilous plants (in which both bill and flower shape evolve to match each other), but by selection on floral shape to filter out less effective insect

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3 195 pollinators (evolving narrow and long corollas and other filtering mechanisms). Hummingbirds are more
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5 196 effective pollinators even of many insect-pollinated plants (Thomson and Wilson 2008), and so selection
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7 197 favors floral morphology that excludes insect visitors when more effective hummingbird pollinators are
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9 198 present. In turn, these elongated flowers with narrow entrances drive selection on hummingbird bills to be
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11 199 thinner and longer to match floral morphology. Once this prominent hummingbird hallmark, the slender
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13 200 bill, evolved, it became a historical contingency that was likely perpetuated through ‘guild coevolution’
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15 201 (see below). Insect exclusion seems to have driven the evolution of narrow flowers and subsequent
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17 202 slender bills, however it seems unlikely that plants that have recently transitioned to rely on hummingbird
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19 203 pollinators exert strong selection on hummingbird bill shape. Instead it is more likely that associated
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21 204 flowers evolve to ‘fit the bill’ (Wilson et al. 2006; Arcos et al. 2019), although rare cases of strong
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23 205 pairwise coevolution undoubtedly occur (Stein 1992; Abrahamczyk et al. 2014; Lagomarsino 2015).
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27 206 Most hummingbird species have bills likely capable of extracting nectar from most trochilophilous plants,
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29 207 regardless of shared coevolutionary history (aka guild coevolution) (Cotton 1998). For example, the
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31 208 Green-backed firecrown (*Sephanoides sephaniodes*) a hummingbird only found in South American
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33 209 latitudes, would be able to feed on the flowers of plants that originated and coevolved with North
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35 210 American hummingbird species, due to similar selective pressures where both hummingbirds and
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37 211 trochilophilous plants occur. In all hummingbird-plant assemblages, there are common inefficient
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39 212 pollinators (like short-tongued bees) that need to be restricted from crawling inside the flower to access
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41 213 the nectar. This is the consensus explanation for why tubular flowers have narrow entrances (too narrow
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43 214 for insect bodies) and elongated corollas (too long for insect mouthparts to access e.g., Rodríguez-Gironés
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45 215 and Llandres 2008; Dalsgaard et al. 2009). While there are many other insect pollinators that could
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47 216 potentially still reach the nectar, hummingbird-pollinated plants have additional barriers at the entrance of
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49 217 the nectar chamber that prevent even long insect mouthparts from accessing the nectar. For example,
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51 218 microtrichia on internal flaps projecting from the internal corolla walls require sufficient physical strength
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to be surpassed; hummingbirds, but not insects, can push their mouthparts through, specifically, their bill tips (Wolf et al. 1972, 1975).

In addition, independent appearances of trochiliphily among plants (see section 1) are common (Wessinger et al. 2019). Plant clades that switch to be hummingbird-pollinated frequently evolve with local hummingbirds feeding on other co-occurring trochiliphilous plants (Serrano-Serrano et al. 2017). These new trochiliphilous clades converge on similar floral traits to take advantage of existing hummingbird pollinators, thus also resulting in selection for these hummingbirds to maintain existing bill morphology to sustain efficient nectar extraction. This pattern of evolution seems to be the norm in hummingbird-plant assemblages (Temeles et al. 2002; Maglianesi et al. 2014; Weinstein and Graham 2017). Nonetheless, extreme cases of bill-corolla matching do occasionally occur, in which hummingbirds have evolved uncommon bill morphologies that often match the lengths and curvatures of the flowers they feed on. Examples of this include the Sword-billed hummingbird (*Ensifera ensifera*) with an exceptionally long bill that matches the elongated corolla of the Passion flowers (*Passiflora spp.*) they visit (Lindberg and Olesen 2001; Abrahamczyk et al. 2014), and the Sicklebill hummingbirds (*Eutoxeres spp.*) with exceptionally curved bills that match the curvature of the *Heliconia* and *Centropogon* flowers they feed on (Stein 1992; Lagomarsino 2015). These extreme cases of bill-corolla matching are likely cases of runaway reciprocal exploitation, in which coevolutionary trends of functional trait matching go beyond what is strictly required to reduce competition on both the bird and the plant sides, if the benefits from a tighter match among the assemblage of species continue to be advantageous.

3) Physical components of hummingbird-plant trait matching and pollination

Our understanding of the selective forces at play during the evolution of bill-corolla matching are greatly improved by considering the physical mechanisms that determine the benefits of stronger matching (Box 1). While in this section we primarily consider trait matching benefits at the level of

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3 245 isolated bird-plant coevolution, in the next section we discuss interference competition and its potential to
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5 246 influence bill shape evolution (thus also affecting coevolutionary processes, see section 4). Thus the
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7 247 mechanistic principles we describe here can be transferred to the level of an assemblage of competing
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9 248 pollinators and plants, providing a more comprehensive explanation for how trait matching evolves.
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13 250 Part of the rationale behind the bill-corolla matching coevolution explanation is that hummingbird
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15 251 individuals of a given species (or sex) should obtain higher net energy gain while feeding on flowers
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17 252 matching their bill length and shape. One complication of this explanation comes from the fact that
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19 253 hummingbirds have tongues that can be extended up to two times their bill length (Rico-Guevara 2017),
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21 254 and it is unknown to which degree this protrusion ability varies across species, especially during nectar
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23 255 feeding. For instance, of two coexisting species in which one has a bill that is half as long as the other (or
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25 256 three-fourths as long as in Fig. 1), an individual from the short-billed species, by extending its tongue,
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27 257 could potentially reach nectar in flowers that ‘match’ the long-bill species better. In fact, many species of
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29 258 hummingbirds visit flowers with corollas longer than their bills (Dalsgaard et al. 2021). Which begs the
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31 259 question, is the difference in bill length sufficient to result in niche partitioning? Three factors need to be
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33 260 considered here, 1) Hummingbird tongues are mostly inert structures that only have motion control at
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35 261 their base (e.g., Rico-Guevara et al. 2019), thus when the tongue is extended inside the flower it behaves
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37 262 as a thread that could get stuck to one of the sides of the corolla, bent among floral reproductive
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39 263 structures, and other vicissitudes. Hummingbird bills are rigid structures that guide the tongue through the
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41 264 length of the corolla. 2) Along the same line, many hummingbird pollinated flowers have nectar chamber
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43 265 barriers that need to be passed in order to access the liquid reward. Hummingbird tongues are too flimsy
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45 266 to transverse these obstacles on their own, therefore, bill tips also provide the final push to access the
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47 267 nectar deep inside the flower. Lastly, 3) longer bills, by probing deeper inside corollas, achieve smaller
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49 268 distances between bill tips and nectar than shorter bills. Smaller bill tip-nectar distances yield greater
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51 269 licking rates (Ewald and Williams 1982), because the tongue needs to travel a shorter distance while
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53 270 reciprocating to collect the liquid, and this in turn results in higher nectar extraction efficiency
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(Hainsworth 1973; Hainsworth and Wolf 1976; Montgomerie 1984; Grant and Temeles 1992; Temeles and Roberts 1993; Temeles 1996). Under this lens, with everything else being equal, bills with a higher match to the flower shape are expected to be more efficient in terms of nectar intake rate (Wolf et al. 1972; Temeles and Kress 2010). However, tests of this hypothesis have produced conflicting results. For instance, we would expect that long-billed species are more efficient on long-tubed flowers and short-billed hummingbirds are more efficient on short-tubed flowers, but experimental results do not support the second prediction (e.g., Hainsworth 1973; Montgomerie 1984; Temeles and Roberts 1993; Temeles 1996). In fact, under experimental conditions, longer-billed birds feed more quickly from longer flowers than shorter-billed birds, but shorter-billed birds do not feed more quickly from shorter flowers than longer-billed ones (Temeles 1996; Temeles et al. 2002, 2009). If long bills can also reach the rewards inside short corollas, minimizing bill tip – nectar distances, and achieving higher energy intake rates (Montgomerie 1984), why doesn't selection always favor longer bills?

Drawbacks associated with longer bills include that longer-billed hummingbirds make more insertion errors when feeding in short, narrow flowers compared to shorter-billed hummingbirds (Temeles 1996). Insertion errors would increase the total time required per floral visit and therefore reduce net energy gain per visit. Similarly, wielding long bills might reduce control of fine adjustments that are required to properly position the bill tip and tongue to transverse the internal floral barriers (when present) that prevent easy access to the nectary and reaching all folds containing nectar. Additionally, longer bills may require longer intraoral transport times given that after offloading the nectar near the bill tip, the liquid needs to be actively transported to the throat (Rico-Guevara 2014). Shorter bills reduce the distance between the body of the bird and substrate surfaces that could be used for support (e.g., feeding while clinging or on the ground). Employing substrates for weight support greatly reduces energy expenditure (hovering is energetically expensive), and thus many hummingbirds regularly perch to perform several consecutive visits, even from the same perching position (e.g., in Asteraceae inflorescences Stiles 2008). Finally, by being closer to the flower, hummingbirds with short bills can more easily perforate the corolla

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3 297 near the nectary to reach nectar and more easily exploit existing holes in the corolla made by other
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5 298 visitors (Lara and Ornelas 2001).
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9 300 Although we have focused on bill length, a suite of traits are involved in bill-corolla trait matching; for
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11 301 example, bill curvature shows trait matching with flower shape (Maglianesi et al. 2014). The extremes of
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13 302 these trait-matching axes (length and curvature) are reflected in their morphological diversification into a
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15 303 novel range of bill shapes (Cooney et al. 2017; Navalón et al. 2021). To showcase morphological
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17 304 extremes, compare the short straight beaks (~ 7 mm) of Purple-backed thornbills (*Ramphomicron*
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19 305 *microrhynchum*) to the extremely long (~ 100 mm) and slightly recurved bills (potentially enhancing
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21 306 visits to pendulous flowers, Stiles 2008) of Sword-billed hummingbirds, to the 90 degree decurved bills
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23 307 of Sicklebill hummingbirds, matching their preferred flowers (Stein, 1992). While hummingbird
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25 308 assemblages are often composed of species with more modest differences in bill shape (but see Rico-
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27 309 Guevara 2008), it is an open question whether there is typically sufficient morphological diversity for
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29 310 trait-derived niche partitioning to develop. Other factors such as phenological and microhabitat overlap,
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31 311 abundance (e.g., Vázquez et al. 2009), and type of competitive interactions (see section 4) need to be
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33 312 considered to achieve a full understanding of the magnitude of the importance of functional trait-matching
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35 313 in determining hummingbird-plant networks. Like bill length, bill curvature shows trait matching with
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37 314 flower shape (Maglianesi et al. 2014). Even if corolla and bill length correspond, if curvature differs,
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39 315 benefits from the length match will be greatly reduced or even nullified for plant and hummingbird.
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41 316 Likewise, benefits will not be fully realized if bill-corolla curvature, but not lengths, match (see
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43 317 discussion on curvature measurement methods in (Rico-Guevara and Araya-Salas 2015). Lastly, the
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45 318 corolla and nectar chamber entrances restrict access also on the basis of bill and tongue thickness (see
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47 319 section 2), and the depth to which the bill can penetrate the corolla depends on its internal configuration.
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49 320 For example, a thicker bill tip and/or tongue could prevent deep access inside the flower because of
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51 321 corollar internal diameter constraints and/or the presence of reproductive structures (stigma and/or
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53 322 anthers) that reduce the bill tip mobility (Smith et al. 1996). The actual bill tip space required for effective

drinking is determined by the bill tip thickness when the bill is opened to receive the tongue full of nectar.

Therefore, the thicker the tongue, the larger the opening has to be to be able to allow for access to the fully loaded tongue (Grant and Temeles 1992). Given the suite of physical traits that have to be considered to characterize bill-corolla functional trait matching, a thorough experimental and quantitative exploration of the reciprocal benefits determined by matching hummingbird bill-flower morphospaces is warranted. Imperfect bill-corolla match does not necessarily restrict access to nectar of thochiliphilous plants (or even other plants e.g., Waser et al. 2018), and so hummingbirds can feed on a variety of floral resources. Characterizing the costs and benefits of physical trait matching, while considering the behavioral and ecological context will be key to understanding how they might drive coevolution.

While we have focused primarily on the pollinator's perspective, from the plant's perspective, the benefits for an individual plant from increased bill-flower matching come from enhanced pollen deposition on pollinator surfaces that ultimately contact a conspecific flower's stigma (cross-pollination, e.g., Betts et al. 2015). In terms of trait matching, there is strong selection on floral morphology that forces the pollinator into a position in which pollen is picked up through contact with the anthers (from the male perspective) and that effectively deposits conspecific pollen onto the stigma (from the female perspective). Reduced bill-flower fit might either result in lower chances of pollen dispersal because the flower becomes a less desirable resource for the pollinator (if it experiences low net energy gain), or in nectar extraction by the pollinator without proper pollen transfer (e.g., robbing or mismatch between floral reproductive organ surfaces and pollinator surfaces, e.g., Betts et al. 2015). The effects on pollen deposition and actual transport (how much of it is able to remain) on a given surface (e.g., forehead) of a pollinator, especially in the face of multiple consecutive deployments, need to be documented to better understand the influence of foraging circuits (see section 4) and mechanisms influencing pollination outcomes. There are many open questions regarding the underlying physical mechanisms involved in successful pollen transfer. For instance, by pressing against the pollinator, does the anther remove previously deposited pollen? Are different structural and/or chemical properties of pollen adaptive in

terms of pollen deposition and transport on the pollinator and transfer to the stigma? Are particular pollinator surfaces adapted for pollen dispersal (e.g., feathers)? Does preening/cleaning remove pollen and how often does this behavior occur between floral visits (e.g., bill rubbing against branches)? These are just a few of the many possible questions relating to mechanisms potentially enhancing or disrupting trait matching, but they stress the importance of quantifying both physical mechanisms and behavioral components.

4) Behavioral components of hummingbird-plant trait matching

While we have considered functional trait matching mostly as a consequence of physical mechanisms, animal behavior at multiple scales plays a powerful role in determining the degree to which physical components contribute to functional trait matching. For example, at the level of individual floral visits, cognitive flexibility and behavioral plasticity in hummingbird lapping rate (e.g., Roberts 1995) and tongue protrusion distance (Rico-Guevara 2017) could enable hummingbirds to functionally adjust their match to different flowers. Thus, even when bill and flower seem morphologically matched, behavioral plasticity could expand the range of matches to some degree. In addition, learning and cognition generally (recently reviewed by González-Gómez and Araya-Salas 2019), preference (related to particular behavioral strategies) and competition (related to abundance of both resources and competitors e.g., Simmons et al. 2019) are potentially powerful behavioral components of functional trait matching evolution. Here we focus on behavioral strategies at the level of landscape (defense and movement among floral resources) and its consequences for the evolution of bill-flower matching in hummingbird-plant assemblages. Optimal foraging theory predicts that hummingbirds should attempt to balance costs and benefits associated with finding and extracting better quality nectar rewards (Heinrich 1975; Stiles 1975; Pyke 2019; Blem et al. 2000). However, to achieve a net positive energy gain, hummingbirds might conceivably visit flowers that are a poor functional match to their bills. As long as the hummingbird achieves a net positive energy gain, a hummingbird may, for example, prefer to visit flowers that are close

together to reduce costs associated with searching, even if the bill-corolla match for some of those flowers is poor. In addition, trochilophilous flowers frequently conceal their nectar deep within the nectary, lacking visual, olfactory, or electrostatic cues associated with the reward (Rocca and Sazima 2010; Lunau et al. 2020; Pauw et al. 2020), unlike, for instance, bee pollinated plants (Clarke et al. 2013; Russell et al. 2018). Consequently, achieving a net positive energy gain from flowers is a probabilistic game that has multiple successful behavioral strategies (Feinsinger and Colwell 1978).

Here we provide new terms to focus on two widely-observed and presumably mutually exclusive behavioral strategies thought to maximize net energy gain, and which have consequences for plant reproductive success: (1) stationary interference (formerly ‘territoriality’), in which individuals stay within a resource patch and use aggressive behaviors to interfere with attempts of different nectarivores to access the patch and (2) traveling exploitation (formerly ‘traplining’), in which individuals forage on resources scattered across the landscape, traveling among foraging areas in a particular sequence (e.g., Stiles 1975; Feinsinger and Colwell 1978, see Kamath and Wesner 2020; Tello-Ramos et al. 2015; Sargent et al. this issue for a discussion on the controversies and semantic problems with the prior terms). These new terms describe both the predominant landscape range and type of competition associated with these ends of the spectrum of behavioral strategies. A stationary interference strategy is expected to be used when tolerating other nectarivores is costly, such as when floral nectar is readily accessible to a diverse assemblage of pollinators (Stiles 1975). This is because interfering with the foraging of competitors is most useful when competitors can access the resource as easily as the interferer, resulting in agonistic interactions collectively known as interference competition (Rico-Guevara et al. 2019). Conversely, when foraging on a specific plant species carries a net positive benefit for only well-matched nectarivores, the focal pollinator is released from the need to defend nectar resources from all possible nectarivores (see Sargent et al. this issue). Net energy gain is thus maximized by visiting only flowers with a good match, regardless of their spatial proximity and thus a traveling exploitation strategy may be favored, in which pollinators visit plants scattered across a broad range (Ohashi and Thomson 2009;

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3 401 Buatois and Lihoreau 2016). Note that this is different from how the term traplining has been applied to
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5 402 foraging circuits independent of the scale at which they occur (Tello-Ramos et al. 2015). From this
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7 403 perspective, traplining can also be performed by a stationary interferer while visiting the flowers in a
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9 404 given patch, while also defending it (Tello-Ramos et al. 2015). In our new terminology, a traveling
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11 405 exploiter, by definition, does not stay in an area to defend the resources within.
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16 407 When hummingbirds restrict access to resource patches via stationary interference, this strategy can shift
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18 408 the realized hummingbird-plant interactions from what would be expected given the fundamental niche
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20 409 distribution based only upon bill-corolla matching. Stationary interference is linked to aggressive
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22 410 behavior, which can also be a strong selective force on hummingbird bill morphology, and thus indirectly
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24 411 drive selection on flower shape and other aspects of a plant's reproductive strategy via coevolution.
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26 412 Indeed, agonistic behaviors are associated with at least some bill traits in the context of intrasexual
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28 413 competition and evolution of intrasexually selected weapons (Rico-Guevara and Araya-Salas 2015; Rico-
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30 414 Guevara and Hurme 2019), and have been proposed to be associated with interspecific competition (Rico-
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32 415 Guevara et al. 2019). For instance, physical confrontations in which the bill is used to contact the
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34 416 opponent could select for 1) longer beaks that provide increased reach to stab an opponent before getting
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36 417 stabbed, 2) straighter beaks that transmit the force from bill base to tip better, 3) thicker beaks that are
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40 419 and 5) stronger bill tips that resist bending forces while biting (e.g., to pluck feathers or physically
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47 422 Stationary interference and traveling exploitation strategies may also influence the benefits for the plant,
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49 423 beyond simple differences in conspecific pollen transfer. For example, stationary interference is likely to
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51 424 reduce pollen dispersal of the defended plants, assuming that displacing other pollinators reduces floral
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53 425 visitation rate, and could also increase heterospecific pollen transfer when interferers visit multiple plant
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55 426 species in the same area (Ashman and Arceo-Gómez 2013). In addition, stationary interference is likely to

reduce outcrossing, as pollen will be mostly transferred among the defended plants (Torres-Vanegas et al. 2019). Traveling exploitation on the other hand involves the pollinator visiting flowers in a discrete sequence across the landscape, thus shaping the direction of pollen movement. As a result, one plant in the visitation sequence may always serve as a pollen donor, while another in the sequence may always serve as a pollen recipient, even if the individual plants are widely separated (e.g., Ohashi and Thomson 2009, but see Torres-Vanegas et al. 2019). Traveling exploitation and stationary interference lie at the extremes of a continuum between exploitative and interference competition (Rico-Guevara et al. 2019) that can select for a variety of behavioral and morphological traits that can directly impact pollination. In an assemblage of spatio-temporally coexisting hummingbirds and hummingbird-visited plants (a pollinator interaction network, see section 5), the foraging links between particular plants and birds, as well as their strength (e.g., the proportion of visits for both parties), depend on pollinator behavior. Likewise, plant and hummingbird abundance and phenology can also affect the links between them and thus the strength of selection (Vázquez et al. 2009). At the community level, additional evolutionary processes need also to be considered in order to link mechanisms and patterns at different ecological scales. For example, character displacement on hummingbirds feeding on coexisting plants can partially explain their astonishing diversity in bill shape and size (Maglianesi et al. 2014). In the same way, intersexual floral resource partitioning is thought to at least in part explain sexual dimorphism in bill morphology (Temeles et al. 2000, 2010). Furthermore, character displacement likely drives the evolution of diverse floral morphologies and pollen deposition and collection strategies among plants (e.g., different lengths of anthers aiming at different body surfaces, lever mechanisms, explosive pollen release, modified petals, etc. (Aluri and Reddi 1995; Rengifo et al. 2006; Temeles and Rankin 2011; LoPresti et al. 2020)).

5) Approaches to studying hummingbird-plant (bill-corolla) trait matching

Inferences about reciprocal specialization between hummingbird bills and the flowers they pollinate are frequently drawn from research on hummingbird-plant interaction networks (examples in Table S1).

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3 453 These studies establish which hummingbird and plant species interact and can generate hypotheses for
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5 454 putative benefits of bill-corolla trait matching (e.g., Maglianesi et al. 2014). While plant-pollinator
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7 455 networks suggest that functional trait matching maintains links among species, the determinants of both
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9 456 pollination and nectarivory at the level of the floral visits are poorly understood. Moreover, evaluating the
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11 457 inferred benefits or drawbacks of particular interactions for plants and pollinators requires a detailed look
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13 458 at what happens during a floral visit. Therefore, to help guide future research, we provide a comparison of
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15 459 different network building methods (Table S1) used to characterize benefits to hummingbird and plant
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17 460 and thus assess the mechanisms enabling putative bill-corolla coevolution. We focus in particular on the
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19 461 value of video, because while some methods effectively characterize which plants and hummingbirds are
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21 462 interacting, as well as the strength of these interactions (e.g., number of visits, their frequency, and the
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23 463 amount of pollen found on the pollinator), only video recordings enable precise characterization of
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25 464 hummingbird behavior on flowers and the interaction between floral reproductive organs and the
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27 465 hummingbird. Recent video footage of plant-pollinator interactions have unveiled pollinator fidelity to
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29 466 their resources even in times of low abundance and a relatively high occurrence of nectar-robbing
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31 467 (Weinstein and Graham, 2017), as well as highlighted the influence of spatial distributions in trait-
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33 468 matching and resulting morphotypes (Sonne et al., 2019).
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39 470 In addition to the importance of quantifying pollination network interactions, it is necessary to understand
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41 471 how they are modulated through a common currency: net energy gain. The main proposed benefit of bill-
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43 472 corolla matching from the pollinator's perspective is an increase in net energy gain (Box 1), which is a
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45 473 consequence of **1**) nectar accumulation in specialized flowers (if competitors cannot access or are
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47 474 morphologically discouraged from accessing the reward, section 4), **2**) reduced access time to the flower
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49 475 entrance and nectary (section 3), and **3**) increased nectar intake efficiency. Measuring nectar
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51 476 replenishment (without affecting floral visitation) and feeding performance in wild flowers was
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53 477 practically impossible until recent technological advances, thus, nectar drinking has been mostly studied
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55 478 under artificial conditions (e.g., Roberts 1995; Collins 2008; Rico-Guevara et al. 2019). In particular,

advances in videography capabilities and affordability (see discussion about costs in Rico-Guevara and Mickley 2017) have been a game changer. Video recordings are comprehensive in capturing both successful and unsuccessful attempts to extract nectar from focal flowers and enabling characterization of alternative behaviors such as nectar robbing. Likewise, in combination with field data (e.g., nectar concentration and flower dimensions) they can provide the means to quantify performance variables involved in evaluating how bill-corolla match shapes net energy gain (e.g., Fig. 2). We present an overview of the video techniques that would be applicable to the study of plant-pollinator and other ecological interactions, and we finish with an example of one out of the many possible combinations of those techniques.

To capture hummingbird fast behaviors (e.g., licking rates up to 17 Hz, Ewald and Williams 1982), we need high-speed video; to resolve movement and contact with the floral sex organs or nectar inside small flowers and tiny nectaries (e.g., the exclusion of insect pollinators or the movement of the bill inside the corolla), we need macro-photography; and to mitigate the influence of the camera on hummingbird behavior, we need tele-photo capabilities (Table S2). Solving these challenges is possible with specialized and field-friendly cameras (e.g., Rico-Guevara and Mickley 2017). We present a combination of backlit-filming, long duration, and high-speed recording techniques that allows us to measure rates of nectar depletion and replenishment in wild flowers. This is key, because to the best of our knowledge, until this point researchers have not had a way to measure nectar extraction, which requires knowing both the uptake rate and the preexisting volume of nectar in the flower. Backlit filming allows estimation of the nectar volume without physically manipulating the flower (e.g., extracting nectar manually) and thus does not affect floral visitation or damage floral tissues (Fig. 3; Table S2). We performed volumetric estimations (Table S3) of the nectar through the visualization of the liquid inside the flower (Video S1) and floral dissections to calculate the internal dimensions of the nectar chamber (Fig. S1). Measuring nectar extraction in unmanipulated wild flowers takes us a step closer to directly testing the bill-corolla matching benefits from the hummingbird's perspective.

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6) Concluding remarks and implications for the study of other coevolutionary systems

Half a century of research on floral foraging by hummingbirds has provided us with a wealth of knowledge about hummingbird nectar preferences (Hainsworth and Wolf 1976; Stiles 1976; Calder 1979; Tamm and Gass 1986; Martínez del Río 1990; Stromberg and Johnsen 1990; Roberts 1996; Blem et al. 1997, 2000; Fleming et al. 2004; Chalcoff et al. 2008), nectar extraction efficiency (Houston and Krakauer 1993; Roberts 1995; Collins 2008), the role of cognition (Healy and Hurly 2013; González-Gómez and Araya-Salas 2019), and optimization of foraging and energetics (DeBenedictis et al. 1978; Hixon and Carpenter 1988; Gass and Roberts 1992; Shankar et al. 2019). Despite the breadth of this literature we still lack a good understanding of the mechanisms underlying bill-corolla matching and their role in maintaining hummingbird-plant interactions as well as driving coevolution. We therefore endeavored to elucidate these gaps in our knowledge via a discussion of the evolution, and physical and behavioral components of bill-corolla matching. To help move this field forward, we have likewise highlighted key questions and methods that we hope will facilitate and expand characterization of functional trait matching for both plant and pollinator (Tables S1, S2).

In particular, we emphasize taking a mechanistic perspective when considering the drivers of adaptations in hummingbird-trochilophilous plant interactions. Specialized videography is uniquely suited to characterize the physical interactions between floral reproductive structures and pollinator surfaces, making it possible to quantify benefits of trait matching for the plant (section 3, Table S2). Likewise, video of hummingbirds drinking from flowers makes it possible to quantify key parameters needed to determine costs and benefits for the birds (Fig. 2), such as energy intake efficiency (Fig. 3), which is one of the drivers of foraging decisions (section 4) and thus reciprocal adaptation. Similarly, a combination of methods (Table S1) can be used to characterize the interaction networks resulting from those foraging decisions. In addition, with videography we can document visits by different hummingbirds to focal

flowers and hummingbirds preventing competitors from accessing those flowers, thus revealing the interplay between exploitative and interference competition. In other words, videography permits us to contrast null expectations of plant-hummingbird interactions based exclusively on bill-corolla matching, with the actual interactions occurring in natural communities and thus the conditions influencing bill-corolla coevolution. Lastly, by combining videography and quantification of nectar extraction performance with the wealth of knowledge about hummingbird aerodynamics and energetics in the context of floral foraging (Sargent et al. this issue), it becomes possible to quantify net energy gain, the key benefit of trait matching for the hummingbird. Altogether, these quantitative and mechanistic approaches make hummingbird-plant interactions a model system for studying the benefits of functional trait matching for both plant and pollinator and, more generally, the drivers of coevolution.

Our proposed framework unites theoretical expectations and empirical observations to improve our understanding of the mechanisms constraining functional trait matching and its evolution. While the methods and framework presented here concern the biomechanics and energetics of hummingbird-flower interactions, they hopefully serve as a template for quantifying costs and benefits in other plant-pollinator systems and ecological interactions. For instance, some of the questions asked about hummingbird-plant interactions, like bill-corolla length-matching, are equivalent to questions asked in bumble bee-plant interactions (tongue-corolla length-matching e.g., Miller-Struttman et al. 2015). Similarly, with slight modifications - such as infrared lights and recording capabilities - equivalent measurements could be collected for a variety of diurnal (e.g., other anthophilous birds, butterflies, bees) and nocturnal (e.g., bats and moths) nectarivores, that sometimes even feed on the same flowers (e.g., Fig. 1A). Additionally, while behavioral plasticity in flower feeding mechanisms potentially strongly affects functional trait matching, we have barely scratched the surface of this subject in a variety of pollination systems, including anthophilous birds, rodents, bats, and butterflies (but see work on moths e.g., Goyret & Raguso 2006; Goyret & Kelber 2011; and work on bees e.g., Russell et al. 2017, 2018; Wei et al. 2020). Finally, biomechanics perspectives are often incompletely developed in other coevolutionary systems, such as the

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557 snake-newt-tetrodotoxin system (e.g., feeding biomechanics that potentially influence the costs of prey
558 consumption) and the anemone-anemonefish system (e.g., anemonefish biomechanics that potentially
559 benefit the host). All in all, the study of physical mechanisms of behavior is an important avenue for
560 reintegrating biological sciences, and will be an active field of research for years to come.
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Figure Legends:

Figure 1. Strategies in hummingbird-plant coevolutionary systems. A) Generalist Indigo-capped hummingbird (*Saucerottia cyanifrons*) visiting a flower of a leguminous tree (*Calliandra spp.*), a plant that also exhibits a generalist strategy (Hernández-Conrique et al. 2007). B) Specialist Black-throated mango (*Anthracothonax nigracollis*) visiting a specialized throbiliphilous plant (*Aphelandra spp.*). Photos taken at the Colibrí Gorriazul Research Station, near Fusagasugá, Colombia, courtesy of Ricardo Zarate.

Figure 2. Variables for quantification of hummingbird drinking performance during visits to wild flowers. Energy intake rate (cal/s) = extraction efficiency (μl/s) * nectar concentration (cal/μl). See text for discussion of licking rate and handling time.

Figure 3. Nectar depletion (in blue and left axis) and extraction rate (in magenta and right axis) for a single visit of a Speckled hummingbird (*Adelomyia melanogenys*) to a flower of *Palicourea sp.* (Video S1). Measurements of nectar pool depletion were performed after every lick (Table S3). Our methods allow for the assessment of feeding performance, at the flower visit level, quantifying variables that have not been possible to measure to date in the wild (e.g., maximum bill insertion, access times, licking rates, liquid collection rates, etc., Fig. 2).



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87x86mm (300 x 300 DPI)

Box 1. Below are the parameters that would ideally be measured to evaluate the benefits to the bird from trait matching via quantification of performance in the wild (Fig. 2). When a hummingbird feeds on a flower that matches its bill shape, it would be expected to obtain a larger net energy gain (calories acquired minus invested) than when feeding on a flower with a poorer match. For example, the hummingbird might be able to more efficiently insert its bill into the flower, reducing its time to access nectar (handling time minus licking time), increase extraction efficiency, and collect a larger reward. By measuring the amount of nectar in the flower and the licking rate, we can calculate the volumetric extraction efficiency ($\mu\text{l/s}$), and with a range of potential values for nectar concentration in a particular flower, we can estimate the energy intake rate (cal/s). While it is not possible to measure the precise nectar concentration consumed in a given floral visit without disturbing the system, the best approximation involves measuring the nectar concentration on the same flower but at a different time, or at the same time from adjacent flowers, or by characterizing nectar concentration variation among flowers and across the relevant temporal scale (e.g. daily fluctuation). Nectar concentration is commonly measured via a refractometer, following extraction of nectar from 'bagged' flowers (preventing visitors from extracting nectar) via microcapillary tubes.

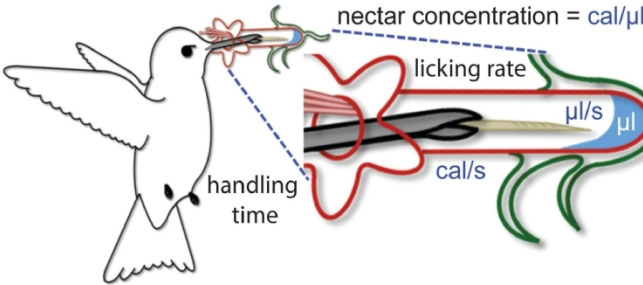


Figure 2. Variables for quantification of hummingbird drinking performance during visits to wild flowers. Energy intake rate (cal/s) = extraction efficiency ($\mu\text{l/s}$) * nectar concentration ($\text{cal}/\mu\text{l}$). See text for licking rate and handling time.

Box 1. Here we present the parameters that would ideally be measured to evaluate the benefits to the hummingbird from trait matching via quantification of hummingbird nectar extraction performance in the wild (Fig. 2).

88x156mm (300 x 300 DPI)

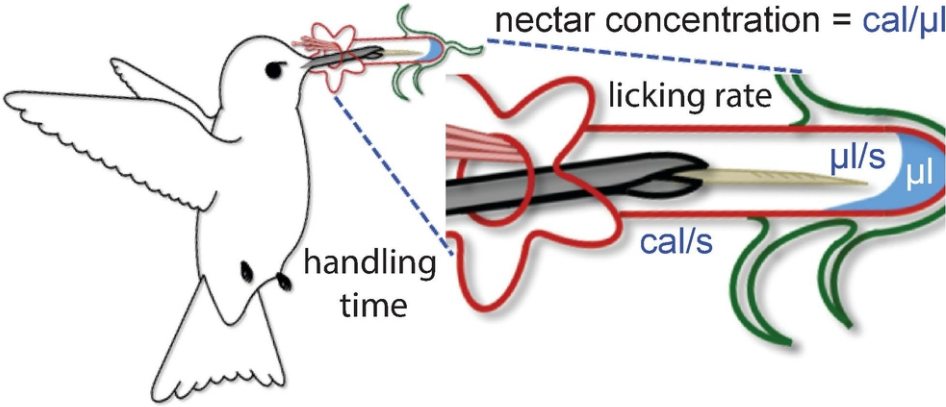


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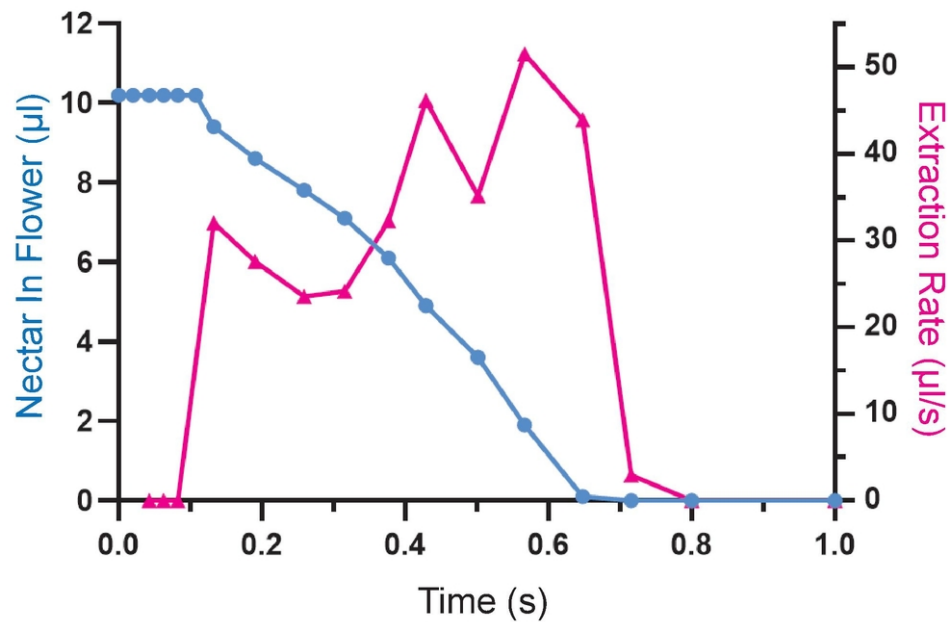


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87x63mm (300 x 300 DPI)