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Functional morphology of hummingbird bill tips: their function as tongue wringers

Alejandro Rico-Guevara^{a,b,*}, Margaret A. Rubega^b

^a Department of Integrative Biology, University of California at Berkeley, CA 94720, USA

^b Department of Ecology and Evolutionary Biology, University of Connecticut, CT 06269, USA

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ABSTRACT

Nectarivores are animals that have evolved adaptations to efficiently exploit floral nectar as the main source of energy in their diet. It is well known that hummingbirds can extract nectar with impressive speed from flowers. However, despite decades of study on nectar intake rates, the mechanism by which feeding is ultimately achieved – the release of nectar from the tongue so that it can pass into the throat and be ingested – has not been elucidated. By using microCT scanning and macro high-speed videography we scrutinized the morphology and function of hummingbird bill tips, looking for answers about the nectar offloading process. We found near the bill tip, in an area of strong lateral compression of internal mandibular width, that the tomia (cutting edges of the bill) are thinner, partially inrolled, and hold forward-directed serrations. Aligned with these structures, a prominent pronglike structure projects upward and forward from the internal mandibular keel. Distal to this mandibular prong, another smaller maxillary prong protrudes downwards from the keel of the palate. Four shallow basins occur at the base of the mandibular prong on the mandibular floor. Of these, two are small basins located proximally and at the sides of the mandibular prong. A third, slightly larger basin is positioned distally to the first two and directly under the maxillary prong. And the fourth basin, the largest, is found more proximally where the bill becomes thicker, as seen from the side. We documented that this group of structures is integrated into the area of the bill where tongue extrusion occurs, and we hypothesize that they function to enhance the nectar release at each lick. We suggest that this “wringer”, operated by bill and tongue movements, helps to move nectar towards the throat.

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1. Introduction

Nectarivores exploit a static, yet periodically replenishable resource that shapes their behavior, ecology, and morphology. Flowers only offer minute amounts of energy packed in small quantities of nectar, and animals that have evolved to feed on them exhibit specialized morphological and behavioral adaptations; including well-known examples such as bees (e.g., [Borrell, 2004](#); [Wu et al., 2015](#); [Zhu et al., 2016](#)) and bats (e.g., [Freeman, 1995](#); [Schondube et al., 2001](#); [Harper et al., 2013](#)), and more obscure ones like spiders (review in [Wildner, 2011](#)), mice (e.g., [Johnson et al., 1999, 2001](#)), and elephant shrews ([Wester, 2011, 2015](#)). Animals have been selected to maximize energy intake per unit time, which is especially important while feeding on tiny amounts of nectar. For

instance, many aspects of the nectar-feeding birds' phenotype have been shaped by the flowers they feed on ([Stiles, 1981](#)). Although nectarivory is commonly viewed as a specialized way of life in birds, repeated independent origins of the nectarivorous lifestyle provide evidence of the wide range of variation in the degree of reliance on nectar across the nectarivore clades, i.e. there are various degrees of specialization for nectarivory in birds ([Paton and Collins, 1989](#)). In the same way, plants employing animal pollinators have a wide range of options, from insects to several kinds of vertebrates; and birds stand out as the main vertebrate pollinators ([Fleming and Muchhala, 2008](#)). At the vertex in which these two continua converge, several cases of plant–bird coevolution have appeared independently, and on several continents ([Stiles, 1981](#)). Various studies have noted strong and repetitive patterns in the bill morphology of avian nectarivores (see review in [Paton and Collins, 1989](#)), and previous authors have assumed that a similar feeding mechanism underlies these convergent morphologies ([Collins, 2008](#); [Köhler et al., 2010](#)). However, despite decades of study of morphological variation in bill size and shape, there is a

* Corresponding author at: Department of Integrative Biology, University of California at Berkeley, CA 94720, USA.

E-mail addresses: a.rico@berkeley.edu, aricog@gmail.com (A. Rico-Guevara).

surprising lack of detailed examination of the morphology of the interior of the bill, where nectar handling actually occurs.

Although the inner structures of hummingbird bills were not taken into consideration, the influence of the morphology of hummingbird bills (Wolf et al., 1972, 1976; Temeles et al., 2009) and tongues (Scharnke, 1931; Weymouth et al., 1964; Hainsworth, 1973) during nectar uptake has received meticulous study. The long and narrow bills of hummingbirds enclose a correspondingly elongated and thin tongue that fills the entire oropharyngeal cavity (Rico-Guevara, 2014). The tongue itself is composed of a pair of extended cylindrical structures firmly attached to each other for almost their entire length (except a bifurcation near the tongue tip); the proximal half of each cylinder is solid while the distal half is hollow and opened by a longitudinal slit, forming a pair of semi-cylindrical grooves (Scharnke, 1931; Weymouth et al., 1964). The walls of these grooves are dissected forming a fringed distal region, sometimes called lamellae; from here on we will refer to these structures as fringes (for further information on tongues of hummingbird, and other avian nectarivores, see Rico-Guevara, 2014). On the basis of the feeding apparatus anatomy, particularly the tongue morphology, Kingsolver and Daniel (1983) developed a widely accepted biophysical model to explain how hummingbirds would collect nectar via capillarity. This model suggested that nectar was removed from the flower by capillary action at the rolled-up bifurcate tongue tips with each lick of the tongue (Hainsworth, 1973; Roberts, 1996; Collins, 2008; Köhler et al., 2010; Kim et al., 2012). Our work has demonstrated that the capillary model needs to be replaced (Rico-Guevara and Rubega, 2011), and we documented intake mechanisms that take advantage of the elasticity of the tongue grooves walls (Rico-Guevara et al., 2015). Nevertheless, all of this research treats only the tongue loading step of the nectar feeding process; here we focus on the next step, the offloading of the tongue inside the bill.

In spite of the recent advances in the understanding of tongue mechanics (review in Rico-Guevara et al., 2015), it is still not clear how the nectar is removed from the tongue (which, after all, functions only to collect the nectar) and passed to the pharynx for ingestion. Scharnke (1931) and others (see review in Böker, 1937) suggested that the nectar uptake is initiated via capillary forces, but later is completed by a vacuum created by the tongue retraction into the oral cavity and the swallowing process. Ewald and Williams (1982) reported compression of the tongue at the bill tip during protrusion, and apparently coordinated movements of the throat with opening and closing of the beak with each lick of the tongue. Although high-speed videography supports the dorso-ventral compression of the tongue while it is being extruded (Rico-Guevara et al., 2015), the mechanistic details of how the morphology of the bill tip interacts with this compression to liberate nectar from the tongue, while retaining it inside the beak, have not been elucidated. Here we describe previously unreported structures in the interior of hummingbird bills, including prongs and basins described in detail in Section 3, that we hypothesize function as a wringer device, an adaptation for nectar feeding.

The implications of the structures presented in this paper will have to be tested by future experiments, but presenting and describing them, along with proposals of their functioning, is a necessary step to incorporate them in future nectar extraction efficiency and energy budgets modeling. The present research is of particular relevance now that we have started to appreciate and understand the diversity and selective pressures involved in the evolution of bill tip morphology among hummingbirds (Rico-Guevara, 2014; Remsen et al., 2015); for instance, the evolution of a dagger-like bill tip (Rico-Guevara and Araya-Salas, 2015) will be in opposition to selection favoring an efficient wringer. The nectar-drinking hypothesis including the wringer device described in the present paper provides testable predictions for future studies, and

can be applied to similar (convergent) traits found in other nectarivores. This hypothesis will facilitate the design of experiments to test other previously proposed hypotheses about nectar intake mechanisms (e.g., Scharnke, 1931; Böker, 1937; Kingsolver and Daniel, 1983; Cheke and Mann, 2008), thereby shedding light on the functional constraints on the evolution of bill morphology in nectar-feeding birds.

2. Materials and methods

We performed analyses of microCT scans (3 species, $n=3$), of high-speed videos from over a hundred visits of hummingbirds to drink nectar from feeders (20 species, $n=35$), and of gross anatomical observations through dissecting microscopes of museum specimens (43 species, $n=352$), in order to obtain complementary information on the form and function of the structures described for the first time in the present paper. For all our methods we focused on the distal half of hummingbird bills; specifically, we studied the maxillary and mandibular rhamphothecae, centering on the tomia, palate, mandibular floor, and underlying bones. Details of each method are provided below.

2.1. High-resolution X-ray computed tomography (microCT)

To examine the three-dimensional arrangement of the structures inside the bill, we used the Xradia MicroXCT scanner of the High-Resolution X-ray Computed Tomography Facility at The University of Texas at Austin. This scanner provided 5-micron voxel resolution, effectively covering the smaller size ranges (<1 cm) of the structures involved in this study. In order to obtain detailed morphological data at the micrometric scale and visualize the tongue soft tissues, we developed a staining protocol by modifying (via trial and error) a common technique for transmission electron microscopy using osmium tetroxide (OsO_4), but without embedding in resin (cf. Metscher, 2009). Recently, a variety of alternative techniques have been used to enhance visualization of soft tissue during microCT imaging, especially by using iodine compounds (reviewed by Gignac et al., 2016). We opted for osmium instead of iodine because, although they both seem to bind to lipids (Gignac and Kley, 2014), the former stabilizes tissue proteins and these proteins do not coagulate during dehydration with alcohol (see below, Hayat, 2000).

We obtained scans for three salvaged specimens, a female ruby-throated hummingbird (*Archilochus colubris*), a male Anna's hummingbird (*Calypte anna*), and a female short-tailed woodstar (*Myrmia micrura*). Their heads were kept in 10% neutral buffered formalin and fixed with a solution containing 2.5% (wt/vol) glutaraldehyde and 2% (wt/vol) formaldehyde in 0.1 M sodium cacodylate trihydrate buffer (pH 7.4 adjusted with NaOH) for 8 h at 4 °C. After two washes in distilled water, the heads were fixed/stained with 2% (wt/vol) OsO_4 in 0.1 M cacodylate buffer water for 4 h at 4 °C. Samples were washed three times in distilled water (20 min apart at 4 °C) and then dehydrated in a graded series (30 min apart) of ethanol solutions (50, 70, 95, and 100% [vol/vol] ethanol–thrice–). The specimens were stored in 100% ethanol at 4 °C. Scans were performed at 70 kV and 10W, with Xradia 0.5 and 4X objectives, and 1 mm SiO_2 , or no filter.

2.2. High-speed videography

In order to visualize the functional interactions among the bill tips and the tongue, we filmed free-living hummingbirds feeding on artificial nectar (18.6% w/v sucrose concentration). We used a high-speed camera (Phantom Miro ex4) running up to 1265 f/s (800 × 600 pixels), with a special high-magnification macro lens (MP-E 65 mm f/2.8 1–5x Macro Photo; Canon USA, Inc.). We focused

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