Evolutionary ecology of interactions between plants and nectar-feeding birds across scales

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2025

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Program Authorized to Offer Degree:

Biology

University of Washington

Abstract

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Plant-pollinator interactions constitute a fantastic system to investigate how ecological and evolutionary processes influence each other. In this dissertation I use honeyeaters (Aves, Meliphagidae), the second-most speciose clade of vertebrate pollinators and an avian family native to Australasia, as a study system to investigate (1) how bird and plant phenotypes interact to determine the outcomes of plant-pollinator interactions and (2) how these interactions can lead to evolutionary consequences such as adaptation, specialization, and coevolution. Chapter 1 begins with a survey of the evidence for convergence in morphology and function across roughly twenty lineages of specialized avian nectarivores. By reviewing the literature on morphological and functional adaptations for nectarivory in these taxa, I found that the feeding apparatus (bill, tongue, hyoid) exhibits the most convergent morphology, and is more commonly modified than the locomotor apparatus (wings and legs) or the digestive and renal systems. This work also illuminated the knowledge gaps that exist in our basic understanding of how birds adapt to nectarivory and provided suggestions for future research.

Chapter 2 focuses on the process of nectar feeding in honeyeaters, as one of the primary biophysical challenges faced by nectar-feeding birds is efficient extraction of small nectar volumes from flowers. This chapter uses a biomechanics approach, including high-speed videography and kinematic analyses, across five species to answer the question of how honeyeaters use their brush-tipped tongues to capture nectar. I found that nectar is primarily captured via surface tension between the bristles at the tip of the tongue, a mechanism called fluid trapping, rather that via fluid flow through the grooved body of the tongue. Using fluid trapping as the primary mechanism of nectar capture could be what allows honeyeaters to visit flowers with a wide range of nectar presentations (i.e., nectar volume, sugar concentration).

Chapter 3 follows by examining the degree to which hyolingual morphology (the size and shape of the tongue and the bony support called the hyoid) varies across honeyeater species, and whether that morphology correlates with the degree of dietary dependance on nectar. This work employs diverse techniques such as linear morphometrics, anatomical characterization, histology, and computer tomography (CT scans) to provide the most comprehensive survey of this type performed in any bird family. I found that there are six distinct tongue types across honeyeaters, some of which are restricted to particular genera that warrant their own biomechanical analysis of feeding due to their seeming morphological incompatibility with nectar feeding via fluid trapping. Additionally, two aspects of tongue morphology – tongue length and the proportion of the tongue that is bristled – were positively correlated with increased reliance on nectar. These macroevolutionary patterns make sense in light of the biomechanical analysis from Chapter 2, as a longer tongue can access a wider range of flowers in the environment and probe deeper into flowers, while a larger bristled portion of the tongue allows more surface area for nectar collection via fluid trapping.

Chapter 4 zooms out beyond the tongue-nectar interface to examine the outcomes of honeyeater-plant interactions in the field. This chapter asks how morphological matching between a honeyeater's bill and a flower determines pollen transfer between flowers (pollen load acquired from anthers and pollen deposited at floral stigmas) and the feeding efficiency of birds (microliters of nectar consumed per second). In this work I found that honeyeater species differ in their pollination and feeding efficiency at flowers, but that a worse bill-flower match was only correlated with less pollen deposition, not with pollen load or feeding efficiency. The finding that increased morphological matching does not facilitate greater feeding and pollination efficiency of honeyeaters is a surprising insight that pushes us to reevaluate the coevolutionary mechanistic rationale of bird pollination systems.

Finally, Chapter 5 uses a community ecology framework to consider how plant traits and interspecific interactions between honeyeater species could influence patterns of bird visitation to plants. This work quantified the honeyeater community composition and nectar resources (as kilojoules of energy per flower and number of flowers produced) at two sympatric species of Australian flowering plants. I found that the two plant species supported significantly different honeyeater communities. The lower-reward plant species (i.e., fewer kilojoules produced per flower and fewer flowers produced) attracted a higher number of small-bodied honeyeater species and was almost never visited by larger species. There were also fewer birds visiting and fewer aggressive interactions at this low-reward plant. These differences in community composition of honeyeater visitors is likely because smaller birds, having lower energetic demands than large species, get relatively more caloric gain from the low-reward plant while avoiding the risk of competition and aggression presented by the high-reward plant species.

In summary, this dissertation studies honeyeater-plant interactions across spatial and temporal scales using an integrative approach. This work has filled several knowledge gaps about honeyeater-plant interactions, such as the fact that honeyeaters drink nectar via fluid trapping and have evolved longer, more bristled tongues as an adaptation to increased nectarivory. Broadly, this dissertation illustrates that interdisciplinary research is necessary to holistically investigate the evolutionary ecology of any animal pollination system and provides methods and a conceptual framework with which to do so.

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Acknowledgments

I would like to thank the Behavioral Ecophysics Lab at the University of Washington for always being a source of kindness and support. I would also like to thank my committee, Alejandro Rico-Guevara, Sharlene Santana, Berry Brosi, and Trevor Branch, for their useful and constructive feedback on this dissertation, which greatly improved the work and helped me grow as a scientist. I would especially like to than my advisor, Alejo Rico-Guevara, for not only being an amazing scientists and mentor, but also an empathetic and kind person who imbues that energy into everything he does.

I would also like to thank Todd and Andrea McWhorter at the University of Adelaide, Roseworthy for helping make Chapter 4 and 5 of this dissertation possible. None of my fieldwork would have happened without Todd and Andrea, who welcomed me into their home, helped me with an incalculable number of logistical difficulties, and made me feel supported even when I was on the other side of the world. I would also like to shout out Rio, Rogue, and Maisie, who provided much fun and laughter.

I would like to thank all of the people and places that have given me funding or logistical support, without whom this work would not have been possible. The undergraduate students who were crucial to the completion of this work – Cassandra Fieldson, Abby Danko, Emma Carney, Shea Molsee, and Max Rossman. Everyone in the UW Biology administrative office that helped with everything from departmental award funding to equipment insurance, thank you for everything you do. The many volunteers and members of the scientific and management committees at Gluepot Reserve in South Australia, where the fieldwork for Chapters 4 and 5 were conducted – Bec Boulton, Ian and Margaret Falkenberg, Deirdre and Ian Kerr, and Tim Pascoe. The curators and collections managers at museums around the world that made the work for Chapter 3 possible – Kevin Epperly, Jeremiah Trimble, Paul Sweet, Chris Milensky, Paul Doughty, and Heather Janetzki. I would also like to thank my funding sources: Ecological Society of Australia Student Research Award, American Ornithological Society Student Research Award, Society of Integrative and Comparative Biology Student Travel Award, UW Graduate School Boeing International Research Fellowship, and the UW Biology Hoag, Edwards, Orians, and Botanical Research Awards.

Finally, I would like to the give the biggest thank you to my friends and family. To the friends I have made in Seattle, especially Christine Nolan, Mariah Wahl, Sonia Grunwald, Jack Litle, David Cuban, and Alyssa Sargent – I would not have made it even one year in this program without you all. Thank you so much for your constant support and affirmation. To Stephanie Camp and Logan Mort-Jones, who have been a constant source of friendship for almost a decade, thank you so much for making the trip out to Seattle to visit us, we love you. To my family, my mom, dad, Alex, Sue, Alex #2, Suzanne, and Tom, I love you all very much and you have fully supported me through all of the crazy decisions I have made to pursue an academic career. Last, but certainly not least, I would like to give my most heartfelt thank you to my wonderful partner Zach and our amazing doghter Pickett. The two of you have filled my life with so much light and joy, and you have been there through the lowest and highest points of this process. I love you both more than I can say, and I dedicate this dissertation to you.

Introduction

Background – A foundational interest in biology is to understand how ecology, the study of organisms interacting with each other and their environment, and evolution, the study of changes in populations over time, influence each other. How do the evolutionary histories of organisms affect their interactions, and how do those interactions in turn affect the evolution of the organisms involved? Plant-pollinator interactions are a fantastic system in which to tackle these questions, as these interactions are ecologically and evolutionarily important for both interaction partners – birds must visit flowers and consume enough floral nectar to meet their high metabolic demands, and plants must receive sufficient pollination services during these visits to reproduce. I am specifically interested in avian pollination systems, of which there are many around the world, such as hummingbirds in the Americas, sunbirds in Africa, and honeyeaters in Australasia. Using avian pollination systems, I aim to understand how birds and plants influence each other's evolution through pollination interactions, and how this can result in adaptation, specialization, and coevolution. I investigate the ways in which bird and plant phenotypes, or traits, can influence the benefits received by the other interaction partner during these interactions, such as how quickly birds can extract nectar from flowers and the degree to which plants receive adequate pollination services; this provides greater understanding of how present-day ecological interactions can have evolutionary consequences and allows for inferences about the evolutionary history of these interactions. I use Australasian honeyeaters (Aves, Meliphagidae) as my study system, and I draw on many fields of biology to investigate these broad themes. Through the culmination of my dissertation, I hope to build a more cohesive conceptual understanding of how ecological interactions between birds and plants result in the evolutionary patterns we can observe across species and throughout time.

Chapter 1 – Many lineages of birds have evolved to feed on nectar and consequently pollinate flowers (Burns et al. 2003; Pauw 2019, Ollerton 2024). Relying on floral nectar as a food source presents several challenges that can place selective pressures on birds. These challenges include navigating plant and floral morphology to extract nectar (Gill and Wolf 1978; Stiles 1981; Carothers 1982; Gass and Roberts 1992; Temeles 1996; Temeles et al. 2002, 2009), moving among nectar resources which can be patchy and temporally unreliable (Keast 1968; Carpenter 1978; Ford and Paton 1985; McFarland 1986; Ramsay 1989; McGoldrick and MacNally 1998; Franklin and Noske 1999; Parachnowitsch et al. 2019; Bogo et al. 2021), and processing high volumes of water while efficiently extracting solutes (Baker and Baker 1983; McWhorter and Martinez del Rio 1999; Gartrell 2000; McWhorter et al. 2003; Nicolson and Fleming 2003, 2014). My first chapter reviews the evidence for convergence, or similarity that has evolved independently in distant lineages, across ten lineages of nectar-feeding birds. This review focuses on the feeding apparatus (bill, tongue, hyoid), the locomotor apparatus (wings and legs), and the digestive and renal systems, aiming to determine whether the morphology (size and shape) and function of these structures are convergently adapted for nectar-feeding across bird lineages. Each of these bodily regions is important at a different point in the feeding/pollination interaction (Gass and Roberts 1992). The feeding apparatus is relevant at the smallest temporal and spatial scale; once a bird has selected which flower to feed at, it must quickly insert the bill and use the tongue to collect nectar. Zooming out, the locomotor apparatus is important for traveling between patches of flowers and for positioning the body to access flowers. Finally, the digestive and renal systems control processes that take place over longer timescales such as satiety, extracting energy from nectar, and maintaining water balance.

The last cross-taxa reviews on this topic were in the 1980s (Stiles 1981; Collins and Paton 1989; Paton and Collins 1989), and since then our knowledge has grown tremendously. By synthesizing this literature, this chapter established that there is stronger evidence for convergence in the morphology of the tongue and bill across nectar-feeding birds than in the other body systems investigated to-date. This pattern can be explained by a number of plausible hypotheses. One hypothesis is that a strong pattern of adaptive evolution in the bill and tongue is due to the fact that efficiently collecting nectar is the most universal selection pressure faced by birds when evolving nectarivory. An alternate hypothesis is that the bill and tongue are simply more labile to evolutionary change than structures with a wider variety of functions, like wings. This work also illuminated the knowledge gaps that exist in our understanding of how birds adapt to nectarivory and provided targeted suggestions for future research.

Chapter 2 – Nectarivorous birds must navigate the internal space of flowers to extract nectar (Gill and Wolf 1978; Stiles 1981; Carothers 1982; Gass and Roberts 1992; Temeles 1996; Temeles et al. 2002). Birds typically consume liquids by scooping them up with the lower bill, but the restrictive nature of flowers and small nectar volumes mean that nectar must be collected using the tongue (Rico-Guevara et al. 2019; Cuban et al. 2022). Accurately describing the biomechanics of nectar feeding provides insight into the ecological interactions between birds and the plants they feed from. Different feeding mechanisms function better under different nectar conditions (e.g., volume, sugar concentration), such that filling mechanisms could dictate the energy consumed when feeding on flowers with different nectar traits (Kingsolver and Daniel 1983; Kim et al. 2011; Heyneman 1983; Herrera 1989). My second chapter uses a biomechanics approach, focused on high-speed videography and kinematic analysis, to answer the question of how honeyeaters use their brush-tipped tongues to efficiently capture nectar. By studying feeding

mechanics in five honeyeater species, I found that nectar is primarily captured via surface tension between the bristles at the tip of the tongue, a mechanism called fluid trapping (Cuban et al 2022). Little nectar flows through the tongue body despite the fact that it is a hollow groove, similar to a straw cut longitudinally. Fluid trapping is less sensitive to nectar volume (Herrera 1989) and sugar concentration (Wei et al 2020) than are flow-based mechanisms, which could be why honeyeaters visit flowers with a large range of volumes and concentrations (Pyke and Waser 1981; Ford et al. 1979; Pyke 1980; Ford and Paton 1985; Paton and Collins 1989).

Chapter 3 – My third chapter follows from the second by examining the morphology of the hyolingual apparatus (tongue and hyoid) from a number of honeyeater species that vary in their reliance on nectar, allowing me to study morphology at an evolutionary timescale and see how it varies with diet. I found that there are six distinct tongue types across honeyeaters, and there are likely some tongue types that require the use of a different mechanism from fluid trapping (as established in Chapter 2). I also found that tongue length and the proportion of the tongue that is bristled were positively correlated with degree of nectarivory across honeyeaters. This makes sense in light of the biomechanical analysis conducted in Chapter 2, as a longer tongue can probe deeper into flowers and/or allow access to a wider range of flowers in the environment, and a larger bristled portion of the tongue allows more surface area for nectar collection via fluid trapping.

Chapter 4 – Animal pollination is often viewed as a mutualism with both parties receiving some benefit from the interaction, and often resulting in coevolution of size and shape between flowers and the pollinator's structures that interact with them (e.g., Castellanos et al. 2004, Johnson and Steiner 1997, Newman et al. 2015, Pauw et al. 2009). Animal pollination also comes with large amounts of pollen loss (Mitchell et al. 2009). One way to reduce pollen loss is to increase the

morphological matching between flower and pollinator so nectar is only accessible when the pollinator is feeding at a specific orientation and depth, ensuring proper contact with floral reproductive structures (Minnaar et al. 2019). In nectar-feeding birds, morphological matching is important when examining patterns of floral visitation. For example, hummingbirds tend to visit flowers that better 'fit' their bill in metrics like length, width, and curvature, presumably because they extract nectar more efficiently at these flowers (Temeles et al. 2009; Maglianesi et al. 2014, 2015; Weinstein and Graham 2017; Janeček et al. 2020; Rico-Guevara et al. 2021; Leimberger et al. 2022). The consensus hypothesis is that, due to its connections to pollination and feeding efficiency, morphological matching has crucial implications for the fitness of both parties in feeding/pollination interactions and this drives bird-plant coevolution (Minnaar et al. 2019; Rico-Guevara et al. 2021; Opedal 2021; Leimberger et al. 2022). This hypothesis remains largely untested, especially in systems other than hummingbirds (but see Collins 2008; Ngcamphalala et al. 2018; Johnson et al. 2020). My fourth chapter addresses this knowledge gap by asking how the morphology of honeyeaters' bills interacts with flower morphology to determine how well the birds pollinate these flowers and how quickly they extract nectar. Working with a collaborator at the University of Adelaide, honeyeaters were caught in the Australian bush and brought into a field lab. In the lab, birds were presented with newly bloomed flowers and these interactions were filmed with high-speed cameras. I found that honeyeater species differ in their pollen transfer and feeding efficiency at these flowers. Surprisingly, and contrary to the expectations from the consensus hypothesis in the literature, the degree of morphological matching between the bird's bill and the flower only affected some outcomes of these interactions. A worse bill-flower match led to less pollen deposition at flowers, but it did not affect the feeding rates of the birds or the ability of birds to acquire a pollen load.

Chapter 5 – Having examined honeyeater-plant interactions at narrow spatial and temporal scales – capturing nectar in a single lick and depositing pollen in a single flower visit – my final chapter zooms out to consider the factors that shape patterns of honeyeater visitation to plants. Interactions between a single bird species and a single plant species do not happen isolation, but rather exist embedded within a larger community that shapes how those interaction partners can engage with each other. This work uses a community ecology approach to not only consider the factors at play in species-specific honeyeater-plant interactions, but the role that interactions between honeyeater species could play in determining patterns of bird visitation to plants. I quantified the avian communities and floral rewards of two sympatric (spatially co-occurring) Australian flowering plant species and I found that the two species attracted significantly different avian communities. Small-bodied honeyeater species disproportionately visited the plant species that produced fewer calories per flower and had fewer flowers available, while larger species either never or almost never visited this plant. This pattern could be occurring for two reasons. First, there were fewer birds visiting and fewer aggressive interactions recorded are the lower-reward plant; this means the lower-reward plant presents less risk of competition and less risk of having to engage in aggressive interactions, especially with larger birds. Second, smaller bird species have lower energetic demands than large species due to their size, such that the caloric reward from the lower-reward plant is relatively greater for a smaller species than a large species and may be sufficient for their energetic needs.

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Chapter 1: Variable evidence for convergence in morphology and function across avian nectarivores

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Published in *Journal of Morphology*: https://doi.org/10.1002/jmor.21513

Abstract

Nectar-feeding birds provide an excellent system in which to examine form-function relationships over evolutionary time. There are many independent origins of nectarivory in birds, and nectar feeding is a lifestyle with many inherent biophysical constraints. We review the morphology and function of the feeding apparatus, the locomotor apparatus, and the digestive and renal systems across avian nectarivores with the goals of synthesizing available information and identifying the extent to which different aspects of anatomy have morphologically and functionally converged. In doing so, we have systematically tabulated the occurrence of putative adaptations to nectarivory across birds and created what is, to our knowledge, the first comprehensive summary of adaptations to nectarivory across body systems and taxa. We also provide the first phylogenetically informed estimate of the number of times nectarivory has evolved within Aves. Based on this synthesis of existing knowledge, we identify current knowledge gaps and provide suggestions for future research questions and methods of data collection that will increase our understanding of the distribution of adaptations across bodily systems and taxa, and the relationship between those adaptations and ecological and evolutionary factors. We hope that this synthesis will serve as a landmark for the current state of the field, prompting investigators to begin collecting new data and addressing questions that have heretofore been impossible to answer about the ecology, evolution, and functional morphology of avian nectarivory.

Introduction

Nectarivory is a foraging niche that has evolved in many vertebrate lineages (e.g., Eifler, 1995; Fleming et al., 2005; Fleming & Muchhala, 2008; Olesen & Valido, 2003), defining nectarivore as "an animal that has specialized to consume floral nectar, which exhibits morphological, physiological and behavioral adaptations for nectar feeding" (Cuban et al., 2022). Throughout their evolutionary history, birds have been prolific in their exploitation of nectar resources (Burns et al., 2003; Pauw, 2019). Based on ancestral state reconstruction (see details in supplementary information), there are approximately 11 independent evolutions of nectarivory across birds when considering species that have >50% of the diet composed of nectar (Figure 1), and approximately 24 independent evolutions when including taxa that have over 30% of the diet composed of nectar (Figure 1, S1). Hummingbirds (Trochilidae) are the oldest lineage of nectar-feeding birds, having split from other Neoaves more than 30 mya (Mayr, 2004, 2007). Fossils of stem-hummingbirds from the Oligocene display traits that we associate with nectarivory in modern hummingbirds (small body size, long, slender and straight bill), suggesting that this lineage has been occupying the niche longer than any other avian group (Louchart et al., 2008; Mayr, 2004, 2007). While hummingbirds are the best-studied avian nectarivores, the majority of nectar-feeding families are found within the songbirds (Figure 1, S1, Pauw, 2019). Although

nectar feeding has evolved many times across birds, most of our information on the physiology, ecology, and evolution of avian nectarivory comes from detailed studies on the three largest radiations of nectar-feeding birds: hummingbirds, sunbirds (Nectariniidae), and honeyeaters (Meliphagidae) (see reviews by Nicolson & Fleming, 2014; Pauw, 2019). While this work has provided a wealth of information about these groups, we lack a survey that encompasses what we know about the functional morphology of avian nectarivory across all lineages and discusses the extent to which convergence is observed, defining convergence as the evolution of similar traits across distantly related lineages (*sensu* Losos 2011).

Nectar-feeding birds face a variety of biophysical challenges that are likely to place selective pressures on their morphology. These challenges include: efficiently extracting nectar that is often concealed within floral structures and can vary dramatically in volume and concentration (Figure 2a; Carothers, 1982; Gass & Roberts, 1992; Gill & Wolf, 1978; Stiles, 1981; Temeles, 1996; Temeles et al., 2002, 2009); moving among nectar resources, which can be patchy and temporally unreliable (Figure 2b; Bogo et al., 2021; Carpenter, 1978; Ford and Paton, 1985; Franklin and Noske, 1999; Keast, 1968; McFarland, 1986; McGoldrick & Mac Nally, 1998; Parachnowitsch et al., 2019; Ramsay, 1989); and processing high volumes of water while efficiently extracting solutes (Figure 2c; Baker & Baker, 1983; Gartrell, 2000; McWhorter et al., 2003; McWhorter & Martinez del Rio, 1999; Nicolson & Fleming, 2003). Because these biophysical challenges are likely to be faced by all avian nectarivores, it is useful to consider whether these challenges have led to the convergent evolution of similar morphological and functional "solutions". In this review we aim to compare the morphology of the feeding and locomotor apparatuses, and the digestive and renal systems, across avian nectarivores to determine whether there is evidence for morphological and functional convergence. While we focus on morphological modifications of external and internal structures expected/reported to be modified for nectarivorous diets, we note that a critical component of adapting to any diet is the ability to physiologically process a given food type. It is likely that the ability to taste sweetness (Baldwin et al., 2014; Toda et al., 2021) and/or the ability to extract energy from nectar (Brown et al., 2010a, 2010b; Napier et al., 2013; Odendaal et al., 2010) are necessary to exploit nectar as a resource. We recognize that physiology and morphology go hand-in-hand when discussing dietary adaptations, and direct readers to Nicolson & Fleming (2014) and McWhorter et al. (2021) reviews on the physiology of avian nectarivory.

The last cross-taxa reviews on the morphology associated with avian nectarivory were written roughly forty years ago (Collins & Paton, 1989; Paton & Collins, 1989; Stiles, 1981). Since these reviews were published, much work has been done expanding our knowledge of the morphology of avian nectarivory, and also the biomechanical and functional roles of that morphology to the process of nectar feeding. No paper has yet synthesized this information across anatomical regions and taxa to establish which taxa and traits we have information for and exhibit convergence, which we lack information for, and what avenues for future research would be most fruitful. Examining morphology associated with nectarivory across many independent evolutions of nectar feeding in birds will provide a basis for understanding the degree to which the biophysical constraints of nectarivory have led to convergent evolution of similar phenotypic solutions. In this review we aim to: 1) synthesize all available information on the functional morphology of nectar-feeding birds; 2) assess the degree to which there is evidence for morphological and functional convergence across avian nectarivores; and 3) illuminate current knowledge gaps and suggest avenues for future research.

2 Literature survey and methods for lineage selection

In our literature survey we aimed to find accounts of morphological adaptations for nectarivory, which requires restricting the list of search taxa to only those lineages that rely routinely on nectar consumption (Figure 1) and excluding those that only take nectar opportunistically. To determine which taxa to focus on in our search, we used the list of nectar-feeding avian lineages identified by Pauw (2019), because it is the most current published list of avian nectarivores and includes many of the nectarivore taxa listed in databases such as EltonTraits (Wilman et al., 2014). Pauw (2019) grouped lineages into high, medium, and low dependence on nectar. We conducted literature surveys looking for morphological adaptations for each of these taxa, but the majority of the literature was restricted to taxa identified by Pauw (2019) as having high and medium nectar dependence. Our literature search was conducted on Google Scholar and Web of Science, using keywords "nectar", "bird", "Aves", "morphology", "anatomy", "feeding", "digestion", "renal", "locomotion", as well as the name of the taxon of interest (e.g., "hummingbird"). There was a tendency among descriptive anatomical papers to use subjective terminology (e.g., long or short, wide or thin) to describe the anatomy of museum specimens. We believe it is important to cite these accounts, even though they have obvious shortcomings in their utility for interspecific comparisons. In the following sections (Feeding Apparatus, Locomotor Apparatus, Digestive and Renal Systems) we conduct cross-taxon comparisons for each aspect of anatomy (e.g., bill, tongue, and hyoid for the Feeding Apparatus) noting which taxa exhibit similar morphological modifications with similar functions.

3 Feeding Apparatus

3.1 Bill

Hummingbird bills are uniformly slender, small in depth from base to tip (Paton & Collins, 1989; Yanega, 2007). Though there are some exceptions (e.g., hermits, *Ramphomicron*; Rico-Guevara et al., 2019), hummingbirds generally have straight, elongated bills, and the longest bills relative to body size across nectarivores (Figure S2; Downs, 2004; Fleischer et al., 2008; Paton & Collins, 1989; Pigot et al., 2020). The primary explanation for the extreme bill elongation seen in hummingbirds is that a long bill helps the tongue to access the nectar deep inside a flower, which is needed because the tongue itself is thin, flexible, and has no muscular control (Rico-Guevara et al., 2021). In addition to guiding the tongue, longer bills bring the tongue closer to the nectar, making it possible to lick faster and feed more efficiently (Rico-Guevara et al 2021). Sunbirds have the second-longest bills relative to body size (Figure S2; Downs 2004; Paton & Collins, 1989) and their bills are slender and attenuated towards the tip, though not as uniformly slender as those of hummingbirds (Paton & Collins, 1989). Honeyeater bills are not as elongated as those of hummingbirds and sunbirds, but they are elongated relative to non-nectarivorous outgroup taxa (Figure S2; Marki et al., 2019; Wooller & Richardson, 1988). Honeyeater bills tend to be wider and deeper than hummingbird or sunbird bills and show strong attenuation towards the tip, but there is extensive variation in width and depth across species (Paton & Collins, 1989).

Hummingbird bill shape is thought to have coevolved with tubular floral corollas (Cotton, 1998; Feinsinger & Colwell, 1978; Leimberger et al., 2022; Rico-Guevara et al., 2021) and hummingbirds have been consistently shown to visit flowers that match their bill shape (e.g., Maglianesi et al., 2014, 2015; Weinstein & Graham, 2017). The evolutionary role of bill-corolla matching in shaping bill morphology is not well established for groups outside of hummingbirds. Many non-hummingbird avian nectarivores, including many sunbirds and honeyeaters, tend to

have decurved bills, which has been hypothesized to be related to their tendency to feed while perching (see Locomotor Apparatus below). A decurved bill is thought to allow greater ease of access to a corolla when approaching from the side and/or below, which is often the case when feeding from a perched position as opposed to hovering, which permits feeding while facing the opening of the corolla (Johnson et al., 2020; Paton & Collins, 1989; Rocca & Sazima, 2010; Temeles et al., 2009; Westerkamp, 1990). There have been many studies examining how corolla traits affect feeding efficiency in hummingbirds (e.g., Collins, 2008; Montgomerie, 1984; Temeles, 1996; Temeles et al., 2002), and relatively few studies for sunbirds (Ngcamphalala et al., 2018) and honeyeaters (Collins, 2008). These studies have demonstrated that hummingbirds, sunbirds, and honeyeaters feed less efficiently (lower volumetric intake rate) at flowers longer than their bills (Collins, 2008; Hainsworth, 1973; Montgomerie, 1984; Ngcamphalala et al., 2018; Schlamowitz et al., 1976), but also that flower length and width interact to affect feeding efficiency such that long, wide flowers and short, narrow flowers can result in similar feeding efficiencies (Ngcamphalala et al., 2018; Temeles, 1996; Temeles et al., 2002). Interestingly, flower orientation has been shown to not affect feeding efficiency in sunbirds (Ngcamphalala et al 2018) and hummingbirds (Collins, 2008; Montgomerie, 1984), but it does affect honeyeaters such that they feed less efficiently on pendulous flowers than erect flowers (Collins, 2008). In order to more completely understand how bill-corolla matching could evolve, studies are needed that simultaneously quantify the fitness consequences of the bird-plant interaction for both the bird and the plant. While it is extremely difficult to measure the actual fitness consequences of pairwise interactions, especially for birds, proxies such as feeding and pollination efficiency can be used to determine whether a set of pairwise bird-plant interactions differ in their cost and benefit.

While less often studied than overall shape, the internal bill morphology of avian nectarivores warrants more detailed investigation. In hummingbirds, internal bill structures are thought to function in the nectar-unloading process. Flexible, forwardly directed, minute tomial serrations in the margins of the upper and lower bill have been hypothesized to serve as wipers to clean the outer surface of the tongue after every lick (Rico-Guevara & Rubega, 2017). Mandibular basins are thought to collect the aliquot of nectar being offloaded after squeezing the tongue upon protrusion (Rico-Guevara & Rubega, 2017). Thirdly, internal prongs in the maxillary roof and mandibular floor have been proposed to help channel the tongue sides into the right place and orientation during tongue wringing (Rico-Guevara & Rubega, 2017). Similarly, in several sunbird species, there are grooves in the palate and mandibular floor (Downs, 2004; Liversidge, 1967), as well as serrations on the maxillary and mandibular tomia (Downs, 2004; Liversidge, 1967). These features may help with nectar offloading in sunbirds as in hummingbirds (Rico-Guevara & Rubega, 2017), but it has not been empirically investigated. No other taxa have been examined for their internal bill morphology.

Out of the remaining avian nectarivore lineages, the flowerpiercers (*Diglossa,* Thraupidae) and the nectarivorous parrots (lorikeets [tribe Loriini], Swift Parrot [*Lathamus discolor*], hanging-parrots [*Loriculus sp.*], kakas [*Nestor sp.*], and parakeets of the genus *Brotogeris*) have the most complete accounts with regards to their bill morphology. Flowerpiercers and nectarivorous parrots interact with flowers differently compared to other avian nectarivores, as they do not tend to insert their bill into the corolla when feeding. Flowerpiercers vary from primarily nectar robbers (Bock, 1985) to those that rob and visit legitimately (i.e., feed through the flower opening and remove/deposit pollen in the process) in equal proportions (Rojas-Nossa, 2007). All species of *Diglossa* have bills that are long and

slender relative to other tanagers (Vinciguerra & Burns, 2021) and are mediolaterally compressed and hooked to varying degrees (Vuilleumier, 1969). The hook is used to hold a flower in place while the lower bill pierces the corolla (Bock, 1985; Schondube & Martinez del Rio, 2003), and species with shorter bills and more curved hooks tend to be primarily nectar robbers (Rojas-Nossa, 2007) while species with longer, less hooked bills both rob flowers and perform legitimate visits (Rojas-Nossa, 2007). Nectarivorous parrots, similarly, do not fit their bill in floral corollas, but rather open their bills wide and repeatedly protract and retract the tongue into and out of the flower. Diet has been shown to be a poor predictor of bill morphology in parrots (Bright et al., 2016), with allometry and phylogeny being more important factors.

Many other nectar-feeding avian groups are consistently described as having bills that are slender, elongate, and decurved. These include the sugarbirds (Promeropidae) (Downs, 2004; Paton & Collins, 1989), Hawaiian honeycreepers (Fringillidae, Figure 3a; Paton & Collins, 1989; Raikow, 1976; Tokita et al., 2017; Ziegler et al., 2002), sunbird asities (Philipittidae; Hawkins, 2020; Hawkins & Bonan, 2020; Winkler et al., 2020) some flowerpeckers (Dicaeidae, Figure 3b; Docters van Leeuwen, 1954; E. Mayr & Amadon, 1947; Morioka, 1992; Rand, 1961), some nectar-feeding icterids (Icteridae; Beecher, 1950, 1951a, 1951b), and Fire-tailed Myzornis *(Myzornis pyrrhoura*, Sylviidae; Rand, 1967). While in some cases the nectarivore-outgroup bill comparisons are striking (e.g., Figure 3a), there are few records that provide morphometrics of nectarivores and outgroups to quantitatively confirm that the nectarivore bills are significantly more slender, elongate, and decurved.

Bills of avian nectarivores have only been superficially studied except in hummingbirds, and although it seems that there are general trends towards elongation (Figure S2) and distal attenuation (Figure 3), there is a large degree of variation in relative proportions, shape, and size, likely due to a combination of factors including phylogeny (e.g., Bright et al., 2016) and the fact that not all lineages interact with flowers in the same way during feeding.

3.2 Tongue

Even though changes in the bill are the most visible and obvious modifications for nectar feeding, it is actually the tongue which is tasked with the extraction of tiny amounts of fluid, and modifications to perform this task efficiently are expected. The hummingbird tongue is supported by the paraglossal at the base and has two keratinous grooves in its distal half and a bifurcated tip where the groove walls are fringed (Figure 3c; Gardner, 1925; Hainsworth, 1973; Lucas, 1891, 1895; Rico-Guevara, 2014, 2017; Scharnke, 1931; Weymouth et al., 1964). In sunbirds, there is considerable variation in tongue morphology (Cheke & Mann, 2008), but in many species the tongue is composed of two keratinous grooves for the majority of its length, the tongue tip is bifurcated and may or may not have a fringe (Downs, 2004; Gardner, 1925, 1927; Liversidge, 1967; Rand, 1967; Scharnke, 1932; Schlamowitz et al., 1976, but see Cheke & Mann, 2008; Gill, 1971). While hummingbird and sunbird tongues are very similar distally (two semi-cylindrical grooves with often fringed ends), a key difference is that the hummingbird tongue grooves transition to solid keratin proximately (i.e., the tongue cannot be used like a straw, e.g., Weymouth et al., 1964) while the sunbird tongue grooves remain hollow and open proximally to the intraoral cavity (e.g., Cheke & Mann, 2008). The honeyeater tongue has curled lateral margins and a groove down the middle; it bifurcates partway down its length and each branch then bifurcates again, forming four grooves that terminate in many fine bristles, such that the tongue is almost always described as brush-tipped (Fleischer et al., 2008; Gardner, 1925; Gartrell, 2000; Liversidge, 1967; McCann, 1964; Paton & Collins, 1989; Wooller & Richardson, 1988). Similarly to the sunbird tongue, the honeyeater tongue grooves remain hollow and open

proximally to the intraoral cavity creating a continuous channel for nectar flow (e.g. Paton & Collins, 1989). The honeyeater tongue has been described as fleshier than that of sunbirds and hummingbirds with musculature extending for roughly two-thirds of its length, however, no active control of the tongue tip is expected given that the distal portions are entirely keratinous (as in hummingbirds and sunbirds) (Gill, 1971; Liversidge, 1967). The tongues of hummingbirds have been shown to have volumetric capacities of 0.8–2.8 μL (Ewald & Williams, 1982; Hainsworth, 1973), while the tongues of sunbirds and honeyeaters have been shown to have capacities of 0.6–2.1 μL and 1.2–20 μL, respectively (Schlamowitz et al., 1976; Paton & Collins, 1989). There seems to be no clear correlation between tongue capacity and body mass within or across any of the families, but this has not been tested empirically; for example, in honeyeaters the tongue of the 10g *Lichmera indistincta* holds 1.2 μL, while the tongue of the 8g *Acanthorhyncus tenuirostris* holds 2 μL. This suggests that either there are specific aspects of tongue morphology that could make nectar capture more efficient across species (e.g., increased number of bristles in honeyeaters), or that species with relatively longer bills, such as *A. tenuirostris* relative to *L. indistincta*, could have relatively longer tongues that can therefore hold greater volumetric capacity (Paton & Collins, 1989); these hypotheses remain to be tested.

Of the remaining avian nectarivore lineages, many exhibit tongue morphology similar to that of honeyeaters, including the sugarbirds (Cheke & Mann, 2008; Downs, 2004; Liversidge, 1967; Scharnke, 1932; Skead, 1967), white-eyes (Chang et al., 2013; Deignan, 1958; Emura et al., 2010; Liversidge, 1967; Moreau et al., 1969), Fire-tailed Myzornis (Rand, 1967), White-eared Sibia (*Heterophasia auricularis*; Chang et al., 2013), some flowerpeckers (Figure 3b; Cheke & Mann, 2008; Docters van Leeuwen, 1954; E. Mayr & Amadon, 1947; Morioka, 1992; Rand, 1961), some nectar-feeding icterids (Beecher, 1950, 1951a, 1951b; Gardner, 1927), the Bananaquit (*Coereba flaveola*, Figure 3d; Gardner, 1925; Lucas, 1894), the Orangequit (*Euneornis campestris*; Gardner, 1925; Lucas, 1894), and the Stitchbird (*Notiomystis cincta*; Driskell et al., 2007). The exceptions to a honeyeater-like tongue morphology are the Hawaiian honeycreepers (Figure 3a), *Toxorhamphus poliopterus* (Melanocharitidae, Figure 3e), the sunbird-asities, *Diglossa* sp., and nectar feeding parrots (Figure 3f). The Hawaiian honeycreepers, *T. poliopterus*, and the sunbird asities have tongues that are wide and fleshy at the base, promptly forming a corneous tube due to medial curling of the lateral tongue margins, and there is often a fringe along the lateral margins and tongue tip, but the tongue is not bifurcated (*T. poliopterus*: Scharnke, 1931; Hawaiian honeycreepers: Beecher, 1951a; Fleischer et al., 2008; Gardner, 1925, 1927; Raikow, 1976; Ziegler et al., 2002; sunbird asities: Moyle et al., 2006; Zubkova, 2019a, but see Prum & Razafindratsita, 1997 for mention of a bifid tongue in *Philepitta castanea*). In *Diglossa,* the tongue is broad and flat at the base and narrows and bifurcates distally, but the depth of the bifurcation varies across taxa (Bock, 1985). Each branch of the split tongue is grooved, as in hummingbirds and sunbirds, but the grooves open ventrally, and there is fraying at the tongue tip (Bock, 1985; Gardner, 1925; Vuilleumier, 1969). Parrot tongues are different from those of hummingbirds and passerines in that they are highly muscular (Holyoak, 1973; Homberger & Brush, 1986; Zweers et al., 1994). In nectar-feeding parrots, the tongue appears to only be modified at the tip. In the tribe Loriini, the dorsal surface of the tongue tip has many papillae, giving it a brush-like appearance (Figure 3f; Emura et al., 2011; Gartrell, 2000; Holyoak, 1973; Homberger & Brush, 1986; Smith, 1975). The unrelated nectar-eating genera *Nestor, Lathamus*, and *Loriculus* also have papillous tongue tips (Holyoak, 1973).

Tongue morphology plays a large role in determining the mechanisms that are both feasible and efficient for nectar extraction. For example, the hummingbird tongue cannot

function as a drinking straw because the grooves are open dorsally, precluding the formation of a pressure gradient (Cuban et al., 2022). While the possible feeding mechanisms are expected to be determined primarily by tongue morphology, it is also relevant to consider the fact that mechanisms differ in efficiency across nectar concentrations and volumes (e.g., capillary filling v. active suction; Cuban et al., 2022; Kim et al., 2011; Wei et al., 2020) due to their varying sensitivity to nectar traits like viscosity and surface tension. The interplay of evolutionary constraint on tongue morphology and the physical traits of nectar likely work together to determine the most efficient nectar loading mechanism out of those that are possible. Many researchers have been interested in studying the biomechanics of the nectar-loading process, how it varies with tongue morphology and nectar traits, and tying that to larger processes like foraging behavior and bird-plant coevolution (e.g., Hainsworth, 1973; Heyneman, 1983; Kim et al., 2011; Kim & Bush, 2012; Kingsolver & Daniel, 1983). The only taxon for which the feeding biomechanics have been empirically investigated is hummingbirds, but there are hypotheses in the literature for many other taxa (reviewed by Cuban et al., 2022). Based on the fact that the hummingbird tongue consists of two tubular grooves, it was originally hypothesized that the tongue functioned like a capillary tube (Kim et al., 2012; Kim & Bush, 2012; Kingsolver & Daniel, 1983; Lucas, 1891, 1895), but based on extensive high-speed videography in wild hummingbirds of many species, we now know that different regions of the hummingbird tongue aid in nectar uptake in highly dynamic ways. The distal-most fringed region acts as a fluid trap (Rico-Guevara & Rubega, 2011), while the elastic nature of the rest of the grooves (which stay outside the liquid) allows them to fill with nectar by an elastic recovery mechanism (Rico-Guevara et al., 2015). Due to morphological similarity and the similarity of their bill and tongue movements, it is possible that sunbirds employ the same fluid trapping and elastic pumping mechanisms as hummingbirds (Rico-Guevara et al., 2019), but this has not yet been studied and warrants further investigation. The honeyeater tongue is thought to function like a paintbrush, with surface tension pulling nectar between the four distal grooves and the terminal bristles (Collins, 2008; Fleischer et al., 2008; Gadow, 1883; Paton & Collins, 1989; Rico-Guevara et al., 2019) and/or as capillary tubes, with menisci pulling nectar within the grooves (Paton & Collins, 1989); these hypotheses have been carried over to other taxa that have a honeyeater-like tongue morphology (Cuban et al., 2022) as well as to nectarivorous parrots (Churchill & Christensen, 1970).

The tongues of most avian nectarivores are keratinous and grooved or tubular (often with one or more bifurcations) with various degrees of bristling at the tip. Parrots are the only documented exception to this, as they have muscular tongues with many papillae at the tip. The common tongue modification found across all avian nectarivores is an increase in the segmentation of the tongue at the tip, whether that be through bristles, grooves, or both.

3.3 Hyoid apparatus

Depending on the corolla length and nectar volume, efficient nectar extraction may require extensive tongue protrusion past the bill tip. In order to feed efficiently on nectar, we would expect the epibranchial bones of the hyoid apparatus (see Rico-Guevara et al., 2019 Figure 17.4a) to be lengthened to increase tongue protrusion capability, and for the muscles that wrap around the epibranchials, the *m. branchiomandibularis* and *m. stylohyoideus* which are responsible for protraction and retraction, respectively, to be well developed (Zubkova, 2019b; Zusi, 2013; Zusi & Bentz, 1984). In hummingbirds, the epibranchial bones, the *m. branchiomandibularis*, and the *m. stylohyoideus* are extremely elongated such that they wrap around the back of the skull and insert in a variety of locations, from above the eyes to past the

craniofacial hinge, depending on the species (Homberger, 2017; Liversidge, 1967; Lucas, 1891; Rico-Guevara, 2014; Weymouth et al., 1964; Zusi, 2013). These modifications to the hyobranchial apparatus allow hummingbirds to protrude the tongue outside the bill up to twice their culmen length (Rico-Guevara, 2017). Sunbirds are the only examined taxon that has a similar hyoid morphology to hummingbirds; in sunbirds the protractor and retractor muscles of the hyoid are lengthened such that they extend around the back of the skull to the frontal bone, inserting above the orbit (Liversidge, 1967; Zusi, 2013). In honeyeaters these muscles are not as elongated as in sunbird and hummingbirds, as they insert on the back of the skull rather than in the orbit (Liversidge, 1967). The functional consequence of the lack of extreme epibranchial and muscular elongation in honeyeaters is demonstrated by the fact that honeyeaters have a more limited tongue protrusion distance (5–20mm, less than or equal to culmen length depending on the species) than hummingbirds (Paton & Collins, 1989; Rico-Guevara, 2017).

In sugarbirds and white-eyes, the protractor and retractor muscles of the hyoid extend to the back of the skull, a similar length to honeyeaters (Liversidge, 1967). In the Hawaiian honeycreepers, the nectar feeding Amakihi (*Chlorodrepanis virens virens*) exhibits elongated epibranchials and associated protractor and retractor muscles compared to the insectivorous Alauahio (*Paroreomyza maculata*; Zweers et al., 1994). In the sunbird-asities, the genus *Philepitta* exhibits no epibranchial elongation compared to non-nectarivorous species in two closely related families, the Pittidae and Eurylaimidae (Zubkova, 2019a). However, out of the two asity genera, *Philepitta* is less nectarivorous than *Neodrepanis* (Wilman et al., 2014), so it is worth investigating hyoid modification in *Neodrepanis* as well.

The hyoid is the part of the feeding apparatus that is least consistently modified for nectarivory, as some taxa exhibit bone and muscle elongation that would be beneficial for nectar feeding while others exhibit minimal elongation or none at all.

4 Locomotor Apparatus

4.1 Body mass

Nectarivory entails relying on flowers that can be variably distributed in the environment and have variable volumes and concentrations of nectar (e.g., Franklin and Noske, 1999; Parachnowitsch et al., 2019; Pyke & Waser, 1981). In order to satisfy its energetic requirements, a bird must be able to not only extract nectar rapidly when at a flower but move among flowers efficiently. The larger the individual, the higher their energetic requirements (Brown et al., 1978), and the more nectar they need to feed on to replenish the energy invested in traveling to these resources; this has led to the expectation that animals subsisting on nectar shall tend to have smaller body sizes (Brown et al., 1978). Hummingbirds are the smallest avian nectarivores, ranging from 2–11g (Bleiweiss, 1998; Collins & Paton, 1989) with the exception of the Giant hummingbird (*Patagona gigas*), which can weigh as much as 31g (Williamson & Witt, 2021).

Many other lineages also exhibit a tendency towards body size < 20g. Sunbirds range from 6–17g (Collins & Paton, 1989; Paton & Collins, 1989), species of *Diglossa* range from 9–22g (Wilman et al., 2014), and flowerpeckers range from 6–12g (Wilman et al., 2014). Hawaiian honeycreepers range in body mass from 10–38g (Tokita et al., 2017) and the two genera of sunbird asity range from roughly 7g (*Neodrepanis* spp.) to 35g (*Philepitta* spp.). Lineages that do not exhibit body sizes regularly <20g are sugarbirds, honeyeaters, and nectar-feeding parrots. Sugarbirds are roughly 30g (Paton & Collins, 1989; Wilman et al., 2014). Honeyeaters exhibit the largest range in body mass, from 8g to over 152g (Collins & Paton, 1989; Gartrell, 2000; Paton & Collins, 1989; Pyke, 1980). Nectarivorous parrots tend to be between 50–200g (Gartrell,

2000; Wilman et al., 2014), with the exceptions of the genera *Vini* (mean: 41g), *Glossopsilla* (mean: 42g), *Charmosyna* (mean: 37g), *Oreopsittacus* (mean: 20g), *Neopsittacus* (mean: 33g), and *Loriculus* (mean: 28g) (Wilman et al., 2014).

With the exception of sugarbirds, honeyeaters, and parrots, avian nectarivores tend to exhibit body size <20g.

4.2 Wing morphology and flight

Hummingbirds are the only bird known to be capable of symmetrical hovering, in which they are able to generate lift on both the downstroke and upstroke of their wingbeats, which allows them to sustain their weight while stationary in mid-air for prolonged periods (Altshuler & Dudley, 2002; Warrick et al., 2012). Hummingbirds tend to have wings of higher aspect ratio (i.e. long and narrow) than other nectarivores, as well as longer wings relative to body size (Collins & Paton, 1989; Warrick et al., 2012). Hummingbirds also have much shorter humeri than other birds (except swifts; Zusi, 2013), which allows for increased downstroke and upstroke velocity at the shoulder joint, facilitating faster movement of the wings to generate more lift during the distinctive figure eight wingtip motion (Böker, 1927; Hedrick et al., 2012; Warrick et al., 2012; Zusi, 2013). Because hummingbirds have extremely elongated phalanges, which make up the majority of the wing's bony support (Böker, 1927; Hedrick et al., 2012; Warrick et al., 2012), much of the supination of the wing during the upstroke originates from the wrist (Hedrick et al., 2012). Additionally, hummingbirds have the largest flight muscles relative to body size of any bird, contributing up to 33% of their body mass (Altshuler & Dudley, 2002; Greenewalt, 1962; Schmidt-Nielsen, 1997; Warrick et al., 2012), and they have a long and deep keel to support these muscles (Zusi, 2013).

A classic paradigm in the avian nectarivore literature is that hummingbirds always hover to feed, while non-hummingbird avian nectarivores always perch to feed (Chang et al., 2013; Fleischer et al., 2008; Pyke, 1980, 1981; D. M. Skead, 1963; Spieth, 1966; Westerkamp, 1990). As hovering is very metabolically expensive (Suarez, 1992; Weis-Fogh, 1975), it was hypothesized there is a mass threshold above which sustained hovering is not a viable form of locomotion, and it was thought that perching may be a better alternative for larger birds (specifically those >9g, see Pyke, 1980, 1981). Studies have shown, however, that the tendency to hover or perch while feeding depends primarily on plant architecture, not on energetic limitations due to body mass (Miller, 1985; Wester, 2013; Westerkamp, 1990). Most hummingbirds perch on any structure that they can reach with their short feet while extracting nectar from a flower (e.g., Feinsinger & Colwell, 1978; Stiles, 2008), and sunbirds and honeyeaters can hover for very brief periods (e.g., <1 minute; Geerts & Pauw, 2009), but it occurs facultatively when a plant does not supply suitable perches (Anderson et al., 2005; Chang et al., 2013; Chen et al., 2019; Ford & Paton, 1977; Geerts & Pauw, 2009; Janeček et al., 2011; Padyšáková & Janeček, 2016; Pyke, 1980; Sejfová et al., 2021; Wester, 2013). To accomplish hovering, sunbirds and honeyeaters use a typical asymmetric flight stroke (lift generated on downstroke only; Norberg, 1990) and generate sufficient lift by increasing wingbeat frequency (Zimmer, 1943). Asymmetric hovering seems to be accomplishable without any morphological adaptation to hovering (Zusi, 2013). No differences have been found in wing morphology of hovering versus perching sunbirds (Louis, 2019). Some speculation has been made that honeyeaters with better flight mobility tend to have longer wings, but Wooller & Richardson (1988) and Marki et al. (2019) did not find differences in relative wing length between honeyeaters and insectivorous Australian passerines to suggest adaptations for nectarivory in the former.

Only hummingbirds, sunbirds, and honeyeaters have been examined for morphological modifications in their flight apparatus related to nectarivory, and only hummingbirds show modifications for nectarivory, specifically for sustained symmetrical hovering flight.

4.3 Leg morphology

While hummingbirds primarily hover when feeding at flowers, they will perch if the plant morphology allows (Miller, 1985; Taylor & White, 2007; Westerkamp, 1990; Zusi & Bentz, 1984). Hummingbirds (alongside their relatives of the "footless" Apodiformes, including swifts) have small legs (Greenewalt, 1990) with greatly reduced muscle mass (Garrod, 1873; Hartman, 1961; Hudson, 1937; Zusi & Bentz, 1984). While hummingbirds have reduced leg musculature, it has been hypothesized that hummingbird species that tend to perch while feeding have correspondingly stronger feet (Feinsinger & Colwell, 1978; Stiles, 2008). While sunbirds and honeyeaters do tend to perch while feeding, there is no evidence that their legs are more adapted to perch than a typical passerine. Wooller and Richardson (1988) found that there were no diet-related differences in tarsus length between honeyeaters and insectivorous Australian passerines. Louis (2019) also found that tarsus length does not vary with locomotor mode (perching or hovering) in sunbirds. Like sunbirds and honeyeaters, all other non-hummingbird avian nectarivores tend to perch while feeding, but there is no evidence that their legs are more adapted to perch than outgroups that perch but do not feed on nectar (e.g., Figure S3). The only exception is Fleischer et al., (2008) noting that nectarivorous Hawaiian honeycreepers have long tarsi and strong feet for perching.

There is no evidence that any avian nectarivores examined have legs that are specifically modified for perching while feeding from flowers.

5 Digestive and Renal Systems

5.1 Crop and gizzard

As the crop is a food storage organ, crop size determines how much nectar can be collected in a given foraging bout, and the time taken to empty the crop is thought to be a key factor determining the frequency of foraging bouts in hummingbirds (Diamond et al., 1986; Karasov et al., 1986). While hummingbirds are often described as having large crops (Carpenter et al., 1991; Weymouth et al., 1964), the degree to which they fill the crop has been shown to depend on their foraging strategy (Carpenter et al., 1991). Territorial hummingbirds with consistent access to resources have been shown to eat smaller, more frequent, meals than intruders, which in turn attempt to decrease altercations with territory owners by crop-loading when they can (Carpenter et al., 1991). Outside of hummingbirds, crop morphology has only been examined in one species of sunbird and in parrots. The Malachite Sunbird (*Nectarina famosa*) lacks a crop, which may improve transit times with direct delivery of nectar from the mouth to the digestive organs, but it would likely come at the expense of food storage and require more frequent, shorter foraging bouts (Mbatha et al., 2002). Nectarivorous parrots have smaller crops than granivorous and frugivorous parrots (Holyoak, 1973; Richardson & Wooller, 1990), but crop size can be plastic and in the Purple-crowned Lorikeet (*Glossopsitta porphyrocephala*), the crop is enlarged when nectar is abundant in the environment (Churchill & Christensen, 1970; Koutsos et al., 2001). There is also some evidence that lorikeets may be able to absorb sugars through their crop, as Churchill & Christensen (1970) found that sugar concentration of nectar decreased from the crop to the gizzard in *G. porphyrocephala*.

The gizzard is the muscular portion of the stomach used for mechanical food breakdown, and its morphology has been examined in several species of honeyeater, flowerpeckers, and

nectarivorous parrots. In honeyeaters, the most nectar-reliant species have smaller, less muscular gizzards than similarly sized insectivorous passerines (Richardson & Wooller, 1986; Wooller & Richardson, 1988). Similarly to honeyeaters, the gizzard is reduced in some flowerpeckers (Docters van Leeuwen, 1954; Mayr & Amadon, 1947) and in nectar-feeding parrots compared to granivorous parrots (Richardson & Wooller, 1990; Schweizer et al., 2014; Smith, 1975). There is variation within nectarivorous parrots, however. The genera *Lathamus*, *Brotogeris*, and *Loriculus* have more robust gizzard musculature than other nectarivorous parrots, which has been attributed to a more insect-heavy diet (Gartrell, 2000; Güntert & Ziswiler, 1972; Richardson & Wooller, 1990; Schweizer et al., 2014).

Overall the crop does not exhibit consistent modifications, with hummingbirds and some parrots having enlarged crops and other taxa having no crop. The gizzard is consistently reduced across the avian nectarivores in which it has been examined.

5.2 Intestine

Birds have shorter digestive tracts and lower nominal intestinal surface area (i.e., excluding villi) than similarly sized, non-flying mammals (Caviedes-Vidal et al., 2007; Lavin et al., 2008; Price et al., 2015) to reduce the weight for flight (as do bats, see Price et al., 2015). Birds also have some of the highest metabolic requirements among vertebrates (Nagy et al., 1999), which they must meet with high rates of feeding and nutrient assimilation. To quickly assimilate nutrients while also having shorter guts and a lower surface area for solute uptake, birds utilize both active and paracellular (passive, between-cell) nutrient transport strategies (Caviedes-Vidal et al., 2007; Lavin et al., 2008; Price et al., 2015). This "leaky gut strategy" allows for more rapid uptake of water-soluble nutrients, such as sugar, down its concentration gradient via diffusion (although reliance on passive transport varies across avian nectarivores, Napier et al., 2008).

While hummingbirds, like other avian nectarivores, will supplement their nectar diet with arthropods to obtain necessary amino acids, vitamins, and minerals (Churchill & Christensen, 1970; Richardson & Wooller, 1990; Rico-Guevara, 2008; Stiles, 1995), the simplicity of nectar seems to have the largest role in shaping their intestinal morphology. As nectar is composed primarily of water and sugar, it requires less digestive effort compared to other foods, such as plant material or seeds, which need substantially more enzymatic and physical action for proper breakdown and assimilation (Battley & Piersma, 2005; Ricklefs, 1996). As such, hummingbirds have short intestines compared to birds in other trophic niches (Lavin et al., 2008), and the lowest intestinal surface area for their body size among avian nectarivores (McWhorter et al., 2021). Despite having short intestines with low surface area, hummingbirds have the greatest sucrase activity per unit of intestinal surface area among avian nectarivores (McWhorter et al., 2021). Honeyeaters have shorter intestines relative to body size than insectivorous Australian passerines (Richardson & Wooller, 1986) and both honeyeaters and sunbirds have less intestinal surface area per unit body mass than non-nectarivorous passerines (McWhorter et al., 2021). While not as prodigious as hummingbirds, honeyeaters and sunbirds have greater sucrase activity per unit of intestinal surface area than passerines of similar body size (McWhorter et al., 2021).

Aside from hummingbirds, sunbirds, and honeyeaters, intestinal morphology has only been examined in flowerpeckers, one species of *Diglossa*, and several nectarivorous parrots. Flowerpeckers have shortened intestines (Docters van Leewuen, 1954). The Cinnamon-bellied flowerpiercer (*Diglossa baritula*) has been examined for its intestinal morphology and sucrase activity. While *D. baritula* is similar to hummingbirds, sunbirds, and honeyeaters in having decreased intestinal surface area (McWhorter et al., 2021), it does not exhibit increased sucrase activity per unit of intestinal surface area (McWhorter et al., 2021). In parrots, the evidence on

changes in intestinal size associated with nectarivory is conflicting. Several studies report that lorikeets have shorter intestines relative to similarly sized frugivorous parrot species (Holyoak, 1973; Richardson & Wooller, 1990), but a macroevolutionary study by Schweizer et al. (2014) was not able to support this finding. Additionally, the Coconut lorikeet (*Trichoglossus haematodus*) was not found to exhibit the trend seen in other nectarivores of low intestinal surface area combined with increased sucrase activity (McWhorter et al., 2021).

Increased sucrase activity in hummingbirds, sunbirds, and honeyeaters correlates with their visitation of plants whose nectar is sucrose-dominated, while generalist nectar-feeding birds lack the ability to process sucrose and visit plants whose nectar is dominated by glucose and fructose, the monosaccharide components of sucrose (Abrahamczyk et al., 2017; Johnson & Nicolson, 2008; McWhorter et al., 2021; Napier et al., 2013; Nicolson & Fleming, 2003; Schondube & Martinez del Rio, 2003a). The fact that flowerpiercers and nectarivorous parrots lack increased sucrase activity indicates that there is nuance to the specialist-generalist physiological divide that is worth investigating (McWhorter et al., 2021). Both flowerpiercers and nectarivorous parrots are morphologically specialized for nectarivory in various ways but can also have relatively omnivorous diets (Wilman et al., 2014), suggesting that future work examining the relationship between the percent of various diet components, including the relative reliance on sucrose and hexose dominated nectars, and physiological indicators of nectar digestive capacity could be enlightening.

Reduced intestinal length and surface area is a common modification across avian nectarivores, with nectar-feeding parrots being the exception. Only hummingbirds, sunbirds, and honeyeaters have been found to have increased sucrase activity to allow for proficient sucrose breakdown.

5.3 Kidneys

To maintain osmotic balance, the kidneys must handle the high levels of water intake that occur from each feeding bout (reviewed in Nicolson & Fleming, 2014). After a foraging bout, hummingbirds absorb all ingested water such that all water must be handled by the renal system. This strategy has been found in two hummingbird species, the Broad-tailed Hummingbird (*Selasphorus platycercus*) and the Green-backed Firecrown (*Sephanoides sephaniodes*) (Hartman Bakken & Sabat, 2006; McWhorter & Martinez del Rio, 1999). Corresponding to this water-handling strategy, hummingbird kidneys have a reduced medullary tissue and fewer loops of Henle (Beuchat et al., 1999; Casotti et al., 1998). These structures are used to concentrate filtrate, and therefore help with water retention during times of water deprivation. Hummingbirds produce large amounts of very dilute urine due to their constant high levels of water intake, and therefore do not retain the kidney tissues needed to produce concentrated urine relative to plasma (Beuchat et al., 1990, 1999; Casotti et al., 1998; Lotz & Martinez del Rio, 2004). Due to the incredible ability of hummingbirds to produce hyper-dilute urine, Lotz & Martinez del Rio (2004) hypothesized that the reptile nephrons in the hummingbird kidney possess a portion called a “diluting segment”. Diluting segments are found in the nephrons of freshwater amphibians and help produce dilute urine by retaining water within the nephron lumen so it can be excreted (Lotz & Martinez del Rio, 2004). The presence of diluting segments in hummingbird kidneys as not been confirmed and warrants further investigation.

Sunbirds and honeyeaters have been shown to handle water in a way that is fundamentally different from hummingbirds. Sunbirds and honeyeaters reduce water absorption by passing some water through the intestine, effectively modulating the amount of processing done by the kidneys (Nicolson and Fleming, 2014); this strategy has been shown in three

passerine nectarivores: the Palestine Sunbird (*Cinnyris oseus*), the White-bellied Sunbird (*Cinnyris talatala*), and the New Holland Honeyeater (*Phylidonyris novaehollandiae*) (McWhorter et al., 2003; Purchase et al., 2013). Beyond hummingbirds, kidney morphology has only been examined in honeyeaters. Within honeyeaters, species that rely more heavily on nectar have relatively less medullary tissue in the kidneys than species that eat more insects (Casotti et al., 1993; Casotti & Richardson, 1992), corresponding with more nectarivorous honeyeaters producing more dilute urine.

While hummingbird and passerines seem to have diverged in their water handling strategy, they have converged on a similar renal morphology due to their production of dilute urine. More passerines need to be investigated, as only honeyeaters have been thus far, but both honeyeaters and hummingbirds show reduction in the kidney medulla.

6 Discussion

6.1 Is there morphological and/or functional convergence across avian nectarivores?

i. Feeding apparatus

Nectarivores tend to have longer bills relative to body size than birds in other dietary groups (Figure S4), and many groups exhibit distal attenuation of the bill. When we examine bill length across avian nectarivores, we see that the tendency towards elongation is driven by extreme elongation in hummingbirds (compare Figure S2 and S4). Many nectarivorous birds have tongues that are largely keratinous (except parrots), are grooved or tubular with some degree of bifurcation, and bristled at the tip (Figure 3). This "segmentation" is thought to enhance the ability of these birds to collect nectar with the tongue by increasing the area to which liquid can adhere, and in most lineages the grooves are hypothesized to be filled via capillary filling. The hyoid apparatus has been examined in relatively few taxa, and some show modifications for increased tongue protrusion (e.g., hummingbirds) while others do not (e.g., sunbird asities).

ii. Locomotor Apparatus

Many taxa, including hummingbirds, sunbirds, Hawaiian honeycreepers, flowerpeckers, sunbird-asities (*Neodrepanis* only) and flowerpiercers, have converged on miniaturization (body mass < 20g). Notable exceptions to this trend are honeyeaters, nectar-feeding parrots, sugarbirds, and sunbirds-asities of the genus *Philepitta*. While multiple groups can hover, only hummingbirds exhibit morphological modifications for symmetrical, sustained hovering flight. There is no quantitative evidence for adaptation to perching being associated with perching to feed on nectar.

iii. Digestive and Renal Systems

The crop is highly variable across avian nectarivores, while the gizzard tends to be reduced. Nectar-feeding birds tend to have reduced intestines (in length and surface area). Hummingbirds, sunbirds, and honeyeaters have short intestines and reduced intestinal surface area but increased sucrase activity, which would help to increase passage rates of food while maintaining sugar absorption. Flowerpeckers and one species of flowerpiercer have a reduced intestine, and the flowerpiercer does not exhibit an increase in sucrase activity compared to other passerines. Nectarivorous parrots exhibit mixed evidence on intestinal length but no increase in sucrase activity. Finally, nectarivores either process all water in the kidneys (hummingbirds) or shunt some through the intestine (honeyeaters and sunbirds). These two strategies converge on a similar kidney morphology: a reduction in medullary tissue, which is responsible for concentrating urine.

iv. Key Takeaways

a. Some traits have clearly converged across avian nectarivores, such as a fringed tongue, while others are highly variable, such as the presence/size of the crop (Table 1). Across nectar feeding birds the most convergent aspects of morphology are an elongated, attenuated bill, a segmented tongue, a tendency towards a small body size, and reduced intestinal length (Table 1). More taxa need to be examined for many traits (e.g., kidney morphology, Table 1), and so more data are needed to confirm these trends and determine whether other aspects of morphology that currently lack strong evidence for convergence are indeed similar across taxa. If we connect the different aspects of anatomy to their corresponding role in nectar feeding, trends in morphological modification can inform us which stages of the process impart the greatest, most universal selective pressures on lineages as they adapt to nectarivory, or about which traits are most liable to change. For example, the bill and tongue are more commonly modified than the hyoid across avian nectarivores, suggesting that fitting the bill into the flower and collecting nectar with the tongue may be more universal limits to efficient nectar extraction than extensive tongue protrusion, which would presumably only be needed if the nectar was difficult to access. Some amount of tongue protrusion (needed for picking up liquid beyond bill tip) could be the ancestral condition for many lineages, based on the fact that many birds conduct intraoral lingual transport of food which requires anterior-posterior movements of the tongue, and the fact that non-nectar based diets utilize protrusion beyond the bill tip, for example lapping juice from fruits (Rico-Guevara et al 2019). If some amount of protrusion is the ancestral condition, there are ecological differences across avian nectarivore systems that could result in selection for increased protrusion capacity being present or not; for example, the fact that hummingbirds tend to feed from long, tubular restrictive flowers with relatively small nectar volumes (Pyke, 1980; Rico-Guevara et al., 2021) while honeyeaters have access to open, cup-shaped flowers and flowers with relatively large nectar volumes (Franklin & Noske, 2000; Pyke, 1980), such that nectar is easier to access and extensive tongue protrusion may not be essential for efficient feeding.

b. While many taxa show the trends in morphological modification summarized above, much variation exists in the ways certain structures are modified (Figure 3, Table 1). We surmise that this could be partly attributed to the disparate ancestral states from which various lineages began (e.g., insectivorous ancestors of hummingbirds (A. Chen et al., 2019) vs. granivorous ancestors of the Bananaquit (Tokita et al., 2017)). In spite of this, here we have compiled information that supports the existence of a few shared trajectories of morphological evolution among nectar-feeding avian lineages (e.g., tendency towards a thinner bill and shorter intestine). If all avian nectarivores had converged in a suite of traits, it would suggest that such a suite of traits constitutes an adaptive peak for nectarivory. Based on this review, extant nectar-feeding lineages do not appear to converge on a single whole-phenotype adaptive peak. Rather, there could be multiple adaptive peaks that are informed by historical contingency and phylogenetic inertia (e.g., an optimal morphology for groups with shared constraints). The idea of multiple adaptive peaks has been supported indirectly for bill morphology by the finding that nectarivores have fast rates of evolution in all bones of the bill, but the relationship between the shape of those bones and diet was weak (Felice et al., 2019); this means that while adapting to nectarivory does cause changes in bill morphology, those changes do not converge on a single shape. It is also necessary to remember that the nectarivore lineages we examined here have different divergence times (e.g., 5-6mya for Hawaiian honeycreepers, Lerner et al., 2011; ~22mya for hummingbirds, McGuire et al., 2014; ~31mya for honeyeaters, Marki et al., 2017), have likely been evolving in the nectarivore niche for variable amounts of time (e.g., Louchart et al., 2008; Mayr, 2004,

2007), and potentially have different rates of morphological evolution, all of which will affect the extent to which we are able to observe them approaching an 'optimum' phenotype for nectarivory.

6.2 Future Directions

i. Intra-familial studies

Here we have established overarching trends in the functional morphology of avian nectarivory, and the next key step is to attempt to elucidate why we see convergence in some traits and divergence in others across families. To do this, future work should aim to investigate the factors driving adaptation to nectarivory in each family of avian nectarivores, as the families vary dramatically in the current and past ecological and evolutionary interactions between birds and plants. For example, honeyeaters exhibit greater variation in their reliance on nectar than do hummingbirds, which is thought to be partially due to Australian and Neotropical differences in climate that affect ecological factors such as intensity and reliability of flowering (Abrahamczyk, 2019; Fleming & Muchhala, 2008; Keast, 1968). The fact that honeyeaters do not have access to abundant and reliable flowers in the environment from year to year has likely been a key factor in their lack of hyperspecialization to nectarivory (Abrahamczyk, 2019; Fleming & Muchhala, 2008), unlike in hummingbirds, and may be important in their different evolutionary trajectories in traits like body size (Fleming & Muchhala, 2008). In terms of body size, nectarivory seems to have had a stabilizing effect on hummingbirds (although this remains to be empirically tested) and an opposite, radiating effect on honeyeaters (Marki et al., 2019), and elucidating the drivers of these different evolutionary responses to nectarivory would be worthwhile.

Another fruitful avenue for future research is investigating the role of phylogenetic inertia relative to diet in the morphological evolution of different bodily systems, as there is already evidence that the former can outweigh the latter in bill evolution (Bright et al., 2016). For example, based on this review one might hypothesize that phylogenetic inertia will have a stronger effect on wing morphology, including aspect ratio and the relative sizes of various bones and muscles, than diet across avian nectarivores. The only group known to show a distinctive wing morphology is the hummingbirds, but some key aspects of wing morphology that allow them to hover, such as a shortened humerus and high aspect ratio wings, are almost certainly plesiomorphic as they are also found in swifts (Zusi, 2013).

In addition to considering factors like ecological and evolutionary history, it will be worthwhile to consider the roles of trade-offs (i.e., compromising one function when adapting for another; Garland, 2014; Roff & Fairbairn, 2007) and evolutionary integration (interdependence among traits; Felice et al., 2018; Goswami et al., 2014; Goswami & Polly, 2010) in shaping morphology within each lineage. For example, while diet has long been thought to be an important factor, if not the most important factor, shaping the evolution of bird bills (Rico-Guevara et al., 2019), new studies are showing that many factors play large roles in shaping bill morphology, including development (Bhullar et al., 2012), allometry (Bright et al., 2016), thermoregulation (Ryeland et al., 2017; Tattersall et al., 2017), and competition for mates (Rico-Guevara & Araya-Salas, 2015; Rico-Guevara & Hurme, 2019). Relevant to avian nectarivores specifically are the findings that allometry and phylogeny are more important than diet in shaping parrot bills (Bright et al., 2016), that bill shape is important for thermoregulation in honeyeaters (Friedman et al., 2017), and that hummingbirds use their bills as weapons to fight for mates (Rico-Guevara & Araya-Salas, 2015).

Once we have a stronger grasp on the factors influencing morphological and functional adaptation to nectarivory within each family of avian nectarivores, we can expand work to

conduct inter-familial phylogenetically informed analyses. With these analyses, we can accurately quantify evolutionary correlations between degree of morphological specialization and nectar feeding, the extent of convergence across families, and the role of nectarivory in driving lineage diversification.

ii. Promising methods and resources for future work

The first step to investigating the topics proposed above is quantifying overall morphology in nectar-feeding lineages, as well as non-nectar-feeding outgroups. Several linear morphometrics, including culmen length, bill width, bill depth, wing length, and tarsus length, are now available for nearly all birds (Tobias et al., 2022). However, if we want to obtain 3D morphological data (e.g., of bill shape) or data on soft tissue structures, such as tongues or digestive organs, more intensive methods are required. Collecting 3D morphological data non-destructively is possible with microCT, which can be coupled with tissue staining in methods such as diceCT (diffusible iodine-based contrast-enhanced computed tomography; Gignac et al., 2016) or with contrast agents such as BriteVu (Lewis et al., 2020), to provide detailed reconstructions of internal anatomy without destruction of the original specimen. The applications of diceCT are particularly exciting, as whole specimens can be stained with iodine (e.g., Early et al., 2020), allowing for quantification of things like the volume of various elements of the digestive tract and of various flight muscles, and the sizes of wing, leg, and skull bones. This is an avenue that would produce an incredible amount of useful data, and while access to a CT scanner can be a technological barrier, these machines are becoming increasingly more common at museums and universities. Furthermore, there is ample literature on the process and important considerations for iodine staining of whole specimens (e.g., Early et al., 2019; Gignac et al., 2016), as well as a large online support community for open source software platforms, like 3D Slicer (Kikinis et al., 2014) and SlicerMorph (Rolfe et al., 2020) that allow for visualization and quantification of morphology from CT scans. Finally, quantifying morphology from 3D models of structures like tongues is challenging, because there are no clear homologous points, which are the basis for traditional geometric morphometrics (Zelditch et al., 2012) and there is dramatic variation in shape across taxa. Methods are being explored for shape quantification that do not rely heavily on traditional landmarking techniques (Bunn et al., 2011; Gardiner et al., 2018; Polly & MacLeod, 2008; Pomidor et al., 2016), and such methods could be useful for quantifying structures that are not easily landmarked, for example quantifying the degree of segmentation in the tongues of avian nectarivores versus outgroups.

Once morphology is quantified, open-source programs and packages can be used to construct a morphospace, quantify whether lineages occupy different adaptive peaks or have converged on a single adaptive peak (Ingram & Mahler, 2013; Mahler et al., 2013), compare models of trait evolution, and assess the evolutionary relationships between morphology and diet/ecological factors (e.g., Pagel & Meade, 2006; Paradis et al., 2004; Paradis & Schliep, 2019; Pennell et al., 2014; Revell, 2012; Stayton, 2015). Phylogenetic comparative methods at this scale are now possible thanks to efforts to produce a comprehensive avian phylogeny (www.birdtree.org, Rubolini et al., 2015) and the fact that family-level phylogenies are becoming increasingly available for all lineages of avian nectarivores (e.g., McGuire et al., 2014 for hummingbirds, Marki et al., 2017 for honeyeaters, Vinciguerra & Burns, 2021 for tanagers).

Finally, in conjunction with improving our understanding of morphological evolution, it is also critical to continue investigating how differences in morphology result in functional differences across avian nectarivores (Cuban et al., 2022), as function is the interface between morphology and the environment, and thus the target of selection (Arnold, 1983). The work done

by Dakin et al. (2018) to analyze the correlation between maneuverability and particular features of the locomotor apparatus (e.g., wing loading) among hummingbirds provides a template for how these morphofunctional studies can be conducted and applied at the macroevolutionary scale. Here, we have demonstrated that avian nectarivory is a fantastic system in which to address many remaining questions at the core of organismal and evolutionary biology, and we hope that this review will inspire future research in this system with the specific goals of understanding macroevolutionary patterns and quantifying the extent to which avian nectarivores have arrived at convergent or alternative morphological and biomechanical "solutions".

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Figures & Tables

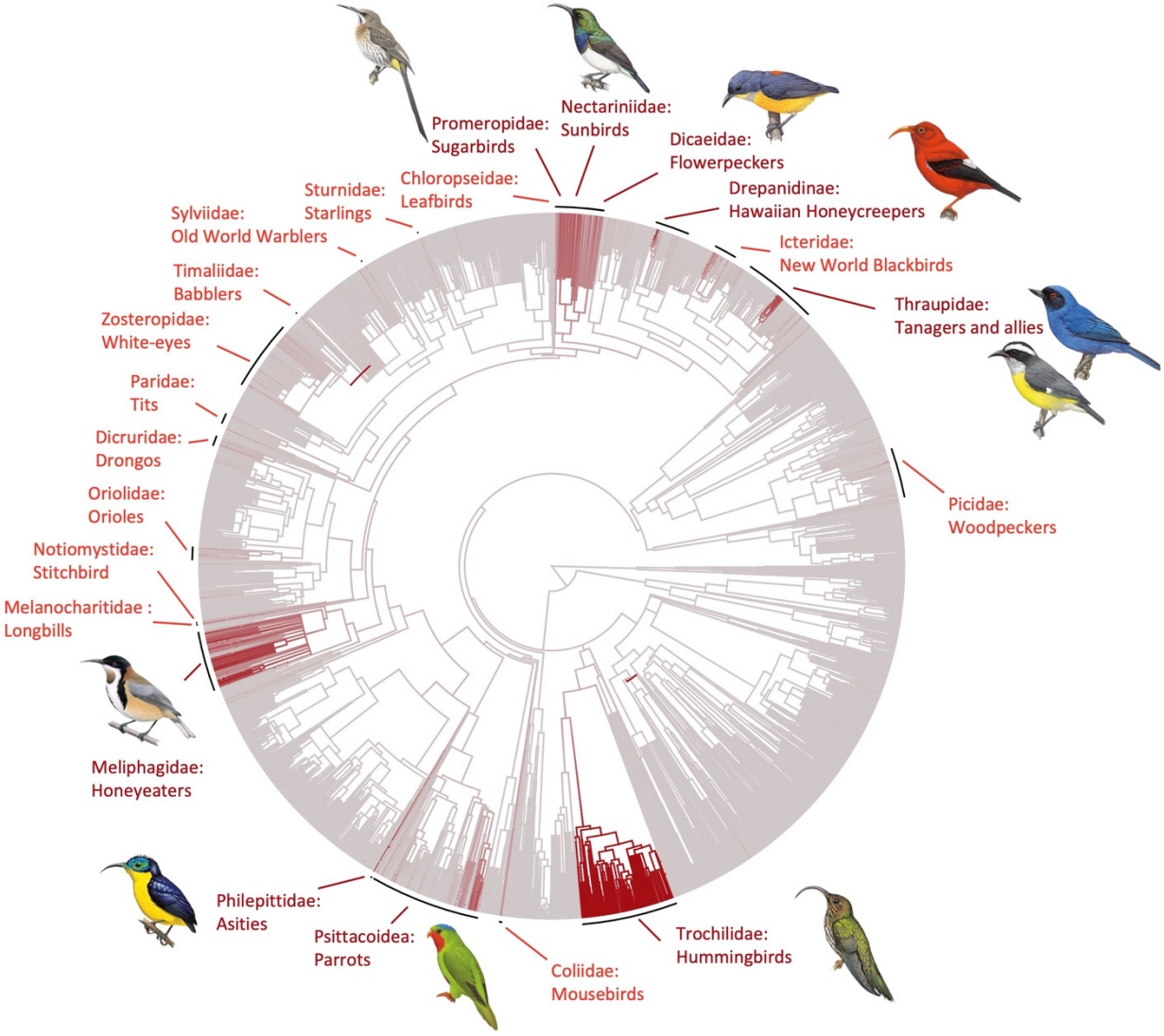

Figure 1. Distribution of nectarivory in birds. Phylogenetic tree from BirdTree (Jetz et al. 2012) trimmed to match the 5296 bird species with diet data in Elton traits database (Wilman et al. 2014). Branches are colored according to the Ancestral State Reconstruction (ASR) of nectarivory. See Supplementary Material for details on ASR. Labels of the clades with species with at least 30% nectarivory are shown in light red. Clade labels in dark red and illustrations of representative species show clades with at least 50% nectarivory. Illustrations of representative species, in clockwise order: *Cinnyris talatala, Dicaeum trigonostigma, Drepanis coccinea, Diglossa cyanea, Coereba flaveola, Eutoxeres aquila, Vini australis, Neodrepanis hypoxantha, Acanthorhynchus tenuirostris,* and *Promerops cafer.* Illustrations are from Birds of the World, Cornell Lab of Ornithology and are not to scale.

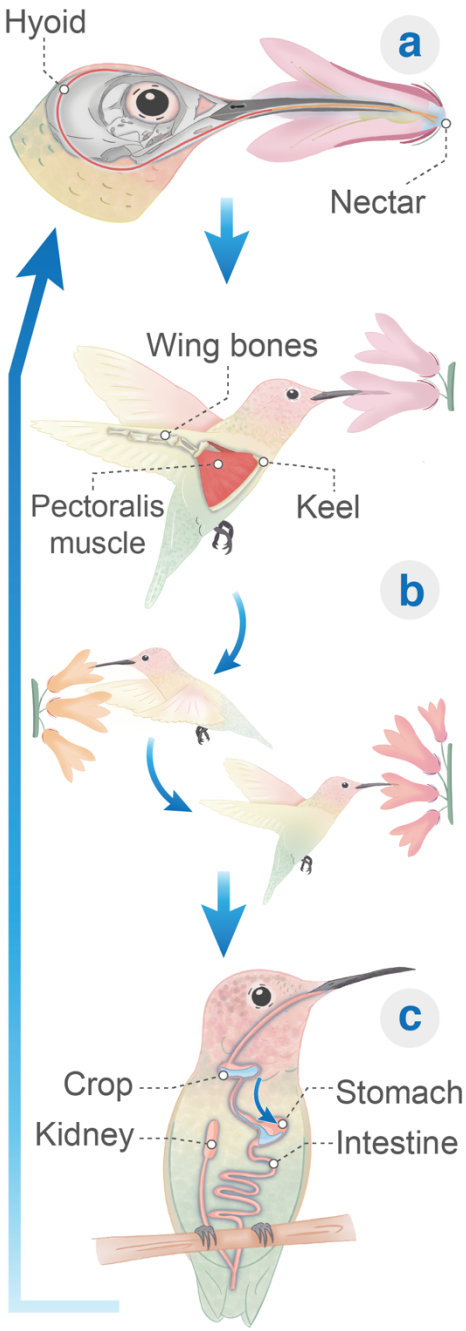

Figure 2. Morphological correlates of different stages of nectar feeding. a) When at a flower, the morphology of the bill, tongue, and hyoid are relevant for extracting nectar. b) When moving between flowers, body mass and wing and leg morphology become relevant for allowing the positioning of the bill at the flower and locomoting between flowers or patches. c) Between foraging bouts, the morphology of the digestive and renal systems will be important for processing a nectar meal before another foraging bout can begin. Hummingbird skull modified from 3D surface model of *Florisuga mellivora* specimen (Yale Peabody Museum). Wing bones and muscles modified from Figures 2 and 3 in Warrick et al. (2012). Digestive and renal anatomy modified from Figures 3 and 4 in Tell et al. (2021).

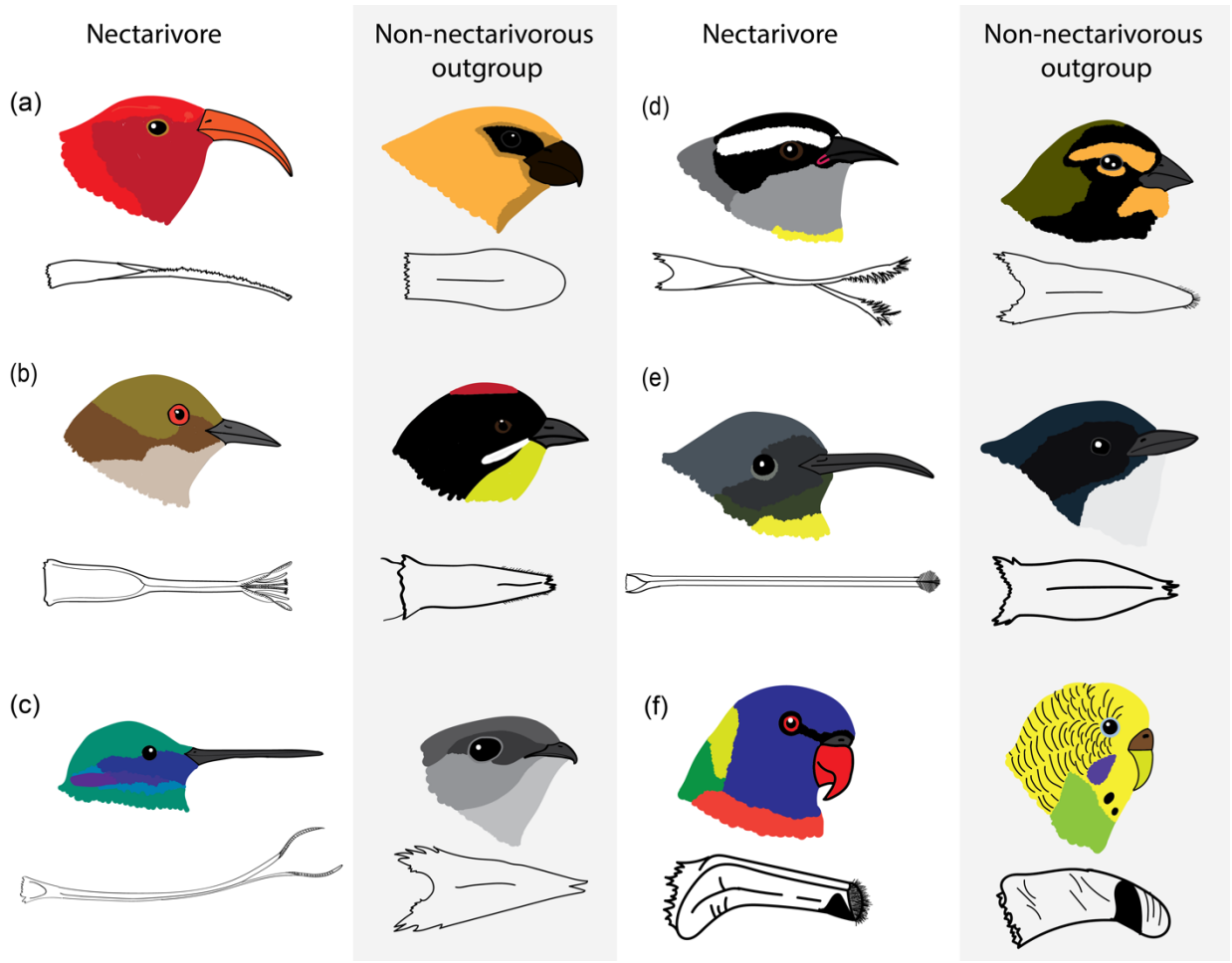

Figure 3. Bill and tongue morphology of nectar-feeding birds compared to a non-nectarivorous outgroup taxon. While all nectar-feeding taxa exhibit some modification to the bill and tongue, the way in which the tongue is modified is not the same such that the resulting morphologies can be disparate. The universal modification is that nectarivore tongues tend to have more partitions than the tongues of non-nectarivores, presumably to increase surface area and facilitate more efficient fluid uptake. The tongue partitions can be grooves, bristles, or both. Images are not drawn to scale. (a) *Vestiaria coccinea* and *Loxoides bailleui* (both Passeriformes, Fringillidae), (b) *Dicaeum nigrilore* and *Prionochilus plateni* (both Passeriformes, Dicaeidae), (c) *Colibri coruscans* (Apodiformes, Trochilidae) and *Aerodramus fuciphagus* (Apodiformes, Apodidae), (d) *Coereba flaveola* and *Tiaris olivaceus* (both Passeriformes, Thraupidae), (e) *Toxorhamphus poliopterus* and *Melanocharis versteri* (both Passeriformes, Melanocharitidae), (f) *Trichoglossus moluccanus* and *Melopsittacus undulatus* (both Psittaciformes, Psittaculidae) Bird images after eBird (https://ebird.org/explore). Tongue images after (a) Pratt 1992, Raikow 1976 and Pratt 1992, (b) Rand 1961 and Morioka 1992, (c) Weymouth et al. 1964 and Lucas 1895, (d) Gardner 1925, (e) Scharnke 1931 and Mayr and Amadon 1947, (f) Homberger and Brush 1986.

Table 1. Avian nectarivore lineages showing various morphological modifications associated with nectarivory, colored by bodily system (purple indicates Feeding Apparatus, green indicates Locomotor Apparatus, orange indicates Digestive and Renal systems). ✓ = evidence that the modification is present in the group, X = evidence that the modification is not present in the group, -- = no evidence about the presence of the modification in the group. For the intestine, L = intestinal length, SA = intestinal surface area, * = elevated sucrase according to 53. Dietary data from Wilman et al. (2014). Number of species from Wilman et al. (2014) as well as other sources cited in the text. Citations are as follows: 1-Paton and Collins 1989; 2-Marki et al 2019; 3-Wooller and Richardson 1988; 4-Bright et al 2016; 5-Docters van Leeuwen, 1954; 6-Mayr & Amadon, 1947; 7-Morioka, 1992; 8-Rand, 1961; 9-Raikow, 1976; 10-Tokita et al., 2017; 11-Ziegler et al., 2002; 12-Beecher, 1950; 13-Beecher 1951a; 14-Beecher 1951b; 15-Vuilleumier, 1969; 16-Downs 2004; 17-Rico-Guevara and Rubega 2017; 18-Rico-Guevara 2017; 19-Weymouth et al 1964; 20-Cheke and Mann 2008; 21-Schlamowitz et al 1976; 22-Liversidge 1967; 23-Fleischer et al 2008; 24-Zubokova 2019a; 25-Bock 1985; 26-Gardner 1925; 27-Holyoak, 1973; 28-Homberger & Brush, 1986; 29-Zweers et al., 1994; 30-Smith, 1975; 31-Homberger, 2017; 32-Rico-Guevara, 2014; 33-Zusi, 2013; 34-Bleiweiss, 1998; 35-Collins & Paton, 1989; 36-Gartrell, 2000; 37-Pyke, 1980; 38-Vinciguerra and Burns 2021; 39-Wilamn et al 2014; 40-Böker, 1927; 41-Hedrick et al., 2012; 42-Warrick et al., 2012; 43-Louis 2019; 44-Greenewalt 1990; 45-Zusi and Bentz 1984; 46-Carpenter et al 1991; 47-Mbatha et al 2002; 48-Richarsson and Wooller 1990; 49-Churchill and Christiensen 1970; 50-Richardson and Wooler 1986; 51-Schwetizer et al 2014; 52-Lavin et al 2008; 53-McWhorter et al 2021; 54-Beuchat et al., 1990; 55-Beuchat et al.,1999; 56-Casotti et al., 1998; 57-Lotz & Martinez del Rio, 2004; 58-Casotti et al., 1993; 59-Casotti & Richardson, 1992.

Taxon	Hummingbirds (Trochilidae)	Honeyeaters (Meliphagidae)	Sunbirds (Nectariniidae)	Nectarivorous parrots (Psittacoidea)	Sugarbirds (Promeropidae)	Flowerpeckers (Dicaeidae)	Hawaiian honeycreepers (Fringillidae)	Sunbird-asities (Philipittidae)	Flowerpiercers (Thraupidae)
Number of species	334	187	122	61	2	40	10	4	17
Dietary nectar (range [mode])	50-100 [90]	10-90 [30]	10-80 [50]	10-100 [50]	60	10-70 [30]	10-90 [30]	20-80 [80]	10-100 [50]
Bill elongated	✓[1]	✓[1-3]	✓[1,16]	X[4]	✓[1,16]	✓[5-8] (only *Dicaeum*)	✓[1,9-11]	--	✓[38]
Bill slender/ tapered	✓[1]	✓[1] (tapered)	✓[1] (tapered)	X[4]	--	--	✓[10]	--	✓[38]
Bill decurved	X[1] (except Phaethornithinae)	✓[1]	✓[1]	X[4]	✓[16]	✓[5-8] (only *Dicaeum*)	✓[1,9-11]	--	X[15]
Internal bill structures	✓[17]	--	✓[16]	--	--	--	--	--	--
Tongue grooved	✓[1,18,19]	✓[1,23]	✓[16,20,21,22]	X[27-29]	✓[16, 20, 22]	✓[5-8, 20] (only *Dicaeum*)	✓[9, 11, 13, 23]	✓[24]	✓[15,25,26]
Tongue bristled	✓[1,18,19]	✓[1,23]	✓[16,20,21,22]	✓[27-30]	✓[16, 20, 22]	✓[5-8, 20] (only *Dicaeum*)	✓[9, 11, 13, 23]	✓[24]	✓[15,25,26]
Hyoid epibranchial & muscular elongation	✓[22, 31-33]	✓[22]	✓[22, 33]	--	✓[22]	--	✓[29]	X[24]	--
Body size <20g	✓[34,35]	X[1,35-37] (some spp. <20g, but many >20g)	✓[1,35]	X[36]	X[1,39]	✓[39]	✓[10] (some spp. <20g, others >20g)	✓[39] (*Neodrepanis*) X[39] (*Philepitta*)	✓[39]
Modified flight apparatus for hovering	✓[33,35,40-42]	X[3,33]	X[33,43]	--	--	--	--	--	--
Modified legs for perching	X[44,45]	X[3]	X[43]	--	--	--	✓[23]	--	--
Enlarged crop	✓[19,46]	--	X[47] (*Nectarina famosa* lacks a crop)	✓[27,48,49] (*Glossopsitta porphyrocephala* has enlarged crop, other spp. do not)	--	--	--	--	--
Reduced gizzard	--	✓[3,50]	--	✓[30,48,51] (only in most nectar reliant species)	--	✓[5,6]	--	--	--
Reduced intestine	✓$_L$[52] ✓$_{SA}$[53*]	✓$_L$[50] ✓$_{SA}$[53*]	✓$_{SA}$[53*]	X$_L$[51] X$_{SA}$[53]	--	✓$_L$[5]	--	--	✓$_{SA}$[53] (*Diglossa baritula*)
Reduced kidney medullary tissue	✓[54-57]	✓[58,59] (more nectar reliant species only)	--	--	--	--	--	--	--

Supplemental Material

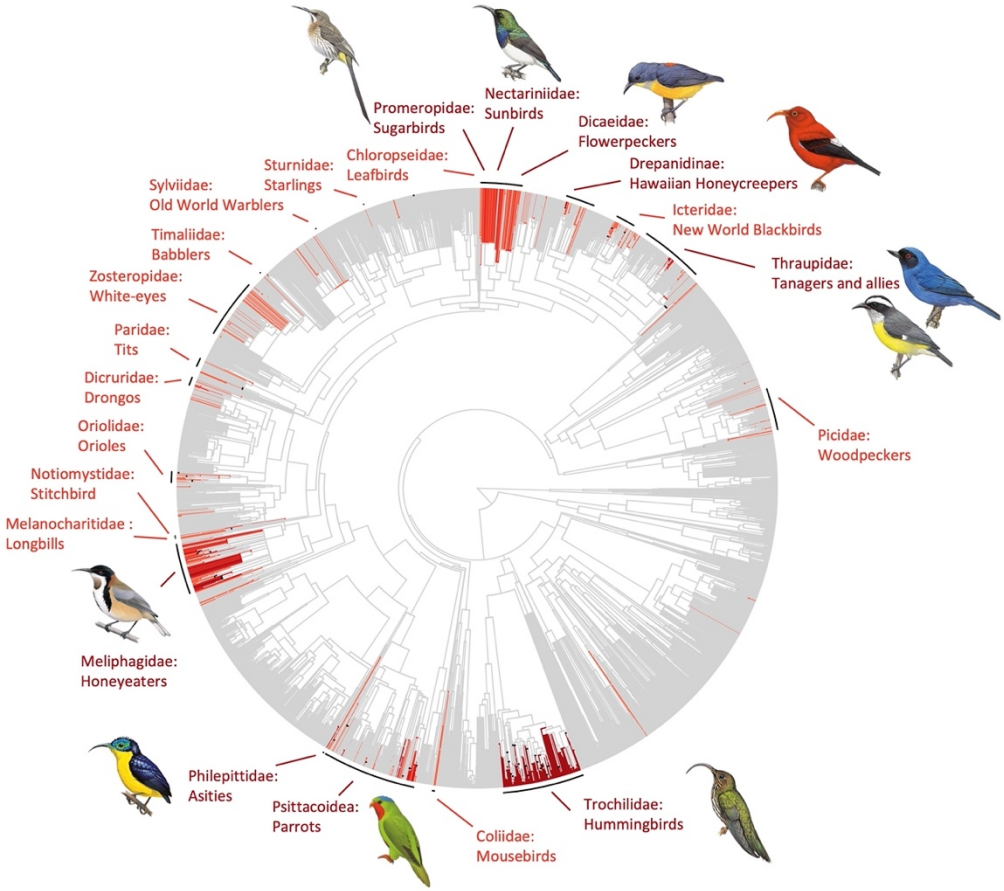

Figure S1. Distribution of nectarivory in extant birds. Phylogenetic tree from BirdTree (Jetz et al. 2012) trimmed to match the 5296 bird species with diet data in Elton traits database (Wilman et al. 2014). Branches of nectarivore species are colored in shades of red according to the percentage of nectar in their diet, divided into four categories: 10-39%, 40-59%, 60-79%, and 80-100%. The lightest red represents the first category, and the darkest the last. We added several species to the Elton traits database and modified nectarivory values of others, see supplementary information for a list of changes. Labels of the clades with species with at least 30% nectarivory are shown in light red. Clade labels in dark red and illustrations of representative species show clades with at least 50% nectarivory. Illustrations of representative species, in clockwise order: *Cinnyris talatala, Dicaeum trigonostigma, Drepanis coccinea, Diglossa cyanea, Coereba flaveola, Eutoxeres aquila, Vini australis, Neodrepanis hypoxantha, Acanthorhynchus tenuirostris,* and *Promerops cafer*. Illustrations are from Birds of the World, Cornell Lab of Ornithology and are not to scale.

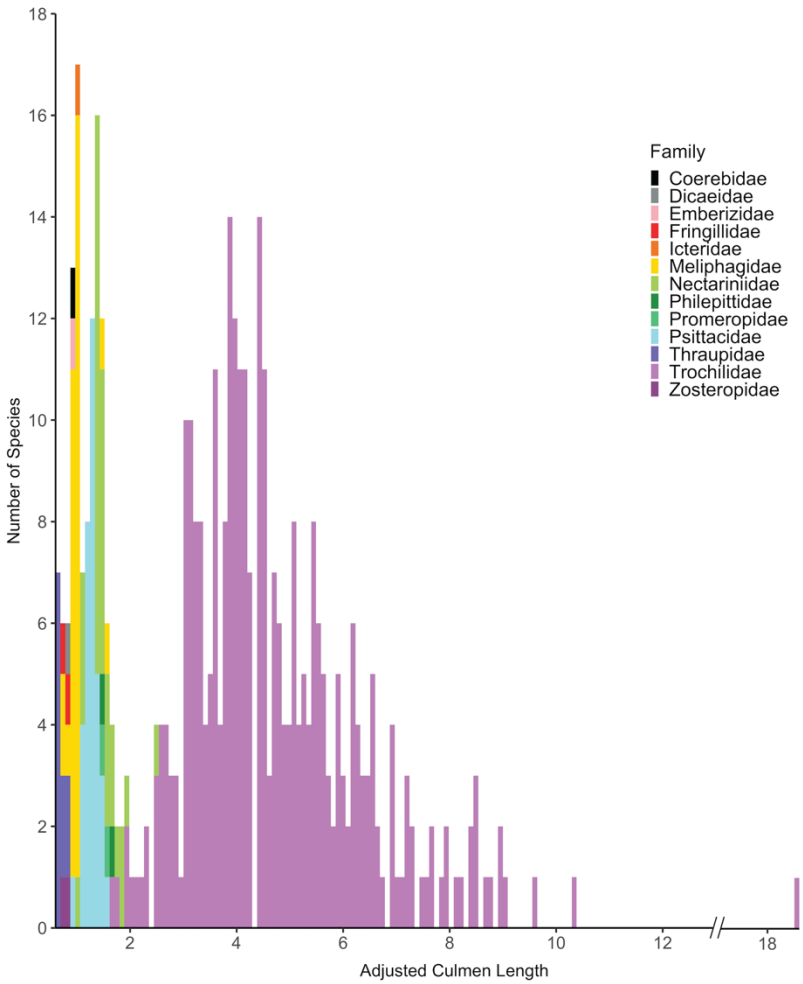

Figure S2. Stacked histogram of adjusted culmen lengths (culmen length/tarsus length) across avian nectarivores. Based on visual examination, the tendency of nectarivores to have a longer bill relative to body size than other dietary groups (compare with Figure S4) is largely driven by hummingbirds (Trochilidae, light purple). Hummingbirds have the longest bills relative to body size, followed by sunbirds (Nectariniidae, light green), sunbird asities (Philepittidae, dark green), sugarbirds (Promeropidae, blue-green), honeyeaters (Meliphagidae, yellow), and nectar-feeding parrots (Psittaculidae, light blue). All nectarivores in the Thraupidae (bright purple), Zosteropidae (dark purple), Fringillidae (red), Icteridae (orange) Coerebidae (black), and Dicaeidae (grey) have adjusted culmen lengths around 1, which is approximately the same value as the other trophic levels (see Figure S4). Morphological data from Tobias et al. (2022).

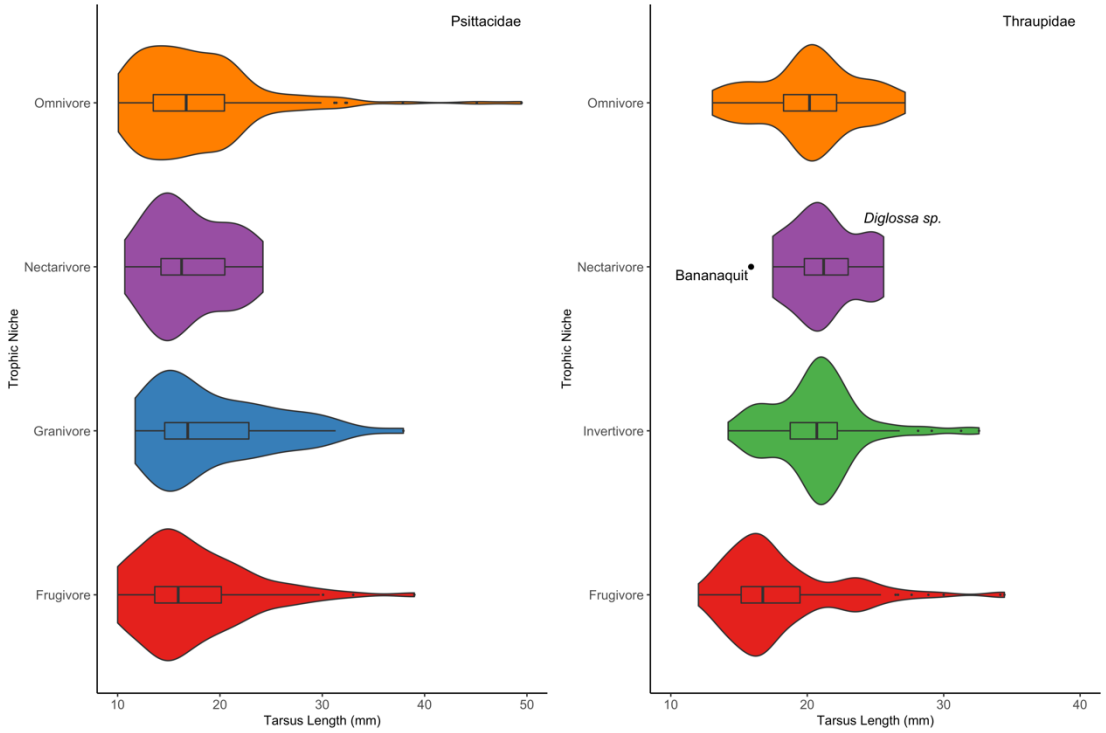

Figure S3. No differences in tarsus length between nectarivores and outgroups in other trophic niches in parrots (Psittacidae) or tanagers (Thraupidae). Violin plots were created using morphological data from Tobias et al. (2022). Trophic niche assignments for each species are from Pigot et al. (2020). Pigot et al. (2020) assigned a species to a trophic niche if >60% of their diet was from that food source.

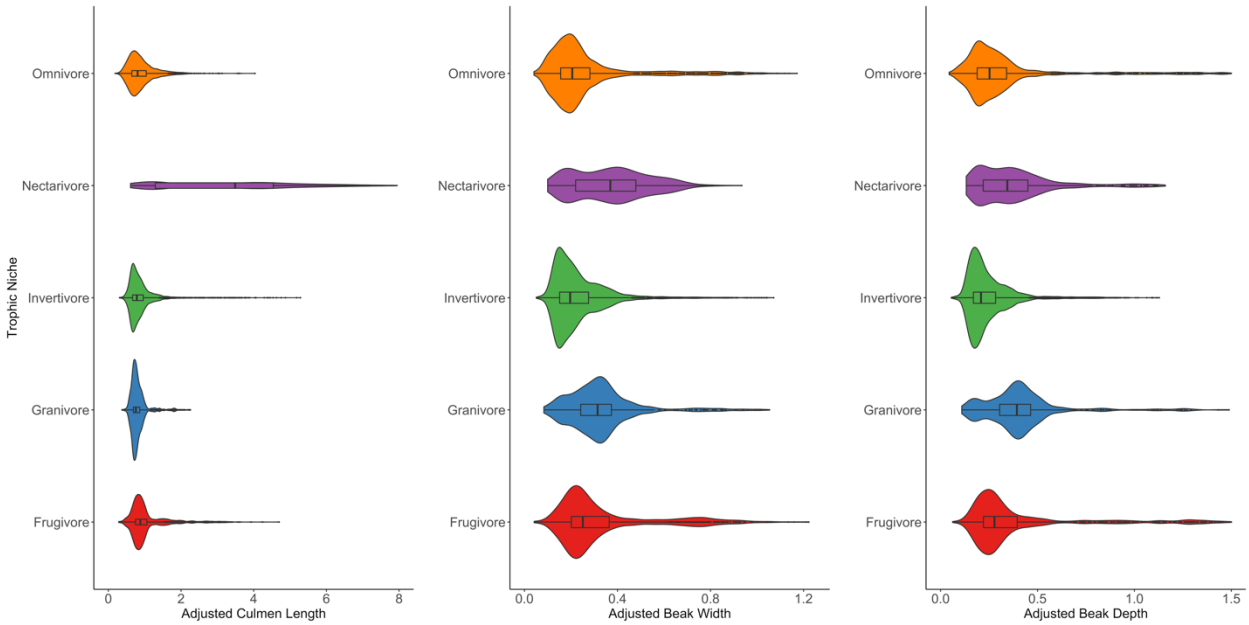

Figure S4. Trends in adjusted culmen length, adjusted beak width, and adjusted beak depth between avian nectarivores and birds in other trophic niches. Values are adjusted for body size by dividing the beak metric by tarsus length. Violin plots were created using morphological data from Tobias et al. (2022). Trophic niche assignments for each species are from Pigot et al. (2020). Pigot et al. (2020) assigned a species to a trophic niche if >60% of their diet was from that food source.

Details on Ancestral State Reconstruction (Figure 1)

We included several species to the Elton traits database and modified the nectarivory values of others, based on information from Birds of The World (Billerman et al. 2022) and other published sources. Changes were as follows: change nectar percentage of two swift species to 0; change two Phasianidae species to 0; change Indicatoridae to 10%; change Ramphastidae to 20%; change Alaudidae to 0; change Pardalotidae to 10%; add Diglossa coerulescens with 50%, 40% for *D. cyanea, D. glauca* and *D. indigotica*, all other Diglossa species with 60%; add *Coereba flaveola* with 50%; add *Modulatrix stictigula* with 10%; change *Gymnoris superciliaris* to 20%; add *Oedistoma pygmaeum* and *Toxorhamphus* species with 30%; add *Colius striatus* with 30% and all other Coliidae species with 20%; change *Cacatua leadbeateri* to 10% and *Lorius garrulus* to 60%; add *Cyanistes caeruleus* with 30%; change hummingbirds with 100% to 90% so there are no species with 100%.

Ancestral State Reconstruction (ASR) was employed using the edited Elton traits continuous measurement of nectarivory (see above), with the phytools package in R (Revell 2012, R Core team 2021). ASR was estimated using Maximum Likelihood with the contMap function in phytools. This function re-roots the tree at each and all internal nodes and calculates the contrast state at the root each time using Felsenstein's (1985) algorithm. States along the edges of the tree are interpolated using equation 2 in Felsenstein (1985).

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Chapter 2: How do honeyeaters drink nectar?

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Published in *Integrative and Comparative Biology*: https://doi.org/10.1093/icb/icad048

Abstract

We investigated the kinematics and biomechanics of nectar feeding in five species of honeyeater (*Phylidonyris novaehollandiae, Acanthagenys rufogularis, Ptilotula penicillata, Certhionyx variegatus, Manorina flavigula*). There is abundant information on honeyeater foraging behaviors and ecological relationships with plants, but there has never been an examination of their nectar-feeding from kinematic and biomechanical perspectives. We analyzed high-speed video of feeding in captive individuals to describe the kinematics of their nectar feeding, with specific focus on describing tongue movements and bill-tongue coordination, and to characterize the mechanism of nectar uptake in the tongue. We found clear interspecific variation in kinematics and tongue filling mechanics. Species varied in lick frequency, tongue velocity, and protrusion and retraction duration, which, in some cases, are relevant for differences in tongue filling mechanisms. We found support for the use of capillary filling in *Certhionyx variegatus* only. By contrast, *Phylidonyris novaehollandiae, Acanthagenys rufogularis, Ptilotula penicillata,* and *Manorina flavigula* employed a modified version of the expansive filling mechanism seen in hummingbirds, as there was dorsoventral expansion of the tongue body, even the portions that remain outside the nectar, once the tongue tip entered the nectar. All species use fluid trapping in the distal fimbriated portion of the tongue, which supports previous hypotheses describing the honeyeater tongue as a 'paintbrush'.

Introduction

One of the greatest difficulties that nectar-feeding birds face is navigating plant morphologies to extract nectar efficiently and without damaging the flower (Gill and Wolf 1978; Stiles 1981; Carothers 1982; Gass and Roberts 1992; Temeles 1996; Temeles et al. 2002). Most birds typically consume liquids by scooping them up with the lower bill (Rico-Guevara et al. 2019), but the restrictive nature of floral corollas and small nectar volumes mean that the scooping strategy is not a feasible method for collecting nectar, therefore nectar-feeding birds must use their tongues to feed (Cuban et al. 2022). Here we investigate how a speciose and ecologically critical clade of nectar-feeding birds, the Australian honeyeaters (Aves, Meliphagidae), use their tongues and bills to capture nectar.

Because many avian nectarivores have tongues that are tubular and/or grooved (Stiles 1981; Paton and Collins 1989; Hewes et al. 2022), it has long been assumed that nectar-feeding birds, including honeyeaters, fill their tongues via capillary action while feeding from flowers (Lucas 1895; Weymouth et al. 1964; Kingsolver and Daniel 1983; Paton and Collins 1989; Kim et al. 2011, 2012; Kim and Bush 2012). The capillary filling hypothesis requires that the tongue capacity (volume of the internal space) either stay consistent, such that the tongue is a static tube of unchanging internal volume, or decrease as it fills (because the walls would be 'zipped' up by the surface tension of the advancing front, Kim et al. 2012) and that there is an observable concave meniscus (Rico-Guevara et al. 2015). These requirements result from the fact that surface tension forces, which are the dominant forces during capillarity, are the ones expected to be the main drivers of the tongue filling mechanism. Extensive high-speed videography work

with hummingbirds has demonstrated that their tongue does not act as a capillary tube during typical feeding conditions, but instead it uses dynamic filling mechanisms (Rico-Guevara and Rubega 2011; Rico-Guevara et al. 2015). Using high-speed videography at up to 2400 frames per second, it has been demonstrated that the tongue tip acts as a dynamic fluid trap (Rico-Guevara and Rubega 2011). Additionally, the tongue grooves outside the nectar fill completely with fluid via a mechanism coined expansive filling which, while it still uses surface tension forces, is largely driven by the elastic energy stored in the tongue as it is compressed between the bill tips during tongue wringing, a process that removes nectar from the tongue as it is being protruded (Rico-Guevara et al. 2015; Rico-Guevara and Rubega 2017; Cuban et al. 2022). An additional critical difference between expansive filling and capillary filling is the rate at which each fills the tongue. Wild hummingbirds lick at rates between 14-17Hz, and expansive filling allows the tongue to fill its entire capacity during each lick (filling rate is 0.5mm/millisecond, Rico-Guevara et al. 2015), while capillary filling is too slow (filling rate is 0.1mm/millisecond, Rico-Guevara et al. 2015), which would result in only partial filling of the grooves at each lick.

A key lesson learned from the hummingbird feeding literature is that there are dynamic processes at play during nectar feeding such that feeding mechanisms cannot be inferred from tongue morphology alone. For all avian nectarivores outside of hummingbirds (e.g., sunbirds, honeyeaters, lorikeets, see Hewes et al. 2022 for complete list), we still know very little about their feeding mechanics because they have not been empirically investigated (Cuban et al. 2022). Accurately describing the drinking mechanics in nectarivorous birds not only improves our understanding of how they overcome peculiar biophysical challenges, but it also provides insight into the ecological interactions between nectarivores and the plants they feed from. Because different tongue filling mechanisms function better under different conditions of nectar presentation, these mechanisms could dictate the energy consumed when feeding on flowers differing in nectar accessibility, volumes, and/or concentrations (Harder 1983, 1986; Herrera 1989; Wei et al. 2020; Rico-Guevara et al. 2021).

As reviewed by Cuban et al. (2022), there are four mechanisms of nectar uptake that have been proposed in the literature for hummingbirds, with only two having been confirmed to occur using techniques like high-speed videography; these mechanisms are hermetic suction, capillary filling, expansive filling (a form of cohesive suction), and fluid trapping. Hermetic suction (*sensu* Cuban et al. 2022) is a process in which an air-filled structure expands and generates enough force to draw in fluid; the structure must be hermetically sealed (i.e., an airtight seal) to generate the suction force. For example, a human drinking through a straw creates an airtight seal around the straw using their mouth/cheeks, and then draws the fluid up the straw through expansion of the lungs. Capillary filling in the context of nectar feeding is a process in which an empty tongue (i.e., no residual nectar inside) is inserted into nectar and a meniscus forms; the surface tension forces at the edges of the meniscus pull the nectar further into the tongue (Cuban et al. 2022). The tongue does not expand during capillary filling, its capacity either stays a constant volume or slightly decreases in volume (Kim et al. 2011). Capillary filling is a completely passive process and has been hypothesized to occur in many groups of avian nectarivores (Cuban et al. 2022). Expansive filling is a process that requires the tongue be compressed before it contacts the nectar. As the tongue is protruded, it is dorsoventrally compressed between the bill tips (tongue wringing *sensu* Cuban et al. 2022), an action that both empties it of nectar from the previous lick and stores elastic energy in the bent tongue walls. As the tongue leaves the bill, it contains a thin film of remnant nectar from the previous lick, which imparts Stefan adhesion forces that keep it compressed as it passes through the air and into the nectar pool (Rico-Guevara et al. 2015); when

the compressed tongue hits the nectar, the stored elastic energy causes it to serially expand anterio-posteriorly and allows it to rapidly fill with nectar (Rico-Guevara et al. 2015). Expansive filling has only been described in hummingbirds and has not been extended to other taxa (Cuban et al. 2022). Finally, fluid trapping is a mechanism originally described for hummingbirds (Rico-Guevara and Rubega 2011) which has been extended to other avian nectarivores (Cuban et al. 2022) to describe the process by which fluid is caught between bristled structures at the distal end of the tongue, similar to how a paintbrush traps fluid between its bristles. Notably, there is no distal to proximal fluid flow into the tongue during fluid trapping and it is a passive process.

Based on morphology alone (review in Hewes et al. 2022), hermetic suction is not likely to be feasible for honeyeater tongues. If honeyeaters used hermetic suction, it would require generating suction forces such that the tongue would function like a drinking straw, but, as in hummingbirds, the honeyeater tongue is open dorsally to the external environment and they lack cheeks or other fleshy structures to create a seal, both of which could preclude the generation of suction within the tongue itself (Cuban et al. 2022). Capillary filling, expansive filling, and fluid trapping could all be used to fill the honeyeater tongue. It is important to note that our current understanding of these mechanisms come solely from work on hummingbirds, and honeyeater tongue morphology differs greatly from that of hummingbirds (Fig. 1). Based on what we know from hummingbirds, expansive filling and capillary filling are mutually exclusive mechanisms. Under these definitions, capillary filling requires an air-filled tongue that remains a constant shape and expansive filling requires that the tongue contain a thin film of fluid and expand dorsoventrally. The hummingbird tongue consists of two grooves that can roll in medially to form a tube that can be compressed and held in a compressed state, while the honeyeater tongue has thicker walls and is composed of wider grooves that cannot be rolled medially to form a tube (Fig. 1). Elastic energy can still be imparted into the honeyeater tongue through bill tip compression, but the morphology of the honeyeater tongue would likely preclude the extreme compression and expansion seen in hummingbirds. This is particularly important for expansive filling and means that expansive filling in honeyeaters might not look exactly the same as expansive filling in hummingbirds. With that as the case, it is possible that capillary and expansive filling are not mutually exclusive for honeyeaters. Because fluid trapping only operates in the distal fringes of the tongue, it is also not mutually exclusive with capillary or expansive filling. While capillary filling, expansive filling, and fluid trapping could be occurring in any combination within the honeyeater tongue, the mechanisms can be distinguished through kinematics and analyses of tongue shape change, as each has a different set of expectations for if/how/when the tongue shape should change when interacting with the fluid (Fig. 2).

Here we aim to describe the general kinematics of honeyeater feeding and to determine which filling mechanism, or combination of mechanisms, honeyeaters are most likely using to load nectar on to the tongue. To investigate feeding kinematics and elucidate the nectar uptake mechanism, we analyzed high-speed videography of nectar-feeding in five species of Australian honeyeaters, the New Holland honeyeater (*Phylidonyris novaehollandiae*), the spiny-cheeked honeyeater (*Acanthagenys rufogularis*), the white-plumed honeyeater (*Ptilotula penicillata*), the pied honeyeater (*Certhionyx variegatus*), and the yellow-throated miner (*Manorina flavigula*).

Methods

a. Animal Care

Birds were captured via mist nets and were kept in captivity for no more than seven hours. While in captivity each bird was kept in its own cage and was given access to Wombaroo nectar mix *ad*

libitum, as well as adequate perches. All cages were kept away from human activity to minimize stress on the birds. All work was done under Deakin University animal ethics approval A87-2011 granted to W.A.B. Mist netting was done at Deakin University Geelong Waurn Ponds Campus under Victoria Department of Sustainability and Environment permit number 10006510 and at the University of New South Wales Fowlers Gap Arid Zone Research Station under New South Wales Park and Wildlife Service permit number SL101085, both granted to W.A.B.

b. Videography

We worked with eight individual adults of five honeyeater species (Table S1) at the Deakin University Geelong Waurn Ponds Campus and at the University of New South Wales Fowlers Gap Arid Zone Research Station, Australia. For *A. rufogularis*, *P. penicillata,* and *C. variegatus* we filmed one adult each, for *P. novaehollandiae* we filmed two adults, and for *M. flavigula* we filmed three adults. For *C. variegatus,* we also filmed one juvenile individual to confirm the unusual tongue kinematics displayed by the adult bird. All individuals were of unknown sex. These species were chosen because they were the most abundant at the two field locations. A flat-sided nectar chamber (to minimize image distortion, see Rico-Guevara and Rubega 2011) filled with variable amounts of nectar at 18% (wt/wt) sucrose concentration was placed in front of the bird's cage, such that the bird could stick its bill out and reach the chamber without straining. This allowed unobstructed visualization of bill and tongue movements, which we filmed at 500 frames per second from the lateral perspective (Fastec TroubleShooter monochrome high-speed camera, Nikon AF-S VR Micro-NIKKOR 105mm f/2.8G IF-ED lens).

c. Kinematic Data Collection and Analysis

Across species we only analyzed videos with relatively consistent bill tip to nectar distance, as that is known to affect licking rate (Ewald and Williams 1982). We first quantified lick duration (s) and lick frequency (Hz) for all species. Then, to better investigate bill and tongue movements and coordination we digitized videos of the eight adults (details of videos selected in Table S1) using FIJI v2.1.0/1.53c (Schindelin et al. 2012). Videos were uploaded into FIJI and the Manual Tracking plugin was used to track the tongue tip, upper bill tip, and lower bill tip. Points were placed every frame of the feeding bout to ensure high temporal resolution of the motions. From this tracking data we measured whether maximal and minimal tongue protrusion and bill tip separation were in phase (occuring at the same time) or out of phase (offset by some amount of time); this allowed us to better understand the coordination of bill and tongue movements. We also measured the duration of tongue protrusion and retraction and used the x-y location data to calculate velocity (mm/s) of the tongue tip during protraction and retraction.

When plotting kinematic data we normalized the feeding bout, which consisted of a series of five licks, from absolute time to percent to facilitate comparisons of kinematics both within and across species; this was necessary because different feeding bouts last different lengths of time. The x-axis was converted from absolute time to percent of the feeding bout in R v4.0.3 (R Core Team 2022).

d. Tongue Thickness Data Collection and Analysis

Under the three possible filling mechanisms the dorsoventral tongue thickness is expected to: 1) capillary filling - not change or decrease (Fig. 2), 2) fluid trapping - increase and remain increased only for the portions submerged in nectar (Fig. 2), or 3) expansive filling - increase and remain increased for the whole tongue, even the portions outside of the nectar (Fig. 2). This can be examined by measuring changes in dorsoventral tongue thickness during a lick the using high-speed videos. Here a "lick" is defined as the act of protruding and retracting the tongue from the mouth. For a given lick, the dorsoventral thickness of the tongue was measured using

StereoMorph v1.6.4 (Olsen and Westneat 2015) to analyze still images captured from videos. We captured still images of each lick starting with the first frame where the tongue tip was visible protruding from the mouth and ending with the last frame where the tongue tip was visible before being fully retracted into the mouth; we captured images every 2 frames. Analysis is restricted to licks where some of the tongue remained outside of the nectar, the bill tip to nectar distance remained relatively constant, and occurred during mid feeding, as the first and last licks of a feeding bout can have reduced protrusion distance (sample size in Table S1). StereoMorph allows for opening successive images while maintaining landmarks between images; it also gives the x-y pixel location of each landmark on the image. These features allowed for retention landmarks, and their absolute distance, from one image to the next. At maximum tongue extension, ten pairs of landmarks were placed equidistantly along the tongue length, from the bill tip to the tongue tip using the digitizeImage() function (Fig. 2C). For each landmark location, a point was placed on the dorsal and ventral sides of the tongue and the absolute distance between landmark pairs was maintained during the entire cycle (method from Rico-Guevara et al. 2015). As the tongue was retracted back into the mouth in the successive frames after maximal tongue protrusion, the proximal-most pairs of landmarks were removed as they passed the bill tip and entered the mouth such that they were no longer visible. We repeated this process for images from tongue protrusion, starting at maximal protrusion and working backwards until the tongue was fully inside the mouth. The scale was set using the length of the side of the nectar chamber (Fig. 2C), and that scale was carried over to all subsequent images that were digitized for that lick.

The distance between dorsal and ventral points in each landmark pair, or dorsoventral tongue thickness, was calculated in R v4.0.3 (R Core Team 2022). The tongue thickness values were converted from pixels to millimeters using the scaling factor calculated in StereoMorph in the digitizing step. The x-axis values were converted from frame number to percent of the lick to facilitate visual comparison across lick sequences and combining data across licks within a species. For each species, we plotted the percent change in dorsoventral tongue thickness between the beginning and end of the lick for each landmark. This plot was qualitatively examined to determine whether the data fit expectations for tongue shape changes under capillary filling, fluid trapping, or expansive filling (Fig. 2).

Results

a. Kinematics

i. Tongue movements

The average lick frequency across species ranged from 7.8 Hz to 14.9 Hz, and all species had licking rates slower than those of hummingbirds, which lick at 14-17Hz (Ewald and Williams 1982). The highest lick frequency and lowest lick duration was measured in *P. novaehollandiae*, followed by *A. rufogularis*, *P. penicillata*, *M. flavigula*, and *C. variegatus* (Table 1). Within each species, licks tended to be very similar (Fig. S1-S5), but across species there were clear differences. For example, the fastest licking species *P. novaehollandiae* showed consistent tongue reciprocation with little to no pausing (Fig. 3, S1) while the slowest species *C. variegatus* showed clear pauses at maximum tongue protrusion (Fig. 3, S2). The remaining species had kinematics more similar to *P. novaehollandiae* (Fig. 3, S3-S5), but with slower licking rates. Differences in licking rate were not explained by differences in the amplitude of tongue protrusion. In terms of absolute protrusion distance, *P. novaehollandiae* had an elevated licking rate but protruded the tongue as far as *A. rufogularis* and *P. penicillata*, and almost twice

the distance of *C. variegatus* and *M. flavigula* (Fig. S1-S5). In terms of tongue protrusion distance relative to bill length, *P. novaehollandiae, A. rufogularis,* and *P. penicillata* protruded the tongue roughly a half to two-thirds of the length of the bill, while *C. variegatus* and *M. flavigula* protruded the tongue roughly a third of the length of the bill. When looking at the velocity of the tongue during feeding, *P. novaehollandiae* had a greater protrusion and retraction velocity than the other species (Fig. 3).

The duration of protrusion and retraction were roughly identical in *P. novaehollandiae, A. rufogularis* and *P. penicillata* (Table S2), indicating a symmetrical path of tongue movement during reciprocation (Fig. 3); the absolute values of those durations were shortest in *P. novaehollandiae* followed by *A. rufogularis* and *P. penicillata* (Table S2). In *M. flavigula* and *C. variegatus* the durations of protrusion and retraction were not identical (Table S2). In *M. flavigula* protrusion was 0.058s $\pm$ 0.0029s while retraction was 0.041s $\pm$ 0.0027s, such that protrusion was roughly 50% longer than retraction (Table S2). In *C. variegatus* protrusion was 0.043s $\pm$ 0.0030s while retraction was 0.086s $\pm$ 0.0057s, such that retraction is twice as long as protrusion (Table S2). Although the videos of the juvenile *C. variegatus* did not satisfy the criteria for landmark analysis, it displayed the same asymmetry in protrusion and retraction as the adult (Fig. S7).

ii. Bill and tongue coordination

In all species the bill tips remained close together as the tongue is initially being protruded, slowly separated as the tongue is protruded further, and were at maximum separation when the tongue was at maximum protrusion, coming together again as the tongue is retracted (Fig. 3). In *P. penicillata* and *M. flavigula*, the maximal and minimal points of tongue protrusion and bill tip separation were in phase (Table S2). In *P. novaehollandiae* and *A. rufogularis* there was evidence for a slight lag in the bill movements compared to the tongue tip, but the nature of the lag is different between the two species. In *P. novaehollandiae* the tongue reached maximal protrusion distance 0.014s $\pm$ 0.0021s before the bill tips, while in *A. rufogularis* the tongue reached minimal protrusion distance 0.016s $\pm$ 0.0033s before the bill tips. In *C. variegatus* there was a larger lag in the bill movements compared to the tongue tip, as the tongue reached maximal protrusion distance 0.033s $\pm$ 0.0065s before the bill tips.

b. Filling mechanism: Fluid movement through tongue

Across all species, a clear meniscus rising up the tongue was only seen in *C. variegatus* and only when the distal-most tip of the tongue was immersed (Video S1). When more than the distal-most tongue tip is submerged, there was never a clear meniscus observed rising up the tongue body in any species (Fig. S6).

c. Filling mechanism: Dorsoventral tongue thickness

When looking at the percent change in dorsoventral thickness along the tongue from the beginning to end of a feeding bout, the patterns were not the same across all species (Fig. 4). *P. novaehollandiae*, *A. rufogularis, P. penicillata* and *M. flavigula* all showed some increase in thickness for all landmarks (Fig. 4), even those that were on portions of the tongue that stayed outside of the nectar at all times; the change increased distally along the length of the tongue, with the most change at the tongue bristles. The adult *C. variegatus* displayed either no change in tongue thickness or a slightly negative change across the majority of the tongue, both for landmarks in and out of nectar (Fig. 4). Additionally, there was only an increase in tongue thickness in the distal fimbriated portions of the tongue (Fig. 4).

Discussion

a. General feeding kinematics

Each individual's bill and tongue movements were very similar across their feeding bouts (Fig. S1-S5). Across species, there are clear differences in these movements, as well as the coordination of the bill and tongue during feeding. *P. novaehollandiae* was found to have the fastest licking rate, while protruding the tongue as far or farther than other species (Fig. 3). In *P. novaehollandiae, A. rufogularis* and *P. penicillata* the duration of tongue protrusion and retraction are roughly identical (Table S2), as are the velocity of the tongue during protrusion and retraction (Fig. 3), indicating a smooth, symmetrical pattern of tongue movement during feeding (Fig. 3). In *M. flavigula* and *C. variegatus* the durations of protrusion and retraction are not identical (Table S2), indicating either an asymmetrical path of tongue movement during reciprocation or a change in tongue velocity between protrusion in retraction. In *M. flavigula* the basis of this asymmetry can be seen in the tongue velocity plot (Fig. 3), where the slope of the line is clearly more gradual during protrusion than during retraction, illustrating the fact that the tongue moves slower during protrusion than retraction even though the movement of the tongue is symmetrical. In *C. variegatus* the explanation for protrusion and retraction duration asymmetry is that the tongue is protruded to its maximum distance and then is left submersed in the nectar and gradually retracted (Fig. 3), a behavior seen in both *C. variegatus* individuals that were digitized (compare Fig. 3 with Fig. S7), resulting in a retraction phase that is roughly twice as long as the protrusion phase (Table S2). Interestingly, *P. novaehollandiae, A. rufogularis,* and *P. penicillata* protrude the tongue further, relative to bill length, than *C. variegatus* and *M. flavigula*; this could be indicative of a functional difference in the length of extensibility of the hyobranchial apparatus across species, but we currently lack any comparative information on the hyobranchial apparatus across honeyeaters. None of the species protrude the tongue as far as hummingbirds, which can extend the tongue up to twice the culmen length (Rico-Guevara 2017).

In all species the bill tips are held very close together while the tongue is being protruded from the mouth. We interpret the fact that the bill tips remain close together upon tongue protrusion (Fig. 3, S1-S5) as evidence for tongue wringing (Ewald and Williams 1982; Rico-Guevara and Rubega 2017; Cuban et al. 2022), a mechanism of emptying the tongue in which the tongue is wrung through the bill tips to remove nectar from the previous lick. If tongue movements and bill tip separation were perfectly synchronized, we would expect that the maximal tongue tip protrusion would align temporally with maximal bill tip separation, and vice versa with minimal tongue tip protrusion and minimal bill tip separation. In *P. penicillata* and *M. flavigula*, the maximal and minimal points of tongue protrusion and bill tip separation are in phase (Table S2), but the bill and tongue maxima and minima are out of phase in *P. novaehollandiae, A. rufogularis,* and *C. variegatus*, which is indicative of interesting kinematic differences.

In *P. novaehollandiae* the tongue reaches maximal protrusion distance 0.014s $\pm$ 0.0021s before the bill tips; the reason for this lag is a sharp increase in bill tip separation as the tongue is being retracted back into the mouth (Fig. S1). In *A. rufogularis* the tongue reaches minimal protrusion distance 0.016s $\pm$ 0.0033s before the bill tips, which is indicative of the fact that the bill takes roughly 0.02s to close once the tongue has been fully retracted into the mouth. In *C. variegatus* the tongue reaches maximal protrusion distance 0.033s $\pm$ 0.0065s before the bill tips because the tongue reaches maximal protrusion while the bill tips are still opening, and the tongue remains extended, slowly being retracted, as the bill tips reach maximum separation, and then the tongue and bill tips quickly close together (Fig. S2).

b. Mechanisms of nectar uptake in the honeyeater tongue

To best determine the mechanisms of nectar uptake in the honeyeater tongue, we must combine our information on general kinematics, changes in dorsoventral tongue thickness, and qualitative information on fluid movement through the tongue. Lick frequency was an uninformative line of evidence, as the lick frequency for all honeyeaters is lower than that for hummingbirds (although *P. novaehollandiae* comes close). It is possible that the slower movement of the honeyeater tongue during feeding, as compared to hummingbirds, is due to functional differences in the feeding apparatus between the two groups but further comparative morphofunctional studies are needed to determine if that is the case.

The tongue thickness data for *P. novaehollandiae*, *A. rufogularis*, *P. penicillata*, and *M. flavigula* (Fig. 4) suggest that there is likely expansive filling occurring, along with fluid trapping at the fimbriated tongue tip. In *C. variegatus* the fact that tongue thickness either does not change or decreases slightly along the tongue body (Fig. 4) suggests that that the most likely mechanism under these circumstances would be capillary filling, but the increase in thickness in the fimbriated region of the tongue (Fig. 4) suggests that there is also fluid trapping.

Our tongue kinematic data and the qualitative assessment of fluid movement described below also support the hypothesis that *C. variegatus* is relying on capillarity for filling the tongue grooves. The kinematic data for all species except *C. variegatus* shows continuous tongue reciprocation with either no or very minimal velocity plateau at maximum protrusion (Fig. 3). In *C. variegatus* the tongue is protruded into the nectar and held immersed while very slowly being retracted (Fig. 3), giving the plateau in the middle of the tongue velocity plot. We interpret this prolonged submersion of the tongue as a way to allow the tongue to fill via capillary rise, because under fluid trapping and expansive filling the tongue would fill with fluid rapidly (Rico-Guevara et al. 2015), likely precluding the need for prolonged submersion of the tongue in nectar. Finally, the only species which displayed a meniscus traveling proximally through the tongue was in *C. variegatus* when the very tip of the tongue was submerged (Video S1). We did not observe a meniscus moving through the tongue in any videos of other species, meaning that there is no visual evidence that nectar actually flows through the tongue body akin to water flowing through a capillary tube. The caveat is that, due to the thickness of the walls of the honeyeater tongue and the lighting restrictions necessary for high-speed videography, the tongue could have been too opaque to allow visualization of a meniscus. Further studies should use methods to improve visualization to confirm the lack of a meniscus, such as dying the nectar or using backlighting. Additionally, to confirm whether the honeyeater tongue is physically capable of functioning like a capillary tube one could examine whether a meniscus forms when dipping museum tongue specimens into sucrose solutions.

Unsurprisingly, all species showed evidence of fluid trapping. The honeyeater tongue has been hypothesized to function like a paintbrush, on account of its bristled distal tip (Paton and Collins 1989). We have demonstrated that from the beginning of a lick to the end, the bristled portion of the tongue increases in dorsoventral thickness (Fig. 4), and visual examination of videos show that it is clearly due to sucrose solution being caught between bristles. We believe that this process involves some of the same hydrodynamic forces as described for fluid trapping in hummingbirds (Rico-Guevara and Rubega 2011), namely surface tension and Laplace pressure. When the bristles are completely submerged in the fluid they relax and expand, and as they are withdrawn from the fluid and gradually pass the nectar-air interface, surface tension and Laplace pressure act to pull the bristles towards each other to form a paintbrush-like tip.

The primary limitation to this work is the number of individuals we were able to film within each species (Table S1). Future studies should conduct these same analyses with more individuals, particularly of *C. variegatus, P. penicillata, and A. rufogularis,* and a wider array of honeyeater species. There is variation in tongue size across honeyeaters (Paton and Collins 1989), and we have demonstrated here that honeyeaters also vary in licking rate. These factors will be relevant to consider when expanding studies of feeding mechanisms to other honeyeaters, as they are likely to affect the ratio of inertial-to-viscous forces at play during feeding and therefore could affect the viability of these filling mechanisms.

c. Relevance for other avian nectarivores

All evidence suggests that there is variability in the mechanism of nectar uptake across the honeyeater species examined. Future work should investigate these differences further, and it would be relevant to consider whether these functional differences result from differences in tongue morphology, as honeyeater tongues vary widely in form (Paton and Collins 1989). While we can infer expansive filling using the landmark technique, expansive filling in honeyeaters is not as pronounced as that in hummingbirds and is harder to discern without landmarking methods. The shape change seen in the honeyeater tongue does not match the expectation for expansive filling exactly (compare Fig. 2 and Fig. 4), and it is possible that this is because of morphological differences between the honeyeater and hummingbird tongue.

The hummingbird tongue is much thinner and more pliable than the honeyeater tongue, and the hummingbird tongue is shaped like two tubes while the honeyeater tongue exhibits a central groove, which is open along the length of the tongue (Hewes at al. 2022). These differences likely mean that the hummingbird tongue can be dorsoventrally compressed more homogeneously along its length, and therefore a larger portion of it can store elastic energy to aid in drawing up fluid. It is possible that some amount of expansive filling is typical for avian nectarivores. With the exception of parrots, avian nectarivores have keratinous tongues (reviewed in Hewes et al. 2022), which have the inherent ability to be compressed and rebound. Additionally, we found that honeyeaters likely employ tongue wringing, as a process to remove nectar from the tongue by squeezing it between the bill tips, as do hummingbirds (Ewald and Williams 1982). Expansive filling may be a common mechanism in avian nectarivores as a result of compressing a keratinous, elastic tongue between the bill tips to rid it of fluid.

d. Ecological implications for honeyeater-plant interactions

The shared use of fluid trapping in the honeyeater species we studied has ecological implications. With fluid trapping, the volume of nectar collected depends on nectar viscosity, retraction speed, and the number of hairs coating the tongue (Nasto et al. 2018), but also on depth of tongue submergence. Thus, differences in the relative contributions of capillary/expansive filling and fluid trapping among honeyeaters are likely to be associated with interspecific differences in favored floral morphology for gathering nectar. If relying largely on fluid trapping, it is important that flower morphology permits a given species to submerge their tongue to a sufficient depth to pick up nectar, and the five species examined here do tend to visit flowers of different sizes (Table 2). It would be interesting to investigate further whether the relative use of fluid trapping versus capillary/expansive filling affect floral preferences. Depending on which mechanism is responsible for the larger volume of fluid capture for a given honeyeater species, differences in nectar properties may also be important component of floral preference. When considering the role that feeding mechanics could play on influencing floral visitation and/or preference, it is key to note that honeyeaters, including the five species sampled here (Table 2), can vary dramatically in their reliance on nectar (Miller et al. 2017); this would

likely mean that any effects of feeding mechanisms on feeding efficiency would likely vary in their ultimate impact on fitness depending on the extent to which nectar is a central component in the diet.

Honeyeater-plant interactions and eco-evolutionary relationships are notably more generalized than those of hummingbirds and their associated plants (Zanata et al. 2017). Australasian flora that are visited and pollinated by honeyeaters tend to have less restrictive morphologies and more easily accessible nectar than hummingbird visited plants (e.g., Ford and Paton 1985; Brown and Hopkins 1995), and they range dramatically in nectar volume and concentration (Paton and Ford 1977; Pyke and Waser 1981). For example, flowers of the genus *Banksia* deposit nectar in thin films along the rachis of their large inflorescence, and previous authors have suggested that a bristled tongue could effectively capture nectar when it's present in such small volumes or thin films (e.g., Paton and Collins 1989).

One limitation to this study is the fact that birds were given access to an essentially unlimited pool of nectar, as is standard in almost all studies of nectar-feeding mechanics. In our laboratory experiments, we observed what we have interpreted as expansive filling plus fluid trapping and capillary filling plus fluid trapping, but it is possible that certain mechanisms are used disproportionally more in nature, rendering the others physically possible but not biologically relevant. For example, if fluid trapping is the only mechanism employed at small nectar volumes or thin films because the only portion of the tongue in contact with nectar is the bristled portion, then the differences in efficiency between capillary and expansive filling may not be relevant when examining questions such as honeyeater energy budgets if flowers like *Banksia* are a large portion of a species' diet. As nectar volume and concentration both play a role in feeding efficiency (Harder 1983, 1986; Herrera 1989; Wei et al. 2020; Rico-Guevara et al. 2021), it would be worthwhile for future studies on honeyeater feeding to incorporate more realistic nectar conditions based on the local flora with which the birds are interacting.

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Figures & Tables

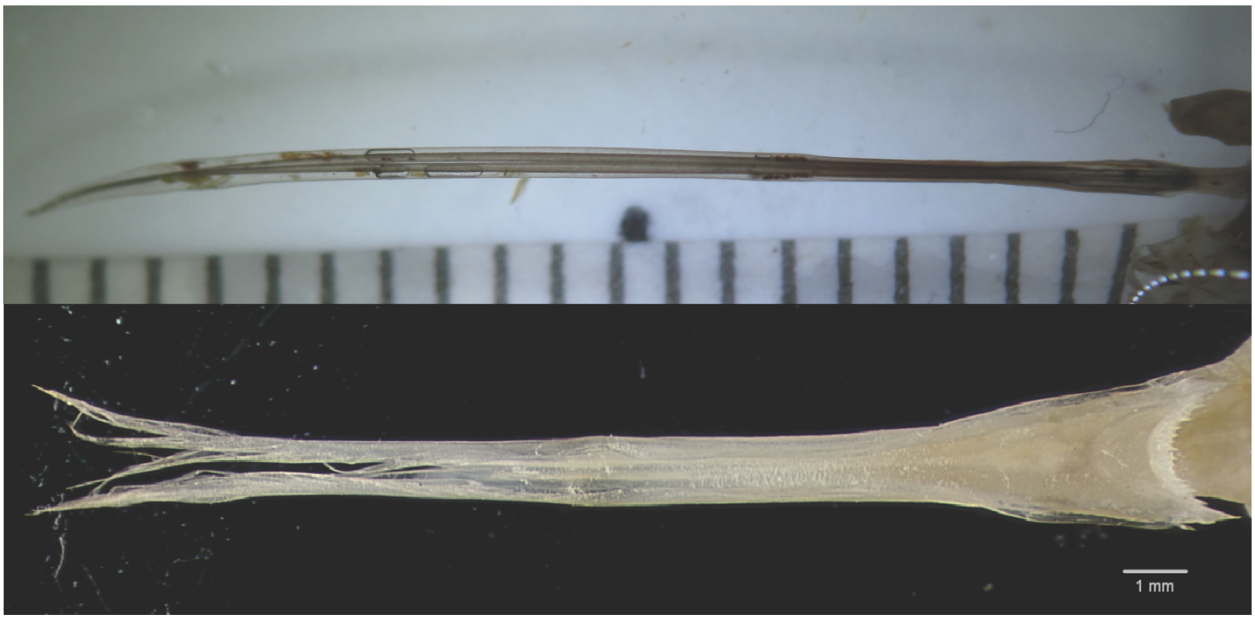

Figure 1. Honeyeater tongues (bottom panel), unlike hummingbird tongues (top panel), have bristled, paintbrush-like tips. Top panel shows an Amethyst-throated sunangel (*Heliangelus amethysticollis*) tongue in dorsal view. Scale is in millimeters. Bottom panel shows the tongue of the white plumed honeyeater (*Ptilotula penicillata*) filmed for this study, also in dorsal view. Both tongues are oriented such that the tongue tip is on the left and the tongue base is on the right.

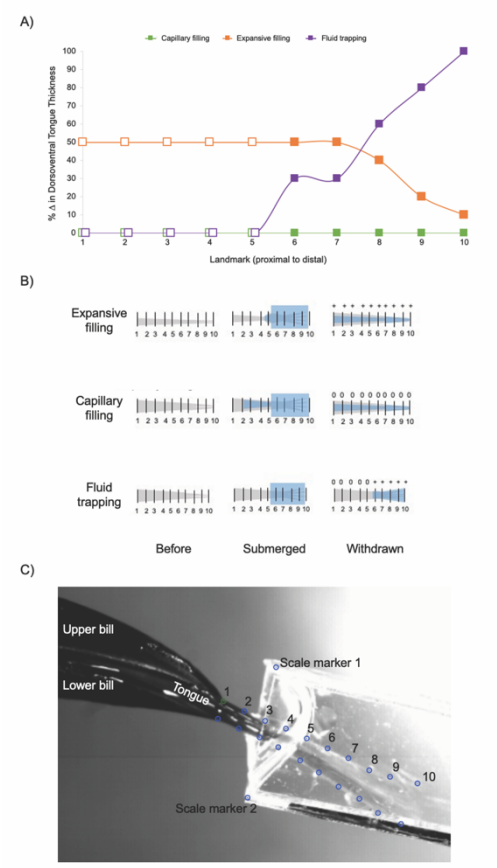

Figure 2. Schematic for expectations of percent change in dorsoventral tongue thickness from beginning to end of the nectar collection portion of a lick for each fluid uptake mechanism. A) Filled squares indicate portions of the tongue that are submerged in nectar, open squares indicate portions of the tongue that are kept outside of the nectar. In capillary filling, no change in thickness is expected for any parts of the tongue (slight negative change is also possible). In fluid trapping, all portions of the tongue that are submerged in nectar increase in thickness and the change is greatest at the tongue tip because of the fluid trapped between the tongue bristles; no change in thickness is expected in the body of the tongue or the portions of the tongue that are kept outside the nectar. In expansive filling the whole tongue expands, even portions of the tongue outside of the nectar, and the change is greater in the tongue body than in the bristles because the elastic recoil acts primarily in the keratinous tongue body. B) Schematic of nectar capture in the tongue under each filling mechanism. For each mechanism, the right panel shows the tongue (grey) before submersion in nectar, the middle panel shows the tongue submerged in the nectar (blue), and the right panel shows the tongue after being removed from the nectar with the nectar imbibed into the tongue shown as blue overlayed on the grey. The numbered lines overlayed on the tongue correspond to the landmarks for measuring dorsoventral tongue thickness, as in the x-axis of panel A. The symbols above each line corresponds to the change in dorsoventral tongue thickness expected at that landmark where 0 = no change and + = increase in thickness. C) Still image at maximal tongue protrusion for a lick from *P. novaehollandiae* in lateral view. The numbers along the tongue indicate the landmark pairs, and correspond to the landmarks on the x-axis of panel A and illustrated in panel B. The scale for each video was set as the distance between the edges of the nectar chamber, labeled here as scale marker 1 and 2.

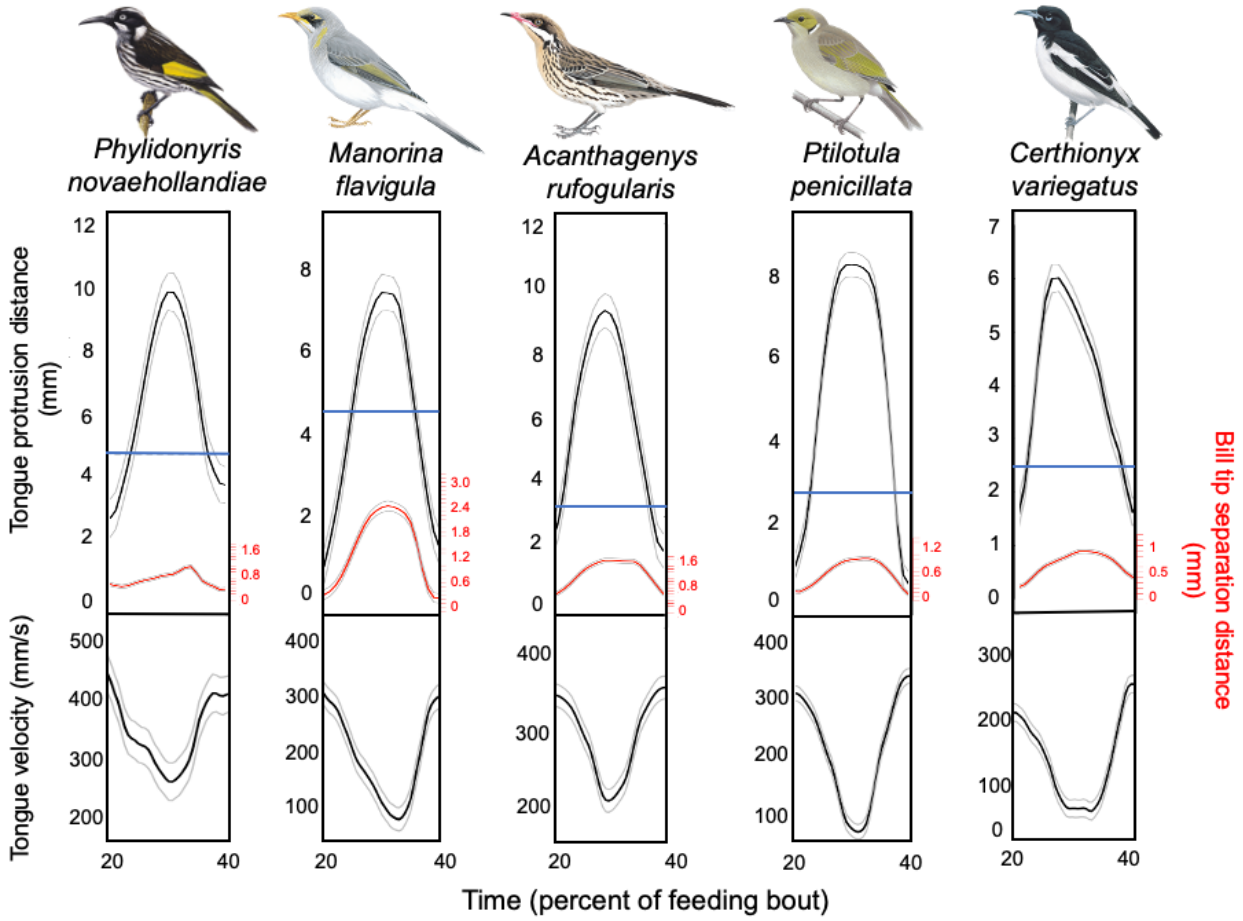

Figure 3. Top panels show average tongue tip (black line) displacement and bill tip separation (red line) against time (shown as percent of the feeding bout) for each species (grey outline is SE). The blue line in each panel indicates the average location of the nectar surface relative to the tongue tip during feeding. Bottom panels show average tongue tip velocity (black line, grey outline SE) against time for each species. Bird images are not to scale. Artist credit: Ren Hathway (*P. novaehollandiae*), Tim Worfolk (*M. flavigula*, *A. rufogularis*), and Jan Wilczur (*P. penicillata*, *C. variegatus*). Illustrations reproduced with permission of Lynx Edicions.

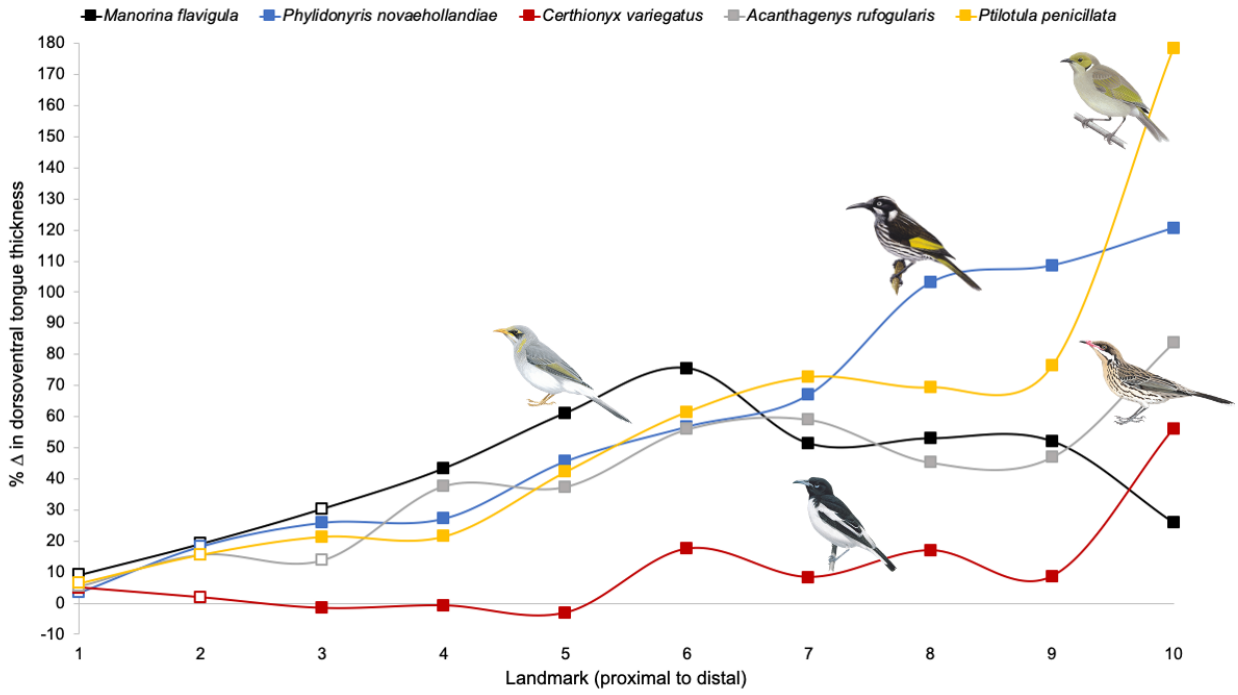

Figure 4. Average percent change in dorsoventral tongue thickness from beginning to end of the nectar collection portion of a lick. Open squares indicate landmarks that are not submerged in nectar, filled squares indicate landmarks that are inside the nectar. Bird images are not to scale. Artist credit: Ren Hathway (*P. novaehollandiae*), Tim Worfolk (*M. flavigula*, *A. rufogularis*), and Jan Wilczur (*P. penicillata*, *C. variegatus*). Illustrations reproduced with permission of Lynx Edicions.

Table 1. Kinematic data for each species. All values are shown as mean ± SE. The number of videos digitized to generate these data are given in Table S1.

Species (number of individuals)	**Lick number**	**Lick duration (s)**	**Lick frequency (Hz)**
Phylidonyris novaehollandiae (2)	11.2 ± 2.1	0.068 ± 0.13	14.9 ± 2.8
Acanthagenys rufogularis (1)	9.6 ± 2.6	0.081 ± 0.021	12.4 ± 3.3
Ptilotula penicillata (1)	11.3 ± 4.0	0.088 ± 0.031	11.4 ± 4.0
Manorina flavigula (3)	7.6 ± 1.7	0.099 ± 0.021	10.0 ± 2.2
Certhionyx variegatus (1)	6.2 ± 1.9	0.13 ± 0.039	7.8 ± 2.3

Table 2. Morphological and diet data for each species. Weight ranges are species averages from Menkhorst et al. (2017). Bill lengths are from videos collected for this study and mean ± SE is shown when multiple individuals were measured per species. Tongue morphometrics from Paton and Collins 1989 (no published data is available for *C. variegatus*). Dietary data is given as percent of the diet and are from Miller et al. (2017); mean length of flower visited is a centered and scaled value, also from Miller et al. (2017).

Species	**Species mass range (g)**	**Bill length (mm)**	**No. of tongue bristles**	**Tongue width (mm)**	**Length of bristled portion of tongue (mm)**	**Nectar**	**Gleaning & sallying for insects**	**Fruit**	**Mean length of flowers visited**
Phylidonyris novaehollandiae	18-28	18.5 ± 0.84	70	1.1	12.9	0.6	0.37	0	1.85
Acanthagenys rufogularis	37-57	18.3	50	1.4	10.3	0.66	0.33	0.11	0.93
Ptilotula penicillata	15-24	12.4	60	0.9	7.6	0.25	0.72	0	0.81
Manorina flavigula	55-64	18.5 ± 0.28	60	1.5	10.2	0.35	0.52	0.01	0.87
Certhionyx variegatus	23-34	17.2	--	--	--	0.39	0.62	0.12	0.99

Supplemental Material

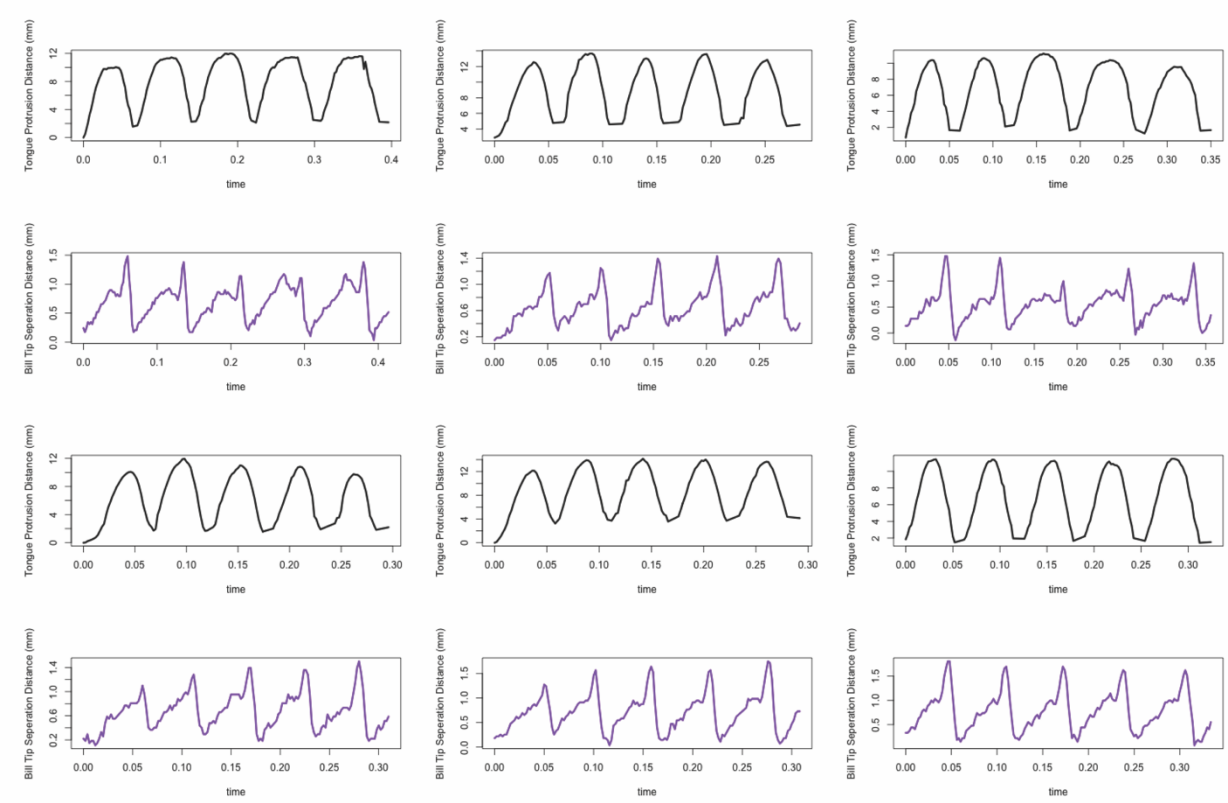

Figure S1. Tongue protrusion distance through time (black lines) and bill tip separation distance by time (purple lines) in six videos analyzed for *Phylidonyris novaehollandiae*.

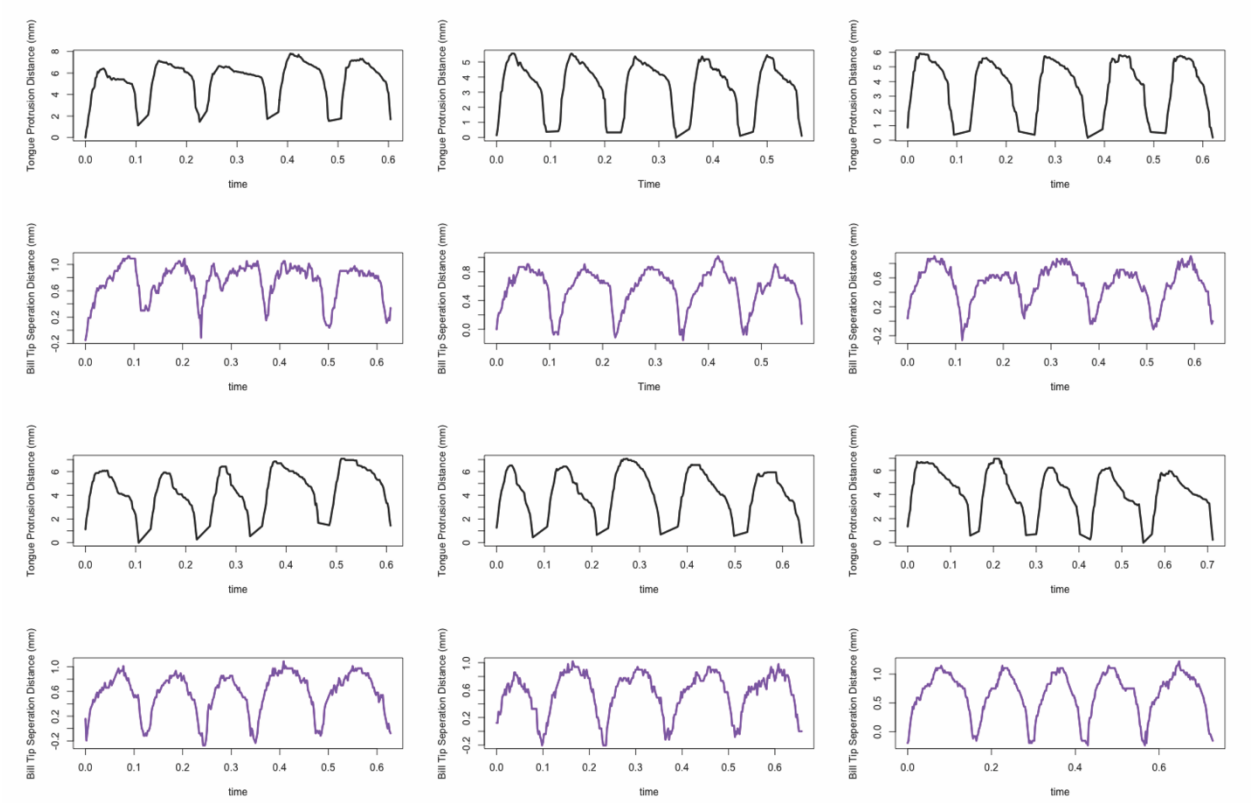

Figure S2. Tongue protrusion distance through time (black lines) and bill tip separation distance by time (purple lines) in six videos analyzed for *Certhionyx variegatus*.

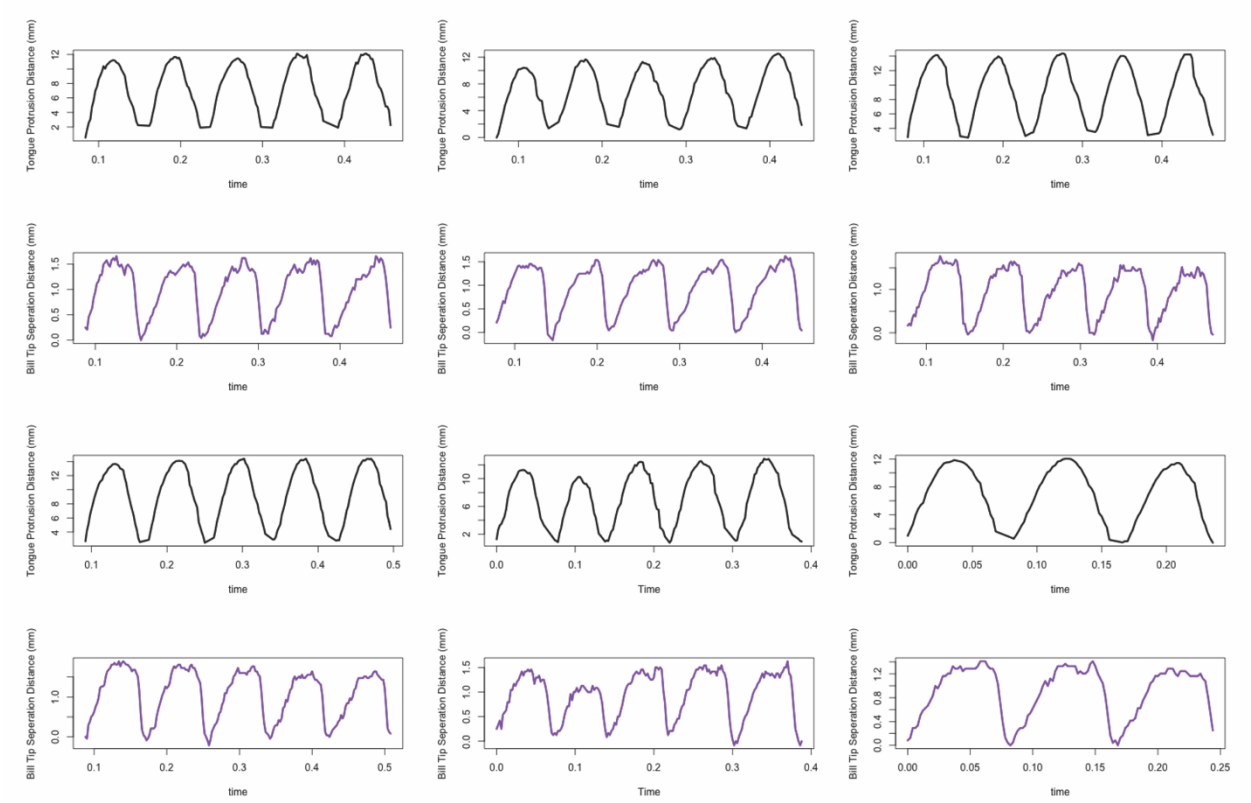

Figure S3. Tongue protrusion distance through time (black lines) and bill tip separation distance by time (purple lines) in six videos analyzed for *Acanthagenys rufogularis.*

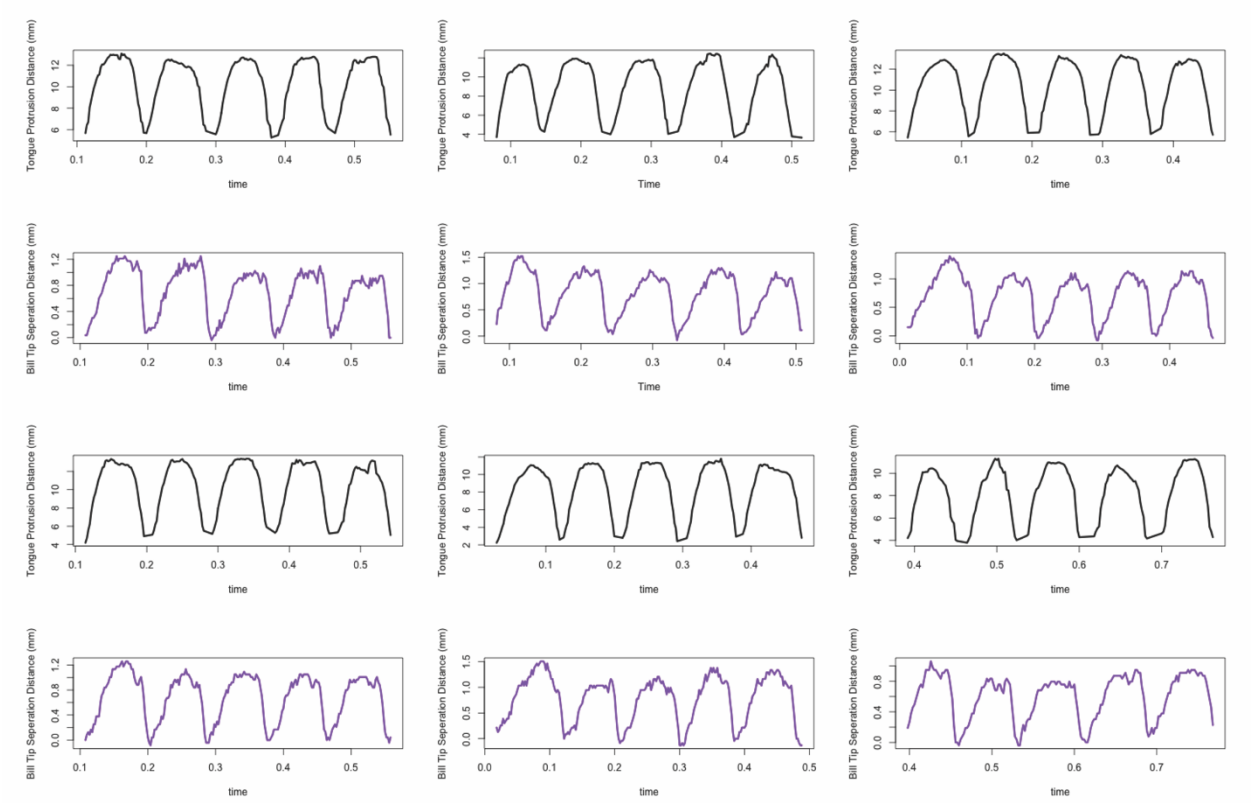

Figure S4. Tongue protrusion distance through time (black lines) and bill tip separation distance by time (purple lines) in six videos analyzed for *Ptilotula penicillata.*

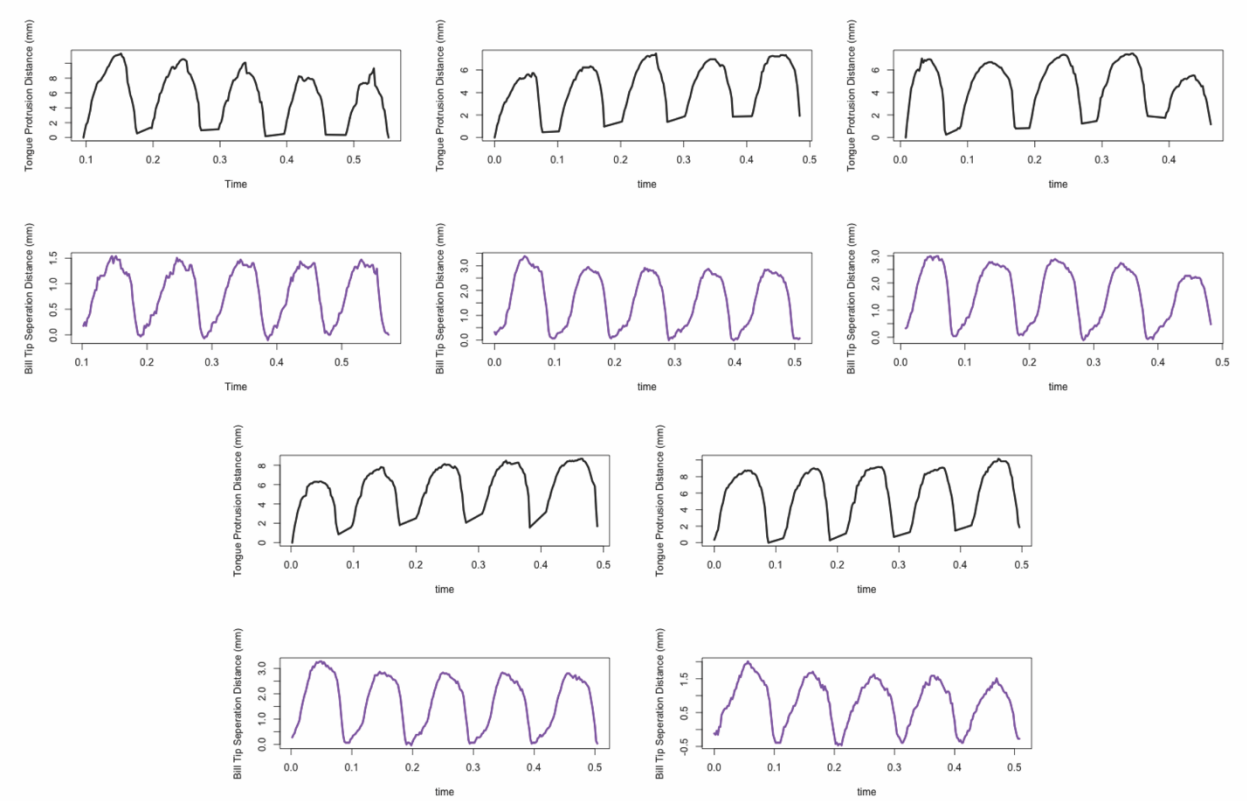

Figure S5. Tongue protrusion distance through time (black lines) and bill tip separation distance by time (purple lines) in five videos analyzed for *Manorina flavigula.*

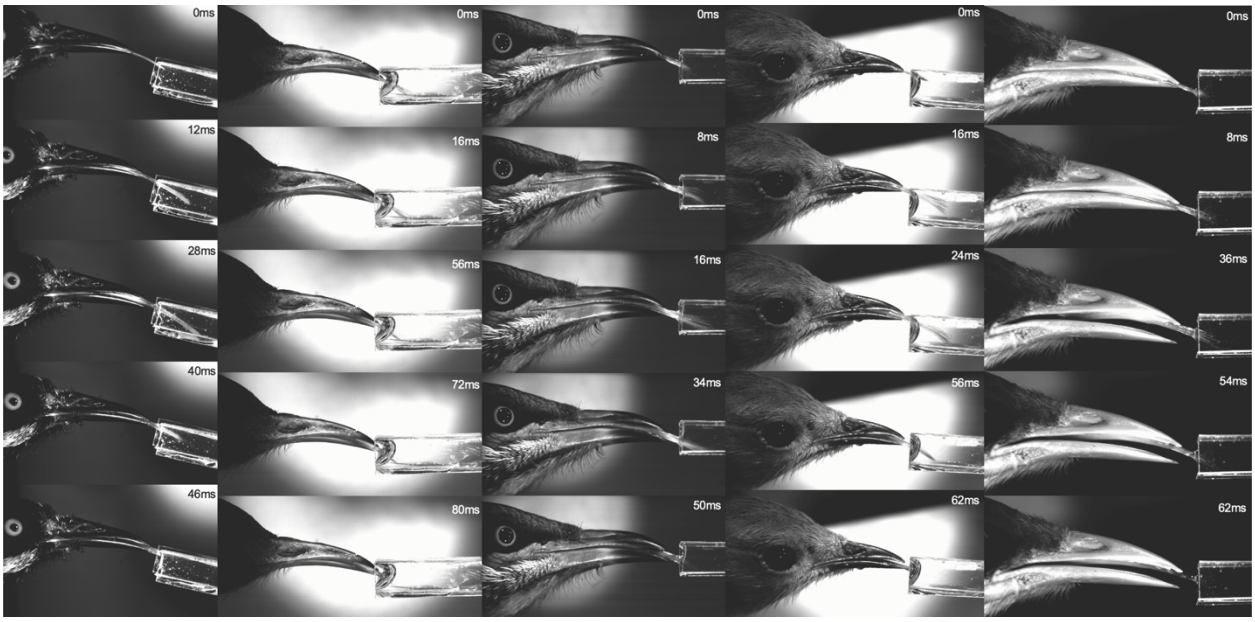

Figure S6. Typical lick for each species, left to right: *Phylidonyris novaehollandiae, Certhionyx variegatus, Acanthagenys rufogularis, Ptilotula penicillata,* and *Manorina flavigula.*

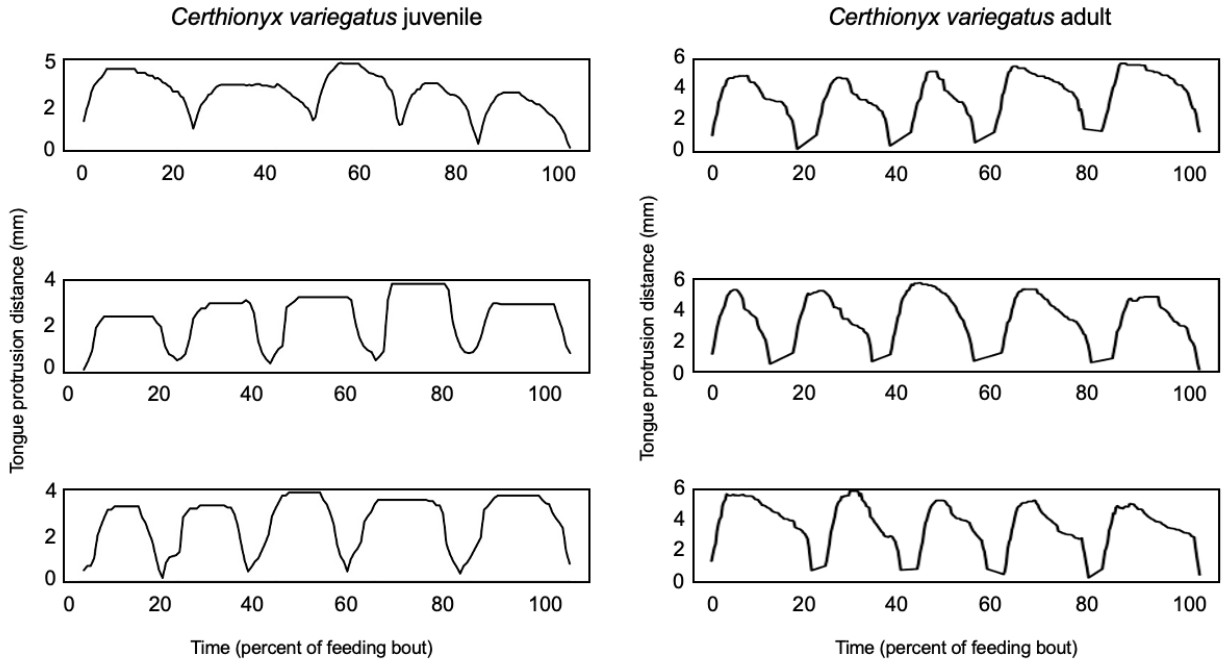

Figure S7. Tongue protrusion distance through time for both *Certhionyx variegatus* individuals. Both individuals display pauses at maximum tongue protrusion that were not detected in any other species.

Table S1. Breakdown, per individual and per species, of data used in kinematic and dorsoventral tongue thickness analyses.

Species	Individual	Number of videos for general kinematic statistics in Table 1 & Table S2	Number of videos for kinematic plots	Number of licks for dorsoventral tongue thickness analysis
Phylidonyris novaehollandiae	1	4	4	12
	2	3	3	7
Acanthagenys rufogularis	1	6	6	16
Ptilotula penicillata	1	6	6	18
Manorina flavigula	1	2	2	6
	2	3	3	9
	3	1	1	3
Certhionyx variegatus	1	6	6	17
	2	0	3	0

Table S2. Values for tongue and bill offset for maxima and minima, as well as difference in duration of protrusion and retraction of the tongue, for each species. *P. novaehollandiae* and *C. variegatus* exhibit phase offset between tongue and bill maxima, while *A. rufogularis* exhibits phase offset between tongue and bill minima (see text). *M. flavigula* and *C. variegatus* exhibit asymmetrical lengths of protrusion and retraction, which is indicative of interesting kinematics (see text). All values are mean ± SE.

Species	**Tongue maxima – bill maxima (s)**	**Tongue minima – bill minima (s)**	**Protrusion duration (s)**	**Retraction duration (s)**	**Protrusion /retraction**
Phylidonyris novaehollandiae	-0.014 ± 0.0021	-0.0050 ± 0.00076	0.034 ± 0.0028	0.029 ± 0.0015	1.2 ± 0.072
Acanthagenys rufogularis	-0.0055 ± 0.00069	-0.016 ± 0.0033	0.042 ± 0.0015	0.039 ± 0.0014	1.1 ± 0.049
Ptilotula penicillata	-0.0051 ± 0.00035	-0.0085 ± 0.0038	0.044 ± 0.0031	0.041 ± 0.0032	1.1 ± 0.12
Manorina flavigula	-0.0071 ± 0.0012	-0.0042 ± 0.0026	0.058 ± 0.0029	0.041 ± 0.0027	1.5 ± 0.13
Certhionyx variegatus	-0.033 ± 0.0065	-0.0064 ± 0.0011	0.043 ± 0.0030	0.086 ± 0.0057	0.51 ± 0.041

Chapter 3: Morphological variability in the honeyeater hyolingual apparatus and its relationship with nectarivory

Amanda E. Hewes, Cassandra Fieldson, Maude W. Baldwin, William Buttemer, and Alejandro Rico-Guevara

Abstract

Honeyeaters (Aves, Meliphagidae) are a speciose clade of nectarivorous birds, and there is immense variation in the degree to which different species within the family rely on nectar. Honeyeater tongues are commonly described as similar to a paintbrush, with this morphology being interpreted as an adaptation for increase nectar extraction efficiency. However, there has been limited work documenting the degree of interspecific variation in tongue morphology across the family or the extent to which that variation correlates with dependance on nectar. This information is also lacking for the hyoid, the structure responsible for moving the tongue in and out of the mouth. We aimed to fill this knowledge gap by examining honeyeater tongues and hyoids from 58 and 38 species, respectively, across the family. We found that there are six distinct tongue types across the Meliphagidae, and that certain genera such as *Acanthorhynchus* sp. and *Phylidonyris* sp. have a unique tongue morphology. Using phylogenetic generalized least square regression, we found that tongue length (relative to bill length) and the proportion of tongue that is bristled positively correlated to degree of nectarivory, while tongue depth (relative to bill depth) and tongue width (relative to bill width) did not. Finally, we found no correlation between relative hyoid length and nectarivory, suggesting that the capacity for tongue protrusion is unrelated to nectar dependence in honeyeaters. Similar studies should be conducted across other groups of avian nectarivores to expand our understanding of dietary ecomorphology beyond the bill, which has been the focus of the majority of research on food handling adaptations thus far.

Introduction

Birds use their cranial apparatus as tools to accomplish many tasks necessary for survival, and efficiently capturing and consuming food is one those critical tasks. The avian bill is often heralded as a classic example of morphological adaptation [e.g., 1,2], because of the intimate links between bill shape, food items consumed, and functional performance in feeding [3–7]. While diet is one factor that shapes bill evolution, there are many other factors that can also be important, such as phylogeny and developmental patterns [8–12], thermoregulation [13–16], and intrasexual combat [17,18]. Therefore, the bill morphology exhibited by a given species or lineage is often best described to be a result of functional tradeoffs between many activities, as well as factors intrinsic to lineages due to development or evolutionary histories [3,13,19]. There is a substantial body of literature that both qualitatively describes and quantitatively tests the evolutionary relationship between bill shape and ecological factors such as diet, but additional components of the avian feeding apparatus have received far less attention in this regard. The hyolingual apparatus (tongue and hyoid) is a vital feeding tools for many birds, as it is crucial for manipulating food in the mouth and moving it backwards towards the esophagus [20–22]. Moreover, in some lineages, the tongue and hyoid apparatus work with the bill to capture food (e.g., nectar feeders, filter feeders) [20–22]. Despite the importance of these structures during feeding, we know relatively little about how diet shapes the morphological evolution of the avian

tongue and hyoid, as indicated by a relative dearth of quantitative macroevolutionary studies of hyolingual apparatus morphology.

There are a number of logistical reasons why there are far fewer studies on tongue and hyoid morphology than bill morphology. The biggest barrier is the limited availability of tongue and hyoid specimens in museum collections. Many studies of bill morphology make extensive use of birds prepared for museum collections. During the preparation of round skins, the most common specimen type in ornithological collections, the tongues and hyoids are typically discarded while the bill remains attached to the skin. In the case of intact bird specimens preserved in ethanol, the bill is often shut making the tongue inaccessible without using expensive and labor-intensive processes like computer tomography (CT) scanning, or through destructive sampling methods like dissection. If a CT scan is logistically possible, one is then presented with the challenge of how to successfully scan structures with little mineralization and therefore low contrast, rendering the tongue tissues nearly impossible to visualize. Avian tongues are typically keratinous, with some lineages such as ducks, geese, penguins, and parrots having highly muscular tongues [20–22] – both of these tissue types often require electron-dense staining to be visible in CT scans [23–27]. If one has access to hyolingual apparatus specimens, the second major barrier is determining what measurements best quantify the shape of the structures. Three-dimensional geometric morphometrics (3D GMM) are a common, comprehensive technique for shape quantification [28,29], especially in macroevolutionary studies of morphology. However, using methods like 3D GMM is more difficult with soft tissue than bone because of the lack of obvious homologous landmarks and the fact that the structure's deformation due to movement and/or preservation artifacts introduces noise to the shape analyses [30,31, but see 32]. This is especially true for tongues and hyoids because they are often smooth and do not have external features that are obviously homologous between specimens.

In order to understand the ways in which the avian tongue and hyoid evolve as a result of dietary ecology, it would be ideal to study a lineage of birds that subsist on a diet which requires integral use of the tongue and hyoid while feeding, and in which that diet is not present in sister taxa. Avian nectarivores are therefore an ideal study system, as there are over 20 independent evolutions of specialized nectarivory in birds and nectar uptake involves extensive use of the tongue and hyoid [33]. Tongue and hyoid morphology influence drinking mechanics and nectar intake rates in avian nectarivores, which determines feeding efficiency and ultimately energy gained when foraging [34,35]. Thus, there is likely to be strong selection on the morphology of these structures in these lineages. Ample descriptive work has shown that all lineages of avian nectarivores have not converged on the same tongue morphology [literature summarized in 33], but the lack of quantitative morphological information has limited our ability to determine whether morphological variation is associated with degree of nectarivory or a result of non-adaptive variation.

Honeyeaters (Aves, Meliphagidae) are an ideal group to address this question, as they are the second-most speciose group of avian nectarivores and exhibit high variability in their reliance on nectar [4,36]. The outgroups to honeyeaters (thornbills, fairy wrens, pardalotes) are largely insectivorous and a shift to nectarivory occurred at the base of the Meliphagidae [4]. Within this family, the range of reliance on nectar is so diverse that some honeyeater species have over 60% of their diet composed of nectar, whereas other species have reverted to full insectivory [4,36]. Previous descriptions of honeyeater tongue morphology have been made [37–39], but they have been limited to fewer than 20 species out of the ~180 species found in the

family [40] and largely present qualitative descriptions and drawings [but see 38]. To our knowledge, there have been no data published on honeyeater hyoid morphology.

We describe the morphology of the tongue across the Meliphagidae, with emphasis on gross morphology and some microscopic anatomy. We then investigate macroevolutionary patterns in hyolingual morphology, specifically testing whether tongue and hyoid morphological variation correlates with the degree of dietary nectar consumption. Because honeyeaters primarily use fluid trapping to collect nectar [41], a method in which surface tension causes an aliquot of nectar to be trapped between bristles or lamellae at the tongue tip, we predicted that the degree of nectarivory would be positively correlated with a longer tongue, a greater proportion of the tongue that is bristled, and a wider and deeper tongue. All of these features would increase the surface area in contact with nectar and should therefore theoretically increase nectar feeding efficiency. Also, as longer hyoid horns are associated with greater tongue protrusion which would be beneficial for nectar feeding [20,33], we predicted a positive correlation between the degree of nectarivory and hyoid horn length. With this study, we aim to broaden our understanding of how the avian feeding apparatus evolves with the transition to a nectar-based diet and tests the potential for the tongue and hyoid to evolve adaptively due to dietary differences.

Methods

Tongue and hyoid morphometrics

We examined preserved tongue specimens of 58 meliphagid species and 13 outgroup (non-nectarivore) species (Table S1) for gross anatomical description and a series of morphometric measurements specified below; the number of specimens per species ranged from 1-10 (Table S1). Only a subset of specimens had the hyoid intact, so 38 meliphagid species and 5 outgroup species were examined for hyoid morphology (Table S2); the number of specimens per species ranged from 1-4 (Table S2). Specimens were from the University of Washington Burke Museum, the Harvard University Museum of Comparative Zoology, the American Museum of Natural History, the Smithsonian, the Western Australian Museum, and the Queensland Museum (Tables S1 and S2). Specimens were kept in collections either in 70% ethanol or dried and tied to the leg of a study skin. Ethanol specimens consisted of the tongue, and sometimes the tongue and hyoid, dissected out during initial preparation of a study skin and kept in ethanol since the initial dissection. To make use of the dried specimens, we rehydrated them using a modified version of the procedure in [42] (see full protocol in File S1). After the rehydration procedure all specimens were kept in 70% ethanol for long term storage. All specimens were kept in a small dish of ethanol during measuring and photographing to prevent desiccation and warping.

We measured the specimens either under a dissecting microscope using a ruler or in ImageJ using photos taken under the microscope with a scale bar. All tongues were measured for total length (from tongue tip to center of the tongue base), bristle length (from tongue tip to the beginning of the bristles or fringes), width (distance between outer tongue walls in dorsal view, taken at the midpoint of the total tongue length), and depth (distance between outer tongue walls in lateral view, taken half way up the tongue) (Figure S1). The portion of the tongue that is bristled was calculated as bristle length/total length of the tongue (Figure S1). Most specimens with the hyoid intact had connective tissue covering the hyoid, making the junction between the ceratobranchial and epibranchial difficult to determine [Figure 17.4 in 20 for anatomical reference]. The length of each hyoid horn was measured as the combined ceratobranchial and epibranchial length (from junction of ceratobranchial with basihyal to the tip of the epibranchial).

This length was measured by holding one end of a thin piece of string to the basihyal-ceratobranchial junction and wrapping the string around the horn, keeping it flush with the ceratobranchial and epibranchial, until it reached the tip of the epibranchial; the string was then removed and laid flat against a ruler and measured in length. If both hyoid horns were intact, then both were measured and a mean value was taken. If only one horn was intact then that single measurement was used. We also took bill measurements from the study skins or whole-body alcohol specimens associated with each specimen, so we could calculate bill/tongue morphometric ratios to account for bill size. We measured exposed culmen length, and bill width and bill depth (including both the maxillary and mandibular rhamphothecae) just behind the nares. For specimens where the round skin could not be measured (12 specimens of 9 species from Smithsonian, see Table S1) we used species mean values from the AVONET database [43] for culmen length, bill width, and bill depth.

Dietary data

To examine the relationship between tongue morphology and diet, we used species-level diet data showing the proportion of feeding bouts that are nectar-feeding (from 0-1). For a species that takes no nectar (i.e, is fully insectivorous), this value would be zero, while for a species that subsists only on nectar this value would be 1. For the majority of species, we used diet data from [36]. For outgroups and species not found in [36], we used data from the EltonTraits database [44]. For the tongue dataset, 42 out of the 71 species (59%) had diet data in [36] (Table S1), and for the hyoid dataset 28 out of the 43 species (65%) had diet data in [36] (Table S2).

Microscopic tongue morphology

While collecting morphometric data we found that there were six distinct tongue types within honeyeaters (see descriptions in Results). Based on these observations, we aimed to study the microscopic tongue morphology from a variety of tongue types to determine if there were internal differences in anatomy. We examined the microscopic tongue morphology of *Ptilotula penicillata* (MCZ Ornithology 364080) which has a Type 1 tongue (see Results) and *Melithreptus lunatus* (UWBM 76699) which has a Type 2 tongue (see Results). We then compared these data with published information on the microscopic tongue morphology of *Acanthorhynchus tenuirostris* (Type 4, see Results) and *Phylidonyris novaehollandiae* (Type 5, see Results) from [39].

We examined the microscopic morphology of the tongue of *Melithreptus lunatus* (UWBM 76699) using histology following the technique used in [39]. We used paraffin histology and implemented the dehydration, embedding, and hematoxylin and eosin-y staining procedures from [45], with the timings modified for the size of our specimen. We then sectioned the tongue at 10-micrometers and viewed and photographed the sections under a Leica DM750 microscope with a Leica EC3 camera attachment at 4X, 10X, and 40X for anatomical description.

The internal structure of the *Ptilotula penicillata* tongue (MCZ Ornithology 364080) was examined using microCT scanning, as it was collected for a separate project examining shape quantification. We used a SkyScan 1173 CT scanner at Museum of Comparative Zoology Digital Imaging Facility at Harvard University, set to 62.5-micron resolution and 40kv, and the tongue was stained with phospho-tungstic acid (PTA) to improve visualization [26]. The 3D reconstruction was done in the software 3D Slicer [46] with the Slicermorph extension [47].

Statistics

Ancestral state reconstruction of tongue types

For all of our phylogenetic comparative analyses, we used the most recent honeyeater time-calibrated molecular phylogeny from [40] pruned to include species in our dataset. While collecting morphometric data we found that there were six distinct tongue types within honeyeaters (see descriptions in Results). Based on these observations, we ran an ancestral state reconstruction analysis using stochastic character mapping to determine what the most likely ancestral tongue shape was for the Meliphagidae, as well as to calculate the number of predicted state changes and time spent in each state across the phylogeny. We used fitMk() in the phytools package v. 1.5-1 [48] to determine whether an unordered equal rates (ER) or all rates different (ARD) model would be best for ancestral state reconstruction, as determined by AIC. Based on the difference in AIC between the ER and ARD models (see Results), we used an ER model for stochastic character mapping. We ran an ancestral state reconstruction using stochastic character mapping [49] in the make.simmap() function in the ape package [50] with an equal rates model and nsim = 1000.

Correlation between hyolingual morphology and diet

We investigated whether tongue types (see descriptions in Results) and linear morphometrics were correlated with the proportion of nectar in the diet. For analyses with linear morphometrics we corrected the linear tongue and hyoid values for bill size by using ratios of tongue and hyoid metrics to bill metrics. We use tongue length/bill length, tongue width/bill width, tongue depth/bill depth, and hyoid length/bill length. Bristle proportion is already a ratio, and so was not size corrected. We calculated species average values for each morphological variable to have a single value for each tip in the phylogenetic tree.

We began by quantifying phylogenetic signal in each variable using Blomberg's K [51]. To test for phylogenetic signal, we used the phylosig() function in the phytools package v. 1.5-1 [48] and set to nsim = 1000. Upon finding that all but one variable (tongue depth) had significant phylogenetic signal, we used phylogenetic ANOVA, phylogenetic PCA (pPCA) and phylogenetic generalized least squares regressions (PGLS) to test our predictions. We used phylogenetic ANOVA to determine whether tongue types vary according to a given species' dietary nectar consumption, as tongue types are discrete states taking one of seven possible values (0-7; see descriptions in Results). We ran the phylogenetic ANOVA using the aov.phylo() function from the geiger package [52] set to nsim = 1000.

To determine the major axes of morphological variation in our bill-size corrected linear morphometrics, we used the phyl.pca() function in the phytools package v. 1.5-1 [48]. Data were centered and scaled prior to the pPCA and two pPCAs were run, one for the tongue morphology only data set (58 meliphagid species and 13 outgroup species) and one for the smaller data set that also included hyoid measurements (38 meliphagid species and 5 outgroup species). We plotted the first three principal components because they explained roughly 80% of the variation (84.29% and 79.68% for tongue only and hyoid, respectively) and color coded the taxa by family to visualize whether there were clear distinctions between meliphagids and outgroup taxa.

After conducting the pPCA for visualization, we used PGLS regressions to examine whether there was a correlation between each bill-size corrected morphological variable and the proportion of nectar in the diet, using Pagel's λ to account for phylogenetic signal [53]. We predicted that the degree of nectarivory would be positively correlated with a longer tongue, a greater proportion of the tongue being bristled, a wider and deeper tongue, and longer hyoid

horns. We used the gls() function in the nlme v 3.1-162 package [54] to run the PGLS for each variable with the correlation structure set to corPagel() and estimating parameters using maximized log likelihood. A second set of PGLS analyses were also run with the raw morphological data (tongue length, tongue width, tongue depth, bristle length, hyoid length) to determine whether the trends hold when there is no correction for bill size.

Results

Gross tongue morphology

There is extensive variation in the shape and structure of the distal portion of the tongue across the Meliphagidae (Fig 1). We found six tongue morphologies via distinct, identifiable features in the distal half of the tongue (Fig 1). The first tongue type is the classic "paintbrush" tongue as described by many previous publications (summarized in [33]). In this tongue type, the central trough of the tongue bifurcates at roughly half the tongue's length and each branch bifurcates again to produce four distal grooves, each of which splits longitudinally many times to form many fine bristles (Fig 1A). The second tongue type is similar to the first, but the central bifurcation does not extend as far up the tongue body and the tongues are sturdier and stiffer (Fig 1B). The third tongue type departs from the paintbrush morphology in that there are minimal or no terminal bristles (Fig 1C). These tongues terminate in four grooves; the outer grooves have fringes (diagonal lacerations along the tissue) along their lateral margins (Fig 1C) and the medial grooves have few or no bristles at their tip. These tongues vary in the number of fringes and relative size of the four grooves, with some species, specifically those of the genera *Plectorhyncha* and *Philemon* (Fig 1C left panel), having four equally sized grooves and other species having two wider lateral grooves and two thinner medial grooves, as seen in the genera *Melilestes* (Fig 1C right panel) and *Myzomela*. Tongue types 1-3 have similar tongue lengths relative to bill length (Fig 2A), bristle proportions (Fig 2B), tongue widths relative to bill width (Fig 2C), and tongue depths relative to bill depth (Fig 2D).

The fourth tongue type is only seen in members of the genus *Acanthorhynchus* (Fig 1D). These tongues are longer relative to bill length than types 1-3 (Fig 3A), but their most distinctive features are the fact that they are composed of only two grooves, rather than the typical four, and there is a cartilaginous supporting rod along ventral surface of each groove (Fig 1D); these features are illustrated in the histological images provided of *Acanthorhynchus superciliosus* in [39]. The distal end of the outer margin of each groove has many fringes (Fig 1D), but this does not extend far proximally (Fig 2B). These tongues are narrower relative to bill width (Fig 2C) and shallower relative to bill depth (Fig 2D) than the other tongue types.

The fifth tongue type is only seen in members of the genus *Phylidonyris* (Fig 1E). Similar to the tongues of *Acanthorhynchus*, these tongues are longer relative to bill length than Types 1-3 (Fig 2A). In *Phylidonyris* the tongue looks similar to those in Type 1, but there are only two grooves and the bristles compose a larger portion of the tongue (Fig 1E and 2B). The presence of only two grooves in tongues of *Phylidonyris* is also shown in the histological images provided in [39] of *Phylidonyris novaehollandiae*. Interestingly, these tongues are wider relative to bill width (Fig 2C) and deeper relative to bill depth (Fig 2D) than most other tongue types, with Type 2 being most similar.

The sixth and final tongue type is seen only in the genus *Epthianura* (Fig 1F), one of the two genera that comprise the Australian chats, an insectivorous clade within the Meliphagidae. These tongues are shorter relative to bill length than the other tongue types seen across meliphagids, being more similar in length to insectivorous outgroups (Fig 2A). These tongues

also they have many small, short bristles on the distal tip (Fig 1F). These tongues bifurcate roughly halfway along their length and they are similar in width to the other tongue types (except Type 4, Fig 2C) but shallower than in the other meliphagids examined, being more similar to insectivorous outgroups (Fig 2D). From the chats, we only found specimens of the genus *Epthianura* in collections, we could not locate any specimens of *Ashbyia*. Based on the drawings and comments of [55] for the genus *Ashbyia*, this morphology does not appear to be consistent between the two genera of chats. The tongue of *Ashbyia* is simpler with no grooves or bristles, being more similar to the tongue of an outgroup insectivore (Fig 1G).

Microscopic tongue morphology

Across the Meliphagidae the proximal half of the tongue is composed of a central trough with raised lingual edges. Viewed in cross section, the trough begins as a "U" shape and transitions to a "W" shape moving proximally to distally (Fig 3 and 4). The distal half of the honeyeater tongue can take many different shapes, as described above, but all of those shapes are formed by tissues made of primarily of keratin. The proximal portion of the tongue contains more tissue types and provides the base for the complex shapes formed at the tip. The proximal half of the tongue is composed of many layers of stratum corneum, or keratinized epithelium (Fig 3B), which are folded inwards at several invagination points (Fig 3C and 3D). These invagination points serve as bifurcation points more distally in the tongue when the central groove splits into two or four grooves (Fig 4A-F). The central core of the tongue contains connective tissue and a cartilaginous rod on either side of the central tongue axis, each of which is dorsoventrally oval shaped in transverse cross sections (Fig 3C and 4E-I). Proximally, these rods grow taller and wider as the tongue itself widens (Fig 3E-F and 4G-I), providing structural support to the tongue base. These rods contain sinuses, which are likely vascular and provide nutrients and oxygen to the connective tissue in the tongue base (Fig 3E-F and 4H-I). These rods and sinuses are found in both *Ptilotula penicillata* and *Melithreptus lunatus* (compare Fig 3 and 4), suggesting that while honeyeaters can have different tongue tip morphologies (*Ptilotula penicillata* is a Type 1 tongue and *Melithreptus lunatus* is a Type 2 tongue), the tongue base is likely to be consistent morphologically at the macro and micro levels of organization.

When comparing the present histological work on *Melithreptus lunatus* with that on *Acanthorhynchus superciliosus* and *Phylidonyris novaehollandiae* [39], there is a notable difference between species in the number of stratum corneum layers and the extent of the invagination of those layers. *Melithreptus lunatus* has a Type 2 tongue shape while *Acanthorhynchus superciliosus* has Type 4 and *Phylidonyris novaehollandiae* has Type 5, but all species show some degree of invagination of the stratum corneum layers, even though the folding is shallow and does not contribute substantially to tongue structure in *Acanthorhynchus superciliosus*. There are more layers of stratum corneum in the tongue walls of *Phylidonyris novaehollandiae* and *Melithreptus lunatus*, while the tongue of *Acanthorhynchus superciliosus* has many fewer layers and is thin walled. The histological images in Collins (2008) show paired cartilaginous rods in *Acanthorhynchus superciliosus* and *Phylidonyris novaehollandiae*, which suggests that those are present in honeyeater tongues regardless of tongue type, however *Acanthorhynchus* is the only genus in which the cartilaginous rods extend to the tongue tip (Fig 1D).

Ancestral state reconstruction of tongue types

The ER model had an AIC of 159.51 and a log-likelihood -78.75, while ARD had an AIC of 195.21 and log-likelihood of -55.60. Given that the ΔAIC was >2 between the two models in favor of the ER model, the ER model was used for stochastic character mapping. Stochastic character mapping trees had 22.17 changes between states on average. Within the Meliphagidae, the most common state was a Type 3 tongue, followed by Types 1 and 2 in roughly equal proportions (Table 1 and Fig 5). From the ancestral insectivorous Type (Type 0) the tongue type ancestral to Meliphagidae is Type 3 (Fig 5). Within the Meliphagidae the most common changes were to Type 1 (Table 1 and Fig 5), and Types 1-3 show higher rates of change than Types 4-6 (Table 1). Type 1 tongues are estimated to have evolved six times (Fig 5), while Type 2 tongues are estimated to have evolved five times (Fig 5). Type 3 tongues are estimated to have evolved three times throughout the tree, at the base of the Meliphagidae from an insectivorous ancestor and in the genus *Melidectes* and in *Caligavis subfrenata* (Fig 5). Types 4-6 are each estimated to have evolved once, as they are restricted to specific monophyletic genera (Fig 5).

Morphological correlations with diet

Tongue types

There was a significant phylogenetic signal in tongue types (Table 2), and there was a significant difference in dietary nectar consumption across tongue types ($p = 0.023$) (Table 3 and Fig 2E). Type 0 (ancestral insectivorous) was associated with the lowest proportion of nectar in the diet ($intercept_0 = 0.031$) along with Type 6 (insectivorous meliphagids, $intercept_6 = 0.069$). Types 1-3 were associated with midrange nectar consumption ($intercept_1 = 0.34$, $intercept_2 = 0.32$, $intercept_3 = 0.42$) and Types 4 and 5 (*Acanthorhynchus* and *Phylidonyris*, respectively) were associated with the highest proportion of nectar in the diet ($intercept_4 = 0.62$, $intercept_5 = 0.57$).

Linear morphometrics

All linear morphometric traits had phylogenetic signal except for tongue depth (Table 2). In the pPCA for the tongue morphology dataset (58 meliphagid species and 13 outgroup species, Table S1), the first three principal components explain 83% of the variation (Table 4 and Fig 6A). In the pPCA for the tongue and hyoid dataset (38 meliphagid species and 5 outgroup species, Table S2) the first three principal components explain and 80% of the variation (Table 4 and Fig 6B). There was no distinct separation between honeyeaters and outgroups in either pPCA (Fig 6A-B). In the tongue only pPCA, PC1 was determined primarily by tongue depth, while PC2 was determined by a combination of tongue length and width, and PC3 was determined by tongue length and bristle proportion (Table 4 and Fig 6A). In the tongue and hyoid pPCA, PC1 was equally determined by tongue length, depth, and width, while PC2 was determined by hyoid length and PC3 was determined by tongue length (Table 4 and Fig 6B).

The only variables that were significantly correlated with the proportion of nectar in the diet (PGLS regression $p < 0.05$) were relative tongue length (Table 5 and Fig 7A) and the proportion of the tongue that is bristled (Table 5 and Fig 7B), and both correlations were positive (Table 5). Relative tongue width and relative tongue depth were not significantly correlated with nectarivory (Table 5 and Figure S2), but honeyeaters did tend to have relatively narrower tongues than outgroups taxa (Fig S2). There was a non-significant negative correlation between relative hyoid length and nectarivory (Table 5 and Figure S2). The trends were the same in the raw data (i.e., tongue length, tongue width, tongue depth, bristle length, and hyoid length not corrected for bill size). Raw tongue length and raw bristle length were significantly positively

correlated with nectarivory, but all other variables were not significantly correlated with nectarivory (Table S3).

Discussion

Functional consequences of variability in honeyeater tongue morphology

The distal half of the honeyeater tongue shows extensive morphological variation between species, which we have classified into tongue types (Fig 1), while the proximal half seems consistent across species (Fig 3 and 4). Honeyeater tongues are typically described as having four grooves that terminate in many fine bristles, being similar to a paintbrush [e.g., 38]. Of the honeyeaters sampled here, there are species that follow this description (Type 1 and Type 2 tongues, Fig 5), but many species that do not. Based on our sample, the ancestral state of the Meliphagidae tongue is estimated to be a Type 3 shape (Fig 5), which is different from the classic 'paintbrush shape' of the Type 1 and Type 2 tongues (Fig 1A-B) in that there are minimal or no terminal bristles (Fig 1C). Rather than terminating in many fine bristles and evoking the shape of a paintbrush, Type 3 tongues maintain four distinct grooves up to the tongue tip and the lateral grooves have fringes along their lateral margins (Fig 1C). Interestingly however, ancestral state reconstructions did find that the most common shape transition was to a Type 1 tongue (Table 1). We also found that tongue Types 4-6 appear to be specific to the genera *Acanthorhynchus, Phylidonyris,* and *Epthianura*, respectively (Fig 5).

The variability in the honeyeater tongues along with the fact that the degree of nectarivory differs significantly across tongue types is intriguing (Table 3 and Fig 2E) and raises the question of whether these differences in tongue shape are functionally significant to the process of nectar feeding. The only study to-date that has investigated the biomechanics of honeyeater feeding included five species: *Phylidonyris novaehollandiae*, *Manorina flavigula*, *Acanthagenys rufogularis*, *Ptilotula penicillata*, and *Certhionyx variegatus* [41]. All five of these species rely primarily on fluid trapping to consume nectar, a process in which the fluid is caught passively between bristles due to surface tension [41]. Four of the species (*Phylidonyris novaehollandiae*, *Manorina flavigula*, *Acanthagenys rufogularis*, and *Ptilotula penicillata*) used some degree of expansive filling (first described in hummingbirds [56]) to load nectar into the grooved body of the tongue, while the fifth species, *Certhionyx variegatus*, used capillary filling [41]. The five species examined in this previous study also have different tongue types. *Phylidonyris novaehollandiae* has a Type 5 tongue, which is again unique to the genus *Phylidonyris*, whereas the remaining species (*Manorina flavigula*, *Acanthagenys rufogularis*, *Ptilotula penicillata,* and *Certhionyx variegatus*) have Type 1 tongues. The fact that all of these species rely primarily on fluid trapping suggests that there are a variety of tongue shapes that can perform this function equally well. Many-to-one-mapping of tongue form to function [57] could explain the evolutionary lability in the honeyeater tongue shape seen across the tree (Fig 5), with 22.17 state changes between tongue types on average across stochastic character mapping runs.

If there is many-to-one-mapping of tongue form to function for the process of fluid trapping, then the most important factors when determining feeding efficiency in species that use this mechanism may be the overall size of the tongue and the nectar properties of the flower the bird is feeding on [39,58]. Importantly, there was nothing notably unique about to tongue of *Certhionyx variegatus* compared to the tongues of the other four species examined in [41] that would facilitate capillary filling versus expansive filling. It is possible that the distinction between those two mechanisms comes from emergent manipulation or deformation of the tongue during the feeding process due to interactions between the tongue and the bill. To test these

hypotheses, fluid dynamics models need to be constructed that quantify the degree to which tongue size, tongue morphology, such as bristle number and length, and nectar properties, like volume and viscosity, affect the volume of fluid captured per lick during the process of fluid trapping.

Convergence with other avian nectarivores

Of the tongue types we identified, the tongues of the genus *Acanthorhynchus* are remarkably convergent with the tongues of hummingbirds and sunbirds; compare with images from [24] and [59], respectively. This is not a surprising finding, as *Acanthorhynchus* is the most nectarivorous genus within the Meliphagidae (Fig 2E). Both hummingbird tongues and *Acanthorhynchus* tongues have two grooves, cartilaginous supporting rods along the entire length of the tongue, and lateral fringes (Fig 1D). The tongues of *Acanthorhynchus* have few bristles at the tongue tip (Fig 1D), making it unlikely that they could use those bristles for substantial fluid trapping. Although the feeding biomechanics of *Acanthorhynchus,* has not been studied, we hypothesize that they might use expansive filling, like hummingbirds [56], or pressure-driven suction, like sunbirds [59], as their primary feeding mechanism instead of fluid trapping. If correct, this would constitute a high degree of morphological and functional convergence.

Previous work alludes to *Acanthorhynchus* likely using a different feeding mechanism than other honeyeaters. When presented with a series of nectar concentrations (10-60% wt/wt) at varying volumes (5,10, 50 microliters), *Anthochaera carunculata* and *Acanthagenys rufogularis* had different responses in their sugar intake rate compared to *Acanthorhynchus tenuirostris* [58]. For all species, the sugar intake rate was highest at 50microliters, but the difference between the 50microliter and 5 and 10 microliter treatments was much larger for *Anthochaera carunculata* and *Acanthagenys rufogularis* than for *Acanthorhynchus tenuirostris* [Figure 2 in 58]. Also, the sugar intake rate peaked at 50% concentration for *Anthochaera carunculata* and *Acanthagenys rufogularis*, but 30% for *Acanthorhynchus tenuirostris* with a sharper decline thereafter [Figure 2 in 58]. These differences could be explained by the different tongue morphology of the three species, beyond differences in tongue size [38,58], and by extension the potential for them to differ in feeding mechanics. *Acanthorhynchus tenuirostris* has similar sugar intake rates between 5, 10, and 50 microliter volumes, while *Anthochaera carunculata* and *Acanthagenys rufogularis* have much higher rates at the 50-microliter volume. This could be due to the fact that *Anthochaera carunculata* and *Acanthagenys rufogularis* have tongues with a larger bristle proportion relative to those of *Acanthorhynchus tenuirostris*, and therefore they can capture a larger aliquot of fluid on each lick using fluid trapping at volumes permitting the bristle portion of their tongue to be largely submerged; when the volume is reduced to 10 or 5 microliters, the intake rate dramatically declines likely due to a reduction in the submersion depth of the bristled portion of their tongue [Table 3 in 38]. *Acanthorhynchus tenuirostris*, on the other hand, does not have a tongue built for fluid trapping and we hypothesize that it uses a method more similar to that of expansive filling [56] or pressure-driven suction [59], which could result in smaller differences in intake rates across variation in volume presentation. The difference in peak intake rate could also be explained by tongue morphology and feeding mechanics. If *Acanthorhynchus tenuirostris* uses a mechanism like that of hummingbirds or sunbirds, it is likely to be more sensitive to nectar concentration (due to the exponential relationship between concentration and viscosity) because it requires more flow of nectar within the tongue body; fluid trapping conversely is likely to be less sensitive to concentration because there is less fluid flow.

Morphological correlates with nectarivory

When comparing linear morphometrics with dietary nectar consumption, we found that a higher degree of nectarivory was correlated with a longer tongue (raw numbers and relative to bill length) and a longer bristle length and greater bristle proportion (Table 5 and S3, Fig 7). This fit our prediction, as a longer tongue allows for greater access to a wider range of floral resources and a tongue with a greater portion bristled means there is a greater surface area to engage in fluid trapping, the main way honeyeaters collect nectar [41]. There is nuance to this, however. As discussed above, the genus *Acanthorhynchus* has minimal bristles on the tongue tip but is highly nectarivorous, perhaps using a different feeding mechanism from fluid trapping.

There was no correlation between tongue width or depth and degree of nectarivory (Table 5 and S3), but honeyeaters generally had narrower tongues than outgroups (Fig S2). This measurement was taken halfway along the length of the tongue, where for many honeyeaters any grooves that are present at the tip have coalesced into a central trough (Fig S1). Although there was no correlation with nectarivory, it is possible that tongue width and depth are constrained by internal bill anatomy, as there is clearly a relationship between tongue width and bill width and between tongue depth and bill depth (Fig S3). Future work examining the *in situ* fit of the tongue within the upper and lower bill using methods like CT scanning would illuminate this and would help begin to unpack the biomechanics of intraoral transport in honeyeaters (*i.e.,* how they move nectar from the tongue to the back of the throat), as it has been recently studied in other groups [59,60].

Though statistically non-significant, there was a negative correlation between nectarivory and relative hyoid length (Table 5 and Fig S2). This suggests that honeyeaters may not modify the feeding apparatus for more efficient nectar feeding through increasing their ability to protrude the tongue further from the mouth. Rather, our results suggest that honeyeaters enhance their feeding apparatus for nectarivory by modifying the tongue only. The distance from the bill tip to the nectar surface has been shown to limit the nectar feeding efficiency of honeyeaters (*Acanthorhynchus superciliosus*, *Phylidonyris novaehollandiae,* and *Lichmera indistincta*) more so than that of hummingbirds (*Campylopterus hemileucurus, Heliodoxa jacula, Lampornis calolaema, Phaethornis guy*) in experiments with artificial flowers [39]. In an interspecific comparison across honeyeaters using experiments with real flowers, a greater bill tip to nectar distance did not equate to lower feeding rate [61]. In fact, the species with a greater bill tip to nectar distance was the species with the largest bill and largest tongue, which allowed for a higher rate of nectar intake per lick and therefore a faster rate of nectar consumption [61]. So, while the finding of no correlation between hyoid length and nectarivory is counter to the general expectations for increase nectar-feeding efficiency (summarized in [33]), it does lend support to the hypothesis that differences in feeding efficiency across honeyeaters are likely due to tongue size. These results concerning hyoid length should be confirmed with CT scans of whole specimens where the hyoid can be seen in context with the cranium and with associated musculature, which will give a more complete functional picture.

Future directions

More studies on drinking mechanics of honeyeaters, especially focused on the honeyeaters with tongue types that have not been studied biomechanically (e.g., genus *Acanthorhynchus*), will help to better elucidate the connections between variability in tongue morphology and variability in tongue function across honeyeaters. Additionally, honeyeaters also subsist on non-nectar foods, such as insects, honeydew, manna, and lerp [62,63], and studies investigating how

variation in tongue morphology affects or correlates to feeding on these sources would be valuable. The robustness of our conclusions would greatly benefit from similar studies of nectarivores outside of the Meliphagidae. There are many qualitative descriptions of tongue morphology, and some on hyoid morphology, across other lineages of avian nectarivores, but quantitative morphological assessments are needed to determine if nectarivory really does result in morphological convergence, or similar vectors of morphological evolution, across avian nectarivores [34,41]. Given the degree of lability in tongue shape found across honeyeaters, it would be interesting to see more studies that investigate whether the hyolingual apparatus, or components of it, evolve as its own evolutionary module in birds, similar to what has been demonstrated for various components of the cranium [64].

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Figures & Tables

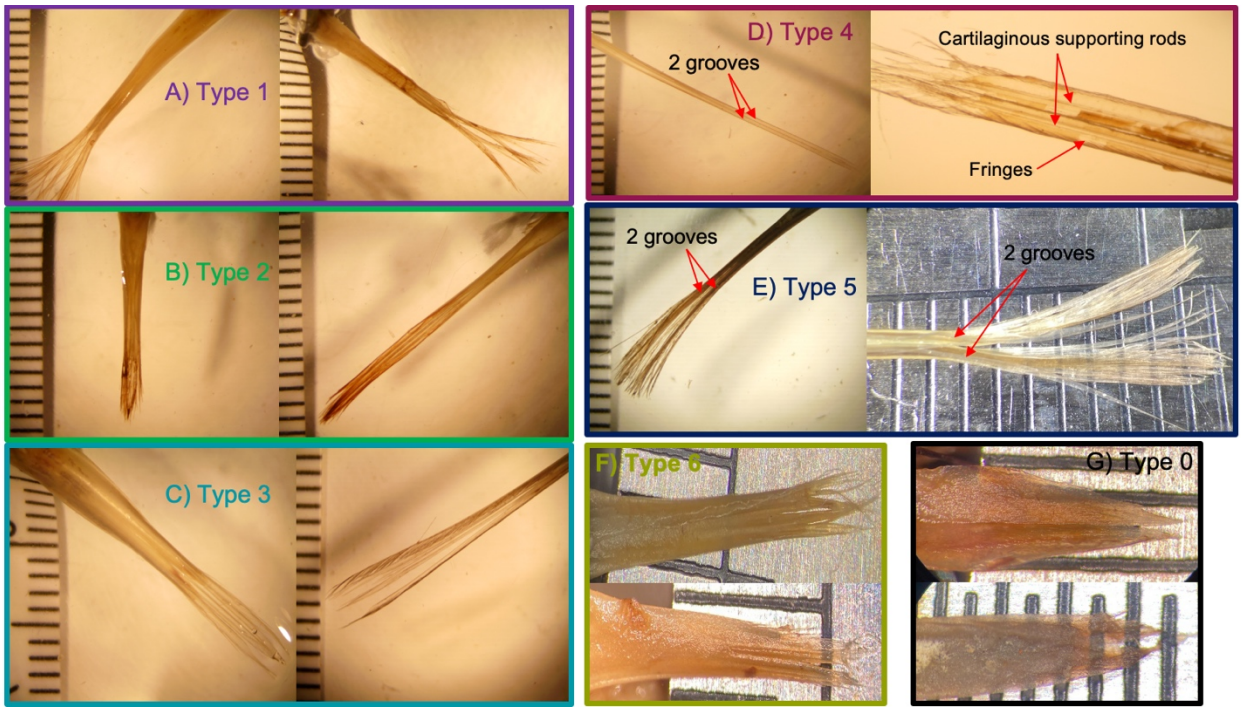


Figure 1. Distinct tongue types found across the Meliphagidae in comparison to outgroup insectivores. A) Type 1 tongues (*Manorina flavigula* (UWBM 57667) and *Ptilotula penicillata* (UWBM 60839)), B) Type 2 tongues (*Melithreptus brevirostris* (UWBM 76602) and *Melithreptus lunatus* (UWBM 76699)), C) Type 3 tongues (*Philemon citreogularis* (UWBM 57671) and *Melilestes megarhynchus* (UWBM 67917)), D) Type 4 tongues (*Acanthorhynchus tenuirostris* (UWBM 76471) and *Acanthorhynchus superciliosus* (UWBM 60869)), E) Type 5 tongues (*Phylidonyris novaehollandiae* (USNM 612648) and *Phylidonyris niger* (QM O.33431)), F) Type 6 tongues (*Epthianura tricolor* (WAM A13975) and *Epthianura aurifrons* (WAM A17143)), G) Type 0 tongues (outgroup insectivores, *Malurus splendens* (WAM A5692) and *Acanthiza apicalis* (WAM A17535)). Arrows in panel D illustrate the distinctive features of having two grooves, cartilaginous supporting rods that extend to the tip of the tongue, and lateral fringes on the distal portion of the tongue. Arrows in panel E illustrate the distinctive feature of having two grooves. Rulers in each image indicate millimeters.

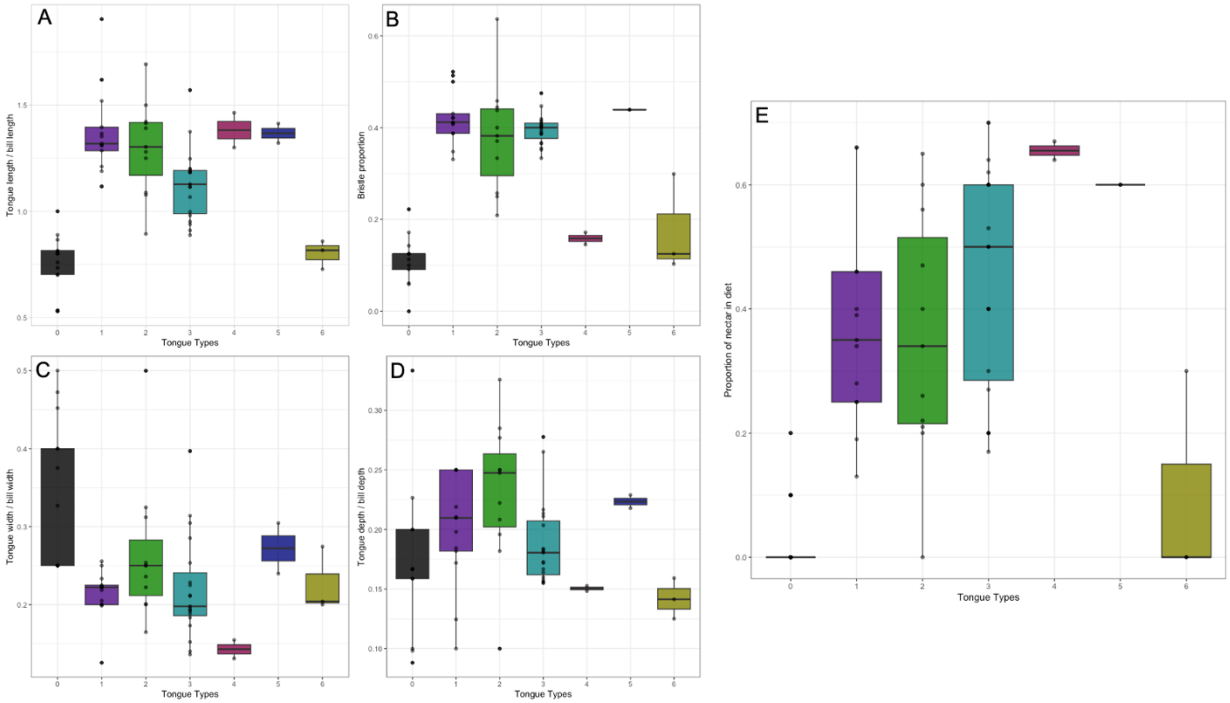


Figure 2. Differences in tongue morphometrics across tongue types found in the Meliphagidae and outgroup insectivores. A) Relative tongue length across tongue types, B) bristle proportion across tongue types, C) relative tongue width across tongue types, D) relative tongue depth across tongue types, E) proportion of dietary nectar across tongue types.

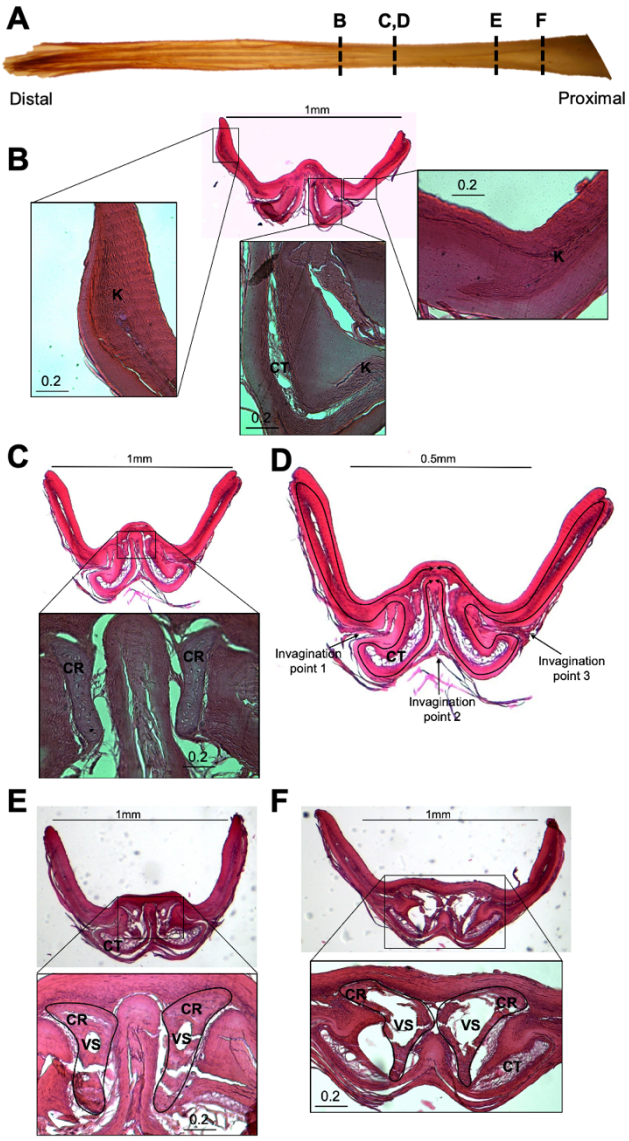


Figure 3. Histology of a *Melithreptus lunatus* tongue sectioned in the transverse plane and stained with hematoxylin and eosin. Panel A shows the location of sections in Panels B-F along the tongue. Panels B-F are arranged proximal to distal and are oriented such that the dorsal side of the tongue is at the top of the image and the ventral side at the bottom. Panel B shows the tongue immediately posterior to the bifurcation into grooves and illustrates the layers of keratin (K) that create the tongue body. Panel C shows the portion of the tongue where cartilaginous supporting rods (CR) become visible and the connective tissue (CT) layer in the center of the tongue thickens. Panel D is a higher magnification image of the section in Panel C, illustrating the invagination points where the keratin layers fold. Panel E shows the portion of the tongue where the vascular sinuses (VS) within the cartilaginous rods (CR) become visible, and where the cartilaginous rods elongate dorsoventrally. Panel F shows the posterior portion of the tongue where the cartilaginous rods (CR) widen to form support for the tongue base and the vascular sinuses (VS) become wider and taller. All sections at 10 microns thick. All scale bars are in mm. Specimen shown is UWBM 76699.

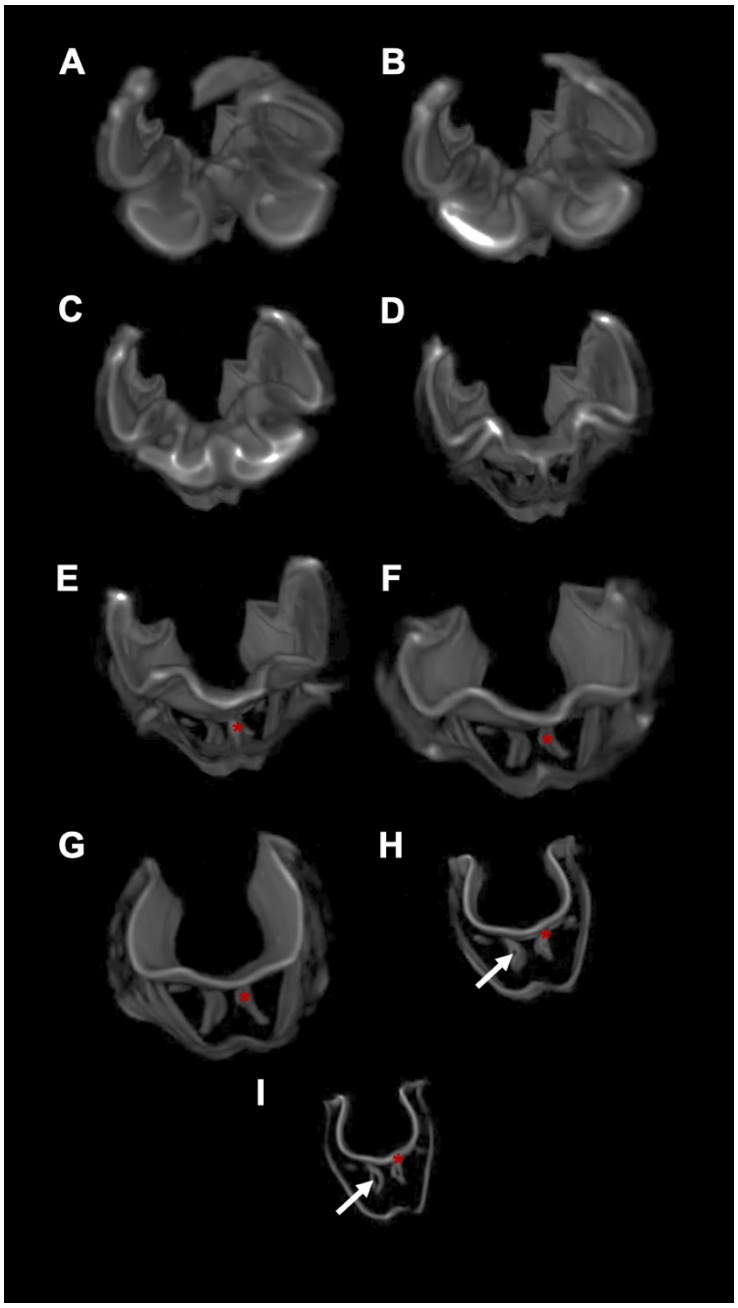


Figure 4. Transverse cut aways from 3D reconstruction of a *Ptilotula penicillata* tongue. Panels A-I are arranged anterior to posterior and are oriented such that the dorsal side of the tongue is at the top of the image. Panels A-C illustrate the four grooves present at the tongue tip. Panel D shows the point immediately posterior to the coalescence of the grooves. Panel E shows the portion of the tongue where cartilaginous supporting rods (red asterisk) become visible. Panels F-G show the tongue widening and the cartilaginous supporting rods (red asterisk) elongate dorsoventrally. Panel H shows the portion of the tongue where the vascular sinuses (white arrow) within the cartilaginous rods (red asterisk) become visible. Panel I shows the posterior portion of the tongue where the vascular sinuses (white arrow) become wider and taller. All panels are at the same scale. Specimen is MCZ Ornithology 364080.

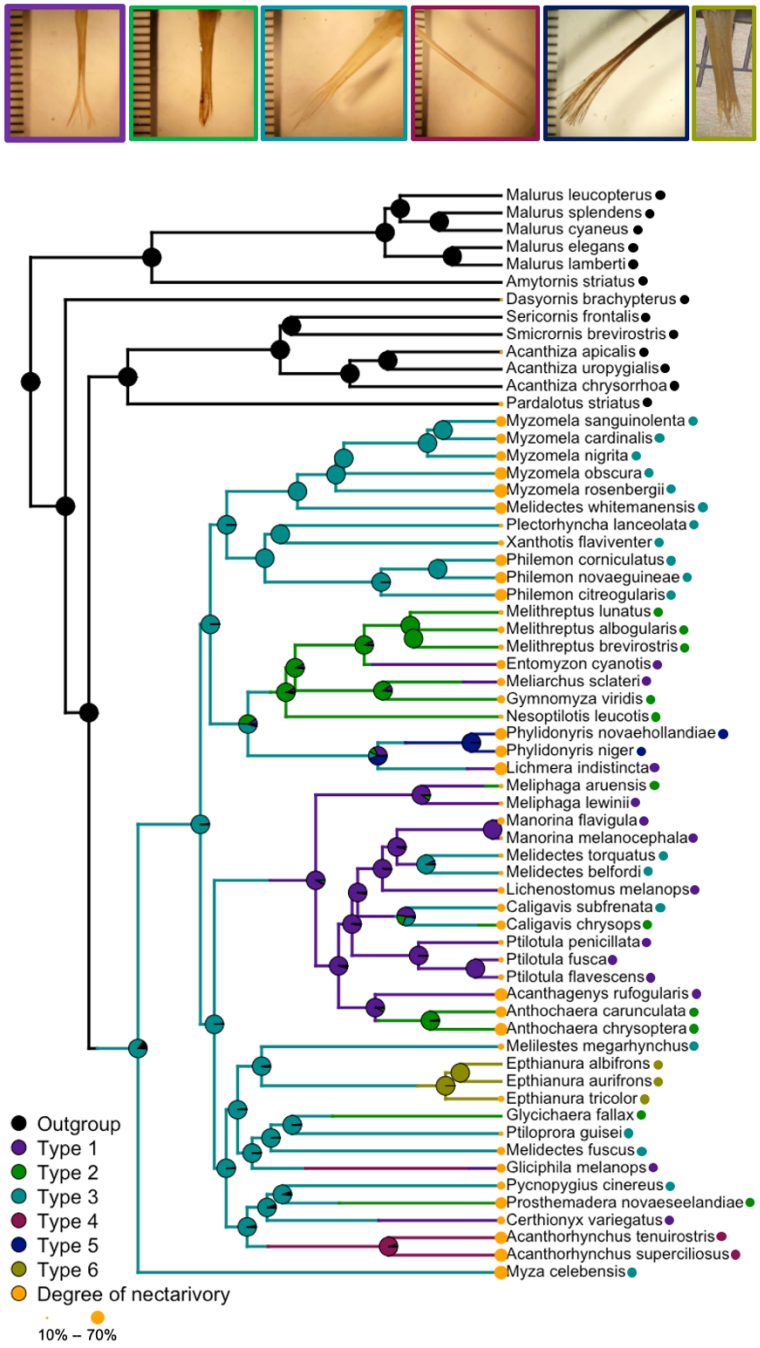


Figure 5. Ancestral state reconstruction of tongue type evolution in the Meliphagidae. Reconstruction determined via stochastic character mapping with equal rates model. Pies show the proportion of simulations in which a given state was selected via MCMC from a posterior distribution for a given node for this run. The color of the circles to the right of species names indiates the tip state. The size of the orange circles at the tips corresponds to the proprotion of nectar in the diet. Tongue images are arranged from Type 1 to Type 6, from left the right. Tongue images are as follows: 1) *Ptilotula fusca* (UWBM 76519), 2) *Melithreptus brevirostris* (UWBM 76602), 3) *Plectorhyncha lanceolata* (UWBM 57795), 4) *Acanthorhynchus tenuirostris* (UWBM 76471), 5) *Phylidonyris novaehollandiae* (USNM 612648).

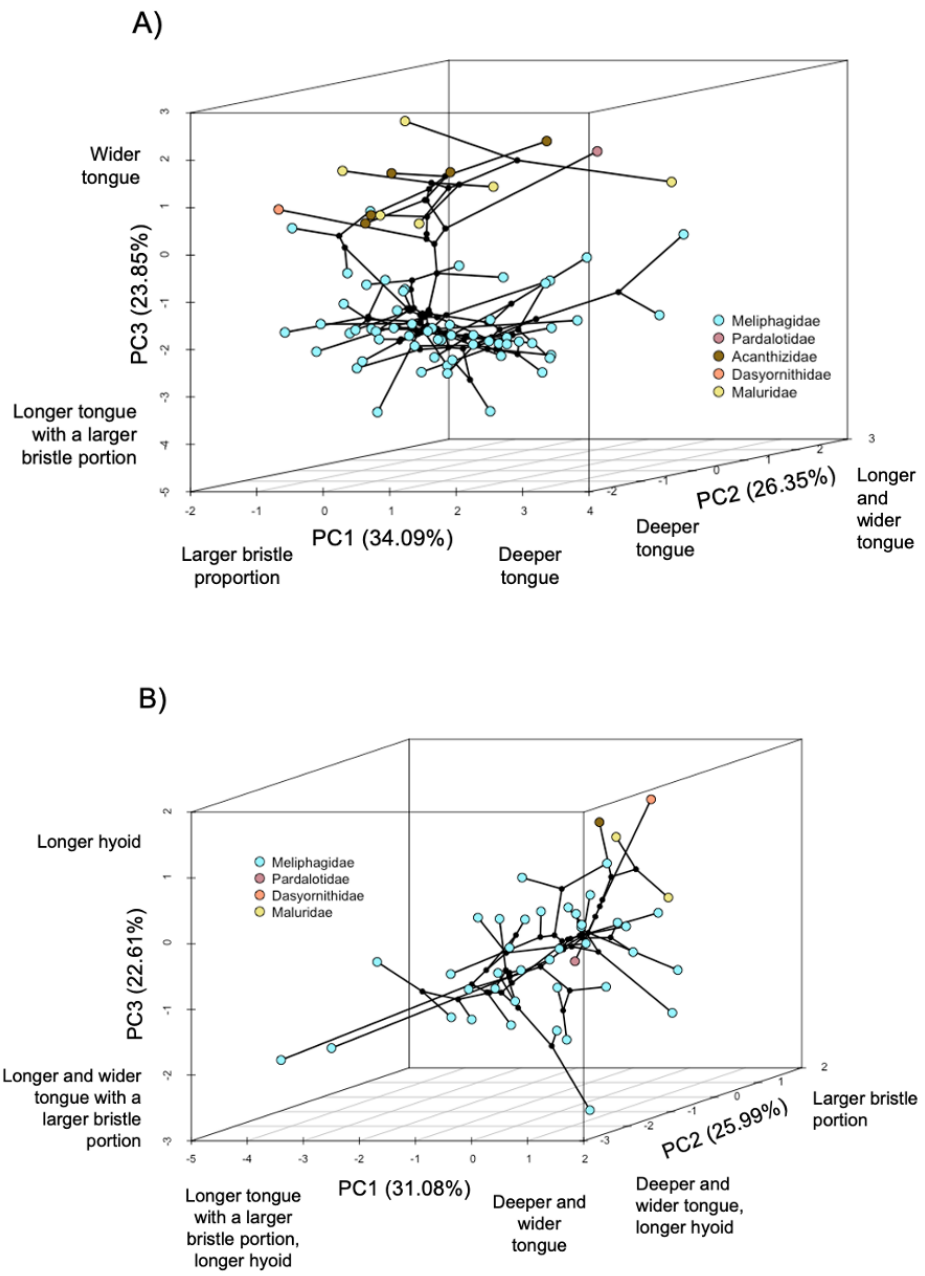


Figure 6. 3D phylogenetic PCA of tongue and hyoid morphology. A) Tongue morphometrics only. B) Tongue and hyoid morphometrics. Labels under axes illustrate the loadings of each morphological variable. See Table 4 for exact loadings.

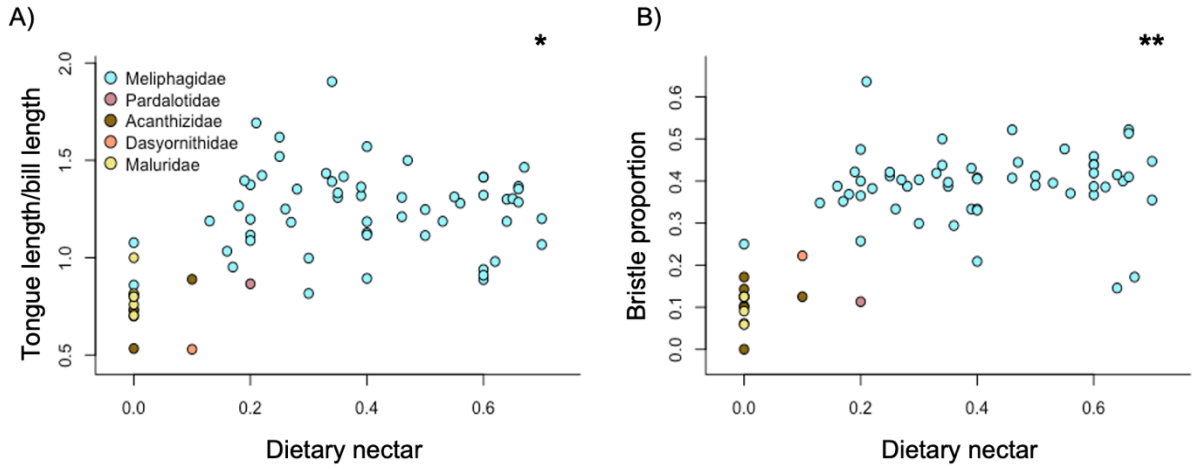


Figure 7. Significant correlations between dietary nectar percentage and tongue morphometrics. A) Dots show tongue length/bill length ~ dietary nectar, B) Dots show bristle proportion ~ dietary nectar. Colors correspond to families. * indicates that the p-value from the PGLS regression was < 0.05, ** that the p-value from the PGLS regression was < 0.01. Model parameters are shown in Table 5.

Table 1. Results of ancestral state reconstruction of tongue types across Meliphagidae and outgroups. Ancestral state reconstruction was run using stochastic character mapping with an equal rates model of state change.

Tongue type (state)	Proportion of time spent in state	Most common state change	
		To	Rate
0	0.28	3	1.14
1	0.14	2	2.87
2	0.13	1	2.14
3	0.38	1	3.11
4	0.028	1	0.17
5	0.019	1	0.57
6	0.024	1	0.19

Table 2. Results of Blomberg's K test for phylogenetic signal. All traits except relative tongue depth show significant phylogenetic signal. Bolded p-values are significant. Significance determined as $p < 0.05$.

Morphological Variable	**Blomberg's K**	**p-value** (H_0: no phylogenetic signal)
Tongue type	2.151	0.001
Tongue length/bill length	1.473	0.001
Bristle proportion (bristle length/tongue length)	1.949	0.001
Tongue depth/bill depth	0.371	0.092
Tongue width/bill width	0.767	0.001
Hyoid length/bill length	0.553	0.033

Table 3. Results of phylogenetic ANOVA of dietary nectar ~ tongue type. There is a significant correlation between dietary nectar consumption and tongue type. Bolded *p*-value indicates significance. Significance determined as $p < 0.05$.

	Df	**Sums of Squares**	**Mean Square**	**F-value**	**p-value**
Tongue type	6	1.976	0.329	12.967	**0.023**
Residuals	56	1.422	0.025		

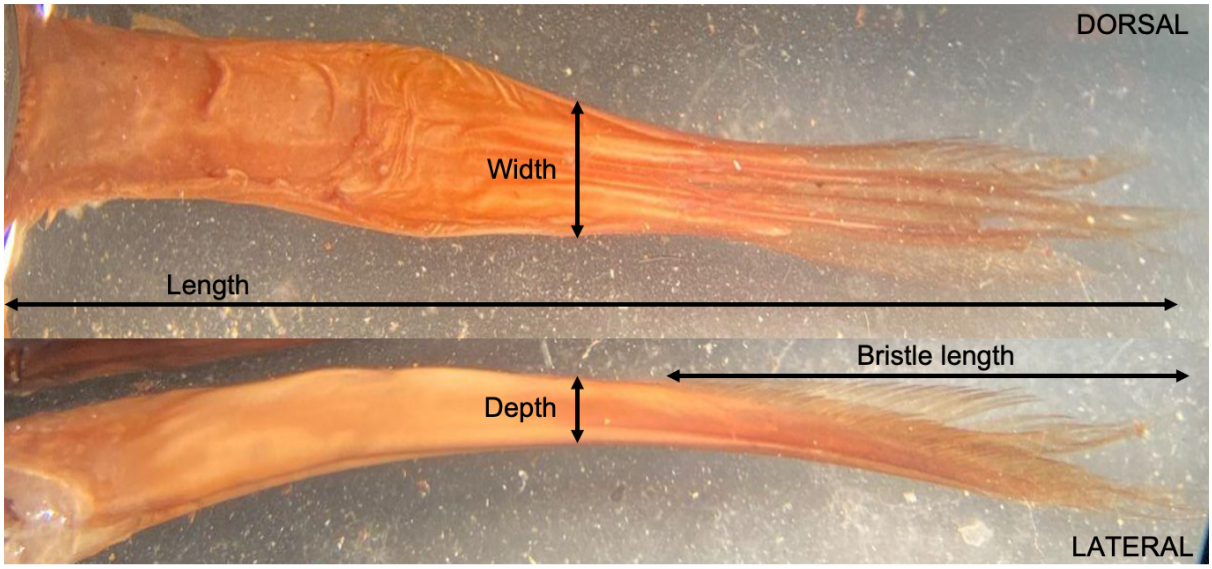
Table 4. Results of pPCA for tongue only and tongue + hyoid morphological datasets. Values in rows show the loadings of each morphological variable in the pPCA. Percentages listed under each principle component show the percent variance explained by that component.

Morphological Variable (tongue only dataset)	**PC1** 34.09	**PC2** 26.35	**PC3** 23.85	**PC4** 13.98
Tongue length/bill length	0.010	0.589	-0.723	-0.362
Bristle portion length	-0.035	0.126	-0.790	0.599
Tongue depth/bill depth	0.995	-0.089	-0.045	-0.005
Tongue width/bill width	0.175	0.889	0.403	0.132
Morphological Variable (tongue + hyoid dataset)	**PC1** 31.08	**PC2** 25.99	**PC3** 22.61	**PC4** 20.57
Tongue length/bill length	-0.636	-0.114	-0.623	-0.299
Bristle portion length	-0.534	0.216	-0.576	-0.088
Tongue depth/bill depth	0.630	-0.431	-0.049	-0.638
Tongue width/bill width	0.611	-0.380	-0.524	0.455
Hyoid length / bill length	-0.538	-0.801	0.225	0.117

Table 5. Results of PGLS analyses for each bill-size corrected morphological variable. Bolded *p*-value indicates significance. Significance determined as $p < 0.05$.

Variable	AIC	Log Likelihood	λ	Slope	Standard Error	t-value	p-value
Tongue length / bill length	-22.119	15.059	0.818	0.299	0.140	2.136	**0.036**
Bristle proportion	-137.584	72.792	0.962	0.193	0.062	3.114	**0.003**
Tongue depth / bill depth	-204.676	106.338	0.224	0.034	0.030	1.099	0.275
Tongue width / bill width	-153.088	80.544	0.805	0.004	0.054	0.082	0.935
Hyoid length / bill length	31.614	-11.807	0.679	-0.478	0.259	-1.845	0.072

Supplementary Material



Philemon novaeguineae (AMNH 5109)

Figure S1. Linear morphometrics measured on all tongues.

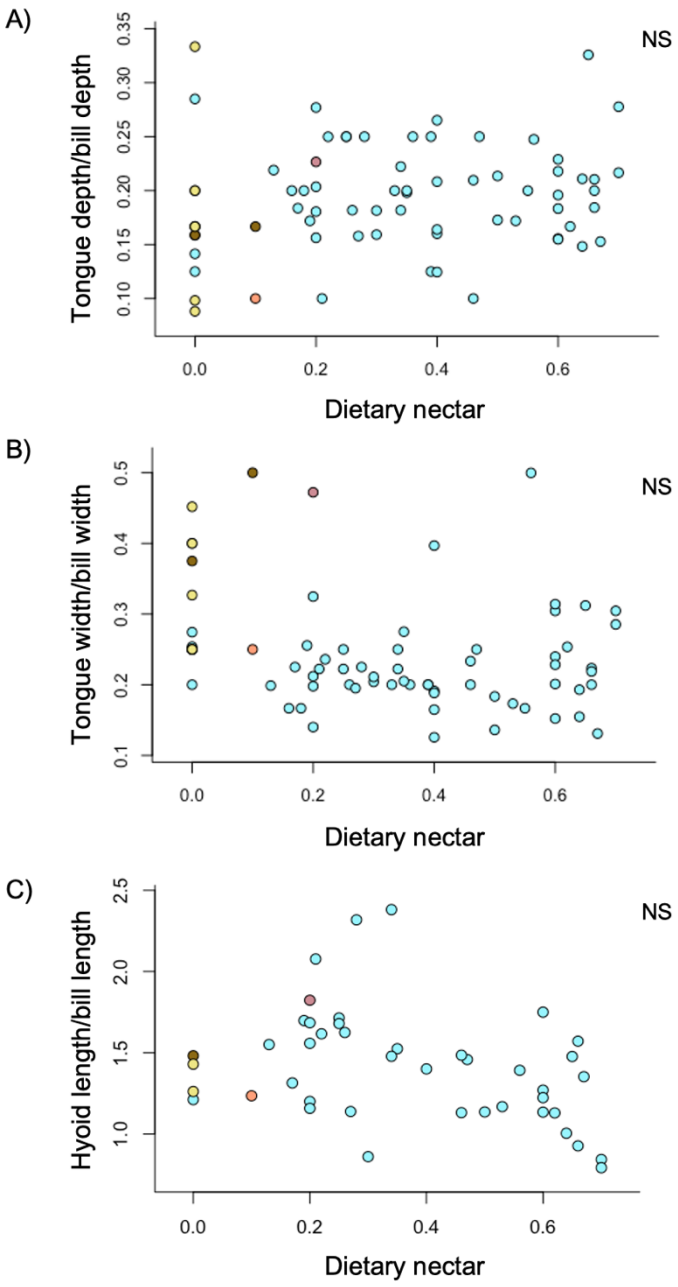


Figure S2. Non-significant correlations between degree of nectarivory and tongue and hyoid morphometrics. A) Relative tongue depth, B) Relative tongue width, and C) Relative hyoid length. Parameters for corresponding PGLS analyses are in Table 5.

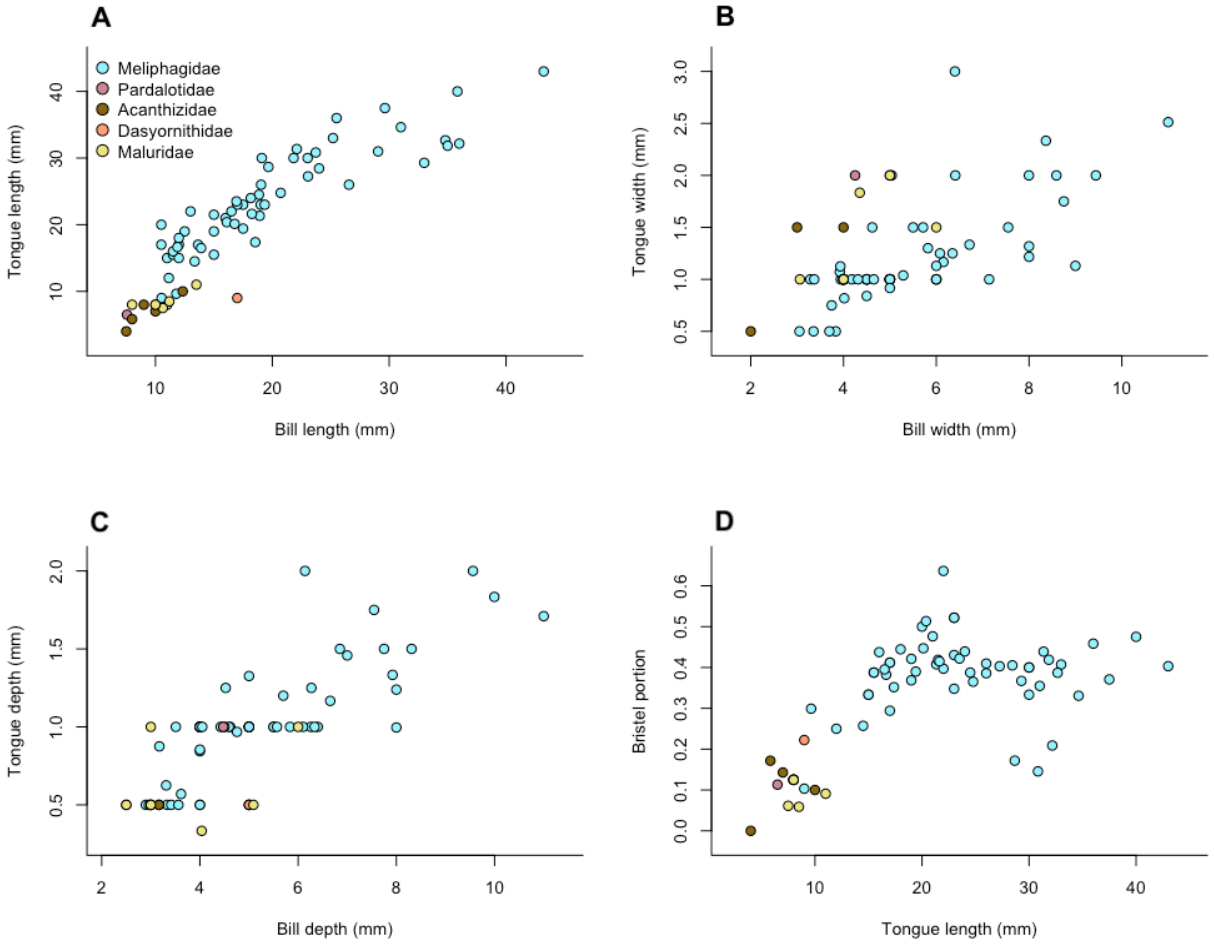


Figure S3. Relationships between corresponding tongue and bill morphometrics. A) Tongue length plotted against bill length. B) Tongue width plotted against bill width. C) Tongue depth plotted against bill depth. D) Bristle proportion plotted against tongue length. Colors indicate families.

Table S1. Complete list of tongue specimens measured for this study. Museum abbreviations are as follows: UWBM = University of Washington Burke Museum, MCZ = Harvard Museum of Comparative Zoology, AMNH = American Museum of Natural History, USNM = Smithsonian, QM = Queensland Museum, WAM = Western Australian Museum, MVZ = UC Berkeley Museum of Vertebrate Zoology. Specimen number is listed in the nomenclature of the museum in which the specimen is housed. Diet source data abbreviations indicate whether data is from Miller et al. (2017) [EM] or Wilman et al. (2014) [ET].

Museum	Specimen #	Species	Family	Preservation type	Diet data source
QM	O.32847	*Acanthagenys rufogularis*	Meliphagidae	Tongue in ethanol	EM
QM	O.33171	*Acanthagenys rufogularis*	Meliphagidae	Tongue in ethanol	EM
MCZ	23-034	*Acanthagenys rufogularis*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-032	*Acanthagenys rufogularis*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-027	*Acanthagenys rufogularis*	Meliphagidae	Rehydrated tongue	EM
WAM	A17764	*Acanthiza apicalis*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A17535	*Acanthiza apicalis*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A34359	*Acanthiza chrysorrhoa*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A48666	*Acanthiza chrysorrhoa*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A48861	*Acanthiza uropygialis*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A14638	*Acanthorhynchus superciliosus*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A37449	*Acanthorhynchus superciliosus*	Meliphagidae	Whole specimen in ethanol	EM
UWBM	60869	*Acanthorhynchus superciliosus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-087	*Acanthorhynchus tenuirostris*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-081	*Acanthorhynchus tenuirostris*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-080	*Acanthorhynchus tenuirostris*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-072	*Acanthorhynchus tenuirostris*	Meliphagidae	Rehydrated tongue	EM
USNM	612652	*Acanthorhynchus tenuirostris*	Meliphagidae	Tongue in ethanol	EM
UWBM	76471	*Acanthorhynchus tenuirostris*	Meliphagidae	Tongue in ethanol	EM
WAM	A34283	*Anthochaera carunculata*	Meliphagidae	Whole specimen in ethanol	EM
USNM	612645	*Anthochaera carunculata*	Meliphagidae	Tongue in ethanol	EM
QM	O.33461	*Anthochaera chrysoptera*	Meliphagidae	Tongue in ethanol	EM
UWBM	76683	*Caligavis chrysops*	Meliphagidae	Rehydrated tongue	EM
WAM	A48670	*Certhionyx variegatus*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A48859	*Certhionyx variegatus*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A14302	*Conopophila rufogularis*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A27168	*Dasyornis brachypterus*	Dasyornithidae	Tongue in ethanol	ET
QM	O.32926	*Entomyzon cyanotis*	Meliphagidae	Tongue in ethanol	EM
UWBM	76649	*Entomyzon cyanotis*	Meliphagidae	Rehydrated tongue	EM

WAM	A48860	*Epthianura albifrons*	Meliphagidae	Whole specimen in ethanol	EM
MCZ	SCO-125	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-124	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-123	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-122	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-121	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-120	*Epthianura albifrons*	Meliphagidae	Rehydrated tongue	EM
WAM	A17143	*Epthianura aurifrons*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A13975	*Epthianura tricolor*	Meliphagidae	Whole specimen in ethanol	EM
QM	O.33352	*Epthianura tricolor*	Meliphagidae	Tongue in ethanol	EM
AMNH	1293	*Epthianura tricolor*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A48669	*Gavicalis virescens*	Meliphagidae	Whole specimen in ethanol	EM
UWBM	60861	*Gliciphila melanops*	Meliphagidae	Rehydrated tongue	EM
MCZ	168130	*Glycichaera fallax*	Meliphagidae	Rehydrated tongue	EM
AMNH	5264	*Gymnomyza viridis*	Meliphagidae	Whole specimen in ethanol	ET
WAM	A48851	*Lichenostomus cratitius*	Meliphagidae	Whole specimen in ethanol	EM
UWBM	76551	*Lichenostomus melanops*	Meliphagidae	Rehydrated tongue	EM
MCZ	168100	*Lichenostomus subfrenatus*	Meliphagidae	Rehydrated tongue	ET
WAM	A34154	*Lichmera indistincta*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A48852	*Lichmera indistincta*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A48853	*Lichmera indistincta*	Meliphagidae	Whole specimen in ethanol	EM
USNM	612736	*Lichmera indistincta*	Meliphagidae	Tongue in ethanol	EM
QM	O.33630	*Malurus cyaneus*	Maluridae	Tongue in ethanol	ET
WAM	A48660	*Malurus elegans*	Maluridae	Whole specimen in ethanol	ET
WAM	A14307	*Malurus lamberti*	Maluridae	Whole specimen in ethanol	ET
QM	O.32656	*Malurus lamberti*	Maluridae	Tongue in ethanol	ET
AMNH	4094	*Malurus lamberti*	Maluridae	Whole specimen in ethanol	ET
WAM	A13982	*Malurus leucopterus*	Maluridae	Whole specimen in ethanol	ET
WAM	A13983	*Malurus leucopterus*	Maluridae	Whole specimen in ethanol	ET
WAM	A5692	*Malurus splendens*	Maluridae	Whole specimen in ethanol	ET
QM	O.33379	*Manorina flavigula*	Meliphagidae	Tongue in ethanol	EM
UWBM	57667	*Manorina flavigula*	Meliphagidae	Rehydrated tongue	EM
QM	O.33486	*Manorina melanocephala*	Meliphagidae	Tongue in ethanol	EM
MCZ	168119	*Melidectes belfordi*	Meliphagidae	Rehydrated tongue	ET

AMNH	5100	*Melidectes fuscus*	Meliphagidae	Whole specimen in ethanol	ET
MCZ	168168	*Melidectes torquatus*	Meliphagidae	Rehydrated tongue	ET
AMNH	563	*Melidectes whitemanensis*	Meliphagidae	Whole specimen in ethanol	ET
USNM	614972	*Melilestes megarhynchus*	Meliphagidae	Tongue in ethanol	ET
UWBM	67917	*Melilestes megarhynchus*	Meliphagidae	Rehydrated tongue	ET
MCZ	167949	*Melilestes megarhynchus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168066	*Meliphaga aruensis*	Meliphagidae	Rehydrated tongue	ET
MCZ	168067	*Meliphaga aruensis*	Meliphagidae	Rehydrated tongue	ET
QM	O.33665	*Meliphaga lewinii*	Meliphagidae	Tongue in ethanol	EM
UWBM	76681	*Meliphaga lewinii*	Meliphagidae	Rehydrated tongue	EM
UWBM	76710	*Melithreptus albogularis*	Meliphagidae	Rehydrated tongue	EM
UWBM	76602	*Melithreptus brevirostris*	Meliphagidae	Rehydrated tongue	EM
WAM	A16588	*Melithreptus gularis*	Meliphagidae	Whole specimen in ethanol	EM
WAM	none	*Melithreptus gularis*	Meliphagidae	Whole specimen in ethanol	EM
QM	O.33644	*Melithreptus lunatus*	Meliphagidae	Tongue in ethanol	EM
MCZ	SCO-162	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-086	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-085	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-084	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-083	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-082	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-078	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
USNM	612646	*Melithreptus lunatus*	Meliphagidae	Tongue in ethanol	EM
UWBM	76699	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
MVZ	KMCR700	*Myza celebensis*	Meliphagidae	Tongue in ethanol	ET
AMNH	595	*Myzomela cardinalis*	Meliphagidae	Whole specimen in ethanol	ET
AMNH	5103	*Myzomela cardinalis*	Meliphagidae	Whole specimen in ethanol	ET
MCZ	167869	*Myzomela nigrita*	Meliphagidae	Rehydrated tongue	ET
MCZ	167870	*Myzomela nigrita*	Meliphagidae	Rehydrated tongue	ET
WAM	A26151	*Myzomela obscura*	Meliphagidae	Whole specimen in ethanol	EM
AMNH	5104	*Myzomela obscura*	Meliphagidae	Whole specimen in ethanol	EM
MCZ	167885	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
MCZ	167882	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
MCZ	167889	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
MCZ	167887	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
QM	O.33439	*Myzomela sanguinolenta*	Meliphagidae	Tongue in ethanol	EM
USNM	612651	*Myzomela sanguinolenta*	Meliphagidae	Tongue in ethanol	EM
UWBM	76729	*Nesoptilotis leucotis*	Meliphagidae	Rehydrated tongue	EM
WAM	A27498	*Pardalotus striatus*	Pardalotidae	Whole specimen in ethanol	ET
QM	O.33400	*Pardalotus striatus*	Pardalotidae	Tongue in ethanol	ET
QM	O.32797	*Philemon citreogularis*	Meliphagidae	Tongue in ethanol	EM
MCZ	SCO-134	*Philemon citreogularis*	Meliphagidae	Rehydrated tongue	EM

MCZ	SCO-130	*Philemon citreogularis*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-055	*Philemon citreogularis*	Meliphagidae	Rehydrated tongue	EM
UWBM	57671	*Philemon citreogularis*	Meliphagidae	Rehydrated tongue	EM
QM	O.32772	*Philemon citreogularis*	Meliphagidae	Tongue in ethanol	EM
QM	O.33381	*Philemon corniculatus*	Meliphagidae	Tongue in ethanol	EM
USNM	612653	*Philemon corniculatus*	Meliphagidae	Tongue in ethanol	EM
UWBM	76697	*Philemon corniculatus*	Meliphagidae	Rehydrated tongue	EM
AMNH	5109	*Philemon novaeguineae*	Meliphagidae	Whole specimen in ethanol	ET
QM	O.33431	*Phylidonyris niger*	Meliphagidae	Tongue in ethanol	EM
WAM	A27418	*Phylidonyris novaehollandiae*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A14500	*Phylidonyris novaehollandiae*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A23038	*Phylidonyris novaehollandiae*	Meliphagidae	Whole specimen in ethanol	EM
USNM	612647	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612648	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612649	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612650	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
UWBM	57795	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-166	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-165	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-164	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	SCO-114	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-037	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-036	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
MCZ	23-026	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
AMNH	5121	*Prosthemadera novaeseelandiae*	Meliphagidae	Whole specimen in ethanol	ET
UWBM	79583	*Prosthemadera novaeseelandiae*	Meliphagidae	Rehydrated tongue	ET
MCZ	168074	*Ptiloprora guisei*	Meliphagidae	Rehydrated tongue	ET
MCZ	168072	*Ptiloprora guisei*	Meliphagidae	Rehydrated tongue	ET
AMNH	570	*Ptiloprora guisei*	Meliphagidae	Whole specimen in ethanol	ET
AMNH	646	*Ptiloprora guisei*	Meliphagidae	Whole specimen in ethanol	ET
WAM	A14300	*Ptilotula flavescens*	Meliphagidae	Whole specimen in ethanol	EM
UWBM	60915	*Ptilotula flavescens*	Meliphagidae	Rehydrated tongue	EM
UWBM	76519	*Ptilotula fusca*	Meliphagidae	Rehydrated tongue	EM
WAM	A27522	*Ptilotula keartlandi*	Meliphagidae	Whole specimen in ethanol	EM
UWBM	60839	*Ptilotula penicillata*	Meliphagidae	Rehydrated tongue	EM
WAM	A13968	*Ptilotula plumula*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A13971	*Purnella albifrons*	Meliphagidae	Whole specimen in ethanol	EM
MCZ	168145	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168147	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168146	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
WAM	A23041	*Sericornis frontalis*	Acanthizidae	Whole specimen in ethanol	ET

WAM	A23029	*Sericornis frontalis*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A23021	*Sericornis frontalis*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A27542	*Smicrornis brevirostris*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A38077	*Smicrornis brevirostris*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A13989	*Smicrornis brevirostris*	Acanthizidae	Whole specimen in ethanol	ET
WAM	A14305	*Stomiopera unicolor*	Meliphagidae	Whole specimen in ethanol	EM
WAM	A13987	*Stomiopera unicolor*	Meliphagidae	Whole specimen in ethanol	EM
QM	O.33432	*Sugomel niger*	Meliphagidae	Tongue in ethanol	EM
QM	O.33431	*Sugomel niger*	Meliphagidae	Tongue in ethanol	EM
MCZ	168180	*Xanthotis flaviventer*	Meliphagidae	Rehydrated tongue	EM
MCZ	168182	*Xanthotis flaviventer*	Meliphagidae	Rehydrated tongue	EM

Table S2. Complete list of tongue+hyoid specimens measured for this study. Museum abbreviations are as follows: UWBM = University of Washington Burke Museum, MCZ = Harvard Museum of Comparative Zoology, AMNH = American Museum of Natural History, USNM = Smithsonian, QM = Queensland Museum, MVZ = UC Berkeley Museum of Vertebrate Zoology. Specimen number is listed in the nomenclature of the museum in which the specimen is housed. Diet source data abbreviations indicate whether data is from Miller et al. (2017) [EM] or Wilman et al. (2014) [ET].

Museum	Specimen #	Species	Family	Preservation type	Diet data source
QM	O.32847	*Acanthagenys rufogularis*	Meliphagidae	Tongue in ethanol	EM
QM	O.33171	*Acanthagenys rufogularis*	Meliphagidae	Tongue in ethanol	EM
UWBM	60869	*Acanthorhynchus superciliosus*	Meliphagidae	Rehydrated tongue	EM
UWBM	76471	*Acanthorhynchus tenuirostris*	Meliphagidae	Rehydrated tongue	EM
USNM	612652	*Acanthorhynchus tenuirostris*	Meliphagidae	Tongue in ethanol	EM
UWBM	57776	*Anthochaera carunculata*	Meliphagidae	Rehydrated tongue	EM
USNM	612645	*Anthochaera carunculata*	Meliphagidae	Tongue in ethanol	EM
QM	O.33461	*Anthochaera chrysoptera*	Meliphagidae	Tongue in ethanol	EM
UWBM	76683	*Caligavis chrysops*	Meliphagidae	Rehydrated tongue	EM
WAM	A27168	*Dasyornis brachypterus*	Dasyornithidae	Tongue in ethanol	ET
UWBM	76649	*Entomyzon cyanotis*	Meliphagidae	Rehydrated tongue	EM
QM	O.33363	*Entomyzon cyanotis*	Meliphagidae	Tongue in ethanol	EM
QM	O.32926	*Entomyzon cyanotis*	Meliphagidae	Tongue in ethanol	EM
UWBM	60861	*Gliciphila melanops*	Meliphagidae	Rehydrated tongue	EM
MCZ	168130	*Glycichaera fallax*	Meliphagidae	Rehydrated tongue	EM
UWBM	76551	*Lichenostomus melanops*	Meliphagidae	Rehydrated tongue	EM
USNM	612736	*Lichmera indistincta*	Meliphagidae	Tongue in ethanol	EM
QM	O.33630	*Malurus cyaneus*	Maluridae	Tongue in ethanol	ET
QM	O.32656	*Malurus lamberti*	Maluridae	Tongue in ethanol	ET
QM	O.33379	*Manorina flavigula*	Meliphagidae	Tongue in ethanol	EM
UWBM	57667	*Manorina flavigula*	Meliphagidae	Rehydrated tongue	EM
QM	O.33486	*Manorina melanocephala*	Meliphagidae	Tongue in ethanol	EM
MCZ	168119	*Melidectes belfordi*	Meliphagidae	Rehydrated tongue	ET
MCZ	168168	*Melidectes torquatus*	Meliphagidae	Rehydrated tongue	ET
USNM	614972	*Melilestes megarhynchus*	Meliphagidae	Tongue in ethanol	ET
MCZ	167949	*Melilestes megarhynchus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168066	*Meliphaga aruensis*	Meliphagidae	Rehydrated tongue	ET
MCZ	168067	*Meliphaga aruensis*	Meliphagidae	Rehydrated tongue	ET
UWBM	76729	*Nesoptilotis leucotis*	Meliphagidae	Rehydrated tongue	EM
UWBM	76681	*Meliphaga lewinii*	Meliphagidae	Rehydrated tongue	EM
QM	O.33665	*Meliphaga lewinii*	Meliphagidae	Tongue in ethanol	EM
UWBM	76710	*Melithreptus albogularis*	Meliphagidae	Rehydrated tongue	EM
UWBM	76602	*Melithreptus brevirostris*	Meliphagidae	Rehydrated tongue	EM
UWBM	76699	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
USNM	612646	*Melithreptus lunatus*	Meliphagidae	Rehydrated tongue	EM
QM	O.33644	*Melithreptus lunatus*	Meliphagidae	Tongue in ethanol	EM

MVZ	700	*Myza celebensis*	Meliphagidae	Tongue in ethanol	ET
MCZ	167869	*Myzomela nigrita*	Meliphagidae	Rehydrated tongue	ET
MCZ	167870	*Myzomela nigrita*	Meliphagidae	Rehydrated tongue	ET
MCZ	167885	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
MCZ	167889	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
MCZ	167887	*Myzomela rosenbergii*	Meliphagidae	Rehydrated tongue	ET
USNM	612651	*Myzomela sanguinolenta*	Meliphagidae	Tongue in ethanol	EM
QM	O.33439	*Myzomela sanguinolenta*	Meliphagidae	Tongue in ethanol	EM
QM	O.33400	*Pardalotus striatus*	Pardalotidae	Tongue in ethanol	ET
QM	O.32797	*Philemon citreogularis*	Meliphagidae	Tongue in ethanol	EM
UWBM	57671	*Philemon citreogularis*	Meliphagidae	Rehydrated tongue	EM
QM	O.32772	*Philemon citreogularis*	Meliphagidae	Tongue in ethanol	EM
UWBM	76697	*Philemon corniculatus*	Meliphagidae	Rehydrated tongue	EM
USNM	612653	*Philemon corniculatus*	Meliphagidae	Tongue in ethanol	EM
QM	O.33381	*Philemon corniculatus*	Meliphagidae	Tongue in ethanol	EM
QM	O.33431	*Phylidonyris niger*	Meliphagidae	Tongue in ethanol	EM
USNM	612649	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612648	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612647	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
USNM	612650	*Phylidonyris novaehollandiae*	Meliphagidae	Tongue in ethanol	EM
UWBM	57795	*Plectorhyncha lanceolata*	Meliphagidae	Rehydrated tongue	EM
UWBM	79583	*Prosthemadera novaeseelandiae*	Meliphagidae	Rehydrated tongue	ET
MCZ	168072	*Ptiloprora guisei*	Meliphagidae	Rehydrated tongue	ET
UWBM	60915	*Ptilotula flavescens*	Meliphagidae	Rehydrated tongue	EM
UWBM	76519	*Ptilotula fusca*	Meliphagidae	Rehydrated tongue	EM
UWBM	60839	*Ptilotula penicillata*	Meliphagidae	Rehydrated tongue	EM
MCZ	168145	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168147	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168146	*Pycnopygius cinereus*	Meliphagidae	Rehydrated tongue	ET
MCZ	168180	*Xanthotis flaviventer*	Meliphagidae	Rehydrated tongue	EM
MCZ	168182	*Xanthotis flaviventer*	Meliphagidae	Rehydrated tongue	EM

Table S3. Results of PGLS analyses for each raw morphological variable. Bolded p-value indicates significance. Significance determined as $p < 0.05$.

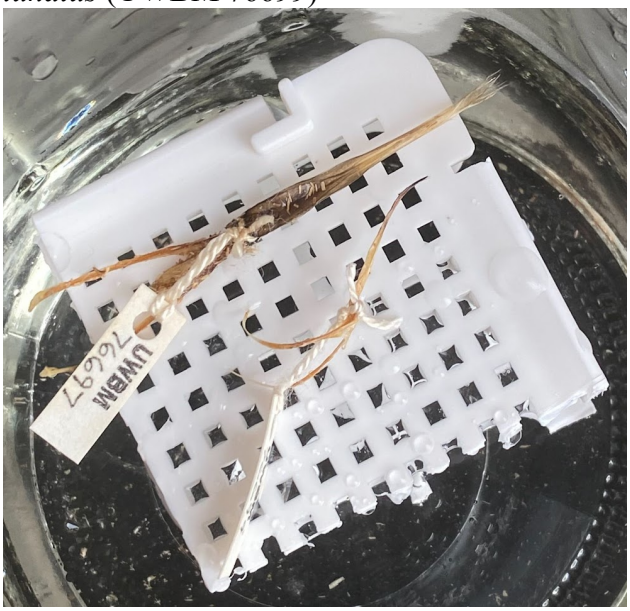
Variable	AIC	Log Likelihood	λ	Slope	Standard Error	t-value	p-value
Tongue length	458.07	-225.04	1.02	6.82	0.00021	32945.20	**0**
Bristle length	360.49	-176.24	1.02	6.79	0.00024	27858.33	**0**
Tongue depth	43.20	-17.60	0.94	0.28	0.23	1.22	0.23
Tongue width	97.31	-44.65	0.94	0.029	0.34	0.086	0.93
Hyoid length	291.91	-141.96	0.90	7.11	6.60	1.08	0.29

File S1. Tongue rehydration procedure. Steps modified from: Singer RA. Are dehydrated specimens a lost cause? A case study to reclaim dehydrated fluid-preserved specimens. Collection Forum. 2014;28(1-2): 16-20. All photos taken by A.E.Hewes

Step	Description	Duration
1	Place a rack at the bottom of a screw top glass jar (jar needs to be sealable) and place tongue(s) on the rack. Pour 200mL of warm thymol solution (10 thymol crystals dissolved in 200mL DI H_2O) into jar and immediately screw on the lid to trap the steam. The tongue(s) should be suspended above, not immersed in, the thymol solution and the container should be sealed.	Leave tongue(s) in sealed jar for 1 week
2	Pour out the thymol solution and remove the rack. Place the tongue(s) in the jar and add 200mL DI H_2O. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
3	Pour out the DI water and replace with 200mL of 10% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
4	Pour out the 10% ethanol and replace with 200mL of 20% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
5	Pour out the 20% ethanol and replace with 200mL of 30% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
6	Pour out the 30% ethanol and replace with 200mL of 40% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
7	Pour out the 40% ethanol and replace with 200mL of 50% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
8	Pour out the 50% ethanol and replace with 200mL of 60% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
9	Pour out the 60% ethanol and replace with 200mL of 70% ethanol. The tongue(s) should be fully submerged in the water and the container should be sealed.	Leave tongue(s) submerged for 24 hours
10	Move tongue(s) to vial of fresh 70% ethanol for permanent storage.	Leave tongue(s) submerged indefinitely

Tongue rehydration procedure continued

Tongues in steam bath – Left: *Philemon corniculatus* (UWBM 76697); Right: *Melithreptus lunatus* (UWBM 76699)



Philemon corniculatus (UWBM 76697) tongue throughout the rehydration process – A) fully desiccated, before rehydration; B) After steps 1-2, partially rehydrated; C) After steps 1-9, fully rehydrated in a dish of 70% ethanol

Chapter 4: Plant-pollinator trait matching affects pollen transfer but not feeding efficiency of Australian honeyeaters (Aves, Meliphagidae)

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Published in *Communications Biology*: https://doi.org/10.1038/s42003-025-07693-w

Abstract
Animal pollination is common among flowering plants. Increased morphological matching between floral and pollinator traits is thought to increase pollen transfer and feeding efficiency, but we lack studies that empirically demonstrate this. Working with Australian honeyeaters, we find that there is positive correlation between bill-corolla matching and pollen deposition at flowers, but no correlation with how efficiently birds can extract floral nectar. The species with the lowest bill-corolla matching deposited the fewest pollen grains but had the highest feeding efficiency, showing that bill-corolla matching expectations were met on the plant side of this interaction but not on the pollinator side. Finally, we find different interspecific patterns of pollen deposition at the scales of a single flower visit versus the landscape, due to differences in patterns of plant visitation. This work illustrates the need for more studies that directly correlate trait matching to fitness proxies of plants and avian pollinators.

Introduction
Animal pollination is a classic example of mutualism[1] ; animal pollinators harvest rewards such as nectar or pollen, while plants receive pollination services that allow them to successfully reproduce. However, animal pollination commonly results in large amounts of pollen loss[2]. In response to selective pressures placed by effective pollinators, floral structures can evolve to be more restrictive (e.g., longer and/or narrower corolla) and can filter access to floral rewards to species that can successfully pollinate[3,4]. When floral morphology is more restrictive, nectar is accessed when a pollinator is feeding in a position that maximizes the likelihood of contact with floral reproductive structures[5–8]. This process reinforces morphological similarity between floral structures and pollinator feeding parts, resulting in plant-pollinator trait matching[3,4]. Trait matching is known to be important in structuring pollination interactions in numerous insect and avian pollination systems[3,9–17] and plant traits that are involved in trait matching tend to have strong signatures of pollinator-mediated selection[18]. Depending on the strength and spatial and temporal variability in pollination selection, trait matching can be the result of, and reinforce, reciprocal coevolution between plants and pollinators[19–21].

In avian pollination systems, hummingbirds in the Neotropics consistently show trait matching with flowers they visit[22–35] and growing evidence suggests that sunbirds in Africa do as well[36,37]. While this pattern has been observed repeatedly in nature, experimental evidence that supports the mechanistic steps by which bill-corolla matching can positively contribute to fitness for both parties, measured via proxies such as feeding efficiency and pollen transfer efficiency, is lacking[34]. Studies that experimentally measure per-visit pollen removal and deposition in avian pollination systems are largely restricted to hummingbirds and these studies have found a positive correlation between bill-corolla matching and pollen transfer success[17,38–41]. However, none of these studies have simultaneously measured feeding efficiency (nectar volume consumed/second). Conversely, many studies have examined how floral traits affect bird nectar extraction but have done so using artificial ‘flowers’ (*i.e.*, plastic tubing) that vary

morphologically in controlled ways (*e.g.*, length or curvature)[42–45]. The few studies of bird feeding rates at real flowers did not measure how much pollen was removed or deposited[24,26,27,46]. We therefore lack studies that simultaneously measure the correlation between bill-corolla matching and fitness proxies for both interaction partners.

Beyond studying pollen removal and transfer in a single flower visit[17,38,39,47], it is important to know the extent to which interspecific patterns in pollen transfer efficiency across bird species will remain consistent when scaling up to landscape-level foraging. Some studies have measured the effect of floral morphology and bill-corolla matching on hummingbird contributions to seed set in wild plants[48], but it is unknown whether interspecific differences are due to differences in pollen transfer efficiency at the level of each flower visit or differences in the number of visits. Spatiotemporal variation in pollinator-mediated selection is likely to be an important determinant of selection on plant morphology[49], as outlined in the geographic mosaic theory of plant-pollinator coevolution[21], so to understand the true evolutionary relevance of bill-corolla matching one would ideally measure its effect at the scale of the flower visit, and then determine whether those effects scale across a visit to an entire plant or multiple plants across the landscape.

Studies on the prevalence and importance of trait matching in pollination interactions are most common in hummingbirds pollination systems (but see [36,37,50,51]). Examining the role of bill-corolla matching in a system with less specialized bird pollinator-plant interactions can provide a broader understanding of these relationships. Honeyeaters, the most common avian pollinators in Australasia[50,52], have more generalized interactions with plants than do hummingbirds[53–55] and are therefore an ideal study system to fill this knowledge gap. While there have been studies examining honeyeater pollination services to native plants[50,56–60], the role of bill-corolla matching in shaping interspecific differences in pollen transfer services has been minimally investigated[50,51] and no study has simultaneously considered fitness proxies for both interaction partners. In this study we examined the role of trait matching, specifically bill-corolla matching, in determining interspecific differences in honeyeater pollen transfer and feeding efficiency at the Australian shrub, *Eremophila maculata* (Scrophulariaceae, spotted emu bush), in the temperate mallee of South Australia. We studied three honeyeater species that constituted >90% of all visits to *E. maculata* in the field. We used high-speed videography of wild-caught birds feeding at *E. maculata* flowers in captivity to simultaneously calculate feeding and pollen transfer efficiency in a controlled, yet real-to-life way. We then combined the laboratory data on pollen deposition with field recordings of honeyeater visitation to *E. maculata* to investigate whether the interspecific differences in pollen deposition at a single flower hold across the landscape. We aimed to answer three questions: 1) Do honeyeater species vary in feeding and pollen transfer efficiency at *Eremophila maculata* flowers, 2) Can that interspecific variation be explained by variation in bill-corolla matching?, and 3) Do interspecific patterns of pollen deposition hold across increasing spatial scales? We predicted there would be a positive correlation between bill-corolla matching and pollen removal, pollen deposition, and feeding efficiency across honeyeater species. Scaling up to the landscape level, we hypothesize that there will likely be interspecific differences in pollen deposition across spatial scales due to interspecific differences in plant visitation. We focus on pollen deposition for this across-scale extrapolation because it is the most likely limiting factor for successful pollination among the variables we measured.

Results

Pollen transfer across honeyeater species

We examined three components of pollen transfer efficiency: 1) the number of pollen grains deposited on the stigma; 2) the duration of contact between the bird's body and the anthers; and 3) the size of the pollen patch on the bird resulting from contact with anthers. We found significant interspecific differences in the number of pollen grains deposited on stigmas (p = 0.004). *A. rufogularis* individuals deposited fewer pollen grains on stigmas than individuals of *Pi. ornata* (Cohen's d = 0.86, p = 0. 017) or *Pu. albifrons* (Cohen's d = 0.82, p = 0. 0013). The number of pollen grains deposited did not significantly differ between *Pu. albifrons* and *Pi. ornata* (Cohen's d = 0.44, p = 0.46) (Figure 1A, Table 1,S1).

There were significant interspecific differences in the duration of anther contact (p = 0.0045) and pollen patch area (p = 0.00057). For duration of anther contact, *Pu. albifrons* had the shortest contact duration, followed by *A. rufogularis*, with *Pi. ornata* having the longest (Table S1). *Pu. albifrons* and *Pi. ornata* were different from each other (Cohen's d = 0.98, p = 0. 011), but neither differed significantly from *A. rufogularis* (*Pi. ornata* - Cohen's d = 0.94, p = 0.054; *Pu. albifrons* - Cohen's d = 0.15, p = 0.97) (Table 1). For area of the pollen patch, *A. rufogularis* individuals had a smaller area of pollen patch than individuals of *Pi. ornata* (Cohen's d = 1.3, p = 0.0008) or *Pu. albifrons* (Cohen's d = 0.71, p = 0.0005), but *Pu. albifrons* and *Pi. ornata* did not significantly differ (Cohen's d = 0.27, p = 0.903) (Figure 1B, Table 1,S1).

Feeding efficiency across honeyeater species

Feeding duration was measured directly from high-speed videos (see Methods). Feeding efficiency was calculated as the microliters of nectar consumed, which was standardized to 30microliters across all trials, divided by feeding duration (see Methods). There were significant interspecific differences in feeding duration (p = 0.0019) and efficiency (p = 0.0018; Figure 1C-D, Table 1,S1). *A. rufogularis* depleted the nectar faster and as a result had a higher feeding efficiency (Figure 1C-D, Table S1), than *Pi. ornata* (feeding duration - Cohen's d = 1.3, p = 0.0018; feeding efficiency - Cohen's d = 1.1, p = 0.0013) and *Pu. albifrons* (feeding duration - Cohen's d = 1.1, p = 0.005; feeding efficiency - Cohen's d = 1.04, p = 0.0075) (Figure 1C-D, Table 1). *Pu. albifrons* and *Pi. ornata* were not significantly different in either feeding metric (feeding duration - Cohen's d = 0.12, p = 0.94; feeding efficiency - Cohen's d = 0.084, p = 0.81) (Figure 1C-D, Table 1).

Bill-corolla matching between honeyeaters and Eremophila maculata

The crux of this study was to investigate whether variability in bill-corolla matching metrics is related to differences in feeding and pollen transfer efficiency across honeyeater species. We quantified bill-corolla matching using several ratios of bill and corolla linear morphometrics (Figure 2). We then used PCA to create a multivariate measure of trait matching that uses all of the ratios measured and handles the inherent correlation between them (Figure 2). We ran one PCA for bird interactions with pollen donor flowers (flowers with anthers intact, see Methods and Figure 3A) and a second PCA for bird interactions with pollen receiving flowers (flowers with anthers removed and stigma only, see Methods and Figure S1A) and we distinguish between these PC scores by noting "pollen donor PC1" and "pollen receiver PC1", "pollen donor PC2" and "pollen receiver PC2", etc. The three honeyeater species were clearly distinguished by PC scores (Figure 3A), and the PCAs for pollen donor and pollen receiver interactions were very similar (compare Figure 3A and Figure S1A). For the pollen donor PCA, PC1 explained 71.7%

of the variation and PC2 explained 15.2% (Figure 3A). For the pollen receiver PCA, PC1 explained 70.7% of the variation and PC2 explained 16.4% (Figure S1A). The loadings of the bill-corolla matching variables were the same for both pollen donor and pollen receiver PCAs (Table S2). In both PCAs, PC1 was dominated by bill-corolla matching length variables and tarsus length, while PC2 was dominated by bill-corolla width matching (Table S2).

A. rufogularis had the lowest PC scores of all three species (Figure 3A), meaning that *A. rufogularis* individuals have the largest bills relative to the size of *E. maculata* flowers out of all three species; this is confirmed when looking at the raw data of ratios between bill and corolla morphometrics (Figure 3B-F). *A. rufogularis* bills are largest relative to *E. maculata* flowers in metrics of length (Figure 3B-E) and width (Figure 3F). Conversely, *Pi. ornata* had the highest PC scores of all three species (Figure 3A), meaning that *Pi. ornata* individuals had the smallest bills relative to the size of *E. maculata* flowers out of all three species (Figure 3A). The raw data of ratios between bill and corolla morphometrics shows that *Pi. ornata* bills are smallest relative to *E. maculata* flowers in metrics of length (Figure 3B-E,) but are a similar size to *E. maculata* flowers width (Figure 3F). Finally, *Pu. albifrons* had scores that were intermediate between those of *A. rufogularis* and *Pi. ornata* (Figure 3A). In some trials the bill of *Pu. albifrons* individuals was larger than the *E. maculata* flower it fed at, while in other trials the bill was smaller than the flower. This is once again seen in the raw data, where *Pu. albifrons* has intermediate values for all metrics of length (Figure 3B-E) and a similar size to *E. maculata* flowers in width (Figure 3F). In the models below where bill-corolla matching is investigated (Figure 4, Table 2), PC scores were converted to absolute value for better interpretability and model fitting. Zero is the mean of each principal component and deviation from zero indicates a larger or smaller bill relative to *E. maculata* flowers (Figure 3A).

Relationship between bill-corolla matching and pollen transfer and feeding efficiency

There was no significant correlation between area of the pollen patch and bill-corolla matching (Table 2). The marginal R^2 (fixed effects only) of the model was very low at 0.0089, showing that bill-corolla matching explains almost none of the interspecific variation in area of the pollen patch. The conditional R^2 of the same model, which considers the fixed effects and the nested random effects of individual within species, was 0.55, which is a substantial increase from the marginal R^2 and shows that much of the variation is explained by the nested random effects of individual within species.

There was a significant correlation between pollen deposition on stigmas and bill-corolla matching (Figure 4, Table 2). The number of pollen grains deposited on *E. maculata* stigmas was negatively correlated with the absolute value of the bill-corolla matching PC1 score (β = -0.37, p = 0.033), with a one unit increase in PC1 score resulting in a 31% decrease in mean pollen deposition (Figure 4, Table 2). The bill-corolla matching PC2 score had a slight negative, but non-significant relationship with pollen deposition (β = -0.18, p = 0.49). Pollen deposition was also positively correlated with the area of the pollen patch picked up from the previous flower visit (β = 0.019, p = 0.019), with a one unit increase in pollen patch area resulting in a 2% increase in mean pollen deposition (Figure 4, Table 2). The marginal R^2 of the model was 0.15, while the conditional R^2 was 0.39 (Table 2).

When examining the correlation with bill-corolla matching, we focused on volumetric uptake rate only as it had the same interspecific differences as total time spent feeding so examining both would be redundant. There was no correlation between feeding efficiency and bill-corolla matching (Table 2). The marginal R^2 of the model was 0.0083 while the conditional

R^2 was 0.35, showing that much of the variation is explained by the nested random effects of individual within species. We did not find a correlation between feeding efficiency and bill-corolla matching (Table 2) but there were interspecific differences (Figure 1D, Table 1). To investigate possible reasons for these differences, we characterized licking rate and found that *A. rufogularis* had the lowest licking rate of the three species, despite having the highest feeding efficiency, while *Pu. albifrons* and *Pi. ornata* had similar licking rates that were higher than those of *A. rufogularis* (Figure 5, Table S1).

Scaling up to the pollination landscape

To determine whether interspecific patterns of pollen deposition from experiments held when considering patterns of foraging behavior, we combined the experimentally derived data on per-flower pollen deposition with camera trap data that quantified the number of flowers probed per visit and the total number of recorded visits. We estimated the per-plant number of pollen grains deposited by each species and the number of pollen grains deposited across the landscape. We found significant interspecific differences in estimated mean pollen deposition per plant visit and in total pollen deposition across the landscape (Figure 6A-B, Table 3). *Pu. albifrons* had the highest estimated mean pollen deposition per plant visit, followed by *Pi. ornata* and *A. rufogularis* and the effect sizes across all pairwise comparisons of species were large (Figure 6A, Table 3). The pattern was different at the landscape scale, where *Pi. ornata* had the highest estimated total pollen deposition, followed by *Pu. albifrons* and *A. rufogularis* (Figure 6B, Table 3). While the pattern differed across scales, the effect sizes across all pairwise comparisons of species were still large at the landscape scale (Figure 6B, Table 3). There was no significant difference across species in the number of flowers probed per visit (Figure 6C, Table 3), but *Pi. ornata* had the greatest number of visits recorded, followed by *A. rufogularis,* and *Pu. albifrons* (Figure 6D, Table 3).

Discussion

In this study we found that honeyeaters differed in their pollen removal, pollen deposition, and feeding efficiency at *E. maculata* flowers. While bill-corolla matching was correlated with pollen deposition on *E. maculata* stigmas, it was not correlated with pollen removal from flowers or with honeyeater feeding efficiency. The presence of a correlation between bill-corolla matching, particularly in bill length relative to corolla length, and pollen deposition on the stigma (Figure 4B) is not unexpected due to the floral morphology of *E. maculata*, where the style and stigma are oriented in the longitudinal plane of the flower (Figure 2). During a given visit, the length of the flower relative to the bill plays a large role in determining where the stigma will make contact with the bird's head (Figure 1E-G). Interspecific differences in pollen patch area are not explained by bill-corolla matching (Table 2), but the location of pollen deposition on the birds' body could have implications for the persistence of that pollen post-anther contact (Figure 1E-G). In all *A. rufogularis* visits the pollen was deposited on the base of the bill, lores (feathers between gape and eye), and/or lower forehead feathers (immediately posterior to the bill; Figure 1E). This matches the findings in the morphological PCA (Figure 3A), which demonstrated that all *A. rufogularis* individuals have bills larger, specifically longer and wider, than the flowers they visited. The bill and surrounding feather patches may be a precarious substrate for pollen adherence and therefore could have been a reason for the smaller pollen patches observed in *A. rufogularis*. This is especially important for honeyeaters as they typically perform a "bill whipping" behavior in which the bill is brushed along the perch after feeding[50] which, if most of

the pollen is on or near the bill as in *A. rufogularis*, could reduce the carried pollen load. In *Pu. albifrons* and *Pi. ornata* the pollen was placed on the crown (Figure 1F-G), because the bill was shorter and narrower (Figure 3) and was probed further inside the flower (Figure 1F-G). The larger and flatter (more horizontally oriented) crown feathers could provide a more secure environment for the pollen grains to adhere to and therefore a large pollen patch could be created, and subsequently more pollen could be carried from the pollen donor flower to the pollen receiver flower. Previous work on honeyeater pollination has shown a similar trend, where pollen is typically deposited on the facial feathers of shorter billed honeyeaters and on the bill of longer billed honeyeaters[50], suggesting that future research on pollen transfer by honeyeaters should investigate the concept of floral adaptive accuracy[7,8] and where on the pollinator body the pollen is being placed. Two previous studies in sunbirds found results that echo what we found in *A. rufogularis*. Among several species of African avian pollinators, the morphologically specialized amethyst sunbird (*Chalcomitra amethystina*) is a worse pollinator of *Aloe ferox* plants than generalized, opportunistic nectar feeding birds such as weavers (*Ploceus ocularis*, *Ploceus cucullatus spilonotus*) and bulbuls (*Pycnonotus tricolor*)[47]. Similarly, in a Mediterranean avian pollinator community the morphologically specialized Palestine sunbird (*Cinnyris osea*) was a worse pollinator of *Anagyris foetida* plants than various species of warblers, bulbuls, and sparrows that visit plants to opportunistically consume nectar[61]. Like *A. rufogularis*, *Ch. amethystina* and *Ci. osea* have bills longer than the flowers they visited, while the generalist species have bills closer in size to, or shorter than these flowers. In these sunbird systems, the generalist birds had to stick their heads deeper into the flower and more pollen was deposited on their head feathers, which could then be carried to another flower[47,61], while the sunbird either had some pollen deposited on the bill's smoother surface or missed the reproductive structures all together.

An alternate, but not mutually exclusive, reason for the low pollen deposition by *A. rufogularis* could be their feeding efficiency. *A. rufogularis* had the highest feeding efficiency and therefore the shortest amount of time in contact with the flower, which could have resulted in the low rate of pollen deposition. The only interspecific difference in anther contact duration was between *Pu. albifrons* and *Pi. ornata* (Table 1), so it might not be that short feeding durations by *A. rufogularis* result in short duration of contact with the anthers, but rather with short duration of contact with the stigma; compounded with the differences in the location of the pollen patch across species, this could explain the low pollen deposition rates by *A. rufogularis.* While we did not find a correlation between bill-corolla matching and feeding efficiency (Table 2), there are other factors that could explain the interspecific differences observed (Figure 1D, Table 1). *A. rufogularis* has a longer tongue than *Pi. ornata* and *Pu. albifrons*[62] and a slower licking rate (Figure 5). *A. rufogularis* may therefore need fewer licks than *Pu. albifrons* and *Pi. ornata* to extract the same amount of nectar because it can capture larger aliquots of nectar with each lick. While bill-corolla matching between the bill and the flower may not be important in determining honeyeater feeding efficiency at *E. maculata*, this variation in efficiency is congruent with differences seen in landscape-scale honeyeater foraging patterns. Larger bodied honeyeater species are known to visit more flowers at a faster rate before moving on to the next plant[51,63], which could be due to increased feeding efficiency on a per-flower basis due to larger tongues, such as we observed in *A. rufogularis*.

Plant and landscape-level foraging patterns are important for determining bird contribution to plant fitness. When examining pollen deposition at various scales, we found that the interspecific patterns of pollen deposition on *E. maculata* stigmas changed from the scale of a

single flower visit, a visit to an entire plant, or across the landscape (Figure 6, Table 3). At the scale of the plant visit, *Pu. albifrons* was estimated to deposit a greater number of pollen grains per plant on average than *Pi. ornata* or *A. rufogularis* (Figure 6A, Table 3); this pattern results from the fact that *Pu. albifrons* individuals had a higher mean pollen deposition per flower than the other two species (though not significantly different from *Pi. ornata*, Table 1,S1) with more high deposition visits (100+ grains deposited, Figure 1A) and is not due to interspecific differences in the number of flowers probed per visit (Figure 6C, Table 3). In contrast, at the landscape level *Pi. ornata* was estimated to deposit a greater number of pollen grains across all *E. maculata* plants observed via camera traps than *Pu. albifrons* or *A. rufogularis* (Figure 6B, Table 3); this pattern results from the fact that *Pi. ornata* individuals were the most frequently observed species at *E. maculata* plants (Figure 6D, Table 3). For self-incompatible plant species like *E. maculata*, the tendency for bird species to visit multiple flowers per plant could reduce the overall pollination efficiency and contribution to seed set of those species. For example, if larger honeyeaters with larger bills remove and deposit less pollen when moving between *E. maculata* flowers, such as *A. rufogularis* did in these experimental trials, and they visit the same number of flowers per plant as other honeyeater species, or more if it is energetically efficient to do so, they may be largely transferring individual-own pollen to stigmas[64]. Existing evidence complicates this simple explanation, however. While not studied in *E. maculata* explicitly, past work on other Australian plants that are pollinated either in-full or in-part by honeyeaters has found high levels of seed paternal diversity. This finding suggests that there are likely other facets of honeyeater behavior in comparison to other pollinator groups (e.g., insects, non-flying mammals) beyond number of flowers probed per visit that affect the extent to which pollen is carried over within and between individual plants during foraging[65]. The distance traveled during foraging, the degree of plant fidelity during foraging, and the propensity to engage in aggressive interactions, which could dislodge pollen from feathers, mix pollen loads between interacting birds, or prevent certain pollinators from accessing flowers, could all be important.

Bill-corolla matching has been minimally considered as a force shaping honeyeater-plant interactions [50,54,55,64]. The current work demonstrates that bill-corolla matching is correlated with differences in pollen deposition across honeyeater species, demonstrating that the role of trait-matching in determining plant-bird interactions should be further investigated in this system. There are several limitations of the present work that should be investigated in further studies, the first being that fitness proxies were measured for both interaction partners rather than actual fitness. While pollen transfer is undoubtedly an important step in the process of pollination and per-visit pollen deposition is a common proxy of pollinator contribution to plant reproduction[66], the relevance of it to ultimate measures of fitness such as seed set and seed genetic diversity vary depending on the breeding system of the plant species[66]. *E. maculata* is an outcrossing, self-incompatible species that has a high pollen/ovule ratio, meaning that many pollen grains are needed for sufficient fertilization of ovules[67]; this does not mean, however, that more pollen on the stigma causally equates to higher seed set and higher plant fitness, especially if birds probe multiple flowers per plant and deposit individual-own pollen. Future studies should quantify pollen limitation and seed set in *E. maculata* in the field to more directly determine the contribution of honeyeaters, and ideally individual honeyeater species, to plant fitness. Additional work should also be done examining differences in honeyeater pollination services between populations of *E. maculata*. The geographic mosaic theory of coevolution established that coevolution, for example that between plants and pollinators, occurs due to a mosaic of selection pressures in space and time between populations of interacting species[19–21] and many

studies support the geographic mosaic theory for plant-pollinator trait matching[11–15,25,33,68]. *E. maculata* is found throughout continental Australia, and while to our knowledge there are no other studies on honeyeater visitation to *E. maculata*, natural history accounts note that honeyeater species such as *Sogumel nigrium* (black honeyeater) and *Certhionyx variegatus* (pied honeyeater) visit the species in the more arid interior portions of the continent[69]. *S. nigrium* and *C. variegatus* are morphologically quite different than the three species examined here, they are smaller and have longer, thinner, more decurved bills[69], and the ecological context between interior Australia and the temperate mallee ecosystem where this study was conducted are also notably different; conducting studies on these populations would allow for more convincing discussions of the presence or absence of trait matching-mediated coevolution between honeyeaters and *E. maculata*.

While much theoretical work on the evolution and maintenance of plant-pollinator relationships and networks has focused on invertebrates due to their tractability for manipulative and large-scale experiments, leveraging the phylogenetic power of the multiple independent origins of avian pollination could provide an ideal study system to address these questions and may produce very different answers, as there are fundamental differences between invertebrate and avian pollination systems[65]. For example, bird-pollinated plants have higher seed paternal diversity than insect-pollinated plants, which has been attributed to a number of life history and behavioral differences between birds and insects including higher mobility and aggression between individuals[65]. Building more bird-plant pollination networks that consider not only visitation rates but the contribution of each bird species to seed set and seed paternal diversity would allow for calculation of network-level metrics like connectance and specialization that are informed by the actual contribution of bird species to plant reproduction[65]. In insect pollination systems these metrics are known to relate to the robustness of plant-pollination interactions to extinction[70], but they are usually only created with pollinator visitation data which does not accurately capture the contribution of each pollinator to plant fitness. Additionally, the increased paternal diversity offered by bird pollination may add additional robustness to bird-plant networks compared to insect networks making them less easily perturbed, even if bird-plant networks differ amongst themselves in metrics like conductance and specialization[54]. Considering the existing evidence documenting the ways in which native honeyeater-plant interactions are already being disrupted by invasive European honeybees[71,72], conducting further work in the honeyeater pollination system is a necessary and time-sensitive endeavor.

Methods

Study site

This study was conducted at Gluepot Reserve in South Australia (-33.762375, 140.124265), which is in the Murray-Darling Depression Bioregion[73] that spans much of southeastern South Australia as well as portions of New South Wales and Victoria. Gluepot is a roughly 54,500-hectare property and was pastoral land from 1877 until it was purchased by BirdLife Australia in 1997 and contains a mix of mallee woodland and casuarina woodland (specifically *Casuarina pauper*), with patches of open shrubland throughout due to the construction of dams (water impoundments for livestock) and the history of grazing. Mallee communities have experienced severe degradation and are cause for conservation concern in Australia[74]. The area encompassing Gluepot Reserve, along with Taylorville Station in the south, Calperum Station to the east and northeast, and Danggali Reserve to the north constitute one of the largest contiguous blocks of

mallee woodland left in Australia[75]. Gluepot is typically dry, receiving 200-250mm of rainfall per year.

Camera trapping

There were no existing data on bird visitation to *E. maculata* flowers for Gluepot Reserve. To monitor bird visitation, we placed 16 cameras at *E. maculata* plants at four sites (four per site) across Gluepot; these sites were 9.2kilometers apart on average, with the closest two sites being 3.5km apart and the furthest two sites being 10.5km apart, and varied in size from roughly 0.5-1 hectare, with varying numbers of *E. maculata* plants per site. Because we were specifically interested in understanding which honeyeaters visited *E. maculata*, we placed our cameras at sites with high numbers of *E. maculata* plants and directing cameras towards plants that were in flower. Three of these sites were at dams surrounded by mallee, with minimal open shrubland. The final site did not have a dam and consisted of a small patch of open shrubland surrounded by mallee.

Cameras were deployed for a total of two weeks in September 2022 and were rotated to new plants within each site after the first week. Cameras were on 24 hours per day and were motion activated, set to record 30-second video clips when triggered. This totaled 5,376 hours of camera deployment and 100 hours of video, which were used to determine which honeyeater species were most frequently visiting *E. maculata* within Gluepot Reserve. All videos were watched by A.E. Hewes at 1x speed to determine the species and number of birds visiting the plant, as well as the number of flowers probed by each individual bird. The three species that were the most common were yellow plumed honeyeaters (*Pituloa ornata*), white fronted honeyeaters (*Purnella albifrons*) and spiny cheeked honeyeaters (*Acanthagenys rufogularis*) (Figure S2), constituting >90% of visits. Because these three species were the most common and exhibited a range of bill morphologies (Table S3), we focused on those species for the rest of the study.

Bird handling and morphometry

We conducted mist netting from 0400-1200, the hours of peak bird activity in these habitats. We chose to mist net in areas of highest honeyeater density, which were also areas with large patches of *E. maculata* and the same areas we had previously placed camera traps. Nets were continuously monitored and birds removed immediately. Upon capture we used clear adhesive tape to clean the bird of existing visible pollen. Each bird was transferred from the point of capture to the field lab in an individual bird bag. After capture, bird morphometrics were measured in-hand before each bird was released into its 60 cm x 60 cm x 60 cm filming cage for acclimation. We measured body mass (g) using a Pesola spring scale and linear morphometrics using calipers. Wing chord (mm) was measured on as folded wing from the carpal joint to the tip of the longest primary feather, tarsus length (mm) was measured from intertarsal joint to the foot, bill length (mm) was measured from the feathers at the base of the bill to the tip of the bill, bill width (mm) was measured on the proximal side of the nares as the distance from one side of the bill to the other, and bill depth (mm) was measured on the proximal side of the nares as the height from the dorsal to ventral bill surface. The mean values ($\pm$ SD) for all morphometrics for each species are given in Table S3.

Each bird was housed individually, and visual barriers were put between cages to minimize stress. No more than two birds were kept in captivity at the same time. Birds were given access to Wombaroo Lorikeet & Honeyeater Food (Wombaroo Passwell, Glen Osmond,

South Australia) and a 20% sucrose solution (wt/wt) *ad libitum*. Birds were given several hours between capture and filming, during which time they were observed to ensure they were moving normally around the cage, preening, and feeding. To help birds acclimate to feeding on flowers while in captivity, we also gave birds access to several emasculated (stamens removed) *E. maculata* flowers filled with 20% sucrose (wt/wt). Any birds that were not feeding after several hours in captivity were released at the point of capture. All birds were caught, filmed, and released in the same day, and no birds were held in captivity for more than 8 hours. Birds were marked on the crown with nail polish before release to prevent reuse if recaptured. We filmed 20 individuals: 9 *Pituloa ornata* (yellow-plumed honeyeater), 8 *Purnella albifrons* (white-fronted honeyeater), and 3 *Acanthagenys rufogularis* (spiny-cheeked honeyeater). All individuals were adults and sex was unknown (all species are sexually monomorphic). We have complied with all relevant ethical regulations for animal use. All work was done under University of Adelaide animal ethics approval S-2022-019 and animal trapping permit E27217-1 issued by the South Australian Department for Environment and Water, and we received permission from the Gluepot Management Committee was obtained prior to conducting field work.

Collecting flowers

The genus *Eremophila* (family Scrophulariaceae) is endemic to Australia, where it is widespread and common in arid environments. Most species of *Eremophila*, including *E. maculata*, are perennial woody shrubs with densely arranged branches of hermaphroditic flowers[76,77]. Across *Eremophila* the flowers consist of 5 petals that vary in their degree of fusion, which tends to correspond with the primary pollen vector as species across the genus are differentially adapted to bird-based pollination (ornithophily) or insect-based pollination (entomophily)[77]. *E. maculata* is an ornithophilous species and this is reflected in its floral morphology; the petals are fused along their entire length except for the lower petal, which is only fused along roughly half its length and is reflexed back to form a lip, and the corolla varies in color but is most typically bright pink with dark pink spots (Figure S3). *E. maculata* is self-incompatible and requires cross pollination from a conspecific to successfully set seed[67,76,77]. *E. maculata* is protandrous, the anthers dehisce before the stigma is completely grown to limit the number of self-pollen grains on the stigma[67,76].

For feeding/pollen transfer trials we needed *E. maculata* flowers that had never been contacted by pollinators. We put pollinator exclusion bags on twelve *E. maculata* plants across three sites of the four sites we had previously placed camera traps, covering clusters of flowers that had not yet bloomed. Bags were checked daily to monitor blooming, particularly anther dehiscence. For flowers that were to be pollen recipients, anthers were clipped off using iris scissors before opening to reduce the chance of self-pollination. For flowers that were to be pollen donors, anthers were allowed to open but only flowers that had opened within the past 24 hours were used in filming to reduce the chance of losing pollen to wind, rain, and other disturbance. The morning of each filming session, we went into the field and removed all viable pollen donor and recipient flowers from the bags on the *E. maculata* plants we were monitoring. Each flower was placed in a clean, covered plastic test tube and the plant ID and flower number were tracked. The tubes were kept in a test tube rack in a refrigerator (4 °C) until they were removed for photographing and filming. Flowers were used at random during filming and each flower was only used one time.

To ground truth whether our data on pollen deposition from experimental trials fell within a reasonable range, we collected flowers from the field to examine their pollen load. We

collected stigmas from *E. maculata* flowers that were unlikely to be visited by pollinators due to the fact that the anthers had not opened and there was no nectar being produced (based on visual inspection of the flower). We also collected stigmas from visited flowers, which were producing nectar and had signs of visitation (some or all pollen removed from anthers). These flowers were collected at random from the *E. maculata* individuals we were observing, and their stigmas were prepared for examination under a microscope using the method described below. Because the pollen deposition data from the experimental trials fell between the range of pollen grains observed from stigmas of unvisited and visited flowers in the field, this supports the efficacy of the flower collection method (*i.e.*, there was no contamination of stigmas prior to the experiment). The sample sizes for number of stigmas from unvisited and visited flowers, as well as for each honeyeater species from the experiments, are given in Table S4.

Videography

Immediately before filming, flowers were photographed with a scale bar from the ventral and lateral perspective by a camera oriented perpendicular to the plane of interest. Floral morphometrics were quantified later in FIJI[78] for calculation of bill-corolla matching (Figure 2). The naturally occurring nectar was removed from each flower using 25 microliter microcapillary tubes and was replaced with 30 microliters of 27% sucrose solution (wt/wt) using a micropipette. The volume and concentration of sucrose solution employed in our experiments was determined by measuring nectar produced by *E. maculata* individuals at Gluepot using a Milwaukee MA871 digital brix refractometer allowing us to simulate the nectar available in an *E. maculata* flower as accurately as possible. Flowers were affixed to the wall of the cage perpendicular to the lateral view camera and were oriented such that they were parallel to the floor of the cage (in a standardized orientation commonly found in the wild *E. maculata* plants). The perch used to approach the flower and feed was set for each individual bird, adjusted during the post-capture acclimation period to ensure that the bird could reach the flower site comfortably, without showing signs of straining or difficulty while feeding.

Each bird participated in five two-part trials and the five trials were done in sequence. First, the bird was presented with a pollen donor *E. maculata* flower (flower that was collected within 24 hrs of dehiscence) and allowed to feed at that flower. The pollen donor flower was then removed from the cage and replaced with a pollen recipient flower (flower that had been emasculated before dehiscence and was stigma-only) and the bird was allowed to feed at that flower. The pollen recipient was subsequently removed from the cage and the process was repeated four more times, for a total of five donor and five recipient flowers per bird. Within pollen donor and pollen receiver groups, flowers were presented randomly with respect to plant ID to each bird (i.e., birds were not only presented with flowers from the same site in which they were captured); this was done to minimize the role of unmeasured site-level effects on flower morphology or traits relevant to pollen transfer (e.g., pollen load) on the parameters measured for this study. After each flower was removed from the cage it was dissected longitudinally and checked for remaining nectar. Any remaining nectar inside the flower was measured using a microcapillary tube. In 100% of trials the 30 microliters of nectar that was artificially placed into the anterior chamber of the corolla (Figure S3) was completely consumed. In 10% of trials there was residual nectar on the surface of the nectary of the corolla (Figure S3). Based on our floral dissections and observations of floral anatomy (Figure S3) we know that access to the nectary of the corolla is physically obstructed by the filaments and ovary such that it is likely inaccessible to birds during feeding. We infer that the occasional appearance of residual nectar in the nectary

represents floral nectar that was not fully removed via capillary tubes due to the constriction above the nectar (Figure S2). Additionally, the 10% of trials where remaining nectar was observed were spread across 8 individuals from all 3 species (SC2, SC3, WF1, WF2, WF4, WF7, YP4, YP5), such that any effect would be unlikely to affect interspecific comparisons.

Each floral interaction was filmed at 800 frames per second from the dorsal and lateral perspectives using two Chronos 1.4 high-speed video cameras with Nikon AF Micro Nikkor 105mm f/2.8 lenses. The high-speed videos were analyzed to measure proxies for pollen transfer and feeding efficiency (see below).

Quantifying pollen acquisition

We measured two variables as proxies of pollen removal from the abovementioned high-speed videos: the duration of contact between the bird and the anthers (*s*) and the area of the pollen patch left on the bird's head (mm^2). Both values were measured using the abovementioned high-speed videos, as the field of view for filming was sufficiently large to capture the entire contact between the bird's head and the anthers. The structure of *E. maculata* anthers also makes contact easy to visualize, as the 4 filaments are elongated such that the anthers extend beyond the opening of the corolla and the anthers are reniform (kidney-shaped) creating a broad, flat surface on which to display pollen (Figure S3). Duration of anther contact was measured using the video analysis software DLTdv8a[79] to count the number of frames where the anthers were seen contacting the bird's body (head and/or bill) and depositing pollen; frame number was converted to seconds dividing by the frame rate (800 fps). The area of the pollen patch left on the bird's head was measured using still images from dorsal-view videos in which the birds head was roughly perpendicular to the filming plane. Images were uploaded into FIJI[78], known floral morphometrics were used to set the image scale in pixels/mm, and the draw polygon tool was used to outline the patch of deposited pollen and measure it in mm^2 .

Quantifying pollen deposition

Pollen deposition on the stigma of recipient flowers was measured using the flowers from the feeding/pollen transfer trials. After the bird's visit, the flower was removed from the filming cage and the style was cut off and placed in a clean 1.2 mL microcentrifuge tube. Stigma samples were kept at room temperature until the end of the filming session for a given individual. Each stigma was flattened on a glass microscope slide and stained using basic fuchsin jelly[80]. The number of *E. maculata* pollen grains on the stigma were counted by viewing slides under an Olympus BX53 microscope. The pollen grains of *E. maculata* are similar in shape to many other genera of the tribe Myoporeae (family Scrophulariaceae) and they can be described as tri-colporate, isopolar, and radiosymmetrical[77]. To identify the pollen grains, we used Chinnock (2007) and the Australian Pollen and Spore Atlas. We did not find pollen grains of any other species in any of the microscope slides.

Because birds participated in five trials within a single day, they could have potentially accumulated some pollen on their body between trials. We chose not to recapture birds and physically remove pollen between trials with brushes or tape because it would have placed more stress on the birds, and because visiting multiple flowers in succession is more similar to what occurs in nature. Before conducting our statistical analyses, we examined whether there was an effect of trial on pollen deposition, and we found no such effect (see Modeling pollen transfer and feeding efficiency section for details, Figure S4).

Quantifying feeding efficiency

Total time spent feeding was measured, along with feeding efficiency in microliters/second, as 30 microliters divided by the duration of the feeding bout in seconds which was measured from the high-speed videos using the video analysis software DLTdv8a[79]. We used forward hyoid movement (visible in the throat, indicative of forward tongue movement) as the marker of the beginning of a feeding bout and used the hyoid coming to rest in the throat, just before the bill was pulled out of the flower, as the marker of the end of a feeding bout; frame number was converted to seconds by dividing by the frame rate (800 fps). Finally, to better characterize differences in feeding efficiency across the three species we also measured licking rate. The number of licks was counted by counting each protrusion/retraction cycle of the tongue, or each cycle of hyoid movement in cases were the tongue was obscured by the floral corolla. The number of licks was counted and divided by the total feeding bout time to get a measurement of licks/second.

Quantifying bill-corolla matching using PCA

We define bill-corolla matching as the ratio between bird and floral linear morphometrics, for example bill length divided by corolla length. The definition of each floral measurement and the corresponding bill-corolla matching metric are given in Figure 2. We focused on metrics that were ratios of width and lengths, as those metrics have been found to be important in previous work on bird-plant feeding interactions[42–44,50], and tarsus length was used in analyses to account for body size[81]. In the raw data, a value of 1 indicates the two structures have the same value for a given bill-corolla matching metric, a value <1 indicates that the floral value is greater than the bird value (*e.g.*, corolla is longer than bill), and a value >1 indicates that the bird value is greater than the floral value (*e.g.*, bill is longer than corolla) (Figure 2B-F).

Because we had a number of bill-corolla matching metrics to investigate (Figure 2), we used a principal component analysis (PCA) to reduce the number of dimensions of the morphological data. We ran one PCA for bird interactions with the pollen donor flowers and a second PCA for bird interactions with the pollen receiving flowers using the prcomp() function in the stats package v 4.2.3[82] in R v 4.2.3[82]. In both PCAs the data were centered and scaled as part of the analysis. We distinguish between these PC scores by noting "pollen donor PC1" and "pollen receiver PC1", "pollen donor PC2" and "pollen receiver PC2", etc. In the models where bill-corolla matching is investigated (Table 2), we used the absolute value of all PC scores (i.e., the magnitude of the score only, not the sign) as it greatly improved the fit of the models and our ability to interpret the results. A PC score of 0 indicates the closest bill-corolla match. The further away from zero the PC score, the more mismatched the bill and corolla are.

Statistics and Reproducibility

Reproducibility

We collected data on 20 individual birds from 3 species: 9 *Pituloa ornata* (yellow-plumed honeyeater), 8 *Purnella albifrons* (white-fronted honeyeater), and 3 *Acanthagenys rufogularis* (spiny-cheeked honeyeater). The complete dataset of feeding/pollen transfer interactions contains 100 bird-flower trials composed of 100 pollen donor visits and 100 pollen recipient visits, totaling 200 individual flower visits.

Modeling pollen transfer and feeding efficiency

All statistical analyses were conducted in R v 4.2.3[82] with alpha =0.05. We began by determining whether there was an effect of trial number on the amount of pollen deposition. Due to the unbalanced number of observations across species, we used the Anova() function with a type III sum of squares from the car package v 3.1-2[83] to determine whether there was a significant effect of trial number, species, or the interaction between trial number and species on the number of pollen grains deposited on *E. maculata* stigmas during experimental trials. As neither trial number nor trial*species were significant (Figure S4), we did not incorporate trial as a predictor in any of the following models. We then used generalized linear mixed effects models (GLMMs) to determine whether honeyeater species differ in metrics of pollen transfer: pollen deposition on stigmas (Mod1), anther contact duration (Mod2), or the area of the pollen patch (Mod3), or in metrics of feeding efficiency: feeding duration (Mod4) or microliters of nectar consumed per second (Mod5). We used GLMMs to answer these questions because we did not expect the data to fit all assumptions of a standard linear model, namely, independence of residuals, as each bird was measured multiple times, and a Gaussian error distribution, as our data was either counts (*e.g.*, number of pollen grains deposited on the stigma) or positive and continuous, with or without zeros (*e.g.*, feeding efficiency). All models were run using the glmmTMB() function in the glmmTMB package v 1.1.7[84].

In Mod1-Mod5 the fixed effect of interest was species and individual bird ID was included as a random effect. We were not interested in intra-individual differences per se but needed to account for them statistically as each bird participated in multiple trials. Pollen deposition on stigmas was modeled using a negative binomial distribution with a log link, due to the fact that the response variable was counts with large variance relative to the mean. Duration of anther contact and area of pollen patch were modeled with a Tweedie distribution with a log link because our response variables were continuous, positive and included zeros, making other distributions such as Gaussian or Gamma inaccurate fits. Finally, feeding duration and feeding efficiency (volumetric intake rate) was modeled using a gamma distribution with a log link, as our response variables were continuous, positive and non-zero.

The fit of all models was checked with model validation operations in the DHARMa package v 0.4.6[85]. In cases where zero inflation affected model fit, a zero-inflation parameter by species was incorporated into the model (Mod2 and Mod3). For all models, the Anova() function in the car package v 3.1-2[83] was used to extract p-values for the fixed effect of "species" and the function contrast() from the emmeans package v 1.8.5[86] was used to extract pairwise p-values across levels of the fixed effect. The cohens_d() function from the rstatix package v 0.7.2[87] to was used to calculate the pairwise effect sizes across levels of the fixed effect. Marginal and conditional R^2 were estimated using the r.squaredGLMM() function from the MuMIn package v 1.47.5[88].

Modeling trait matching and the correlation with pollen transfer and feeding efficiency

Having established that honeyeater species differ in bill-corolla matching using PCA and in metrics of pollen transfer and feeding efficiency in Mod1-Mod5, we then used GLMMs to determine whether bill-corolla matching is correlated with area of the pollen patch (Mod6), pollen deposition on the stigma (Mod7), or microliters of nectar consumed per second (Mod8).

In Mod6-8 the explanatory variable of interest was trait matching, but we needed to account for multiple measurements per individual and for the fact that individuals belong to distinct species, so we included the random effects of individual nested within species. Mod6

examines the relationship between the area of the pollen patch the birds had after visits to pollen donor flowers their trait matching with those flowers. We used pollen donor PC1 and pollen donor PC2 as predictors to quantify as trait matching, as the first two PCs explain over 85% of the variance. Mod7 examines the relationship between the number of pollen grains birds deposited on the stigma of pollen receiver flowers and their trait matching with those flowers, as well as the size of the pollen patch they had removed at their previous pollen donor flower visit; in this model we used pollen receiver PC1 and pollen receiver PC2 as predictors to quantify trait matching, as the first two PCs explain over 85% of the variance. Mod8 examines the relationship between birds' volumetric uptake rate of nectar during visits to both pollen donor and pollen receiver flowers and their trait matching with those flowers; we used PC1 and PC2 from pollen donor and pollen receiver PCs. The fit of all models was checked with model validation operations in the DHARMa package v 0.4.6[85]. The Anova() function in the car package v 3.1-2[83] was used to extract p-values for the fixed effects. Marginal and conditional R^2 were estimated using the r.squaredGLMM() function from the MuMIn package v 1.47.5[88].

Scaling up to the pollination landscape

The experiments described above quantify pollen deposition in a single flower visit, but our camera trap data showed that these three species have different visitation frequencies to *E. maculata* plants in the field. We combined our data on visitation frequency and number of flowers probed per plant from camera traps with data on pollen deposition from pollen transfer experiments to determine how these three species differ in their estimated pollen deposition at the scale of an entire plant visit and visiting multiple plants across the landscape. For all visits by *A. rufogularis, Pi. ornata,* and *Pu. albifrons* recorded on camera traps, we used a permutation approach where a random value for pollen deposition, sampling from the values measured in pollen transfer experiments, was assigned to a given camera trap observation. Pollen deposition values were only sampled within each species and values were sampled with replacement, as there are more camera trap observations than pollen transfer trials per species. Once pollen deposition values were assigned to each camera trap observation, the number of pollen grains deposited during the entire plant visit was determined by multiplying the number of pollen grains assigned to that observation by the number of flowers probed during that observation, as counted by A.E.Hewes when examining camera trap videos. The mean number of pollen grains deposited per visit per species was then calculated for that permutation run (Figure 6A). The number of pollen grains deposited across the landscape was determined by summing all per-visit pollen deposition estimates per species for each permutation run (Figure 6B). We ran 100 permutations to generate a distribution of pollen deposition estimates for each species.

Due to the unbalanced number of observations across species, we used the Anova() function with a type III sum of squares from the car package v 3.1-2[83] to determine whether there were interspecific differences in average number of pollen grains deposited per visit and total pollen grains deposited across the landscape, using the data generated from this permutation approach, as well as the number of flowers probed per visit from our raw camera trap data. We then used the pairs() function from the emmeans package v 1.8.5[86] to conduct a TukeyHSD post-hoc test to calculate p-values for each pairwise species comparison and the cohens_d() function from the rstatix package v 0.7.2[87] to calculate the effect sizes of each pairwise species comparison (Table 3).

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Figures & Tables

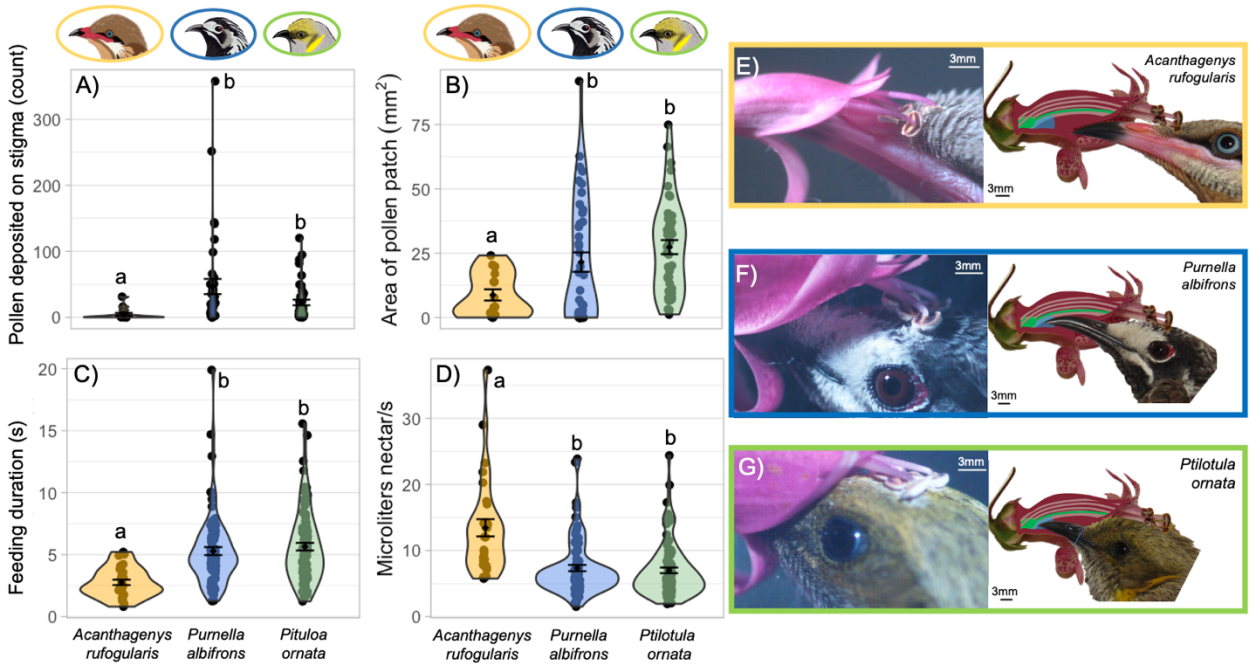
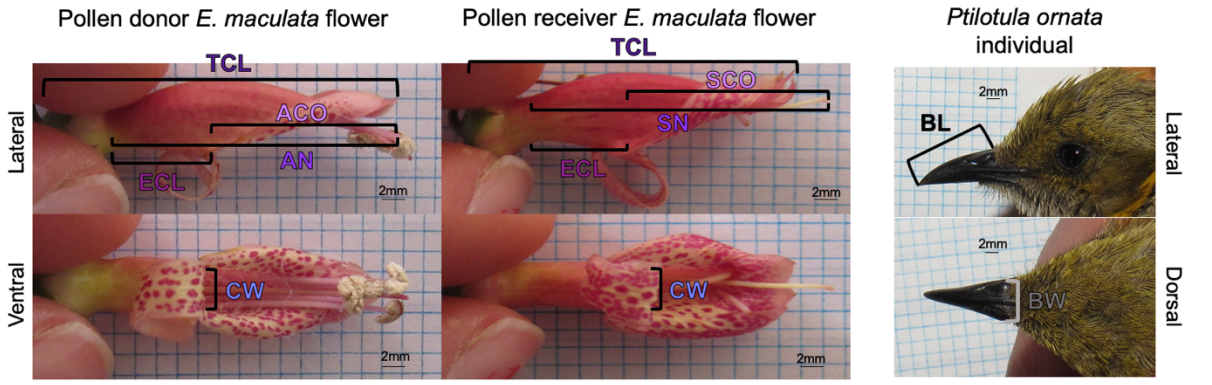


Figure 1. Patterns in pollen transfer and feeding efficiency across honeyeaters. A-D) Differences measured in pollen transfer efficiency (A,B) and feeding efficiency (C,D) across honeyeater species. *A. rufogularis* deposited (A) and removed (B) significantly less pollen than *Pu. albifrons* or *Pi. ornata*, but the latter two species were not significantly different. *A. rufogularis* fed faster (C) and therefore had higher volumetric uptake rate (D) than *Pu. albifrons* or *Pi. ornata*, but the latter two species were not significantly different. E-G) Left: still images from high-speed videos showing pollen deposition on each species, Right: bird species (to scale) overlaid on cross sections of *Eremophila maculata* flowers, illustrating differences in bill insertion depth, which in turn affects pollen deposition location and the distance the tongue must travel to access the nectar pool; these differences played a role in determining the interspecific differences illustrated in A-D. While *A. rufogularis* has a greater bill tip-to-nectar distance than *Pu. albifrons* or *Pi. ornata*, it also has a larger tongue and can therefore likely capture more nectar per lick (see Discussion). In A-D the mean of each sample is indicated with a diamond and the error bars show ± 1 standard error. Letters over each violin plot show which species are significantly different. Results from modeling are shown in Table 1 and summary statistics are shown in Table S1. Bird illustrations above panels A & B are by Kindall Murie (to scale).



Floral trait	Description	Corresponding bird trait	Bill-corolla matching metric
Total corolla length (**TCL**)	Tip of corolla to base of corolla, lateral view	Bill length (**BL**)	**BL / TCL**
Effective corolla length (**ECL**)	Corolla opening to nectary, lateral view	Bill length	**BL / ECL**
Corolla width (**CW**)	At corolla opening, distance from one internal wall of corolla to the other, ventral view	Bill width (**BW**)	**BW / CW**
Distance from anthers to corolla opening (**ACO**) – measured for pollen donor flowers only	Base of furthest-most anther to corolla opening, lateral view	Bill length	**BL / ACO**
Distance from anthers to nectary (**AN**) – measured for pollen donor flowers only	Base of furthest-most anther to nectary, lateral view	Bill length	**BL / AN**
Distance from stigma to corolla opening (**SCO**) – measured for pollen receiver flowers only	Stigma to corolla opening, lateral view	Bill length	**BL / SCO**
Distance from stigma to nectary (**SN**) – measured for pollen receiver flowers only	Stigma to nectary, lateral view	Bill length	**BL / SN**

Figure 2. Quantifying bill-corolla matching**.** Floral and bird morphometrics taken to quantify bill-corolla matching. There are no expectations for a particular number that indicates best matching between bill and flower, for example a bill length to total corolla length (BL/ TCL) ratio of 1, because the matching is an emergent property of the bill-flower interaction and not all bill-corolla matching metrics can simultaneously equal the same value.

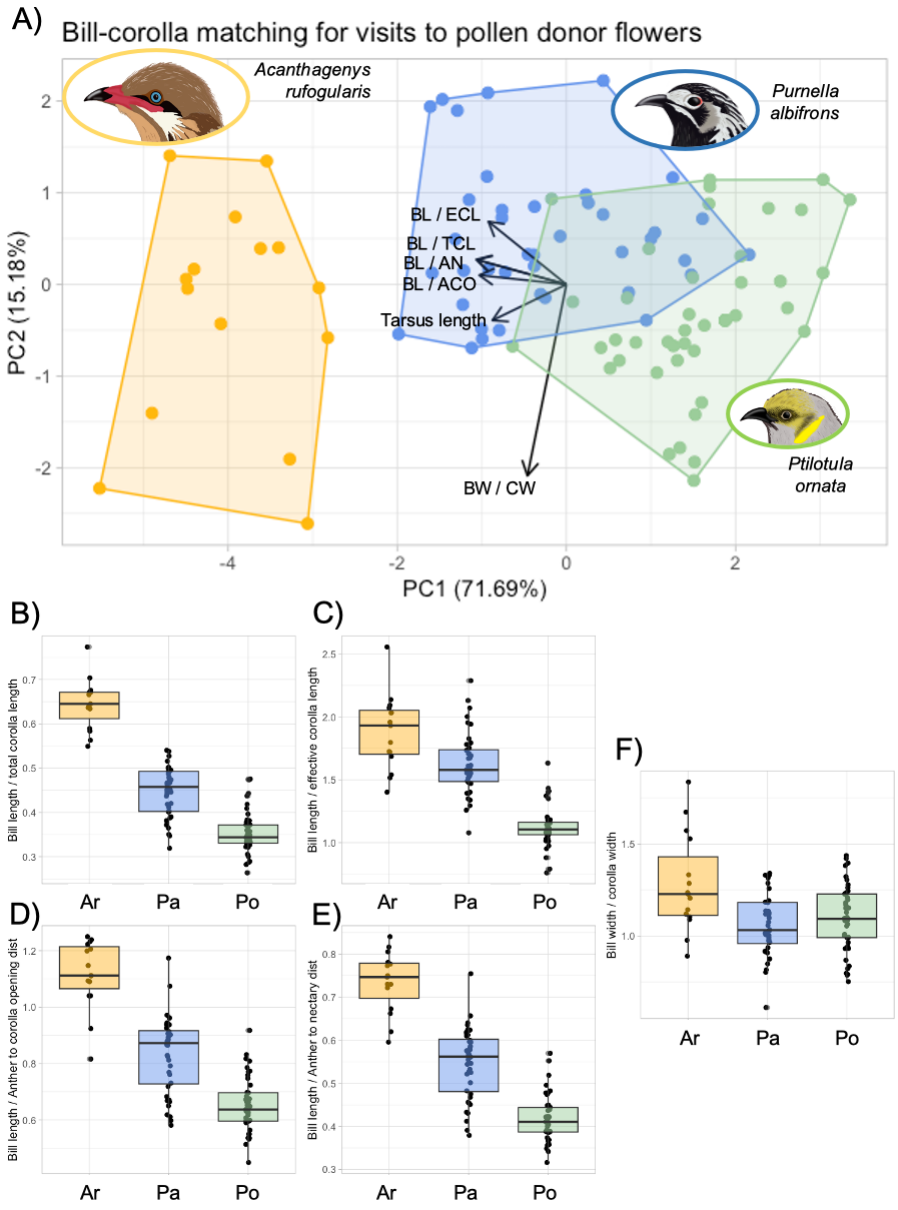


Figure 3. PCA and raw data of bill-corolla matching metrics**.** A) PCA of bill-corolla matching metrics measured from honeyeater visits to pollen donor *Eremophila maculata* flowers. The loadings of each bill-corolla matching variable are illustrated with arrows. B-F) Raw data of bill-corolla matching metrics showing the same trends visualized in the PCA. In panel A abbreviations are as follows: BL / TCL = bill length / total corolla length, BL / ECL = bill length / effective corolla length, BW / CW = bill width / corolla width, BL / ACO = bill length / distance from anthers to corolla opening, BL / AN = bill length / distance from anthers to nectary, BL / SCO = bill length / distance from stigma to corolla opening BL / SN = bill length / distance from stigma to nectary. See Table S2 for PCA loadings and Figure 2 for descriptions of all bill-corolla matching variables. See Figure S1 for PCA of bill-corolla matching metrics measured from honeyeater visits to pollen receiver flowers. Bird illustrations (to scale) are by Kindall Murie.

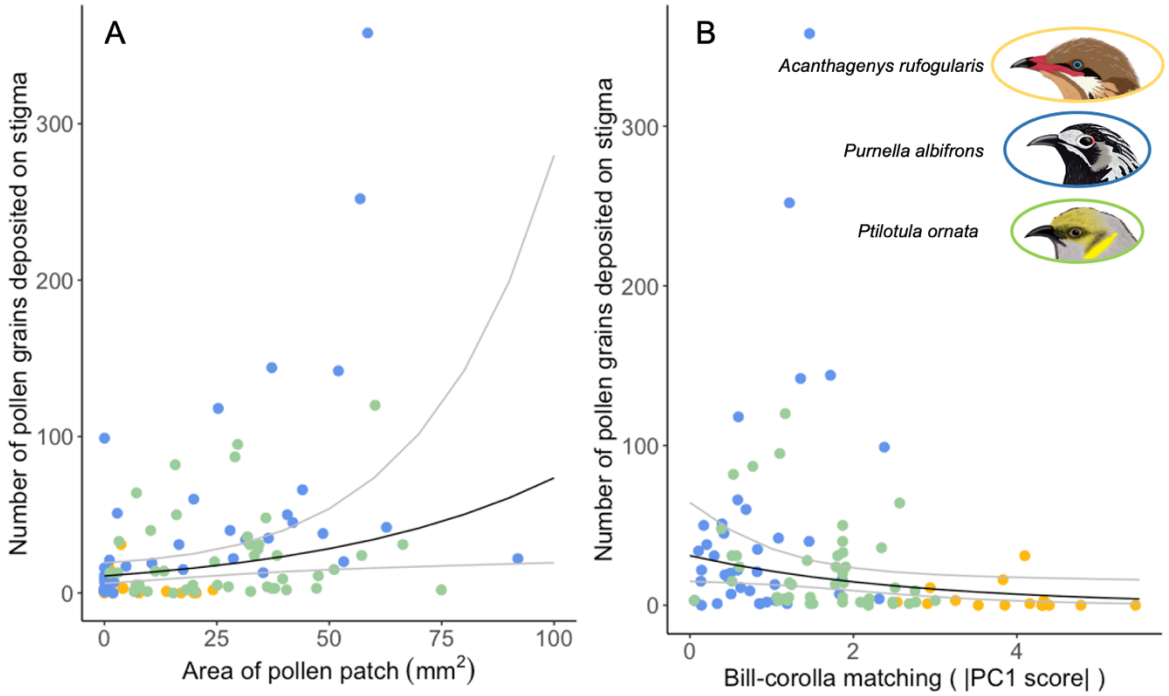


Figure 4. Factors that best explain pollen deposition of honeyeaters at *Eremophila maculata*. Predictors that best explain the number of pollen grains deposited on *Eremophila maculata* stigmas: area of the pollen patch (A) and pollen receiver |PC1 score| (B). The area of the pollen patch is positively correlated with pollen deposition, while |PC1 score| is negatively correlated with pollen deposition, meaning that has the bill-corolla matching worsens (primarily in length) the amount of pollen deposited is reduced. See Table 2 for full statistical results. Solid black lines are predictions made with the ggpredict() function while holding one predictor constant at its mean value. Grey lines show 95% confidence intervals around those predictions. Bird illustrations (to scale) are by Kindall Murie.

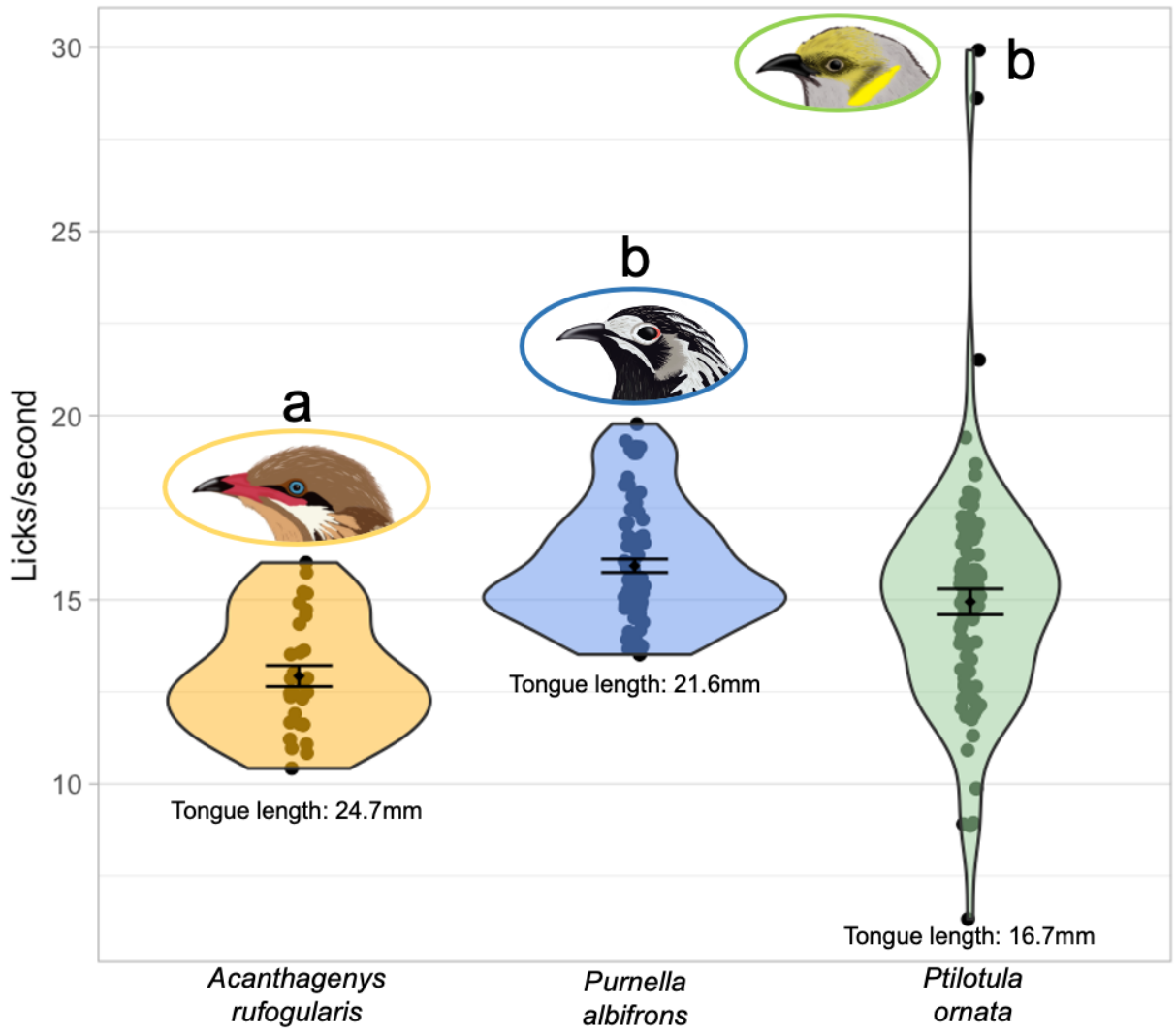


Figure 5. Differences in licking rate across honeyeaters**.** Licking rates of *A. rufogularis*, *Pu. albifrons* and *Pi. ornata* at *Eremophila maculata* flowers measured using high-speed videography. Letters denote which species are significantly different. Tongue lengths are provided for reference from Paton and Collins (1989). Bird illustrations (to scale) are by Kindall Murie.

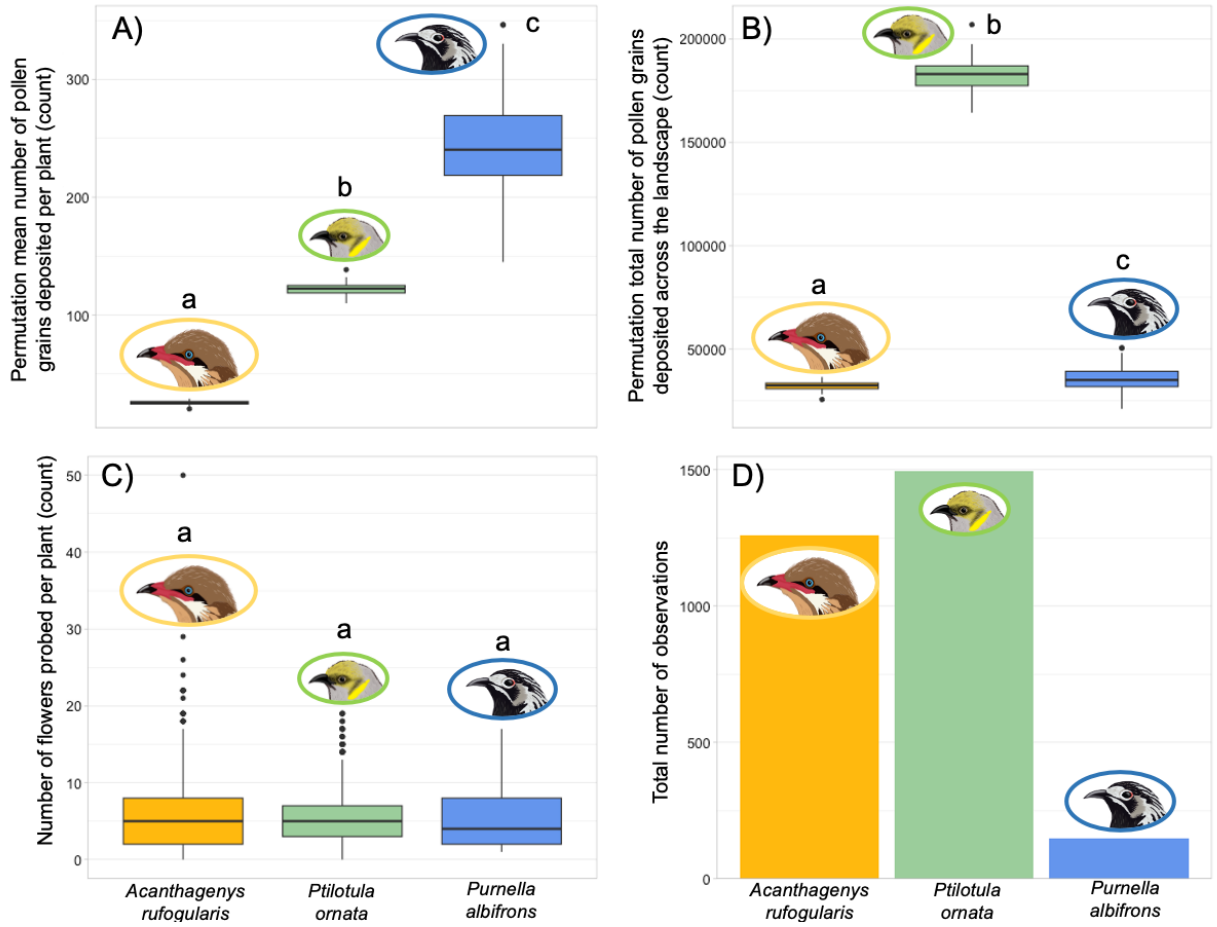


Figure 6. Differences in plant visitation and estimated landscape-scale pollen deposition across honeyeaters**.** Estimates of A) mean pollen deposition per species during a visit to a single *Eremophila maculata* plant and B) total pollen deposition across all *Eremophila maculata* plants that were monitored with camera traps. Differences cannot be explained by the number of flowers probed per plant by each species (C) but is largely due to a combination of differences in the total number of visits to *Eremophila maculata* plants (D) and difference in the ability to deposit pollen (Figure 1A). In plots A and B each data point is the mean estimated pollen deposition from a single permutation run, and the distribution shown for each species is the mean estimated pollen deposition from all 100 permutation runs. Letters above boxplots denote which species are significantly different. Results from permutations and statistical analysis are shown in Table 1. Bird illustrations (to scale) are by Kindall Murie.

Table 1. Interspecific differences in pollen transfer and feeding efficiency across honeyeaters**.** Outputs of models examining interspecific differences in pollen transfer and feeding metrics across honeyeater species. Regression coefficients are provided as raw values (on the scale of the link function) and exponentiated to be on the response scale. Raw regression coefficients can be interpreted as the expected change in the ln(counts) for every 1 unit increase in X. Transformed regression coefficients can be interpreted as a multiplicative factor by which the mean of Y changes for every 1 unit increase in X. Significant p-values are bolded. *Ar = Acanthagenys rufogularis, Pa = Purnella albifrons, Po = Ptilotula ornata*

Model	Response	Fixed effects			P-value		Effect size (Cohen's d)	Marginal R^2 (trigamma)	Conditional R^2 (trigamma)
		Effect *Pairwise levels*	**Raw regression coefficient (link scale)**	**Transformed regression coefficient (response scale)**	**Fixed effect**	**Pairwise levels**			
1	Number of pollen grains deposited on stigma	**Species**	-1.68	0.187	**0.004**	**0.0027**	0.82	0.11	0.19
		Ar – Pa	-1.27	0.281		**0.031**	0.86		
		Ar – Po							
		Pa – Po	0.407	1.5		0.38	0.44		
2	Anther contact duration (s)	**Species**	-0.0615	0.94	**0.0045**	0.97	0.15	0.14	0.27
		Ar – Pa	-0.584	0.558		0.054	0.94		
		Ar – Po							
		Pa – Po	-0.523	0.593		**0.011**	0.98		
3	Area of pollen patch (mm^2)	**Species**	-1.18	0.306	**0.00057**	**0.0005**	0.71	0.25	0.36
		Ar – Pa	-1.11	0.331		**0.0008**	1.3		
		Ar – Po							
		Pa – Po	0.0783	1.08		0.903	0.27		
4	Feeding duration (s)	**Species**	-0.634	0.53	**0.0019**	**0.0050**	1.1	0.18	0.40
		Ar – Pa	-0.683	0.505		**0.0018**	1.3		
		Ar – Po							
		Pa – Po	-0.0488	0.952		0.94	0.12		
5	Feeding efficiency (µL/s)	**Species**	0.601	1.82	**0.0018**	**0.0075**	1.04	0.17	0.37
		Ar – Pa	0.691	1.99		**0.0013**	1.1		
		Ar – Po							
		Pa – Po	0.0898	1.09		0.81	0.084		

Table 2. Correlations between bill-corolla matching and pollen transfer and feeding efficiency**.** Outputs of models examining correlation between bill-corolla matching and pollen transfer and feeding efficiency. Regression coefficients are provided as raw values (on the scale of the link function) and exponentiated to be on the response scale. Raw regression coefficients can be interpreted as the expected change in the ln(counts) for every 1 unit increase in X. Transformed regression coefficients can be interpreted as a multiplicative factor by which the mean of Y changes for every 1 unit increase in X. Significant p-values are bolded.

Model	**Response**	**Fixed effects**			**P-value**	**Marginal R^2 (trigamma)**	**Conditional R^2 (trigamma)**
		Effect	**Raw regression coefficient (link scale)**	**Transformed regression coefficient (response scale)**			
6	Area of pollen patch (mm^2)	**|PC1|**	0.031	1.03	0.84	0.0089	0.55
		|PC2|	-0.16	0.85	0.36		
7	Number of pollen grains deposited on stigma	**Area of pollen patch**	0.019	1.02	**0.019**	0.15	0.39
		|PC1|	-0.37	0.69	**0.033**		
		|PC2|	-0.18	0.84	0.49		
8	Feeding efficiency (µL/s)	**|PC1|**	0.043	1.04	0.48	0.0083	0.35
		|PC2|	-0.015	0.99	0.81		

Table 3. Interspecific differences in estimated pollen deposition across the landscape**.** Summary statistics and results from statistical analysis of pollen deposition at multiple scales. Summary statistics shown as mean ± SE (except for number of visits). Number of visits and flowers probed per plant are raw data from camera traps. Pollen deposited per plant and pollen deposited across landscape are estimates from the permutation analysis. Significant p-values are in bold. *Ar = Acanthagenys rufogularis, Pa = Purnella albifrons, Po = Ptilotula ornata*.

Species	**Number of visits**	**Number of flowers probed per visit**			**Permutation mean number of pollen grains deposited per plant (count)**			**Permutation total number of pollen grains deposited across the landscape (count)**		
Acanthagenys rufogularis	1258	5.6 ± 0.12			25.6 ± 0.163			32210 ± 205		
Ptilotula ornata	1495	5.4 ± 0.092			122 ± 0.463			182570 ± 692		
Purnella albifrons	146	5.2 ± 0.31			245 ± 3.98			35804 ± 581		
Species pair		*ANOVA* F value, p-value	*Tukey* Estimate, p-value	*Cohen's d*	*ANOVA* F value, p-value	*Tukey* Difference, p-value	*Cohen's d*	*ANOVA* F value, p-value	*Tukey* Difference, p-value	*Cohen's d*
Ar–Po		1.94, 0.144	0.260, 0.19	0.0654	3335, **<2e-16**	-95.6, **<0.0001**	-26.6	27910, **<2e-16**	-148889, **<0.0001**	-28.2
Ar–Pa			0.458, 0.38	0.113		-218, **<0.0001**	-9.51		-2893, **0.0002**	-0.781
Po–Pa			0.198, 0.83	0.0541		-122, **<0.0001**	-5.28		145996, **<0.0001**	24.2

Supplementary Material

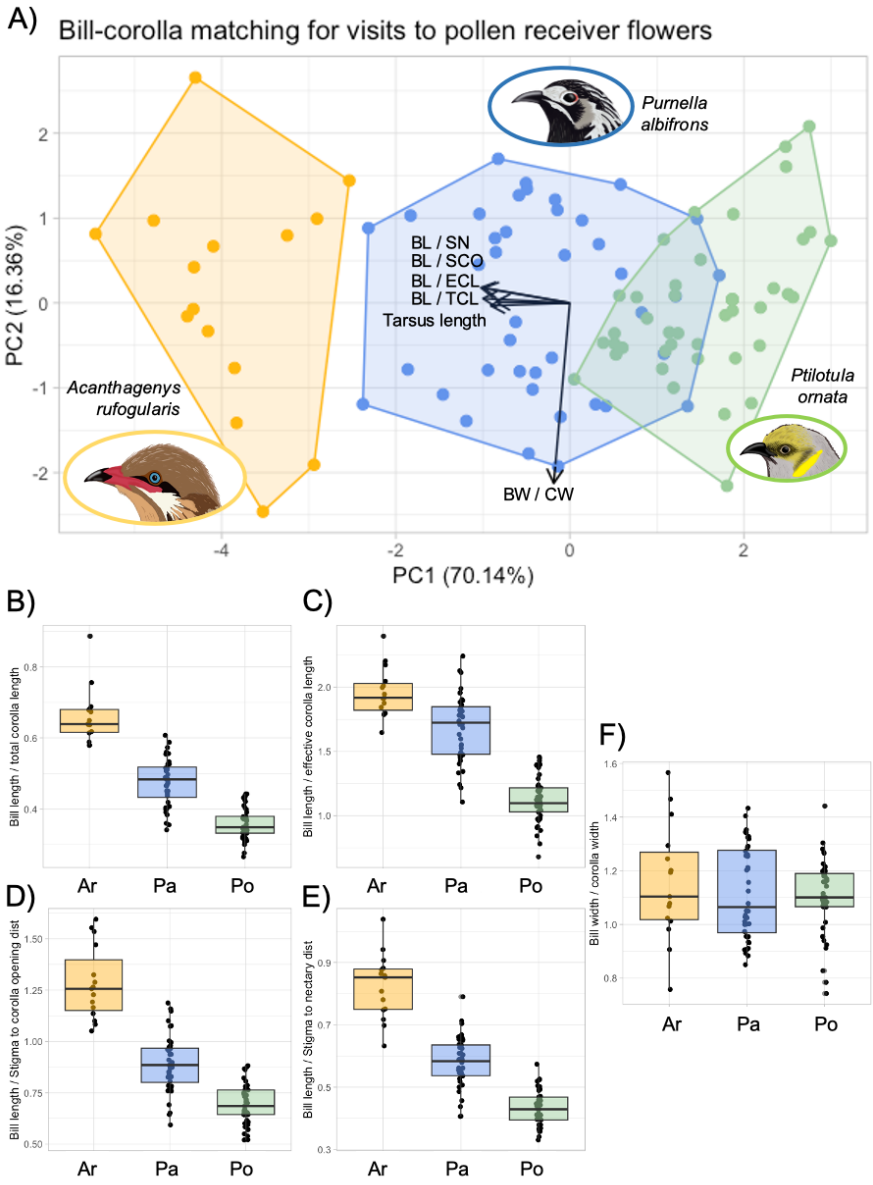


Figure S1. A) PCA of bill-corolla matching metrics measured from honeyeater visits to pollen receiver *Eremophila maculata* flowers. The loadings of each bill-corolla matching variable are illustrated with arrows on both plots. B-F) Raw data of bill-corolla matching metrics showing the same trends visualized in the PCA. In A abbreviations are as follows: BL / TCL = bill length / total corolla length, BL / ECL = bill length / effective corolla length, BW / CW = bill width / corolla width, BL / ACO = bill length / distance from anthers to corolla opening, BL / AN = bill length / distance from anthers to nectary, BL / SCO = bill length / distance from stigma to corolla opening BL / SN = bill length / distance from stigma to nectary. See Figure 2 for descriptions of all bill-corolla matching variables, Table S2 for PCA loadings, and Figure 3A for PCA of bill-corolla matching metrics measured from honeyeater visits to pollen donor flowers. Bird illustrations (to scale) are by Kindall Murie.

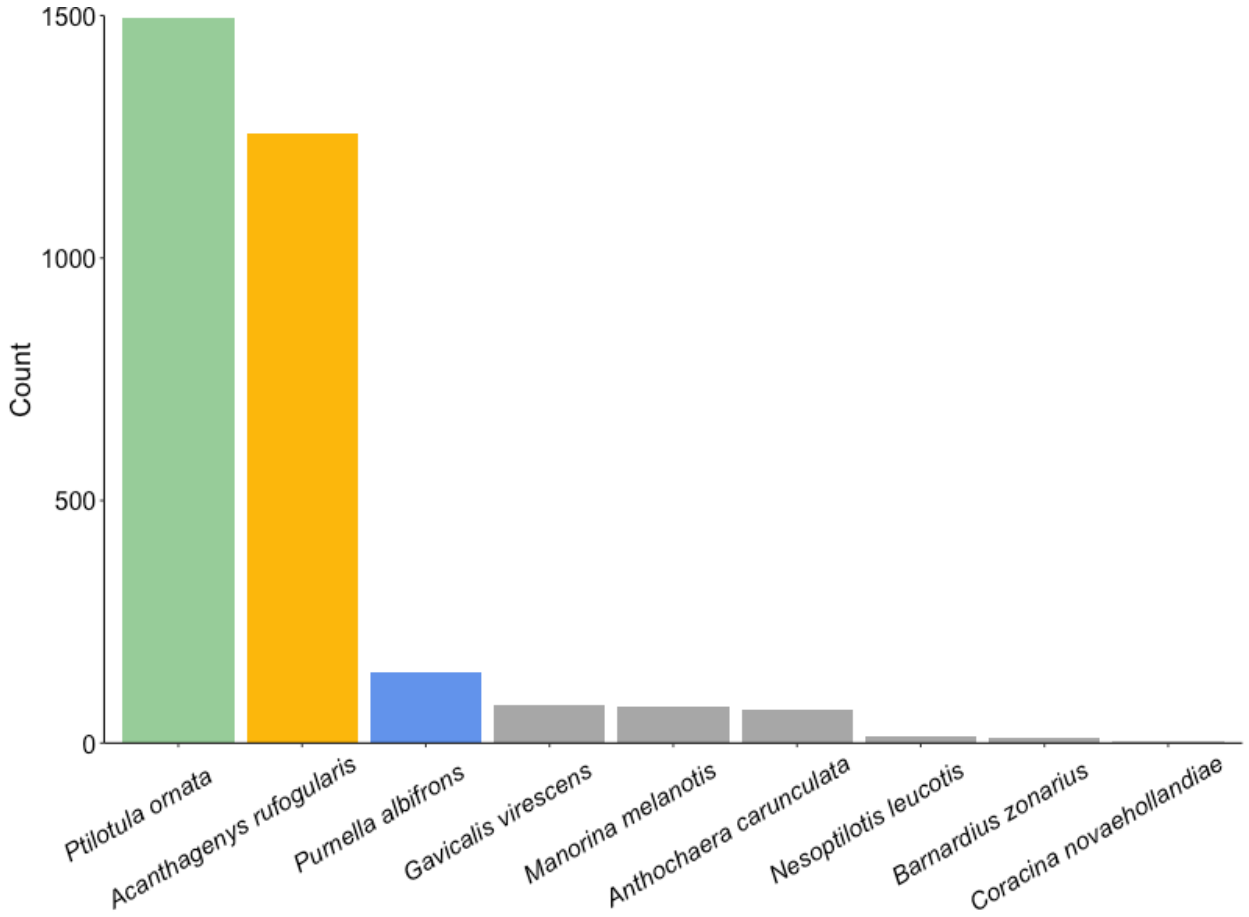


Figure S2. Visitation of all bird species to *Eremophila maculata* plants recorded on camera traps in the field.

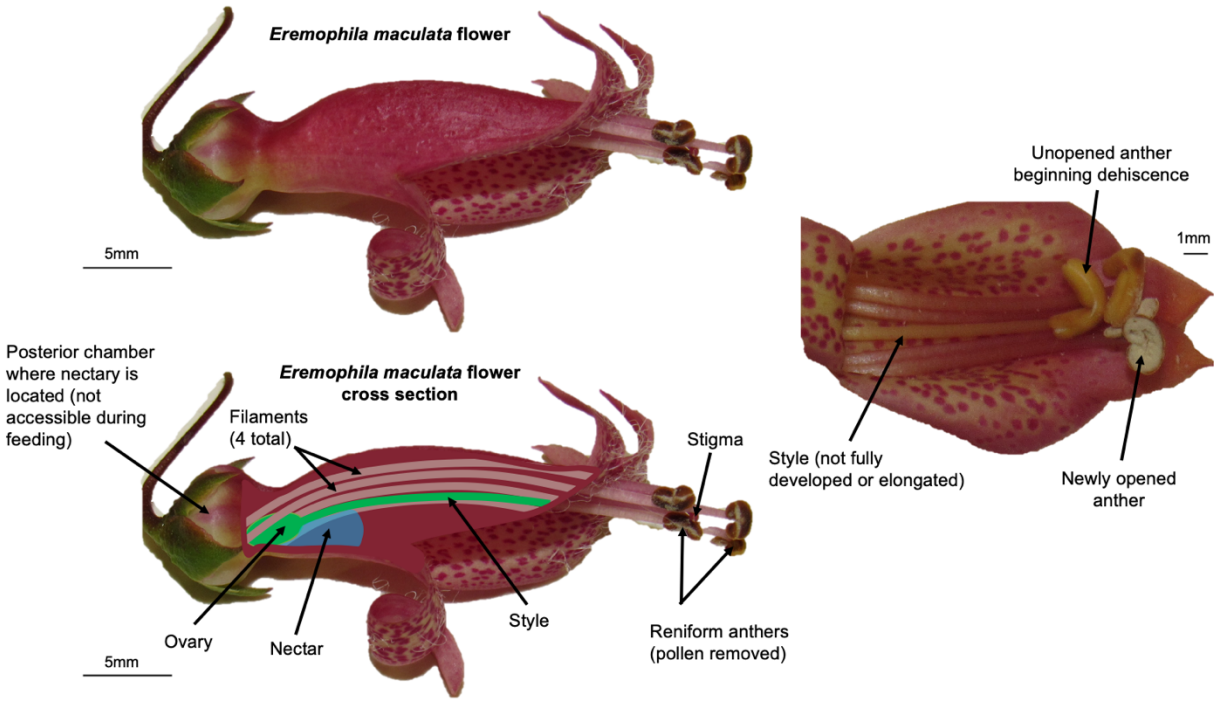


Figure S3. Relevant anatomical features of *Eremophila maculata* flowers. Blue shading shows where naturally occurring nectar pools for consumption by pollinators, after being produced by secretary pores of nectary (Chinnock 2007b) in the posterior chamber behind the ovary. The nectar shown would have been removed via capillary tube and replaced with a sucrose solution before being used in feeding/pollen transfer experiments.

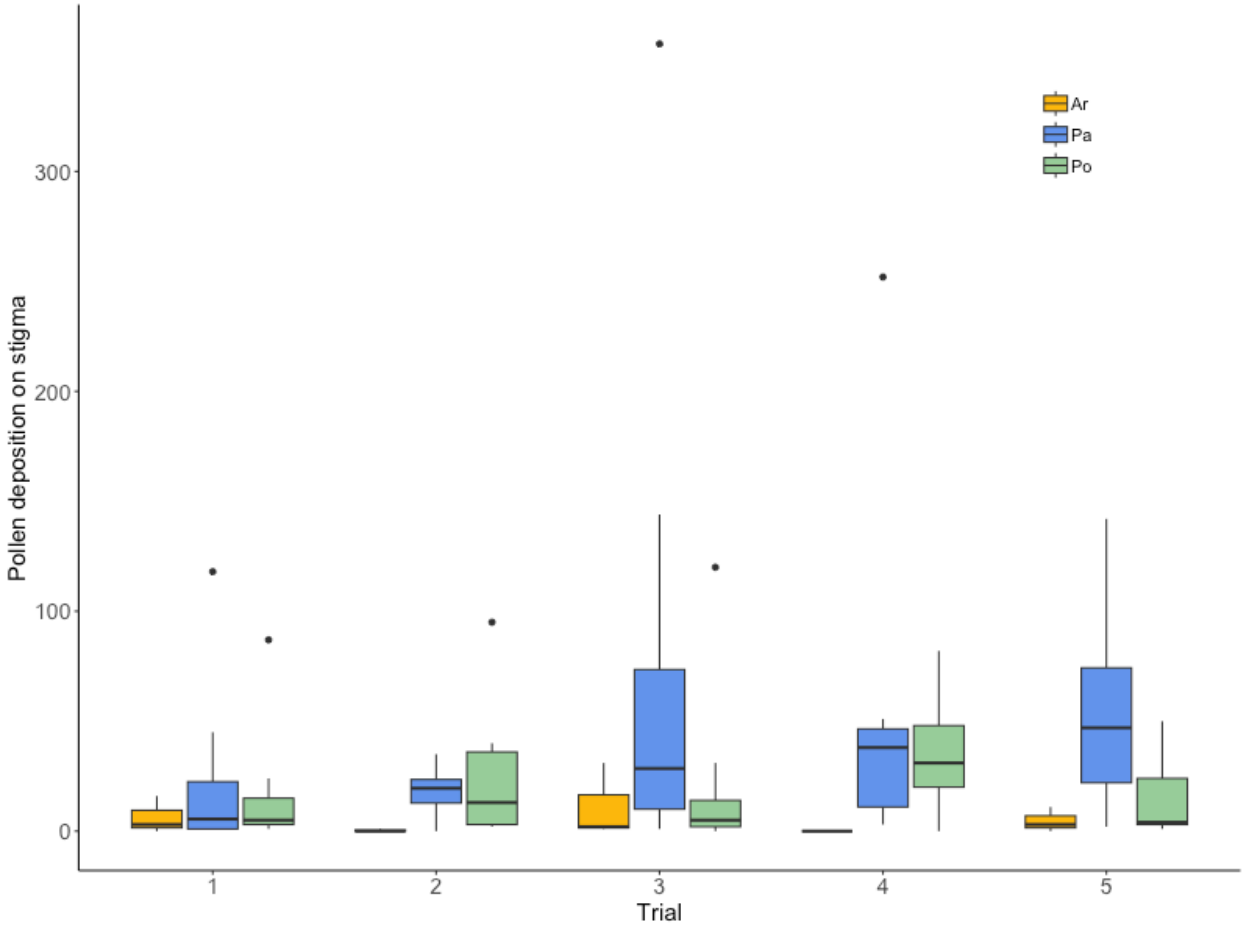


Figure S4. Pollen deposition on *Eremophila maculata* stigmas for each trial. There was no significant relationship between pollen deposition and trial number (F statistic = 0.581, p-value = 0.677), or between pollen deposition and the interaction of species and trial number (F statistic = 0.656, p-value = 0.729). Ar = *Acanthagenys rufogularis,* Pa = *Purnella albifrons*, and Po = *Ptilotula ornata*.

Table S1. Pollen transfer and feeding metrics across species (mean ± SE).

Species	Pollen deposition on stigma (count)	Anther contact duration (s)	Pollen patch area (mm^2)	Feeding duration (s)	Feeding efficiency (µL/s)	Licking rate (licks/s)
Acanthagenys rufogularis	4.5 ± 2.2	2.1 ± 0.38	8.8 ± 2.2	2.8 ± 0.22	13.4 ± 1.3	12.92 ± 0.18
Pituloa ornata	22.5 ± 4.2	4.4 ± 0.46	27.4 ± 2.7	5.6 ± 0.31	7.0 ± 0.43	14.95 ± 0.35
Purnella albifrons	46.7 ± 11.6	1.8 ± 0.31	21.6 ± 3.8	5.3 ± 0.33	7.3 ± 0.47	15.92 ± 0.18

Table S2. Results of PCAs for pollen donor and pollen receiver flower interactions. The loadings for each bill-corolla matching trait on each principle component are shown in rows, while the variance explained by each principle component is shown at the top of each column. The abbreviations for the bill-corolla matching variables in each row are defined in Figure 2.

Pollen donor PCA	**Principle component (proportion of variance)**	**PC1 (71.7%)**	**PC2 (15.2%)**	**PC3 (7.8%)**	**PC4 (4.0%)**	**PC5 (1.1%)**	**PC6 (0.17%)**
	BL / TCL	-0.4654	0.1175	0.0687	-0.1184	0.8650	0.0517
	BL / ECL	-0.4068	0.3027	-0.4725	-0.6194	-0.3184	0.1859
	BW / CW	-0.2035	-0.9210	-0.3168	-0.0945	0.0288	-0.0162
	BL / ACO	-0.4544	0.0442	-0.0766	0.6483	-0.1900	0.5739
	BL / AN	-0.4701	0.1199	-0.1117	0.3022	-0.1715	-0.7946
	Tarsus length	-0.3860	-0.1733	0.8083	-0.2858	-0.2900	0.0425
Pollen receiver PCA	**Principle component (proportion of variance)**	**PC1 (70.1%)**	**PC2 (16.4%)**	**PC3 (8.1%)**	**PC4 (3.6%)**	**PC5 (1.6%)**	**PC6 (0.26%)**
	BL / TCL	-0.4659	0.0243	-0.1389	0.1605	0.8582	0.0272
	BL / ECL	-0.4241	-0.0153	-0.6006	0.4888	-0.4112	-0.2262
	BW / CW	-0.0860	-0.9926	0.0119	-0.0826	-0.0018	0.0214
	BL / SCO	-0.4606	0.0830	0.0762	-0.6551	-0.0992	-0.5798
	BL / SN	-0.4765	0.0841	-0.0544	-0.3152	-0.2356	0.7798
	Tarsus length	-0.3956	0.0055	0.7817	0.4473	-0.1702	-0.0584

Table S3. Honeyeater morphometrics. Tongue morphometrics are from Paton and Collins (1989), all others are average values ± SD measured from individual birds used in the present study.

Species (n)	Bill length (mm)	Bill width (mm)	Bill depth (mm)	Wing chord (mm)	Tarsus length (mm)	Capture mass (g)	Tongue length (mm)	Tongue width (mm)	Tongue depth (mm)
Acanthagenys rufogularis (3)	19.93 ± 2.11	5.55 ± 0.96	5.62 ± 0.54	110 ± 4.36	25.11 ± 0.19	45 ± 2.0	24.7	0.88	0.78
Purnella albifrons (8)	13.36 ± 1.32	4.52± 0.36	4.29 ± 0.37	75 ± 4	17.76 ± 1.27	15.11 ± 1.89	21.6	0.83	0.59
Ptilotula ornata (9)	10.59 ± 1.03	4.81 ± 0.15	4.24 ± 0.20	79.9 ± 5.26	17.56 ± 0.92	16.91 ± 2.0	16.7	0.93	0.73

Table S4. Sample size information for pollen deposition analyses

Treatment	**Number of individuals (plant or bird)**	**Total number of stigmas sampled**
Acanthagenys rufogularis	3	15
Purnella albifrons	8	40
Ptilotula ornata	9	45
Unpollinated baseline (anthers unopened, unbagged)	8	26
Pollinated baseline (anthers opened, unbagged)	15	61

Chapter 5: Honeyeater visitation patterns at *Eremophila maculata* and *Grevillea huegelii* in the semi-arid mallee of South Australia

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Abstract

Many birds consume floral nectar either opportunistically or as a large part of their diet. Documenting the factors driving patterns of bird visitation to sympatric plant species is important for understanding the potential for birds to place selective pressures on floral and nectar traits over evolutionary time. In this work we describe the avian communities that visit two sympatric flowering plants in the semi-arid mallee of South Australia, *Eremophila maculata* (Scrophulariaceae) and *Grevillea huegelii* (Proteaceae), and characterize aspects of floral display, nectar rewards, and interspecific interactions between avian species that could be influencing the composition of those communities. We found that *E. maculata* and *G. huegelii* attract and support significantly different avian communities, and that *E. maculata* is visited by more birds and a wider variety of species than is *G. huegelii*. Additionally, the avian community of *E. maculata* is dominated by larger species, while the community of *G. huegelii* is mostly smaller species. These patterns can be explained, at least in part, by two factors: 1) *E. maculata* plants produce more flowers with more kilojoules of nectar per flower than *G. huegelii* and therefore may be the preferred resource of larger-bodied species, and 2) *E. maculata* plants have higher visitation rates and are the site of more aggressive interactions, both of which could deter smaller-bodied bird species looking to avoid interference competition and aggression.

Introduction

Honeyeaters (Aves, Meliphagidae) are a critically important part of the Australian ecosystem, specifically in their capacity as pollinators of many native and endemic plants (Paton and Ford 1977). Part of understanding how these mutualistic interactions evolve and persist is understanding what factors structure and drive the community composition, visitation rates, and energy gain of honeyeaters interacting with various plants.

Honeyeaters have been shown to most frequently visit plants that offer the highest energy reward per foraging bout and to forage at plants that offer sufficient nectar reward to offset their estimated energy spent foraging (Pyke 1980; Ford 1981; Paton 1982; Ford and Paton 1982; Collins 1985). However, the ability to maximize net energy gain is greatly affected by interspecific interactions among honeyeater species. Interspecific aggression can affect which honeyeater species visit which plants, as larger-bodied honeyeater species can competitively exclude smaller species from higher reward, more concentrated resources (Ford 1979, 1981; Ford and Paton 1982; Collins 1985; Armstrong 1991). The ability to maximize net energy gain also depends on the energetic reward provided by plants, which is known to influence honeyeater foraging behavior (Ford and Paton 1977; Pyke 1980; Collins and Briffa 1983; Collins 1985), but the roles of sugar, amino acid, and micronutrient composition of nectar in shaping honeyeater foraging behavior have not been examined.

Much of the previous work on honeyeater visitation to flowers has focused on woodlands and heathlands (e.g., Paton and Ford 1977; Ford and Paton 1977, 1982; Ford 1981; Paton 1982; Collins and Briffa 1983; Collins 1985; Brown and Hopkins 1995). There is relatively much less information on honeyeater visitation to flowers in mallee habitats (but see Ford and Paton 1976;

Elliot et al. 2012), and specifically to flowers other than those of the eponymous mallees – multi-trunk trees of the genus *Eucalyptus* that grow to be 2m-8m tall (Menkhorst and Bennett 1989; Clarke et al. 2021). The floristic composition of the understory in mallee habitats can be complex, and often contains a variety of sclerophyllous shrubs that are important sources of nectar and seeds for animals (Menkhorst and Bennett 1989; Clarke et al. 2021). Many of the flowering plants found in mallee habitats, including the mallee themselves, produce flowers that are critical nectar resources for honeyeaters but do not have flowers that are overtly identifiable as ornithophilous (bird-pollinated). Most mallee species (e.g., *E. incrassata, E. socialis*) produce small, white, staminiferous flowers that are cup-shaped such that the nectar is easily accessible by birds and insects (e.g., Bond and Brown 1979; Ford et al. 1979; Paton 1986). On the contrary, *Grevillea huegelii* and *Eremophila maculata* are common plants in the mallee understory and produce flowers that are classically ornithophilous.

Grevillea huegelii, commonly called the comb grevillea, produces flowers with a red perianth that are gullet shaped (Paton 1986); these flowers are arranged in an inflorescence, with multiple inflorescences produced per individual (Wrigley and Fagg 1989). *Eremophila maculata*, commonly called the spotted emu bush, produces many large tubular flowers that are pink on the exterior and yellow with pink spots on the interior (Chinnock 2007a). Both *Grevillea* and *Eremophila* are specious genera endemic to Australia, are known to be important nectar resources for honeyeaters across the continent (Ford et al. 1979), and include species that rely on birds for some or all of their pollination (Hermanutz et al. 1998; Richardson et al. 2000; Kalinganire 2000; Smith 2002; Chinnock 2007b). However, we know very little about the nectar resources provided by *Grevillea huegelii* and *Eremophila maculata* specifically, or the composition of honeyeater communities that visit them in mallee habitats.

The aim of the current work is to study honeyeater visitation to *Eremophila maculata* and *Grevillea huegelii* in the semi-arid mallee of South Australia. We recorded honeyeater visitation to plants and measured a variety of features of plant phenotype during the spring of 2022 in an effort to 1) describe *G. huegelii* and *E. maculata* as food sources for honeyeaters, 2) describe the composition of the avian community that visits both species, and 3) estimate energy gain per visit for each avian species at both *G. huegelii* and *E. maculata*. Based on the patterns of nectar resource use by honeyeater communities described in the literature, we hypothesized that larger-bodied honeyeater species would be the most common visitors at *E. maculata*, which we predict will provide greater nectar rewards and therefore provide birds greater per visit energy gain than *G. huegelii*.

Methods

Study site

This study was conducted at Gluepot Reserve in South Australia (-33.762375, 140.124265) which, along with Taylorville Station in the south, Calperum Station to the east and northeast, and Danggali Reserve to the north constitute one of the largest contiguous blocks of mallee woodland left in Australia (Clarke et al. 2021). The area that constitutes Gluepot Reserve was pastoral land from 1877-1997, at which time it was purchased by BirdLife Australia. Gluepot is in the Murray-Darling Depression Bioregion, which spans much of southeastern South Australia as well as portions of New South Wales and Victoria. Gluepot Reserve is semi-arid, receiving 200-250mm of rainfall per year, and consists of mallee and casuarina woodland, with patches of open fields throughout due to the construction of dams and the history of grazing. The mallee woodland itself is represented by Triodia Mallee, Chenopod Mallee, and Heathy Mallee, as

described by (Clarke et al. 2021), reflecting the diversity in understory composition across the reserve.

Bird visitation

We monitored bird visitation to *E. maculata* and *G. huegelii* using Moultrie M-880 motion activated trail cameras set to record 30 second video clips, and clips were recorded successively if a bird visit lasted longer than 30 seconds. For *E. maculata*, we monitored visitation at four sites across the reserve that had clusters of *E. maculata* plants. We placed four cameras at each site (Figure 1A, 16 cameras total) for two weeks, rotating the cameras to new plants within each site (5-15 meters away from the original plant) after the first week. Because *G. huegelii* occurs in much lower numbers, is much less densely distributed, and flowers more slowly than *E. maculata*, it took longer to sufficiently document bird visitation. For *G. huegelii*, we monitored visitation at three sites across the reserve that had clusters of *G. huegelii* plants. We placed four cameras at each site (Figure 1A, 12 cameras total) for a month and cameras were not rotated between plants. The combined sampling effort for all camera trapping was 8,064 camera hours.

All videos were examined by eye by AEH, EC, AD, SM, and MR to document the species visiting, the number of flowers each individual bird probed during its visit, and whether any flowers were probed more than once. All species identifications were double checked by AEH. In successive videos where it was clearly the same bird moving about the plant, those videos were counted as a single visit for that individual. Honeyeaters do not possess any individual-level markings, so identification of individuals was not possible. Unless it was clear that one individual appeared in multiple successive videos, individuals in each video were assumed to be different. All species observed are sexually monomorphic, so sex is unknown. The video recordings were not high enough resolution to all for visualization of a gape or juvenile versus adult plumage, so age is also unknown.

Aggressive interactions

Aggressive interactions were scored from camera trap videos by AEH. Aggressive interactions were defined as those in which one bird physically attacked another bird and either completely displaced it from the plant or made it flee and occupy a different spot on the plant. The species that initiated and received the aggression was recorded for each interaction.

Floral morphology

Floral morphometrics were measured for flowers that were mature; for *E. maculata* these are flowers where the anthers had opened and the stigma was elongated past the anthers, while for *G. huegelii* these are flowers where the pollen presenter is fully exposed and covered in pollen (the pollen presenting surface is also the stigmatic surface in *Grevillea*). We measured corolla width and length, as well as various aspects of the floral reproductive structures. For *E. maculata* we measured the distance from the anthers to the corolla opening, the distance from the anthers to the nectary, the distance from the stigma to the corolla opening, and the distance from the stigma to the nectary. For *G. huegelii* we measured the distance from the pollen presenter/stigma to the nectary, the distance from the base of the pollen presenter to the nectary, and the pollen presenter protrusion length. Due to the fact that *E. maculata* plants were more abundant on a per-site basis than *G. huegelii*, more individuals could be measured at each site. For *E. maculata* we collected data on floral morphometrics for 32 plants (8 individuals per site). For *G. huegelii* we collected data on floral morphometrics from 18 plants (6 individuals per site).

Flowering phenology

We measured flowering phenology on the same plants we deployed camera traps to obtain an estimate of the number of flowers available per plant. On each plant we denoted the corners of a 30cm x 30cm square with small, inconspicuous markers (white paper clips) affixed to branches. A photo was taken of that square every week from September through November 2022, using the markers to line up the corner of the photo frame. In each photo the number of buds, open flowers, senesced flowers, young fruits (green colored), and mature fruits (maroon colored) were counted using the point count tool in ImageJ (Schindelin et al. 2012). For *E. maculata* we collected data on flowering phenology for 16 plants (4 individuals per site). For *G. huegelii* we collected data on flowering phenology from 12 plants (4 individuals per site).

Nectar traits

Nectar volume was measured using calibrated microcapillary tubes as standing crop (μL) and production rate (μL/hour) for both *E. maculata* and *G. huegelii.* Standing crop was measured as the volume of nectar present in flowers at dawn when no birds were observed visiting flowers, while nectar production rate was measured by emptying flowers of nectar, bagging them to prevent pollinator visits, and then measuring the volume produced 24 hours later. For *E. maculata*, standing crop was measured for 16 plants (4 plants per site), with multiple flowers measured per plant (see Table S1 for sample sizes), and nectar production rate was measured for 8 plants (4 plants at 2 sites), with multiple flowers measured per plant (see Table S5 for sample sizes). For *G. huegelii*, standing crop and nectar production rate were measured for 11 plants (4 plants at 2 sites and 3 plants at the final site), with multiple flowers measured per plant (see Table S1 for sample sizes).

Nectar concentration was measured as wt/wt (g sugar/g solution) on a Milwaukee MA871 digital Brix refractometer; this refractometer had a minimum sample volume of 1mL, so samples were pooled across flowers within each plant individual to measure sugar concentration. For *E. maculata*, nectar concentration for 16 plants (4 plants per site), with multiple pooled samples (pooled across flowers) per plant (see Table S1 for sample sizes). For *G. huegelii*, nectar concentration was measured for 11 plants (4 plants at 2 sites and 3 plants at the final site), with multiple pooled samples (pooled across flowers) per plant (see Table S1 for sample sizes). After the sugar concentration of these pooled samples was measured, sugar assays were performed for each plant to determine the proportion of sucrose, glucose, and fructose in the nectar. Samples were pooled again within plants and diluted so the total sugar concentration was within the assay range of the kits (n = 15 plants for *E. maculata*, n = 9 plants for *G. huegelii*). Sucrose and glucose were measured using a colorimetric assay (ab65334, Abcam, Cambridge, United Kingdom), following the manufacturer's instructions. Fructose was measured using a fluorometric assay (ab241022), following the manufacturer's instructions and including the use of the 'clean-up' reagent to remove glucose interference. All assays were read on a CLARIOstar Plus plate reader (BMG LABTECH in Ortenberg, Germany).

Estimating energy gain

We estimated energy gain for each bird species on a per-visit basis. For every camera trap that was deployed we counted the number of flowers probed during a single visit by every bird. We then combined this data on probe count with estimates of kilojoules of energy in the nectar of flowers of *E. maculata* and *G. huegelii.* To calculate the average kilojoules per flower at each camera trapping site, we first calculated the mean nectar concentration. We then converted from

Brix (wt/wt) to g sugar/μL using the density of sucrose solution (Bolton et al. 1979). We then multiplied by the mean per flower standing crop nectar volume at each site to calculate g sugar/per flower. Finally, we converted to kJ/flower using the caloric value of 1g sugar (17kJ/gram for all sugars).

We also examined the kJ gained per visit when accounting for differences in metabolic costs across the species examined, which is more similar to net energy gain. For honeyeaters, which have elevated basal metabolic rates (BMR) compared to related passerines (McNab 2016), we used the mass-specific metabolic rate equation from (McNab 2016) of $BMR = 5.97*mass^{0.729}$. For non-honeyeaters, we used the mass-specific metabolic rate equation from (McKechnie and Wolf 2004) of $BMR = 0.0346*mass^{0.669}$. Mean body masses for each species were taken from (Menkhorst et al. 2017).

Statistics

Community composition between *E. maculata* and *G. huegelii*

We computed a series of metrics to characterize composition of the avian communities of *E. maculata* and *G. huegelii*. For both plants we calculated the species richness and Shannon's diversity at each sample site using the specnumber() and diversity() functions in the vegan package v 2.6-4 (Oksanen et al. 2025). We then combined data across sampling sites for both plants to calculate the total and relative abundance of each bird species at *E. maculata* and *G. huegelii*. To determine if the communities of *E. maculata* and *G. huegelii* were significantly different in composition, we used the adonis2() function from the vegan package v 2.6-4 (Oksanen et al. 2025) set to 10000 permutations and using a Morisita-Horn distance calculation in the vegan package v 2.6-4 (Oksanen et al. 2025).

Floral and nectar traits

We used the lm function from the stats package v 4.2.3 (R Core Team 2023) to run linear models to determine whether *G. huegelii* and *E. maculata* were significantly different in corolla length and width and in nectar volume, concentration, and production rate. We then used the anova function from the stats package v 4.2.3 (R Core Team 2023) to extract the model results and p-values. Model validation tests were run using the var.test and shapiro.test functions from the stats package v 4.2.3 (R Core Team 2023), as well as QQ-plots.

Estimating energy gain

We first aimed to determine whether the three estimates of energy gain (kilojoules consumed per visit, the metabolically corrected kilojoules consumed per visit, or the number of flowers probed per visit) differed between visits to *G. huegelii* and visits to *E. maculata.* Model validation tests on linear models showed that the residuals were not normally distributed, so non-parametric statistics were used. A Kruskal-Wallis test was performed using the kruskal.test function from the stats package (R Core Team 2023) to examine the effect of plant species on energy intake.

Next, we filtered the dataset to include only the five species that visited both plants (*Acanthagenys rufogularis, Ptilotula ornata, Purnella albifrons, Nesoptilotis leucotis, Barnardius zonarius*), removing species that only visited either *E. maculata* or *G. huegelii*. With this subset of the data, we performed a Kruskal-Wallis test for each predictor to assess the effect of plant species on estimates of energy gain, using the kruskal.test() function from the stats package (R Core Team 2023). Finally, to assess whether these differences were the same across all five species that visited both *E. maculata* and *G. huegelii*, we tested the relationship between

each predictor and the interaction of plant species*bird species using a Bonferroni correction for multiple comparisons in the dunnTest() function in the FAS package (Beyhum and Striaukas 2024).

Results

Community composition

The number of camera trap recordings per site along with the species richness and Shannon's diversity are given in Table S2. There was almost an order of magnitude more visits recorded at *E. maculata* (3,147) than at *G. huegelii* (334) and there was a significant difference in the avian community between *E. maculata* and *G. huegelii* (F-value = 5.47, p-value = 0.032). The species richness tended to be higher at *E. maculata* than *G. huegelii* (Table S2) and the most abundant species at both *E. maculata* and *G. huegelii* was *Ptilotula ornata* (Table S3). After *Pt. ornata, E. maculata* was most often visited by *Acanthagenys rufogularis* and *Purnella albifrons* (Table S3), while *G. huegelii* was most often visited by *Purnella albifrons* and *Melithreptus brevirostris* (Table S3). *E. maculata* was visited numerous times by the critically endangered black eared miner *Manorina melanotis* (Table S3). Both species were visited by the opportunistic nectarivore *Barnardius zonarius* (Psittacidae) though *E. maculata* more often than *G. huegelii* (Table S3). *G. huegelii* was also visited by *Pardalotus striatus* (Pardalotidae) and *E. maculata* was also visited by *Coracina novaehollandiae* (Campephagidae, Table S3). Three honeyeater species were recorded visiting *E. maculata* but never recorded at *G. huegelii* were *Gavicalis virescens, Manorina melanotis,* and *Anthochaera carunculata* (Table S3). The only honeyeater that was recorded at *G. huegelii* but never recorded at *E. maculata* was *Melithreptus brevirostris* (Table S3).

Aggressive interactions

At *G. huegelii*, 10 aggressive interactions were recorded, which constituted 3.3% of all visits (Table 1). At *E. maculata,* 146 aggressive interactions were recorded, which constituted 4.6% of all visits (Table 2). At *G. huegelii*, the majority of the aggressive interactions were *Ptilotula ornata* attacking conspecifics (6), followed by *Pt. ornata* attacking *Purnella albifrons* (2), *Pu. albifrons* attacking *Pt. ornata* (1), and *Acanthagenys rufogularis* attacking *Pt. ornata* (1) (Table 1). At *E. maculata* the majority of the aggressive interactions were also *Pt. ornata* attacking conspecifics (77), followed by *A. rufogularis* attacking conspecifics (19), *A. rufogularis* attacking *Pt. ornata* (24), *Pt. ornata* attacking *Pu. albifrons* (11), and *A. rufogularis* attacking *Pu. albifrons* (6), with one or two interspecific aggressive events recorded among other species (Table 2). We did not observe any failed attempts at aggression (i.e., the individual that initiated the aggression ends up being displaced) or any 'ties' where an attempted displacement was unsuccessful and both individuals remained on the plant.

Floral and nectar traits

E. maculata and *G. huegelii* are quite different in their flowering phenology. Individuals of *E. maculata* produced far more buds and flowers than individuals of *G. huegelii* (Figure 1B-C). *E. maculata* flowers had corollas that were significantly longer (F-value = 802.2, p-value < 2.2e-16) and wider (F-value = 39.58, p-value = 2.01e-9) than those of *G. huegelii* (Figure 1D-I, Tables S4-S5). *E. maculata* flowers also produced a greater volume of nectar per flower (F-value = 70.31, p-value = 6.2e-16) and had a higher rate of nectar production (F-value = 137.6, p-value < 2.2e-16), but the sugar concentration (wt/wt) was lower than in flowers of *G. huegelii* (F-value =

13.47, p-value = 3.4e-04) (Figure 2, Table S1). *E. maculata* nectar was hexose dominant and was composed of 3.93 ± 0.65% sucrose, 55.67 ± 1.44% glucose and 40.40 ± 1.69% fructose (comparable values as reported in Amato et al. 2021), while *G. huegelii* nectar was sucrose dominant and was composed of 94.38 ± 0.59% sucrose, 2.97 ± 0.34% glucose and 2.65 ± 0.29% fructose (Figure 3, Table S6).

Estimating energy gain

When examining differences in energy gain estimates for the entire avian community, all three metrics were significantly different (Figure 4A-D, Table 3) and were significantly higher for visits to *E. maculata* (Figure 4A-D, Table 3). The same trend was found when sub-setting the avian community to only include species recorded at both *E. maculata* and *G. huegelii* (Figure 4E-H, Table 3). For the subset data, this pattern was driven by difference in the per-visit metrics for *Ptilotula ornata, Purnella albifrons,* and *Nesoptilotis leucotis* (Figure 4E-H, Table 3). *Acanthagenys rufogularis* and *Barnardius zonarius* did not differ in any metrics between their visits to *E. maculata* and *G. huegelii* (Figure 4E-H, Table 3), which may be due to the fact that both species had visitation heavily skewed towards *E. maculata* with relatively very few visits to *G. huegelii* (Table S7).

Discussion

Floral resources provided by G. huegelii* and *E. maculata

We found that *G. huegelii* and *E. maculata* offer significantly different nectar resources (Figure 2, Table S1). *E. maculata* flowers are larger (Figure 1D-I, Table S4-S5) and produce larger volumes of a more dilute nectar at a faster rate (Figure 2A-C, Table S1) than flowers of *G. huegelii*. *E. maculata* nectar is hexose dominant while *G. huegelii* nectar is sucrose dominant (Figure 3). It is unlikely that sugar composition played a role in determining which honeyeaters visited *E. maculata* versus *G. huegelii,* as even though honeyeaters vary in their expression of the sucrase enzyme (McWhorter et al. 2021), they seem to have near 100% assimilation efficiency of sucrose, glucose, and fructose (Napier et al 2013). It is interesting that *E. maculata* was visited many times by two opportunistic nectarivores, *Barnardius zonarius* and *Coracina novaehollandiae*, as most of the sugars present in the nectar are hexoses and therefore do not require the sucrase enzyme, which is most likely lacking in these non-specialized opportunistic nectarivores, to be assimilated (McWhorter et al. 2021).

Avian communities supported by G. huegelii* and *E. maculata

We found that *G. huegelii* and *E. maculata* support significantly different avian communities in this semi-aird mallee ecosystem and that *E. maculata* tended to have higher species richness (Table S2) and supported a larger number of birds than *G. huegelii* (Table S3). Another distinctive difference in the avian community of *E. maculata* and *G. huegelii* are the size of the birds (Menkhorst et al. 2014; Miller et al. 2017). Of the eleven avian species recorded on camera trap (Table S3), there were only three species that had similar visitation rates to both *G. huegelii* and *E. maculata* and they were all moderately sized honeyeater species (*Ptilotula ornata* – 15.25g*, Purnella albifrons* – 18.25g*, Nesoptilotis leucotis* – 24g) (mean species masses from Menkhorst et al. 2014). When removing the species that visited both plants, the avian community of *E. maculata* contained honeyeater species that are larger in body size, including *Acanthagenys rufogularis* (47g)*, Manorina melanotis* (50g)*, Gavicalis virescens* (29.5g) and*, Anthochaera carunculata* (107g), and well as the opportunistic nectarivores *Barnardius zonarius* (145g) and

Coracina novaehollandiae (117.5g). Of these larger species, *Acanthagenys rufogularis* was only recorded at *G. huegelii* nine times and *Barnardius zonarius* was recorded at *G. huegelii* once. Contrastingly, the avian community that was exclusive to *G. huegelii* consisted of the small-bodied *Melithreptus brevirostris* (13.5g), with one visit recorded from the opportunistic nectarivore *Pardalotus striatus* (12g). Notably, *Melithreptus brevirostris* and *Pardalotus striatus* were never observed at *E. maculata.*

Factors shaping community composition

The differences in the avian community between *E. maculata* and *G. huegelii* are likely due to an interplay of resource availability, interspecific interference competition, species-specific nectar dependence, and net energy gain (modulated by metabolic costs). Birds had higher estimated per-visit energy gain at *E. maculata* than *G. huegelii* (Figure 4B, Table 3). This is probably due to the fact that *E. maculata* had a larger number of flowers available (Figure 1B-C) and higher kilojoules per flower (Figure 2D), so birds probed more flowers per visit (Figure 4A) and got more energy from each flower (Figure 2B, Table 3) when visiting *E. maculata*. If the energetic opportunity of feeding at *E. maculata* is greater than that of feeding at *G. huegelii* (Figure 4B-D, Table 3), the standing crop of nectar across all *E. maculata* plants is high enough to support all species, and all species can equally access the floral nectar in *E. maculata* flowers, then one would expect all bird species to preferentially feed at *E. maculata.* This then begs the question of why some species split their visits between *E. maculata* and *G. huegelii,* and why some only visited *G. huegelii* despite the fact that it is an inferior resource. This can be explained by several non-mutually exclusive hypotheses. The first plausible explanation is interference competition between honeyeater species, which can be observed in the form of aggressive interactions that interrupt a foraging bout to displace birds from plants or make them move to a different portion of the plant. Honeyeaters are known to partition resources by body size (Ford 1979). Larger-bodied species were more common at *E. maculata*, as were aggressive interactions initiated by large species. While the proportion of visits where aggression was observed was not much different between *G. huegelii* (3.3%, Table 1) and *E. maculata* (4.6%, Table 2), the threat of aggression from larger species was only present at *E. maculata*; this could act to deter smaller-bodied species and create the observed pattern in which the middle-sized species, *Ptilotula ornata, Purnella albifrons,* and *Nesoptilotis leucotis,* split their visits between *E. maculata* and *G. huegelii*, and the smallest species, *Melithreptus brevirostris*, never visited *E. maculata.*

Another factor that could create the size discrepancy observed between the *G. huegelii* and *E. maculata* honeyeater communities is species-specific nectar dependance and metabolic costs. The honeyeater species that visited *E. maculata* tend to consume nectar as 50-70% of their diet (Miller et al. 2017) and be larger, hence they have higher metabolic costs. These larger-bodied honeyeater species need to obtain more energy per unit time to meet their higher metabolic demands, making *E. maculata,* with its higher per-flower nectar rewards and greater number of available flowers, the preferred resource and worth aggressively displacing others. The honeyeater species that visited *E. maculata* and *G. huegelii* tend to consume nectar as 20-70% of their diet and be mid-sized, meaning that relative to the larger honeyeater species their metabolic costs will be lower. This could offer the medium-bodied honeyeater species more flexibility, feeding at *E. maculata* when possible and at *G. huegelii* when necessary. The one honeyeater species that was unique to *G. huegelii*, *Melithreptus brevirostris*, has a relatively low proportion of nectar in the diet at 26% (Miller et al. 2017) and would be expected to have the lowest metabolic costs due to its small size. The low reliance on nectar of *Melithreptus*

brevirostris, coupled with its small body size and concomitantly lower metabolic costs, could mean that *G. huegelii*, while it produces fewer kJ/flower, offers sufficient caloric reward while also offering a lower risk of aggression making it a profitable resource to visit.

Summary and Future Directions

We observed significant differences in the avian communities supported by *E. maculata* and *G. huegelii*, and we hypothesize that the difference in those communities is driven by a combination of 1) the dramatic difference in nectar rewards produced by *E. maculata* and *G. huegelii* relative to the different metabolic costs of different species, and 2) the higher risk and presence of interference competition and aggression at *E. maculata* compared to *G. huegelii*. Future work should aim to more directly link community composition at different plant species to net energy gain, especially through the lens of interference competition. Through the combined use of techniques like feeder-mask respirometry to more accurately quantify metabolic rates in flight (Welch 2011) and remote tracking to describe movement patterns (Williamson and Witt 2021), questions surrounding the role of competition and aggression in structuring community composition can be answered more thoroughly. Additionally, while the concept of plant-pollinator trait matching (i.e., morphological similarity between flowers and the mouthparts of pollinators) has been investigated in numerous hummingbird studies and shown to explain, at least in part, hummingbird visitation patterns to plants (Dalsgaard et al. 2021; Rico-Guevara et al. 2021; Leimberger et al. 2022), the concept has not been applied to honeyeater systems despite suggestions that it may be informative (Miller et al. 2017). Future work should aim to study honeyeater-plant interactions in a network context (e.g., Thompson 2006; Bascompte and Jordano 2007; Zanata et al. 2017), and determine the extent to which morphological similarity in metrics like bill and corolla length, bill and corolla width, and bill and corolla curvature can explain honeyeater visitation patterns.

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Figures & Tables

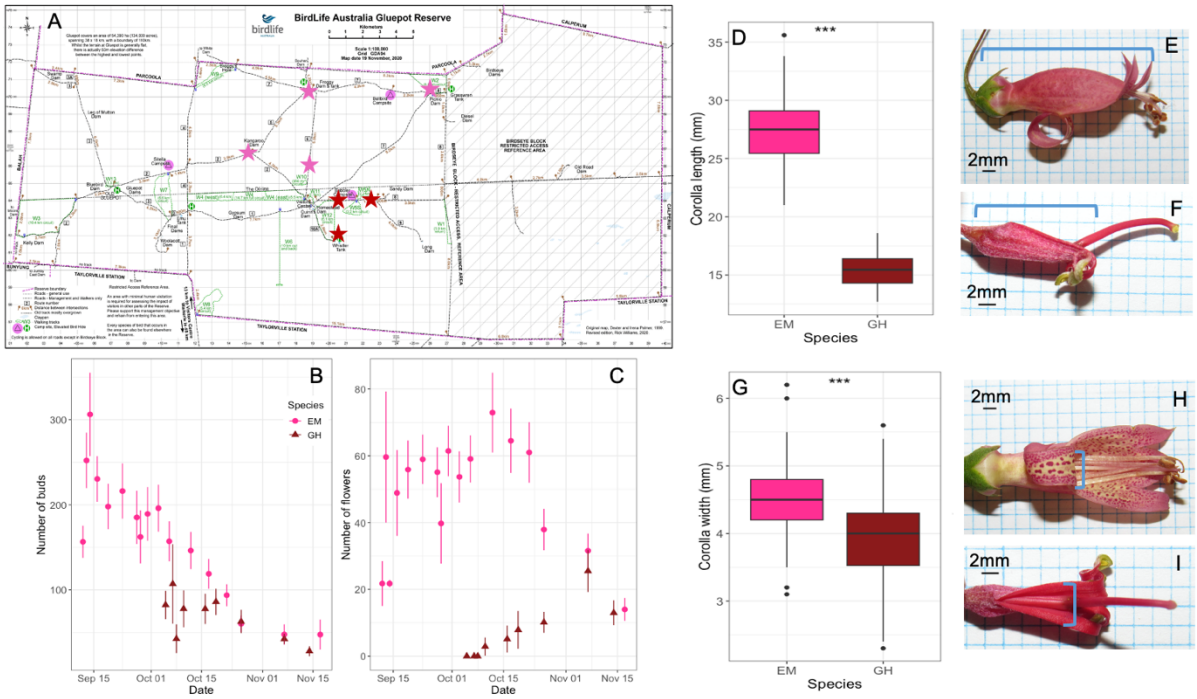

Figure 1. A) Sites where camera traps were deployed and flower data were collected. Pink stars indicate *Eremophila maculata* sites, red stars indicate *Grevillea huegelii* sites. Base map prepared by BirdLife Australia. B-C) Flowering phenology of *Eremophila maculata* and *Grevillea huegelii* throughout the duration of sampling. Points show mean value across all plants at all sites for each date. Error bars show standard error. D-F) Differences in corolla length between *Eremophila maculata* and *Grevillea huegelii*. Blue brackets show how the linear morphometric was measured. G-I) Differences in corolla width between *Eremophila maculata* and *Grevillea huegelii*. Blue brackets show how the linear morphometric was measured. *** = $p < 0.001$.

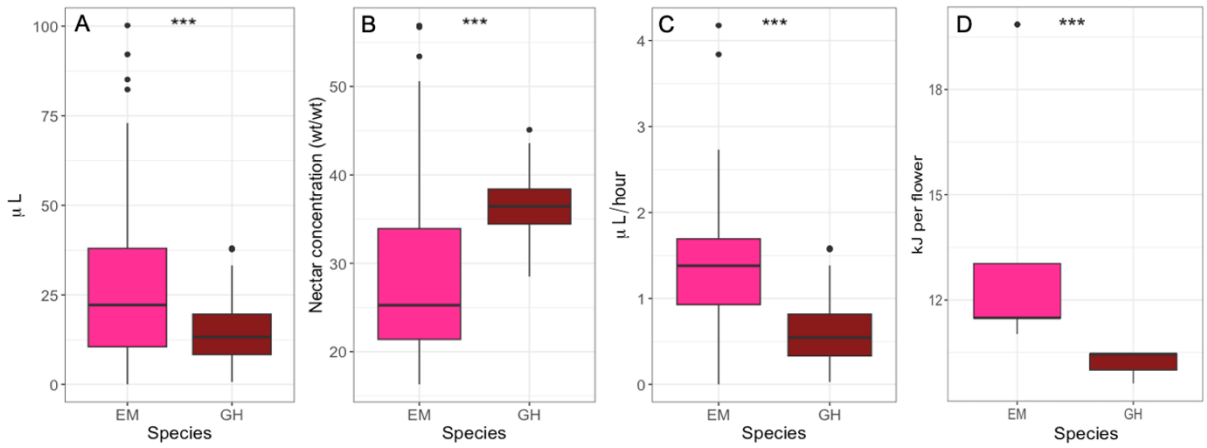

Figure 2. Differences between *Eremophila maculata* and *Grevillea huegelii* in A) standing crop nectar volume per flower, B) sugar concentration of floral nectar, C) nectar replenishment rate per flower, and D) kilojoules per flower provided in nectar. *** = $p < 0.001$.

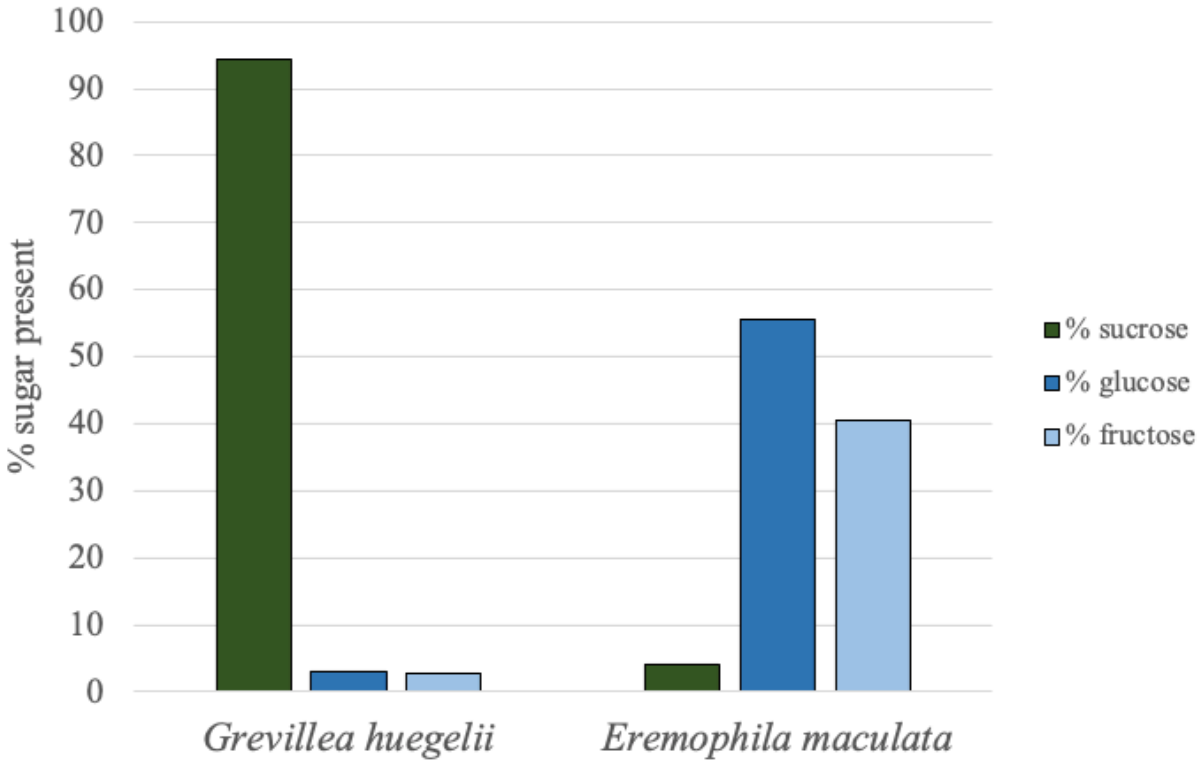

Figure 3. Floral nectar composition for *Grevillea huegelii* and *Eremophila maculata.*

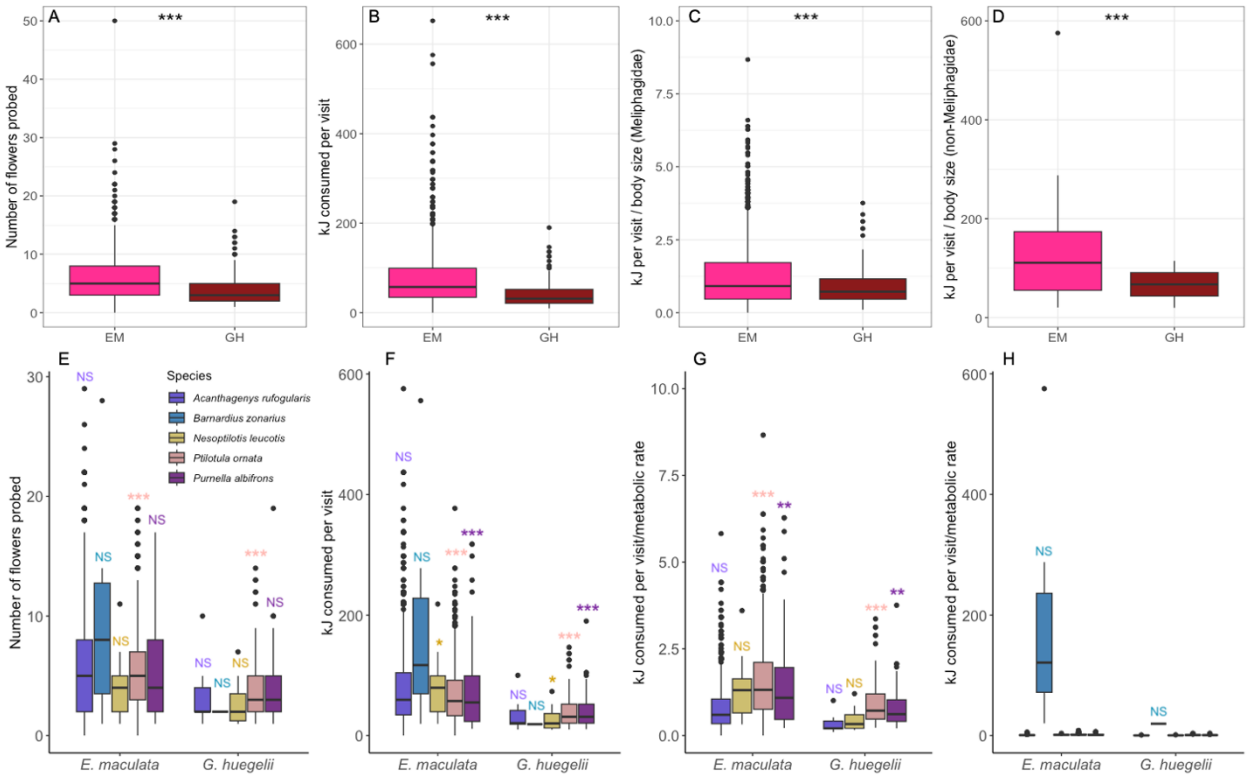

Figure 4. A-D) Effect of plant species on estimates of energy gain for entire avian community for visits *Eremophila maculata* versus visits to *Grevillea huegelii*. Statistics are in Table 3. kJ per visit/metabolic costs was seperated into Meliphagidae and non-Meliphagidae for visulatizion, in analysis they were lumped together (Table 3). E-H) Results of Dunn test for comparison of energy gain between *Eremophila maculata* and *Grevillea huegelii* for birds that visited both species. Results for kJ consumed per visit/metabolic rate are shown seperately for *Barnardius zonarius* (right panel) because the values are much larger than those for honeyaters. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = non-significant.

Table 1. Aggressive interactions recorded at *Grevillea huegelii*

		Receiver	
Aggressor	**Species** (abundance)	*Purnella albifrons*	*Ptilotula ornata*
	Acanthagenys rufogularis	0	1
	Purnella albifrons	1	0
	Ptilotula ornata	2	6
Total number of aggressive interactions recorded		10	
Proportion of visits at *Grevillea huegelii* where aggression was observed		10/334 = 3.3%	

Table 2. Aggressive interactions recorded at *Eremophila maculata*

		Receiver					
Aggressor	**Species** (abundance)	*Barnardius zonarius*	*Anthochaera carunculata*	*Gavicalis virescens*	*Acanthagenys rufogularis*	*Purnella albifrons*	*Ptilotula ornata*
	Barnardius zonarius	1	0	0	0	0	0
	Manorina melanotis	0	0	0	1	0	0
	Anthochaera carunculata	0	1	0	2	0	0
	Acanthagenys rufogularis	0	0	1	19	6	24
	Purnella albifrons	0	0	0	1	2	0
	Ptilotula ornata	0	0	0	0	11	77
Total number of aggressive interactions recorded		146					
Proportion of visits at *Eremophila maculata* where aggression was observed		146/3147 = 4.6%					

Table 3. Results of Kruskal-Wallis tests for the effect of plant species on estimates of energy gain for entire avian community, and for subset of avian community shared between *Eremophila maculata* and *Grevillea huegelii*. For the shared species only, species-specific results are from Dunn tests to examine the interaction of bird species*plant species. (z-value reported first, followed by p-value with Bonferroni adjustment for multiple comparisons). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Entire avian community							
Parameter	**KW chi-squared**		**KW p-value**				
Number of flowers probed per visit	66.352		3.772e-16***				
kJ consumed per visit	172.72		<2.2e-16***				
kJ consumed per visit /metabolic rate	27.953		1.243e-07***				
Shared species only							
Parameter	**KW chi-squared** EM v. GH	**KW p-value** EM v. GH	***Acanthagenys rufogularis*** EM v. GH	***Barnardius zonarius*** EM v. GH	***Nesoptilotis leucotis*** EM v. GH	***Ptilotula ornata*** EM v. GH	***Purnella albifrons*** EM v. GH
Number of flowers probed per visit	49.438	2.047e-12***	0.988, 1	1.58, 1	1.31, 1	5.62, 8.48e-07***	1.92, 1
kJ consumed per visit	136.19	<2.2e-16***	2.97, 0.136	2.09, 1	3.33, 0.0396*	8.74, 1.07e-16***	4.11, 0.00182**
kJ consumed per visit /metabolic rate	27.881	1.29e-07***	2.31, 0.95	0.00565, 1	3.1, 0.0869	8.12, 2.15e-14***	3.65, 0.0116*

Supplementary Material

Table S1. *Eremophila maculata* and *Grevillea huegelii* nectar traits. All values are mean +/- standard error within each sampling site.

Species	**Site**	**Nectar concentration**		**Nectar volume**		**Nectar production rate**	
		No. of plants (# of pooled samples)	**Brix (wt/wt)**	**No. of plants (# of flowers)**	**μL**	**No. of plants (# of flowers)**	**μL/hour**
Eremophila maculata	Airstrip	4 (32)	23.1 ± 0.756	4 (50)	25.7 ± 2.46	Not measured	
	Froggy Dam	4 (23)	31.7 ± 1.57	4 (89)	21.3 ± 1.89	4 (36)	1.3 ± 0.138
	Kangaroo Dam	4 (27)	21.5 ± 0.642	4 (32)	28.9 ± 3.8	Not measured	
	Picnic Dam	4 (34)	36.8 ± 1.69	4 (65)	27.3 ± 1.98	4 (46)	1.41 ± 0.0841
Grevillea huegelii	Track 8	4 (4)	37.1 ± 1.69	4 (41)	13.7 ± 1.27	4 (36)	0.547 ± 0.0569
	Walk 10	3 (8)	34.9 ± 1.22	3 (88)	14.1 ± 0.748	3 (53)	0.583 ± 0.0445
	Walk 5	4 (10)	36.9 ± 1.69	4 (98)	14.3 ± 0.762	4 (68)	0.616 ± 0.0411

Table S2. Distribution of observations across all sites monitored as well as species richness and alpha diversity, measured using Shannon's diversity index, at each site. For *Eremophila maculata*, we quantified bird visitation using four camera traps per site for two weeks, rotating the cameras to new plants within each site after the first week. For *Grevillea huegelii*, we quantified bird visitation using four camera traps per site for a month, and cameras were not rotated.

Plant species (total number of records)	**Site**	**Number of bird observations**	**Percent of total**	**Species richness**	**Shannon's Diversity Index**
Eremophila maculata (3147)	Airstrip	775	24.63	6	0.924
	Froggy Dam	529	16.81	4	0.903
	Kangaroo Dam	1156	36.73	6	1.01
	Picnic Dam	687	21.83	8	1.38
Grevillea huegelii (334)	Track 8	86	25.75	5	1.04
	Walk 10	13	3.89	5	1.44
	Walk 5	235	70.36	6	1.05

Table S3. Abundance and relative abundance of bird species observed visiting *Eremophila maculata* and *Grevillea huegelii* across all sites

Species	*Eremophila maculata*		*Grevillea huegelii*	
	Species abundance	**Relative abundance**	**Species abundance**	**Relative abundance**
Ptilotula ornata	1495	0.475	201	0.602
Acanthagenys rufogularis	1258	0.400	9	0.0269
Purnella albifrons	146	0.0464	76	0.228
Gavicalis virescens	78	0.0248	0	0
Manorina melanotis	75	0.0238	0	0
Anthochaera carunculata	68	0.0216	0	0
Nesoptilotis leucotis	15	0.00477	10	0.0299
Barnardius zonarius	10	0.00318	1	0.00299
Coracina novaehollandiae	2	0.000636	0	0
Melithreptus brevirostris	0	0	36	0.108
Pardalotus striatus	0	0	1	0.00299

Table S4. *Eremophila maculata* floral morphometrics. All values are mean +/- standard error within each sampling site. Values are shown in millimeters.

Site (number of flowers measured)	Corolla length	Corolla width	Distance from anthers to corolla opening	Distance from stigma to corolla opening	Distance from anthers to nectary	Distance from stigma to nectary
Airstrip (48)	27.1 ± 0.373	4.44 ± 0.0813	11.6 ± 0.281	14.5 ± 0.389	20.2 ± 0.353	22.6 ± 0.414
Froggy Dam (36)	26.4 ± 0.462	4.66 ± 0.0799	14.4 ± 0.354	16.1 ± 0.324	21.8 ± 0.439	23.7 ± 0.314
Kangaroo Dam (24)	28.4 ± 0.461	4.65 ± 0.11	13.4 ± 0.469	16.9 ± 0.379	22.3 ± 0.54	25.8 ± 0.531
Picnic Dam (36)	27.7 ± 0.602	4.49 ± 0.0793	12.5 ± 0.481	15.6 ± 0.376	20.6 ± 0.494	23.3 ± 0.407

Table S5. *Grevillea huegelii* floral morphometrics. All values are mean +/- standard error within each sampling site. Values are shown in millimeters.

Site (number of flowers measured)	Corolla length	Corolla width	Distance from base of pollen presenter to nectary	Distance from tip of pollen presenter to nectary	Pollen presenter length
Track 8 (18)	15.4 ± 0.318	3.76 ± 0.175	10.2 ± 0.159	19.6 ± 0.384	13.1 ± 0.251
Walk 10 (15)	15.7 ± 0.444	3.83 ± 0.177	10.2 ± 0.494	19.6 ± 0.49	13.7 ± 0.256
Walk 5 (21)	15.5 ± 0.284	4.22 ± 0.146	9.41 ± 0.295	18.8 ± 0.393	13.3 ± 0.163

Table S6. Results of assays for sugar assay of *Eremophila maculata* and *Grevillea huegelii*

Species	Plant ID	Number of pooled flower samples combined to run assay	% Sucrose	%Glucose	% Fructose
GH	GH1	1	Error – out of range of assay		
GH	GH2	1	93.060	4.353	2.587
GH	GH4	1	92.948	3.617	3.435
GH	GH5	1	93.358	3.388	3.254
GH	GH7	4	97.026	1.405	1.570
GH	GH8	3	95.698	2.007	2.295
GH	GH10	1	95.564	2.653	1.784
GH	GH12	3	Error – out of range of assay		
GH	GH13	2	92.018	3.788	4.194
GH	GH15	1	96.250	1.939	1.811
GH	GH17	4	93.448	3.610	2.942
EM	EM02	3	4.412	56.478	39.110
EM	EM04	4	2.511	54.345	43.143
EM	EM08	3	5.579	63.542	30.879
EM	EM10	2	4.015	56.371	39.614
EM	EM12	1	5.001	61.883	33.116
EM	EM14	2	0.560	57.927	41.513
EM	EM15	3	4.367	56.505	39.128
EM	EM17	3	1.577	56.830	41.593
EM	EM20	2	6.948	52.517	40.536
EM	EM21	2	1.584	39.407	59.009
EM	EM23	2	4.611	50.175	45.214
EM	EM25	1	1.788	55.193	43.019
EM	EM27	3	4.391	59.383	36.226
EM	EM30	4	1.396	58.370	40.234
EM	EM32	3	10.194	56.126	33.680

Table S7. Species means (+/- SE) for the number of flowers probed per visit, the kilojoules consumed per visit, and the kilojoules consumed per visit when accounting for metabolic rate (kJ/met). n = the number of camera trap observations for that species

Species	Plant species	n	Number of flowers probed per visit	kJ per visit	kJ/met
Acanthagenys rufogularis	EM	1258	5.66 +/- 0.122	80.1 +/- 1.95	0.810 +/- 0.0197
	GH	9	3.33 +/- 0.943	33.9 +/- 9.49	0.343 +/- 0.0961
Anthochaera carunculata	EM	68	5.9 +/- 0.55	73.9 +/- 7.88	0.41 +/- 0.0438
	GH	0	--	--	--
Barnardius zonarius	EM	10	9.5 +/- 2.52	168 +/- 50.9	174 +/- 52.7
	GH	1	2	19.2	19.9
Coracina novaehollandiae	EM	2	3.5 +/- 1.5	38.6 +/- 16.5	46 +/- 19.7
	GH	0	--	--	--
Gavicalis virescens	EM	78	5.92 +/- 0.446	118 +/- 8.86	1.67 +/- 0.126
	GH	0	--	--	--
Manorina melanotis	EM	75	6.48 +/- 0.482	84.2 +/- 6.77	0.814 +/- 0.0655
	GH	0	--	--	--
Melithreptus brevirostris	EM	0	--	--	--
	GH	36	3 +/- 0.295	30.7 +/- 3.01	0.772 +/- 0.0757
Nesoptilotis leucotis	EM	15	4.13 +/- 0.682	82 +/- 13.5	1.35 +/- 0.224
	GH	10	2.7 +/- 0.633	27.8 +/- 6.71	0.459 +/- 0.111
Pardalotus striatus	EM	0	--	--	--
	GH	1	2	20.9	115
Ptilotula ornata	EM	1495	5.4 +/- 0.0924	67.4 +/- 1.19	1.55 +/- 0.272
	GH	201	3.87 +/- 0.178	40 +/- 1.85	0.919 +/- 0.0425
Purnella albifrons	EM	146	5.21 +/- 0.311	74.4 +/- 5.23	1.47 +/-0.103
	GH	76	4.12 +/- 0.338	42.3 +/- 3.43	0.836 +/- 0.0679