Floral development in an Andean bellflower (*Centropogon granulosus*, Campanulaceae) and pollination by Buff-tailed Sicklebill (*Eutoxeres condamini*)

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#### Author Contributions

M.B. and Q.C. designed the study, and D.A., J.J., and M.B. conducted the field work. M.B. analysed the data and wrote the first manuscript draft. All authors contributed to writing and editing the final manuscript.

#### Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

#### Abstract

The Andean bellflowers (‘centropogonids,’ Lobelioidae) have experienced some of the highest speciation rates ever recorded in the angiosperms. Their rapid radiation is correlated, in part, with transitions in floral morphology, which is thought to have initiated and maintained reproductive isolation. For effective floral isolation, we expect floral shape to filter the local pollinator community such that interspecific pollen transfer is minimized. In the centropogonids, species with extremely curved flowers (‘eucentropogonids’) are predicted to be pollinated exclusively by one of two parapatric species of Sicklebill hummingbirds (*Eutoxeres*). But whereas pollination by the northernmost species (*E. aquila*) is relatively well-documented, very little is known of its southern congener, *E. condamini*. In this study we focused on the floral biology of *C. granulosus*, a eucentropogonid thought to be pollinated by *E. condamini* in the south portion of its range. We confirm that *E. condamini* is a visitor to *C. granulosus*, and that visitation is necessary for fruit development. We also establish preliminary evidence for the congruence between floral phenology and hummingbird foraging behaviour in this system. While we find support for the role of floral morphology as a source of divergence in the centropogonids, we also lay the groundwork for quantifying phenological ecotypes for future comparative studies in this mega-diverse clade of Andean bellflowers.

#### Introduction

   Plant-pollinator co-evolution is an important biotic component of rapid Andean radiations. During Pleioscene orogeny, numerous angiosperm clades diversified as they migrated and adapted to novel Andean environments ([Gentry, 1982](#ref-gentry_1982)). In these vicariant montane populations, floral traits may drift, evolve via pleiotropy, or adapt to a pre-existing pollinator community ([Harder and Johnson, 2009](#ref-harder_2009); [Smith, 2016](#ref-smith_2016)). Subsequently, divergence in floral phenotype can be reinforced when reciprocal adaptation between the plant and pollinator promotes floral isolation ([Grant, 1949](#ref-grant_1949); [Thompson, 2005](#ref-thompson_2005)). This process is generally framed as special case of ecological speciation ([Gómez et al., 2009](#ref-gomez_2009); [Althoff et al., 2014](#ref-althoff_2014)), and is increasingly supported as a critical component of Andean floristic diversity (e.g. in *Costus* L., [Kay et al., 2005](#ref-kay_2005); *Lupinus* L., [Hughes and Eastwood, 2006](#ref-hughes_2006); bromeliads, [Givnish et al., 2014](#ref-givnish_2014); centropogonids, [Lagomarsino et al., 2016](#ref-lagomarsino_2016); epiphytic orchids, [Pérez-Escobar et al., 2017](#ref-perez_2017)). However, while phenotypic and phylogenetic evidence generally supports the model of co-evolutionary diversification, the underlying microecological processes (sensu [Dellinger, 2020](#ref-dellinger_2020)) are comparatively understudied. For example, the pollination syndrome concept is widely used in macroevolutionary models to assign pollination modes to understudied species (e.g. correlated evolution of floral traits and most effective pollinator, [Dellinger, 2020](#ref-dellinger_2020)). These models are powerful because a small sample of ecological data can be used to infer evolutionary patterns for an entire clade. Subsequently, there is a need to ‘close the loop’ by testing these predictions via field work focused on previously understudied taxa. These data can then be used to validate and update macroevolutionary models, thus iteratively refining our understanding of rapid plant radiations.

   Here we investigated whether microecological processes (i.e. biotic interactions, floral development) support the theory of pollinator-mediated speciation in the mega-diverse Andean bellflowers (Campanulaceae): *Centropogon* C.Presl, *Siphocampylus* Pohl, and *Burmeistera* H.Karst & Triana. These genera form a South American clade of Lobelioideae known as the ‘centropogonids,’ a group arising in the last five million years and diversifying into over 550 species ([Lagomarsino et al., 2014](#ref-lagomarsino_2014), [2016](#ref-lagomarsino_2016)). This rapid radiation has been attributed to increased speciation rates driven by Andean uplift and the repeated evolution of hummingbird and bat pollination ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)). The Andean clade *Centropogon* section *Centropogon* ([McVaugh, 1949](#ref-mcvaugh_1949)) or ‘eucentropogonids’ (38 spp., [Lagomarsino et al., 2014](#ref-lagomarsino_2014)) are distinct because of their elongated, hook-shaped flower tube (Figure 1), and the presence of a scale made of fused hairs that serves as a lever to deposit pollen (Figure S1). Both traits are thought to be adaptations for pollination by Sicklebill hummingbirds (*Eutoxeres*), two parapatric species of large Hermits (Phaethornithinae) named for their dramatically curved bill (Figure 1). In addition to eucentropogonids, the unique bill shape of *Eutoxeres* match the curved flowers of *Heliconia* subgen. *Stenochlamys* Baker and *Heliconia* subgen. *Griggsia* L.Andersson (Zingiberales). The Hermits diverged early in the evolutionary history of hummingbirds (~26 Ma, [McGuire et al., 2014](#ref-mcguire_2014)), and represent one of two major foraging types, the trapliners (cf. territory holders, [Janzen, 1971](#ref-janzen_1971); [Sargent et al., 2021](#ref-sargent_2021)). *Eutoxeres* diverged 21.5 Ma, and probably co-evolved extreme curvature with lowland *Heliconia*, before the uplift of the Andes ([Stein, 1987](#ref-stein_1987); [Abrahamczyk et al., 2017](#ref-abrahamczyk_2017)). During a recent period of major Andean uplift (~5 Ma) *Centropogon* colonized and radiated into novel montane habitat, with eucentropogonids evolving floral curvature, enabling pollination by *Eutoxeres* ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)). This may have meditated the elevational expansion of *Eutoxeres*, and explains their persistence beyond the range of *Heliconia* ([Stein, 1987](#ref-stein_1987); [Abrahamczyk et al., 2017](#ref-abrahamczyk_2017)). At present, *Eutoxeres* has the largest elevational range of any Hermit clade — it can be found in its ancestral range, the Amazonian lowlands, as well as the foothills and montane forests of the Andes up to 3300 m ([Hinkelmann and Boesman, 2020](#ref-hinkelmann_2020)). However, the physiological pre-adaptations that allowed Sicklebills to expand upslope, and not other Hermits, remains unknown ([Stiles, 2004](#ref-stiles_2004)).

   A recent analysis of floral trait macroevolution confirms that eucentropogonids exhibit a divergent floral phenotype indicative of Sicklebill pollination ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)). One of the hypotheses generated by this finding is that White-tipped Sicklebill (*Eutoxeres aquila*, Costa Rica to northern Peru) and/or Buff-tailed Sicklebill (*E. condamini*, northern Peru to Bolivia) are visitors and pollinators of the various eucentropogonid species. Furthermore, because this pollination system is presumably specialized, we expect additional aspects of the pollination syndrome (e.g. phenology) to reflect adaptation to *Eutoxeres* behaviour. Indeed, previous studies have progressively supported White-tipped Sicklebill as a specialized pollinator of *Heliconia* and eucentropogonids: this hummingbird visits and pollinates the aforementioned plants ([Stiles, 1985](#ref-stiles_1985); [Gill, 1987](#ref-gill_1987); [Maglianesi et al., 2015](#ref-maglianesi_2015); [Morrison and Mendenhall, 2020](#ref-morrison_2020)), its bill curvature matches the curved corollas of these plants more than its relatives ([Maglianesi et al., 2014](#ref-maglianesi_2014); [Sonne et al., 2019](#ref-sonne_2019)), and its local abundance is correlated with seasonal patterns of flowering ([Stiles, 1985](#ref-stiles_1985)). In contrast, very little is known of its southern congener, *E. condamini*. Like *E. aquila*, its curved bill appears to be adapted to feed from eucentropogonids. However, there is only one published record of visitation to a species from this clade (*Centropogon gamosepalus* Zahlbr., [Stein, 1987](#ref-stein_1987)) and the effects on plant fitness are not known. Further evidence of co-adaptation, such as congruence of foraging and phenological modes, are missing.

   Thus, the goal of this study is to further test the theory of pollinator-meditated diversification in the eucentropogonids by examining the microecology of the Andean bellflower *Centropogon granulosus* C.Presl and Buff-tailed Sicklebill in southeastern Peru (Figure 1). We structured our approach by asking: (1) Is Buff-tailed Sicklebill a visitor of *C. granulosus*? (2) Does Sicklebill visitation affect the fitness of *C. granulosus*? and (3) Is the phenology of *C. granulosus* consistent with adaptation to the foraging mode exhibited by Sicklebills? While other eucentropogonid species are found in this region ([Stein, 1987](#ref-stein_1987)), we focus on *C. granulosus* as it has been previously studied with respect to *E. aquila* pollination ([Stiles, 1985](#ref-stiles_1985)), and is locally abundant, providing a tractable system for study. *E. condamini* is the only Sicklebill species present at this latitude (Figure 1); its congener (*E. aquila*) occurs further north, from the Loreto region of northern Peru to the Cordillera Central of Costa Rica ([Hinkelmann and Boesman, 2020](#ref-hinkelmann_2020)).

#### Materials and Methods

*Field Site*

   We based our field work at the Gallieto de las Rocas Lodge situated at ~1350 m a.s.l in the Kosñipata Valley, Cusco Region, Peru (-13.055, -71.548 DD, Figure 1). Research Permit No. 0441-2017 was administered by the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR). The field site is situated within the cloud forests of the Yungas ecoregion on the eastern slope of the Peruvian Andes. Local mean annual rainfall and temperature is 2631 mm and 19.1 °C, respectively ([Salinas et al., 2011](#ref-salinas_2011)).

Figure 1. Top left: Elevational heatmap of Peru and neighbouring countries. Units are meters above sea level. Map generated using tmap v.3.3-2 (Tennekes, 2018). Study site location is marked with a cross. Top right: Montane cloud forests of the eastern Andes (San Pedro, Peru). Bottom: C. granulosus., an understory vine often flowering at edges and treefall gaps.

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*Pollinator Observations*

   We deployed five camera traps (Hyperfire HC600, Reconyx Inc.) near *Centropogon granulosus* vines located in a previous survey of the area ([Boehm et al., 2018](#ref-boehm_2018_a)). Camera traps were checked for new captures every 12 hours. If no floral visitors were recorded within three days, the camera traps were moved to different *C. granulosus* individuals. Where floral visitors were recorded, we attempted targeted (manual) video recording to better document visitation behaviour. Camera traps were active continuously from August 17 to September 20, 2017. Monitored locations are listed in Table S1.

*Pollinator Exclusion Experiment*

   Following the methods of Sun et al. ([2017](#ref-sun_2017)), we constructed and deployed wire cages covering one inflorescence each from six *Centropogon* individuals (Figure S2). Wire cages prevent hummingbirds from accessing the flowers while allowing invertebrates to move freely. An additional ten inflorenscences were tagged and monitored as controls. Using the control flowers, we defined four stages of floral development (Table S2). These stages were used to quantify and compare the developmental trajectories of the control and pollinator-excluded flowers. Monitoring of a flower stopped when (1) berry development completed (stage D), (2) the flower died prematurely, or (3) the study period ended. Daily observations were recorded between August 17 and September 20, 2017. Floral development data was analysed in R v.4.0.2 ([R Core Team, 2017](#ref-R_2017)) and organized using here v.0.1 ([Müller, 2017](#ref-muller_2017)) and tidyverse v.1.3.0 ([Wickham et al., 2019](#ref-wickham_2019)) . All data and R scripts are available at (Dryad link).

   Flowering stage data was staggered because each flower developed independently. The data was also fragmented because some samples abscissed prematurely due to herbivory or weather. This type of data was treated as ‘right-censored,’ i.e. some flowers exited the study before reaching maturity ([Allison, 2014](#ref-allison_2014)). Similarly, we collected data from flowers that had already progressed past the initial stages (‘left censored’). Although manually aligning the developmental data is possible, for convenience we used a multiple sequence alignment protocol to automate the process. To do this, we first sorted the sequence fragments into ‘early’ (stages 1 and 2) or ‘late’ (stages 3 and 4) types. We then aligned all fragments of one type through ClustalW implemented in the R package msa v.3.9 ([Bodenhofer et al., 2015](#ref-bodenhofer_2015)) with a neutral (identity) substitution matrix. Aligned sequences were then used to compute a consensus sequence for the ‘early’ and ‘late’ stages — this was done using the ConsensusSequence() function in DECIPHER v.2.14.0 ([Wright, 2015](#ref-wright_2015)). The consensus was computed with a 15% threshold (the maximum proportion of sequences not represented at any given position). The consensus ‘early’ and ‘late’ sequences were then spliced to give the full consensus sequence of floral development. This process was repeated separately for both the control and pollinator-excluded data.

   We then conducted an event history analysis ([Allison, 2014](#ref-allison_2014)) to compare the probability of post-anthesis survival between pollinator-excluded and control flowers. First, we counted the number of days elapsed from anthesis (Stage C) through berry development (Stage D) for each flower, and pooled the data for control ( = 61) and pollinator-excluded ( = 29) plants. Daily survival probability (from anthesis) was calculated for each treatment using the survival package v.3.1 ([Therneau, 2015](#ref-therneau_2015)). Differences in survival probability were evaluated by a log-rank test ([Peto and Peto, 1972](#ref-peto_1972)) using the survival::survdiff() function.

*Flowering mode*

   To model flowering phenology of *C. granulosus*, we fit linear models to the rate of anthesis and senescence for each inflorescence that produced at least five flowers ( = 6 controls, = 5 pollinator excluded). We also examined whether pollinator-exclusion affected flower production and the timing of the male and female phases of anthesis. Linear models were evaluated in broom v.0.7.6 ([Robinson et al., 2021](#ref-robinson_2021)), or where applicable, mixed-effects models were fit by lmerTest v.3.1-3 ([Kuznetsova et al., 2017](#ref-kuznetsova_2017)). Cohen’s standardized effect () was calculated in effectsize v.0.4.4 ([Ben-Shachar et al., 2020](#ref-shacar_2020)). Model parameters are fully detailed in the Supplementary Materials.

#### Results

*Floral Visitors*

Camera trap recordings and *in situ* observations confirm Buff-tailed Sicklebill as a visitor to *Centropogon granulosus* flowers (Figure 2, Figure S3, video footage available in Dryad Repository). We recorded 12 visits to six *C. granulosus* individuals over 32 days (Table S3). Visitation tended to occur from 5:20 to 10:40 in the morning (=9), and 12:40 to 16:30 in the afternoon (=3), though these patterns may have been affected by our activity in the area. A total of seven flowers were probed from six *C. granulosus* individuals, i.e. a second visit was recorded to an inflorescence as flowers opened sequentially. We observed feeding both by perching on the lignified inflorescence (=3), as well as hovering (=9). We also recorded two instances of Sicklebills approaching and inspecting inflorescences without open flowers. Wedge-billed hummingbird (*Schistes geoffroyi*) was also recorded nectar robbing *C. granulosus*. From opportunistic camera trapping we recorded five visits per day to the same inflorescence (Table S4). Further details can be found in Boehm ([2018](#ref-boehm_2018_b)).



Figure 2. Pollination of C. granulosus by Buff-tailed Sicklebill (E. condamini).

   Reviewing still frames from the video recording reveals an interesting feeding problem posed by the sharply curved flowers of *C. granulosus*. The hook-shape forces the hummingbird to hover below the corolla opening, tilt its head backwards so that it is facing skyward, at which point it can insert the tip of its bill into the flower aperture (Figure S3). The remainder of the bill is further inserted by tilting the head back to resting position while lifting itself to eye-level with the corolla opening. Due to the unique morphology and orientation of *C. granulosus* flowers, this hovering maneuver is likely performed only by *Eutoxeres*. We note that once the bill is inserted, the throat and crown are covered by the ventral and dorsal corolla lobes, respectively.

   Two additional vertebrates, a murid (Muridae) and a long-nosed bat (Glossophaginae) were recorded near the inflorescences, but not observed to interact with the plant directly (Figures S4, S5). We note this because it is unknown how the fleshy berries are dispersed, though we documented signs of frugivory (Figure S6).

   Numerous invertebrates occupied or visited the flowers of *C. granulosus* in this study. As found in previous studies, we observed ants ([Stein, 1992](#ref-stein_1992)), mites ([Naskrecki and Colwell, 1998](#ref-naskrecki_1998)), and dipterids ([Weiss, 1996](#ref-weiss_1996)) in or on the flowers of this species. Though *Anelosimus* spiders (Araneae) are known to build webs scaffolded by *Centropogon coccineus* (Hook.) Regel ex B.D.Jacks. ([Nentwig and Christenson, 1986](#ref-nentwig_1986)), we observed unidentified Aranids inside of the floral tubes. We also recorded a larval leptidoptran inhabiting a flower (Figure S7), and an anthophilid (bee) collecting pollen from the anther scale (Figure S8).

*Floral development and pollinator exclusion*

   Hummingbird-exclusion did not affect the total number of flowers produced (=0.782, =0.282, =0.08). Caged inflorescences produced 11.2 ± 4.6 flowers whereas controls produced 12.2 ± 8.1 flowers (mean ± SE). We note that these numbers reflect flowering over the lifespan of the experiment; the upper limit of flower production for a single inflorescence is not known, though we counted up to 68 peduncle scars on an individual not included in this study (Figure S9). While *C. granulosus* flowers generally open sequentially, temporal overlap is not uncommon (Figure S10).

   Linear models accurately described flowering rate (Figure S11): the average adjusted was 0.934 ± 0.047, with marginal variation in between individuals (=0.117, =1.73, =0.58). Flowering rate (slope) varied between individuals (=0.037, =2.45, =0.82), but not between treatments (=0.273, =1.17, =0.39). For the control plants, the average rate was one anthesis event per 3.44 ± 0.12 days.

   The flowers of *C. granulosus* are dichogamous: for the control plants, the male and female phases lasted 2.12 ± 0.19 and 2.73 ± 0.19 days, respectively (Figure S12). Whereas pollinator exclusion marginally shortened the male phase (=0.079, =1.59, =0.23), there was no effect on the duration of the female phase (=0.603, =0.523, =0.07).

   We identified and described four discrete stages of floral development in *C. granulosus* (Table S2). Delimiting floral development into four stages generated a consensus developmental sequence (per treatment) that mapped at least 85% of the phenology data collected in this study. These four stages were used to compare differences in development between control and pollinator-excluded flowers (Figure 3). Between treatments, floral development is comparable from stages A (bud development) to C (anthesis). However, control flowers spend an additional 18 days developing berries, while hummingbird-excluded flowers did not produce berries. Event history analysis showed that, following anthesis, daily survival probability decreased at similar rates. However, at 13 days post-anthesis, hummingbird-excluded flowers abscise, while control plants continued to produce berries (Figure 4). Overall, the survival curves differed significantly (=0.0336, =4.51, =2.07).

Figure 3. Developmental trajectories for the flowers of C. granulosus, and effects of pollinator exclusion. The four stages labeled on the x-axis have statistical support as discrete developmental units and are described in Table S2. Sub-stages (e.g. B1, B2, etc.) are descriptive and used for convenience only. Note that hummingbird-excluded plants did not reach the D2 stage

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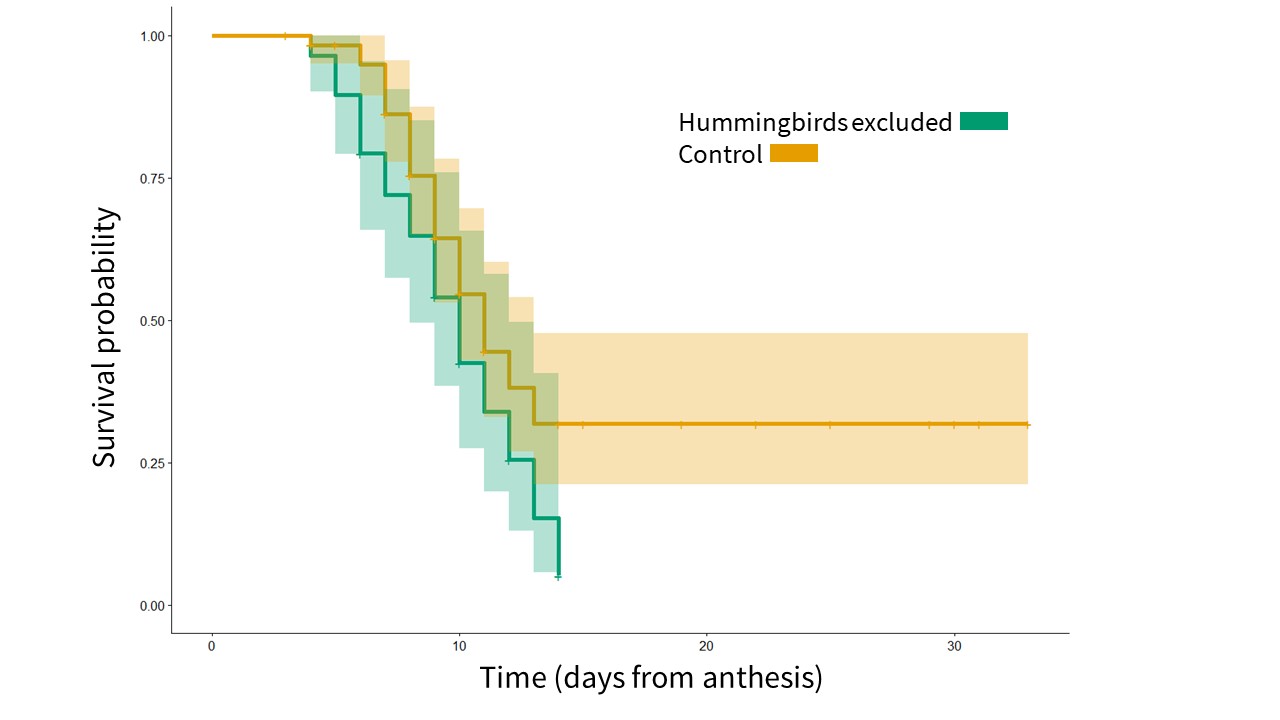


Figure 4. Post-anthesis survival analysis of C. granulosus flowers, and effects of pollinator-exclusion. Shaded regions are 95% confidence intervals.

#### Discussion

*Buff-tailed Sicklebill is a pollinator of* C. granulosus

In this study we photo-documented Buff-tailed Sicklebill visitation to *C. granulosus*, and demonstrated that hummingbird visitation is required for berry development. We interpret the inability of hummingbird-excluded flowers to produce berries as owing to a lack of pollination.

   Eucentropogonid pollen collected from White-tipped Sicklebill (*Eutoxeres aquila*) has been identified in previous studies ([Maglianesi et al., 2015](#ref-maglianesi_2015)), including *C. granulosus* ([Morrison and Mendenhall, 2020](#ref-morrison_2020)). And while *Centropogon* pollen has been identified from Buff-tailed Sicklebills from this study site ([Dyck-Chan et al., 2021](#ref-dyck-chan_2020)), species-level pollen identification for this clade remains difficult. Confirmation of eucentropogonid pollen on Buff-tailed Sicklebill (*E. condamini*) would help to bolster the evidence presented here.

   During visitation, the face of *E. condamini* is inserted into the corolla tube so that the crown and throat are covered by the petal lobes. This is facilitated by the exceptionally inflated corolla opening characteristic of the eucentropogonids ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)). While narrow corolla apertures are thought to promote specialization ([Temeles et al., 2002](#ref-temeles_2002)), the evolution of curvature might relax selection for corolla width. Conversely, because *E. condamini* tilts its head backwards during bill insertion, it may not be able to see the corolla opening; thus a narrow corolla width could negatively affect pollination if the barriers to accessing nectar are too high.

*Steady State Flowering and Traplining*

*C. granulosus* exhibits a linear flowering rate befitting the ‘steady state’ ecotype described by Gentry ([1974](#ref-gentry_1974)). It is one of several phenological modes that are thought to have evolved via competition for pollinators, and represents an axis of niche partitioning that contributes to tropical plant diversity ([Gentry, 1974](#ref-gentry_1974); [Kessler et al., 2020](#ref-kessler_2020)). Indeed, most hummingbird species exhibit foraging behaviour that is adapted either to steady state or ‘cornucopia’ flowering (sensu [Gentry, 1974](#ref-gentry_1974)), with few species able or willing to visit plants of both types ([Kessler et al., 2020](#ref-kessler_2020)). However, beyond qualitative descriptors, there is a need for a quantitative framework to better define and classify phenological ecotypes. For example, quantifying flowering rates of centropogonids could allow robust statistical classifications of phenological ecotypes. This would help to move the field towards understanding the evolution of phenological modes as mechanisms of divergence.

   The designation of ‘steady state’ implies a general mode of flowering, however the nuances of this strategy are not well known. In *C. granulosus*, there is a degree of coordination in the anthesis rate and the male-female transition rate. As a new flower opens, the initial male phase lasts 2.12 days. As the flowering rate is 3.44 days, there is a small but potentially effective window of 1.32 days where the female phase is protected from receiving pollen from a flower of the same inflorescence. Given the visitation frequency of *E. condamini* (1-2 per day), these temporal patterns might promote out-crossing while minimizing the risk of a pollinator visit without open flowers. Additionally, we found that several aspects of flowering were not affected by pollinator exclusion: the total number of flowers produced, the rate of flowering, and duration of the male/female phases of anthesis. Whether this suite of traits is adaptive (i.e. they improve chances of pollination), or incidental is an unexplored aspect of the steady state ecotype.

   Sicklebills were not marked and our ability to comment on individual behaviour is limited. Nonetheless, the visitation rates support the notion that Buff-tailed Sicklebill is a trapliner. More specifically, this species appears to exhibit traveling exploitation (sensu [Sargent et al., 2021](#ref-sargent_2021)), i.e. individuals travel long distances without defending resources, and competition for floral nectar is indirect. We make this designation based on the observations that (1) these hummingbirds have not been recorded defending static territories, and (2) individual food plants are visited 1-2 times per day for brief (seconds) periods of foraging or inspection. This is in contrast to the multiple visits per day made by the nectar robber *Schistes geoffroyi*.

   Traplining is widely viewed as an adaptation to steady state flowering ([Kessler et al., 2020](#ref-kessler_2020)). Hummingbird traplines can reach several kilometers in length ([Volpe et al., 2014](#ref-volpe_2014)) and can increase pollen transfer between plants at these scales ([Torres-Vanegas et al., 2019](#ref-torres_2019)). While the foraging behaviour of trapliners suggests resource specialization, the traplines of *Eutoxeres* and the consequent effects on *Centropogon* reproduction are avenues yet explored. As trapline architecture is further elucidated, a synthesis of the morphological (e.g. bill shape), behavioural (e.g. foraging), and physiological (e.g. flight mechanics) traits associated with specialization in hummingbirds would provide an exciting extension of the pollination syndrome concept — we encourage a continued focus on field-based pollination ecology for this reason.

*Perching versus Hovering*

In contrast to previous accounts of Sicklebill visitation to *Centropogon* ([Stein, 1987](#ref-stein_1987)), we observed hovering in addition to perching. While floral orientation in some hummingbird-pollinated plants may have evolved to exclude non-hovering visitors ([Colwell et al., 2021](#ref-colwell_2021)), hovering is one of the most energetically expensive modes of locomotion ([Suarez and Gass, 2002](#ref-suarez_2002)). Recent work has found that short-billed hummingbird species have repeatedly evolved claws that improve their ability to perch ([Colwell et al., 2021](#ref-colwell_2021)). Conversely, long-billed species tend to hover to feed, supporting the idea that long (and sometimes curved) tubular flowers evolve in response to selection for pollinator specialization (reviewed in [Boehm et al., 2021](#ref-boehm_2021)).

   Foraging mode also appears to correlate with flight ability, whereby trapliners generally have smaller flight muscles and are less agile than territorial hummingbirds ([Sargent et al., 2021](#ref-sargent_2021)). Hover-pollination of *C. granulosus* may then present an exceptional challenge for *Eutoxeres*, a hummingbird adapted to feed from *Heliconia* using the floral bract as a perch. This relative difficulty in hovering might further contribute to selection for increased corolla width in eucentropogonids (discussed above).

   We speculate that the inflorescences of *C. granulosus* are lignified primarily to support and orient flowers, and are only opportunistically used by Sicklebills as perches. This is because open flowers are, on the majority, oriented to face away from the stem on long peduncles (Figure 1). This is in contrast to Sicklebill visits to *Heliconia* made at the same study site: flowers are oriented so that the opening is aligned with the perch (i.e. floral bract, Figure S13). We note that the nectar robber *Schistes geoffroyi* exclusively hovers when visiting *C. granulosus*, and appears to exhibit traveling exploitation at a smaller spatial scale than *Eutoxeres*.

*Concluding Remarks*

The pollination syndrome concept has been remarkably successful at using limited *a priori*  information to infer the pollination mode of understudied taxa ([Dellinger, 2020](#ref-dellinger_2020)). In the centropogonids, coding pollination mode for a clade with incomplete field records, and hundreds of species, has necessitated the use of the pollination syndrome framework. Consequently, macroevolutionary models of floral trait evolution can generate informed estimates of plant-pollinator relationships for rare or difficult-to-study species. Focused efforts on field work can then serve as an invaluable means to validate these models and the pollination syndrome concept that underlies its predictions.

   Confirming Sicklebill visitation in *C. granulosus* raises myriad questions. For instance, given that eucentropogonids are so diverse and widespread in southeastern Peru, is *E. condamini* the pollinator of all of these species? Furthermore, what morphological and phenological traits, if any, prevent interspecific pollen transfer within the eucentropogonids? Through making observations of floral visitors, we were surprised to learn that another hummingbird (*Schistes geoffroyi*) is a nectar robber of *C. granulosus* exhibiting traveling exploitation. If and how *Eutoxeres* interacts with competitors, and the consequences for pollination in *Centropogon* offers a promising avenue for future research.

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