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

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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 the evolution of 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*). Whereas pollination by the northernmost species (*E. aquila*) is relatively well-documented, very little is known about 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. Using camera traps stationed at flowering individuals, and a pollination exclusion experiment, we confirm that *E. condamini* is a visitor to *Centropogon granulosus*, and that visitation is necessary for fruit development. We also establish preliminary evidence for the congruence between phenological ecotype and hummingbird foraging behaviour in this system. Our results support the role of floral morphology as a source of divergence in the centropogonids, and also lays the groundwork for quantifying phenological flowering types for future comparative studies in this mega-diverse clade of Andean bellflowers.

#### Introduction

   Plant-pollinator coevolution 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 a 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 coevolutionary 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 model 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 phenology) 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* sect. *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 S2). In addition to eucentropogonids, the unique bill shape of *Eutoxeres* match the curved flowers of some *Heliconia* (Zingerberales, [Stein, 1987](#ref-stein_1987)).

   The Hermits (36 spp.) diverged early in the evolutionary history of hummingbirds (~26 Ma, [McGuire et al., 2014](#ref-mcguire_2014)) and are generally found in Neotropical lowland and lower montane forest. Hermit hummingbirds have relatively long bills and tails, and represent one of two major foraging types, traplining (cf. territory holding, [Janzen, 1971](#ref-janzen_1971); [Sargent et al., 2021](#ref-sargent_2021)). *Eutoxeres* diverged 21.5 Ma, and probably coevolved extreme bill 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 Andean orogeny (~5 Ma) centropogonids 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 in the foothills and montane forests of the Andes up to 3300 m ([Hinkelmann and Boesman, 2020](#ref-hinkelmann_2020)).

   A recent analysis of floral trait macroevolution confirms that eucentropogonids exhibit a divergent floral morphology 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 the exclusive pollinators of the eucentropogonids. 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 eucentropogonid (*Centropogon gamosepalus* Zahlbr., [Stein, 1987](#ref-stein_1987)), and further details on the extent of mutualism have not yet been studied (e.g. effects of visitation on fruit set and seed production).

   Furthermore, because this pollination system is presumably specialized, we expect additional aspects of the pollination syndrome, specifically phenology, to reflect adaptation to *Eutoxeres* behaviour. In addition to the seasonal flowering trends documented by Stiles ([1985](#ref-stiles_1985)), phenological patterns at finer temporal scales (i.e. days) might also conform to the daily foraging habits of *Eutoxeres*. Considering that *E. aquila* is a trapliner ([Stiles, 1985](#ref-stiles_1985)), the ‘phenological ecotype’ model (hereafter ‘flowering type’) of Gentry ([1974](#ref-gentry_1974)) would suggest that eucentropogonids exhibit ‘steady state’ flowering congruent with low, but regular, daily visitation rates by pollinators (compared to, e.g. synchronous flowering in ‘cornucopia’ and ‘big bang’ events). Because hummingbird communities tend to partition into foraging modes ([Feinsinger and Colwell, 1978](#ref-feinsinger_1978); [Stiles, 1985](#ref-stiles_1985)) we expect steady state flowering to be incompatible with the foraging behaviours of territorial hummingbirds, thus further promoting floral isolation in the eucentropogonids. As with floral shape, flowering types are thought to evolve either via competition for pollination or selection against interspecific pollen transfer ([Primack, 1985](#ref-primack_1985); [Rathcke and Lacey, 1985](#ref-rathcke_1985); [Kessler et al., 2020](#ref-kessler_2020)). Therefore, accurately assigning flowering types in the context of pollinator foraging modes will be key to examining the evolution of this trait in the centropogonids, and assessing the role of phenology in coevolutionary diversification.

   Although the flowering types of some centropogonids have been described qualitatively, e.g. *Centropogon valerii* Standl. ([Colwell et al., 1974](#ref-colwell_1974)), *Centropogon solanifolius* Benth. ([Weiss, 1996](#ref-weiss_1996)), the ‘ecotype’ framework of Gentry ([1974](#ref-gentry_1974)) considers two continuous variables, flowering duration () and rate (), which have been used previously to calculate the *maximal display size* () of an inflorescence or individual ([Harder and Johnson, 2005](#ref-harder_2005)). We propose that to categorize flowering types, the anthesis rate () should be examined for linearity, where we expect steady state species to exhibit a constant daily flowering rate, whereas cornucopia and big bang species would flower non-linearly ([Gentry, 1974](#ref-gentry_1974)). To this end, the *average deviation from linearity* metric ([Kroll et al., 2000](#ref-kroll_2000)) may be useful in developing a reproducible, quantitative framework for assigning Gentry’s ([1974](#ref-gentry_1974)) phenological ecotypes (see: Methods).

   Therefore, 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 (*Eutoxeres condamini*) in southeastern Peru. Specifically, we ask: (1) Is Buff-tailed Sicklebill a visitor of *C. granulosus*? (2) Does Sicklebill visitation affect the reproductive success of *C. granulosus*? and (3) Is the flowering type of *C. granulosus* consistent with adaptation to the presumed foraging mode of Buff-tailed Sicklebill?

#### Materials and Methods

*Focal Species*

*Centropogon granulosus* is an understory, vining subshrub with abruptly curved, tubular flowers. The flowers are bright red or orange, with yellow deltate lobes, and are positioned vertically on lignified, terminal inflorescences ([Stein, 1987](#ref-stein_1987); [Lagomarsino et al., 2014](#ref-lagomarsino_2014)). This species is both the most widespread and variable eucentropogonid, occurring from southern Nicaragua to Bolivia. Consequently, the collections representing this species are not monophyletic ([Lagomarsino et al., 2014](#ref-lagomarsino_2014)), and may be reorganized in future treatments of Lobelioideae. The species examined here conforms to *Centropogon granulosus* subsp. *granulosus* (*sensu* [Stein, 1987](#ref-stein_1987)). Although 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.

   Very little is known of Buff-tailed Sicklebill: Stein ([1987](#ref-stein_1987)) described *Eutoxeres* as “not particularly rare, [but] seldom seen”. Beyond occurrence records and taxonomic treatments, scant natural history information is available for this hummingbird (e.g. nesting biology, foraging behaviours, mating). What is presumed to be known of this species tends to have been inferred from its relatively well studied congener, White-tipped Sicklebill. *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)).

*Field Site*

   We based our field work at the Cock-of-the-Rock (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 stationed at the transition of lower montane forest and cloud forest within 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. Coloured cells represent 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)). Cameras were mounted onto nearby trees using bungee cord, typically at a distance of 1-2 meters from an inflorescence and at a height equivalent to the inflorescence. 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 and floral development*

   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 S3). Wire cages prevent hummingbirds from accessing the flowers while allowing invertebrates to move freely. An additional ten inflorescences were marked with cardstock tags tied to the stem and monitored as controls. Using the control flowers, we defined eight stages of floral development (Table S2, Figure 3). 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 H), (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).

   Some flowering stages were not completely observed due to herbivory or weather. Similarly, monitoring of some flowers began with the current stage partially completed. This type of data is ‘right censored’, i.e. the true durations of these stages are greater than was observed ([Allison, 2014](#ref-allison_2014)). To account for censoring, we fit parametric survival functions ([Allison, 2014](#ref-allison_2014)) to the stage duration data. This allowed an estimation of the median duration () for each stage (), i.e. the number of days elapsed in stage before the daily probability of transitioning to stage surpassed 50%. Survival functions and median stage durations were estimated from the Gompertz distribution ([Ricklefs and Scheuerlein, 2002](#ref-ricklefs_2002)) using flexsurv v.2.0 ([Jackson, 2016](#ref-jackson_2016)).

   To reconstruct floral development from the censored dataset, we used the median stage durations and 95% confidence intervals (CI) estimated from the survival analysis above. For each treatment, we cumulatively summed the median stage durations to approximate the number of days elapsed between stages A and H. We accounted for error propagation, i.e. the uncertainty of each in influencing the 95% CI of , by summing the 95% CIs in quadrature ([Ku, 1966](#ref-ku_1966)).

*Flowering type*

   To characterize the flowering type of *C. granulosus*, we used broom v.0.7.6 ([Robinson et al., 2021](#ref-robinson_2021)) to fit linear models to the number of flowers produced through time. Models were fit for each inflorescence that produced at least five flowers ( = 5 for each treatment). The slope of each linear regression was interpreted as the anthesis rate. To assess linearity, we used lin.eval v.0.1.2 ([Shrivastav, 2019](#ref-shrivastav_2019)) to fit linear and polynomial (>1) curves to the anthesis rate. This method uses the *average deviation from linearity* ([Kroll et al., 2000](#ref-kroll_2000)), to determine if non-linear fits have significantly lower residuals than a linear regression.

#### Results

*Floral Visitors*

Camera trap recordings and *in situ* observations confirm Buff-tailed Sicklebill as a visitor to *Centropogon granulosus* flowers (Figure 2, Figure S4, video footage available in Dryad Repository). We recorded 12 visits to six *C. granulosus* individuals over 34 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. *E condamini* feeds 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* by piercing the corolla tube at the base. Over the course of two days, a camera trap recorded five visits per day to the same inflorescence (Table S4). Further details of *S. geoffroyi* behaviour can be found in Boehm ([2018](#ref-boehm_2018_b)). No other hummingbirds were recorded visiting these flowers.

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

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 and 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 S4). 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 S5, S6). We note this because it is unknown how the fleshy berries are dispersed, though we documented signs of frugivory (Figure S7).

   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 lepidopteran inhabiting a flower (Figure S8), and a stingless bee (Meliponini) collecting pollen from the anther scale (Figure S9).

*Pollinator exclusion and floral development*

   We identified and described eight stages of floral development in *C. granulosus* (Table S2, Figure 3). During the first stage (A), the flowers are small buds, and have not yet developed curvature. In stages B to D, the flowers elongate and form their characteristic hook shape — nearly all floral curvature is developed here. Stages E and F are defined by the staminate and pistillate phases of anthesis, respectively. Following anthesis, the flowers wilt (Stage G), and if pollinated, form berries (Stage H, see Table S2 for further details of each stage).

   These eight stages were used to compare developmental differences between control and pollinator-excluded flowers (Figure 3). Between treatments, the progression of floral development is comparable from stages A (bud development) to E (anthesis). However, control flowers spend 24.2 ± 4.47 days (median ± 95% CI) developing berries, while all but two hummingbird-excluded flowers did not produce berries.

*Flowering rate*

   Hummingbird exclusion did not affect the total number of flowers produced (=0.782, =0.282, =0.15). 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 (34 days); the upper limit of flower production for a single inflorescence has not been determined, though we counted 68 peduncle scars on an individual not included in this study (Figure S10).

   Linear models accurately described flowering rate (Figure 4): all anthesis rates were fit better by linear models than polynomials ( < 0.05). Flowering rate (slope) varied among inflorescences (=0.027, =2.70, =1.91), but not between treatments (=0.200, =1.40, =0.99). The average rate was one anthesis event per 3.23 ± 0.12 days for control plants and 4.60 ± 0.07 days for pollinator-excluded plants. The highest flowering rate was documented in a control plant at 1.88 days, nearly twice the rate of the next fastest individual (Figure 4). When the fast inflorescence is removed, the mean flowering rate for control plants is 3.94 ± 0.03 days. The lowest flowering rates were 7.22 days and 7.06 days, both in pollinator-excluded plants.

Figure 3. Developmental trajectories for the flowers of C. granulosus, and effects of pollinator exclusion. The eight stages labeled on the x-axis are described in Table S2. The y-axis represents the number of days required to reach each stage.

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Figure 4. Flowering rate in C. granulosus. The x-axis represents the number of days elapsed since the first flower opened. The y-axis counts the cumulative number of flowers opened since t=0, not the number of flowers open simultaneously.

Figure 4. Flowering rate in C. granulosus. The -axis represents the number of days elapsed since the first flower opened. The -axis counts the cumulative number of flowers opened since =0, not the number of flowers open simultaneously.

#### 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 due 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 Sicklebill at 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 ([Westerkamp, 1990](#ref-westerkamp_1990); [Rico-Guevara et al., 2021](#ref-ricoguevara_2021)).

   In contrast to previous accounts of Sicklebill visitation to *Centropogon* ([Stiles, 1985](#ref-stiles_1985); [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); [Altschuler et al., 2004](#ref-altschuler_2004)) and is avoided when perches are available ([Westerkamp, 1990](#ref-westerkamp_1990)). Recent work has found that short-billed hummingbird species have repeatedly evolved large 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 ([Temeles et al., 2019](#ref-temeles_2019); [Boehm et al., 2021](#ref-boehm_2021)).

   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 tend to face away from the stem on long peduncles (Figure 1). This is in contrast to *E condamini* visits to nearby *Heliconia*, which has flowers oriented so that the aperture is aligned with the perch (i.e. floral bract, Figure S11).

*Pollinator exclusion and floral development*

   Plants with pollinators excluded are less likely to produce berries. We note that berry development occurred in 12% of pollinator-excluded flowers, though these fruits were aborted after ~18 days. This consistent with the glasshouse experiements of Stein ([1987](#ref-stein_1987)), who reported that 18% of self-fertilized *C. granulosus* produced viable fruit. Given that *E. condamini* was the only hummingbird to legitimately visit *C. granulosus*, we conclude that Buff-tailed Sicklebill is a highly specialized pollinator, whose visitation is required for the completion of the *C. granulosus* life cycle. However, we found that other aspects of floral display were not affected by pollinator exclusion: the total number of flowers produced per inflorescence, and the rate of flowering. Of the latter, we note that one control plant exhibited an accelerated flowering rate at twice the magnitude of the average. This was not accompanied by shortened anthesis duration, suggesting that this individual experienced increased geitonogamy. While geitonogamy is generally viewed as a trade-off for floral display size, it may also be a plastic trait providing reproductive assurance ([Huang et al., 2020](#ref-huang_2020)). Whether this observation is incidental or indicates the potential for adaptive plasticity of flowering rates is an interesting and unexplored aspect of the steady state flowering type.

*Steady State Flowering and Traplining*

*C. granulosus* exhibits a linear flowering rate befitting the ‘steady state’ phenological type described by Gentry ([1974](#ref-gentry_1974)) as “..[the production of] a few flowers a day over an extended period of time (usually a month or more)”. It is one of several phenological modes representing an axis of niche partitioning that is thought to contribute 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 flowering types. Because the steady state strategy implies a linear flowering rate, anecdotal observations of flowering type can be further tested using the linearity metric implemented here.

   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. Broadly, the behaviours exhibited by *E. condamini* are in accordance with the foraging behaviours documented for White-tipped Sicklebill in Costa Rica ([Stiles, 1985](#ref-stiles_1985)). However, the fine-scale daily movements of *Eutoxeres* (and Hermits generally) have not yet been studied — at present, comparative analyses are constrained by our rudimentary understanding of the natural history of these pollinators.

*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 to validate these models and the pollination syndrome concept that underlies its predictions. Furthermore, because Sicklebills exhibit traplining, it is possible that specialization operates not only through the unique corolla shapes of the eucentropogonids, but also the steady state flowering type exhibited in *C. granulosus*. We hope that through further studies of Sicklebill-pollinated *Centropogon*, we might better understand the contribution of flowering type and phenology in the rapid diversification of the Andean flora.

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