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

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**Submission Type**: Brief Communication

**Abstract**: 211 words

**Main Body**: 3908 (including figure captions)

**Figures**: 4 (Additional Figures in the Supplementary Materials)

**Tables**: In Supp. Mat. only

**Keywords**: *Centropogon*, *Eutoxeres*, floral development, pollination, Sicklebill, traplining

**Manuscript received**

**Revision accepted**

**Short title** Floral development and pollination of *C. granulosus*

#### Acknowledgements

Access to field sites was made possible by the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR) of Peru, and Daniel Blanco and the generous staff of the Cock-of-the-Rock Lodge, San Pedro, Peru. The faculty and staff at the Universidad Nacional de San Antonio Abad del Cusco (UNSAAC) herbarium graciously assisted with the permit application process. B. Freeman and M. Scholer organized ground transport to the field sites. J. Fahr gave their expert identification for Figure S3 via iNaturalist. We thank A. Sargent and R. Colwell for feedback and discussions that greatly improved the manuscript. M.M.A.B. acknowledges financial support from a Hesse Research Award in Ornithology, the Heliconia Society International, the University of British Columbia, and the Natural Sciences and Engineering Research Council (NSERC) of Canada Post-Graduate Scholarship program.

* NSERC information for QC and JJ

#### 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 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 floral phenology 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 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 (Julians FigureXXX). 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 are generally found in Neotropical lowland and lower montane forest. The 36 species of Hermits described 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 uplift (~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 pollinators of the various eucentropogonid species. 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) should also conform to the daily foraging habits of *Eutoxeres*. Considering that *E. aquila* is a trapliner ([Stiles, 1985](#ref-stiles_1985)), we can use the ‘phenological ecotype’ model of Gentry ([1974](#ref-gentry_1974)) to predict that eucentropogonids do not flower simultaneously in ‘cornucopia’ events, but should rather exhibit a ‘steady state’ strategy that is adapted to low, but regular, daily visitation rates. 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 incongruent with the foraging behaviours of territorial hummingbirds. Although flowering rates of some centropogonids have been described qualitatively, e.g. *Centropogon valerii* ([Colwell et al., 1974](#ref-colwell_1974)), *Centropogon solanifolius* ([Weiss, 1996](#ref-weiss_1996)), to date, flowering type has not yet been studied as a continuous trait. As with floral shape, flowering types are thought to evolve via competition for pollinators ([Gentry, 1974](#ref-gentry_1974); [Kessler et al., 2020](#ref-kessler_2020)). Thus, quantifying flowering rates in the context of pollinator foraging modes will be key to examining trait evolution across the centropogonids, and assessing the role of phenology in co-evolutionary diversification.

   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 (*Eutoxeres condamini*) in southeastern Peru (Figure 1). Specifically, we ask: (1) Is Buff-tailed Sicklebill a visitor of *C. granulosus*? (2) Does Sicklebill visitation affect the reproductive success of *C. granulosus*? (3) Is Buff-tailed Sicklebill visitation consistent with traplining behaviour? and (4) Is the flowering phenology of *C. granulosus* consistent with adaptation to the foraging mode exhibited by 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)). 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.

   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 situated 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 when possible, 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 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 inflorescences were marked with cardstock tags tied to the stem and monitored as controls. Using the control flowers, we defined six 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 F), (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 were staggered because each flower developed independently. The data were also fragmented because some samples wilted or 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 A to C) or ‘late’ (stages D and F) 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 20% 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 F) 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 characterize flowering phenology of *C. granulosus*, we used broom v.0.7.6 ([Robinson et al., 2021](#ref-robinson_2021)) to fit linear models to the anthesis rate for each inflorescence that produced at least five flowers ( = 6 controls, = 5 pollinator excluded). The adjusted of the model fit was used to assess linearity. Comparisons of between individuals and treatments were made using XXX.

To test for effects of pollinator exclusion on the duration of the male and female phases of anthesis, we fit generalized linear mixed effects models (GLMMs) in lme4 v.1.1-26 [XXX, Supp. Mat.]. We used fitdistrplus 1.1-5 [XXX] to examine skewness and distribution families for the GLMMs, and effectsize v.0.4.4 ([Ben-Shachar et al., 2020](#ref-shacar_2020)) to compute Cohen’s .

Tests and model parameters are further 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 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. No other hummingbirds were recorded visiting these flowers. Over the course of two days, a camera trap 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 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 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 lepidopteran inhabiting a flower (Figure S7), and an anthophilid (bee) collecting pollen from the anther scale (Figure S8).

*Flowering rate and pollinator exclusion*

   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 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 0.949 ± 0.036, with marginal variation in among inflorescences (=0.117, =1.73, =1.15). Flowering rate (slope) varied among inflorescences (=0.037, =2.45, =1.63), but not between treatments (=0.273, =1.17, =0.78). The average rate was one anthesis event per 3.44 ± 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 1.79 days, nearly twice the rate of the next fastest individual (Figure S11). When the fast inflorescence is removed, the mean flowering rate for control plants is 4.12 ± 0.03 days. The lowest flowering rates were 7.22 days and 7.06 days, both in pollinator-excluded plants.

*Floral development and pollinator exclusion*

   The flowers of *C. granulosus* are protandrous: for the control plants, the male and female phases lasted 2.12 ± 0.23 and 2.73 ± 0.26 days, respectively (Figure S12). Pollinator exclusion did not have a significant effect on the duration of the male phase (=0.217, =1.23, =0.33) or female phase (=0.784, =0.27, =0.07, Figure S12).

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

   Delimiting floral development into six stages generated a consensus developmental sequence (per treatment) that mapped at least 80% of the phenology data collected in this study. These six stages were used to compare differences in development between control and pollinator-excluded flowers (Figure 3). Between treatments, the rate of floral development is comparable from stages A (bud development) to C (anthesis). However, control flowers spend an additional 21 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 for both treatments. However, at 13 days post-anthesis, hummingbird-excluded flowers wilt, while control plants continued to develop into a fruiting phase and produce berries (Figure 4). Before berry development, the survival curves are comparable (=0.974, =0.01, =0.03). When berry development is considered, the survival curves differ significantly (=0.034, =4.51, =2.07).

Figure 3. Developmental trajectories for the flowers of C. granulosus, and effects of pollinator exclusion. The six stages labeled on the x-axis have statistical support as discrete developmental units and are described in Table S2. Stage B is represented by three substages that are grouped together; these substages are descriptive and used for convenience only. The y-axis represents the number of days required to reach each stage. Note that hummingbird-excluded plants do not reach stage F

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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.

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 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 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 ([Rico-Guevara et al., 2021](#ref-ricoguevara_2021)).

*Perching versus Hovering (natural history)*

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](#ref-suarez_2002) ([2002](#ref-suarez_2002)); doug] and is avoided when perches are available ([**westerkamp\_1990?**](#ref-westerkamp_1990)). 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 are predicted to have smaller flight muscles and are less maneuverable than territorial hummingbirds ([Sargent et al., 2021](#ref-sargent_2021)). Hover pollination of *C. granulosus* may then present an exceptional challenge for *Eutoxeres*; these hummingbirds have large feet presumably adapted for using the floral bracts of *Heliconia* as a perch while feeding ([Feinsinger and Colwell, 1978](#ref-feinsinger_1978); [Colwell et al., 2021](#ref-colwell_2021)). 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 makes twice as many daily visits as *E. condamini*. For these reasons, we expect *S. geoffroyi* to exhibit traveling exploitation at a smaller spatial scale than *Eutoxeres*, whereby a smaller number of scattered plants are visited more frequently, but not continuously defended as part of a static territory ([Sargent et al., 2021](#ref-sargent_2021)). It should also be noted that strongly curved bills are a feature of nectivorous African sunbirds (FAMILY) which perch (often on thickened peduncles) to feed [2020 paper on experimental perching].

*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 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 flowering types. For example, quantifying flowering rates of centropogonids could allow robust statistical classifications of phenological flowering types. 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 have rarely been considered in the context of dichogamy. 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 outcrossing while minimizing the risk of a pollinator visit without open flowers.

   We found that several aspects of flowering were not affected by pollinator exclusion: the total number of flowers produced, the duration of the female phase, and the rate of flowering. Of the latter, we note that one control plant exhibited an accelerated flowering rate (Figure S11) at twice the magnitude of the average. 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. We also observe that the male phase shortens by ~25% in response to pollinator exclusion. This is in contrast to *Centropogon talamancensis* Wilbur and *Centropogon solanifolius* Benth. studied in Costa Rica, where experimental pollen removal shortened the male phase ([Koptur et al., 1990](#ref-koptur_1990); [Weiss, 1996](#ref-weiss_1996)). In their study of proandry in *Campanula americana* L., Evanhoe and Galloway ([Evanhoe and Galloway, 2002](#ref-evanhoe_2002)) observed a ~20 hour delay between pollen removal and the onset of the female phase: they hypothesized that the delay aligns the female phase to coincide with daily peaks in pollinator activity. Thus, pollinator-excluded plants in this study might be reconsidered as “controls” whereby plants visited by *Eutoxeres* have modified (delayed) onset of the female phase in order to coincide with visitation patterns inherent in traplining behaviour. Of course, very little is yet known of traplining in hummingbirds, and

The floral longevity model of Shoen and Ashman [@-shoen\_1995] predicts that the male phase should shorten when fitness accrual (i.e. pollen removal) is high. Further work will be needed to determine if the patterns observed here are the result of strong selection for the onset of the female phase, e.g. for a plant not receiving po

Because further work with a greater sample size will be needed to

Indeed, ([**shoen\_1995?**](#ref-shoen_1995))

   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*. 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, interspecific comparisons are limited by a lack of data.

   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 not 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.

*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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