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

**Author Names**: Mannfred M.A. Boehm, David Guevara Apaza, Jill E. Jankowski, Quentin C.B. Cronk

**Corresponding Author**: [mannfred.boehm@ubc.ca](mailto:mannfred.boehm@ubc.ca)

**Author affiliations**: Department of Botany, University of British Columbia, 3156–6270 University Boulevard, Vancouver, BC, Canada, V6T 1Z4.

Biodiversity Research Centre, University of British Columbia, 2212 Main Mall, Vancouver, BC, Canada, V6T 1Z4.

Facultad de Ciencias Biológicas, Universidad San Antonio Abad del Cusco, Av. de La Cultura 773, Cusco 08000, Peru.

Department of Zoology, University of British Columbia, 4200–6270 University Boulevard, Vancouver, BC, Canada, V6T 1Z4.

**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 and J. Ascher gave their expert identifications via iNaturalist for Figures S4 and S8, respectively. 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) 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 of Gentry ([1974](#ref-gentry_1974)) would predict that eucentropogonids exhibit a ‘steady state’ strategy that is adapted to low, but regular, daily visitation rates (compared to, e.g. flowering simultaneously in ‘cornucopia’ 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 incongruent with the foraging behaviours of territorial hummingbirds. Although flowering rates 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)), 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*? and (3) Is the flowering phenology 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)). 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.

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](#ref-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.

*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 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 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 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)).

   Finally, we tested for effects of pollinator exclusion on the duration of the staminate (stage E) and pistillate (stage F) phases of anthesis, by fitting generalized linear mixed effects models (GLMMs) in lme4 v.1.1-26 ([Bates et al., 2015](#ref-bates_2015)). We used fitdistrplus 1.1-5 ([Delignette-Muller and Dutang, 2015](#ref-delignette_2015)) to examine distribution families for the GLMMs, and effectsize v.0.4.4 ([Ben-Shachar et al., 2020](#ref-shacar_2020)) to compute Cohen’s . Most censoring occurred at the early and late stages of development, thus, this analysis required discarding only 12 (23%) and 20 (20%) censored observations from the pollinator excluded and control data, respectively.

*Flowering rate*

   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 coefficient of determination, , of the model fit was used to assess linearity. Individual and treatment driven variation in was tested by ANOVA.

#### 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 a stingless bee (Meliponini) collecting pollen from the anther scale (Figure S8).

*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.

   The flowers of *C. granulosus* are protandrous: for the control plants, the staminate (stage E) and pistillate (stage F) 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 staminate phase (=0.217, =1.23, =0.33) or pistillate phase (=0.784, =0.27, =0.07, Figure S12).

*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 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 1.88 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.

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.

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.

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 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](#ref-suarez_2002) ([2002](#ref-suarez_2002)); 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 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).

*Pollinator exclusion and floral development*

   We found that several aspects of floral display were not affected by pollinator exclusion: the total number of flowers produced per inflorescence, the duration of the staminate and pistillate phases, 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.

   It is noteworthy that the staminate and pistillate phases were not affected by pollinator exclusion. This is in contrast to studies of *Centropogon talamancensis* Wilbur ([Koptur et al., 1990](#ref-koptur_1990)) and *Centropogon solanifolius* Benth. ([Weiss, 1996](#ref-weiss_1996)), where experimental pollen removal shortened the staminate phase — a trait that is thought to improve male fitness in the presence of intrasexual competition for pollination [lloyd\_1982; shoen\_1995]. In *Lobelia cardinalis* L. ([Devlin and Stephenson, 1984](#ref-devlin_1984)) and *Campanula americana* L. ([Evanhoe and Galloway, 2002](#ref-evanhoe_2002)), experimental pollen removal also shortens the staminate phase; however, in natural populations of *L. cardinalis* visited by hummingbirds, this phenomenon is not observed because repeated visits over several days are required to remove the available pollen ([Devlin and Stephenson, 1985](#ref-devlin_1985)). In contrast, natural populations of bee-pollinated *C. americana* exhibit the same shortened staminate phase observed in plants with pollen removed experimentally ([Evanhoe and Galloway, 2002](#ref-evanhoe_2002)). Taken together, these studies point to variation in sexual phase longevity that is driven by efficiency of pollen removal which itself may be influenced by pollinator identity. Because pollen was not experimentally removed in this study, it is possible that either *C. granulosus* has not experienced selection to modify sexual phase longevity in response to pollen removal, or that single visits by Buff-tailed Sicklebill do not remove enough pollen to initiate this trait.

\*\*\*Maybe floral traits were not affected because shortening the display in response to pollination might negatively affect floral display. Because there is usually only one-two flowers open at a time, any shortening of the male/female phase might negatively affect floral display.

\*\*\*Maybe there is strong selection against geitonogamy, and increasing the male phase would encourage geitonogamy if the female phase cannot be delayed.

\*\*\*C. granulosus produces berries from ~18% of selfed flowers (Stein 1987). This points to why sexual phase durations would not be altered in response to pollination (might increases geitonogamy)

*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 the centropogonids could allow robust statistical classifications of phenological flowering types and tests of correlated evolution with pollination mode, morphology, geography, etc. If steady state flowering is interpreted as conforming to a linear model – as we have assumed here – then deviations from this phenological type might be quantified by the coeffiecient of determiniation, , where steady state species would have a relatively higher compared to those exhibiting cornucopia flowering. Pairing such measurements with rates of pollinator visitation would move the field towards understanding the evolution of phenological modes as mechanisms of divergence.

   In *C. granulosus*, there is a degree of coordination in the anthesis rate and the staminate -> pistillate transition rate. As a new flower opens, the initial staminate 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 pistillate phase is protected from geitonogamy. 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.

*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.

   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.

# References

Abrahamczyk, S., Poretschkin, C., and Renner, S.S. (2017). Evolutionary flexibility in five hummingbird-plant mutualistic systems: Testing temporal and geographic matching. Journal of Biogeography *44*, 1847–1855.

Allison, P.D. (2014). Event history and survival analysis: Regression for longitudinal event data (Thousand Oaks, CA: SAGE publications Inc.).

Althoff, D.M., Segraves, K.A., and Johnson, M.T. (2014). Testing for coevolutionary diversification: Linking pattern with process. Trends in Ecology & Evolution *29*, 82–89.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software *67*, 1–48.

Ben-Shachar, M.S., Lüdecke, D., and Makowski, D. (2020). effectsize: Estimation of effect size indices and standardized parameters. Journal of Open Source Software *5*, 2815–2892.

Boehm, M.M.A. (2018). Biting the hand that feeds you: Wedge-billed hummingbird is a nectar robber of a sicklebill-adapted Andean bellflower. Acta Amazonica *48*, 146–150.

Boehm, M.M.A., Scholer, M.N., Kennedy, J.J.C., Heavyside, J.M., Daza, A., Guevara-Apaza, D., and Jankowski, J.E. (2018). The Manú Gradient as a study system for bird pollination. Biodiversity Data Journal *6*.

Boehm, M.M.A., Jankowski, J.E., and Cronk, Q.C.B. (2021). Plant-pollinator specialization: Origin and measurement of curvature. In Review.

Colwell, R.K., Betts, B.J., Bunnell, P., Carpenter, F.L., and Feinsinger, P. (1974). Competition for the nectar of *Centropogon valerii* by the hummingbird *Colibri thalassinus* and the flower-piercer *Diglossa plumbea*, and its evolutionary implications. The Condor *76*, 447–452.

Colwell, R.K., Rico-Guevara, A., Sustaita, D., Yanega, G., Fucikova, K., and Rangel, T. (2021). An adaptive tradeoff concealed by allometry: Foot size and bill length are negatively correlated among hummingbirds. In Review.

Delignette-Muller, M.L., and Dutang, C. (2015). fitdistrplus: An R package for fitting distributions. Journal of Statistical Software *64*, 1–34.

Dellinger, A.S. (2020). Pollination syndromes in the 21st century: Where do we stand and where may we go? New Phytologist *228*, 1193–1213.

Devlin, B., and Stephenson, A. (1984). Factors that influence the duration of the staminate and pistillate phases of *Lobelia cardinalis* flowers. Botanical Gazette *145*, 323–328.

Devlin, B., and Stephenson, A. (1985). Sex differential floral longevity, nectar secretion, and pollinator foraging in a protandrous species. American Journal of Botany *72*, 303–310.

Dyck-Chan, L., David, S., and Jankowski, J.E. (2021). Resource specialization and range overlap of tropical hermit hummingbirds. In Revision.

Evanhoe, L., and Galloway, L.F. (2002). Floral longevity in *campanula americana* (Campanulaceae): A comparison of morphological and functional gender phases. American Journal of Botany *89*, 587–591.

Feinsinger, P., and Colwell, R.K. (1978). Community organization among neotropical nectar-feeding birds. American Zoologist *18*, 779–795.

Gentry, A.H. (1974). Flowering phenology and diversity in tropical Bignoniaceae. Biotropica *6*, 64–68.

Gentry, A.H. (1982). Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? Annals of the Missouri Botanical Garden *69*, 557–593.

Gill, F.B. (1987). Ecological fitting: Use of floral nectar in *Heliconia stilesii* Daniels by three species of hermit hummingbirds. The Condor *89*, 779–787.

Givnish, T., Barfuss, M., Ee, B. van, Riina, R., Schulte, K., Horres, R., Gonsiska, P., Jabaily, R., Crayn, D., Smith, J., et al. (2014). Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. Molecular Phylogenetics and Evolution *71*, 55–78.

Gómez, J., Perfectti, F., Bosch, J., and Camacho, J.P.M. (2009). A geographic selection mosaic in a generalized plant–pollinator–herbivore system. Ecological Monographs *79*, 245–263.

Grant, V. (1949). Pollination systems as isolating mechanisms in angiosperms. Evolution *3*, 82–97.

Harder, L.D., and Johnson, S.D. (2009). Darwin’s beautiful contrivances: Evolutionary and functional evidence for floral adaptation. New Phytologist *183*, 530–545.

Hinkelmann, C., and Boesman, P.F.D. (2020). Buff-tailed Sicklebill (*Eutoxeres condamini*), version 1.0. In Birds of The World, J. del Hoyo, A. Elliott, J. Sargatal, D.A. Christie, and E. de Juana, eds. (Ithaca, NY: Cornell Lab of Ornithology),.

Hughes, C., and Eastwood, R. (2006). Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences *103*, 10334–10339.

Jackson, C. (2016). flexsurv: A platform for parametric survival modeling in R. Journal of Statistical Software *70*, 1–33.

Janzen, D.H. (1971). Euglossine bees as long-distance pollinators of tropical plants. Science *171*, 203–205.

Kay, K.M., Reeves, P.A., Olmstead, R.G., and Schemske, D.W. (2005). Rapid speciation and the evolution of hummingbird pollination in neotropical *Costus* subgenus *Costus* (Costaceae): Evidence from nrDNA ITS and ETS sequences. American Journal of Botany *92*, 1899–1910.

Kessler, M., Abrahamczyk, S., and Krömer, T. (2020). The role of hummingbirds in the evolution and diversification of Bromeliaceae: Unsupported claims and untested hypotheses. Botanical Journal of the Linnean Society *192*, 592–608.

Koptur, S., Dávila, E., Gordon, D., McPhail, B.D., Murphy, C.G., and Slowinski, J.B. (1990). The effect of pollen removal on the duration of the staminate phase of *centropogon talamancensis*. Brenesia *33*, 15–18.

Ku, H.H. (1966). Notes on the use of propagation of error formulas. Journal of Research of the National Bureau of Standards *70*, 263–273.

Lagomarsino, L.P., Antonelli, A., Muchhala, N., Timmermann, A., Mathews, S., and Davis, C.C. (2014). Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae). American Journal of Botany *101*, 2097–2112.

Lagomarsino, L.P., Condamine, F.L., Antonelli, A., Mulch, A., and Davis, C.C. (2016). The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). New Phytologist *210*, 1430–1442.

Lagomarsino, L.P., Forrestel, E.J., Muchhala, N., and Davis, C.C. (2017). Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade. Evolution *71*, 1970–1985.

Maglianesi, M.A., Blüthgen, N., Böhning-Gaese, K., and Schleuning, M. (2014). Morphological traits determine specialization and resource use in plant–hummingbird networks in the Neotropics. Ecology *95*, 3325–3334.

Maglianesi, M.A., Blüthgen, N., Böhning-Gaese, K., and Schleuning, M. (2015). Functional structure and specialization in three tropical plant–hummingbird interaction networks across an elevational gradient in Costa Rica. Ecography *38*, 1119–1128.

McGuire, J.A., Witt, C.C., Remsen Jr, J., Corl, A., Rabosky, D.L., Altshuler, D.L., and Dudley, R. (2014). Molecular phylogenetics and the diversification of hummingbirds. Current Biology *24*, 910–916.

McVaugh, R. (1949). Studies in South American Lobelioideae (Campanulaceae) with special reference to Colombian species. Brittonia *6*, 450–493.

Morrison, B.M., and Mendenhall, C.D. (2020). Hummingbird–plant interactions are more specialized in forest compared to coffee plantations. Diversity *12*, 126.

Müller, K. (2017). here: A simpler way to find your files. R Package Version 0.1 *https://CRAN.R-project.org/package=here*.

Naskrecki, P., and Colwell, R.K. (1998). Systematics and host plant affiliations of hummingbird flower mites of the genera *Tropicoseius* Baker & Yunker and *Rhinoseius* Baker & yunker (Acari: Mesostigmata: Ascidae) (Lanham, MD: Entomological Society of America).

Nentwig, W., and Christenson, T.E. (1986). Natural history of the non-solitary sheetweaving spider *Anelosimus jucundus* (Araneae: Theridiidae). Zoological Journal of the Linnean Society *87*, 27–35.

Pérez-Escobar, O.A., Chomicki, G., Condamine, F.L., Karremans, A.P., Bogarı́n, D., Matzke, N.J., Silvestro, D., and Antonelli, A. (2017). Recent origin and rapid speciation of Neotropical orchids in the world’s richest plant biodiversity hotspot. New Phytologist *215*, 891–905.

R Core Team (2017). R: A language and environment for statistical computing (Vienna, Austria: R Foundation for Statistical Computing).

Ricklefs, R.E., and Scheuerlein, A. (2002). Biological implications of the weibull and gompertz models of aging. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences *57*, B69–B76.

Rico-Guevara, A., Hurme, K.J., Elting, R., and Russell, A.L. (2021). Bene‘fit’ assessment in pollination coevolution: Mechanistic perspectives on hummingbird bill-flower matching. Integrative and Comparative Biology *https://doi.org/10.1093/iob/oby006*.

Robinson, D., Hayes, A., and Couch, S. (2021). broom: Convert statistical objects into tidy tibbles. R Package Version 0.7.6 *https://CRAN.R-project.org/package=broom*.

Salinas, N., Malhi, Y., Meir, P., Silman, M., Roman Cuesta, R., Huaman, J., Salinas, D., Huaman, V., Gibaja, A., Mamani, M., et al. (2011). The sensitivity of tropical leaf litter decomposition to temperature: Results from a large-scale leaf translocation experiment along an elevation gradient in Peruvian forests. New Phytologist *189*, 967–977.

Sargent, A., Groom, D., and Rico-Guevara, A. (2021). Locomotion and energetics of divergent foraging strategies in hummingbirds: A review. Integrative and Comparative Biology *https://doi.org/10.1093/icb/icab124*.

Smith, S.D. (2016). Pleiotropy and the evolution of floral integration. New Phytologist *209*, 80–85.

Sonne, J., Zanata, T.B., Martı́n González, A.M., Cumbicus Torres, N.L., Fjeldså, J., Colwell, R.K., Tinoco, B.A., Rahbek, C., and Dalsgaard, B. (2019). The distributions of morphologically specialized hummingbirds coincide with floral trait matching across an Andean elevational gradient. Biotropica *51*, 205–218.

Stein, B.A. (1987). Systematics and evolution of *Centropogon* subg. *Centropogon* (Campanulaceae: Lobelioideae). Ph.D. Dissertation. Washington University, St. Louis, WA.

Stein, B.A. (1992). Sicklebill hummingbirds, ants, and flowers. BioScience *42*, 27–34.

Stiles, F.G. (1985). Seasonal patterns and coevolution in the hummingbird-flower community of a Costa Rican subtropical forest. Ornithological Monographs *36*, 757–787.

Suarez, R.K., and Gass, C.L. (2002). Hummingbird foraging and the relation between bioenergetics and behaviour. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology *133*, 335–343.

Sun, S.-G., Huang, Z.-H., Chen, Z.-B., and Huang, S.-Q. (2017). Nectar properties and the role of sunbirds as pollinators of the golden-flowered tea *Camellia petelotii*. American Journal of Botany *104*, 468–476.

Temeles, E.J., Linhart, Y.B., Masonjones, M., and Masonjones, H.D. (2002). The role of flower width in hummingbird bill length-flower length relationships. Biotropica *34*, 68–80.

Temeles, E.J., Liang, J., Levy, M.C., and Fan, Y.-L. (2019). Floral isolation and pollination in two hummingbird-pollinated plants: The roles of exploitation barriers and pollinator competition. Evolutionary Ecology *33*, 481–497.

Tennekes, M. (2018). tmap: Thematic maps in R. Journal of Statistical Software *84*, 1–39.

Thompson, J.N. (2005). The geographic mosaic of coevolution (Chicago, IL: University of Chicago Press).

Weiss, M.R. (1996). Pollen-feeding fly alters floral phenotypic gender in *Centropogon solanifolius* (Campanulaceae). Biotropica *28*, 770–773.

Westerkamp, C. (1990). Bird-flowers: Hovering versus perching exploitation. Botanica Acta *103*, 366–371.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., et al. (2019). Welcome to the tidyverse. Journal of Open Source Software *4*, 1686.