Floral phenology of an Andean bellflower (*Centropogon granulosus*, Lobelioideae) 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**: 245 words (max 250 for AmJBot)

**Main Body**: 3777 (including figure captions; 4000 max for AmJBot)

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

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

**Keywords**: *Centropogon*, *Eutoxeres*, phenology, pollination, Sicklebill, specialization, trapline

**Manuscript received**

**Revision accepted**

**Short title** Floral phenology 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) and the Vargas (CUZ) herbarium both graciously assisted with the permit application process. B. Freeman and M. Scholer organized ground transport to the field sites. J. Fahr, J. Ascher, and Y. Delago gave their expert identifications for Figures S5, S6, and S9 via iNaturalist. We thank L. Lagomarsino, A. Sargent, and R. Colwell for feedback and discussions that greatly improved the manuscript. M.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. Q.C. and J.J. are supported by the NSERC Discovery Grants program.

#### AUTHOR CONTRIBUTIONS

M.B. and Q.C. designed the study, and D.G., 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

**Premise of the study**: The Andean bellflowers comprise a highly diverse recent radiation, mediated in part by the evolution of different pollination niches. One diverse clade has evolved with extremely curved floral tubes (‘eucentropogonids’) and these species are predicted to be pollinated exclusively by one of two parapatric species of Sicklebill hummingbirds (*Eutoxeres*). In this study we focused on the floral biology of *Centropogon granulosus*, a eucentropogonid thought to be exclusively pollinated by *E. condamini* in southeastern Peru.

**Methods**: Using camera traps and a pollination exclusion experiment, we determined the visitors to *C. granulosus*, and the effect of hummingbird-exclusion on fruit development. Because phenological types may also mediate floral specialization, we quantified anthesis rates in the context of pollinator behaviour.

**Key Results**: *E. condamini* was the only legitimate pollinator of *C.granulosus* during this study, and visitation is necessary for fruit development. We estimate an average anthesis rate of one flower per 3.94 ± 0.03 days. Based on the inflorescence of one individual, we approximate a flowering season lasting 9 months. Flowering rates were unequivocally linear and conformed to the ‘steady state’ phenological type. Over the course of >1800 hours of monitoring we recorded 12 *E. condamini* visits totaling 42 seconds, indicating traplining behaviour.

**Conclusions**: As predicted by its floral morphology, *C. granulosus* is exclusively pollinated by Buff-tailed Sicklebill. We also present preliminary evidence for the congruence between phenological type and hummingbird foraging behaviour as a driver of specialization in this mega-diverse clade of Andean bellflowers.

#### INTRODUCTION

The Andean bellflowers (Lobelioideae) of *Centropogon*, *Siphocampylus*, and *Burmeistera* (the ‘centropogonids’) comprise over 550 species emerging in the last five million years ([Lagomarsino et al., 2016](#ref-lagomarsino_2016)). This rapid diversification is correlated, in part, with the repeated evolution of pollination by bats and hummingbirds ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)). For some centropogonids, pollinator shifts are concomitant with pollinator specialization ([Lagomarsino and Muchhala, 2019](#ref-lagomarsino_2019)), i.e. floral traits appear to be adapted for pollination by the most effective pollinator and/or exclusion of other animal taxa ([Armbruster, 2017](#ref-armbruster_2017)). It is through this process that the evolution of specialization can influence patterns of plant diversification (reviewed in [Kay and Sargent, 2009](#ref-kay_2009); [Armbruster, 2014](#ref-armbruster_2014)). Thus, elucidating the ecology and natural history of putatively specialized pollination systems will continue to shape our understanding of rapid plant radiations.

   One diverse clade of *Centropogon*, the ‘eucentropogonids’ (38 spp.), evolved after what is apparently a single unique shift to pollination by Sicklebill hummingbirds (*Eutoxeres*, [Lagomarsino et al., 2017](#ref-lagomarsino_2017)). Almost all members of this clade are predicted, on the basis of their strongly curved corolla tubes, to be pollinated by Sicklebills (Fig. 1, [Stein, 1987](#ref-stein_1987)). This is thought to enable coexistence between Sicklebill-pollinated species, and those pollinated by straight-billed hummingbirds and bats ([Lagomarsino et al., 2017](#ref-lagomarsino_2017)), though it is unknown if or how sympatric eucentropogonids maintain floral isolation (but see: [Muchhala and Potts, 2007](#ref-muchhala_2007)). In this study we focus on the pollination of *Centropogon granulosus* C.Presl by Buff-tailed Sicklebill (*Eutoxeres condamini*). We test the hypothesis that *C. granulosus* is solely pollinated by Sicklebills, and has functional characters (floral morphology and flowering phenology) that facilitate this.

*C. granulosus* is an understory, vining subshrub with abruptly curved, tubular flowers (Fig. 1). The flowers are bright red to orange, with yellow triangular lobes, and are positioned vertically on lignified, terminal inflorescences ([Stein, 1987](#ref-stein_1987); [Lagomarsino et al., 2014](#ref-lagomarsino_2014)). The anthers are fused into a hardened scale that acts as a trap door: the majority of a flower’s pollen is released with only a single push of the anther scale. 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 in Costa Rica with respect to pollination by *Eutoxeres aquila* ([Stiles, 1985](#ref-stiles_1985)), and is locally abundant, providing a tractable system for study.

*Eutoxeres* is comprised of two parapatric species of Sicklebill hummingbirds, that together, adhere to the geographic distribution of *Centropogon* ([Abrahamczyk et al., 2017](#ref-abrahamczyk_2017)). White-tipped Sicklebill (*Eutoxeres aquila*) occurs from Costa Rica to northern Peru, while Buff-tailed Sicklebill (*E. condamini*) occurs from northern Peru to Bolivia. Previous studies have supported White-tipped Sicklebill as a specialized pollinator of eucentropogonids and some *Heliconia* spp. *E aquila* has been documented as a pollinator of *Centropogon* and *Heliconia* species with curved corolla tubes ([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 other co-occurring Hermits ([Maglianesi et al., 2014](#ref-maglianesi_2014); [Sonne et al., 2019](#ref-sonne_2019)). Further, its local abundance is correlated with seasonal flowering of *C. granulosus* in Costa Rica ([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 suited 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 thought to be 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 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). Moreover, we expect steady state flowering to provide insufficient daily nectar to territorial hummingbirds so that these plants are only visited by traplining species.

   Because hummingbird species generally adhere to a single foraging mode ([Feinsinger and Colwell, 1978](#ref-feinsinger_1978); [Stiles, 1985](#ref-stiles_1985); but see [Sargent et al., 2021](#ref-sargent_2021)), phenological types may be effective filters of the local pollinator community, further promoting floral specialization in the eucentropogonids. As with floral shape, phenological 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 phenological 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 pollinator shifts.

   Although the phenological 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 ‘phenological type’ 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 phenological 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 types (see: Methods).

   The goal of this study is to test the hypothesis that eucentropogonids are uniquely specialized for pollination by Sicklebill hummingbirds, specifically the less known Buff-tailed Sicklebill, by examining the floral phenology and pollination of *Centropogon granulosus* 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 phenological type of *C. granulosus* consistent with adaptation to the presumed foraging mode of Buff-tailed Sicklebill, i.e. does *C. granulosus* exhibit steady state flowering?

#### MATERIALS AND METHODS

***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, Fig. 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. A: Pollination niches within hummingbird-adapted centropogonids. K_{tot} is ‘total curvature’ in degrees as defined in Boehm et al (Boehm et al., 2021); measurement protocols are described in the Supp. Mat. All photos used with permission from their owners. Top left: Eugenes fulgens (photo: Ronald E. Thill). Top centre: Phaethornis guy (photo: Mike Hooper). Top right: Eutoxeres condamini (photo: Julian Heavyside). Bottom left: Centropogon talamancensis (photo: Nancy Overholtz). Bottom centre: Centropogon solanifolius (photo: Terry Gosliner). Bottom right: Centropogon granulosus (photo: Josh Vandermeulen). Evidence for pollination in each species pair are described in (from left to right): Colwell 1973 (1973); Snow 1977 (1977); this study. B: 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. C: Montane cloud forests of the eastern Andes (San Pedro, Peru).

Figure 1. A: Pollination niches within hummingbird-adapted centropogonids. is ‘total curvature’ in degrees as defined in Boehm et al ([2021](#ref-boehm_2021)); measurement protocols are described in the Supp. Mat. All photos used with permission from their owners. Top left: Eugenes fulgens (photo: Ronald E. Thill). Top centre: Phaethornis guy (photo: Mike Hooper). Top right: Eutoxeres condamini (photo: Julian Heavyside). Bottom left: Centropogon talamancensis (photo: Nancy Overholtz). Bottom centre: Centropogon solanifolius (photo: Terry Gosliner). Bottom right: Centropogon granulosus (photo: Josh Vandermeulen). Evidence for pollination in each species pair are described in (from left to right): Colwell ([1973](#ref-colwell_1973)); Snow ([1977](#ref-snow_1977)); this study. B: 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. C: Montane cloud forests of the eastern Andes (San Pedro, Peru).

***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_2018a)). 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 (Fig. 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, Fig. 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 were 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 G. 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)).

***Phenological type***—

   To characterize the phenological 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. A separate model was 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 (Figs. 2 and S4, video footage available in Dryad Repository). 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. Given that *Eutoxeres* is active within an ~11 hour daily window, the total monitoring effort is ~1870 hours (5 camera traps 34 days 11 hours per day). Within this time we recorded 12 visits to six *C. granulosus* individuals, totaling 42 seconds of *E. condamini* observation (Fig. S12, Table S3). Ten of the 12 records were single, brief visits (≤3 seconds) that occurred once in the day — two additional records were made when a second visitation was observed on the same day. 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_2018b)). No other hummingbirds were recorded visiting these flowers.

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

Figure 2. Visitation 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 (dorsal head flexion), at which point it can insert the tip of its bill into the flower aperture (Fig. S4). The remainder of the bill is further inserted by tilting the head back to a forward-facing 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 (Figs. S5, S6). We note this because it is unknown how the fleshy berries are dispersed, though we documented signs of frugivory (Fig. 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. We observed unidentified Aranids inside of the floral tubes, and note that *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 also recorded a larval lepidopteran inhabiting a flower (Fig. S8), and a stingless bee (Meliponini) collecting pollen from the anther scale (Fig. S9).

***Pollinator exclusion and floral development***—

   We identified and described eight stages of floral development in *C. granulosus* (Table S2, Fig. 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 produce berries (Stage H, Table S2).

   These eight stages were used to compare developmental differences between control and pollinator-excluded flowers (Fig. 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 no hummingbird-excluded flowers produced 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 scars on the peduncle of an individual not included in this study (Fig. S10).

   Linear models accurately described flowering rate (Fig. 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 inflorescences and 4.60 ± 0.07 days for pollinator-excluded inflorescences. The highest flowering rate was documented in a control plant at 1.88 days, nearly twice the rate of the next fastest individual (Fig. 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 (green) versus the control treatment (orange). The bars represent the median duration spent in each stage. 95% CIs are estimates of when a stage could end. At stage E, where some individuals begin, others will have already finished and moved onto stage F (see ‘Results’ for descriptions of the eight stages). No fruits (stage H) were produced by plants with pollinators excluded.

Figure 3. Developmental trajectories for the flowers of C. granulosus, and effects of pollinator exclusion (green) versus the control treatment (orange). The bars represent the median duration spent in each stage. 95% CIs are estimates of when a stage could end. At stage E, where some individuals begin, others will have already finished and moved onto stage F (see ‘Results’ for descriptions of the eight stages). No fruits (stage H) were produced by plants with pollinators excluded.

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. Each line shows the flowering rate of an individual belonging to the exclusion treatment (green) or control group (orange).

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. Each line shows the flowering rate of an individual belonging to the exclusion treatment (green) or control group (orange).

#### 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., submitted), hovering is one of the most energetically expensive modes of locomotion ([Suarez and Gass, 2002](#ref-suarez_2002)) 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., submitted). 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)). 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 pedicels (Fig. 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, Fig. S11). Whether floral orientation promotes specialization in the eucentropogonids is an understudied aspect of pollination in this clade (but see: [Fenster et al., 2015](#ref-fenster_2015) for a study of *Silene*).

***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 types. Because the steady state strategy implies a linear flowering rate, anecdotal observations of phenological type can be further tested using the linearity metric implemented here.

   Despite the continental breadth of the *C. granulosus* complex, there is a striking similarity in seasonal flowering duration across its range. Stiles ([1985](#ref-stiles_1985)) recorded a 9 month (~270 days) flowering season of *C. granlosus* in Costa Rica. Considering the peduncle with 68 pedicle scars (see: Results), and the mean flowering rate of of 3.94 ± 0.03 days (controls), we approximate that this inflorescence produced flowers for 268 ± 2.04 days. Not only is this a remarkably long flowering season for one individual, but this conserved or convergent phenological type operates similarly between disparate populations pollinated by either species of *Eutoxeres*.

   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 at most 1-2 times per day for brief periods (seconds) 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.

#### DATA AVAILABILITY

All data and scripts are available as an RStudio Project at: <https://github.com/mannfred/centropogon_eutoxeres>. A Dryad submission will be prepared in coordination with the editorial office.

# References

Abrahamczyk, S., C. Poretschkin, and S. S. Renner. 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. SAGE publications Inc., Thousand Oaks, CA.

Armbruster, W. S. 2014. Floral specialization and angiosperm diversity: Phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB Plants* 6.

Armbruster, W. S. 2017. The specialization continuum in pollination systems: Diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology* 31: 88–100.

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

Boehm, M. M. A., J. E. Jankowski, and Q. C. B. Cronk. 2021. Plant-pollinator specialization: Origin and measurement of curvature. *The American Naturalist*: https://doi.org/10.1086/717677.

Colwell, R. K. 1973. Competition and coexistence in a simple tropical community. *The American Naturalist* 107: 737–760.

Colwell, R. K., B. J. Betts, P. Bunnell, F. L. Carpenter, and P. Feinsinger. 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.

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

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

Fenster, C. B., R. J. Reynolds, C. W. Williams, R. Makowsky, and M. R. Dudash. 2015. Quantifying hummingbird preference for floral trait combinations: The role of selection on trait interactions in the evolution of pollination syndromes. *Evolution* 69: 1113–1127.

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

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.

Harder, L. D., and S. D. Johnson. 2005. Adaptive plasticity of floral display size in animal-pollinated plants. *Proceedings of the Royal Society B: Biological Sciences* 272: 2651–2657.

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

Kay, K. M., and R. D. Sargent. 2009. The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics* 40: 637–656.

Kessler, M., S. Abrahamczyk, and T. Krömer. 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.

Kroll, M. H., J. Præstgaard, E. Michaliszyn, and P. E. Styer. 2000. Evaluation of the extent of nonlinearity in reportable range studies. *Archives of Pathology & Laboratory Medicine* 124: 1331–1338.

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

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

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

Lagomarsino, L. P., and N. Muchhala. 2019. A gradient of pollination specialization in three species of Bolivian *Centropogon*. *American Journal of Botany* 106: 633–642.

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

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

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

Muchhala, N., and M. D. Potts. 2007. Character displacement among bat-pollinated flowers of the genus Burmeistera: Analysis of mechanism, process and pattern. *Proceedings of the Royal Society B: Biological Sciences* 274: 2731–2737.

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

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

Primack, R. B. 1985. Patterns of flowering phenology in communities, populations, individuals, and single flowers. The population structure of vegetation, 571–593. Springer.

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

Rathcke, B., and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* 16: 179–214.

Ricklefs, R. E., and A. Scheuerlein. 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., K. J. Hurme, R. Elting, and A. L. Russell. 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., A. Hayes, and S. Couch. 2021. broom: Convert statistical objects into tidy tibbles. *R package version 0.7.6* https://CRAN.R-project.org/package=broom.

Salinas, N., Y. Malhi, P. Meir, M. Silman, R. Roman Cuesta, J. Huaman, D. Salinas, 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., D. Groom, and A. Rico-Guevara. 2021. Locomotion and energetics of divergent foraging strategies in hummingbirds: A review. *Integrative and Comparative Biology* https://doi.org/10.1093/icb/icab124.

Shrivastav, V. 2019. Lin.eval: Perform polynomial evaluation of linearity. *R package version 0.1.2* https://CRAN.R-project.org/package=lin.eval.

Snow, B. K. 1977. Feeding behavior of two hummingbirds in a Costa Rican montane forest. *The Wilson Bulletin* 89: 613–616.

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

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

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

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 C. L. Gass. 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., Z.-H. Huang, Z.-B. Chen, and S.-Q. Huang. 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., J. Liang, M. C. Levy, and Y.-L. Fan. 2019. Floral isolation and pollination in two hummingbird-pollinated plants: The roles of exploitation barriers and pollinator competition. *Evolutionary Ecology* 33: 481–497.

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

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

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., M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemund, et al. 2019. Welcome to the tidyverse. *Journal of Open Source Software* 4: 1686.