Floral development and pollination of *Centropogon granulosus* (Campanulaceae) in an Andean cloud forest

**Author Names**: Mannfred M.A. Boehm, David Guevara Apaza + others

**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.

**Submission Type**: Note

**Abstract**: (200 max for Botany)

**Main Body**: (4200 max for Botany)

**Figures**: # Figures

**Keywords**: (3-6 for Botany)

#### Acknowledgements

Put Acknowledgements here.

#### Introduction

The Andes comprise the highest mountains in the western hemisphere and are one of the most biodiverse regions on Earth (Myers et al., [2000](#ref-myers_2000); Hoorn et al., [2018](#ref-hoorn_2018)). With a mean elevation of 4000 m, the Andes encompass a wide environmental gradient ranging from warm Amazonian rainforests, to dry, montane grasslands. During Andean orogeny, numerous plant clades were either uplifted (Heads, [2019](#ref-heads_2019)) or migrated and diversified into the newly formed niche space, e.g. *Lupinus* L. (Fabaceae, Hughes and Eastwood, [2006](#ref-hughes_2006)), centropogonids (Lobelioideae, Lagomarsino et al., [2016](#ref-lagomarsino_2016)), epiphytic orchids (Epidendroideae, Pérez-Escobar et al., [2017](#ref-perez_2017)). Today, about 10% of global angiosperm diversity is found in the Andes eco-region (Joppa et al., [2011](#ref-joppa_2011)).

   Plant-pollinator co-evolution offers a powerful model of the rapid diversification of the Andean flora (e.g. Kay et al., [2005](#ref-kay_2005); Lagomarsino et al., [2017](#ref-lagomarsino_2017); Serrano-Serrano et al., [2017](#ref-serrano_2017)). As a plant species expands into a novel montane environment, 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 (sensu Grant, [1949](#ref-grant_1949)). However, despite compelling phenotypic and phylogenetic evidence, many putative cases of pollinator-driven plant diversification lack direct observations of floral visitation. In this study, we aimed to test the theory of hummingbird-mediated diversification in the eucentropogonids (sensu Lagomarsino et al. ([2014](#ref-lagomarsino_2014))) by recording pollinator visits, performing a pollinator exclusion experiment, and synthesizing evidence from related studies.

   Here, we focus on the Andean bellflower *Centropogon granulosus* C.Presl and buff-tailed sicklebill, *Eutoxeres condamini* Bourcier (Phaethornithinae). The Andean bellflowers (*Centropogon* C. Presl), along with *Siphocampylus* Pohl and *Burmeistera* H.Karst & Triana form a South American clade of Campanulaceae known as the ‘centropogonids’ (Lagomarsino et al. ([2014](#ref-lagomarsino_2014))). The centropogonids arose in the last 5 million years and have diversified into over 550 species. This rapid radiation has been attributed to increased speciation rates driven by the Andean uplift, simultaneous with decreased extinction rates during periods of global cooling (Lagomarsino et al., [2016](#ref-lagomarsino_2016)). The Andean clade *Centropogon* section *Centropogon* (McVaugh 1949) or the ‘eucentropogonids’ (38 spp., Lagomarsino et al 2014) are distinct because of their elongated, hook-shaped flower tube, and the presence of a scale made of fused hairs that serves as a lever to deposit pollen. Both traits are likely adaptations to facilitate pollination by Sicklebill hummingbirds (Stein, [1987](#ref-stein_1987)). Generally, the elevational distribution of the eucentropogonids is mid- to high-montane (Figure 1).

   Sicklebill hummingbirds (*Eutoxeres* Reichenbach) constitute two species of large hermits (Phaethornithinae) that are defined by their dramatically curved bill. Their unique bill shape is likely an adaptation to specialize on those *Centropogon* and *Heliconia* with similarly curved flowers. The hermits diverged early in the evolutionary history of hummingbirds (~26 Ma, McGuire et al., [2014](#ref-mcguire_2014)), and represent one of two major ecotypes of hummingbirds: the trapliners. There are currently 36 extant hermit species (McGuire et al., [2014](#ref-mcguire_2014)). *Eutoxeres* diverged 21.45 Ma, and like other hermits, probably co-adapted to *Heliconia* before major uplifts of the Andes (Abrahamczyk et al., [2017](#ref-abrahamczyk_2017)). At present, *Eutoxeres* has the largest range of any hermit hummingbird—it can be found in its ancestral range, the Amazonian lowlands, as well as the foothills and mid-montane habitats of the Andes up to 2800 m (**???**).

   Because *Heliconia* and the hermit hummingbirds existed well before the emergence of the eucentropogonids, the leading evolutionary scenario posits that sicklebills and *Heliconia* subgenus *Stenochlamys* and/or *Heliconia* subgenus *Griggsia* co-evolved extreme bill and flower curvature in lowland Amazonia, before the uplift of the Andes (Stein, [1987](#ref-stein_1987); **???**). During a recent period of major Andean uplift, ~5 Ma, *Centropogon* colonized and radiated into the novel montane habitat, with some species adapting to sicklebill pollination. This meditated the elevational expansion of sicklebills, and explains why they are able to exist outside of the range of *Heliconia*. However, the physiological pre-adaptations that allowed sicklebills to expand, and not other hermits, remains unknown (**???**). This scenario presents the possibility of a plant-hummingbird interaction cline, with *Centropogon* and *Eutoxeres* co-evolving in the absence of *Heliconia* at medium to high elevations (Figure 1), and *vice versa* in the lowlands. The distribution of putatively sicklebill-pollinated *Centropogon* and *Heliconia* suggests that above ~1500 m, there are few Heliconia-Sicklebill interactions (Figure 2).

   While trait-matching and anecdotal reports strongly suggest that sicklebills are pollinators of *Centropogon*, empirical evidence remains absent. While the pollination syndrome concept has proven useful in inferring pollinators from floral phenotypes (but see: **???**), a complete theory of pollinator-mediated plant diversification ultimatey requires *in situ* observations of visitation, and some evidence of pollination. Moreover, without a dedicated focus on the organisms’ natural history, it cannot be ruled out that yet-unknown floral visitors directly or indirectly interact with the pollinator. All said, we aim to provide some missing fundamental information on this fascinating and increasingly well-studied pollination system.

#### Materials and Methods

*Floral Development* In this study we focused on the pollination biology of *Centropogon granulosus* C.Presl. While other eucentropogonid species were present at the study site (Figure 1), *C. granulosus* was the most readily observed.

*Pollinator Observations* We based our field work at the Gallieto de las Rocas Lodge, in the Kosñipata Valley, Peru (-13.055, -71.548 decimal degrees). During August and September (2017) we deployed camera traps near at five individual *Centropogon granulosus* vines. If no floral visitors were recorded within three days, the camera traps were moved to different *Centropogon* individuals, totaling n=???. Where floral visitors were recorded, we performed targeted (manual) video recording to better document visitation behaviour.

*Pollinator Exclusion Experiment*

   Following the methods and designs of Sun et al. ([2017](#ref-sun_2017)), we constructed and deployed wire cages covering one inflorescence each from six *Centropogon* individuals. Wire cages prevent hummingbirds from contacting the flowers while allowing invertebrates to move freely. An additional ten inflorenscences were monitored as controls. Each inflorescence produced on average 11 and 13 flowers, respectively. We made daily records of the developmental stage of each flower using the data collected above. Monitoring for a sample stopped when 1) berry development initiated (Stage XXX), 2) the flower died prematurely, or 3) the study period ended. Flowers that did not reach berry development were treated as right-censored (Allison, [2014](#ref-allison_2014)). All observations were made between August 17 and September 20, 2017.

   Flower development data was averaged to calculate a mean developmental trajectory per inflorescence. Data was imported and organized using the packages here v0.1 (Müller, [2017](#ref-muller_2017)) and tidyverse v1.3.0 (Wickham et al., [2019](#ref-wickham_2019)) in R v3.6.3 (R Core Team, [2017](#ref-R_2017)). We conducted an event history analysis using the survival package v3.1 (Therneau, [2015](#ref-therneau_2015)) to compare the probability of reaching berry production between pollinator-excluded and untreated flowers.

<https://www.emilyzabor.com/tutorials/survival_analysis_in_r_tutorial.html>

#### Results:

* In studying the development and pollination of *C. granulosus* flowers, we noted some natural history traits yet published elsewhere. First, the blah blah..
* Figure 2. Developmental stages of Centropogon.
* Figure 3. Pollination by Sicklebills
* Discuss things that live in or around the flowers (ants (cite Stein and LauraLago), mites (cite MacGregor), arachnids)

# 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 (SAGE publications).

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.

Heads, M. (2019). Passive uplift of plant and animal populations during mountain-building. Cladistics *35*, 550–572.

Hoorn, C., Perrigo, A., and Antonelli, A. (2018). Mountains, climate and biodiversity (John Wiley & Sons).

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.

Joppa, L.N., Roberts, D.L., Myers, N., and Pimm, S.L. (2011). Biodiversity hotspots house most undiscovered plant species. Proceedings of the National Academy of Sciences *108*, 13171–13176.

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.

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.

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.

Müller, K. (2017). Here: A simpler way to find your files.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature *403*, 853–858.

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).

Serrano-Serrano, M.L., Rolland, J., Clark, J.L., Salamin, N., and Perret, M. (2017). Hummingbird pollination and the diversification of angiosperms: An old and successful association in Gesneriaceae. Proceedings of the Royal Society B: Biological Sciences *284*, 20162816.

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

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

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.

Therneau, T.M. (2015). A package for survival analysis in s.

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.