

# Drivers of selection on flower traits in a spatio-temporal context

Dr. Sissi D. Lozada Gobilard

## Summary and problem statement

Most flowering plants depend on animal pollinators for sexual reproduction. Different pollinators interact with plants in different ways, thus driving patterns of selection on floral traits, mating systems, and indirectly affecting plant gene flow, genetic variation, and evolutionary potential. Natural selection on flower traits are not driven only by pollinators, but also by abiotic environmental variables. Furthermore, the composition of local assemblages of co-flowering species can change pollinator behavior (foraging patterns) and plant-plant interactions (e.g., competition, facilitation), indirectly affecting selection of a focal species. To disentangle these different factors, studies are needed of plant-pollinator interactions in a community context, replicated along environmental gradients. I apply for funds supporting the establishment of a long-term project assessing the biotic and abiotic drivers of variation in selection along time and space in a tropical ecosystem. The requested budget will contribute to the first two field campaigns to start data collection on plant-pollinator interaction along an altitudinal gradient (~1500m up to 3000m) and during wet and dry seasons in the tropics of Bolivia. Data collected during my postdoctoral position will allow me to produce the research outputs needed for me to qualify as a PI following my current position, as well as to establish a long-term study system.

## Background and motivation

Changes in pollinator-mediated selection pressures are expected to drive divergence in relevant plant traits<sup>1,2</sup>. Agents of selection can be biotic (e.g., pollinators, herbivores) or abiotic (e.g., climate). Previous studies have shown that differences in selection among populations can be predicted based on functionally distinct pollinators (e.g.,<sup>3,4</sup>), but also other kinds of environmental changes such as water and nutrients, can affect selection of flower traits<sup>5</sup>. Altitudinal gradients are associated with extensive environmental variation in space and time, making them ideal systems to study variation in pollinator- and environment-mediated selection. Environmental variation along altitudinal gradients is commonly associated with changes in the taxonomical and functional diversity of plants, pollinators and their interactions<sup>6-8</sup>. Similarly, climatic seasonality causes differences in plant-pollinator interactions<sup>7,9</sup>. Changes in floral traits both with elevation and among seasons highlight the importance of studying plant-pollinator interactions considering spatiotemporal factors<sup>10</sup>. However, to which extent selection is driven by abiotic (i.e., seasons, climate) and biotic factors (i.e., pollinators, co-flowering plant species) remains unclear. In this project, we propose to disentangle the direct and indirect selective agents (biotic and abiotic) acting on floral traits along an altitudinal gradient and between seasons (wet and dry) in a tropical system (Figure 1A).

**Question:** To what extent do biotic and abiotic environmental factors drive selection in floral traits across altitudes and seasons?

**Hypothesis:** We hypothesize that predominance of selection drivers (abiotic and biotic) would change along altitudes and between climatic seasons. **High altitudes** are expected to be associated to a decrease in pollinator availability or shift in pollinator guilds towards inefficient pollinators<sup>11</sup> due to harsh abiotic conditions (i.e., low precipitation), enhancing environmental-mediated selection on flower traits<sup>12</sup>. While lower altitudes are associated with more favorable conditions (pollinators, nutrients), and therefore selection on flower traits might be mainly driven by pollinators. At the same time, **climatic seasonality** can determine plant phenology, flower and pollinator abundance. Higher diversity of pollinators during the wet season is expected to be linked to a higher specialization towards certain floral traits<sup>13,14</sup> (but see<sup>15</sup>), possibly strengthening pollinator-mediated selection during wet compared to dry season. We hypothesize that at high altitudes where the conditions are hard, selection of flower traits would be mainly mediated by the environment, with an enhanced effect on dry season compared to the wet. While, at low favorable altitudes, pollinator-mediated selection would dominate, and would be enhanced during the wet season (Figure 1B).

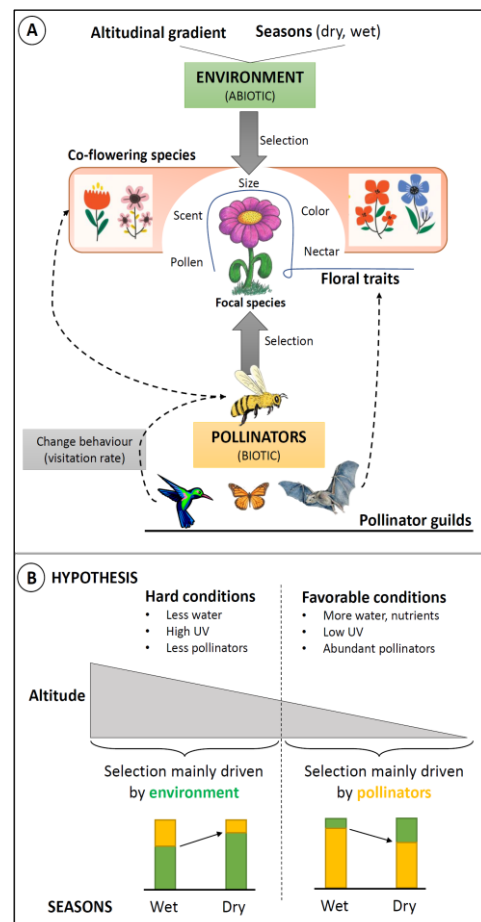


Figure 1. A: Conceptualization. B: Hypothesis.

## **Project description**

Studies on selection usually focus mainly on one or few species. Here we propose to implement a more integrated approach. We wish to investigate plant-pollinator interactions and their consequences for plant evolution along an altitudinal gradient and during wet and dry seasons in Yungas Bolivia combining pollinator observations (visitation rate), pollinator-related traits (e.g., nectar, pollen, flower size) and plant mating systems. ***First***, at a community level, we will focus on plant-pollinator interactions and how plant and pollinator functional traits are affected by biotic and abiotic factors. ***Second***, we will select representative genera to in more detail the selection drivers of flower traits comparing patterns of pollination reliability, trait distributions and mating systems across altitudes and seasons. And ***third***, from these species, depending on their life history and ploidy level, we will select one representative species to study later their population genetics, connectivity and dispersal distributions.

## **Methods**

***Study sites:*** We will set up a monitoring of plant and pollinator communities and their interactions along an altitudinal gradient at the Sillutinkara trail located at the Cotapata PN-ANMI (Parque Nacional-Area Natural de Manejo Integrado) in Yungas, Bolivia. This National Park is a hotspot of biodiversity with an altitudinal variation from ~1500m up to 3000m, and mainly characterized by three forest types: Sub-montane forest (<1500m), humid montane forest (1500-2400m) and cloudy forest (2400-3000m). These forests include species of families like Moraceae, Euphorbiaceae, Bromeliaceae, Ericaceae, Melastomataceae, Rubiaceae, Leguminosae, and Araliaceae, among others. These preliminary results will be the basis for a long-term project at a larger scale.

***Sample collection:*** During the wet (Nov-Jan) and dry (Jun-Aug) season of 2022-2023 we will carry out field campaigns along the altitudinal gradient in Yungas, Bolivia. We will establish 3 study sites per type of forest (see above) with a total of nine. In each of these points, we will establish 3-4 transects and record all the flowering animal-pollinated species and observe pollinator visitation rate. Pollinators will be identified during the observations or collected with a net for later identification. In the case of larger pollinators (birds, bats), photographs will be used for identification. After the first campaign of observations, we will identify potential genus or species commonly occurring along the gradient to be considered as a focal plant. In this focal plant we will measure pollinator-related traits (i.e., size, color, nectar, scent), pollinator visitation and perform experiments to assess the mating systems. In order to assess dependence on pollinators, and the degree of self-compatibility, we will establish 4 experimental treatments: **1)** Open flowers exposed to pollinators. **2)** Flower buds emasculated (no stamens) as negative control. **3)** Hand pollination within the same flower **4)** Hand pollinated flowers using pollen of flowers of the same plant (geitonogamy). Fruits and seeds will be later collected. At each point, data loggers will be set to monitor climatic variables (e.g., temperature, humidity, precipitation).

***Data analysis:*** We will assess the magnitude and direction of selection acting on floral traits per population across altitudes and seasons following <sup>3</sup>. Plant fitness will be based on pollinator visitation rate, as well as fruit and seed set. A selection gradient approach will be implemented to include all floral traits across altitudes and seasons.

## **Available resources/Budget comments**

I apply for funds supporting the set-up of this long-term project studying the selective agents driving floral trait variation in plant communities. After the first two campaigns, the collected data will serve for at least a couple of publications in peer-reviewed journals. I have already established collaboration with colleagues at the Universidad Mayor de San Andrés and Herbario Nacional de Bolivia, and the project will benefit from the help and expertise of local botanists and zoologists based in La Paz, Bolivia. The initial support from the Royal Physiographic Society will be essential to start this project, as I do not currently have other grants for this work.

## **Career implications and future prospects**

Funding for this project will be important for the research needed to establish myself as a future PI at Lund University or other Swedish institutions. The requested funds will allow me to test the described hypothesis and initiate a longer project in Bolivia. Networking opportunities and exchange of knowledge will be established during the project, increasing my possibilities to develop an independent scientific career keeping contact to my homeland. The data collected during the upcoming field season will help strengthen further funding applications.

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