

For more than a decade, scientists have stopped seeing plants as isolated organisms, thus recognizing how much influence, for example, microbes have on their functioning. More attention is now being paid to biotic interactions between plants and microbes, as it has become clear that these interactions affect the physiology and development of the former, as well as their ecology and distribution. Historically, pollination was seen as a two-way interaction between plants and pollinators, but over time here too, it was recognized how many other organisms influence the ecology and evolution of this relationship. The floral microbiome, understood as a community of bacteria and fungi, plays a key role in plant health, growth, and reproductive success. For example, it can affect the attractiveness of flowers to pollinators by altering their scent, floral rewards, or appearance itself. The family Orchidaceae Juss. is one of Earth's most diverse groups of plants. According to various estimates, it has between 25,000 and 40,000 species worldwide, and the variety of floral forms and colors of its representatives seems unlimited. Even though orchids have attracted the attention of both hobbyists and scientists for centuries, the relationship between orchids, the floral microbiome, and pollinators has never been explored. One of the more interesting pollination syndromes is ornithophily or pollination by birds. It has evolved several times in many plant groups, including orchids. It is particularly important in mountain ecosystems and when adverse weather conditions cause a decline in the activity of other pollinators, such as bees. Bird-pollinated flowers are often red, campanulate, and secrete large amounts of dilute sucrose-rich nectar. In addition, they lack features associated with other pollination syndromes, such as scent or nectar trails. Bird pollination is thought to have originated from bee pollination, but the mechanism of this transition remains poorly studied. Learning and understanding what factors and to what extent influenced this phenomenon is therefore a milestone in modern research on orchid pollination. The numerous specialized communities of bacteria and fungi that populate flowers can influence their appearance (phenotype) and interactions with pollinators. It is believed, for example, that microbial metabolites can signal the presence or quality of rewards offered to pollinators. Understanding the relationship between the composition of the floral microbiome and pollination systems can bring new and previously unknown insights into the mechanism of shifts between pollination syndromes. The presented project aims to characterize and compare the microbiome of melittophilous and ornithophilous flowers, verify experimentally whether the detected microorganisms can change the chemical composition of the scent and nectar, and whether infecting flowers with the microbiome from flowers representing different pollination syndromes would attract other types of pollinators. Thus, the main focus of the project is to test the following hypotheses: I) The composition of the floral microbiome in orchid species pollinated by bees and birds differs significantly. II) The change in the microbiome composition will alter floral attractants in orchids (scent/nectar). III) Switching the microbiome between bee-pollinated and bird-pollinated flowers will cause changes in the type of animals that visit them. Ornithophily occurs almost exclusively in tropical and subtropical regions with constant availability of nectar-rich flowers that provide food reserves for birds, so Costa Rica was chosen as the project site. A total of 10 species from the well-studied subtribe Maxillariinae Benth. were chosen as study objects. The research includes a field and laboratory component. During this first one, plant material will be collected for floral attractant tests to be able to characterize what rewards a species offers to pollinators and what their composition is. In addition, scent samples will be collected. The floral microbiome will then be sampled. The final stage will be to conduct field observations to characterize what animals visit the flowers of the species under study. The laboratory part of the project will involve studying the microstructure of flower segments (scanning electron microscopy) and their ultrastructure (transmission electron microscopy and histochemical staining). This will allow us to characterize the rewards offered by the species, confirm whether and where production and release of floral secretions (including nectar and fragrance) occur, and what their chemical composition is. Barcoding will be used to find out the composition of the microbiome, while the scent will be examined using gas chromatography-mass spectrometry. The final component of the project will be to see whether infecting bird-pollinated flowers with the microbiome of bee-pollinated flowers and vice versa, will change the profile of animals visiting them, as well as the chemical composition of nectar and scent. We expect that the microbiome in both syndromes will differ significantly and that the swiping of the microbiome will result in a change in the chemical composition of nectar and scent. The above research will allow us to better understand the pollination biology of Neotropical orchids and determine how the microorganisms that populate the flowers affect it. This, in turn, is a key element in developing effective species conservation plans for these remarkable plants.