Evaluating hybridization in the genus *Kohleria* (Gesneriaceae)

Katherin Arango Gómez
Research group "Ecología y Diversidad Vegetal" Universidad del Valle, Cali, Colombia

Hybridization is a prevailing process in nature that implies the genetic exchange between organisms genetically different and, in consequence, can influence processes like speciation, adaptation and extinction by introducing novel traits that may favor the survival of the organisms (Soltis & Soltis, 2009). In this sense, studying hybrid individuals could provide fundamental evidence to understand the evolutionary history of lineages and provide insights about the capacity of populations and species to respond to environmental disruptions (Soltis & Soltis, 2009; Hamilton & Miller, 2015; Vallejo-Marín & Hiscock, 2016). In fact, it has been suggested that in environments suffering changes as rapidly as the current ones, the adaptive characters that can be introduced into populations via hybridization, more specifically introgression, can become key factors to ensure the conservation of certain groups of organisms (Hamilton & Miller, 2015; Cronk & Suarez-Gonzalez, 2018).

Hybridization in Gesneriaceae seems to be a frequent phenomenon (Ellstrand, et al. 1996), and has been evaluated in genera such as *Columnea* (Smith et al., 2017), *Cyrtandra* (Johnson et al., 2015) and *Ramonda* (Lazarević, et al. 2014); these studies have used tools like morphometry and molecular data to assess the genetic flow among parental species and hybrids. Likewise, interspecific and intraspecific hybridization in the genus *Kohleria* have been extensively reported (Wiehler, 1983; Kvist & Skog, 1992), although the hybrids seem to occur mostly in cultivated populations, and studies focused on evaluating the process in wild populations are still lacking (Kvist & Skog, 1992).

My undergraduated thesis, entitled "Taxonomy and delimitation of species and hybrids in *Kohleria* (Gesneriaceae) with molecular and morphometrics data" (Arango et al. 2019, thesis in preparation for publication) and directed by Drs. Alejandro Zuluaga and Laura Clavijo, sought to clarify the taxonomy of the genus from morphological and geographical data. Additionally, to study hybridization in *Kohleria*, I used morphometric data to detect the presence of hybrids in a secondary forest in Sevilla, Valle del Cauca, Colombia, where *K. inaequalis* var. *inaequalis, K. spicata, K. trianae,* and *K. warszewiczii* are sympatric. My morphometric analyses included 26 quantitative floral characters from 111 individuals and Principal Components (PCA) and Cluster Analyses, with a hybrid selection criterion based on intermediate morphologies (e.g., Johnson et al., 2015; Lazarević, et al. 2014; Léveillé-Bourret et al., 2014; Matos et al., 2016; McIntosh et al., 2014).

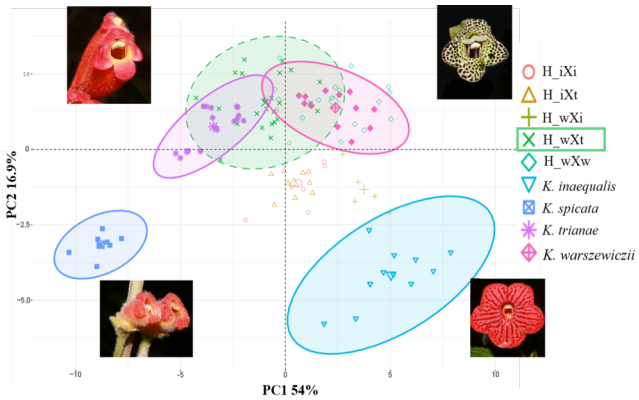

Figure 1. PCA results from Arango et al. (2019). *Kohleria trianae* (purple asterisk), *K. warszewiczii* (pink diamonds), *K. inaequalis* var. *inaequalis* (light blue triangles), and *K. spicata* (dark blue squares). In green, individuals from a putative hybrid between *K. trianae* x *warszewiczii* (H_wXt). Notice the isolated location for *K. spicata*.

My results showed clear intermediate morphologies (Fig. 1), suggesting the presence of hybridization in the study area. However, for some hybrid individuals, the relationship with

the potential parental species was unclear, and further studies are necessary. Various processes like, for instance, introgression between hybrids and parental species, may be acting in this area and could explain such morphologies that deviate from the intermediate (Soltis & Soltis, 2009). Finally, *K. spicata* never grouped with others species or hybrids in our analyses (Fig. 1), what may suggests that *K. spicata* is not interbreeding with the others in the evaluated locality, possibly because the small size of the flower causes the pollen to be placed on a very distinct location of the pollinator, compare to the other species, and therefore decreasing the interbreeding likelihood. However, it is evident that to improve our understanding of the dynamics occurring among the species of *Kohleria* when they grow in sympatry, we need to combine this morphological evidence with molecular data, particularly because processes like backcrossing or the participation of more than two species can lead to hybrids that are difficult to detect based only in morphology (Soltis & Soltis, 2009). For this reason, I plan to use molecular data along with the morphometry to better understand the role of hybridization in the maintenance or blur of the species boundaries in *Kohleria*.

To achieve this goal, I will collaborate with Dr. Eric H. Roalson (School of Biological Science, Washington State University), who is working on phylogenomics of *Kohleria*, and who will provide access to his laboratory and cover lab and sequencing costs. The samples that I will evaluate correspond to silica-dried leaf material from the species and potential hybrids present in the *Kohleria* hybrid swarm of Sevilla (Arango et al., 2019). Our sample size for parental species will be 5 to 8, including samples from the hybrid swarm and allopatric populations. The method selected to evaluated hybridization is Restriction-Site Associated DNA sequencing (RADseq), an approach successfully applied in the detection of hybrids in other genera (Gramlich et al, 2018; Khan et al., 2020). Subsequently, the iPyrad pipeline (Eaton & Overcast, 2020) will be used to align the loci, remove non-homologous/problematic areas, and discover SNP's; and the program STRUCTURE 2.3.4 (Pritchard et al., 2000) will allow the characterization of the genetic structure of the hybrids and species in the hybrid swarm. This data will be greatly useful to test my findings on hybridization occurring in *Kohleria* based on morphology, and to answer the following questions: is there a species with a greater tendency to hybridize than others when they grow in sympatry? Are there species less likely to hybridize? Does hybridization in *Kohleria* behave as a mechanism that could eventually blur the boundaries? or, despite the constant mixing of genetic material, do species in sympatry maintain their genetic integrity?

Despite hybridization being frequently mentioned and that the unusual morphologies given by this process had contributed to an enormous taxonomic confusion in the history of *Kohleria*, it has never been fully studied, so our understanding of such natural dynamics and the evolution and ecology of the genus are still incipient and required. Moreover, my visit to Dr. Roalson's lab will allow me to acquire important skills in the implementation of laboratory technics and data analysis, and get acquainted with the systematic studies that they perform in his lab to gain new skill necessary for my future postgraduate studies, which I wish to carry out on this subject and in Gesneriaceae. Therefore, It is with great interest that I am submitting this application requesting a financial support of USD$ 2,000 from the IAPT Research Grant to cover travel and living expenses while visiting Dr. Roalson's lab.

Literature cited

Arango, K., Clavijo, L. & Zuluaga, A. (2019), "Taxonomía y delimitación de especies e híbridos en *Kohleria* (Gesneriaceae) con datos moleculares y morfometría", Undergraduate thesis (Unpublished), Universidad del Valle, Cali.

Cronk, Q.C. & Suarez-Gonzalez, A. (2018), "The role of interspecific hybridization in adaptive potential at range margins", *Molecular Ecology.* Vol. 27, pp.4653–4656.

Eaton, D.A.R. & Overcast, I. (2020), "ipyrad: Interactive assembly and analysis of RADseq datasets", *Bioinformatics*, DOI: https://doi.org/10.1093/bioinformatics/btz966

Ellstrand, N., Whitkus, R. & Rieseberg, L. (1996), "Distribution of spontaneous plant hybrids", *Proceedings of the National Academy of Sciences of the United States of America*, National Academy of Sciences, Vol. 93 No. 10, pp. 5090–5093.

Gramlich, S., Wagner, N.D. & Hörandl, E., (2018), "RAD-seq reveals genetic structure of the F2-generation of natural willow hybrids (*Salix* L.) and a great potential for interspecific introgression", *BMC Plant Biology,* Vol. 18, 317.

Hamilton, J.A. & Miller, J.M., (2015), "Adaptive introgression as a resource for management and genetic conservation in a changing climate", *Conservation Biology,* Societty for Conservation Biology, Vol. 30, pp. 33-41.

Johnson, M., Price, D., Price, J. & Stacy, E. (2015), "Postzygotic barriers isolate sympatric species of *Cyrtandra* (Gesneriaceae) in Hawaiian montane forest understories", *American Journal of Botany*, Vol. 102 No. 11, pp. 1870–1882.

Khan, G., Franco, F.F., Silva, G.A.R., Bombonato, J.R., Machado, M., Alonso, D.P., Ribolla, P.E.M., Albach, D.C. & Moraes, E.M. (2020), "Maintaining genetic integrity with high promiscuity: Frequent hybridization with low introgression in multiple hybrid zones of *Melocactus* (Cactaceae)", *Molecular Phylogenetics and Evolution,* Elsevier Inc., Vol. 142, DOI: https://doi.org/10.1016/j.ympev.2019.106642

Kvist, L. & Skog, L. (1992), "Revision of *Kohleria* (Gesneriaceae)", *Smithsonian Contributions to Botany*, Vol. 79, pp. 1–83.

Lazarević, M., Rakić, T. & Šinžar-Sekulić, J. (2014), "Morphological differences between the flowers of *Ramonda serbica*, *R. nathaliae* and their hybrid", *Botanica Serbica*, Vol. 38 No. 1, pp. 91–98.

Léveillé-Bourret, É., Bailleul, S.M., Cayouette, J. & Joly, S. (2014), "Testing Hybridization Hypotheses with Morphometry: the Case of Eastern American Arctic Species of *Potentilla* sect. Niveae (Rosaceae)", *Systematic Botany*, American Society of Plant Taxonomists, Vol. 39 No. 1, pp. 193–204.

Matos, J., Juan, A., Agulló, J. & Crespo, M. (2016), "Morphological features, nuclear microsatellites and plastid haplotypes reveal hybridization processes between two sympatric *Vriesea* species in Brazil (Bromeliaceae)", *Phytotaxa*, Vol. 261, pp. 58–74.

McIntosh, E., Rossetto, M., Weston, P. & Wardle, G. (2014), "Maintenance of strong morphological differentiation despite ongoing natural hybridization between sympatric species of *Lomatia* (Proteaceae)", *Annals of Botany*, Vol. 113 No. 5, pp. 861–872.

Pritchard J.K., Stephens, M. & Donnelly, P. (2000). "Inference of Population Structure Using Multilocus Genotype Data", *Genetics,* Vol. 155, pp. 945-959.

Smith, J., Clark, J., Amaya-Márquez, M. & Marín-Gómez, O. (2017), "Resolving incongruence: Species of hybrid origin in *Columnea* (Gesneriaceae)", *Molecular Phylogenetics and Evolution*, Elsevier Inc., Vol. 106, pp. 228–240.

Soltis, P. & Soltis, D. (2009), "The role of hybridization in plant speciation", *Annual Review of Plant Biology*, Vol. 60 No. 1, pp. 561–588.

Vallejo-Marin, M. & Hiscock, S.J., (2016), "Hybridization and hybrid speciation under global change", *New Phytologist*, Tansley Review, Vol. 211, pp. 1170-1187.

Wiehler, H. (1983), "A synopsis of the neotropical Gesneriaceae", *Selbyana*, Vol. 6 No. 1, pp. 1–219.