Species Boundaries in Two Northern California Monkeyflowers

Jacob A. Ewald and Christopher T. Ivey

California State University, Chico, Department of Biological Sciences

jewald@mail.csuchico.edu

Introduction:

- Recently diverged taxa maintain species boundaries via one or more reproductive barriers [1].
- Annual wildflowers *Mimulus guttatus* and *Mimulus glaucescens* overlap in range and interbreed in the greenhouse but rarely hybridize in nature [2].
- Previous research examined 14 potential reproductive barriers but did not demonstrate complete reproductive isolation [3, 4].
- Thus, either unmeasured reproductive barriers exist, or species boundaries are semipermeable between the two taxa.

Study Taxa:

Mimulus guttatus & M. glaucescens are closely-related monkeyflower species. The species have nearly identical floral morphology and are only distinguishable by vegetative traits [5].

Mimulus guttatus:

- *M. guttatus* possesses two bracts subtending each inflorescence.
- *M. guttatus* possesses trichomes on leaves and bracts.

Mimulus glaucescens:

- *M. glaucescens* possesses one single, circular bract subtending each inflorescence.
- Trichomes are absent on the bracts of *M. glaucescens*.

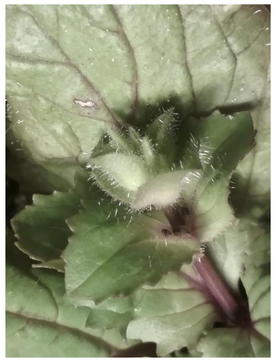

Fig 1. *M. guttatus* bract pair covered in trichomes.

Fig 2. Single *M. glaucescens* bract exhibiting distinctive circular shape.

Results:

Morphological measurements suggest that hybrids and backcrosses possess intermediate traits between *M. guttatus* and *M. glaucescens.*

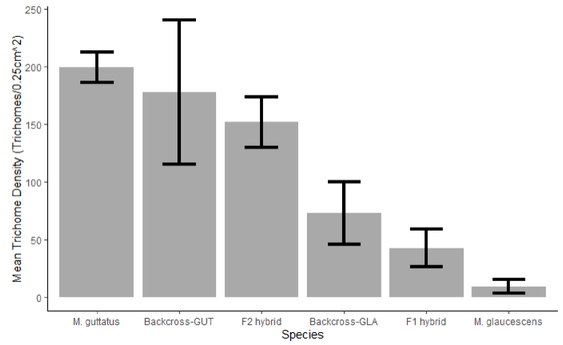

Fig. 3. Mean trichome density of *Mimulus* species, hybrids, and backcrosses. Bars represent 95% confidence intervals.

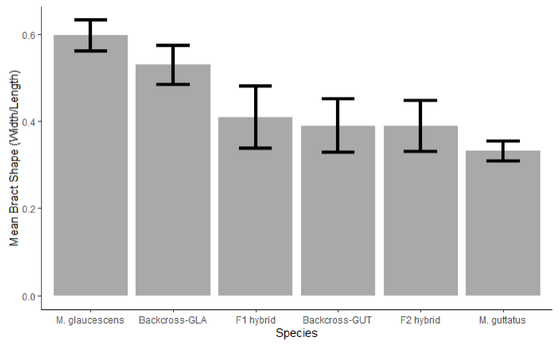

Fig 4. Mean bract shape of *Mimulus* species, hybrids, and backcrosses. Bars represent 95% confidence intervals.

Preliminary fastSTRUCTURE results suggest that little, if any, genetic admixture has occurred between *M. guttatus* and *M. glaucescens* in Butte Creek Canyon, CA.

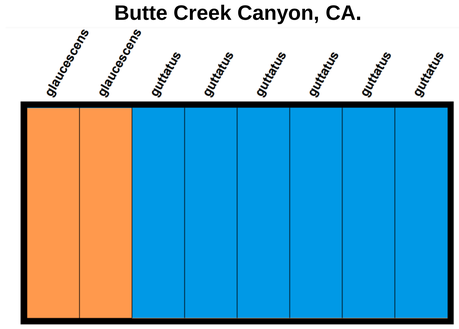

Fig. 5. Preliminary fastSTRUCTURE results from six *M. guttatus* and two *M. glaucescens* individuals collected from four sympatric populations in Butte Creek Canyon, CA. Each column represents one *Mimulus* individual, symbolized by the estimated proportions of *M. guttatus* and *M. glaucescens* genetic ancestry present in each individual. *M. guttatus* ancestry (blue) and *M. glaucescens* ancestry (orange) are clearly separated, with no genetically admixed individuals identified in this study.

Methods:

Morphological Analysis:

- We conducted morphological measurements on greenhouse-grown *M. guttatus*, *M. glaucescens*, F1 hybrid, F2 hybrid, and backcrossed individuals.
  Measurements included trichome density (trichomes per 0.25cm^2) and bract shape (bract width / bract length).

Statistical Analysis:

- We evaluated differences in morphology between *M. guttatus*, *M. glaucescens*, hybrids, and backcrosses using Analysis of Variance (ANOVA).

Genetic Analysis:

- We collected *M. guttatus* and *M. glaucescens* bract tissue samples from individuals located in Butte Creek Canyon, a known sympatric zone for the two species.
- We then extracted DNA from bract tissues and sent the samples to the UC Davis Genome center for whole-genome sequencing (2x coverage).
- Using fastSTRUCTURE [6], we analyzed SNPs for evidence of genetic admixture. We used DISTRUCT [7] to visualize results.

Significance:

- In contrast to the strong and often redundant reproductive barriers in other *Mimulus* sister taxa [8], the species boundaries between *Mimulus guttatus* and *Mimulus glaucescens* are either much weaker or perhaps incomplete [3, 4].
- F1 and F2 hybrids possess intermediate morphological traits compared to their parent species, suggesting quantitative inheritance. Still, field identification of hybrids is difficult.
- Preliminary genetic data suggests that genetic introgression is not occurring. More genetic analysis with a larger sample size and additional sympatric populations would strengthen these findings.

Acknowledgements/Literature Cited:

Mark Leigh kindly provided *Mimulus* cultivation assistance. Dr. Colleen Hatfield graciously lent out crucial field equipment. Additionally, the following groups have generously provided financial support for this project: California Botanical Society; California Native Plant Society; California State University, Chico; Center for Water & the Environment; Northern California Botanists; Friends of the Chico State Herbarium. We are truly grateful for the tremendous support.

1. Mayr, E. 1942. *Systematics and the origin of species.* Columbia University Press, New York.
2. Vickery, Jr., R. K. 1964. Barriers to gene exchange between members of the Mimulus guttatus complex (Schrophulariaceae). *Evolution* 18: 52 – 69.
3. Bergmann, J. (2013). *Prezygotic reproductive isolation between Mimulus guttatus and Mimulus glaucescens.* Chico, Calif.]: [California State University, Chico].
4. Habecker, N. (2012). *Ecogeographical and intrinsic postzygotic isolation between Mimulus Glaucescens and Mimulus Guttatus.* Chico, Calif.]: [California State University, Chico].
5. Baldwin, B., Goldman, D., & Vorobik, L. (2012). *The Jepson manual: Vascular plants of California* (2nd ed.). Berkeley, Calif.: University of California Press.
6. Raj, A., Stephens, M., & Pritchard, J. K. (2014). fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics (Austin),* 197(2), 573–589.
7. Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. *Mol. Ecol. Notes* 4:137–138.
8. Ramsey, J., Bradshaw, H., & Schemske, D. (2003). Components of Reproductive Isolation Between the Monkeyflowers Mimulus lewisii and M. cardinalis (Phrymaceae). *Evolution,* 57(7), 1520-1534.