

INTRODUCTION

- Speciation generally involves divergence in resource use and in mating signals and mate preferences, all generating reproductive isolation (Kirkpatrick & Ravigné 2002).
- However, before speciation is complete, hybridization can reverse the process and fuse diverging populations. Thus, whether and how hybridization occurs between recently diverged species is a key question.
- This study aims to test whether viable hybrid offspring are produced when members of the *E. binotata* species that diverged ca. 10,000 years (Hsu et al. 2018) come into close proximity.
- In Wisconsin there are two members of the *E. binotata* complex that use the same host plant, Viburnum *lentago*. These two species can be distinguished by color and pattern as nymphs or by the dominant frequency of mating signals as adults.
- We set out to create a controlled mechanism to provide ample room for reproduction, protection, and the ability to closely observe mating pairs.
- We began construction by testing materials and designs to determine what kind of capsule would best fit the plants, allow access to the mating pairs, and not hinder the survival or observation of either plant or treehopper.
- Our final product makes use of clear deli cups, mesh fabric, superglue, and rubber bands that are assembled around a stem of potted host plant exemplar, into which experimental pairs are placed.



A method for close observation of hybrid pairings in plant-feeding insects

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METHODS

- We collected *Enchenopa* nymphs near UWM, and reared them on potted Viburnum lentago host plants at the greenhouse.
- When males and females matured, we paired them on Viburnum lentago host plants at the greenhouse using reusable capsules.
- Capsules were custom built from materials readily available in the lab. ullet
- containers using a scalpel, both bottoms were sanded to a rough finish and fused together using Gorilla clear superglue.
- The top of the capsule was created using a 32 oz. deli lid that had the center removed, this was used to support a 10"x10" piece of mesh. Additionally a rube band was placed around the mesh as a precaution should the lid fail.
- The bottom of the capsule called for a full deli lid, two ¼" holes were drilled centered approximately one inch from each edge, in addition twelve smaller holes 1/16th diameter were drilled from the inside out.

CONSTRUCTION





Bottom centers were removed from two 32 oz. Polypropylene Clear Deli

Deli lid, center removed to provide

- Mesh screen provides airflow and
- Rubber band added for security
- Two deli cups, bottoms removed
- Bottom deli lid of capsule has: -Two ¼" holes to provide anchoring points for capsule onto plant -Several small drainage holes on the

DISCUSSION

- used outside.

REFERENCES

• This simple and inexpensive design allows for easy replication and maintenance. Constructing these reusable capsules will aid the Rodriguez lab with future experiments and has the potential for expansion into other research projects.

• Several variations were designed and tested before this version was finalized and built in large quantity. • Each cup takes about 10 minutes to build, including cutting, gluing, and drying.

• Because of the simplicity of design and materials used these chambers should last several seasons before needing repairs or being recycled.

• Future variations of this design can improve on water drainage, currently the design is made to withstand greenhouse conditions. By expanding water draining capabilities these capsules can be

• Kirkpatrick, M., & Ravigné, V. (2002). Speciation by Natural and Sexual Selection: Models and Experiments. *The American Naturalist*, 159(S3), S22–35.

• Hsu, Y.H., Cocroft, R.B., Snyder, R.L., & Lin, C.P. (2018). You stay, but I Hop: Host shifting near and far co-dominated the evolution of Enchenopa treehoppers. *Ecology and Evolution*, 8(4), 1954–1965.