

# A natural experiment untangling the roles of geography and diet on seed beetle speciation

Marielle Krivit and Geoffrey Morse, Department of Biology, University of San Diego

## Introduction

Half of the world's multicellular diversity is found within plant-insect interactions. One explanation is that certain insect and plant lineages are driven by coevolutionary relationships. This evolutionary 'arms race' often leads to specialization and subsequent speciation within many insect taxa. Seed beetles (Coleoptera: Chrysomelidae: Bruchinae) impose a particularly intense selective pressure on plants by directly consuming plant offspring, contributing to widespread specialization between seed beetle species and specific host plants.<sup>1,2</sup> Thus, it is highly unusual to come across seed beetles that are considered relative generalists.<sup>3</sup> The Great Plains seed beetle, *Acanthoscelides fraterculus*, presents a mystery as it has been reared from host plants within the *Astragalus*, *Oxytropis*, and *Glycyrrhiza* genera.



Figure 1. *Acanthoscelides fraterculus* shown laterally (left) and dorsally (right).



Figure 2. Host plants *Oxytropis sericea* (left) and *Glycyrrhiza lepidota* (right).

## Methods

Figure 3 displays the generalized localities sampled from in Colorado. At each site seed pods were collected from each host plant species and *A. fraterculus* populations were reared in the lab. Samples of beetles from each population were then photographed in detail before destructive genomic DNA extraction and isolation. DNA was quantified using fluorometric quantitation, amplified with PCR, and sequenced using sanger sequencing. Sequences were aligned alongside all prior Morse lab *A. fraterculus* samples, and patterns of genetic structure were analyzed using maximum likelihood Bayesian phylogenetic trees.<sup>4</sup>



Geographic association alternative

*A. fraterculus* likely a generalist

Host plant association alternative

*A. fraterculus* likely cryptic or a specialist

★	<i>Astragalus</i>
▲	<i>Oxytropis</i>
●	<i>Glycyrrhiza</i>

Figure 3. Generalized display of two potential association outcomes. Seven sites in Colorado were sampled in July 2019. Similar shapes represents populations of *A. fraterculus* reared from the same host plant. Similar colors represent populations that are closely genetically related.

## Discussion

There is a great deal of genetic diversity within the sampled *A. fraterculus* populations. Preliminary results support host associated genetic clades for *A. fraterculus* populations reared from *Oxytropis sericea* and *Glycyrrhiza lepidota* sites in Colorado, which is a strong indication of cryptic speciation. Other populations show some genetic clades grouped by both host plant and geographic location, as well as high genetic similarity between individuals reared from distant localities, indicating higher rates of gene flow and generalist tendencies among these groups. Results also display many polytomies which could indicate an ongoing process of evolution, but more resolution is needed.

## Continuation

Additional research should be conducted to make more substantial claims about the population structure of *A. fraterculus*. I am working on implementing whole genome sampling using Restriction-site Associated DNA sequencing (RADseq) to detect high-resolution genetic variation across large numbers of individuals.<sup>5</sup> Doing so will allow for cryptic species groups to be more precisely identified. Additional morphological comparisons may also be conducted using high resolution photographs and illustrations catalogued alongside each specimen's genetic information. This would allow for robust assessments on *A. fraterculus* population makeup to determine whether this species is a specialist, generalist, or cryptic species.

## References

- <sup>1</sup> Ehrlich, P. R., and R. H. Raven. 1961. Butterflies and Plants: A Study in Coevolution. *Evolution* 15:359-608.
- <sup>2</sup> Farrell, B. D. 2019. "Inordinate Fondness" Explained: Why are There so Many Beetles? *Science* 322:269-270.
- <sup>3</sup> Fox, L. R., & Morrow, R. A. 1981. Specialization: Species property or local phenomenon? *Science*, 211, 887-889.
- <sup>4</sup> Britschard, J. K., M. Stepien, and R. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- <sup>5</sup> Stolt, R. D., S. Beesham, R. J. Hohenlohe, E. J. Johnson, and W. J. Crisler. 2011. SNP Discovery and Genotyping for Evolutionary Genetics Using RAD Sequencing. *Methods in Molecular Biology* 772:157-179.

## Results

Figure 4a. Maximum likelihood Bayesian phylogenetic tree of *A. fraterculus* populations distinguished by locality. Color indicates locality region.

Figure 4b. Maximum likelihood Bayesian phylogenetic tree of *A. fraterculus* populations distinguished by host plant. Color indicates host species.

Figure 4c. Map of populations of *A. fraterculus* collected at sites across the Great Plains. Colors indicate shared host species.

