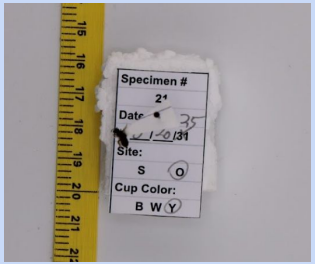
Improving DNA Extraction and Amplification Techniques in Native Arizona Bees

Question

Will our DNA sequencing success rate increase by modifying transects and changing the PCR primer?

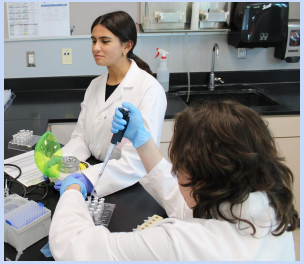
Results

We had a higher success rate with sequencing smaller bees this year however, there still is room for improvement.



Experiment

We collected bees and extracted DNA and amplify CO1 gene to sequence the samples and identify the species.



Conclusion

We were more successful with the new primer but, we could try soaking entire sweat bees beforehand to see if it improves the success rate.

Why Bees Are Important

- Roughly 90% of the world's plant species are pollinated by animals, many are bees.
- Bees are important to agriculture as they are the main pollinators for crops.
- Bee populations are declining due to habitat loss, pesticides, and climate change.

Figure 1: Flower observed in the Chiricahua mountains.

Importance of Research

- Southeastern Arizona is believed to have the highest bee species richness on Earth.
- Bees are the only pollinators of specific plants in AZ.
- More research is greatly needed in many areas of bee conservation.

Figure 2: Students collecting bee specimens in the Chiricahua mountains.

Research Question

Will changing our PCR primers and suspending our bee collection cups from fence posts improve our success rate with collecting and DNA barcoding bees?

Figure 3: Student performing PCR process

Figure 4: Students placing bee collection cups.

Hypothesis

- We feel that changing the PCR primers will cause a higher success rate because they were tested by the Biotech project at UofA and found to yield better bee DNA sequencing results over with many bee types.
- We feel that suspending the bee collection cups from fence posts will give us a better bee collection rate because it will keep animals from interfering with the cups.

Figure 5: Javelina photo. Javelinas have been responsible for the majority of disturbance to sampling transects in past experiments.

Methods:

Phase 1: **Collection of Bees**

- Collected bees from cups filled with soap water. (cups were 3 colors: yellow, blue, and white)
- The location of the samples were from a drainage area and near the school pollinator garden.
- Cups were collected every week from August until the end of October.

Phase 2: Extract DNA from Bee Samples

- Extracted and purified DNA from bees using spin columns.

Phase 3: Amplified CO1 gene using PCR

- Ran Polymerase Chain Reaction to amplify the CO1 gene.
- This year we used the BeeCox1 PCR primer.

Figure 6: Students conducting bee DNA extraction.

Results of DNA Extraction

Of our 150 Bee samples we successfully amplified 32 Bee specimens.

(Figure 7: Images of electrophoresis results, wells 40-60)

Results of DNA Extraction

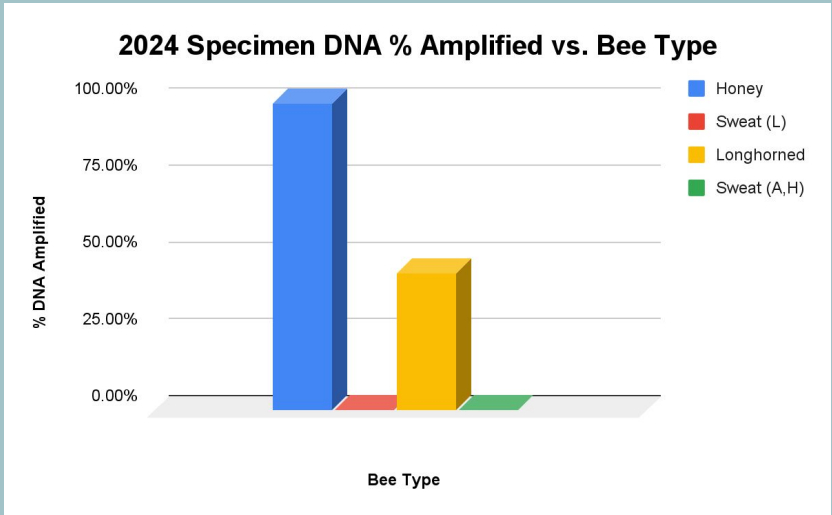


Figure 8: Results of bee amplification 2024.

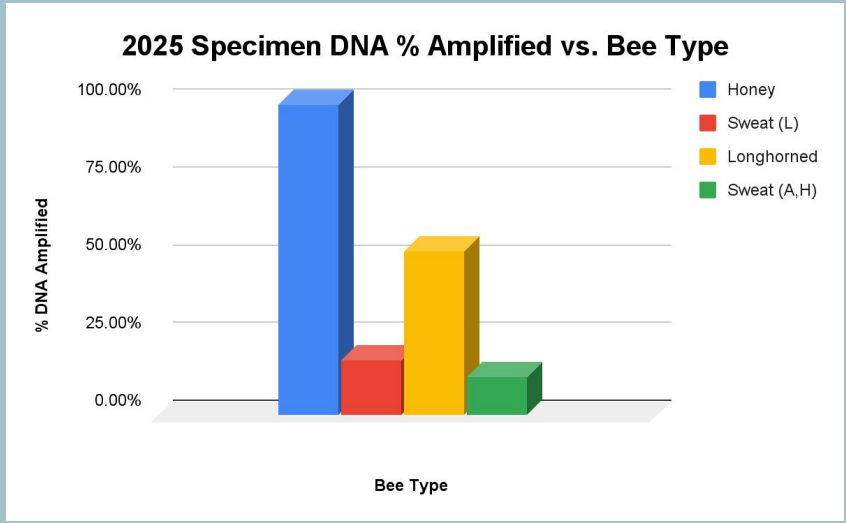


Figure 9: Results of bee amplification 2025.

Results of DNA Extraction 2025

- This year, we had a higher success rate with extracting and amplifying DNA from smaller bee varieties.
- We had a similar success rate to last year with amplifying DNA of larger bee varieties.

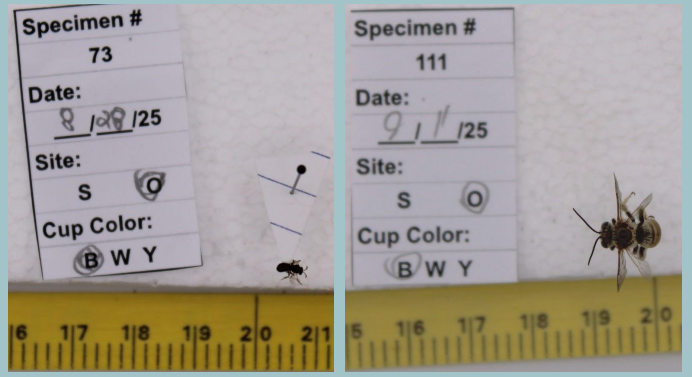


Figure 10: Image of *Lasioglossum* Sweat bee and Longhorned bee collected in 2025.

Results of DNA Sequencing

Figure 11: Electropherogram showing DNA read results for specimen 65.

Results of DNA Sequencing

- 15 of the 32 specimens that were sent for DNA sequencing yielded results that were high enough quality to be used for identification.
- We used the Barcode of Life Database to search for DNA matches in each of the 15 specimens.
- This year we collected bees belonging to the *Apis, Agapostemon, Halictus, Lasioglossum,* and *Mellisodes* genuses.

Figure 12: Image of Bee #88, identified as *Agapostemon femoratus*.

Bees Identified by DNA Sequencing:

Figure 13: Bee specimen #39, species name; *Apis mellifera.*

Figure 14: Bee specimen #97 species name; *Lasioglossum microlepoides.*

Conclusion:

- Overall we did improve from the results last year compared to this year.
- We may be able to increase our DNA extraction success with small bees rate in the future by leaving the entire bee in extraction buffer overnight.

Figure 13: Students gathering bee collection cups.

Successes

The new PCR primer was more effective at sequencing a wider variety of bees than previous years.

Suspending cups from collection cups was very effective at deterring wildlife. No cups were disturbed throughout the sampling season.

Needs Improvement

The success rate was still low in many specimens. We need to continue to modify our DNA extraction / amplification protocols to improve our success rate.

Conclusion 2:

Interesting Encounters:

We obtained a high quality DNA sequence from Bee specimen #120 . There were no matches for it using BOLD. Using other databases, there were no close credible matches. This could mean that this species has yet to be documented on Bioinformatics databases.

Figure 14: Unknown bee specimen #120.

Works Cited

1. Winfree, R. (2010). The conservation and restoration of wild bees. Annals of the New York academy of sciences, 1195(1), 169-197.
2. Campbell, C. (2020). An approach for creating site-specific planting palettes to support pollinators in the sky islands. Air, Soil and Water Research, 13, 1178622120950269.