Evaluating metagenetic eDNA techniques for the characterization of floral-associated arthropod communities

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Introduction

- **Background:** Current survey protocols for arthropod communities are time-consuming and require experienced observers. Metagenetic analysis of environmental DNA (eDNA) provide an efficient, non-invasive, and non-lethal approach (Thomsen & Sisgaard, 2019).
- Diverse arthropod assemblages interact with flowers, and floral surfaces have been shown to harbor arthropod DNA. Honey bee-collected pollen may represent an additional eDNA substrate. Regardless of substrate, there is uncertainty about which genetic markers best characterize floral-associated arthropod communities.
- **Objectives:**
  - Assess the sensitivity of three different genetic markers for characterizing pollinator communities at the genus level
  - Test whether honey bee-collected pollen eDNA provides for more sensitive pollinator detections relative to flower eDNA samples
  - Compare the results of eDNA-derived pollinator detections against traditional aerial net-derived detections.
- **Findings:** Our results demonstrate that bee communities can be detected with eDNA techniques and that the choice of marker and substrate substantially influences detection. Future improvements to our methods are required, but eDNA surveys appear well-suited to characterize diverse pollinator communities and provide novel sampling perspectives within plant-pollinator networks.

Methods

- Flower and pollen samples were collected in 2020 from two central Ohio sites during August and September.
- After sequencing, forward and reverse COI and 16S reads were merged. Only forward reads of 28S were used due to the length of this marker. Semi-global VSEARCH alignment (Rognes et al., 2016) was used to query sequences against the reference database of each marker.
- Arthropod reference databases were created by trimming NCBI sequences to the target amplicon region of each marker, using MetaCurator v1.0.1 (Richardson et al., 2020).
- We compared the genus-level richness of eDNA detections per sample across each of the three markers, as well as between pollen and flower substrates using non-parametric Kruskal-Wallis tests.

Marker	Total Reference Sequences	Orders Represented	Families Represented	Genera Represented	Species Represented	Anthophila Genera Represented	Anthophila Species Represented
16S	72,939	101	1,564	14,275	34,014	76	457
COI	606,750	117	2,095	28,975	104,245	272	1,572
28S	44,660	96	1,338	13,123	24,729	385	1,144

Table 1: Taxonomic completeness of each associated reference sequence database.

Results

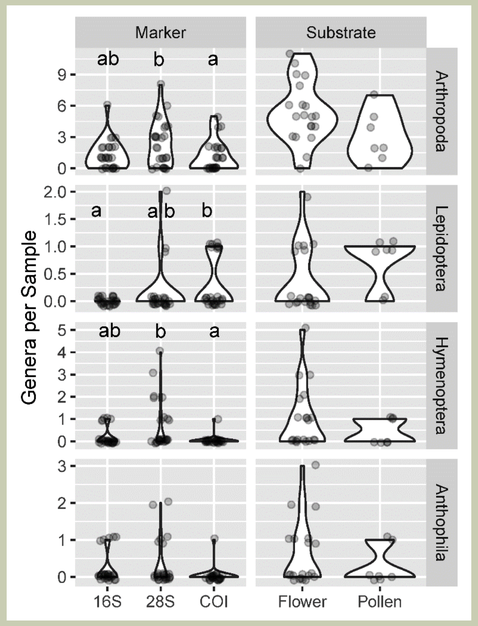

Figure 1: Violin plots depicting the richness of genera detected by marker and substrate. For comparisons exhibiting at least one significant difference, groups with different letters were found to be significantly different according to post hoc pairwise comparison.

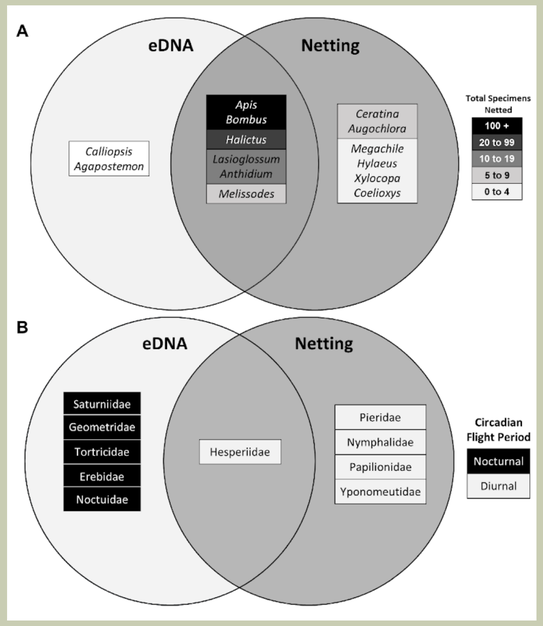

Figure 2: Bee genera (A) and Lepidopteran families (B) detected by molecular eDNA analysis, netting surveys, and both methods.

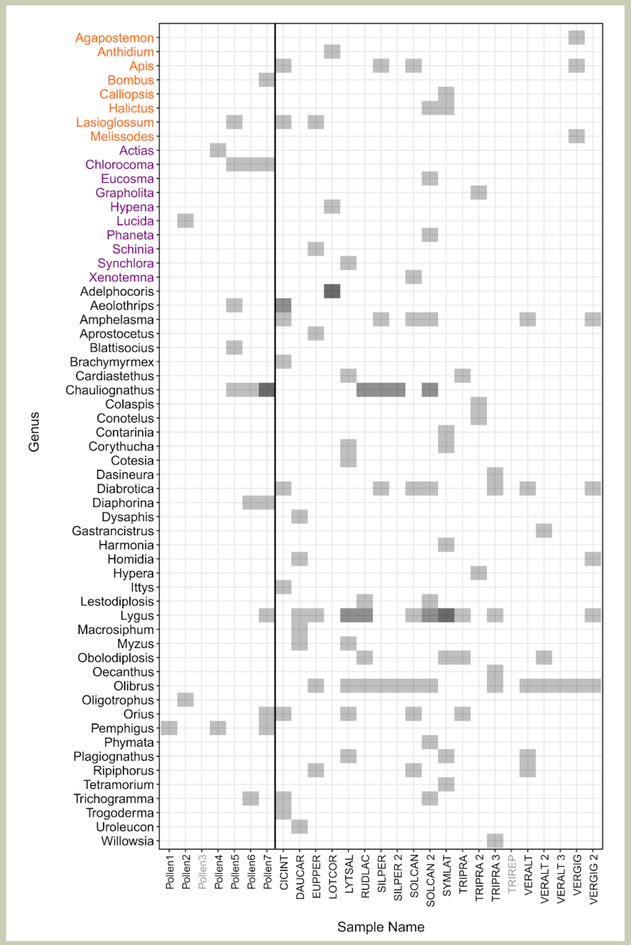

Figure 3: Genus-level eDNA detections for each pollen and flower sample; with number of markers indicated by tile color. Purple and orange labels denote Lepidoptera and Anthophila, respectively.

Discussion

- Richness of Anthophila genera per sample was not different between markers ($H = 4.197$, $p = 0.123$), but richness of all Arthropoda genera per sample varied significantly ($H = 6.053$, $p = 0.048$).
- The strongest evidence for differences between pollen and flower eDNA sources was observed for the taxonomic groups Arthropoda ($H = 3.275$, $p = 0.070$) and Lepidoptera ($H = 2.897$, $p = 0.089$).
- Traditional netting detected 12 Anthophila genera, in comparison to eight detected using eDNA methods, and a total of 14 with both methods combined. Significantly more genera per sample was detected in all four taxonomic groups with netting.
- Results indicate that the choice of metagenetic marker strongly influences the inferences that can be made and that bee-collected pollen contains traces of eDNA from the broader pollinator community. Our data generally suggest that future refinements will be needed before eDNA methods will be field ready as a sensitive complementary tool relative to traditional survey methods.

References

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