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Applying Pollen DNA Metabarcoding to the Study of Plant–Pollinator Interactions

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Abstract

Background: Current knowledge of plant-pollinator visitation networks is based primarily on visual sightings of bees coming and going from various flowers in one location and can lack both accuracy and time depth. When pollen from bees is identified to provide information on prior foraging, plant-pollinator networks become more detailed. Further advantages in efficiency and accuracy can be achieved when this is coupled with DNA metabarcoding to identify mixed-species pollen batches and high-throughput sequencing. In our experiment we used dual-indexing DNA metabarcoding with the *rbcL* and ITS2 regions in order to gather data and recreate a plant-pollinator network in forests being managed for biofuels production.
Results: We were able to successfully construct a quantitative plant-pollinator network using DNA metabarcoding.
Significance: This work demonstrates that non-quantitative DNA metabarcoding can be used in constructing quantitative plant-pollinator networks. This also demonstrates the increased efficiency and cost-effectiveness of DNA metabarcoding for mixed-species pollen batches over traditional barcoding or visual sighting methods.

Background

Plant-pollinator networks play a crucial role in the maintenance of biodiversity, and assessing the impact of land management and change on these networks with accurate, high-throughput methods is essential. Current knowledge of plant-pollinator networks is based primarily on visual sightings of bees visiting flowers in one location and can lack both accuracy and time depth.[1] Plant-pollinator networks become more detailed when pollen from bees is identified to provide information on prior foraging, but shortcomings still exist. The traditional method of pollen identification, visual identification under a microscope, involves huge time costs and lacks high taxonomic resolution.[2]

DNA metabarcoding, the simultaneous identification of multiple species in a mixture using high-throughput sequencing, is particularly applicable to pollen samples. Although expensive, costs can be reduced by using unique indexed primer combinations and combining samples in a single run.[3] In this study we successfully used dual-indexing DNA metabarcoding in order to recreate plant-pollinator networks in managed forests as proof of concept for use in managed and changing ecosystems.

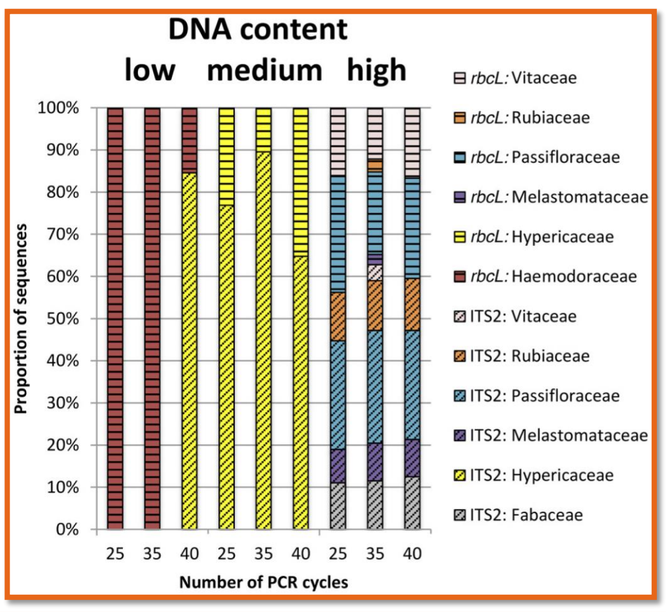

Figure I: Proportion of sequencing reads for family-level taxonomic identifications from a sample with high DNA content, moderate DNA content, and low DNA content, with varying numbers of PCR cycles. Sequencing reads from *rbcL* are horizontally striped, sequencing reads from ITS2 are diagonally striped, and different families are represented in different colors. Image and modified caption from Bell *et al*. 2017.

Methods

- Bees were collected from longleaf pine forests with 13 individual sites of 7 different land management types
- DNA was isolated and the genes *rbcL* (primers: *rbcL2* and *rbcLaR*) and ITS2 (primers: ITS-S2F and ITS4R) were amplified using primers appended with MiSeq-specific adapters and index sequences. Following PCR and purification, DNA concentrations were measured and samples were pooled in equal concentrations and sequenced using Illumina MiSeq
- Taxonomic identification followed a modified version of the bioinformatics pipeline of Sickel *et al.*, to include *rbcL* data
- We constructed the network using only the data from the ITS2 data as no clear method exists for combining the two data sets and the ITS2 marker possesses greater database coverage and taxonomic resolution
- The strength of interactions were quantified based on interaction frequency rather that proportions of sequencing reads, due to variations in copy number of *rbcL* and ITS2

Results

- We were able to identify 78% and 55% of all plants to at least genus level with ITS2 and *rbcL* respectively
- The resulting bipartite network contained 37 pollinators and 51 plant taxa based on the ITS2 taxonomic classification

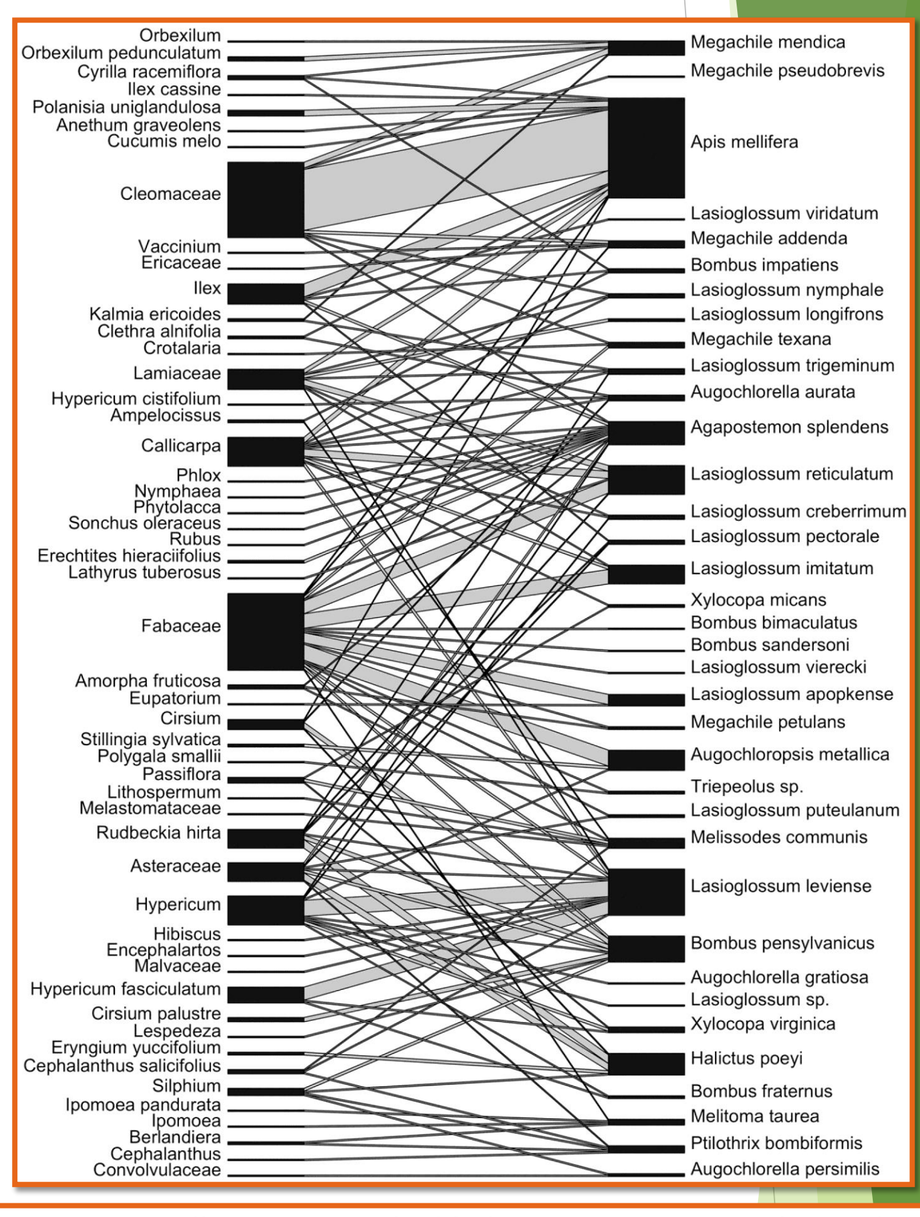

Figure II: Bipartite pollination network of 37 bee specimens (top nodes) to the 51 plant taxa present in their pollen loads based on ITS2 taxonomic classification (bottom nodes). Interactions were pooled within plant and bee taxa. Links between plants and pollinators are represented with lines whose width is proportional to the number of interactions while the width of the nodes represents total abundance of that taxon across all of its interactions. Image and caption from Bell *et al*. 2017.

Discussion and Conclusions

We were successfully able to identify mixed-species pollen loads collected from bees using DNA metabarcoding and demonstrated that constructing quantitative plant-pollinator networks is possible. While there are some circumstances when visual identification of pollen is more appropriate, DNA metabarcoding has a number of clear advantages compared with microscope identification. These include increased taxonomic resolution, a distinct time advantage, and much less hands-on work.

There remain a number of factors to be taken into account when applying these techniques in the future. These include choice of barcoding markers, methods of quantification, the effect of increased PCR cycles on low concentration samples, the presence of false positives due to contamination given the ubiquity of pollen, and the possible exclusion of rare species in an attempt to avoid false positives. Despite these concerns, DNA metabarcoding has many advantages over visual identification for pollination network reconstruction. New tools such as this one are needed given the importance of understanding changes in these networks due to anthropogenic climate change and global pollinator declines.

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References: 1. Bosch, J., Martín González, A. M., Rodrigo, A., & Navarro, D. (2009). Plant–pollinator networks: adding the pollinator's perspective. *Ecology Letters*, 12(5), 409-419. **2.** Bell, K. L., de Vere, N., Keller, A., Richardson, R., Gous, A., Burgess, K. S., & Brosi, B. J. (2016). Pollen DNA barcoding: current applications and future prospects. *Genome*, 59(9), 629–640. **3.** Sickel, W., Ankenbrand, M. J., Grimmer, G., Holzschuh, A., Härtel, S., Lanzen, J., ... & Keller, A. (2015). Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach. *BMC Ecology*, 15(1), 20. **4.** Bell, K. L., Fowler, J., Burgess, K. S., Dobbs, E. K., Gruenewald, D., Lawley, B., … & Brosi, B. J. (2017). Applying pollen DNA metabarcoding to the study of plant–pollinator interactions. *Applications in Plant Sciences*, 5(6), 1600124. doi:10.3732/apps.1600124