Poster 784

Using next generation and modified PCR approaches to DNA barcode *Drosophila* species and their parasitoid wasps in the eastern U.S.A.

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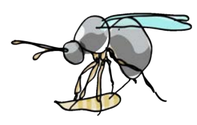
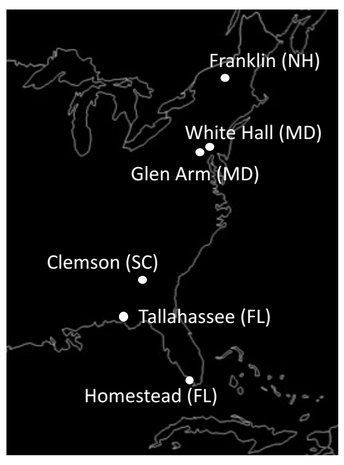


Abstract

The goal of the project for which we developed these methods is to investigate host-parasitoid trophic relationships among *Drosophila* flies and their parasitoid *Leptopilina* wasps in fruit orchards in the eastern United States. Ultimately the project will provide information on species richness, relative abundance of hosts and parasitoids, and species associations, including specificity and frequency. We had difficulty barcoding many specimens of *Leptopilina* wasps using traditional COI barcoding methods, due, we hypothesize, to co-amplification of remnant *Drosophila* host DNA along with the wasp DNA. PCR products of these mixed CO1 sequences could not be cleanly sequenced using Sanger methods. We therefore tested methods for teasing apart the DNA sequences of host and parasitoid. We designed primers for a short variable region of 28S that amplifies in both taxa. Using these primers we amplified the region from parasitized *Drosophila* pupae and empty pupal cases and developed next-gen libraries from the amplification products. Adapters and sample-specific indexes were ligated to each amplification product and equal amounts of DNA from each was then run on a nextgen platform. Sequences from each indexed sample were compared to reference sequences for both fly and wasp. We were able to recover sufficient sequences from each sample so that both fly and parasitoid could be identified. Next steps include optimizing the method using the traditional barcode marker COI. In addition, we developed a method of PCR using blocking primers that allowed for direct amplification and Sanger sequencing of the barcode region from both fly and wasp. We developed two sets of blocking primers: one to block the amplification of fly DNA so that the wasp could be cleanly amplified, and the reciprocal set. Using two PCR reactions with these sets of primers for each pupal specimen, we successfully amplified both host and parasitoid COI.

Background



1A.

1B.

Figure 1A. Map of the US eastern seaboard showing collection localities.
Figure 1B. Sizes of *Drosophila* host and *Leptopilina* parasitoid (relative to a US dime).

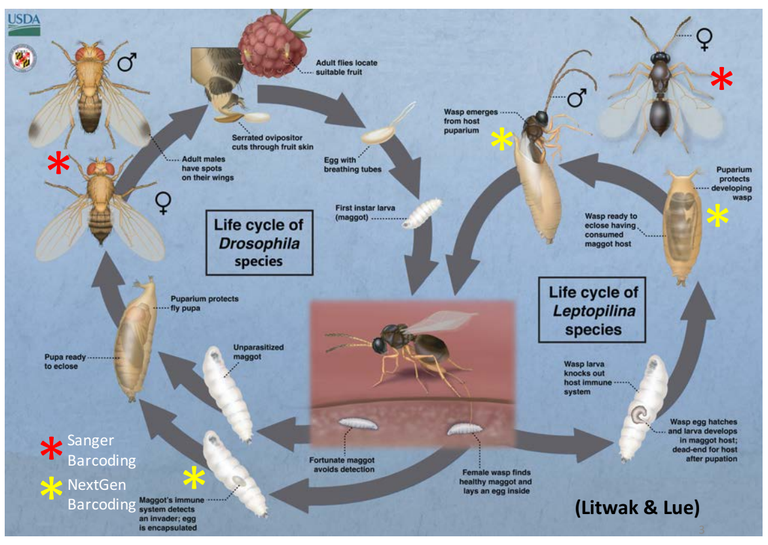


Figure 2. Chart of the life cycles of the *Drosophila* host and *Leptopilina* parasitoid wasp. Life stages sampled in this project are marked with an asterisk. We conducted typical Sanger sequencing barcoding on adults of all species (marked in red). We sampled various life stages for the nextgen barcoding study: adult wasps with encapsulated wasp eggs, which occur when the host's immune system successfully halts wasp development; parasitized pupae, including those which contained a nearly full-grown wasp; and empty puparia – what's left over after the wasp emerges.

As part of a larger study examining host-parasitoid community structure in fruit orchards in the eastern US, one goal is to identify host fly species and their parasitoids. Understanding which species of parasitoid are host-specific and which are generalists is key. In the locations studied, we identified 32 species of *Drosophila* and at least 6 species of the parasitoid *Leptopilina* using morphology. Traditional COI barcoding was difficult possibly in some instances due to co-amplification of both host and parasitoid. We therefore turned to nextgen and other PCR methods to obtain clean barcodes of one (blocking PCR) or both (nextgen) species.

Methods and Results

We used 32 parasitized specimens that amplified for COI using traditional methods. These included adult flies with encapsulated parasitoid eggs, parasitized pupae, fully developed parasitoid wasps still in the puparium, adult wasps, and empty puparia. Samples were amplified using a "universal" COI primer developed from figitid and *Drosophila* sequences. Cleaned PCR products were amplified using Nextera-tailed dual-indexed. After clean-up and quantification, the library was run on a Miseq using a V3 600 Miseq kit. The resulting data was cleaned of primers and short sequences and fastq files for each sample produced. Sequences in each sample were then identified by comparison to our 61 sequence reference database.

Both wasp and fly sequences were recovered from all specimens except the adult wasps. The ratio of wasp sequence to fly sequence varied with the "volume" of wasp parasitizing the fly (Figure 3). The wasp identified in all samples was *Leptopilina boulardi* and hosts were eight species of *Drosophila* (see Figure 4) – the most common being *D. melanogaster*. An additional species of parasitoid wasp, the braconid *Asobara sp.,* was identified from one pupa.

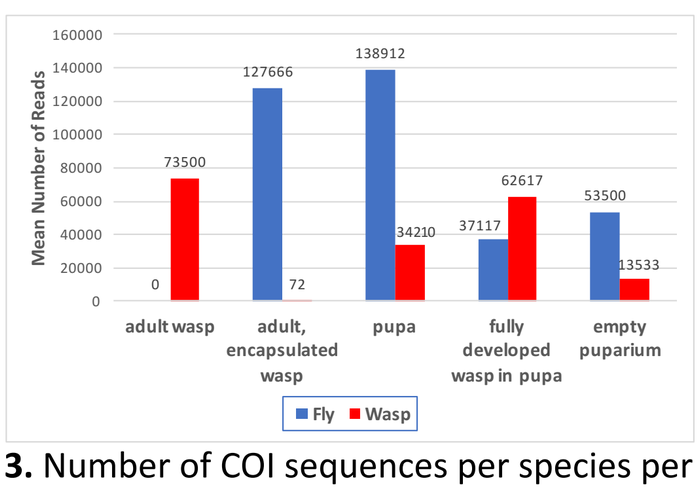


Figure 3. Number of COI sequences per species per specimen. The number of wasp sequences increases with the ratio of wasp:fly mass. Some life stages are more amenable to nextgen barcoding than others because of variable read number.

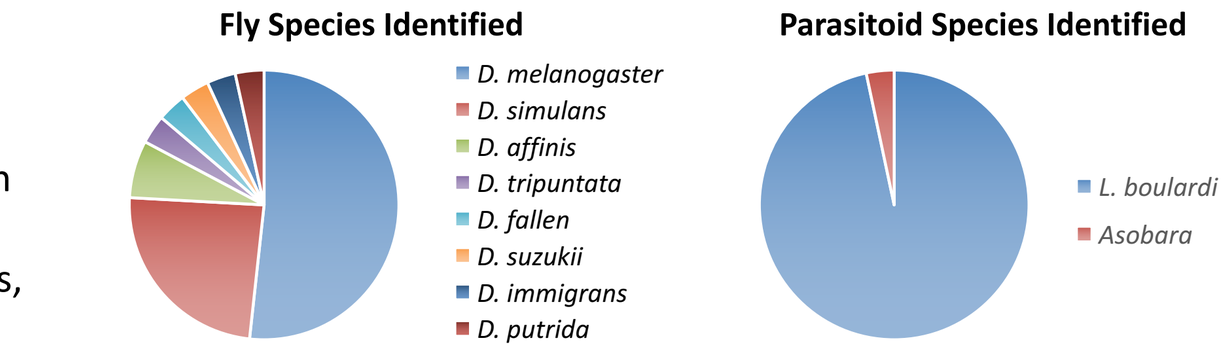
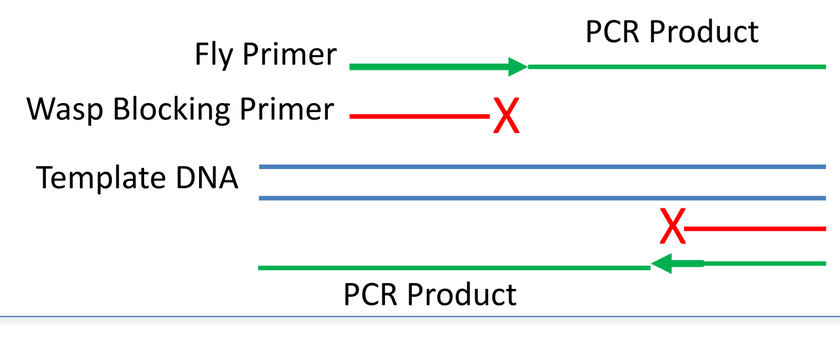


Figure 4. Species identified in the study. 100% of specimens were parasitized by *Leptopilina boulardi* and one specimen had an additional parasitoid, *Asobara sp.*

PRODUCING SPECIES SPECIFIC PCR USING BLOCKING PRIMERS

We hypothesized that barcoding failures were due to co-amplifi - cation of both wasp and fly. To counter this, we designed blocking primers (Vestheim & Jarman 2008): primers with a 3' spacer 3C, which prohibits extension. We found a site downstream of the 5' end of the Folmer fragment that was variable enough to design specific primers. For both fly and wasp we designed F and R primers and added the 3' spacer to the blockers. The fly barcode is then obtainable by using the wasp blocking primer and fly primer in a 10:1 ratio. And vice versa for the wasp barcode.

To Generate Fly CO1 Sequence:



References

Lue, CH, AC Driskell, J Leips, ML Buffington. 2016. Review of the genus *Leptopilina* (Hymenoptera, Cynipoidea, Figitidae, Eucoilinae) from the eastern United States, including three newly described species. *J of Hymenoptera Research*. 53, 35-76.

Vestheim, H., S.N. Jarman. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples – a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology,* 5, 12.

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