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 COI sequences could not be clearly 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. Using these primers we amplified the region from parasitized *Drosophila* pupae. 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 fly sample, we successfully amplified both host and parasitoid COI.

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 *Drosophila* and *Drosophilia* sequences. Cleaned PCR products were amplified using Nextera-tailed dual-indexed. After cleanup 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.

References

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