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Preliminary Study Examining the Presence of a *Wolbachia* Endosymbiont in Winter Moth Populations

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Introduction

The winter moth (*Operophtera brumata*) is a geometrid native to Europe. It invaded New England just over a decade ago (1). The larvae defoliate juvenile leaves of many hardwood and fruit-bearing plants (Figure 1). Fruit yields and home ornamentals suffer from this defoliation. *O. brumata* forms an endosymbiosis with the bacterium, *Wolbachia pipientis*. Infection by *W. pipientis* can create reproductive problems (feminization and cytoplasmic incompatibility) (2). The phenotypic effects of the relationship with *O. brumata* are unclear, but up to 25% of populations are infected (personal communication, N. Havill, USDA Forestry Service). The aim of this study was to test Rhode Island, Massachusetts and European moths for the presence of *W. pipientis*. Understanding the infection rate of populations can help us trace the invasion back to its origins in New England and Europe.

Figure 1 (left). Moth larvae eating juvenile leaf tissue of a hardwood tree (April-May)
Figure 2 (right). Adult moths inhabiting a hardwood tree. Males can fly, while females are wingless (December)

Methods

I. Sample Collection

- *O. brumata* DNA extracts (8 Massachusetts & 8 Europe; gift of N. Havill)
- *Wolbachia* positive control sample (gift of J. Andersen)
- Pheromone trapped specimens (Figure 2; Wakefield, RI; 41.452696, -71.535842)

II. Primer Design

We created PCR primer sets for *O. brumata* and *W. pipientis* on NCBI based on their recently sequenced genomes (3).

- *O. brumata* primer sets: *IDH & cycY* (4)
- *W. pipientis* primer sets: *wsp* & *16S* (5,6)

III. Troubleshooting

- Switched DNA extraction methods from a squish buffer method (7) to a Qiagen DNeasy Blood and Tissue kit (Hilden, Germany)
- Switched to New England BioLabs (NEB) OneTaq reagents (Ipswich, MA) to optimize PCR conditions

IV. Polymerase Chain Reaction (PCR) & Gel Electrophoresis

Based on Zhou et al., 2003. Per 25uL reaction: 17.375uL H2O, 5uL 5X NEB OneTaq buffer (final concentration 1.8 mM Mg), 0.5uL dNTP's (10.0nM each), 0.5uL of each 10uM primer set, 1uL of sample, and 0.125uL NEB OneTaq. Thermal cycling conditions were as follows: 1min @ 94˚C, 1min @ 52˚C or 55˚C (depending on primer), 1min @ 68˚C, 35 cycles. We ran gels using 0.5X TBE on 1.5% agarose for roughly 30min.

V. PCR purification, Sequencing, & Analysis

- NEB Monarch PCR purification kit (Ipswich, MA)
- Submitted DNA for sequencing to URI Genomic Sequencing Center
- Sequences aligned with CLC Sequence Viewer 7 (Cambridge, MA)

Results

Experimental Design

We designed PCR primers for conserved *O. brumata* genes to test whether the DNA could be amplified (4). We also designed *Wolbachia* PCR primers (5, 6), to test for the presence of the bacterium.

European Samples

Of the 8 European samples received, one amplified. A 12.5% infection rate is consistent with findings by our collaborator (personal communication, N. Havill, USDA Forestry Service). The positive sample was from the Republic of Georgia and amplified both *O. brumata* and *Wolbachia* genes (Figure 3). Its *wsp* and *16S* genes were sequenced and aligned with reference sequences from NCBI and pubmlst.org, a database created to analyze genomic diversity amongst prokaryotes (8).The *wsp* gene sequence was highly conserved at 99.2% identity to *wsp* allele 577 and 100% identity to alleles wsp460 & wsp385 in pubmlst.org (0.0 e-value) (Figure 4). The *16S* gene sequence of the Georgia sample was highly conserved (99% identity; 0.0 e-value) with the *W. pipientis 16S* ribosomal gene (U23709.1) logged in Genbank (Figure 5).

Massachusetts Samples

O. brumata genes *IDH* and *cycY* were amplified in 5 of 8 samples (62.5%), indicating high quality DNA. No *Wolbachia* genes were amplified in the same samples. This yielded a 0% infection rate of *W. pipientis* in Massachusetts *O. brumata* samples.

Rhode Island Population

O. brumata genes amplified in 14 of 17 samples (82.4%) and no *Wolbachia* genes amplified. This yielded a 0% infection rate of *W. pipientis* (Figure 6), which were collected from one population in Wakefield, RI.

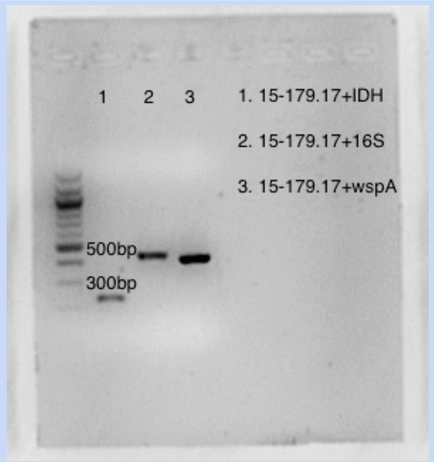

Figure 3. Amplification of *O. brumata* (*IDH*) and *W. pipientis* (*16S* & *wsp*) genes in the Republic of Georgia sample (15-179.17). Band sizes are consistent with expected amplification products based on the published sequences (3).

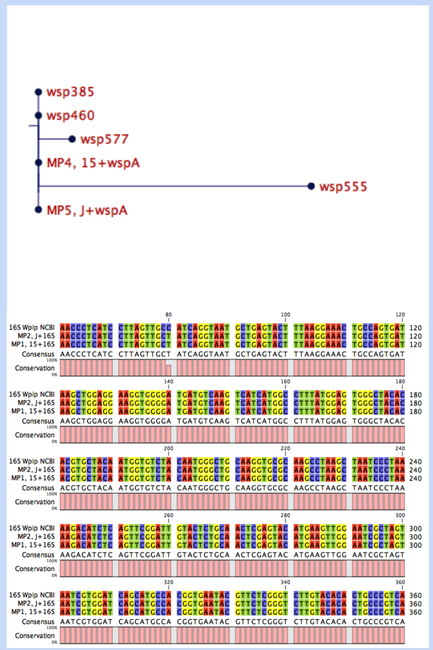

Figure 4 (top). Tree diagram comparing the *wsp* amplified sequences for the Republic of Georgia (MP4), the positive control from J. Andersen (MP5), and three *wsp* alleles from pubmlst.org. wsp385, wsp460, MP4, and MP5 are all 100% conserved, while wsp577 and wsp555 have nucleotide differences.
Figure 5 (bottom). Alignment of 16S sequences: Republic of Georgia (MP1; bottom), positive control from J. Andersen (MP2; middle), and the top BLAST hit for the Georgia sample (16S Wpip NCBI; top). The positive control and Georgia samples are100% conserved, while the NCBI sequence differs by one nucleotide.

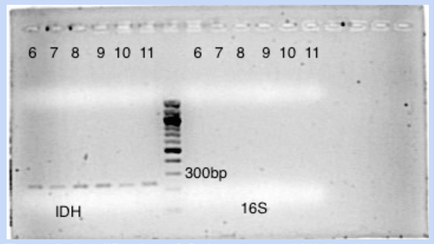

Figure 6. Amplification of *O. brumata* and *Wolbachia* genes in Rhode Island samples. Left : *IDH*. Right: *Wolbachia 16S* (no amplification). These results indicate no bacterial DNA present.

Discussion

The data collected from our samples is preliminary, but gives a glimpse into the population structure of *O. brumata* in both New England and Europe. The infection rate in Europe was similar to that found in North America (personal communication, N. Havill, USDA Forestry Service), which indicates a constant vertical transfer of the endosymbiont.

The 0% infection rates we observed from New England populations do not give us a completely accurate representation of the population structure in the region. A 25% infection rate was observed in other New England populations using a very large sample size (1200 individuals; personal communication, N. Havill, USDA Forestry Service). Our sample size for these populations was low. This was magnified due to troubleshooting problems, poor DNA quality from shipped MA samples, and time restrictions. Testing more samples from these areas would give a more accurate representation of the *W. pipientis* infection of *O. brumata* in New England.

The next phase of the project will be to amplify and sequence more samples for *Wolbachia* genes. Additional samples are in progress. We can also determine the bacterial strain type by comparing the sequences to references in the database, pubmlst.org (8). The strains will give a scope of the genetic differences in these endosymbionts throughout the world. From here, we can analyze the European strain(s) to see whether the same ones appear in North America. The bacterium essentially acts as a tracking device to follow the spread of the invasive species. This is a hypothetical outline of where the project can take us in the future.

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