DEVELOPMENT OF RESTRICTION ENZYME ANALYSES TO DISTINGUISH WINTER MOTH FROM BRUCE SPANWORM AND HYBRIDS BETWEEN THEM

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ABSTRACT

Elkinton et. al. recently completed a survey of northeastern North America for the newly invasive winter moth, *0perophtera brumata* L. The survey used traps baited with the winter moth pheromone, which consists of a single compound also used by Bruce spanworm, *O. bruceata* (Hulst), the North American congener of winter moth. Our traps filled with both species, and they are difficult to tell apart because wing characters are unreliable. Eidt et. al. showed that the two species could be distinguished based on different shapes of the male genitalia. However, we found that many moths in the region where the two species co-occurred had intermediate genitalia, suggesting that they might be hybrids. To develop a more reliable identification, we extracted DNA and amplified and sequenced the barcoding gene, cytochrome oxidase subunit I (COI) and showed that the two species differed by 7.4 percent of their nucleotides. To distinguish hybrids, we amplified and sequenced the nuclear gene glucose-6- phosphate dehydrogenase (G6PD). We found the two species differed by six nucleotides in the G6PD fragment that we sequenced of which three were completely reliable. Hybrids were heterozygotic at these nucleotides. Sequencing costs are prohibitive ($10-$20 per moth) when large numbers need to be identified as in a regional survey. Here we report a restriction enzyme assay that distinguishes the two species and F1 hybrids between them that greatly lowers the cost of obtaining an ID.

Based on the COI sequence previously obtained by Elkinton et al., we demonstrated expected fragments for the digest with Sac I enzyme for the winter moth samples (~ 241 and 453 bp). The Bruce spanworm samples don't have a Sac I enzyme site; therefore, the bands size are at ~ 694 bp as an uncut PCR product. Because COI is a mitochondrial gene identifying the maternal line, it is not sufficient to distinguish the hybrids between these two species.

To identify hybrids, Elkinton et al sequenced the nuclear gene glucose 6 phosphate dehydrogenase (G6PD) and found six nucleotides out of the 233 bp G6PD fragment that reliably distinguished between the two species. Hybrids (at least F1 hybrids) were heterozygotic at all six sites. Here we demonstrated a G6PD restriction enzyme assay based on the Taq I enzyme that cut the 233 bp G6PD fragment for Bruce spanworm into two fragments at ~ 179 and 54 bp. The winter moth G6PD sequence doesn't have this enzyme site so the band size is at ~ 233 bp as uncut PCR product. The hybrid samples, having the DNA from winter moth and Bruce spanworm parents, had all three fragments with the size of 233, 179, and 54 bp. Running the two assays together allowed one test to confirm the species ID with the other. In the case of hybrids, the G6PD test would identify the hybrid and the COI test would identify the female parent.