Protocol for collecting winter moth and other Operophtera in traps baited with winter moth pheromone.

November 06

J. Elkinton

Over view

We are attempting a world-wide survey of winter moth and other species of *Operophtera* that are attracted to the same pheromone compound.(Z,Z,Z-1,3,6,9-Nonadecatetraene) identified by Roelofs in 1982. These species include winter moth, *O brumata* and, in North America, Bruce spanworm, *O. bruceata* and perhaps others. We hope to place out the pheromone traps this November/December. Flight is just beginning here in Massachusetts and lasts until the first week of January. In more northerly locations flight may end in early November. We have sequenced the CO1 gene from these two species from eastern US and this has proved to be a completely reliable method for distinguishing between them. The next step is to sequence this and other genes from *Operophtera* specimens collected world-wide. With this effort we may be able to determine where our winter moth comes from, the degree of relatedness between species and also the degree to which winter moth and Bruce spanworm are hybridizing.

If you are willing to help we will send you a small number (1-5) of pheromone traps including lures, and a DDVP strip to kill the moths that enter the trap. We will provide directions for assembling and placing the traps. Our primary objective is to collect up to 50 dead moths in good condition from each trap from which we will extract mitochondria DNA. A secondary objective is to obtain live or recently killed individuals (within 24 h) preserved in alcohol from which we will extract nuclear DNA.

Procedures

1. Assemble the Universal moth traps (directions enclosed), place the DDVP (Vapona) strip in bottom of trap and the baited rubber septum in the small container at the top of the trap.
2. Hang the trap from a branch in a forest that contains host trees for winter moth (oaks and maples) or other Operophtera. We have noted that flight tends to reach peak on warm evenings after a cold spell
3. Data collection: Remove moths from the traps at your convenience and when at least 10 have been captured. For each trap record the trap location (Country , State or Province, precise location, i.e. street address or equivalent and GPS coordinates if that is feasible). Record when the trap was placed and when the moths were retrieved. I will prepare a data sheet and e-mail it to all cooperators.
4. When you sample the traps remove dead moths and place up to 50 moths per trap in a paper bag (provided with traps), then fold the top over and staple it shut and place the envelopes somewhere for the moths to dry out if they are damp. Label the envelopes clearly with trap location or ID number. When the moths are dry please ship the moths to Joe Elkinton (address below) for ID and DNA analysis. For shipping he envelope should be placed in a small box so that the moths will not get crushed in shipping with packing material so the envelope will not rattle around too much.
5. As a second priority we would like to collect some live moths (or very recently killed) in alcohol. Often when you open the trap there will be a few live ones not yet killed by the Vapona. Please put up to 20 moths in one or more vials with alcohol. (95% ethyl alcohol is best but any alcohol

will do). Alcohol is considered a hazardous material and is illegal to ship without proper packaging and certification, so please pour off the alcohol (re-cap the vial) immediately before placing the vial in the zip-lock bag with cushioning material (provided) for shipment. This should be adequate for preserving the specimens during the short time they are in the mail. Please make sure the vials are labeled with trap ID or location info.

6. Assuming we can figure out how to do this I am happy to pay for any shipping costs. I can send my UPS account number for example.

Send to my address:

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