USE OF MOLECULAR PROBES TO DETECT PARASITES AND RETROTRANSPOSONS IN GYPSY MOTHS

John H. Werren[1] and Thomas O'Dell[2]
[1]Department of Biology , University of Rochester, Rochester, NY 14627
[2]USDA Forest Service, Northeastern Forest Experiment Station,
51 Mill Pond Rd., Hamden, CT 06514

ABSTRACT

Retrotransposon screen: Gypsy moth families containing straggling and nonstraggling individuals were divided into categories of straggling, medium, and nonstraggling individuals, from which DNA was extracted. Four families were tested by southern hybridization and probing with ribosomal sequences designed to detect R1 and R2 retrotransposon insertions. Results showed no differences between stragglers and nonstragglers in the proportion of insertions in their ribosomal genes. The average proportion of insertions was 30%.

An initial screen from one family indicated that an amplification of ribosomal genes had occurred among stragglers; however, this was not observed in the other three families. It can be concluded that variable expression of R1 and R2 retrotransposons is not a likely cause of straggling in Gypsy Moths. Experiments were performed to test the effectiveness of the *Compsilura* total genomic probe for detecting parasitization in gypsy moths by that fly parasitoid. For one experiment, 3rd instar and 4th instar larvae were exposed to *Compsilura* females until stung. These were then shipped (1-2 days) to the Werren laboratory. Samples were divided into three groups. One was frozen immediately at -70 C, the second group developed on media for one day (25 C) prior to freezing, and the third group developed for two days. These were then individually homogenized and probed using radionucleotide labelled *Compsilura* total genomic DNA. Forty individuals were used in each group. Approximately 73% of third instar larvae yielded parasites and approximately 78% of 4th instar larvae yielded parasites. Most of these produced 1 parasite per larva, although multiple parasites did emerge in a few cases.

DNA probing results closely match rearing results for larvae which had fed for 1-2 days post stinging. However, there was a significant drop-off in detection of parasites in 3-4 day post stung larvae. There are two possible explanations for this drop-off. First, parasite larvae may be growing less rapidly than the gypsy moth larvae, resulting in a "dilution" of the parasite DNA below the level of sensitivity of the particular probe. A second explanation may be that shipping of samples caused some lethality of parasites within the hosts, and that decreasing detection with time represents degradation of parasite DNA.

So far, results are very promising that a simple molecular probe can be used to detect parasitization of gypsy moths. However, issues relating to sensitivity of the technique for detecting parasitization need to re resolved.