Woolly adelgid nymph

Population Genetics of the Hemlock Woolly Adelgid

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Introduction

The hemlock woolly adelgid (*Adelgis tsugae*), accidentally introduced from Asia, has been responsible for widespread eastern hemlock (*Tsuga canadensis*) decline in the northeast and threatens to alter ecosystem structure and function in heavily infested areas. The insect was found in northwestern North America in the 1920s (Annand 1924), near Richmond, VA in the 1950s (Souto *et al* 1996), in southern New England in 1985 (McClure 1990), and in Portsmouth, NH by 2001 (Anonymous 2001). As the insect migrates unpredictably northward, several questions have been raised, including the apparent 'resistance' of some hemlocks to adelgid attack (Souto *et al* 1996), as well as the documented cold hardiness of individual adelgids. Recently performed common garden experiments suggest that the ability to withstand cold temperatures has a genetic component (Butin unpublished).

Using AFLPs (amplified fragment length polymorphism) and possibly microsatellite markers we hope to:

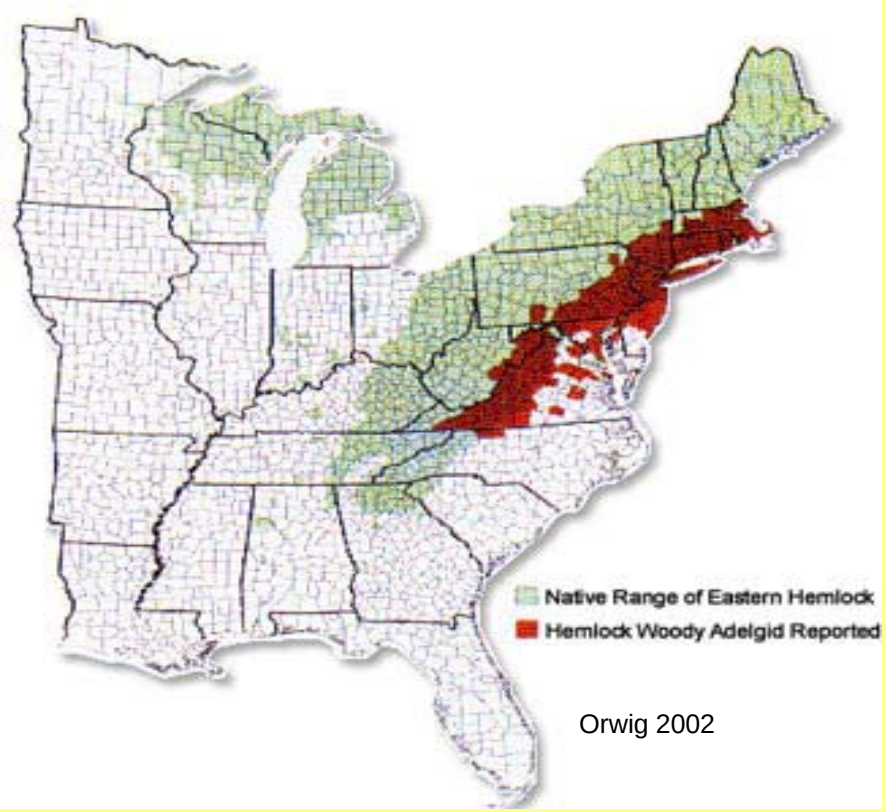
1) Document the level of genetic variation in the woolly adelgid throughout the introduced range

2) Characterize variation among different spatial scales such as individual branch, tree, hemlock stand, region, and entire range

3) Determine if differences exist between cold hardy and cold susceptible individuals

4) Provide information on adelgid dispersal patterns and size of initial founder populations.

Figure 1: Distribution of Eastern Hemlock and the Hemlock Woolly Adelgid



Orwig 2002

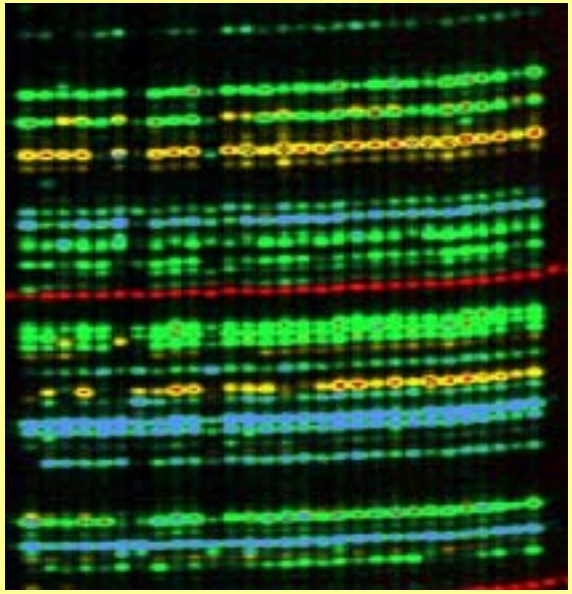
Methods

In order to determine if variation exists and if it is arranged spatially in any predictable pattern, a total of 38 adelgids from 8 locations (Figure 2) were analyzed using AFLPs. This method selectively amplifies portions of the genome, and differences are inferred from varying band sizes among individuals. Fifteen primer sets were used.

digestion of genomic DNA with 2 restriction enzymes and ligation of linkers to resulting fragments PCR amplification of linker ligated DNA selective PCR amplification using several primer sets (linker + 3 additional base pairs) visualization of bands on polyacrylamide gel construction of phenogram using Paup v4.0

Figure 2: Adelgid collection localities

Figure 3: AFLP gel
The results of three primer sets are shown; green, yellow, and blue bands are different primer combinations; red is a size standard.



Results/ Conclusions

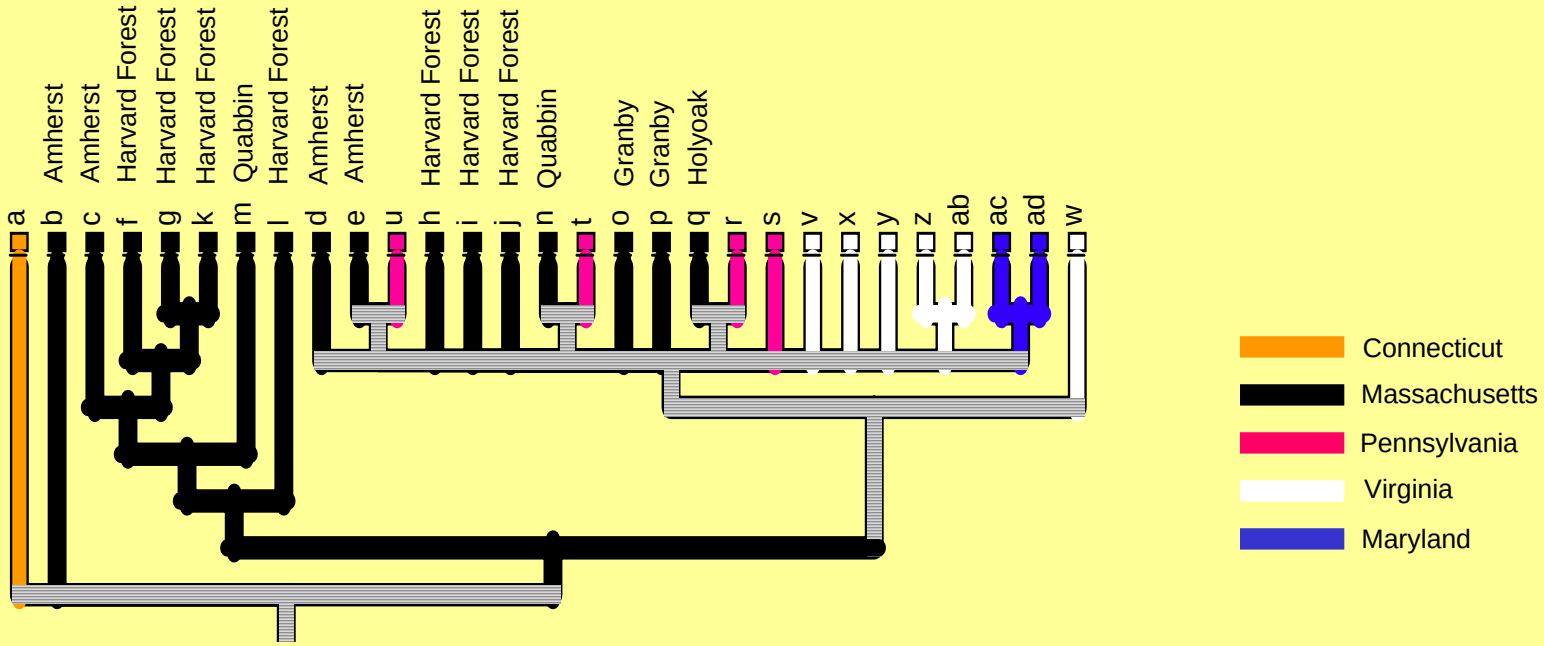
The fifteen primer pair combinations generated 387 unambiguous AFLP markers. Less than 15%, or about 60 were polymorphic.

There were no fixed differences between individuals from different localities.

Variation appears to correspond somewhat with geography, however a much larger sample size is needed to confirm/refute this finding. Interestingly, different clones were found at the smallest spatial scale- adjacent trees in Amherst and in Harvard Forest.

The 15 primer pairs used did not distinguish between cold hardy and cold susceptible individuals. This does not indicate that such differences do not exist, as further screening may reveal differences between these groups. In future studies, collections to address this question will not simply compare individuals collected in northern vs. southern locations, but will take different climatic zones into consideration.

These preliminary data show that woolly adelgids in the northeast are remarkably similar as was found by Sánchez (2002) using allozyme data. In order to address questions of dispersal, additional primer sets will be used in combination with the sets already run showing the most variation. D-loop, a highly variable region within the mitochondrial genome, and microsatellites may also provide useful information at the population level. We believe this method has enormous potential to characterize the diversity and population history of adelgids in the introduced range, and hope to include adelgids from the west coast in future analyses.



Preliminary phenogram constructed using parsimony in Paup v4.0 with data from 15 primer sets and 29 individuals. Letters a-ad represent individual adelgids.

References

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