

Initial Estimates of the Number of Distinct Populations of Hemlock Woolly Adelgid in the Eastern United States Using Isoenzyme Profiles

Vicente Sánchez

USDA Forest Service, Northeastern Center for Forest Health Research
Hamden, CT

Abstract

Enzyme electrophoretic analysis was used to test the hypothesis that hemlock woolly adelgid in the East are genetically uniform. Adelgids from infested eastern hemlock collected in Virginia, New Jersey, and Connecticut were screened at each of seven putative enzyme loci. The isozyme profiles for four of the enzymes were typical of homozygous and invariant loci for adelgids from the three sites. The remaining isozymes exhibited profiles expected of groups with heterozygous origin but the putative alleles for the adelgids were fixed across all three sites. This initial estimate of large-scale genetic diversity in hemlock woolly adelgid suggests that populations of this insect in the Eastern United States are genetically uniform. If eastern populations of adelgid actually have little or no genetic differentiation between them, the low variability could delay pesticide resistance or favor the breeding of resistant hemlock.

Keywords:

Genetic diversity, enzyme electrophoretic analysis, isozyme profiles.

Introduction

Like most Aphidoidea, the hemlock woolly adelgid (*Adelges tsugae* Annand) has a complex lifecycle including morphological polymorphisms and host alternation. In North America, hemlock (*Tsuga* spp.) trees host three parthenogenetic generations of this adelgid, one of which is a winged morph that in its native range migrates to a spruce (*Picea* spp.) host on which it lays the eggs of the sexual generation. Sexual reproduction in the United States is presumably absent since no suitable primary spruce host is reported in North America. The lack of genetic recombination through sexual reproduction suggests that contiguous groups of hemlock woolly adelgid in the United States are likely genetically uniform. However, other factors such as multiple introductions or uneven selection pressures can promote genetic diversity in geographically distributed populations.

Large-scale measures of genetic diversity in populations of Aphidoidea have been derived using estimates obtained from characterizing macromolecules such as heritable enzymes, or allozymes (Loxdale and Brookes 1989). Electrophoresis is used to separate these enzymes, their reaction products are stained, and the colored precipitates from the macromolecular characters are used for

analysis (Murphy et al. 1990). Enzyme profiles can be useful for detecting variation in asexually reproducing species since populations from the same maternal lineage would be distinct. Since the hemlock woolly adelgid probably reproduces only asexually in North America, multiple introductions would be expected to produce distinct lineages of genetically uniform populations in the absence of recombination events.

The distribution of hemlock woolly adelgid hosts in North America is disjunct, with western hemlock (*T. heterophylla*) restricted to the Pacific Northwest, while eastern hemlock (*T. canadensis*) is relatively contiguous along the temperate east coast. Consequently, eastern populations of the hemlock woolly adelgid experience differing climatic pressures as they spread along eastern hemlock habitats between 35° and 45°N latitude. Evaluating heritable characters of the hemlock woolly adelgid along such north-south gradients is one way of determining their genetic diversity and estimating the number of distinct populations. This report describes the result of an initial enzyme electrophoretic analysis of adelgids collected from three sites along the Atlantic coast of the United States.

Materials and Methods

The hemlock woolly adelgid samples were collected from infested eastern hemlock at sites in extreme and central portions of the adelgid's current range along Atlantic Coast States. Branches were clipped from trees in late June 2000 from Olean in Giles County, Virginia (VA), and Walpack in Sussex County, New Jersey (NJ), and in June 2001 from Hamden in New Haven County, Connecticut (CT). The branches were kept moist and refrigerated during transport to the laboratory, where they were culled to sections bearing viable adelgid infestations. Adelgids were left on the selected branches to reduce the likelihood of specimen damage, put into labeled double-layered zip lock freezer bags, and stored at -80°C until analysis.

Preliminary runs indicated that single adelgids provided insufficient sample to resolve enzymes on the traditional starch gels used in isozyme analysis. Consequently, a newer method developed by Helena Laboratories¹ using cellulose acetate membranes as the electrophoresis substrate and requiring far less sample was used.

Adelgids collected from the three sites were at similar stages of development but infestation levels varied, as did apparent host health. The branches contained mostly adult progrediens with eggs, with the VA samples having a higher proportion of early instars than the NJ or CT samples.

In preparation for analysis, an apparently healthy and intact adelgid was removed from a branch, placed in the well of a chilled Helena sample plate with 3 ml of homogenization buffer (10% Sucrose with 1% Triton-X100), ground until no recognizable fragments remained. The well was then filled with an additional 9 ml aliquot of buffer. Samples were kept over ice until loaded onto membranes.

¹Use of trade names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

Electromorph patterns were initially investigated for 30 enzyme systems using either a Tris Glycine (pH 8.0) or Citric Acid Morpholine (pH 7.0) buffer for resolving the isozymes. Initially, 10 insects per site were used to determine resolvability, then the best resolving systems were used to examine an average of 30 individuals per site. Two reference lanes of CT adelgids (VA when CT was sampled) were included in runs as internal controls to insure comparability among membranes. After loading the samples onto cellulose acetate membranes, the membranes were put in electrophoresis tanks and run at 200 volts for 15 minutes at room temperature. Membranes were then removed and stained, generally as agar overlays, across a battery of best-resolving putative enzyme loci using formulations from Sánchez and Cardé (1999) with some modifications from Herbert and Beaton (1993). After membranes had optimally stained, these zymograms were qualitatively scored using visible enzyme profiles for putative allele assignments, then photographed and samples stored.

Results

The initial survey of 30 enzyme systems using the two buffer systems revealed seven putative allozyme systems with sufficient resolution for analysis (Table 1). The system could not be simplified to one buffer, as the pH of each buffer was important and each resolved only a subset of the isoenzymes. The Tris Glycine buffer gave resolution in four potential loci (Aspartate Aminotransferase, Fumarase, Malate Dehydrogenase, Triose Phosphate Isomerase), while the Citric Acid Morpholine was needed for resolution of the remaining isoenzyme systems (Esterase, Glucose Phosphate Isomerase, Phosphogluconate Dehydrogenase).

Table 1. Qualitative Allelic Profiles of 30 Hemlock Woolly Adelgids From Each of Three Eastern Sites

Enzyme Locus	Alleles Present by Locality [§]		
	Virginia	New Jersey	Connecticut
Aspartate Aminotransferase	1	1	1
Esterase	1	1	1
Fumarase	1 and 2	1 and 2	1 and 2
Glucose Phosphate Isomerase	1	1	1
Malate Dehydrogenase	1 and 2	1 and 2	1 and 2
Phosphogluconate Dehydrogenase	1	1	1
Triose Phosphate Isomerase	1 and 2	1 and 2	1 and 2

[§] Most common allele classified as 1.

The profile patterns for these likely allozymes were observed to be homozygous and invariant across four of the enzyme loci evaluated. The three remaining putative loci produced patterns qualitatively similar to profiles expected of groups with heterozygous origin, but fixed nonetheless across all three sites. Allele frequencies across all loci and sites were fixed for each allozyme resolved. Similarly, no rare or private alleles that could distinguish the adelgid's collection site were recovered at any locus.

Discussion

The initial estimates of large-scale genetic diversity between three populations of hemlock woolly adelgid revealed no evidence of differences among putative allozyme profiles, suggesting that the adelgid in the Eastern United States is genetically homogenous. As aphids are parthenogenetic (Lees 1966), so too are adelgids and probably also apomictic which would result in homogametic females identical to their mother. This would provide a clonal quality to adelgids in the eastern United States that is consistent with these findings. In future analyses, adelgids will be included from additional eastern and western North American sites, as well as from Asia.

Variation also can arise from mutations in neutral genetic information not under as stringent selection as allozymes. The more than 50-year time interval since the adelgid arrived in the Eastern United States is considered sufficiently long for changes to have accumulated in neutral characters. A concurrent study for distinguishing hemlock woolly adelgid populations is investigating variation of such heritable characters through an analysis of randomly amplified genetic sequences.

The hemlock woolly adelgid was first recovered from hemlock in the Eastern United States in Virginia in 1951, though samples are known from the Pacific Northwest as early as 1924, and it continues to spread throughout eastern hemlocks. The possibility that eastern populations of adelgid have little or no genetic differentiation between them could be advantageous for pest management because a restricted gene pool could mean a lower likelihood of developing pesticide resistance, predator avoidance mechanisms, or developing cold tolerance, and a higher likelihood of developing host-resistant hemlock stocks.

Acknowledgments

Adelgid samples were kindly collected and provided from Virginia by James "Rusty" Rhea, USDA Forest Service; from New Jersey by Mark Mayer, New Jersey Department of Agriculture; and from Connecticut by Michael E. Montgomery, USDA Forest Service.

References

Herbert, P.D.N. and M.J. Beaton. 1993. *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Technical Bulletin, Helena Laboratories, Beaumont, Texas.

- Loxdale, H.D. and C.P. Brookes. 1989. Use of genetic markers (allozymes) to study the structure, overwintering and dynamics of pest aphid populations, pp. 231-270. In Loxdale, H.D. and J. den Hollander, (eds.). *Electrophoretic Studies on Agricultural Pests, Systematics Association*, Special Volume No. 39. Oxford: Claredon.
- Lees, A.D. 1966. The control of polymorphisms in aphids. *Advances in Insect Physiology* 3: 207-277.
- Murphy, R.W.; J.W. Sites, Jr.; D.G. Buth; and C.H. Haufler. 1990. Proteins I: Isozyme electrophoresis, pp. 45-126. In Hillis, D.W. and C. Moritz, (eds.). *Molecular Systematics*. Sinauer, Massachusetts.
- Sánchez, V. and R.T. Cardé. 1999. Isozyme patterns and their inheritance in the tachinid *Compsilura concinnata*. *Journal of Heredity* 90:568-573.