

Range-Wide Assessment of Genetic Variation and Structure in Eastern Hemlock, an Imperiled Conifer, Using Microsatellites

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Hemlock woolly adelgid



Eastern hemlock forest

Introduction

Eastern hemlock (*Tsuga canadensis*) (Figure 1) is an ecologically important tree species experiencing severe mortality across much of its North American distribution, caused by the exotic hemlock woolly adelgid (*Adelges tsugae*) (HWA).

To guide gene conservation strategies for this imperiled conifer and to better understand its genetic architecture, we conducted the first range-wide genetic variation study for eastern hemlock (Figure 2).



Figure 1: Eastern hemlock is an important species in riparian areas; this stand is at South Mountains State Park, N.C.

Objectives

We amplified 13 microsatellite marker loci isolated from eastern hemlock and Carolina hemlock (*T. caroliniana*) (Josserand *et al.* 2008; Shamblin *et al.* 2008) across 1,180 trees from 60 eastern hemlock populations (~20 per population) to:

- (1) Identify areas of high and low genetic variation,
- (2) Compare genetic variation in peripheral disjunct and main range populations,
- (3) Assess regional differences in genetic variation to better understand its recent phylogeographical history, and
- (4) Compare genetic variation in populations currently threatened by HWA with those not yet in the infested range.

Figure 2: Sampled populations of eastern hemlock; the dashed line depicts the maximum extent of the Wisconsinian glaciations, ca. 18,000 years ago.

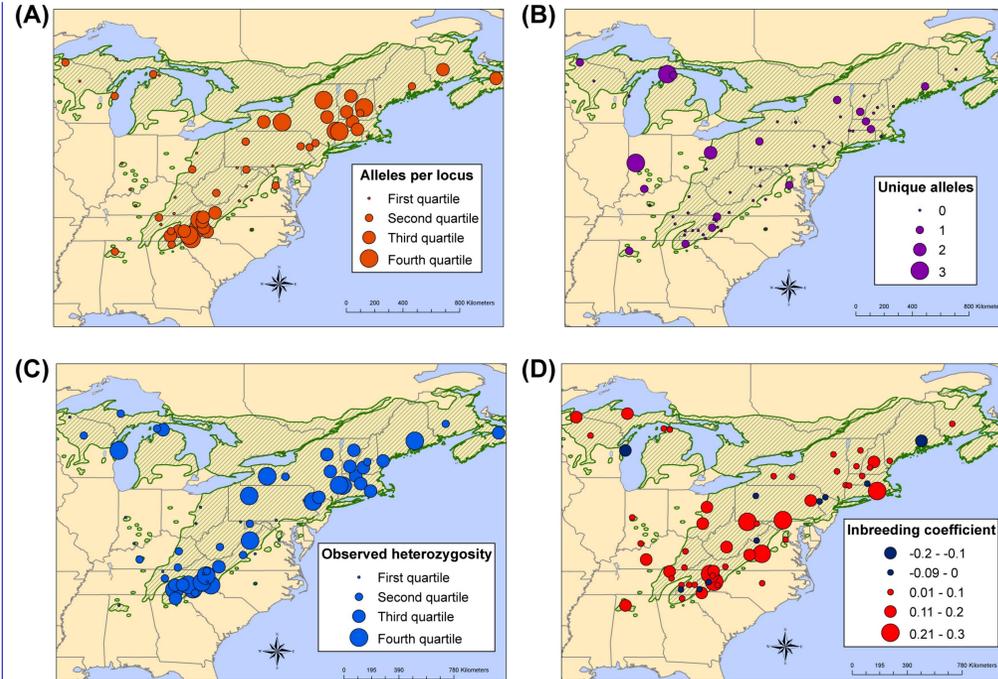
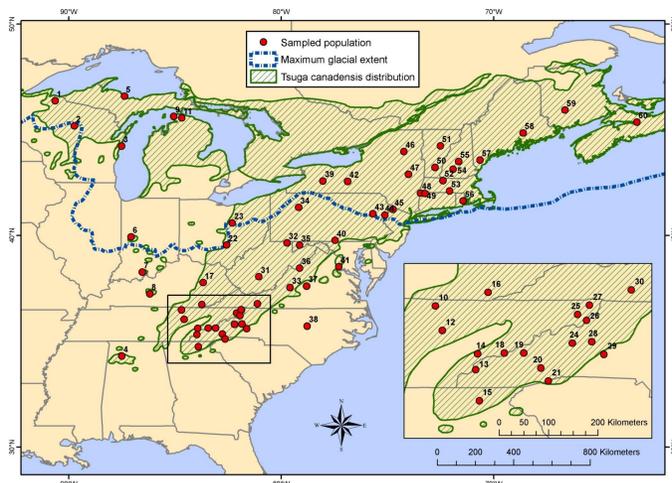


Figure 3: Eastern hemlock population classifications of (A) mean rarefaction estimate of alleles per locus, (B) unique alleles, (C) observed heterozygosity, and (D) inbreeding coefficient (F_{IS}), based on 13 polymorphic microsatellite loci.

Analyses

We used FSTAT 2.9.3.2 to calculate standard genetic variation metrics across the range of the species and for each population.

To test for significant differences between the means of populations (1) north and south of the maximum glacial extent, (2) in or not in counties infested with HWA, and (3) within and disjunct from the main range, we conducted an exact two-sample Wilcoxon rank-sum test using the NPAR1WAY procedure in SAS 9.2.

We used the spatial Bayesian clustering program TESS 2.3.1 to determine the number and composition of distinct gene pools.

Results

- ❖ Contrary to expectations, populations in formerly glaciated regions are not less genetically diverse than in the southern refugial region (Figure 3).
- ❖ Disjunct populations are less genetically diverse than the main range, but some are highly differentiated or contain unique alleles (Figure 3).
- ❖ Many populations exhibit moderate inbreeding (Figure 3D).
- ❖ Much variation exists in areas both infested and uninfested by HWA.
- ❖ The Bayesian clustering analysis indicates the presence of five gene pools in the southern part of the range, but only one across most of the northern populations (Figure 4).

Discussion

Strong and often unexpected patterns of genetic variation exist across eastern hemlock's range. The cluster results suggest that glacial refugia existed in the South, but populations there were not more diverse, as expected.

These patterns, and the widespread inbreeding across the range, may be explained by a range-wide population decline that occurred about 5,000 years ago, and is attributed to a catastrophic pest outbreak. Significantly, southern populations were more inbred than northern ones, consistent with a more dramatic decline in the South than in the North.

As expected, we found that isolated populations were generally less diverse, but that they were often more differentiated and contained rare alleles.

While populations in HWA-infested areas were not more genetically diverse, the southern part of the range includes all the inferred gene pools and has experienced severe HWA mortality.

Gene Conservation Implications

Efforts to conserve eastern hemlock genetic material should emphasize capturing:

- ❖ Broad adaptability that occurs across the range of the species;
- ❖ Variability within regions with the highest allelic richness and heterozygosity, such as the Southern Appalachians and New England; and
- ❖ Variability within disjunct populations that are genetically distinct and/or contain rare alleles.

References

Josserand, S.A.; Potter, K.M.; Echt, C.S.; Nelson, C.D. 2008. Isolation and characterization of microsatellite markers for Carolina hemlock (*Tsuga caroliniana*). *Molecular Ecology Resources*. 8(6):1371-1374.
Shamblin, B.M.; Faircloth, B.C.; Josserand, S.A. [and others]. 2008. Microsatellite markers for eastern hemlock (*Tsuga canadensis*). *Molecular Ecology Resources*. 8(6):1354-1356.



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