RANGEWIDE ASSESSMENT OF GENETIC STRUCTURE AND VARIATION IN EASTERN HEMLOCK (*TSUGA CANADENSIS*), AN IMPERILED CONIFER, USING MICROSATELLITE MARKERS

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Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is an ecologically important species experiencing extensive mortality caused by the invasive hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand). We conducted the first rangewide molecular marker population variation study for eastern hemlock to **(1)** help guide gene conservation efforts for the species, **(2)** assess the genetic effects of isolation on peripheral disjunct populations, and **(3)** better understand the biogeographical processes that shaped the genetic architecture of the species. This study encompasses 60 eastern hemlock populations, of which eight are disjuncts, half are from areas north of the maximum extent of the Wisconsinian glaciation, and 38 are in counties infested with HWA. We used 13 highly polymorphic and consistent nuclear microsatellite loci in the analysis after screening 42 loci isolated from eastern hemlock, Carolina hemlock (*Tsuga caroliniana* Engelm.) and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.).

We found moderate levels of genetic differentiation among populations, with approximately 6 percent of the variation among, rather than within, populations. Overall observed heterozygosity was less than expected heterozygosity, and most populations had relatively high levels of inbreeding. The species appears to have two main centers of genetic variation, in the southern Appalachians and in New England. Unique alleles, however, were present in the highest numbers in western populations, including disjuncts in Indiana and Kentucky. Interior populations were more significantly more diverse than disjunct populations by nearly every measure. Disjuncts, however, on average had more private alleles. Levels of genetic variation in Northern and Southern populations were not generally significantly different, although Southern populations were, on average, significantly more inbred than northern populations. There was little difference in genetic variation between populations in counties infested and uninfested by HWA, although uninfested had more unique alleles on average.

A spatially explicit Bayesian clustering analysis of individual trees using TESS suggested that the species contains five gene pools. Strong geographic patterns were apparent in the arrangement of these gene pools, with all five gene pools present in southern populations, and only one dominant in northern populations. These findings suggest that one or more Pleistocene glacial refuge existed in the South, with a main post-glacial movement first into the Northeast and then into the Great Lakes region. A separate migratory path may have existed to the populations west of the southern Appalachian crest.

The results suggest the following: **(1)** Efforts to conserve the genetic variation of eastern hemlock should focus on the areas with the highest allelic richness and heterozygosity (the southern Appalachians and New England) and on other areas with high numbers of unique alleles. **(2)** Gene conservation activities also should target disjunct populations. While these are more inbred and less genetically diverse, several also contain high numbers of unique alleles or represent gene pools rare in the interior of the range. **(3)** It is not too late to conserve eastern hemlock genetic variation, given that much genetic variation exists in locations that have not yet been impacted by HWA.