Carolina hemlock foliage and cones

Population Isolation Results in High Inbreeding and Differentiation in Carolina Hemlock (*Tsuga caroliniana*)

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Carolina hemlock forest

Figure 1: ***Carolina hemlock is typically found on rocky upland soils; this stand is in Linville Gorge, N.C.***

Introduction

Carolina hemlock (*Tsuga caroliniana*) (**Figure 1**) is a rare conifer species that exists in small, isolated populations. This Southern Appalachian endemic is declining as a result of infestation by the exotic hemlock woolly adelgid (HWA)(*Adelges tsugae*).

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 Carolina hemlock (**Figure 2**).

Objectives

We amplified 16 microsatellite marker loci isolated from Carolina hemlock (Josserand *et al.* 2008) across 439 trees from 29 populations to:

(1) Identify areas of high and low genetic variation,

(2) Evaluate genetic variation in peripheral disjunct and core range populations,

(3) Assess regional differences in genetic variation to better understand its recent phylogeographic history, and

(4) Compare genetic variation in populations with high and low levels of HWA infestation.

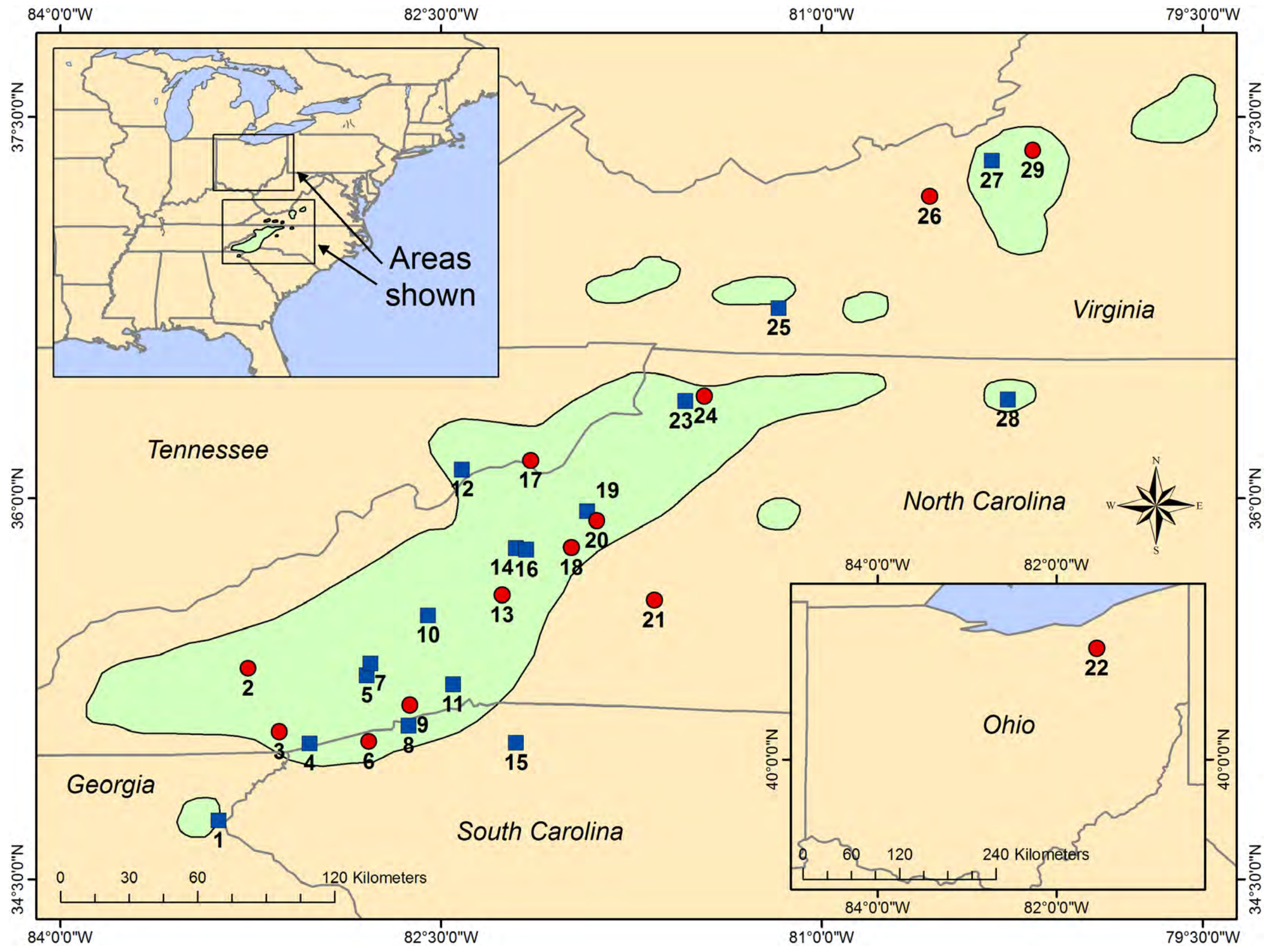

Figure 2: ***Sampled populations of Carolina hemlock. Blue populations (squares) are those from which seeds have been collected for gene conservation.***

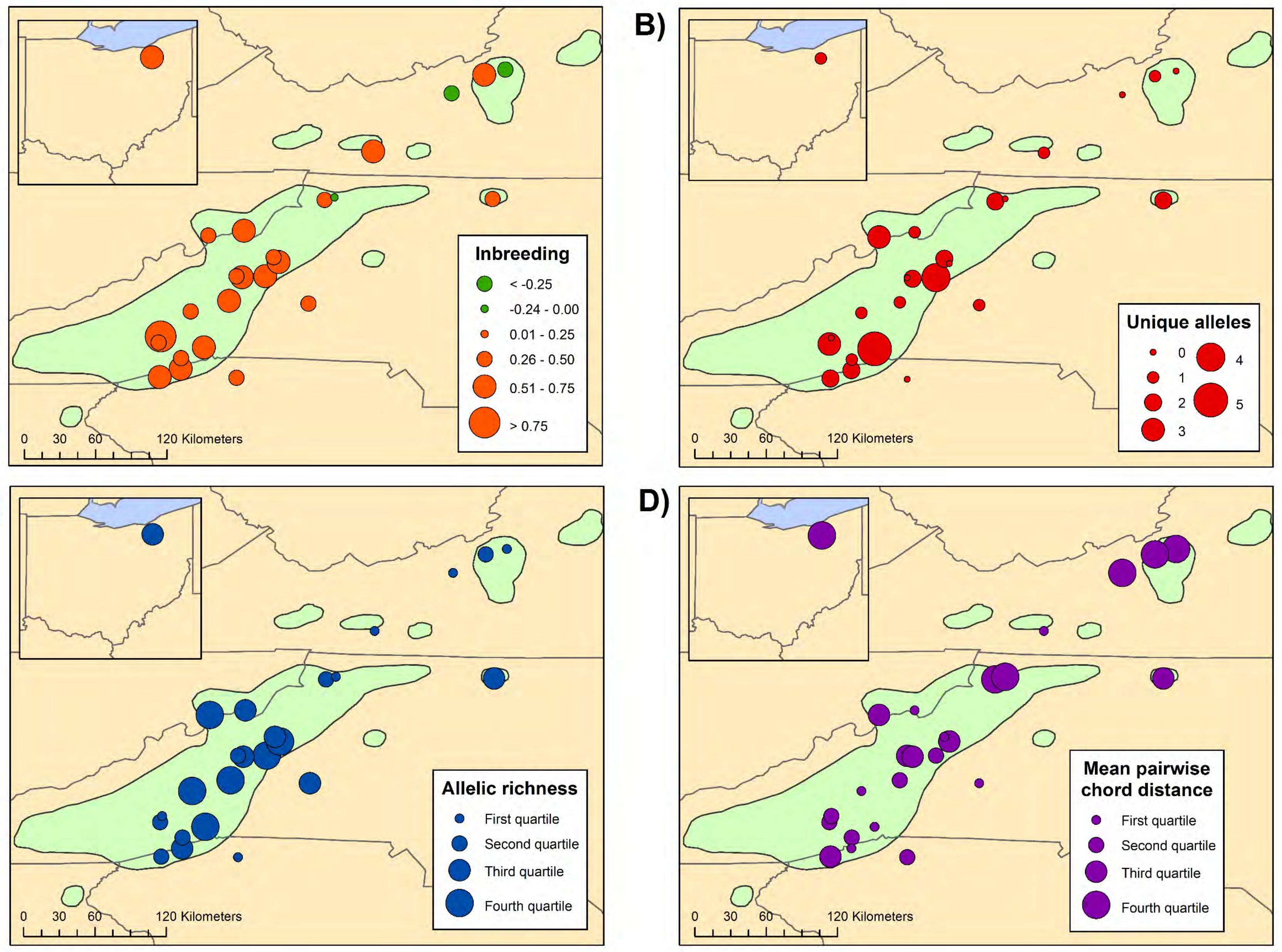

Figure 3: ***Carolina hemlock population classifications of (A) inbreeding coefficient (F_{IS}), (B) unique alleles, (C) allelic richness, and (D) mean pairwise genetic chord distance for each population, based on 16 polymorphic microsatellite loci.***

Analyses

We used GenAlEx 6.501 and FSTAT 2.3.9.2 to calculate standard genetic variation metrics across the range of the species and for each population.

We tested for significant differences between the means of populations **(1)** within and disjunct from the core range, **(2)** having more or less than 50% HWA infestation, and **(3)** having or not having a previously completed seed collection, using an exact two-sample Wilcoxon rank-sum test in SAS 9.2.

We used the spatial Bayesian clustering program STRUCTURE 2.3.4 to determine the number and composition of distinct gene pools.

Results

❖ Populations are highly differentiated ($F_{ST} \approx 0.48$), with little estimated gene flow (0.92 migrant per generation).

❖ Most populations exhibit high levels of inbreeding (**Figure 3A**).

❖ Southern populations have higher genetic diversity (**Figure 3B, 3C**).

❖ Disjunct populations are less genetically diverse than core range populations, but some are highly differentiated (**Figure 3D**).

❖ The Bayesian clustering analysis inferred three gene pools, one in the northern part of the range and two in the southern part (**Figure 4**).

Discussion

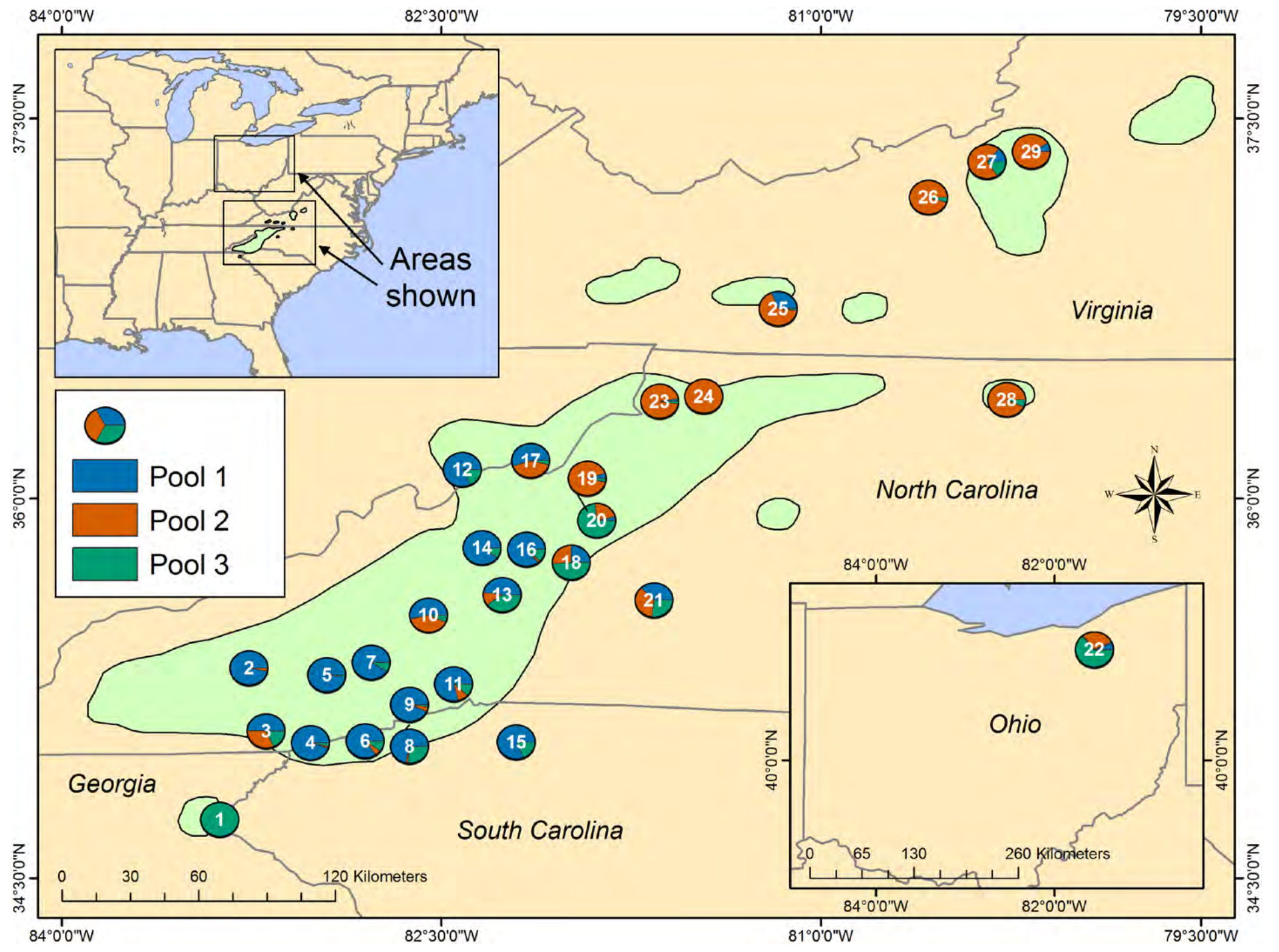

Figure 4*: The proportion, within each Carolina hemlock population, of inferred ancestry from the genetic clusters defined using STRUCTURE.*

Carolina hemlock's high inbreeding is not surprising given that it consists of small and relatively isolated populations, even in the core of its range. Differentiation among populations is surprisingly high for a conifer with wind-dispersed pollen, however.

Inter-population gene flow appears to be a rare event in Carolina hemlock, in part because its populations are small and widely dispersed. Pollen susceptible to desiccation also may be a factor. Carolina hemlock populations may not exceed a threshold of gene flow needed to avoid genetic drift and inbreeding.

As expected, marginal populations are generally less diverse than core-range populations, but are often more differentiated. No diversity differences exist between populations with high and low levels of HWA , and between those with and without completed seed collections. Finally, the genetic clustering results suggest the Cuyahoga Valley outlier population in Ohio was likely planted.

Gene Conservation Implications

❖The fact that nearly all Carolina hemlock populations are highly inbred emphasizes the necessity of quickly and effectively preserving the species' genetic diversity.

❖The high levels of differentiation among populations, and the commonness of alleles unique to populations, underscore the importance of ensuring that gene conservation efforts represent as many populations as possible.

References

Josserand, S.A.; K.M. Potter; C.S. Echt; C.D. Nelson. 2008. Isolation and characterization of microsatellite markers for Carolina hemlock (*Tsuga caroliniana*). *Molecular Ecology Resources*. 8(6):1371-1374.
Campbell, A.R.; K.M. Potter; S.A. Josserand; and R.M. Jetton. *In preparation*. Assessing range-wide population genetic structure and diversity of Carolina hemlock (*Tsuga caroliniana*), an imperiled Southern Appalachian endemic conifer.

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