

Prioritizing conservation seed banking locations for imperiled hemlock species using multi-attribute frontier mapping

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Abstract Hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA) is an invasive forest insect sweeping across the native range of eastern (*Tsuga canadensis* [L.] Carr.) and Carolina (*Tsuga caroliniana* Engelm.) hemlocks, threatening to severely reduce eastern hemlock extent and to push Carolina hemlock to extirpation. HWA poses a significant threat to these eastern US natives, now infesting hemlocks across 19 states and more than 400 counties. For the long-term preservation of the species, *ex situ* genetic resource conservation efforts such as seed collection, storage, and adelgid-resistant hemlock breeding may all be necessary. To ensure the efficient and effective application of these efforts, it will be necessary to prioritize locations within the native ranges, because it is logistically impossible to apply these efforts to all populations. To build upon 12 years of seed banking for eastern and Carolina hemlock, we applied a novel approach for incorporating multiple dissimilar data sets into a geographic prioritization of areas for the most effective and efficient conservation of genetic diversity. The approach involves integration of geographic information systems with the multi-attribute frontier mapping technique to

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identify locations across the ranges of these two imperiled species most in need of conservation actions. Specifically, our assessment incorporated four genetic diversity parameters, a climate component, a measure of population disjunctiveness, a measure of local hemlock abundance, and seed collection density to prioritize areas of eastern and Carolina hemlock occurrence for *ex situ* gene conservation. For each species, the result was a mapped index of locations prioritized by the combined significance of these factors. For eastern hemlock, this assessment assigned the highest priority to disjunct populations and to some areas within the main-body range. With Carolina hemlock, disjunct northern populations and central main-body locations received the highest prioritization. Our prioritization approach could be applied similarly to other species facing pressure from invasive pests or other environmental threats.

Keywords Eastern hemlock · Carolina hemlock · Gene conservation · Genetic diversity · Multi-criteria evaluation · Climate change

Introduction

Eastern hemlock (*Tsuga canadensis* [L.] Carr.) and Carolina hemlock (*Tsuga caroliniana* Engelm.) are widespread and economically important conifer tree species native to the eastern United States, currently suffering extensive mortality caused by an exotic insect. Carolina hemlock tends to occur in isolated populations on mountain bluffs and dry ridges in North Carolina, South Carolina, Georgia, Tennessee, and Virginia, although the current range could be a relict of a once more widespread distribution (Jetton et al. 2008). Though its native range is entirely encompassed by that of eastern hemlock, Carolina hemlock is more closely related to Asian hemlock species (Havill et al. 2008). Eastern hemlock's range is much more extensive, and the species can be found in mid-story canopies from northern Georgia and Alabama in the south, to Nova Scotia in the north. The western edge of the range also extends as far west as Minnesota. It is a canopy-dominant species in parts of its range. Both hemlock species are shade tolerant and long-lived, providing a thick duff layer, dense shade, slow rates of nitrogen cycling, and habitats where hundreds of plant and animal communities are adapted to survive (Galatowitsch et al. 2009).

Eastern and Carolina hemlock have experienced extensive mortality since the introduction of the hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand), an invasive aphid-like insect. Although first noted in Richmond, Virginia in 1951 (McClure 1989; Souto et al. 1996), HWA was likely introduced prior to this date directly from Japan (Havill and Salom 2014). Since its introduction HWA has spread to 447 counties across 19 states (USDA Forest Service 2017), including 403 in the native U.S. range of eastern hemlock (58% of 696 U.S. counties) and the entirety of Carolina hemlock's distribution. HWA is parthenogenetic—meaning it is capable of asexually reproducing without fertilization—and individual females can produce more than 300 eggs per year. Driven by its parthenogenetic nature and high egg production, it is capable of killing its host in as little as four years (McClure et al. 2003). HWA is easily transported by humans, birds, and the wind often via manmade landscape corridors or riparian areas, and crawler generations are active when human recreation and bird migration are likely to be highest (McClure 1990; Koch et al. 2006).

Genetic diversity studies along with gene conservation are critically important strategies for maintaining hemlock on the landscape, as hemlock mortality soars and limits to the spread of HWA remain elusive (Oten et al. 2014). Since 2003, Camcore (international tree breeding and conservation program in the Department of Forestry and Environmental Resources at North Carolina State University) and the United States Department of Agriculture (USDA) Forest Service have worked to secure eastern and Carolina hemlock genetic diversity via seed collection and long-term storage from 750 mother trees across 76 populations of eastern hemlock and 168 mother trees across 24 populations of Carolina hemlock—approximately 2.5 million seeds in total (Hodge et al. 2017). Additionally, more than 2000 seedlings have been planted in genetically diverse seed orchards in Chile, Brazil, and North Carolina (Jetton et al. 2013; Oten et al. 2014). The primary concept behind the installation of *ex situ* seed orchards is the availability of diverse genetic material for *in situ* breeding and restoration once successful adelgid management techniques are in place (Oten et al. 2014). Similar to disease resistance-breeding for American chestnut (*Castanea dentata* (Marsh.) Borkh.), hemlock host-resistant breeding methods may come to include hybridizing native hemlock trees with a resistant relative from western North America or Asia and then backcrossing the offspring with the pure species (Rhea and Jetton 2010). Additionally, locating naturally occurring HWA-resistant eastern and Carolina hemlocks is of critical importance for gene conservation, as it may be possible to breed these individuals with pure species individuals, and then exclude undesirable genes from non-native hemlocks (Oten et al. 2014).

Resources for seed collection and other conservation actions are necessarily limited for these two imperiled hemlock species, as they are for most species in need of conservation. In such situations, it is necessary to apply rational, systematic, and defensible prioritization approaches to efficiently allocate scarce conservation resources (Bottrill et al. 2008). One such effort has already been applied on a relatively small scale, identifying priority eastern hemlock conservation areas on the Allegheny Plateau of Pennsylvania and New York (Johnson et al. 2016). We here describe and apply a novel approach for prioritizing eastern and Carolina hemlock populations for conservation efforts—especially *ex situ* conservation actions—across the entire range of each species, to ensure the effectiveness and efficiency of conservation efforts directed at these species. In particular, we demonstrate a combinatorial method, known as the multi-attribute frontier approach (Yemshanov et al. 2013), that allows us to rank areas of occurrence for each species according to the combined significance of several geospatially referenced (i.e., mapped) criteria. Unlike other combinatorial methods used in multi-criteria evaluation (e.g., linear weighted averaging or the application of weights elicited from experts), the multi-attribute frontier approach does not require prior information or judgment about the relative importance of the criteria. Instead, each area of interest is ranked objectively against the other areas by considering values for all of the criteria simultaneously.

This prioritization assessment relies heavily on recent studies of eastern hemlock (Potter et al. 2012) and Carolina hemlock (Campbell 2014) that identified patterns of genetic diversity within the species. Both studies employed microsatellite markers to assess genetic diversity and identify possible within-species genetic lineages for targeting of seed collections across the species' respective ranges. Results from both studies included the detection of high levels of inbreeding and low genetic diversity range-wide. Similar to that of eastern hemlock in the study conducted by Potter et al. (2012), Carolina hemlock exhibited genetic clustering that was possibly indicative of one or more glacial refugia (Campbell 2014).

Climate change is of particular concern for species like eastern and Carolina hemlock because of their slow growth rates, limited climatic and geographic preferences, and poor seed dispersal. HWA range expansion as a result of climate change puts eastern hemlock populations at significant risk; all Carolina hemlock populations are already at risk from HWA. Fortunately, adelgid range expansion is currently limited in the northern latitudes of the eastern hemlock range by average annual minimum temperatures below -15 to -30 °C depending on the time of year (Parker et al. 1999; Costa et al. 2004). Unfortunately, according to NOAA/GFDL CM2.1, UKMO HadCM3, and DOE/NCAR PCM general circulation model (GCM) projections, HWA is expected to infest the entire northeastern U.S. region under IPCC A1 emission scenarios by the end of the century (Paradis et al. 2008). This is notable as pests respond to changing climate much more rapidly than the forests—and likely the host species—they infest (Logan et al. 2003). Projected rates of climate change are also expected to accelerate—so much so that *in situ* genetic adaptation of hemlock populations to new climate conditions is unlikely (Jump and Penuelas 2005; Heller and Zavaleta 2009). Modeling climate changes across the entire ranges of the two hemlock species can inform emphasis areas for conservation efforts. If followed by sampling, gene conservation, and adaptation measures, modeling could also limit the chance of species range reduction or extinction due to climate change (Sgro et al. 2011).

In order to prioritize locations of the eastern and Carolina hemlock range for gene conservation, this study utilized four genetic diversity metrics—allelic richness, percent of polymorphic loci, observed heterozygosity, and inbreeding—from the genetic diversity studies conducted by Campbell (2014) and Potter et al. (2012). Using the spatially explicit multi-attribute frontier model (Yemshanov et al. 2013) and geographic information systems (GIS), interpolated genetic diversity data were combined with downscaled GCMs of average annual extreme minimum temperature; the degree of isolation of peripheral disjunct populations; diameter of sampled hemlock trees, as a measure of local abundance; and seed collection density to identify and prioritize hemlock locations for further genetic diversity studies and seed collection efforts.

Methods

Genetic diversity

Twenty-nine Carolina hemlock populations were sampled throughout the native range by Campbell (2014) during the summer of 2013. Sixteen microsatellite loci were amplified from each of 439 trees, making it the most extensive Carolina hemlock genetic diversity study performed thus far. Populations that were planted or that had a very small sample size were not included in the spatial analysis. The remaining data were stored in a shapefile containing 24 point-geometry features (Fig. 1). Each feature contains the genetic diversity attributes of a single sampling location.

Sixty eastern hemlock populations were sampled throughout the species' U.S. range from 2006 to 2009 as described by Potter et al. (2012). For this study, thirteen microsatellite loci were amplified across 1180 trees. The resulting data were stored in a shapefile containing 60 point-geometry features with genetic diversity attributes (Fig. 2). From the Campbell (2014) and Potter et al. (2012) studies, four genetic diversity metrics

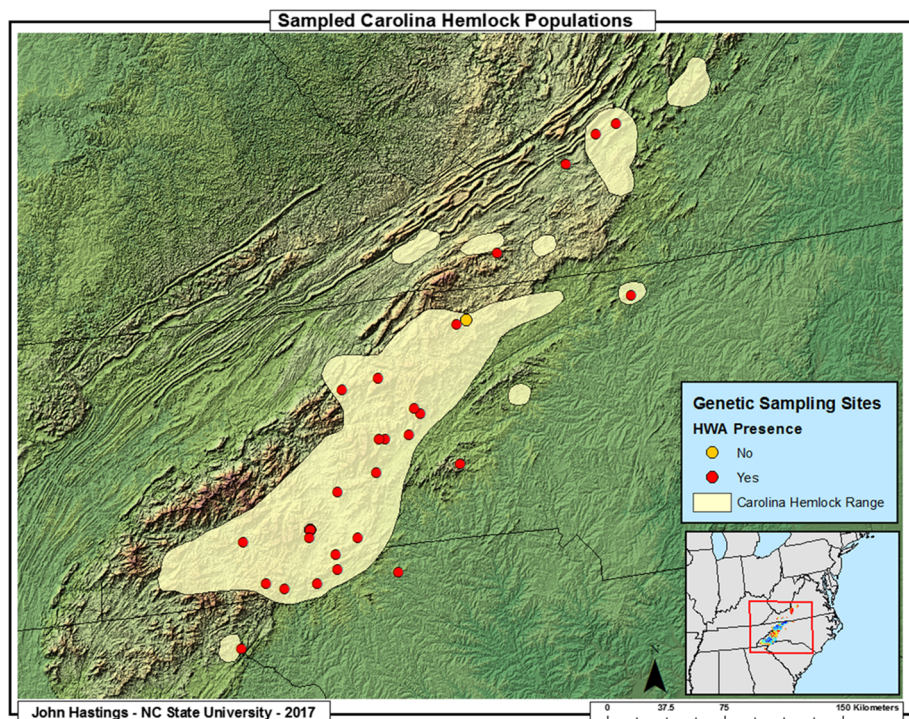


Fig. 1 Carolina hemlock seed collection locations as sampled by Campbell (2014). Aside from the modeled parameters, low sampling distribution in the southwestern sector should contribute to at least one sampling location in the area

were selected as significant to future sampling: allelic richness, percent polymorphism, observed heterozygosity, and inbreeding (F_{IS}).

To generate a spatially explicit prioritization index for hemlock throughout the eastern United States, the four genetic diversity parameters were combined with a minimum temperature difference from projected GCMs, a parameter accounting for geographic population disjunctiveness, existing hemlock seed collection density, and mean diameter of hemlock trees (a species-specific local dominance measure).

The genetic diversity metrics, as well as seed collection density and mean diameter, were interpolated using ordinary kriging with a stable semivariogram in ArcGIS 10.3 (ESRI 2014), producing six kriging results for each species (one for each parameter). Two genetic diversity sampling locations of eastern hemlock in Canada informed that species' interpolation, but the results are shown only for the U.S. range of the species. The purpose of this interpolation was to assign values to areas based on proximity to other points and the number of points within the neighborhood. A standard search neighborhood with a maximum of 6 and a minimum of 3 sampling points was used in conjunction with a 1-sector circle—meaning the points within the circle were not divided into different sectors. These neighborhood restrictions were implemented to account for low sampling density. Each of the kriging results was converted to raster format before being clipped to the U.S. range boundary of eastern or Carolina hemlock.

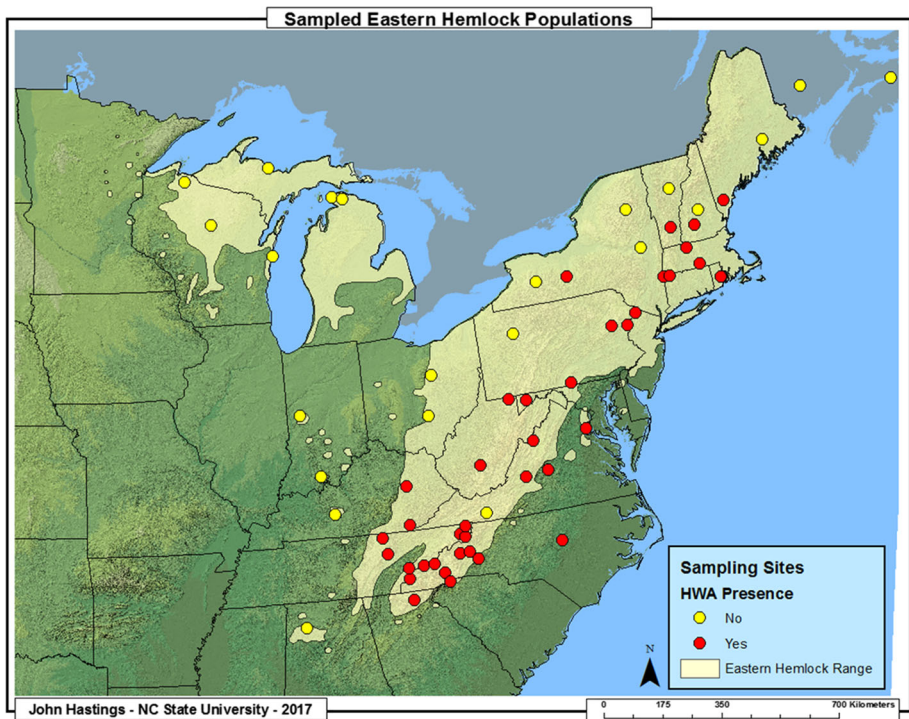


Fig. 2 Eastern hemlock seed collection locations as sampled by Potter et al. (2012). Low sampling distribution in the northern and western sectors contribute to high prioritization in those areas

Minimum temperature difference

A minimum temperature metric from the University of Idaho Gridded Surface Meteorological Data (UofI METDATA) was used to evaluate historical climate observations across the eastern and Carolina hemlock ranges. The UofI METDATA database combines PRISM climate datasets with 4 km gridded regional-scale reanalysis data based on LNDAS-2 daily-gauge readings from 1979—present. The data are then validated against a network of weather stations including RAWs, AgriMet, AgWeatherNet, and USHCN-2 (Abatzoglou 2013).

The average annual extreme minimum temperature metric was also adopted to project minimum temperature from 2020—2039 using the Multivariate Adaptive Constructed Analogs (MACA) statistical downscaling method of the University of Idaho. Under this method, 20 GCMs were downscaled from Coupled Model Inter-Comparison Project 5 (CMIP5) future Representative Concentration Pathways (RCPs): RCP 8.5 greenhouse gas trajectory (Abatzoglou 2013). The RCP 8.5 emissions scenario was used because it is more consistent with the current greenhouse gas emissions trajectory (Sanford et al. 2014). The historical dataset and the projected dataset for average annual extreme minimum temperature came in the form of NetCDF, a set of machine-independent data formats that support array-oriented scientific data (Rew et al. 1997).

The RCP 8.5 emissions trajectory was projected for the years 2020—2039 using the 20 GCM ensemble average. The ensemble approach was used because averaged results from

different members of an ensemble may provide more robust estimates of climate change than individually tested GCMs (IPCC-DDC 2011). A difference layer was then calculated by subtracting averaged historical minimum temperature observations from the averaged RCP 8.5 projection in degrees Celsius (°C). Under high emission scenarios such as RCP 8.5, more northerly latitude temperatures are projected to increase as much as 3 °C. The projected 5 °C temperature increments for RCP 8.5 were calculated from historical minimum temperature observations (Online Resources 1 and 2).

Disjunct populations

To account for the degree of isolation of peripheral disjunct populations of both species, we measured the distance of each pixel within these populations to the edge of the contiguous main-body range of the species, as defined by E.L. Little's distribution maps for the two species (United States Geological Survey 1999). The degree of disjunct isolation is an important conservation criterion because among-population genetic differentiation is expected to increase from the center of a species' geographic range to its periphery (Eckert et al. 2008), and the conservation of peripheral disjunct populations may afford the best opportunity for conserving rare alleles (Gapare et al. 2005). At the same time, small and isolated populations of trees often experience a loss of genetic diversity as a result of genetic drift and inbreeding (Jaramillo-Correa et al. 2009), risk factors that increase their risk of extirpation under changing environmental conditions (Willi et al. 2006). In eastern hemlock, isolated disjunct populations were found to be less genetically diverse than main range populations for several measures of genetic variation, but were more genetically distinct and contained more unique alleles on average, emphasizing the need to represent peripheral populations in gene conservation efforts (Potter et al. 2012).

Existing seed collections

A collaborative effort between the USDA Forest Service and Camcore, an international tree breeding and conservation program at North Carolina State University, has focused since 2003 on conserving the genetic resources of both Carolina hemlock and eastern hemlock (Jetton et al. 2013). This effort has resulted in the long-term storage of seeds from 750 eastern hemlock and 168 Carolina hemlock mother trees from 76 and 24 populations, respectively. An *ex situ* conservation prioritization assessment for these species should place a higher emphasis (without an exclusive focus) on areas in which previous seed collection efforts have been limited. We therefore used the georeferenced locations of existing mother trees from both tree species to create spatial surfaces of sampling intensity.

This was accomplished by first intersecting the tree point locations with a lattice of hexagons covering the eastern United States, each approximately 200 km² in area, and then summing the number of seed tree locations separately for eastern and Carolina hemlock within each hexagon. The centroids of these hexagons were then used in simple kriging, with 90 points used as neighbors for eastern hemlock (four layers of hexagons) and 42 points for Carolina hemlock (three layers of hexagons).

Hemlock abundance

Eastern hemlock and Carolina hemlock are not evenly distributed across their ranges, with higher densities in some locations and lower densities in others. Because tree species are

more difficult to locate in areas with low densities and because populations in these areas are likely to have lower genetic diversity (Hamrick et al. 1992; Myking 2002), this prioritization assessment emphasizes locations with higher densities of the hemlock species. We determined hemlock absolute abundance using a local species-specific measure of dominance, the average plot-level diameter of hemlock stems, determined using information from the national Forest Inventory and Analysis (FIA) program of the USDA Forest Service. The FIA program collects information from a nationally consistent sampling protocol of approximately 140,000 forested plots across the conterminous United States and coastal Alaska, with each plot representing 2428 ha of land (Woudenberg et al. 2010; Bechtold and Patterson 2005). Given the spatially balanced FIA sampling design, these data should provide unbiased measures of abundance.

Similar to seed collection density, we created a spatial surface of abundance for both eastern hemlock and Carolina hemlock using simple kriging of plot means within 200 km² hexagons. As before, we used 90 hexagon centroids as neighbors for eastern hemlock and 42 centroids for Carolina hemlock.

Multi-attribute frontier mapping

We implemented a method for combining multiple geospatial data sets into a single (i.e., one-dimensional) output via the multi-attribute frontier approach. Technical aspects of the approach are described fully in Yemshanov et al. (2013), and an executable utility is available by request from the authors. Briefly, under this approach, each observation (map location) is ranked in comparison to all other observations. Individual map locations (or cells in a raster map) are depicted as points in an n -dimensional cloud, where n is the number of mapped parameters, or criteria, being used to arrange the points. The coordinates of each point within this cloud correspond to its values for each of the n criteria. The range of possible values of each criterion must be ordered clearly, and typically from lowest to highest, so that the outermost boundary of the point cloud can be determined. This outermost boundary represents the multi-attribute efficient frontier of the point cloud; no point in the frontier could be assigned a higher value for one criterion without lowering its value with respect to one or more of the other criteria. In other words, for the set of N points comprising an n -dimensional cloud, the points in the multi-attribute efficient frontier represent a subset, N' , that is non-dominated by the remainder of the population, $N-N'$ (Koch et al. 2016).

To utilize this approach, all eight of our mapped parameters were snapped to the same spatial extent. Carolina hemlock was assessed at a resolution of 2-km because of its relatively small extent, while eastern hemlock was assessed at a resolution of 10-km because of its broad geographic distribution. Each location (i.e., raster map cell) is represented by a vector of its values for each parameter, and in turn, these values serve as the spatial coordinates for a corresponding point in the n -dimensional cloud. During the ranking process, the points (locations) on the outermost convex boundary of the cloud receive the highest value. Those points are then temporarily set aside from the cloud and another iteration is run to find the points on the outermost boundary of the remaining (and now smaller) cloud, and so on until all points are assigned a rank value. After all of the points are ranked in this manner, their assigned rank values are mapped back to the original geographic space, and the resulting aggregated map is rescaled between 0 (corresponding to the lowest rank values) and 1 (the highest rank values).

Parameter correlations

When using multi-criteria evaluation methods, parameter independence should be considered (Malczewski 2000). Doing so ensures proper weighting is assigned to each parameter. In this case, parameters are not presumed to be independent of one another but rather are limited in their codependence, i.e. they are preferentially dependent. To assess the relationship between all eight parameters, we ran a spatial correlation test using R statistical computing software. Upon evaluation of the correlation matrices (Table 1), we found insufficient evidence to remove any variables from the eastern hemlock assessment, as the correlations among parameters were generally low. For Carolina hemlock, we removed the genetic diversity variables of allelic richness and percent of polymorphic loci because they were reasonably highly correlated ($r > 0.8$) with each other and with other genetic diversity parameters.

Results

Eastern hemlock

The results of the prioritization analysis for eastern hemlock are shown in Fig. 3. Most notably, eastern hemlock priority is given to disjunct populations. Locations in the main-body part of the range are sporadically featured with areas of high priority. The western Great Lakes states showed comparatively limited priority, as did the eastern range edge through New Jersey, New York, Connecticut, and Rhode Island. The farthest northern and southern reaches of the main-body range including Maine and the Southern Appalachians demonstrated limited priority as well (Fig. 3).

In the case of eastern hemlock, the interpolation showed that inbreeding was high range-wide, consistent with a positive mean inbreeding coefficient value of 0.073 (Potter et al. 2012) (Online Resource 3). That study also found that nearly 100% of all amplified loci were polymorphic, and detected high allelic richness in the southern Appalachian Mountains as well as throughout New England (Online Resources 4 and 5). Moreover,

Table 1 Pairwise parameter correlations

	Dia	Seed	Dist	AR	F_{IS}	HO	PP	Temp
Dia	–	–0.066	–0.086	0.343	–0.201	0.290	0.289	0.212
Seed	–0.202	–	0.038	–0.163	–0.159	–0.059	–0.090	0.393
Dist	–0.365	0.417	–	–0.264	0.056	–0.254	–0.176	–0.109
AR	0.066	–0.832	–0.516	–	–0.063	0.363	0.486	–0.104
F_{IS}	0.585	–0.370	–0.865	0.452	–	–0.702	–0.121	–0.480
HO	–0.081	–0.680	–0.480	0.827	0.362	–	0.536	0.122
PP	0.362	–0.775	–0.741	0.815	0.774	0.732	–	–0.231
Temp	–0.399	0.276	0.869	–0.413	–0.833	–0.383	–0.604	–

Upper diagonal eastern hemlock. *Lower diagonal* Carolina hemlock

Dia mean tree diameter, *Seed* seed collection density, *Dist* degree of disjunct isolation, *AR* allelic richness, *F_{IS}* inbreeding, *HO* observed heterozygosity, *PP* percent polymorphism, *Temp* minimum temperature difference

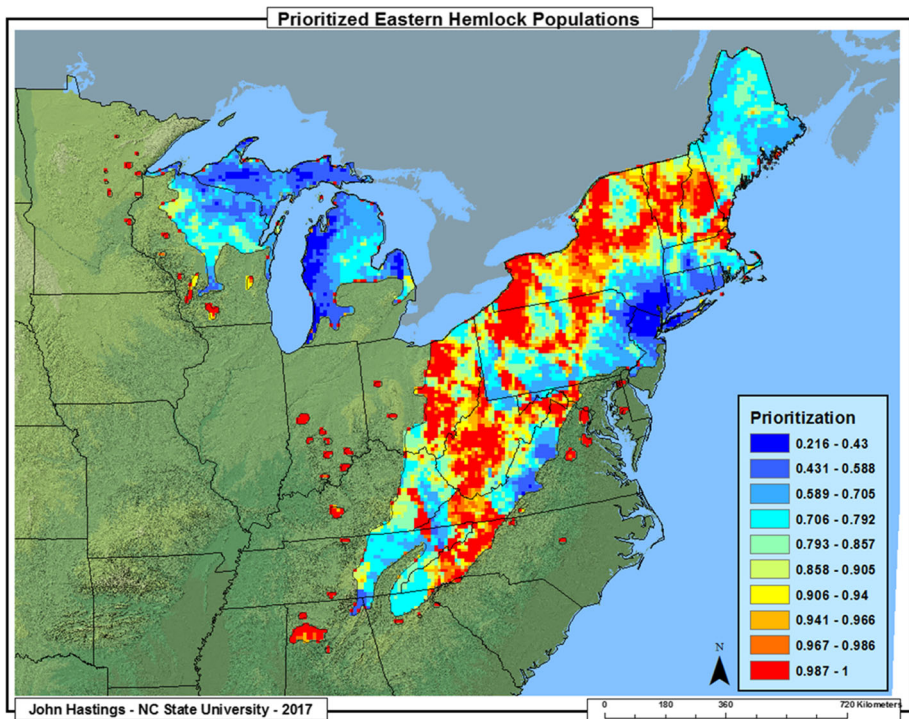


Fig. 3 Eastern hemlock populations prioritized based on the combination of weighted climatic and genetic parameters. Many populations of highest priority—those most significant for genetic diversity and seed collection efforts—are not yet infested by hemlock woolly adelgid. Highest priority areas also see relatively low sampling density

observed heterozygosity was high in pockets of the south and in northern parts of the range, while disjunct populations generally had low heterozygosity (Online Resource 6). Minimum winter temperature projections demonstrated a gradient of greater expected temperature change in the northern reaches and minimal temperature difference in the southern reaches (Online Resource 7). Disjunct populations to the west of the central main-body range were the farthest from the main-body range and received the highest value for degree of disjunctiveness (Online Resource 8). Seed collection density was highest in the southern Appalachians and northwest Pennsylvania (Online Resource 9). The mean diameter of eastern hemlock trees was highest in the northern region of the main-body range from New York to Maine (Online Resource 10).

Carolina hemlock

The results of the prioritization analysis for Carolina hemlock are shown in Fig. 4. A large area of high priority was located in the center of the main-body range for Carolina hemlock. While the largest disjunct population, in Virginia, has the highest concentration of high-priority cells, disjunct populations exhibited high overall priority. Locations in the northern and southern edges of the main-body range displayed the lowest priority (Fig. 4); however, disjunct populations in these areas were exempt from this low priority.

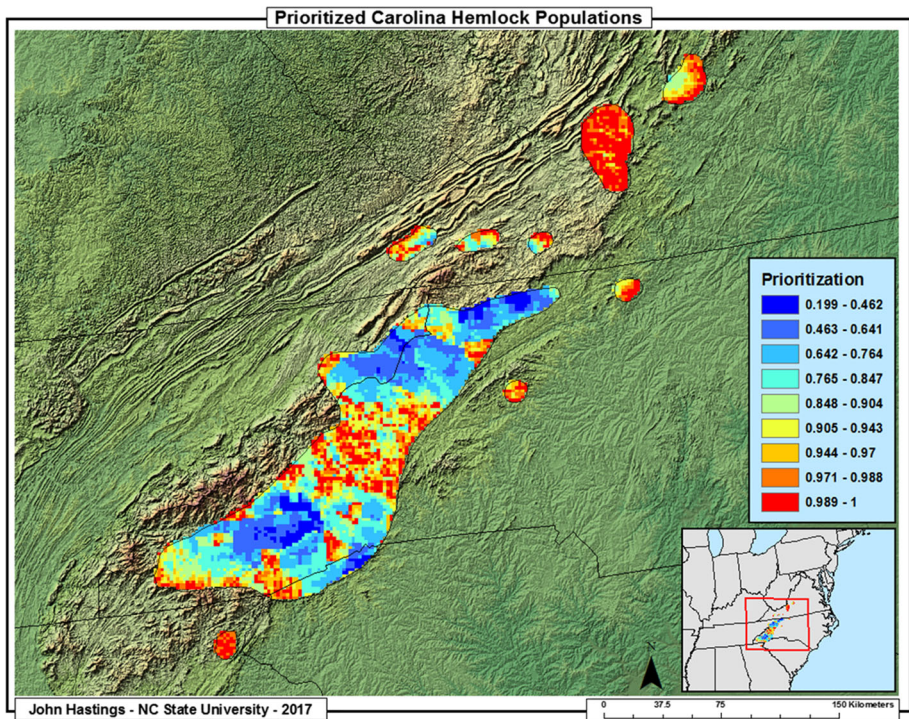


Fig. 4 Carolina hemlock populations prioritized based on the combination of weighted climatic and genetic parameters. Many populations of highest priority are currently infested by hemlock woolly adelgid. Highest priority areas have the highest sampling density and are centrally located in the main-body range

The interpolations showed that inbreeding was higher in the southern Carolina hemlock range and was lower in northern disjunct populations (Online Resource 11). Observed heterozygosity was also high in the central main-body range (Online Resource 12). As with eastern hemlock, minimum winter temperature demonstrated a gradient of greater change in the northern reaches and minimal difference in the southern reaches of the Carolina hemlock range (Online Resource 13). Disjunct populations to the north of the central main-body range were the farthest from the main-body range and received the highest value for degree of disjunctiveness (Online Resource 14). Seed collection density was highest in the northern and southern reaches of the main-body range and disjunct populations (Online Resource 15). Mean diameter of Carolina hemlock trees was highest in the southernmost region of the main-body range in Georgia and North Carolina (Online Resource 16).

Discussion

The kind of genetic conservation prioritization analysis we present here is a critically important response to destructive pests like HWA and a potential solution for the efficient and effective application of limited conservation resources in the face of an uncertain future. Regardless of species imperilment, genetic diversity studies can help inform conservation strategies so that genetic diversity is maintained in the presence of a pest attack

and other threats. Additionally, genetic diversity provides a basis for adaptation and resilience to the growing number, variety, and frequency of environmental stresses to which tree species are exposed (Schaberg et al. 2008), including pest and pathogen infestation. The results from this study will help locate genetically and climatically significant portions of the eastern and Carolina hemlock ranges, especially in the northern part of the eastern hemlock range where climate change is the greatest threat and where hemlocks are currently uninfested by the adelgid.

Eastern hemlock

The results of our analysis indicate that disjunct eastern hemlock populations are prime targets for further genetic diversity studies and gene conservation (Fig. 3). Likewise, high priority areas within the main-body range are also strong candidates for seed banking and conservation efforts.

All disjunct populations should receive conservation consideration, as disjunct populations sampled by Potter et al. (2012) were found to be genetically distinct from main-range populations. In general, such geographic outliers should be candidates for conservation activities (Yanchuk and Lester 1996) because individuals in small populations may have lower fitness as a result of environmental stress and inbreeding, which can substantially increase the probability of population extinction, especially as environmental conditions change, because of their relative isolation (Willi et al. 2006). Minimum temperature difference also has a strong influence on the overall prioritization results and clearly shows that northern locations are more at risk from rising temperatures. It does not imply southern hemlock locations are not at great risk of climate change, but rather indicates the necessity of gene conservation efforts in the north where HWA is currently limited by cold winter temperatures but may not be in the future. To date, a majority of seed collections have occurred in the south, thus offering an additional reason that northern locations should be prioritized for additional sampling (Jetton et al. 2013).

Finally, it is important to note that variability in genetic diversity sampling density for eastern hemlock populations represents a important limitation for this analysis. Differences in sampling density in the range-wide genetic diversity analysis of the species (Potter et al. 2012) have a profound effect on the ability to interpolate regions similarly. In the northeastern region where the genetic diversity sampling density is lower, the interpolation had a tendency to draw from sampling locations that are further away, stretching the results across a larger area. In the south where the range narrows, the sampling locations are much closer together. This changes the pattern of the prediction effort, which tends to be high in areas with limited sampling locations and low in areas where sampling is dense. These sampling issues are accounted for in the methodology, in which a neighborhood restriction limits the number of points included in the interpolation to a minimum of three and a maximum of six. Northeastern main-body locations should receive emphasis for further gene conservation and seed collection efforts, based on comparatively low genetic diversity sampling density and relatively high values for all four of the genetic diversity variables. Many of the high priority locations are in areas that are not currently infested by HWA; however, they should receive priority given the risks of climate change, cold adaptation in HWA, and the pest's likely expansion into these areas in the future.

Carolina hemlock

It was previously hypothesized that the southern part of the Carolina hemlock range would be more genetically diverse than the northern range due in part to the inferred existence of a Pleistocene refuge south of the Appalachian Mountains (Potter et al. 2010). Campbell (2014), however, found that there was no spatial correlation between latitude and genetic diversity in Carolina hemlock. Our results show clustering of medium-high genetic diversity in the central main-body range—including high observed heterozygosity—potentially suggesting the existence of a Pleistocene refuge nearer to this part of the range. The results suggest that much of the central main-range body should receive emphasis for conservation efforts.

The northern disjuncts show significantly lower inbreeding values than southern populations, which is contrary to the “southern richness and northern purity” hypothesis. This hypothesis expects that recolonizing populations descended from subsets of the genotypes present in the refugial population often undergo founder effects and genetic bottlenecks (Hewitt 1999), and thus would have the lowest genetic diversity and the highest levels of inbreeding. Neither is spatially apparent in our study of Carolina hemlock. Because nearly all populations of Carolina hemlock are isolated, the entirety of the range is at risk in a changing climate because they are generally less fit as a result of inbreeding (Willi et al. 2006).

Though more comprehensive and with a higher sampling density per unit of range area than eastern hemlock, further sampling of Carolina hemlock remains vital. Four populations sampled by Campbell (2014) were omitted from this analysis because they lacked sufficient sample size, all having four or fewer samples. This highlights the need to further sample populations that encompass sufficient numbers of trees for statistical analyses, including in areas that are disjunct from the main range of the species.

Genetic diversity implications and future efforts

The overall spatial trends revealed by these analyses align with the results of previous genetic diversity studies. Low genetic diversity in eastern hemlock (Potter et al. 2008, 2012) and Carolina hemlock (Campbell 2014), coupled with generally high levels of inbreeding across both species, highlight the need for quick and decisive action to assess the genetic diversity of previously undersampled areas and to fully protect the genetic composition of each species.

Because of the profound effect cold temperature has on HWA mortality (Hansen 1991; Skinner et al. 2003; Costa et al. 2004; Dukes et al. 2009; Preisser et al. 2014), modeling climate effects across the entire hemlock range can identify areas most at risk of future HWA infestation and can therefore help inform decisions about where to focus conservation efforts. Butin et al. (2005) explained that there are many incentives to defining the range limits of HWA, including better allocation of resources for pest management. Using minimum temperature model results to provide insight into how future temperature will differ may additionally help promote genetic diversity efforts in species that may be affected by climate warming.

Notably, our study is a novel demonstration of the utility of the multi-attribute frontier approach—which was developed originally for use in pest risk analysis and mapping—for the alternate purpose of prioritizing areas based on multiple criteria, including key indicators of genetic diversity and anticipated effects of climate change. This framework could

be applied to other at risk tree species as well, including those identified in species-level conservation assessments that categorize and prioritize species based on threat vulnerabilities (e.g., Potter et al. 2017, this issue). Two species threatened by insects and their respective pathogens that seem suitable for such an analysis are butternut (*Juglans cinerea* L.), which is being killed by butternut canker (*Sirococcus clavigignenti-juglandacearum*) (Rink 1990), and green ash (*Fraxinus pennsylvanica* Marshall), which is being decimated by emerald ash borer (*Agrilus planipennis* Fairmaire) (Tanis and McCullough 2015).

Genetic diversity studies such as this are an integral step in collecting and preserving as much genetic diversity as possible for these two imperiled hemlock species. As outlined by Jetton et al. (2013), the main objectives of ongoing genetic resource conservation for eastern and Carolina hemlock are to establish additional seed orchards within and outside of the United States and to complete additional seed collections within areas that have high genetic diversity but that have not yet been adequately sampled.

The results of this spatial prioritization will guide future genetic diversity and seed collection endeavors by Camcore and the USDA Forest Service by establishing exactly which areas should be emphasized in seed collection, banking, and preservation. Additionally, these results expand population prioritization beyond genetic diversity to include the potential effects of climate change. The implications of further sampling will expand our understanding of the genetic diversity of the species and therefore help achieve our overall goals for their conservation.

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