*Invasive Plant Science and Management*

**www.cambridge.org/inp**

# Temporal changes in genetic diversity reveal small-scale invasion dynamics of the eastern redcedar (*Juniperus virginiana* var. *virginiana*) in the Lakeside Daisy State Nature Preserve in Ohio

Hannah M. Hartman[1] and Oscar J. Rocha[2]

[1]Graduate Student, Department of Biological Sciences, Kent State University, Kent, OH, USA and [2]Associate Professor, Department of Biological Sciences, Kent State University, Kent, OH, USA

## Research Article

**Cite this article:** Hartman HM and Rocha OJ (2023) Temporal changes in genetic diversity reveal small-scale invasion dynamics of the eastern redcedar (*Juniperus virginiana* var. *virginiana*) in the Lakeside Daisy State Nature Preserve in Ohio. Invasive Plant Sci. Manag **16**: 160–169. doi: 10.1017/inp.2023.23

Received: 8 February 2023
Revised: 30 July 2023
Accepted: 19 August 2023
First published online: 29 August 2023

**Associate Editor:**
Marie Jasieniuk, University of California, Davis

**Keywords:**
Diffusion; encroachment; gene flow; genetic structure; long-distance dispersal; microsatellite markers; range expansion

**Corresponding author:**
Hannah M. Hartman;
Email: hhartma8@kent.edu

## Abstract

Eastern redcedar (*Juniperus virginiana* L. var. *virginiana*; hereafter ERC) is a native species currently invading open areas and grasslands outside of its original range in the United States. We studied ERC's invasion patterns in the Lakeside Daisy State Nature Preserve (LDSNP), a short grass prairie located on the Marblehead Peninsula in Ohio, examining the changes in the genetic diversity and structure of the encroaching population. We investigated the relative importance of long-distance dispersal versus diffusion in the invasion of this short grass prairie by ERC. We use eight microsatellite marker loci to infer gene flow from external sources versus within-population recruitment. We found that the older trees in this preserve were less than 50 yr old, indicating that the population was established between 1970 and 1980. When we grouped trees into five age categories of 10-yr increments, we found that the allelic diversity, as indicated by the average number of alleles per locus, increased as the age of the trees decreased. We also found that not all loci were in Hardy-Weinberg equilibrium, probably due to the arrival of new variants in the preserve. Moreover, heterozygosity remained high, with an excess of heterozygotes in all age groups ($F = -0.163 \pm 0.046$). Principal coordinate analysis showed two distinct groups of trees in the LDSNP. Analysis of the cryptic population structure of the ERC trees using STRUCTURE revealed four ancestral clusters in the ERC population. All ancestral clusters are present in all age groups, suggesting that all trees sampled are derived from an admixed population. Furthermore, the high observed heterozygosity and lack of inbreeding in this dioecious species maintained all ancestral clusters over time. Overall, our findings indicate that ERC encroachment of the LDSNP results from multiple and reiterated gene flow events from the edge of the range through animal-mediated seed dispersal.

## Introduction

There is ample evidence that native and nonnative species invasions have similar impacts on biodiversity (Sagoff 1999; Schnelle 2019; Simberloff et al. 2012; Yazlik and Ambarli 2022). While most invasive species are not native, there are native species that have become too abundant and have been implicated as drivers of recent extinctions (Blackburn et al. 2019). Some native invaders became problematic after intracontinental movements outside their native ranges into new suitable habitats. However, other species expand their ranges from the edges and become increasingly dominant because of their prolific fruit production, seed dispersal by birds and mammals, anthropogenic disturbances, overgrazing of pastures, and tolerance of environmental extremes (Aththanayaka et al. 2023; Schnelle 2019). Range expansion by woody native plants has been documented globally (Gettys and Schnelle 2018; Negi et al. 2021; Schnelle 2019; Ward et al. 2022).

Woody encroachment by native species is an important issue in grasslands globally resulting from overgrazing by cattle, anthropogenic interventions, and reduced fire frequency (Briggs et al. 2002; Eldridge et al. 2011; Ratajczak et al. 2012; Ward 2020). Encroachment of woody plants into grasslands reduces the species diversity and changes the quality and quantity of light reaching the understory (Ratajczak et al. 2012). In addition, woody encroachment modifies nutrient cycling, ecosystem productivity, soil chemistry, natural disturbance regimes, and local hydrology (Donovan et al. 2018; Eldridge et al. 2011; Ratajczak et al. 2012; Ward 2020). Climate change may also exacerbate the problem, as the increase in global $CO_2$ levels may benefit $C_3$ woody species more than $C_4$ grasses, allowing for further grassland transition to shrubland (Eldridge et al. 2011; Schnelle 2019; Tunnell et al. 2004).

Eastern redcedar (*Juniperus virginiana* L. var. *virginiana*; hereafter ERC) is a widespread native tree in North America (Ward 2021). ERC is currently expanding its range out of its

© The Author(s), 2023. Published by Cambridge University Press on behalf of the Weed Science Society of America. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2023.23

### Management Implications

Woody encroachment is an important issue in grasslands globally. Although ecologists are usually more concerned with invasions by alien species into a foreign habitat, native woody species can aggressively invade grasslands. The encroaching of native woody species, like *Juniperus virginiana* var. *virginiana* (eastern redcedar; hereafter ERC), threatens native flora, reduces species diversity, changes the quality and quantity of light reaching the understory, and affects ecosystem functions. To understand how native species expand their ranges, managers should know whether population growth is driven by the reproductive activity of previously established trees or by the continual arrival of propagules from distant source populations. If seeds are transported successfully over long distances, rapid dispersal of invasions from small satellite populations can occur and increase the rate of range expansion for the species. On the other hand, if seeds travel only short distances, managers can focus control efforts on the edge of the range to prevent the establishment of woody encroachers in intact grassland ecosystems. As clearing satellite populations before individuals become sexually mature is the most cost-effective form of management, understanding range expansion patterns provides a framework for better prioritization of management efforts.

In the case of ERC, our data indicate that encroachment results primarily from local seed production and intermediate-distance seed dispersal by animals, with occasional arrival of propagules via long-distance dispersal facilitated by humans. Therefore, adequate management requires consistent monitoring of grasslands to detect and eradicate newly established foci before initiating seed production with a particular focus on grasslands near other populations of ERC. Because the youngest recorded cone-producing female tree in our study was 6 yr old, managers could survey grasslands and eradicate ERC every 5 yr to most efficiently prevent the establishment of new ERC stands. Furthermore, managers should restrict the use of known invasive woody plants like ERC for windbreaks and landscaping. In addition, we recommend that managers limit seed source usage in nurseries, avoiding using seeds of distant origin. Limiting seed sources minimizes the risk of intraspecific hybridization among different ecotypes, which may broaden the range of environmental conditions suitable for the species and thus promote the local expansion of their ranges.

traditional habitat and into open areas like grasslands (Briggs et al. 2002; Vasiliauskas and Aarssen 1992; Ward 2021). It is commonly used as a windbreak surrounding agricultural fields and can aggressively colonize neighboring grasslands, rapidly transforming them into closed-canopy ERC forests (Briggs et al. 2002; Donovan et al. 2018). Although ERC's historical niche is limestone soils and cliffsides, it can survive in many environments and thrives in xeric environments where competition with other plants is reduced (Lawton and Cothran 2000; Sangüesa-Barreda et al. 2021; Ward 2020). In addition, ERC is an animal-dispersed species, and seeds can be dispersed away from the mother tree, allowing further movement into new areas (Holthuijzen and Sharik 1985; Horncastle et al. 2004). Like other successful invaders, ERC trees become sexually mature early in their lives; female ERC trees produce many seed-containing fleshy cones when they are 10 yr old, and males develop pollen-producing cones when they are 6 yr old, leading to rapid reproduction and dispersal (Aronson et al. 2007; Van Haverbeke and Read 1976; Wickert et al. 2017; K Shvach, personal communication). Moreover, increasing severe droughts brought on by climate change could give ERC an even more decisive competitive advantage over native grassland plant species (Kaskie et al. 2019).

One area affected by ERC invasion is Lakeside Daisy State Nature Preserve (LDSNP). Located in Ohio on the Marblehead Peninsula on the shore of Lake Erie, LDSNP is a unique preserve, including extensive wetlands and a small prairie traditionally dominated by short grasses and wildflowers (Figure 1). This preserve was likely historically maintained by its xeric environment and shallow soil, limiting competition with the native flora by other plant species. Observation of historical satellite imagery reveals clumps of established trees in the 1990s, just shortly after the preserve's founding in 1988 (Ohio Department of Natural Resources n.d.). Possible reasons for the establishment of ERC trees at LDSNP could include urbanization of the peninsula and subsequent landscaping of privately owned land using ERC or anthropogenic disturbance caused by limestone quarrying at the site. Additionally, ERC trees populate the sides of highways and hills surrounding LDSNP and could be dispersing into the preserve from these areas. ERC's affinity for xeric environments where competition with other plants is low and shade is minimal has allowed this population to rapidly colonize the prairie at LDSNP following its introduction (Lawton and Cothran 2000; Sangüesa-Barreda et al. 2021; Ward 2020).

Grasslands like LDSNP are considered among the most endangered ecosystems globally (Kaskie et al. 2019; Leis et al. 2017). Changes in plant community composition linked to woody encroachment can modify the belowground biomass of these ecosystems, reducing native grasses' soil-stabilizing effects (Watson et al. 2019). In semiarid environments like grasslands, soil stabilization is critical to prevent erosion and retain the little available water (Eldridge et al. 2011; Kaur et al. 2020; Knapp et al. 2020). Additionally, the fragmentation of grasslands resulting from anthropogenic influence and woody species encroachment can result in biodiversity losses (Leis 2017). Up to 330 million ha of grasslands were experiencing shrub encroachment in 2011, negatively affecting many of the economic and ecological resources that grasslands possess (Eldridge et al. 2011).

Two theoretical models describe how invasions typically occur: outward diffusion from the edge of the range and long-distance dispersal followed by local expansion (Auld and Coote 1980; Campbell and Dooley 1992; Gorchov et al. 2014). Diffusion of plant species is characterized by seed and pollen traveling short distances from the edge of the range and may result in local adaptation due to isolated reproduction in different areas (Auld and Coote 1980; Campbell and Dooley 1992; Keller et al. 2017). On the other hand, long-distance dispersal may have a homogenizing effect on genetic diversity, because it allows for sharing of genetic material through pollen and seeds between geographically separate areas of the range (Barriball et al. 2015; Campbell and Dooley 1992). Additionally, long-distance dispersal may lead to an increased rate of spread, as new adult trees in an area can facilitate the deposition of more seeds by providing perches for birds, the main dispersers of some woody encroachers (Higgins et al. 2000; Holthuijzen and Sharik 1985; Horncastle et al. 2004). If seeds are transported successfully over long distances, range expansion can occur even more rapidly for the species (Moody and Mack 1988). It is therefore essential to identify patterns of a range expansion in order to model and predict them more accurately. Better prediction of spread rate and pattern can give managers the

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

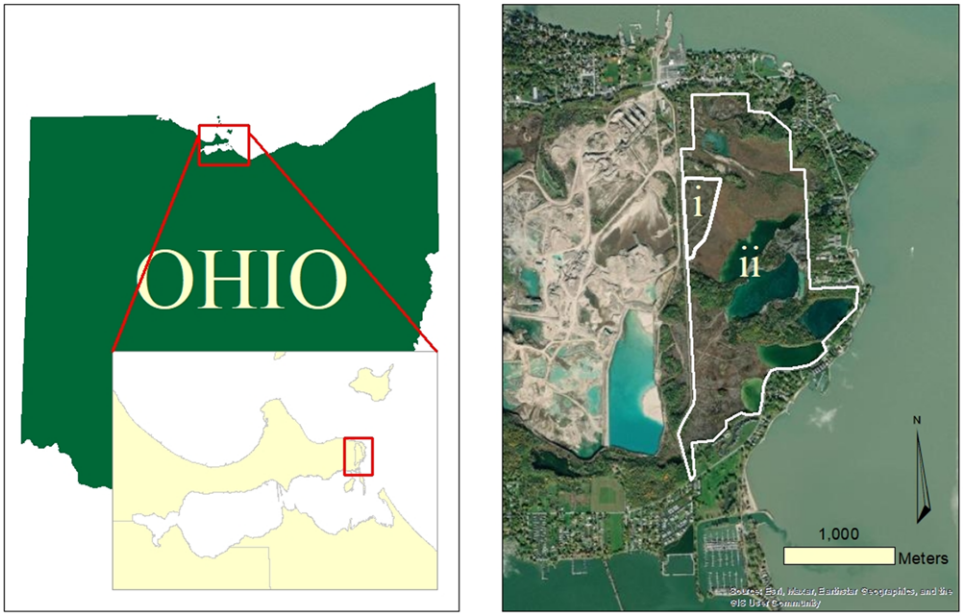



**Figure 1.** Lakeside Daisy State Nature Preserve is located on the eastern end of Marblehead Peninsula in northern Ohio. The sampling area (i), where the population of encroaching *Juniperus virginiana* var. *virginiana* is located, is approximately 22 acres out of the entire (ii) 136-acre preserve. Created using ArcGIS (ESRI 2011).

ability to target management efforts on new colonies before they expand further (Gorchov et al. 2014; Higgins et al. 2000; Moody and Mack 1988).

Using neutral genetic markers provides one way to infer the historical seed dispersal rates and patterns (Hamrick and Trapnell 2011). Dispersal patterns of seeds and pollen affect the genetic diversity and structure of plant populations, which is a driver of plant evolution and determines the population's response to selection pressures (Chybicki and Oleksa 2018; Sork and Smouse 2006). Therefore, studying the changes in the genetic structure over time can provide a more detailed understanding of how a newly established population of ERC persists over time (Céspedes et al. 2003; Roser et al. 2017). We suggest that higher genetic diversity allows the population to respond to environmental variation and may affect the success of ERC trees in different environmental conditions (Fuchs et al. 2013; Gonzales et al. 2009). We used genetic similarity among ERC trees over a temporal scale in the LDSNP to address the question: Is ERC's pattern of range expansion driven by diffusion from the edge of the range or by long-distance dispersal events? Answering this question will help us estimate patterns of dispersal and colonization of ERC and inform a model of ERC's range expansion in grasslands and other open areas. Such models can help determine where to prioritize control measures and proactively manage expanding populations (Donovan et al. 2018; Kaskie et al. 2019).

## Materials and Methods

### Study Site

The LDSNP is at the eastern end of the Marblehead Peninsula on Lake Erie in Ohio, USA (41.53°N, 82.73°W) (Figure 1). The 55-hectare preserve was established to protect the only naturally occurring population of the federally threatened lakeside daisy (*Tetraneuris herbacea* Greene) in the United States and includes an old limestone quarry of the Marblehead geological series (Ohio Department of Natural Resources n.d.). Our study focuses on the 9-ha parcel of prairie habitat with a high presence of ERC (Figure 1, i), because much of the rest of the 155-ha preserve (Figure 1, ii) is made up of wetlands where ERC does not have a dominant presence. ERC presence in this parcel of prairie habitat threatens the native flora of LDSNP, including *T. herbacea*. Mean annual precipitation ranges from 686 to 914 mm, and mean annual air temperature ranges from 7 to 11 C.

### Sampling Protocol

Leaf samples from 189 ERC trees from LDSNP were collected and stored in resealable plastic bags in a −20 C freezer until DNA extraction. We sampled 170 ERC trees using a grid-like pattern from the north to the south end of the 9-ha sampling area. Eleven sampling transects run from east to west every 50 m, and we collected leaf tissue from the closest tree every 10 m along each transect. The transect length varied, as we stopped the transect when we reached the wetland where ERC trees were absent. Overall, the sampled area had a perimeter of 1,389 m, with transect two being the longest (24 trees sampled). Tree height, coordinates, presence or absence of female cones, and diameter at breast height (DBH) for tall trees or base diameter for short trees were noted. The youngest tree found bearing female cones, indicating sexual maturity, was determined to be 6 yr old.

Additionally, 9 large trees found in a clump in one portion of the 9-ha sampled area and 10 large trees along the roadside on the edge of the sampling area were targeted as candidates for founding members of the population at LDSNP. These trees were sampled outside the grid sampling scheme, but they were sampled in the same way and included in our data analysis together with the rest of the trees sampled. LDSNP is located in the less-developed eastern end of the Marblehead Peninsula and is surrounded by a town with private residences (Figure 1). Large trees can be seen in the town of Marblehead, on other islands close to LDSNP, and along the highways in northeast Ohio, but these were located on privately owned property and therefore could not be sampled.

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

**Table 1.** Locus name, oligonucleotide primer sequences, repeat motifs, and PCR product size range for each of the seven microsatellite loci for genetic analysis of *Juniperus virginiana* var. *virginiana* and one microsatellite locus developed for *Juniperus communis*.

| Locus ID | Forward primer (5′-3′) | Reverse primer (5′-3′) | Repeated motif | Product size range |
|---|---|---|---|---|
| | | | | —bp— |
| JV1 | AATGTTCGATCCATTAAAGAGG | TTATAGCATTGGCTGCATTTAG | (ATA)5 | 380–430 |
| JV2 | AGTCTAATTTTGGGCATGATAG | GTTGGCTAAATCTTCCCTGTT | (TAT)5 | 270–310 |
| JV3 | CACTCAACCTAGTCAAGATCCA | TCAATTTGAAGAAACATGACTG | (TTA)5 | 420–470 |
| JV4 | TCACCATTTCTCGAGGTATTAG | ACTCCACCAATAGACTAGGAGC | (TAT)6 | 380–430 |
| JV5 | TCTTGTGCAACATACTTCCTTC | AAGAAGTTGAAAGCTAAGTGGG | (CTT)5 | 380–430 |
| JV6 | TTTCCAGGACTTGTTGTCATAG | CATGTTACACCTACCATTCCAC | (CTT)7 | 150–210 |
| JV7 | ACACCTCAGAAAATGGAATGAC | TTAGCCACTAGTTCCAATGATG | (ATT)7 | 120–170 |
| JC1 | TGTGTTTATTCTCCCCATCT | CCCCCAGTTATTCTAAACATT | (CA)20 | 121–147 |

Wood core samples were collected using an increment borer from 45 trees for which a DBH measurement was taken; meanwhile, stem cross sections were collected from 50 trees that had a base diameter taken. Cores and cross sections were frozen in a −20 C freezer until processed. The annual rings in each tree core or stem cross section were counted under a dissecting microscope to determine the age of each tree. A core or cross section could not be obtained for all trees, as some trees were missing the tags that were placed during the initial sampling and could not be reliably located using GPS coordinates. Linear relationships between stem diameter and age of the tree, based on growth ring count, were used to estimate the age of those trees for which we did not have a core (41 tall trees) or stem cross section (53 small trees). ERC growth rates are highly variable across sexes and microsite conditions, especially once trees are sexually mature (Quinn and Meiners 2004), so this estimation may have resulted in miscalculation of the age of trees and may have contributed to the disproportionate sample sizes of each age group. The trees were grouped into five age groups of 10-yr increments rather than on a continuous age scale to help account for any error resulting from age estimation.

### DNA Extraction and Microsatellite Analysis

Total genomic DNA was extracted from the leaf tissue of each ERC tree using a modification of the CTAB protocol described by Doyle and Doyle (1987). Subsequently, all DNA samples were diluted to approximately 10 ng $\mu l^{-1}$ for polymerase chain reaction (PCR) amplification. PCR was performed using eight microsatellite markers, seven of these were developed in our lab for genetic analysis of ERC and one was developed for genetic analysis of common juniper (*Juniperus communis* L.) (Michalczyk et al. 2006) (Table 1). PCR was performed in a final volume of 10 µl, containing approximately 15 ng of genomic DNA, 10 mM Tris buffer with KCl, pH 8.8, 1.88 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, and 1 unit of Taq polymerase using a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA). The PCR was conducted using a touchdown annealing approach to improve the specificity of primer binding to the target DNA. Annealing temperature decreased by 1 C every three cycles from 60 to 56 C during the first 15 cycles, then annealing temperature remained at 55 C for the remaining 30 cycles. The thermocycling profile consisted of initial denaturation at 94 C for 2 min, followed by 15 cycles of 94 C for 30 s, 60 to 56 C for 30 s, 72 C for 30 s, followed by 30 cycles of 94 C for 30 s, 55 C for 30 s, 72 C for 30 s, a final extension of 72 C for 3 min, and a holding temperature of 5 C. All forward primers were labeled using the fluorescent dyes FAM or TET. The PCR product size was determined using an ABI 3730 DNA sequencer (Applied Biosystems, Waltham, MA, USA) at MC Lab (San Francisco, CA, USA). The program Peak Scanner was used to determine the multilocus genotype of all trees.

### Temporal Variation in Genetic Diversity

The program GenAlEx (v. 6.4; Peakall and Smouse 2006) was used to determine the levels of genetic variation found within and among ERC trees from different age categories. This program was used to calculate common indicators of genetic diversity such as the number of alleles per locus ($N_a$), effective number of alleles ($N_e$), number of unique alleles in each age group, and observed and unbiased expected heterozygosity ($H_o$ and $H_e$, respectively). $N_e$ indicates the number of equally frequent alleles that it would take to achieve the same $H_e$ in each age group, and alleles unique to an age group are present only in that age group. Deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were determined using Genepop (v. 4.7; Raymond and Rousset 1995; Rousset 2008).

We also used GenAlEx to estimate Wright's $F$ coefficients and to conduct an analysis of molecular variance (AMOVA) to assess the distribution of genetic diversity among ERC established each decade to determine levels of differentiation between age groups (Peakall and Smouse 2006). Because genetic diversity estimates are positively correlated with sample size, we obtained 30 random subsamples of 10 individuals for age groups 10 to 19, 20 to 29, and 30 to 39 to compare to the 0 to 9 and 40 and greater (40+) age groups in order to account for the disparity in sample size among age groups in our study (Ward and Jasieniuk 2009). After random subsampling, we calculated $N_a$, $N_e$, the number of unique alleles, $H_o$, $H_e$, and pairwise genetic differentiation index ($F_{st}$) among all age groups. In addition, we conducted one-way ANOVAs using the 30 random samples taken from age groups 10 to 19, 20 to 29, and 30 to 39 to determine whether there were significant differences in the means of $N_a$, $N_e$, and $H_o$ among groups. We use pairwise post hoc Tukey tests among age groups for these three diversity indicators. We also used GenAlEx to conduct principal coordinate analysis (PCoA) to visualize the genetic relationships among trees of different age groups.

### Genetic Structure

The genetic structure of ERC was analyzed with the use of Bayesian model–based clustering with the program STRUCTURE (v. 2.3.4; Hubisz et al. 2009; Pritchard et al. 2000). This analysis was conducted to determine whether there are changes in the genetic structure among age groups. This program predicts the most likely number of subpopulation clusters for the populations sampled and recalculates $F$-statistics. All STRUCTURE runs used a burn-in length of 100,000 followed by 1,000,000 Markov chain Monte

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

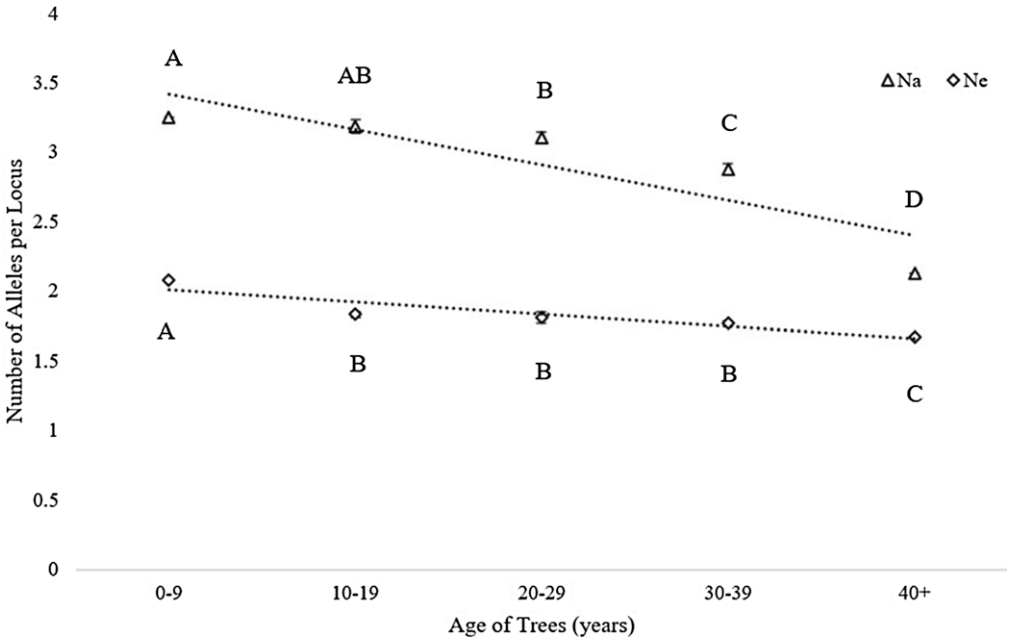



**Figure 2.** Average number of alleles ($N_a$) and average number of effective alleles ($N_e$) per locus for all age groups of *Juniperus virginiana* var. *virginiana* estimated from 30 random subsamples of age groups 10–19, 20–29, and 30–39. Values for $N_a$ and $N_e$ for age groups 0–9 and 40+ were calculated using all individuals ($N = 9$ and $N = 5$, respectively). Sample size disparity among age groups may impact $N_a$ and $N_e$ values. Error bars correspond to standard errors resulting from ANOVA. Letters indicate significantly different means determined using Tukey's honest significant difference (HSD) pairwise comparisons.

Carlo repetitions. A graphic representation of the results generated by STRUCTURE was presented using the program DISTRUCT (v. 1.1; Rosenberg 2003). Options were selected to allow admixture, assume independence among loci, and ignore population affiliations when defining clusters. To determine the likeliest number of subpopulation clusters ($K$), we followed the methodology proposed by Evanno et al. (2005). All probable $K$ values were run 20 times to obtain $\Delta K$, which is an ad hoc measure based upon the second-order rate of change of the likelihood function with respect to each $K$ value (Evanno et al. 2005). According to this procedure, the modal value of the $\Delta K$ can be used as an indicator of the number of ancestral population clusters in the area. The program STRUCTURE HARVESTER (v. 6.0; Earl and von Holdt 2012) was used for calculating parameters of Evanno et al. (2005).

## Results and Discussion

### *Levels of Genetic Diversity*

The results of this study showed that the genetic diversity of a population of ERC in LDSNP is increasing over time. We found a total of 63 alleles among the 189 ERC trees examined using eight microsatellite loci. After random subsampling, average $N_a$ values ranged from 2.125 ± 0.227 in the 40+-yr-old group to 3.250 ± 0.453 in the 0- to 9-yr-old group (Figure 2; Table 2). $N_e$ values showed a similar trend, with averages ranging from 1.671 ± 0.153 in the 40+-yr-old group to 2.079 ± 0.221 in the 0- to 9-yr-old group (Figure 2; Table 2). There is a significant overall effect of age group on $N_a$, $N_e$, and $H_o$ (MANOVA, Wilks's lambda = 0.1188, $F(8, 288) = 68.46$, $P < 0.0001$). ANOVA and Tukey's honest significant difference (HSD) tests revealed significant differences in mean values of $N_a$ and $N_e$ between different age groups of ERC ($N_a$: $F(4, 145) = 197.515$; $P < 0.0001$) ($N_e$: $F(4, 145) = 46.538$; $P < 0.0001$) (Figure 2). Our findings indicate that, considering all trees with no subsampling, the average number of unique alleles per tree increased from 0 in the oldest group to 0.44 in the youngest group (Table 3). When random subsampling was conducted in age groups with more than 10 individuals, we found that the number of unique alleles in each age group increased from 0.033 unique alleles in the 40+-yr-old group to 3.667 unique alleles in the 10- to 19-yr old group (Table 2). Although there were no unique alleles in the 40+-yr-old age group when considering all trees in the data set, our random subsampling of the data set revealed that there is there a low likelihood of finding a unique allele among the oldest group of trees (unique allele = 0.033 ± 0.033) (Tables 2 and 3). $N_e$ values increased only slightly among all ages, but the difference between the mean $N_e$ values of the youngest group and the older groups was significant (Figure 2). These findings suggest that, although there is a greater number of unique alleles found in younger populations, the new alleles are in low frequency within all populations, suggesting the arrival of new variants over time (Table 2).

Our analysis also showed that two of the eight loci are not in HWE in the sample as a whole, namely, JV4 and JV10 (Hardy-Weinberg exact test P-values = 0.0014 ± 0.0014 and 0.0095 ± 0.0050, respectively). Departure from HWE may occur due to various causes, including purifying selection, inbreeding, population substructure, copy-number variation, or genotyping error. For example, deviations from HWE are likely to occur in expanding populations and structured populations such as ERC in the LDSNP (Chen et al. 2017; Meisner and Albrechtsen 2019). Moreover, genotype frequency deviation in finite random-mating populations may also result from the difference between the gene frequencies of male and female gametes, which is determined by two independent causes: the gene frequency difference between male and female parents and the sampling error due to the finite number of offspring (Wang 1996). Given that this population was recently established and that seeds from nearby trees continue to

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

**Table 2.** Sample size after subsampling ($N$), number of alleles ($N_a$), number of effective alleles ($N_e$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), number of unique alleles, and fixation index ($F$) across all loci for each *Juniperus virginiana* var. *virginiana* age group.

| Age group | $N$ | $N_a$ | $N_e$ | $H_o$ | $H_e$ | Unique allele | $F$ |
|---|---|---|---|---|---|---|---|
| 0–9 | 9 | 3.250 (±0.453) | 2.079 (±0.221) | 0.581 (±0.119) | 0.467 (±0.075) | 3.467 (±0.202) | −0.241 (±0.129) |
| 10–19 | 10 | 3.188 (±0.439) | 1.833 (±0.214) | 0.484 (±0.143) | 0.405 (±0.062) | 3.667 (±0.273) | −0.155 (±0.082) |
| 20–29 | 10 | 3.104 (±0.475) | 1.811 (±0.189) | 0.472 (±0.098) | 0.413 (±0.063) | 3.467 (±0.243) | −0.119 (±0.111) |
| 30–39 | 10 | 2.879 (±0.369) | 1.772 (±0.151) | 0.527 (±0.097) | 0.401 (±0.057) | 2.833 (±0.254) | −0.273 (±0.095) |
| 40+ | 5 | 2.125 (±0.227) | 1.671 (±0.153) | 0.458 (±0.107) | 0.361 (±0.066) | 0.033 (±0.033) | −0.245 (±0.117) |
| All trees | 189 | 4.075 (±0.406) | 1.810 (±0.069) | 0.493 (±0.040) | 0.414 (±0.024) | 0.7000 (±0.287) | −0.163 (±0.046) |

**Table 3.** Number of unique alleles present in each locus (JV1–JV7, JC1) for each *Juniperus virginiana* var. *virginiana* age group and total number of unique alleles per age group, considering all individuals in each age group.

| Age group | $N$ | JV1 | JV2 | JV3 | JV4 | JV5 | JV6 | JV7 | JC1 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 0–9 | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 4 |
| 10–19 | 95 | 0 | 1 | 0 | 4 | 1 | 1 | 6 | 2 | 13 |
| 20–29 | 48 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 4 |
| 30–39 | 32 | 0 | 1 | 0 | 0 | 2 | 0 | 2 | 0 | 5 |
| 40+ | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

arrive at LDSNP, it is reasonable to expect that the population will not be in equilibrium.

Similarly, there are indications of linkage disequilibrium among loci JV4 and JV7 (P-values = 0.0256 ± 0.0064) and loci JV11 and JV8 (P-values = 0.0460 ± 0.0127). Disequilibrium in expanding populations can also result from the arrival of individuals from different provenances or regions with unusual genomic patterns, which can distort the inference of the genetic structure of the population (Liu et al. 2022). Furthermore, we found that pairs of loci showing linkage disequilibrium were inconsistent among age groups.

The results of this study help to elucidate the historical patterns of encroaching of ERC in the LDSNP and provide insight into how dispersal and genetic admixture can contribute to its range expansion. Our findings show that despite the size of the study area, there is considerable genetic diversity in the ERC population. Furthermore, our findings suggest that genetic diversity has continued to build over the last four to five decades. Because genetic diversity is essential for population survival and adaptation to changing environments, our findings suggest that increasing genetic diversity during ERC invasion of open areas could be a critical driver for its range expansion.

Ward et al. (2008) reviewed published studies that examined the changes in allelic diversity of alien species expanding in their new range. The invasion processes of nonnative species imply an initial founder effect into a novel range; therefore, the new populations typically have lower diversity relative to populations in their native ranges (Uller and Leimu 2011; Ward et al. 2008; Zhao et al. 2013; Zimmermann et al. 2010). Moreover, environmental filtering also causes diversity loss in alien species after arrival at their new locations, further lowering genetic diversity (Dar et al. 2020; Dormontt et al. 2014; Vyšniauskienė et al. 2011). In contrast, native species that become invasive do not always experience loss of genetic diversity due to environmental filtering (Negi et al. 2021). Native invasive species, like ERC, have more sources of genetic diversity close to their invasive range than most nonnative invasive species and are therefore more likely to experience intraspecific hybridization with other ecotypes, which allows the species to cope with stresses resulting from invading new habitat (Castillo et al. 2021; Glisson and Larkin 2021; Negi et al. 2021).

Changes in the genetic diversity of native species increasing their ranges by invading new environments have received little attention. Our results show that changes in genetic diversity can occur within a short time, with the average number of alleles per locus increasing by 65% in three decades (Table 2; Figure 2). Excoffier et al. (2009) examined the genetic consequences of range expansion. They proposed that range expansions could promote the surfing of rare variants into newly occupied territories. Our findings revealed the accumulation of unique low-frequency alleles in the LDSNP as time progressed (Tables 2–4). As expected for native species experiencing range expansion, our findings indicate that the newly established population was not isolated from other nearby populations, which explains the arrival of additional genetic diversity in the population. We have observed nearby populations of ERC on the Marblehead Peninsula, with some less than 1 km away, and on surrounding islands, including Kelley's Island, approximately 8 km away.

### Genetic Structure

The AMOVA revealed that only 1% molecular variance was among age groups. Our analysis also showed that genetic differentiation among age groups increased slightly as the age difference between age groups increased (Table 5). We found that $H_o$ was highest in the youngest age group ($H_o = 0.581 \pm 0.119$) and lowest in the oldest age group ($H_o = 0.458 \pm 0.107$) (Table 2). While this trend is interesting, it is possible that the smaller sample size of the oldest group contributed to the lower $H_o$ value (Ward and Jasieniuk 2009). On the other hand, individuals in the older age groups may have died off over time; therefore, we may see an underrepresentation of genetic diversity for that age group. Although the $H_o$ values do not show a clear trend over time, our analysis using ANOVA and Tukey's HSD test revealed significantly different mean $H_o$ values for the 0 to 9, 10 to 19, 20 to 29, and 40+ age groups, but the 30 to 39 age group was not significantly different from either the 20 to 29 or 40+ age groups ($F(4, 145) = 60.117$; $P < 0.0001$). The significant increase of $H_o$ seen in the youngest two age groups further indicates the increase in genetic diversity in younger trees. Overall, we saw an excess in heterozygosity and a low level of differentiation among age groups ($F_{st} = 0.037 \pm 0.009$)

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

**Table 4.** List of unique allele size (bp) found in each *Juniperus virginiana* var. *virginiana* age group, considering all individuals in each age group.

| Locus | 0–9 | 10–19 | 20–29 | 30–39 |
|---|---|---|---|---|
| JV1 | 403 | 0 | 0 | 0 |
| JV2 | 0 | 270 | 294 | 285 |
| JV3 | 0 | 0 | 403 | 0 |
| JV4 | 0 | 367 | 0 | 0 |
| | | 373 | | |
| | | 376 | | |
| | | 409 | | |
| JV5 | 0 | 377 | 0 | 374 |
| | | | | 380 |
| JV6 | 0 | 141 | 156 | 0 |
| JV7 | 110 | 113 | 134 | 149 |
| | 167 | 116 | | 173 |
| | | 143 | | |
| | | 158 | | |
| | | 161 | | |
| | | 182 | | |
| JC1 | 119 | 123 | 0 | 0 |
| | | 129 | | |

**Table 5.** Pairwise $F_{st}$ values demonstrating similarity between pairs of age groups of *Juniperus virginiana* var. *virginiana*.

| Age group | 0–9 | 10–19 | 20–29 | 30–39 | 40+ |
|---|---|---|---|---|---|
| 0–9 | 0.000 | | | | |
| 10–19 | 0.035 | 0.000 | | | |
| 20–29 | 0.034 | 0.024 | 0.000 | | |
| 30–39 | 0.042 | 0.024 | 0.020 | 0.000 | |
| 40+ | 0.037 | 0.026 | 0.024 | 0.024 | 0.000 |

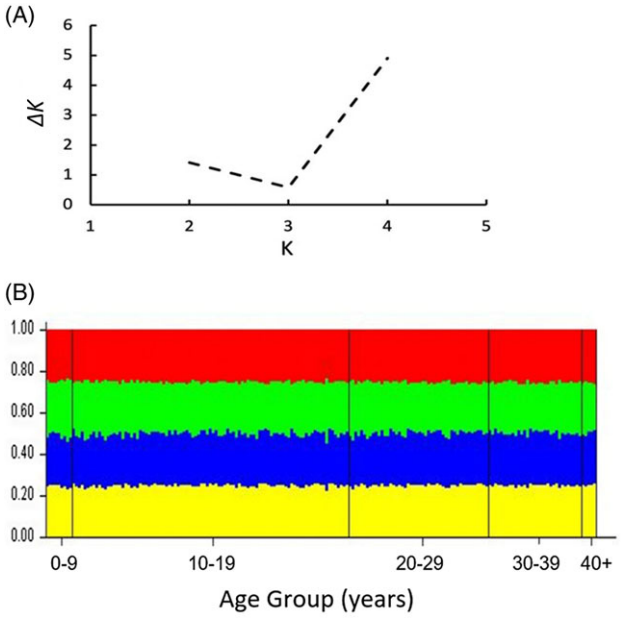



**Figure 3.** STRUCTURE estimates of cryptic population structure of the *Juniperus virginiana* var. *virginiana* in the Lakeside Daisy State Nature Preserve. (A) Calculation of the second-order rate of change ($\Delta K$), determined by the modal peak. The modal peak for natural populations is at $K = 4$. (B) STRUCTURE plot of ancestral subpopulations from the natural populations, with different colors representing the four population clusters and each line on the *x* axis representing a single individual arranged in age groups, with the percent of its genome identified by the *y* axis (Cluster 1: yellow; Cluster 2: blue; Cluster 3: green; Cluster 4: red).

(Table 2). An excess in heterozygosity in a population indicates little biparental inbreeding is occurring in a population. Accompanied by the fact that ERC is a dioecious species, it is likely that an influx of new lineages is invading LDSNP.

We used the Bayesian model-cluster program STRUCTURE to describe the genetic structure among age groups. Our analysis showed that the genetic structure could be best described by four genetically distinct clusters (K = 4) (Figure 3A). However, our analysis showed that all four clusters are present in all age groups, and all clusters showed a similar contribution to the genetic diversity of all age groups (Figure 3B). Moreover, this finding suggests that all individuals originated from previously admixed populations of the four clusters. Despite the similar contribution of all lineages in all age groups, a PCoA shows two distinct groups of trees when the first two axes are plotted (Figure 4). It is worth noting that individuals from all age groups are represented in both groups in the PCoA in similar proportions (Figure 4). Principal coordinates 1 and 2 explain 16.8% and 11.3% of the variation, respectively.

Genetic diversity in invasive plant populations accumulates through multiple introductions, gene flow, mutation, and hybridization among plants of different origins (Carr et al. 2019; Espeland 2013; Gaskin and Schaal 2002; Jeschke and Starzer 2018). However, the results of the STRUCTURE analysis show that all individuals at LDSNP contain the same admixture of four genetic clusters, suggesting that all individuals are derived from the same admixed source population. In addition, the high heterozygosity and the lack of inbreeding maintains the four genetically distinct clusters, as ERC is a dioecious wind-pollinated species.

Seed dispersal outside a local area has been documented for numerous invasive species (Horncastle et al. 2004; Martinod and Gorchov 2017; Nathan et al. 2008). This type of seed dispersal is especially common among species with fruiting phenologies that mature their fruits in the late fall (Barriball et al. 2015; Bartowitz and Orrock 2016; McNeish and McEwan 2016). ERC trees initiate fruit maturation in September and bear mature fruits through the winter months in LDSNP (K Shvach, personal communication). These fruits are available to be dispersed by a diverse array of birds and mammals over the winter when resources are scarce (Horncastle et al. 2004). Avian dispersers of ERC can disperse seeds short and intermediate distances from the source and are primarily resident or nomadic birds, traveling with no regular pattern to follow resource availability (Holthuijzen and Sharik 1985; Horncastle et al. 2004; K Shvach, personal communication). Additionally, small mammals are likely to carry seeds short distances (<100 m), while medium-sized mammals may spread seeds longer distances (1 km or more) (Horncastle et al. 2004). ERC can be distributed by humans as well through use of the tree for landscaping and windbreaks and are often distributed very long distances from the seed source, resulting in long-distance dispersal (Donovan et al. 2018). Therefore, the behavior of ERC's seed dispersers could result in the colonization of LDSNP by ERC seeds from within LDSNP and from surrounding populations from varying distances. This seed dispersal pattern likely explains ERC's rapid encroachment of LDSNP and the increase in allelic diversity and heterozygosity in the younger age groups.

Once introduced to a new suitable habitat, ERC can quickly dominate through its strong competitive abilities, similar to many nonnative invasive plants (Briggs et al. 2002; Donovan et al. 2018). ERC thrives in environments where competition with other plants for sunlight is low and can tolerate many moisture levels, especially xeric environments (Hamati 2022; Ward 2020). Additionally, birds are more likely to deposit seeds near perching sites, making previously

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

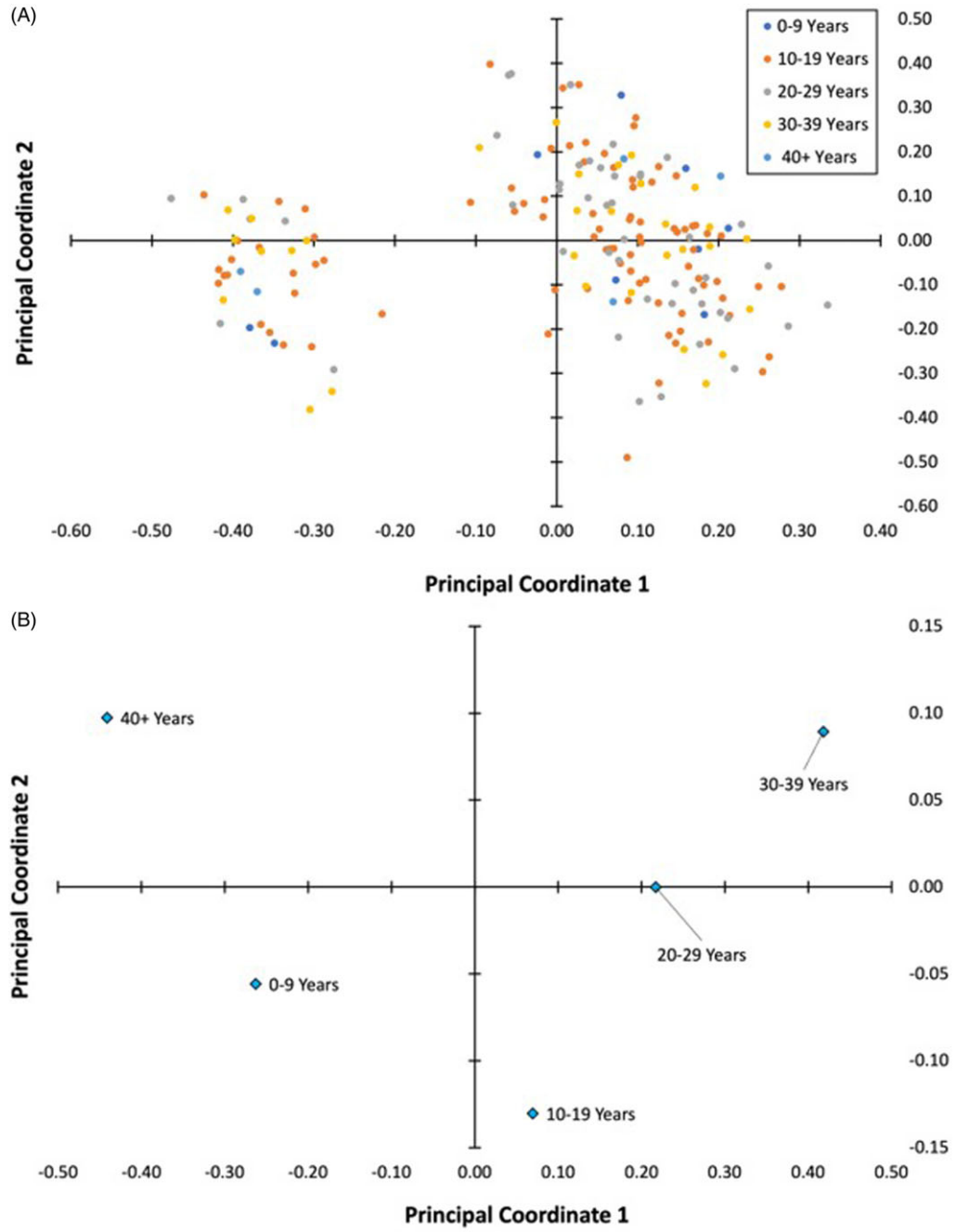



**Figure 4.** The principal coordinate analysis (PCoA) of (A) individual *Juniperus virginiana* var. *virginiana* and (B) age groups at Lakeside Daisy State Nature Preserve based on eight polymorphic microsatellite markers.

colonized open areas more likely to be sites of future seed deposition than undisturbed open areas (Higgins et al. 2000; Holthuijzen and Sharik 1985; Horncastle et al. 2004). When long-distance dispersal occurs, ERC becomes established in a new area, and the acceleration of invasion quickly increases (Moody and Mack 1988). Therefore, it is imperative to prevent long-distance dispersal and establishment of ERC into undisturbed grasslands to maintain the integrity of the ecosystem and prevent further invasion.

## Conclusions

Overall, we found that the invasion pattern of the native ERC has similarities and differences from that of nonnative invasive plants. First, contrary to most nonnative invasive plants, there was an increase in allelic diversity in the ERC population at the LDSNP in just a few decades. This finding follows the predictions of Excoffier et al. (2009); the new alleles are rare (low frequency). Moreover, the results of our STRUCTURE analysis and trends in genetic diversity also suggest sustained gene flow from small clusters of trees neighboring LDSNP originating from previously admixed source populations now found along highways, farmland, and in yards in and around Marblehead. Because ERC is a dioecious tree, outcrossing promotes admixture among clusters, increasing the range of environmental conditions where it can thrive. Hybridization among different ecotypes is often a condition that facilitates invasion of both nonnative and native invaders (Castillo et al. 2021; Ellstrand 2009; Vilà et al. 2000).

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

Finally, while we could not identify a clear advancing front, the level of admixture observed in all ages in our analysis suggests that diffusion from newly established foci trees through seeds dispersed at short and intermediate distances drives ERC's range expansion. Additionally, anthropogenic distribution resulting from using ERC for landscaping can result in the long-distance dispersal of trees from different origins, which in turn facilitates hybridization and further invasion of grasslands and open areas. The new allelic variants seen in the younger individuals in our data set are present at low frequencies and are likely to result from introgression with external sources in this admixed population through pollen and seed dispersal from neighboring populations. Specifically, in the case of LDSNP, ERC has likely arrived through a combination of animal- and human-induced means from neighboring populations and has subsequently disseminated through the preserve's prairie from those introductions. At LDSNP, ERC is quickly dominating the dry, open areas of the preserve but is being outcompeted in wetter areas.

It is imperative that managers control the spread of ERC to new areas in order to prevent the establishment of these fast-growing populations. To forestall the establishment of new stands of ERC most effectively, managers should eliminate satellite populations before individual trees are able to reach sexual maturity. Additionally, humans have the responsibility to prevent long-distance dispersal of invasive plants through anthropogenic means like landscaping. By preventing the long-distance dispersal of ERC, managers can limit the amount of intraspecific hybridization occurring among different ecotypes with the goal of limiting genetic diversity and potential for evolution of beneficial traits in invasive plants.

**Acknowledgments.** The authors are thankful for and acknowledge the contribution of Katie Shvach for her company and help in our fieldwork. We also thank William Hass for his comments on the previous version of this article. We thank the anonymous reviewers of our article for their time spent reviewing drafts and for their many insightful comments. Finally, we also thank the National Science Foundation (grant no. DEB 2214146 to OJR), the Graduate Student Senate at Kent State University, and the Art and Margaret Herrick Aquatic Ecology Research Facility Student Research Grant (to HMH) for funding this work. The authors declare no conflict of interest and they have no relevant financial or non-financial interests to declare.

## References

Aronson MFJ, Handel SN, Clemants SE (2007) Fruit type, life form and origin determine the success of woody plant invaders in an urban landscape. Biol Invasions 9:465–475

Aththanayaka CP, Siyasinghe DP, Prakash SL, Bloch CP, Surasinghe TD (2023) Native and exotic plant invasions vary across habitat types and anthropogenic disturbances in a tourism-heavy protected area. Biol Invasions 25:411–429

Auld BA, Coote BG (1980) A model of a spreading plant population. Oikos 34:287–292

Barriball K, McNutt EJ, Gorchov DL, Rocha OJ (2015) Inferring invasion patterns of *Lonicera maackii* (Rupr) Herder (Caprifoliaceae) from the genetic structure of 41 naturalized populations in a recently invaded area. Biol Invasions 17:2387–2402

Bartowitz KJ, Orrock JL (2016) Invasive exotic shrub (*Rhamnus cathartica*) alters the timing and magnitude of post-dispersal seed predation of native and exotic species. J Veg Sci 27:789–799

Blackburn TM, Bellard C, Ricciardi A (2019) Alien versus native species as drivers of recent extinctions. Front Ecol Environ 17:203–207

Briggs JM, Hoch GA, Johnson LC (2002) Assessing the rate, mechanisms, and consequences of the conversion of tallgrass prairie to *Juniperus virginiana* forest. Ecosystems 5:578–586

Campbell DR, Dooley JL (1992) The spatial scale of genetic differentiation in a hummingbird-pollinated plant: comparison with models of isolation by distance. Am Nat 139:735–748

Carr AN, Hooper DU, Dukes JS (2019) Long-term propagule pressure overwhelms initial community determination of invader success. Ecosphere 10:1–20

Castillo ML, Schaffner U, van Wilgen BW, Manuel Montaño N, Bustamante RO, Cosacov A, Mathese MJ, Le Roux JJ (2021) Genetic insights into the globally invasive and taxonomically problematic tree genus *Prosopis*. AoB Plants 13:1–13

Céspedes M, Gutierrez MV, Holbrook NM, Rocha OJ (2003) Restoration of genetic diversity in the dry forest tree *Swietenia macrophylla* (Meliaceae) after pasture abandonment in Costa Rica. Mol Ecol 12:3201–3212

Chen B, Cole JW, Grond-Ginsbach C (2017) Departure from Hardy Weinberg equilibrium and genotyping error. Front Genet 8:1–6

Chybicki IJ, Oleksa A (2018) Seed and pollen gene dispersal in *Taxus baccata*, a dioecious conifer in the face of strong population fragmentation. Ann Bot 122:409–421

Dar T, Bhat BA, Khuroo AA, Verna S, Islam SU (2020) Genetic diversity and population structure of an invasive plant species differ in two non-native regions with differing climate invasion success. Nord J Bot 38:1–9

Donovan VM, Burnett JL, Bielski CH, Birgé HE, Bevans R, Twidwell D, Allen CR (2018) Social–ecological landscape patterns predict woody encroachment from native tree plantings in a temperate grassland. Ecol Evol 8:9624–9632

Dormontt EE, Gardner MG, Breed MF, Rodger JG, Prentis PJ, Lowe AJ (2014) Genetic bottlenecks in time and space: reconstructing invasions from contemporary and historical collections. PLoS ONE 9:1–11

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JF, Whitford WG (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. Ecol Lett 14:709–722

Ellstrand NC (2009) Evolution of invasiveness in plants following hybridization. Biol Invasions 11:1089–1091

Espeland EK (2013) Predicting the dynamics of local adaptation in invasive species. J Arid Land 5:268–274

ESRI (2011) ArcGIS Desktop: version 10.8.2. Redlands, CA: Environmental Systems Research Institute

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. Annu Rev Ecol Evol Syst 40:481–501

Fuchs EJ, Robles T, Hamrick JL (2013) Spatial distribution of *Guaiacum sanctum* (Zygophyllaceae) seedlings and saplings relative to canopy cover in Palo Verde National Park, Costa Rica. Rev Biol Trop 61:1521–1533

Gaskin JF, Schaal BA (2002) Hybrid *Tamarix* widespread in U.S. invasion and undetected in native Asian range. Proc Natl Acad Sci USA 99:11256–11259

Gettys LA, Schnelle MA (2018) The natives are restless: proceedings from the ASHS Invasive Plants Research Interest Group 2017 and 2018 Workshops. HortTechnology 29

Glisson WJ, Larkin DJ (2021) Hybrid watermilfoil (*Myriophyllum spicatum* x *Myriophyllum sibiricum*) exhibits traits associated with greater invasiveness than its introduced and native parental taxa. Biol Invasions 23:2417–2433

Gonzales E, Hamrick JL, Smouse PE, Trapnell DW, Peakall R (2009) The impact of landscape disturbance on spatial genetic structure in the Guanacaste tree, *Enterolobium cyclocarpum* (Fabaceae). J Hered 101:133–143

Gorchov DL, Castellano SM, Noe DA (2014) Long-distance dispersal and diffusion in the invasion of *Lonicera maackii*. Invasive Plant Sci Manag 7:464–472

Hamati S (2022) Ecophysiology of *Juniperus virginiana* Encroachment in Ohio. Ph.D dissertation. Kent, OH: Kent State University. 244 p

Hamrick JL, Trapnell DW (2011) Using population genetic analyses to understand seed dispersal patterns. Acta Ecolog 37:641–649

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/inp.2023.23

Higgins SI, Richardson DM, Cowling RM (2000) Using a dynamic landscape model for planning the management of alien plant invasions. Ecol Appl 10:1833–1848
Holthuijzen AM, Sharik TL (1985) The avian seed dispersal system of eastern redcedar (*Juniperus virginiana*). Can J Bot 63:1508–1515
Horncastle VJ, Hellgren EC, Mayer PM, Engle DM, Leslie DM (2004) Differential consumption of eastern redcedar (*Juniperus virginiana*) by avian and mammalian guilds: implications for tree invasion. Am Midl Nat 152:255–267
Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332
Jeschke JM, Starzer J (2018) Propagule pressure hypothesis. Pages 147–153 *in* Jeschke JM, Heger T, eds. Invasion Biology: Hypotheses and Evidence. Wallingford, Oxfordshire, UK: CAB International
Kaur R, Joshi O, Will RE (2020) The ecological and economic determinants of eastern redcedar (*Juniperus virginiana*) encroachment in grassland and forested ecosystems: a case study from Oklahoma. J Environ Manag 254:1–8
Kaskie KD, Wimberly MC, Bauman PJ (2019) Rapid assessment of juniper distribution in prairie landscapes of the Northern Great Plains. Int J Appl Earth Obs Geoinf 83:1–9
Keller SR, Chhatre VE, Fitzpatrick MC (2017) Influence of range position on locally adaptive gene–environment associations in *Populus* flowering time genes. J Hered 109:47–58
Knapp AK, Chen A, Griffin-Nolan RJ, Baur LE, Carroll CJW, Gray JE, Hoffman AM, Li X, Post AK, Slette IJ, Collins SL, Luo Y, Smith MD (2020) Resolving the Dust Bowl paradox of grassland responses to extreme drought. Proc Natl Acad Sci USA 117:22249–22255
Lawton RO, Cothran P (2000) Factors influencing reproductive activity of *Juniperus virginiana* in the Tennessee Valley. J Torrey Bot Soc 127:271–279
Leis SA, Blocksome CE, Twidwell D, Fuhlendorf SD, Briggs JM, Sanders LD (2017) Juniper invasions in grasslands: research needs and intervention strategies. Rangelands 39:64–72
Liu Q, Wu D, Wang C (2022) Identification of genomic regions distorting population structure inference in diverse continental groups. Quant Biol 10:287–298
Martinod KL, Gorchov DL (2017) White-tailed deer browse on an invasive shrub with extended leaf phenology meets assumptions of an apparent competition hypothesis. AoB Plants 9:1–14
McNeish RE, McEwan RW (2016) A review on the invasion ecology of Amur honeysuckle (*Lonicera maackii*, Caprifoliaceae) a case study of ecological impacts at multiple scales. J Torrey Bot Soc 143:367–385
Meisner J, Albrechtsen A (2019) Testing for Hardy-Weinberg equilibrium in structured populations using genotype or low-depth next generation sequencing data. Mol Ecol Resour 19:1144–1152
Michalczyk IM, Sebastiani F, Buonamici A, Cremer E, Mengel C, Ziegenhagen B, Vendramin GG (2006) Characterization of highly polymorphic nuclear microsatellite loci in *Juniperus communis*. L. Mol Ecol Notes 6:346–348
Moody ME, Mack RN (1988) Controlling the spread of plant invasions: the importance of nascent foci. J Appl Ecol 25:1009–1021
Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. Trends Ecol Evol 23:638–647
Negi VS, Maletha A, Pathak R, Maikhuri RK (2021) Expansion of a native species and its impacts on alpine ecosystems, Indian Himalaya. Biologia 76:889–899
Ohio Department of Natural Resources (n.d.) Lakeside Daisy State Nature Preserve. ohiodnr.gov/wps/portal/gov/odnr/go-and-do/plan-a-visit/find-a-property/lakeside-daisy-state-nature-preserve. Accessed: January 7, 2022
Peakall R, Smouse PE (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Quinn JA, Meiners ST (2004) Growth rates, survivorship, and sex ratios of *Juniperus virginiana* on the New Jersey piedmont from 1963 to 2000. J Torrey Bot Soc 131:187–194
Ratajczak Z, Nippert JB, Collins SL (2012) Woody encroachment decreases diversity across North American grasslands and savannas. Ecology 93: 697–703
Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249
Rosenberg NA (2003) District: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138
Roser LG, Ferreyra LI, Saidman BO, Vilardi JC (2017) Ecogenetics: an R package for the management and exploratory analysis of spatial data in Landscape Genetics. Mol Ecol Resour 17:241–250
Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8:103–106
Sagoff M (1999) What's wrong with exotic species? Report from the Institute for Philosophy & Public Policy 19:16–23
Sangüesa-Barreda G, García-Cervigón AI, García-Hidalgo M, Rozas V, Martín-Esquive JL, Martín-Carbaja J, Martínez R, Olano JM (2021) Vertical cliffs harbour millennia-old junipers in the Canary Islands. Ecology 103
Schnelle MA (2019) Native woody plants of the southern United States with weedy or invasive tendencies: a review of common offenders. HortTechnol 29:567–570
Simberloff D, Souza L, Nunez MA, Barrios-Garcia N, Bunn W (2012) The natives are restless, but not often and mostly when disturbed. Ecology 93:598–607
Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. Landscape Ecol 21:821–836
Tunnell SJ, Stubbendieck J, Huddle J, Brollier J (2004) Seed dynamics of eastern redcedar in the mixed-grass prairie. Great Plains Res 14:129–142
Uller T, Leimu R (2011) Founder events predict changes in genetic diversity during human-mediated range expansions. Global Change Biol 17:3478–3485
Van Haverbeke DF, Read RA (1976) Genetics of Eastern Redcedar. U.S. Forest Service, Division of Forest Economics and Marketing Research. Washington, DC: U.S. Government Printing Office
Vasiliauskas SA, Aarssen LW (1992) Sex ratio and neighbor effects in monospecific stands of *Juniperus virginiana*. Ecology 73:622–632
Vilà M, Weber E, Antonio CMD (2000) Conservation implications of invasion by plant hybridization. Biol Invasions 2:207–217
Vyšniauskienė R, Rančelienė V, Žvingila D, Patamsytė J (2011) Genetic diversity of invasive alien species *Lupinus polyphyllus* populations in Lithuania. Žemdirbystė Agric 98:383–390
Wang J (1996) Deviation from Hardy-Weinberg proportions in finite populations. Genet Res 68:249–257
Ward D (2020) Shade is the most important factor limiting growth of a woody range expander. PLoS ONE 15:1–20
Ward D (2021) Shade affects fine-root morphology in range-encroaching eastern redcedars (*Juniperus virginiana*) more than competition, soil fertility and pH. Pedobiologia 84:1–8
Ward D, Pillay T, Mbongwa S, Kirkman K, Hansen E, Van Achterbergh M (2022) Reinvasion of native invasive trees after a tree-thinning experiment in an African savanna. Rangeland Ecol Manag 81:69–77
Ward SM, Gaskin JF, Wilson LM (2008) Ecological genetics of plant invasion: what do we know? Invasive Plant Sci Manag 1:98–109
Ward SM, Jasieniuk M (2009) Review: sampling weedy and invasive plant populations for genetic diversity analysis. Weed Sci 57:593–602
Watson PA, Alexander HD, Moczygemba JD (2019) Coastal prairie recovery in response to shrub removal method and degree of shrub encroachment. Rangeland Ecol Manag 72:275–282
Wickert KL, O'Neal ES, Davis DD, Kasson MT (2017) Seed production, viability, and reproductive limits of the invasive *Ailanthus altissima* (Tree-of-Heaven) within invaded environments. Forests 8:226
Yazlik A, Ambarli D (2022) Do non-native and dominant native species carry a similar risk of invasiveness? A case study for plants in Turkey. NeoBiota 76:53–72
Zhao J, Solís-Montero L, Lou A, Vallejo-Marín M (2013) Population structure and genetic diversity of native and invasive populations of *Solanum rostratum* (Solanaceae). PLoS ONE 8:1–9
Zimmermann H, Ritz CM, Hirsch H, Renison D, Wesche K, Hensen I (2010) Highly reduced genetic diversity of *Rosa rubiginosa* L. populations in the invasive range. Int J Plant Sci 171:435–446

Downloaded from https://www.cambridge.org/core. IP address: 87.49.42.83, on 01 Oct 2026 at 23:23:27, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/inp.2023.23