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Chronosequence of invasion reveals minimal losses of population genomic diversity, niche expansion, and trait divergence in the polyploid, leafy spurge

Thomas A. Lake[1], Ryan D. Briscoe Runquist[1], Lex E. Flagel[12], and David A. Moeller[1]

Affiliations

[1] Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, USA

[2] Gencove, Long Island City, New York, USA

Emails and ORCID

Thomas Lake: lakex055@umn.edu, 0000-0002-8836-5164

Ryan Briscoe Runquist: rbriscoe@umn.edu, 0000-0001-7160-9110

Lex Flagel: flag0010@umn.edu, 0000-0003-2106-4196

David Moeller: moeller@umn.edu, 0000-0002-6202-9912

Correspondence

Thomas A. Lake and David A. Moeller

Department of Plant and Microbial Biology, University of Minnesota, St. Paul, Minnesota, USA

Email: lakex055@umn.edu, moeller@umn.edu

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ABSTRACT

Rapid evolution may play an important role in the range expansion of invasive species and modify forecasts of invasion, which are the backbone of land management strategies. However, losses of genetic variation associated with colonization bottlenecks may constrain trait and niche divergence at leading range edges. The spatial and temporal scales over which adaptation contributes to invasion dynamics remains unresolved. We leveraged detailed records of the ~130 year invasion history of the invasive polyploid plant, leafy spurge (*Euphorbia virgata*), across ~500km in Minnesota, U.S.A. We examined the consequences of range expansion for population genomic diversity, niche breadth, and the evolution of germination behavior. Using genotyping-by-sequencing, we found some population structure in the range core, where introduction occurred, but panmixia among all other populations. Range expansion was accompanied by only modest losses in sequence diversity, with small, isolated populations at the leading edge harboring similar levels of diversity to those in the range core. The climatic niche expanded during most of range expansion, and the niche of the range core was largely non-overlapping with the invasion front. Ecological niche models indicated that mean temperature of the warmest quarter was the strongest determinant of habitat suitability and that populations at the leading edge had the lowest habitat suitability. Guided by these findings, we tested for rapid evolution in germination behavior over the time course of range expansion using a common garden experiment and temperature manipulations. Germination behavior diverged from early to late phases of the invasion, with populations from later phases having higher dormancy at lower temperatures. Our results suggest that trait evolution has contributed to niche expansion during invasion and that distribution models of future invasion may underestimate invasion potential without accounting for evolution.

Key Words (6): adaptation, colonization bottlenecks, plant invasion, population genetic structure, range shift, rapid evolution

61

Introduction

Invasive species experience considerable changes to genetic variation during the process of introduction and subsequent invasion (Lee 2002; Suarez and Tsutsui 2008). In particular, it has been well-documented that founder effects and genetic drift can cause substantial losses of genetic diversity during the colonization process (Dlugosch and Parker 2008; Uller and Leimu 2011). Following initial establishment, further losses of variation may occur during range expansion. However, the magnitude of changes in genetic variation depends upon the number of introductions and the severity of population bottlenecks (Nei et al. 1975; Uller and Leimu 2011; Welles and Dlugosch 2019). Such changes in genetic variation early in the invasion process may influence the capacity for adaptation, forecasts of range expansion, and subsequent management decisions.

Following colonization, some non-native species exhibit rapid population growth and dispersal into new environments (Sakai et al. 2001). The process of invasion is often highly variable, involving repeated founder events and density-dependent population growth, especially Allee effects (Melbourne and Hastings 2009; Sullivan et al. 2017). In addition to affecting the speed of invasion, these population fluctuations can influence levels of genetic diversity and structure across an invaded range (Austerlitz et al. 1997; Excoffier 2004). For example, range expansion is expected to cause a reduction in allelic richness and heterozygosity with increasing distance from the origin of expansion (Slatkin and Excoffier 2012; Peter and Slatkin 2013). The prevalence of drift during invasion may also cause populations to depart from migration-drift equilibrium, resulting in a lack of isolation-by-distance (Wright 1943; Slatkin 1987, 1993; Hutchison and Templeton 1999). Last, mutations arising at the range edge may rise in frequency due to genetic drift, “surf” along the expanding front, and travel long distances (Klopfstein et al. 2006; Excoffier and Ray 2008). Models of allele surfing indicate that rapid range expansion can produce clinal variation in allele frequencies and increase the frequency of loci with private alleles (Klopfstein et al. 2006; Excoffier and Ray 2008; Goodsman

et al. 2014). Such clines can emerge for any type of mutation (beneficial, neutral, deleterious), and therefore could reflect drift and/or selection (Lehe et al. 2012; Peischl et al. 2013; Koski et al. 2019). Overall, the prevalence of drift during range expansion has the potential to influence the capacity for adaptation as organisms encounter novel environments, particularly when functionally-important allelic variation is lost.

An increasing body of evidence suggests that rapid phenotypic evolution can be important to the process of range expansion (Colautti and Barrett 2013; Hodgins et al. 2018; Selechnik et al. 2019; Ma et al. 2020). Forecasts of range expansion in invasive species can underpredict the potential extent of invasion if they assume a species does not evolve or adapt over short time scales (Chardon et al. 2020; Collart et al. 2020). A recent meta-analysis indicated that the signature of local adaptation in invasive species was at least as strong as in native species, even when accounting for variation in life history (Oduor et al. 2016). Invasive species frequently expand across strong environmental gradients and into novel niche space (Atwater et al. 2018; Bates and Bertelsmeier 2021). Such niche expansion may require adaptive evolution at the invasion front (Chown et al. 2015; Moran et al. 2017; Hodgins et al. 2018). For example, purple loosestrife rapidly diverged in flowering time and plant size during invasion across latitudinal gradients in growing season length (Colautti and Barrett 2013). Despite evidence of rapid evolution in some systems, range expansion may not involve any changes in the organism's niche if the only limit to spatial expansion is dispersal and time. As such, the apparent expansion of the climate niche with invasion may not actually involve the evolution of ecologically-important traits. While tests of local adaptation within an invaded range remain few, there is growing appreciation that rapid evolution is likely to shape the trajectory of range expansion in non-native species (Hodgins et al. 2018; Woods and Sultan 2022).

Phenotypic evolution during range expansion may be caused by spatially-variable selection and/or neutral processes (e.g., spatial sorting) (Keller and Taylor 2008; Colautti and Lau 2015; Hodgins et al. 2018). Adaptive evolution, in particular, may be paramount to the

114 invasion process if selection in response to novel environments results in trait changes that
115 enhance a species' capacity to establish in new habitats (Prentis et al. 2008; Williams et al.
116 2016; Hodgins et al. 2018; Woods and Sultan 2022). While reciprocal transplant experiments
117 are the gold standard for testing for adaptation, the translocation of invasive species for these
118 experiments is subject to ethical concerns and legal restrictions in many areas. Alternatively,
119 researchers have started to use ecological niche models (ENMs) to identify important
120 environmental gradients that span from optimal to marginal habitat, such as from a range core
121 to edge. Predictions are then made about traits that may promote adaptation to the novel
122 environments found in marginal habitats (Searcy and Shaffer 2016; Dixon and Busch 2017;
123 Capblancq et al. 2020; Morente-López et al. 2022). Finally, common garden experiments can
124 determine whether the putative traits under selection have differentiated across the key
125 environmental gradients identified by ENMs. Taken together, this series of approaches can
126 provide insight into the role of adaptation in the process of niche expansion at leading range
127 edges.

128 Among invasive plant species, polyploidy is prevalent (Pandit et al. 2011) and can
129 influence the process of range expansion (Van de Peer et al. 2021). The frequency of polyploid
130 species increases with higher latitudes, lower temperatures, and seasonally drier environments
131 (Brochmann et al. 2004; Rice et al. 2019). Direct effects of polyploidy on physiological,
132 morphological, and phenological traits may facilitate niche shifts (Glennon et al. 2014; Blaine
133 Marchant et al. 2016; Brittingham et al. 2018; Wang et al. 2022) and preadapt polyploids to new
134 environments (Treier et al. 2009; Lachmuth et al. 2010). Polyploidy may also influence the
135 capacity for adaptation during range expansion as new environments are encountered (te Beest
136 et al. 2012; Baniaga et al. 2020). Although genetic drift during range expansion can cause
137 losses of genetic diversity, drift may have less severe effects (e.g., inbreeding depression) in
138 polyploids relative to diploids when there is polysomic inheritance (Moody et al. 1993; Soltis and
139 Soltis 2000). Despite numerous polyploid invaders, they have been the subject of few studies

because of substantial challenges with the application of evolutionary genetic analyses that were developed for diploids (Rutland et al. 2021).

In this study, we used a well-documented chronosequence of invasion to examine the consequences of range expansion for population genomic diversity, climatic niche breadth, and the evolution of germination behavior in the polyploid, leafy spurge (*Euphorbia virgata*). We focused on one area of introduction to southwestern Minnesota, U.S.A and subsequent range expansion to the north and east. We were interested in examining the severity of losses of genetic diversity following introduction and its potential consequences for invasion, particularly since existing species distribution models predict a low probability of range expansion at the current leading range edge (Lake et al. 2020). Introduction to this region occurred in the 1890s in southwestern Minnesota with subsequent range expansion to northeastern Minnesota (ca., 500 km), where populations are currently rare, isolated, and small. Based on historical occurrence datasets, we defined a range core, area of early expansion, area of late expansion, and invasion front.

First, we sampled populations along the gradient from core to invasion front using two sampling schemes to quantify population genomic diversity and structure (using reduced representation sequencing). Samples of multiple individuals from 14 populations (population samples) allowed us to quantify changes in sequence diversity among populations over the course of range expansion. Samples of single individuals from 157 populations (landscape samples) allowed us to test for fine-scale population structure (e.g., isolation-by-distance) over the time series of range expansion. Second, we tested whether range expansion involved niche expansion - i.e., occurred into novel climatic environments. Third, we developed an ecological niche model (ENM) to test whether habitat suitability declines from range core to invasion front and to determine which environmental gradients are most strongly associated with high versus low habitat suitability. Warm season temperature had the greatest positive contribution to habitat suitability. Because temperature is known to modulate germination behavior in leafy

spurge, and because establishment in new habitats is dependent upon successful germination timing, we focused on this trait for common garden experiments. Past work has also suggested that shifts in seed dormancy might facilitate invasion at leading range edges (Mathias and Kisdi 2002; Travis et al. 2021). We examined the responses of seeds from early versus late in invasion to five temperature regimes in a common garden experiment. We specifically tested whether there was an interaction between geographic region (early vs. late invasion stages) and temperature regime, which would indicate divergence in germination behavior over the course of invasion.

Methods

Biology and invasion of leafy spurge

Species biology

Leafy spurge has invaded nearly two million acres across the northern tier of the United States and southern Canada (Duncan et al. 2004). While it is most commonly found in dry, open sites with well-drained soils (e.g., prairies), it can occasionally occur in seasonally wet meadows and riparian areas (Selleck et al. 1962). Leafy spurge impacts rangelands and natural habitats by competitively displacing native species (Hein and Miller 1992). When damaged, plants exude a toxic white latex that deters grazing (Lym and Kirby 1987; Lym 1998).

Leafy spurge spreads locally via rhizomes and ballistic seed dispersal (Morrow 1979). Longer distance dispersal has been proposed to occur via animals or agricultural machinery (Pemberton 1988; Lacey et al. 1992). Seeds germinate in spring or may remain dormant in soil for at least two years (Hanson and Rudd 1933; Selleck et al. 1962). Flowers are insect pollinated and the mating system is primarily outcrossing (Selleck et al. 1962).

Leafy spurge is an auto-allohexaploid that likely originated from hybridization between closely-related *Euphorbia* species, although the progenitor species are not yet known (Schulz-Schaeffer and Gerhardt 1989; Riina et al. 2013). It has been the subject of several genomic

192 investigations but lacks a full genome assembly and annotation (Chao et al. 2005; Horvath et al.
193 2015, 2018; West et al. 2023).
194
195 **Invasion history**
196 One introduction of leafy spurge occurred into southwestern Minnesota ca., 1890
197 purportedly via contaminated grains imported from southern Russia (Batho 1932; Hanson and
198 Rudd 1933; Dunn 1985). Following introduction, the range expanded to eastern South Dakota
199 by ca., 1902 (Bakke 1936), eastern North Dakota by ca. 1909 (Hanson and Rudd 1933), and
200 southern Manitoba and Saskatchewan by the 1920s (Batho 1932; Selleck et al., 1962). Hanson
201 and Rudd (1933) documented in detail the distribution of leafy spurge across Minnesota and
202 neighboring regions, providing a baseline for understanding the timeline of subsequent range
203 expansion. By the late 1970s, leafy spurge had become common throughout grasslands of the
204 north-central plains (Dunn 1979, 1985). Invasion of the boreal forest region of northeastern
205 Minnesota began in the 1940s and 1950s with isolated occurrences (Lakela 1965) and
206 populations were not common until the 1990s. This invasion front has persisted with limited
207 expansion further northeast.
208
209 **Delineating the timeline of range expansion**
210 We digitized the earliest known point record map (Hanson and Rudd 1933) using ArcGIS
211 Pro (ESRI, 2022). We then applied empirical Bayesian kriging to produce a continuous density
212 surface that represented the density of populations in the north-central plains. From this density
213 surface, we applied an equal-interval threshold to demarcate a range core, area of early
214 expansion, area of late expansion, and invasion front that corresponded to four density
215 categories across Minnesota and surrounding states (Figure 1). We verified these demarcations
216 with published accounts of the invasion history (described above).
217

Population genetic diversity and structure

Sampling and sequencing

In 2019, we collected leaf tissue from six individuals in each of 14 populations distributed evenly across Minnesota (*hereafter:* population samples; Figure 1A). In addition, we collected tissue from one individual in each of 157 populations distributed relatively evenly across Minnesota, eastern South Dakota, eastern North Dakota, and western Wisconsin (*hereafter:* landscape samples; Figure 1B). We sampled tissue from individuals that were at least five meters apart to minimize collecting from the same genet and placed tissues immediately in silica for preservation until DNA extraction (Table S1).

We extracted DNA using QIAGEN DNeasy Plant Mini Kits (QIAGEN Inc.). Dual-indexed GBS (genotyping-by-sequencing) libraries were created using the BamHI + NsiI enzyme combination. All libraries were pooled and sequenced on an Illumina NovaSeq System (Illumina Inc., San Diego, CA, USA) with 1x100-bp sequencing. Once sequenced, the reads were demultiplexed and balanced with a mean quality score ≥ Q30 for all libraries. We filtered low-quality bases using Trimmomatic (Bolger et al. 2014) and used Stacks v.2.5.9 (Rochette et al. 2019) to build loci *de novo* (i.e., without aligning reads to a reference genome). Overall, we obtained 510 million reads across the 241 samples (599,386 – 3,376,078 of raw reads per individual). Mean read depth per locus ranged from 14x to 26x (Supplementary Methods).

We called SNPs using polyRAD v.1.6 (Clark et al. 2019), a Bayesian algorithm designed for polyploid GBS data. PolyRAD estimates the genotype probabilities for each individual from read depth distributions with ploidy level as a prior. First, we filtered our dataset using the H_{ind}/H_e statistic to cull likely paralogous loci (Clark et al. 2022). Next, we estimated posterior probabilities for each genotype using the 'IterateHWE' function with Hardy-Weinberg equilibrium as the prior (Gerard and Ferrão 2020; Clark et al. 2022). For each individual at each locus, we exported the most probable genotype for subsequent analyses (Supplementary Methods).

We implemented a second filtering step to account for potential biases caused by homoeologous loci present in our dataset. Because leafy spurge is an auto-allohexaploid (Schulz-Schaeffer and Gerhardt 1989), we expect homoeologous loci to have a 2:1 allelic ratio (e.g. AAAABB genotype; Horvath et al. 2018). Indeed, we identified a peak in the minor allele frequency spectrum around 0.33 (Figure S1). We removed loci with a minor allele frequency above 0.26 from our dataset because they are likely to have an excess of homoeologous genotypes (Figure S1). While essential, this second filtering step limits our capacity to understand absolute levels of sequence diversity. However, our primary goal was to examine changes in sequence diversity over the course of invasion rather than absolute quantities. After filtering, 3,176 loci remained for downstream analyses.

Population structure

We performed an analysis of molecular variance (AMOVA) to quantify the proportion of genetic variation partitioned among populations, among individuals within populations, and within individuals (Excoffier et al. 1992; Meirmans 2012, 2020). We estimated genetic variance components using the rho statistic (Ronfort et al. 1998) and determined significance using permutation tests (n= 999) using the R package poppr v 2.8.6 (Kamvar et al. 2015). Further, we checked for clonality among samples using the 'clonecorrect' function in (Kamvar et al. 2015).

We assessed population structure using the Bayesian clustering algorithm STRUCTURE v.2.3.4 (Pritchard et al. 2000). We ran the analysis for 500,000 Markov Chain Monte Carlo iterations with a 50,000-run burn-in period, specifying an admixture model with the assumption of uncorrelated allele frequencies. We used 'structure_threader' in Python 3 (Pina-Martins et al. 2017) to parallelize runs across clusters (K = 1 – 10). We determined the most plausible number of clusters using the Evanno Delta K method (Evanno et al. 2005) and STRUCTURE HARVESTER web v.0.6.94 (Earl and vonHoldt 2012).

We performed principal component analyses (PCA) to examine population structure using GENODIVE v.3.0.4 (Meirmans 2020). We performed PCA separately for the population samples and the landscape samples.

We tested for isolation by distance (IBD) (Wright 1943) in the population samples by estimating genetic differentiation as G_{ST} (Dufresne et al. 2014) using GENODIVE v.3.0.4 (Meirmans 2020). We also tested for IBD in the landscape samples by calculating Nei's genetic distance (D) (Nei 1972) (Meirmans 2020). We subset the landscape samples according to successive stages of range expansion and tested for IBD within each subset (i.e., within range core, then successively including areas of early expansion, late expansion, and invasion front). We tested for a relationship between genetic and geographic distance matrices using Mantel tests with 9,999 permutations in the R package 'adegenet' v.2.1.8 (Jombart 2008).

Tests for changes in population genetic diversity during range expansion

For each of the 14 population samples, we estimated observed heterozygosity (H_o; gametic heterozygosity: which corrects for potential overestimates of heterozygosity in polyploids by calculating the fraction of heterozygotic gametes for each genotype) (Moody et al. 1993), the inbreeding coefficient (G_{IS}) (Meirmans et al. 2018), and the number of private alleles (P) (Kamvar et al., 2014). We also estimated Tajima's *D* (Tajima 1989) using DNASp v 6.0 (Rozas et al. 2017) to gauge if populations have an excess or deficit of rare alleles, which can be indicative of population expansion following a bottleneck (negative *D*) or sudden population contraction (positive *D*), respectively. Because we filtered loci with higher minor allele frequencies, Tajima's *D* should be biased to lower values.

We tested for changes in population genetic parameters (H_o, G_{IS}, P, and Tajima's *D*) as populations dispersed beyond the range core (Table 1). For each statistic separately, we used a multiple linear regression that included latitude, longitude, and their interaction as independent variables with the R package 'car' (Weisberg, 2019). As all late expansion and invasion front

294 populations are located either north or east of the range core, latitude and longitude describe
295 the northern and eastern invasion spread, respectively. In the model of private alleles, we
296 identified the 'Winona' population as an outlier using diagnostic plots of residuals (Figure S2), so
297 we removed it from the analysis.
298
299 **Niche breadth and habitat suitability**
300 **Environmental data**
301 We downloaded three bioclimatic variables at a 30 arcsecond resolution (~1 km) from
302 Worldclim (http://worldclim.org/version2): minimum temperature of the coldest month (Bio 6),
303 mean temperature of the warmest quarter (Bio 10), and precipitation of the warmest quarter (Bio
304 16). These three variables provide biologically meaningful axes of climate variation that are
305 relevant to key life history stages (Figure S3) (Petitpierre et al. 2017; Chapman et al. 2017).
306 Specifically, minimum cold temperatures are important for plant physiological responses to
307 overwintering and cold tolerance (Chapman et al. 2017), whereas the temperature of the
308 warmest season acts on seed germination, plant growth, and phenological transitions
309 (Wolkovich et al. 2013). Precipitation in the warmest season also affects plant growth and
310 reproduction, with lower precipitation associated with reduced growth and increased drought
311 stress in northern temperate ecosystems (Petitpierre et al. 2017; Gorton et al. 2019).
312
313 **Tests for niche differentiation during range expansion**
314 We tested for climatic niche differentiation between the range core, early expansion, late
315 expansion, and invasion front using the 'ecospat' R package (Di Cola et al. 2017). We sampled
316 the total extent of the background environmental space with a principal components analysis
317 using 1500 random points drawn from a bounding box centered on Minnesota (Latitude: min:
318 43°N, max: 50°N; Longitude: min: -98°W, max: -89°W). We then used the landscape sample

localities to calculate the niche boundaries and density of occurrence for each portion of the range within the environmental PCA space.

For all pairwise comparisons of the range core, early expansion, late expansion, and invasion front, we quantified four measures of niche differentiation. First, we used Schoener's *D* to quantify the similarity in niche by incorporating both niche breadth and density (Warren et al. 2008). Values of *D* can vary from 0 (no overlap) to 1 (complete overlap). For each pair of regions, we then calculated what proportion of the combined niche space represented niche stability, expansion, and unfilling. In this framework, if 'A' represents the older potion of the range (e.g. range core) and 'B' represents the more recent portion of the range (e.g. invasion front), niche stability is the proportion of niche B that overlaps A, niche expansion is the proportion of niche B that does not overlap A, and niche unfilling is the proportion niche A that does not overlap B.

We used permutation tests to determine if values of Schoener's *D*, expansion, stability, and unfilling were equivalent between the range core, early expansion, late expansion, and invasion front. In the niche equivalency tests, the data were pooled and then randomly assigned to one group of the pairwise range comparisons for 999 permutations. For each permutation, we computed all four statistics. We rejected the null hypothesis of niche equivalency if the observed Schoener's *D* value was less than 95% of permuted *D* values. Similarly, we rejected niche equivalency based on the combined niche space if observed stability was less than 95% of permuted values and observed niche expansion or unfilling was greater than 95% of permuted values.

Ecological niche model

We developed an ecological niche model (ENM) using MaxEnt v.3.4.3 (Merow et al. 2013; Phillips et al. 2017) with the 'dismo' package in R (Hijmans et al. 2022). Our goal was identify environmental gradients that could potentially drive phenotypic evolution during range

345 expansion (Elith and Leathwick 2009; Araújo et al. 2019; Morente-López et al. 2022). We built
346 ENMs with the same bioclimatic variables and in the same bounding box as the analyses of
347 niche differentiation. We used occurrence records from our tissue collection sites and 10,000
348 background points. We excluded threshold and hinge features during the model building
349 process as the preliminary models that included these features tended to be overspecified. We
350 used five-fold cross-validation to assess model performance; data were randomly partitioned
351 into five equal groupings and 80% of data were used for training and 20% were used for
352 evaluation. Model predictions are a mean of the five cross-validated models.

353 We evaluated models using AUC and sensitivity, which were calculated using the
354 withheld dataset. AUC characterizes model discrimination ability and ranges between 0 and 1,
355 with higher values indicating greater model performance and a value of 0.5 indicating that model
356 discrimination is no better than random (Phillips and Dudík 2008). Sensitivity quantifies the
357 proportion of correctly identified positives. We calculated sensitivity where the sum of the true
358 positive rate and true negative rate was maximized (threshold = 0.53). We then used the
359 variable permutation importance and percent contribution to identify which environmental
360 variable had the greatest contribution to habitat suitability. We also visualized response curves
361 of each environmental variable to ensure models were not overspecified.

362 To test for divergence in germination behavior during range expansion, we focused on
363 warm season temperature since it most strongly affected predicted habitat suitability in the
364 ENM. Populations from early in the invasion had high predicted habitat suitability and higher
365 warm season temperatures (all above 20°C) compared to those from later in the invasion, which
366 had lower predicted habitat suitability and lower warm season temperatures (all below 20°C;
367 Fig. 6A,B). The other environmental variables in our ENM did not differ consistently between
368 early versus late in invasion (Figure S5). Guided by these results, we divided the 14 populations
369 for which we had seed collections into two groups: early (n=8) versus late (n=6) in invasion and
370 exposed them to five temperature regimes (see below).

371

372 **Tests for differentiation in germination behavior during range expansion**

373 We collected seeds from 14 populations (8 – 24 maternal families/pop) in 2019 (Table
374 S6). Seeds were collected from individuals that were at least 5 meters apart. We stored and
375 after-ripened seeds in an indoor environment for two years prior to the germination experiment
376 (Wicks and Derscheid 1958). Seeds were pooled within populations prior to applying
377 treatments. We were unable to collect seeds from every population used in the population
378 genetic survey because some had already dispersed seeds prior to our collection effort.

379 We examined the effects of temperature regime and source geographic region (early vs.
380 late in invasion) on germination. Five temperature regimes were designed to mimic the full
381 range of variation in daytime and nighttime temperatures during the spring and summer in this
382 region (14 hour day /10 hour night periods: 15/5 °C, 20/10 °C, 25/15 °C, 30/20 °C, and 35/25
383 °C). The experiment was conducted in five successive rounds in two growth chambers
384 (Conviron Inc.). We conducted each temperature regime twice – i.e., once in each growth
385 chamber - to control for growth chamber effects.

386 For each round, we placed ten seeds per population in 60 x 15mm polystyrene Petri
387 dishes containing 2 mL sterile distilled water, lined with one Whatman #1 filter paper, and
388 sealed with parafilm. Each population was replicated three times per chamber for a total of 42
389 dishes per treatment per chamber or 84 dishes per round. Dishes were wrapped in aluminum
390 foil to block light, which can inhibit germination (Selleck et al., 1962). Every 24 hours we
391 recorded the number of germinated seeds (emergence of radicle) per dish. After each treatment
392 period ended, we tested whether ungerminated seeds were viable by soaking bisected seeds in
393 a 1% Tetrazolium solution for 24 hours (Verma and Majee 2013). Red staining of tissues
394 indicates that seeds are viable. For analyses of germination, we included the number of
395 germinated seeds out of the total number of germinated plus viable (but ungerminated) seeds.

We tested for the effects of temperature regime (categorical), source geographic region, and their interaction on germination probability using a mixed-effects model with a binomial family. Experimental round and population were included as random effects. We evaluated significance with Type III tests. All models were run using the 'mixed' function in the 'afex' package (Singmann et al. 2016) in R v.4.0.2 (R Development Core Team, 2015). We used linear contrasts to test for differences in germination between geographic regions for each temperature regime category individually.

Results

Population genetic consequences of range expansion

Analysis of molecular variance (AMOVA) revealed significant partitioning of genetic variance among populations (13.7%; $P < 0.001$), among individuals within populations (8.4%; $P < 0.001$), and within individuals (77.8%; $P < 0.001$) (Table S2). No clonal genotypes were detected in the dataset. STRUCTURE indicated that the optimal number of clusters was three (K = 3) (Table S3). All individuals were assigned primarily to one cluster regardless of where the population was found in the invasive range (i.e., core, early expansion, late expansion, invasion front). There was some evidence of population structure in the other two clusters; however, there was no clear geographic pattern (Figure 2).

The PCA did not indicate substantial population structure across the invaded range based on either population or landscape samples. For the population samples, there was some evidence of differentiation among three populations in or near the range core (Figure 3; PC1 and PC2 explained: 6.8% and 5.9% of variance, respectively). The PCA of landscape samples did not reveal a relationship between genetic similarity and geography over the time course of range expansion (Figure 3; PC1 and PC2 explained 1.7% and 1.3% of variance, respectively).

There was no evidence of isolation by distance (IBD) for either population ($R^2 = 0.02$; $P = 0.418$; Table 2; Table S4) or landscape samples ($R^2 = 0.05$; $P = 0.676$; Table S4). We also did

not detect IBD when landscape samples were subset according to the four invasion phases that we defined (Figure 4).

Range expansion from the core area of invasion was accompanied by only modest changes in genetic diversity. Heterozygosity declined modestly from the core to the invasion front, as indicated by a significant interaction of latitude and longitude ($P = 0.013$; Table 3). However, the number of private alleles, Tajima's *D*, and the inbreeding coefficient did not change over the course of range expansion (Table 3).

Niche differentiation during range expansion

The climatic niche expanded during invasion. Relative to the range core, the early expansion niche represented a sizable increase in niche breadth (Figure 5A; Table S5; overlap = 0.43; $P < 0.01$; expansion = 0.37; $P < 0.01$; stability = 0.63; $P < 0.01$). When comparing the early expansion and late expansion niches, there was similar evidence for a niche shift (Figure 5B; Table S5; overlap = 0.36, $P < 0.01$; expansion = 0.39, $P < 0.01$; stability = 0.61, $P < 0.01$). Between the late expansion niche and the invasion front, the null hypothesis of niche equivalency was not rejected (Table S5); the invasion front niche was contained within the late expansion niche (Figure 5C). When comparing range core to invasion front, there was near-zero niche overlap (Figure 5D; Table S5; overlap = 0.03, $P < 0.01$; stability = 0.06, $P < 0.01$) and high expansion (Table S5; expansion = 0.94, $P < 0.01$). Niche differences between the core and invasion front were most apparent along environmental axes related to temperature of the warmest quarter and minimum temperature of the coldest month, rather than precipitation (Figure S4).

Ecological niche model

The model AUC (0.79) and sensitivity (0.75) metrics indicated moderately high discrimination and accuracy (Figure 6A). The variable response curves indicated the ENM was

not overspecified (Figure S5). The mean temperature of the warmest quarter constituted the most important variable for predicted habitat suitability (percent contribution = 81.5%; permutation importance = 67.8%) and warmer temperatures were largely associated with an increase in habitat suitability (R = 0.67; Figure S6). The minimum temperature of the coldest month had the second highest importance (percent contribution = 11.2%; permutation importance = 17.9%) and was modestly associated with increased habitat suitability (R = 0.31; Figure S6). Precipitation of the warmest quarter had the lowest importance (percent contribution = 7.3%; permutation importance = 14.3%) and was weakly correlated with habitat suitability (R = 0.02; Figure S6).

Populations from early versus late in invasion were distinct along the mean temperature of the warmest quarter axis with a disjunction at 20°C. This temperature also distinguished highly suitable from less suitable habitat in the ENM (Figure 6A & 6B).

Evolution of germination behavior during range expansion

Populations from early versus late in invasion responded differently to temperature regimes (source geographic region x temperature regime interaction: P = 0.003; Table S7; Figure 6C). Although linear contrasts between geographic regions did not differ significantly for any particular temperature treatment regime, there was a trend toward populations from late in invasion having a lower germination probability in the lower temperature treatments (Table S8). Germination probability increased with temperature for both geographic regions and plateaued (P < 0.001; Table S7; Figure 6C).

Discussion

Rapid evolution is increasingly recognized as an important process contributing to the range expansion of invasive species (Prentis et al. 2008; van Boheemen et al. 2019; Clements and Jones 2021). However, our understanding of the temporal and spatial scale over which

niche and trait divergence contribute to invasion at leading range edges remains unresolved. Our study took advantage of a well-documented invasion history to synthesize the consequences of recent range expansion for population genomic diversity, niche breadth, and germination behavior, a trait important in the colonization of new habitats. We found that leafy spurge populations experienced only modest losses in sequence diversity over the chronosequence of invasion. Range expansion involved climatic niche expansion and ecological niche models suggested that warm season temperature had the strongest influence on habitat suitability. Populations differentiated in germination behavior in response to temperature, with leading edge populations having increased dormancy at low temperatures. Our results suggest that evolution during range expansion may be important to consider in the development of models forecasting range shifts under current and future climates.

Loss of genetic diversity during range expansion may have fitness consequences and limit adaptive capacity (Lee 2002; Dlugosch and Parker 2008; Clements and Jones 2021). We found that range expansion was only accompanied by minimal losses in heterozygosity but no changes in inbreeding coefficient or the number of private alleles. Prevalent long-distance dispersal from the expanding core to the invasion front could reintroduce allelic variation lost due to bottlenecks in the colonization process. Historical data on leafy spurge range expansion suggests that long-distance, anthropogenic dispersal was likely occurring throughout the invasion process (Selleck et al., 1962; Dunn, 1979). Admixture among independent introductions could also contribute to within-population diversity, especially when there is genetic divergence between sources of introductions (Keller and Taylor 2008; Uller and Leimu 2011). We observed some divergence in PCA space among several populations in or near the range core, with populations from the remainder of the range occupying intermediate PCA space. These patterns similarly suggest that gene flow has been substantial among populations despite introduction less than 140 years ago. Leafy spurge is an auto-allohexaploid, and the minor allele frequency spectrum revealed a peak at 0.33, indicating that homoeologous variants

could confound inferences. By filtering out variants with a minor allele frequency (MAF) > 0.26, we avoided the possibility of detecting spurious population structure, but we also lost some power given that some higher MAF variants may be truly allelic. As such, we believe that our approach was conservative, but not overly so, given the substantial biases that can arise in polyploid datasets.

In addition to gene flow during invasion, polyploidy may be an important factor influencing losses of genetic variation during colonization bottlenecks and the capacity for range expansion. Polyploids often maintain higher levels of genetic variation (Otto and Whitton 2000) and there is some evidence that phenotypic plasticity is greater in synthetically produced autopolyploids (Mattingly and Hovick 2023). These factors have been used to explain why polyploids may be better invaders than diploids (Pandit et al. 2011). Leafy spurge is an auto-allohexaploid, suggesting that its higher N_e should facilitate the maintenance of genetic diversity within populations and minimize divergence among them. Polyploidy has also been suggested to increase the capacity for adaptive evolution (Otto and Whitton 2000), which could also contribute to success as an invader. It is important to recognize that losses of diversity in DNA sequences do not necessarily translate to losses of variation in quantitative traits (Reed and Frankham 2001). Nevertheless, it is possible that polyploidy contributed to rapid invasion in leafy spurge, but more work is needed to distinguish the contribution of polyploidy from other factors.

Accumulating evidence suggests that invasive plant species frequently undergo climatic niche shifts during range expansion (Medley 2010; Atwater et al. 2018; van Boheemen et al. 2019; Bates and Bertelsmeier 2021); but see (Petitpierre et al. 2012; Liu et al. 2020). Consistent with past findings, we observed climatic niche expansion throughout most of range expansion, except from the late expansion region to the invasion front. From an ENM, we found that warm season temperature had the strongest influence on habitat suitability and therefore may be one source of divergent selection from range core to invasion front. Of course, climate change has

already caused poleward shifts in plant species distributions through climatic niche matching (Parmesan 2006; Clements and Ditommaso 2011; Parmesan and Hanley 2015). Therefore, some range expansion may simply involve dispersal to already climatically suitable habitats. However, adaptation may be necessary for continued range expansion, especially where the species is already at its climatic niche limit (Clements and Ditommaso 2011). In leafy spurge, minimal range expansion has been observed in the last 30+ years and populations remain very small/low density at the invasion front, suggesting that populations have reached climatic niche limits. Moreover, ENMs suggest that invasion front populations have very low habitat suitability. Therefore, our results are inconsistent with the hypothesis that the leading edge is highly suitable but expansion is limited by dispersal. Instead, our results suggest that responses to divergent selection may be important for persistence at the leading range edge and for further range expansion.

Divergence in trait expression at a leading range edge can be driven by local adaptation, phenotypic plasticity, and/or maternal environmental effects (Des Roches et al. 2017; Westerband et al. 2021). Phenotypic plasticity is considered important during early stages of invasion because it allows introduced populations to establish in a broader range of environmental conditions (Sexton et al. 2002; Richards et al. 2006; Funk 2008; Lande 2015). In germination traits, plasticity could represent a means of habitat selection and niche construction (Donohue 2003, 2005) by which leafy spurge in the leading edge germinates optimally at the onset of spring conditions (warmer temperatures in northern latitudes). Likewise, rapid evolution during range expansion can result from selection on loci that influence dormancy and/or germination timing (Clements and Ditommaso 2011; Hodgins et al. 2018; Clements and Jones 2021). We found increased dormancy at lower temperatures in leading edge populations. One possibility is that germination at colder temperatures exposes seedlings to more unpredictable environments (e.g., late season frost) and thus that selection favored germination later in the season for leafy spurge at its northern range limit. Interestingly, other work has suggested that

552 reduced dormancy evolves at leading range edges (Tabassum and Leishman 2018), contrary to
553 our findings. Although warm season temperature is most strongly associated with habitat
554 suitability and relevant to germination in leafy spurge, it is also possible that other variables
555 influenced the evolution of germination behavior. Overall, our results suggest that range
556 expansion involved niche expansion and that the evolution of germination timing may have been
557 important in establishment at the leading range edge.

558 Early life history transitions are thought to be under strong selection because of their
559 cascading effects on later life stages (Baskin and Baskin 1971; Marks and Prince 1981;
560 Donohue 2002, 2005). In plant populations, the environmental conditions at the time of
561 germination can alter the strength and direction of natural selection on postgermination traits
562 (Donohue et al. 2010; D'Aguillo and Donohue 2023). In turn, this can affect the competitive
563 environment, resource availability, and density-dependent selective agents experienced by
564 populations (Donohue et al. 2010). While we did not investigate postgermination traits, it would
565 be valuable to investigate whether germination timing in leafy spurge influences performance at
566 later life history stages, especially in leading edge populations.

567 Our results suggest that even over the course of a fairly rapid invasion losses of
568 genomic variation may be minimal. In leafy spurge, this may have occurred because of
569 substantial gene flow during invasion and/or polyploidy. Regardless of the mechanism, higher
570 levels of genetic variation can challenge management when genotypes vary in their responses
571 to eradication measures (Gaskin et al. 2020). We also found that trait divergence may have
572 contributed to climatic niche expansion and thus to the spatial extent of invasion. Forecasts of
573 continued invasion typically rely on species distribution models (SDMs), which rarely take into
574 account evolution. As such, models may fail to predict the complete extent of range expansion,
575 or the severity of range infilling. Evolution-free SDMs are likely still valuable for management
576 planning over meaningful spatial and temporal scales in many systems. However, in systems

where local adaptation is extensive, forecasts of range shifts with climate change may require the construction of regional SDMs that account for evolution.

Tables

Table 1. Locality information and genetic diversity metrics (observed heterozygosity, Ho; inbreeding coefficient, G_{is}; number of private alleles, and Tajima's D) for 14 leafy spurge population samples from four phases of range expansion (see Figure 1).

Range Position	Population Genetic Samples	Latitude	Longitude	Observed Heterozygosity (Ho)	Inbreeding Coefficient (Gis)	Private Alleles	Tajima's D
Core	Blue Earth	44.16	-94.09	0.068	0.123	27	-0.037
	Lyon	44.33	-95.82	0.067	-0.017	9	-0.020
	Meeker	44.94	-94.64	0.07	-0.017	4	0.205
Early Expansion	Becker	46.88	-96.05	0.068	0.116	10	-0.052
	Big Stone	45.52	-96.55	0.067	0.127	9	-0.028
	Polk	47.75	-96.25	0.07	0.097	11	-0.058
Late Expansion	Aitkin	46.98	-93.72	0.068	0.138	27	0.122
	Anoka	45.29	-93.13	0.071	-0.026	7	0.101
	Crow Wing	46.38	-94.22	0.065	0.081	8	0.001
	Winona	44.04	-91.62	0.071	0.156	42	-0.167
Invasion Front	Duluth	46.76	-92.11	0.066	0.12	23	-0.034
	Koochiching	48.60	-93.40	0.066	0.089	10	-0.008
	Pine	46.04	-92.36	0.069	0.138	21	0.000
	St. Louis	47.72	-91.97	0.065	0.046	6	0.068

Table 2. Pairwise estimates of genetic differentiation measured as G_{st} for 14 leafy spurge populations from four phases of range expansion (see Figure 1). Darker versus lighter shading of cells indicates higher versus lower values of pairwise G_{st}.

		Blue Earth	Lyon	Meeker	Becker	Big Stone	Polk	Aitkin	Anoka	Crow Wing	Winona	Duluth	Koochiching	Pine	St. Louis
Core	Blue Earth														
	Lyon	0.176													
	Meeker	0.215	0.266												
Early Expansion	Becker	0.126	0.173	0.208											
	Big Stone	0.125	0.171	0.209	0.112										
	Polk	0.118	0.171	0.205	0.115	0.119									
Late Expansion	Aitkin	0.124	0.187	0.216	0.126	0.127	0.127								
	Anoka	0.187	0.243	0.277	0.177	0.198	0.185	0.197							
	Crow Wing	0.134	0.188	0.219	0.116	0.128	0.126	0.136	0.201						
	Winona	0.084	0.134	0.181	0.081	0.084	0.081	0.095	0.153	0.096					
Invasion Front	Duluth	0.126	0.163	0.203	0.110	0.116	0.118	0.128	0.179	0.124	0.080				
	Koochiching	0.136	0.197	0.225	0.139	0.138	0.139	0.152	0.213	0.153	0.099	0.137			
	Pine	0.132	0.188	0.204	0.122	0.128	0.130	0.138	0.201	0.135	0.094	0.121	0.153		
	St. Louis	0.178	0.240	0.249	0.169	0.177	0.178	0.177	0.252	0.124	0.143	0.171	0.199	0.182	

Table 3. ANOVA testing for a relationship between four metrics of sequence diversity and geography (latitude, longitude, and their interaction) for 14 population samples. Bold indicates p-value less than 0.05.

Model	Heterozygosity (Ho)			Private Alleles			Tajima's D			Inbreeding (Gis)		
Term	Df	F	P	Df	F	P	Df	F	P	Df	F	P
Latitude	1	2.37	0.158	1	0.07	0.803	1	0.24	0.636	1	1.41	0.266
Longitude	1	0.48	0.506	1	1.29	0.285	1	1.22	0.298	1	0.04	0.846
Latitude x Longitude	1	9.49	**0.013**	1	0.84	0.382	1	0.02	0.887	1	0.48	0.508

Figures

Figure 1. Map of the invasion history and tissue collection sites for 14 population samples and 157 landscape samples of leafy spurge. The invasion history was derived from Hanson & Rudd (1933) and used to delineate four phases of invasion: core, early expansion, late expansion, and invasion front.

Figure 2. Cluster assignment probability from STRUCTURE analyses (K = 3) for population and landscape samples. Each bar represents one individual, and populations are separated by black lines.

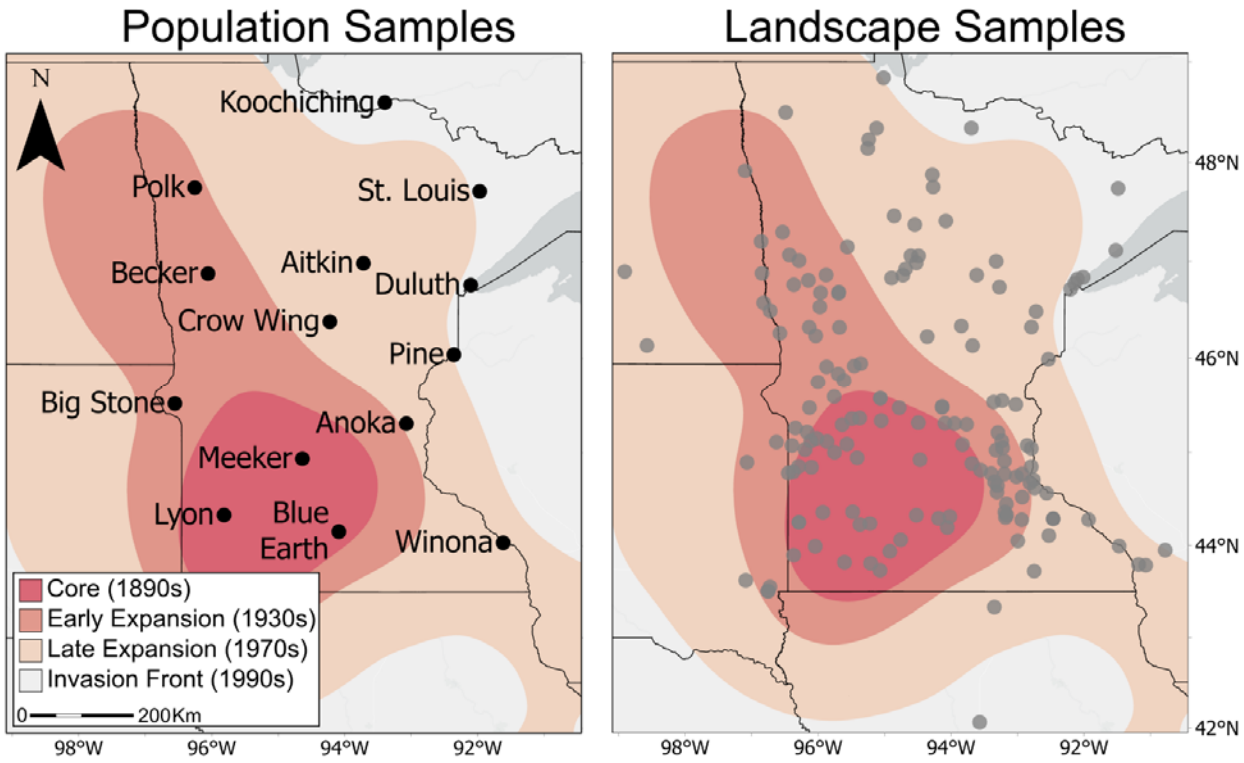
Figure 3. Principal components analysis (PCA) bi-plots for population genomic data from 14 population samples (n = 6 per population) and 157 landscape samples (n = 1 per population).

Figure 4. Isolation by distance (IBD) displayed as scatterplots of genetic distance versus geographic distance for A) population samples (A) and B-E) landscape samples (B-E). For landscape samples, we subset individuals for analyses by successive stages of invasion: B) range core (B), C) range core plus early expansion (C), D) range core, early, plus late expansion ranges (D), and E) all samples from across the four phases (E).

Figure 5. Niche overlap during range expansion in climate niche space. The extent of the background environment is outlined in black (solid = total niche space; dashed = 90% of extent). A) Core (dark pink) versus early expansion (light pink), B) Early expansion (light pink) versus late expansion (beige), C) Late expansion (beige) versus invasion front (grey), D) Core (dark pink) versus invasion front (grey). In all panels, the arrow represents the direction of shift in the centroid of niche space.

Figure 6. Ecological Niche Model (ENM) habitat suitability projections for seed source populations and germination probability for source regions. A) Habitat suitability projection from leafy spurge ENM. Predicted habitat suitability ranges from 0 (purple) to 1 (yellow). The red line demarcates the boundary of 20°C for mean temperature of the warmest quarter. Seed source populations for the germination experiment are marked and colored yellow (>20°C) or purple (>20°C) depending on their mean temperature of the warmest quarter. B) Variable response curve for the mean temperature of the warmest quarter. The vertical red bar denotes 20°C. Vertical yellow bars correspond to seed source populations from the warmer south and vertical purple bars correspond to source populations from the cooler north. C) Mean germination probability (± SE) by temperature treatment (X axis) for seed source regions. The warmer southern region is shown in yellow and the cooler northern region is shown in purple.

663 Figure 1.



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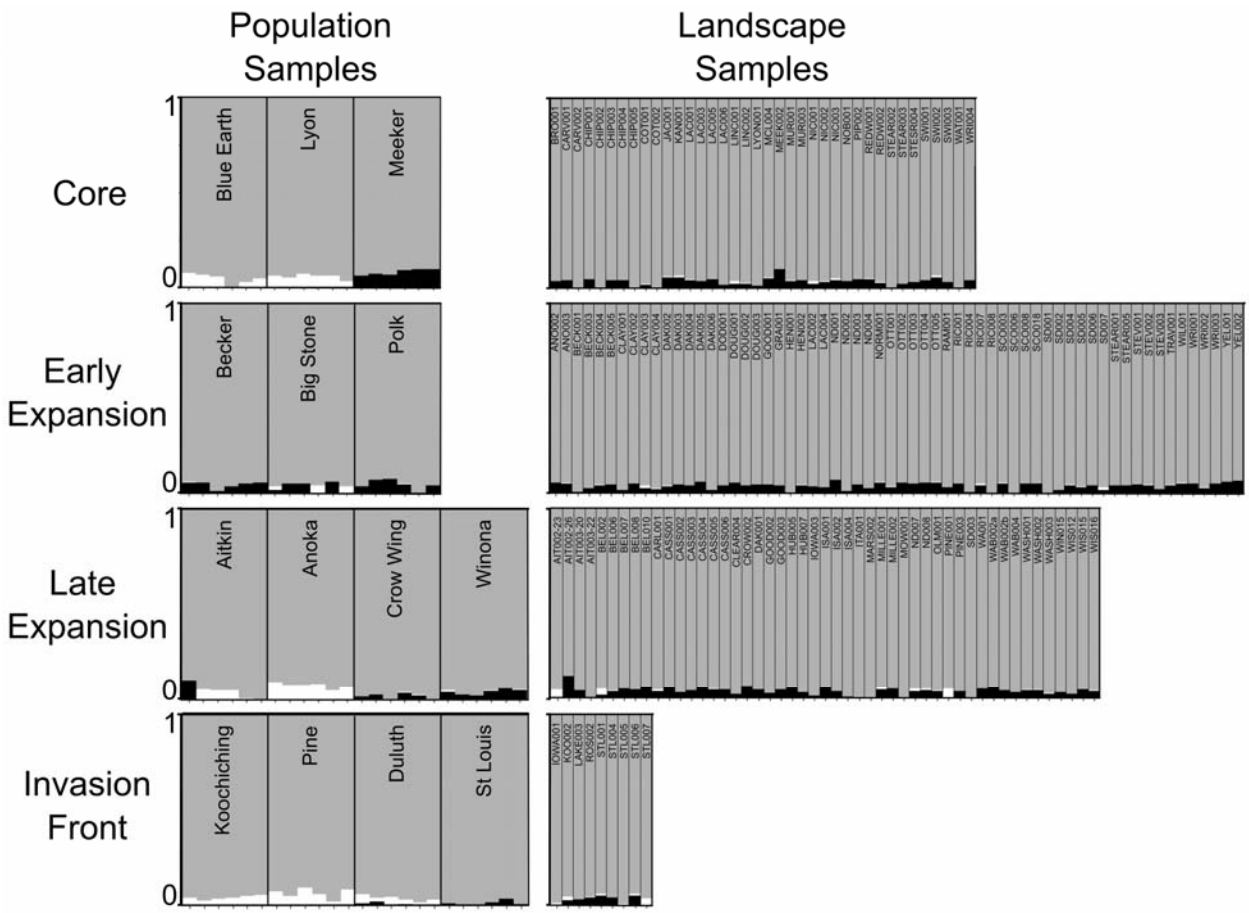

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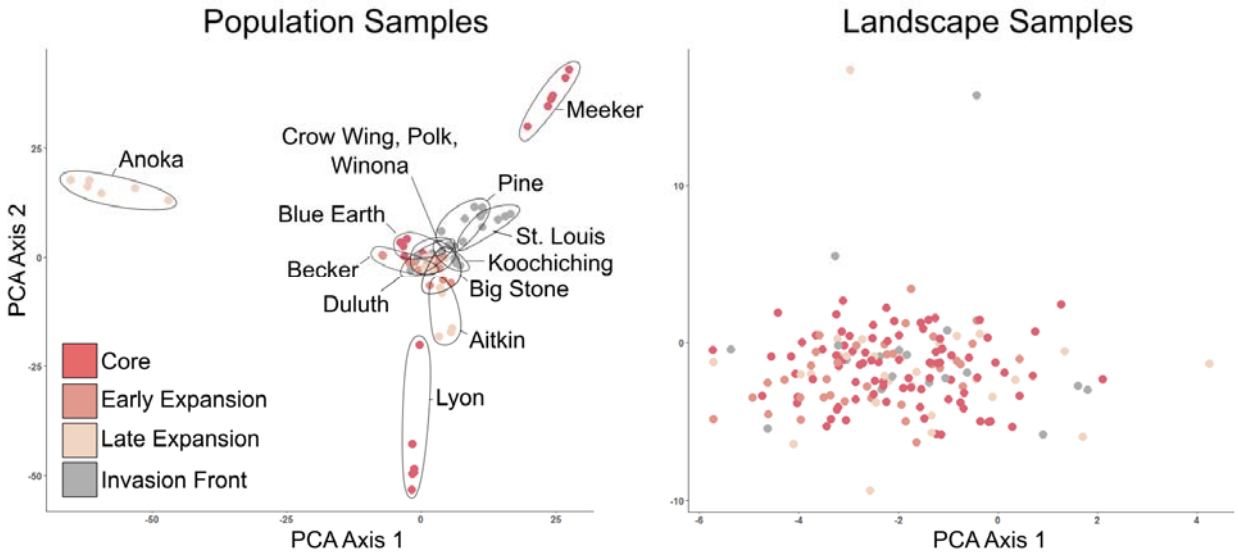

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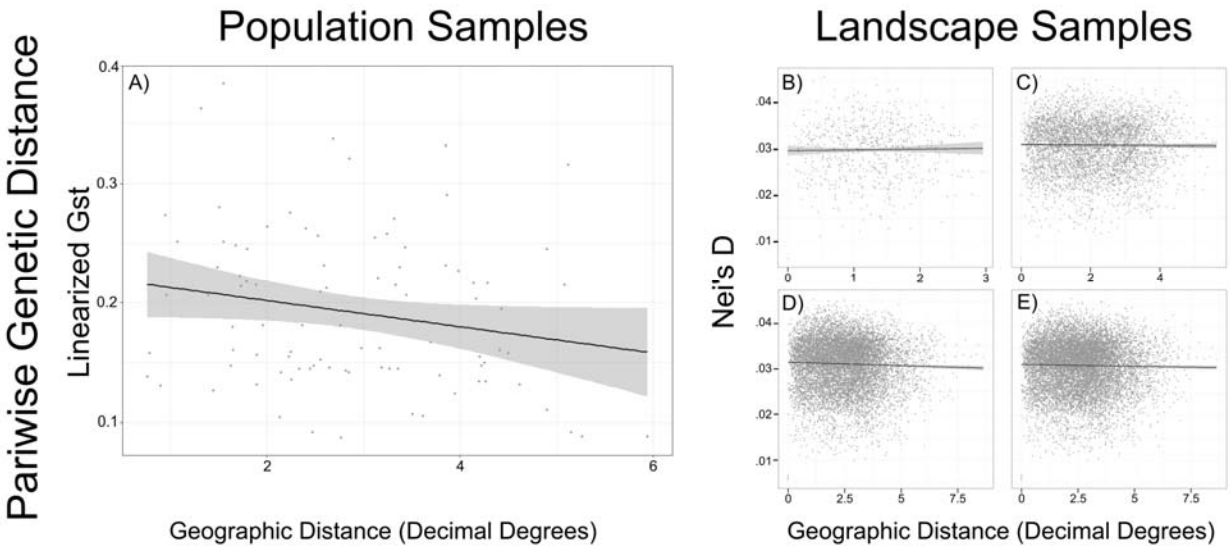

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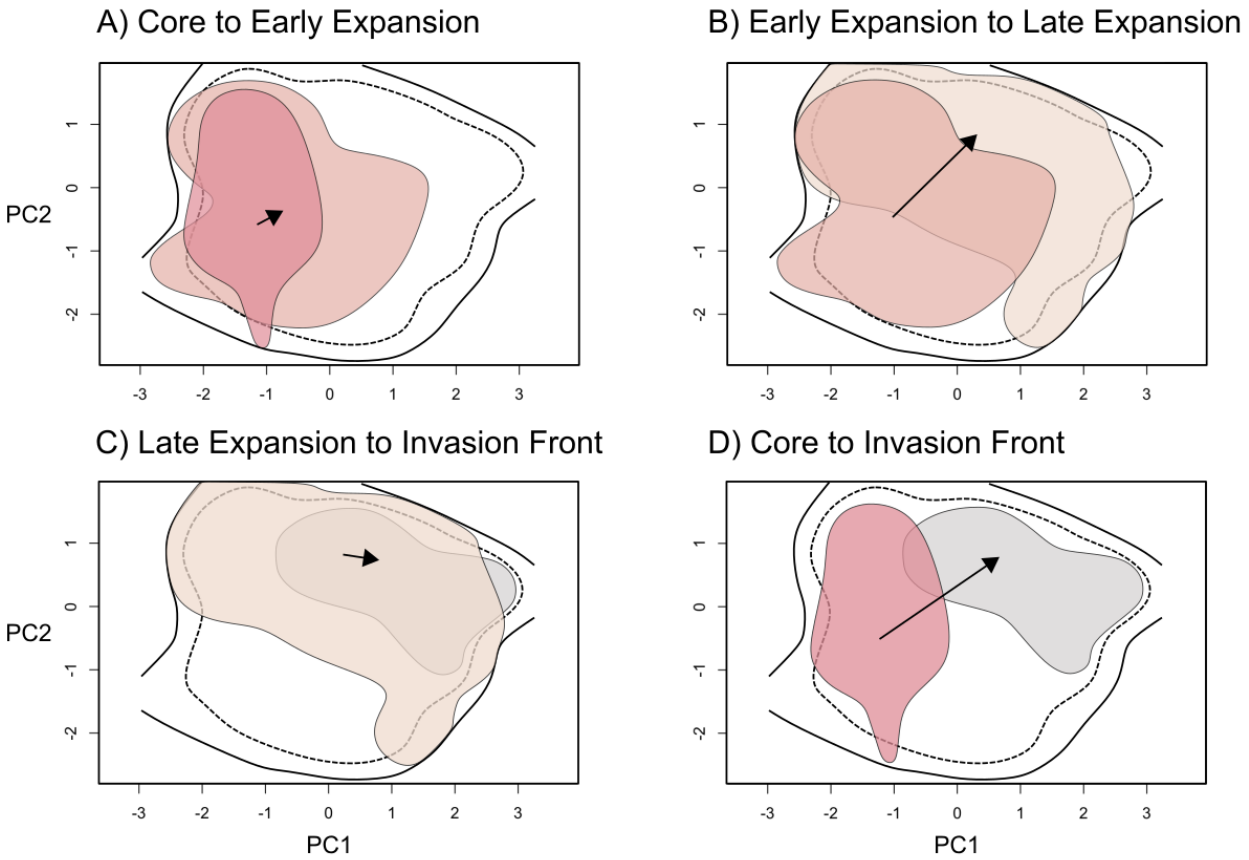

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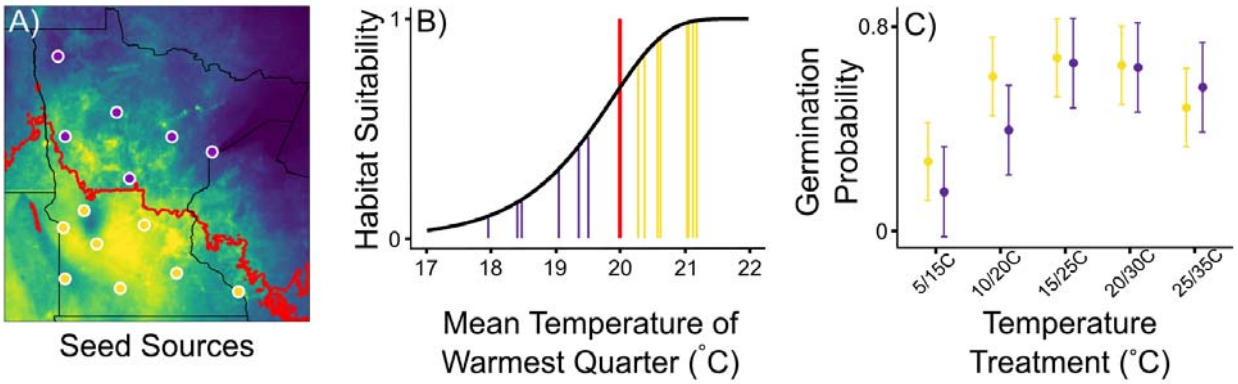
728 Figure 6.



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Statements and Declarations

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