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A lack of population structure characterizes the invasive *Lonicera japonica* in West Virginia and across eastern North America[1,2]

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Abstract. Invasive plant species cause massive ecosystem damage globally yet represent powerful case studies in population genetics and rapid adaptation to new habitats. The availability of digitized herbarium collections data, and the ubiquity of invasive species across the landscape make them highly accessible for studies of invasion history and population dynamics associated with their introduction, establishment, spread, and ecological interactions. Here we focus on *Lonicera japonica*, one of the most damaging invasive vine species in North America. We leveraged digitized collections data and contemporary field collections to reconstruct the invasion history and characterize patterns of genomic variation in the eastern USA, using a straightforward method for generating nucleotide polymorphism data and a recently published, chromosome-level genome for the species. We found an overall lack of population structure among sites in northern West Virginia, USA, as well as across sites in the central and eastern USA. Heterozygosity and population differentiation were both low based on FST analysis of molecular variance, principal components analysis, and cluster-based analyses. We also found evidence of high inbreeding coefficients and significant linkage disequilibrium, in line with the ability of this otherwise outcrossing, perennial species to propagate vegetatively. Our findings corroborate earlier studies based on allozyme data, and suggest that intentional, human-assisted spread explains the lack of population structure, as this species was planted for erosion control and as an ornamental, escaping cultivation repeatedly across the USA.

Key words: genomics, honeysuckle, invasion genomics, invasion history, population genetics, SNP

Invasive species cause billions of dollars in damage to habitats in the USA and around the globe (Simberloff 2013). Yet, they provide important case studies in ecosystem dynamics and rapid evolution to new environments (Lawson-Handley *et al*. 2011). Traditional understanding of genetic diversity within invasive species focused on single introductions, subsequent genetic bottlenecks, and hypothesized low genetic diversity in the invasive range compared to that in the native range (see Tsutsui *et al*. 2000, Lee 2002, Frankham 2005, Estoup *et al*. 2016). While this was the case for many invasive species, the application of molecular markers has consistently identified similar or even higher levels of genetic variation in invasive populations compared to those in the native range (*e.g.,* Frankham 1997, Kolbe *et al*. 2004). More recently, many studies have identified multiple introductions over space and time in invasive species, including admixture among originally isolated allele pools, and "bridgehead" introductions, whereby invasions occur in successive stages across regions or continents (Dlugosch and Parker 2008, Keller and Taylor 2010, van Boheemen *et al*. 2017, Vallejo-Marín *et al*. 2021). Thus, the picture emerging from molecular genetic studies of invasive species is often more complex than "traditional" hypotheses of invasion, and represents the interplay between history, dispersal, breeding system, source and recipient habitats, and several other factors (Sakai *et al*. 2001; Sutherland *et al*. 2021).

Information from digitized collections databases provides a useful tool for reconstructing invasion

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routes and history, and trait variation over space and time, while physical collections provide genomic resources for spatiotemporal analysis of variation (*e.g.,* Gallinat *et al*. 2018, Barrett *et al*. 2022, Bieker *et al*. 2022, Heberling 2022). In parallel, advances in genomic sequencing (RAD-seq, GBS, low-coverage whole genome sequencing, sequence capture, multiplexed amplicon sequencing) provide increased power over previous methods (allozyme variation, organellar gene/spacer sequencing, microsatellites) for studies of genetic variation and population structure, with broader representation of the genome for detecting both neutral and adaptive variation (Chown *et al*. 2015, Hamelin and Roe 2020, North *et al*. 2021). In plant biology, many of these technological advances have focused on crops or threatened/endangered species; relatively fewer have focused on invasive species (Barrett 2015, Hohenlohe *et al*. 2020, North *et al*. 2021).

Lonicera japonica Thunb. is one of the most aggressive, invasive vines in North America, yet is surprisingly not well studied from a genetic perspective across its globally invasive range, including North America (Schierenbeck 2004). This species forms dense mats, climbs trees and shrubs, outcompetes native vines and understory species, and causes tree mortality (Leatherman 1955, Evans 1984, Hardt 1986, Dillenberg *et al*. 1993). Traditionally, this species has been planted as a means of erosion control and as an ornamental, from which it is hypothesized to have escaped cultivation repeatedly. *Lonicera japonica* is highly attractive to diverse pollinators, with large nectar rewards, and the seeds are dispersed locally by birds and mammals, and by humans over greater distances (Luken 1996). *Lonicera japonica* is an obligate outcrosser but also propagates vegetatively by rerooting from stems, forming clonal ramets in many places (Leatherman 1955). At least 12 cultivars are known, but "Hall's honeysuckle" is believed to be the most common and prolific, and further hypothesized to be the major player in invasion across the USA (Schierenbeck 2004).

Studies based on allozyme electrophoresis revealed low levels of genetic diversity in the southeastern USA (Schierenbeck *et al*. 1995, Schierenbeck 2004), yet genome-scale data and analysis are yet to be applied to quantify patterns of diversity in this species. A chromosome-level genome was recently published (Pu *et al*. 2020), providing a powerful resource for population genomics. The genome assembly consisted of nine chromosome-level scaffolds, and was 843.2 Megabases in length, of which ~60% was composed of repetitive elements, including over 180,000 microsatellite regions. In addition, economical, technologically straightforward methods have recently been published based on sequencing of inter-simple sequence repeat amplicons (ISSR) for the generation of single nucleotide polymorphisms (SNPs), which use microsatellite DNA as priming sites (*e.g.,* MIG-seq, Suyama and Matsuki 2015; and ISSR-seq, Sinn *et al*. 2021). These methods allow technically straightforward, PCR-based genome-scale assessments of population-level variation that were not possible previously. Thus, the tools and resources for the study of invasion genomics are now at hand for numerous species, including *L. japonica*.

Our objectives were two-fold: 1) Mapping the invasion history of *L. japonica* in the USA using digitized herbarium specimen information, and 2) quantifying contemporary patterns of genetic diversity and population structure across the eastern USA using genomic data. We sampled 166 individuals across 16 localities in eastern North America (with a focus on northern West Virginia), and employed a straightforward, amplicon-based protocol (MIG-seq, or multiplexed inter-simple sequence repeat genotyping) to quantify genomic variation in *L. japonica*. Our analysis yielded >1,500 SNPs and revealed an overall lack of population structure for this invasive species in the eastern USA, suggesting a highly admixed gene pool.

Materials and Methods. RECONSTRUCTING INVASION HISTORY WITH HERBARIUM RECORDS. We created an animation using database records from herbarium specimens collected over the past two centuries. Specimen information was accessed through the Global Biodiversity Information Center (GBIF 2023) with the R package rgbif v.3.7.5 (Chamberlain *et al*. 2023), and the animation was created in R following Barrett *et al*. (2022). A static representation was also created across six time slices of 30 years each, from 1880–2020 (except for the latter slice, which was 20 years from 2000–2020). Code for the animation and static maps (and the figures themselves) can be accessed via GitHub (Supplemental Material, File S1).

Table 1. Sampling locality and basic diversity information. N = sample size, *Fis* = inbreeding coefficient, *Ia* and *r-barD* = metrics of linkage disequilibrium with associated *P*-values based on 999 permutations (*P*-values < 0.05 are in boldface).

Locality	Latitude	Longitude	N	*Fis*	*Ia*	*P-value*	*r-barD*	*P-value*
Allegheny, PA	40.53315	−79.781593	6	0.663	1.015	0.76	0.010	0.67
Arboretum, Monongalia, WV	39.644729	−79.976635	54	0.772	3.333	**0.01**	0.009	**0.01**
Cheat Lake, Monongalia, WV	39.6715	−79.847774	14	0.71	1.192	0.91	0.006	0.84
Deckers Creek, Monongalia, WV	39.628758	−79.949796	10	0.716	0.982	0.69	0.007	0.61
Durham, NC	36.024027	−78.924629	6	0.712	2.003	0.26	0.017	0.22
Fayette, PA	39.797474	−79.794005	3	0.637	2.031	0.17	0.023	0.17
Jackson, WV	38.82314	−81.719958	3	0.589	7.534	**0.02**	0.076	**0.02**
Lawrence, PA	41.02137	−80.446194	6	0.687	1.597	**0.04**	0.012	**0.03**
LittleFalls, Monongalia, WV	39.553667	−80.015538	8	0.693	0.855	0.62	0.006	0.55
Life Sciences Bldg., Monongalia, WV	39.63786	−79.95392	5	0.611	0.798	0.1	0.007	0.08
Morgantown, Monongalia, WV	39.645545	−79.980235	20	0.736	5.659	**0.01**	0.018	**0.01**
Oktibbeha, MS	33.450835	−88.918471	3	0.644	−0.964	0.97	−0.012	0.96
Preston, WV	39.65972	−79.791026	7	0.663	3.832	**0.02**	0.019	**0.01**
Shelby, TN	35.143333	−89.986183	5	0.662	1.056	0.05	0.011	0.04
Star City, Monongalia, WV	39.683194	−79.961472	8	0.689	1.618	**0.04**	0.012	**0.04**
Uffington, Monongalia, WV	39.587624	−80.002644	6	0.668	0.678	0.25	0.007	0.2

Sampling, DNA Extraction, and MIG-seq. Whole green leaves were collected from 166 individuals at 16 localities in the eastern and midwestern USA (Table 1). At each sampling site, leaf samples were collected at least 10 m apart to avoid collecting tissue from the same ramet. One individual from each locality was pressed as a voucher specimen and deposited at the West Virginia University Herbarium. A large number of individuals were collected at the West Virginia University Earl Core Arboretum and in the City of Morgantown, WV; smaller samples were collected at other localities locally in WV and more broadly across the eastern USA (Table 1). An ethanol-sterilized marker cap was used to punch an equal area of tissue (1 cm diameter) from each leaf, avoiding the midvein. The CTAB DNA extraction procedure (Doyle and Doyle 1987) was used to isolate genomic DNAs, using a modified 96-well extraction protocol. Briefly, samples were stored in 2 ml screw-cap tubes, frozen in liquid nitrogen, and pulverized with 3 mm steel bearings. DNA concentrations were measured with a plate reader (broad-range assay; Tecan Group, Ltd., Zurich, Switzerland) and diluted to 20 ng/µl in TE buffer (pH 8.0). MIG-seq amplicons were produced following the procedure in Suyama and Matsuki (2015) but modified for dual indexing. PCR conditions were as follows: 98 °C 5 min, followed by 30 cycles of 98 °C (30 sec), 48 °C (30 sec), and 72 °C (90 sec), with a final extension at 72 °C for 5 min. PCR products were then diluted 1:50 in sterile PCR water, and used in a second round of PCR to add dual-indexed barcodes (Supplemental Material, File S2). Cycle conditions were as follows: 15 cycles of: 98 °C (10 sec), 54 °C (15 sec), and 72 °C (1 min). PCR products were then quantified via NanoDrop spectrophotometry (Thermo Fisher, Waltham, Massachusetts, USA) and pooled at equimolar ratios. A single, two-sided PCR cleanup/size selection was conducted with Quantabio SparQ beads (Beverly, Massachusetts, USA) at bead to sample ratios of 0.8× and 0.56×. The resulted size-selected library pool was quantified with an Agilent Bioanalyzer (Santa Clara, California, USA) and with quantitative PCR, and sequenced at the West Virginia University Genomics Core Facility on two runs of an Illumina MiSeq using v.3 chemistry for 2 × 300 bp reads.

Read Processing, Mapping, and SNP Calling. Reads were processed using a dedicated pipeline designed for amplified ISSR fragments (Sinn et al. 2022). Briefly, reads were trimmed using BBDuk (Bushnell 2023) and mapped to an indexed reference sequence with BBMap (Bushnell 2023) (here, the *Lonicera japonica* genome, NCBI BioProject accession no. PRJNA794868; Pu *et al*. 2020). Resulting BAM alignment files were sorted, and PCR duplicates were removed with v.1.7-13 and Picard v.3.0.0, respectively (Danecek *et al*. 2021, Broad Institute 2023). BAM files were then analyzed with GATK4 v.4.2, specifically using GATK's "Best Practices" filters, using HaplotypeCaller to realign around indels (van der Auwera *et al*. 2013, Poplin *et al*. 2017, van der Auwera and O'Connor 2020). The resulting

variants, called across all samples, were output as a .vcf file. This file was further filtered on missing data (for sites and individuals) and minor allele frequencies (removing minor allele sites with frequency < 0.05) and thinned to keep only a single SNP per locus (minimum distance = 1,000 bp) with TASSEL5 v.5.0 (Bradbury *et al*. 2007) and PLINK v1.90b6.24 (Purcell *et al*. 2007).

POPULATION DIVERSITY AND STRUCTURE ANALYSES. Population genetic analyses were conducted with SambaR v.1.08 (De Jong *et al*. 2021), adegenet v.2.1.0 (Jombart 2008), hierfstat v.0.5.11 (Goudet 2005), Poppr v.2.9.3 (Kamvar *et al*. 2014), and SNPRelate v.1.32.2 (Zheng *et al*. 2012), following Sinn *et al*. (2021). Inbreeding coefficients (*Fis*) were calculated in SambaR and population differentiation (*Fst*) metrics were calculated with Poppr. Analysis of molecular variance was performed with hierfstat, testing the significance of the components of variation with 999 permutations. Principal components analysis (PCA) was conducted with SNPRelate. Heterozygosity values (observed, *Ho*, and expected, *He*) for each locality were calculated with SambaR and Poppr. Discriminant analysis of principal components (DAPC; Jombart *et al*. 2010) was conducted in adegenet, choosing "k," or the number of ancestral genomic population clusters, using the Bayesian Information Criterion to select among different k-values. The cross-validation method in adegenet was used to choose the optimal number of principal components in the analysis.

An additional population structure analysis was conducted with ParallelStructure (Besnier and Glover 2013) via the CIPRES web portal (Miller *et al*. 2010), for k = 1–8, under a correlated allele frequency model with 100,000 burn-in steps and 100,000 recorded steps for each of 10 replicates per k-value. Results were evaluated with the Evanno *et al*. (2005) "delta-k" method to select the optimal k-value, or number of ancestral population clusters. All results were evaluated with StructureHarvester (Earl and von Holdt 2012), and ancestry plots generated with Clumpak (Kopelman *et al*. 2015). A multilocus genotype network was also constructed with adegenet. Finally, a dendrogram based on Nei's genetic distance was created, grouping by locality, with the "aboot" function in Poppr, with 1,000 bootstrap pseudoreplicates. All plots were created with the ggplot2 v.3.4.1 (Wickham 2016), ggpubR v.0.6.0 (Kassambara *et al*. 2020), and pheatmap v.1.0.12 (Kolde 2023) packages for R.

Results. INVASION HISTORY. Plotting of historical herbarium records over six time slices revealed a rapid colonization of *Lonicera japonica* across the eastern USA (Fig. 1; Supplemental Material, File S3). By 1880, this species was present around New York City, in upstate New York, and in northern Virginia and Maryland. By 1910 it had spread to Pennsylvania, the Carolinas, Florida, Georgia, Arkansas, Missouri, Texas, and as far west as California (a single record, near Lake Tahoe on the Nevada border). By 1940 the spread continued in the northeastern USA, southeastern USA, midwestern USA, and within California. By 1970 it had spread northward into New England and the northern Midwest, and into southern California, Arizona, and New Mexico. By 2000 it had invaded nearly all of the eastern USA except Maine, most of California, and it had spread into Colorado, Oregon, and Idaho by 2020.

GENETIC DIVERSITY. After filtering and linkage disequilibrium thinning, 1,571 codominant SNP markers remained across 166 individuals, with 43.37% missing data (Supplemental Material, File S4). Both observed and expected heterozygosity (*Ho* and *He*, respectively) were low across all sampling localities, with a mean $Ho = 0.0319$ and $He = 0.1204$. Mean inbreeding was relatively high (overall $Fis = 0.7347$). *Ho* ranged from 0.1–0.15, while *He* ranged from 0.2–0.45, and *Ho* was lower than *He* at all sampling localities (Fig. 2). *Ho* and *He* were highest at the Morgantown, Monongalia, WV and Preston, WV localities, and lowest at the Life Sciences Building and Decker's Creek sites (Monongalia, WV) localities; these four localities are all within a 20 km radius. Analysis of linkage disequilibrium after clone correction revealed significant values of *Ia* and *r-barD* at six localities (Table 1): Arboretum (Monongalia, WV), Jackson (WV), Lawrence (PA), Morgantown (Monongalia, WV), Preston (WV), and Star City (Monongalia, WV; Table 1).

POPULATION STRUCTURE. Analysis of molecular variance revealed a lack of overall population structure, with percentages of variation between localities = 0.38%, among individuals within localities = 0.31%, and within individuals = 99.3%, though none of the components of variation was significant. Principal components analysis of SNP data revealed an overall lack of

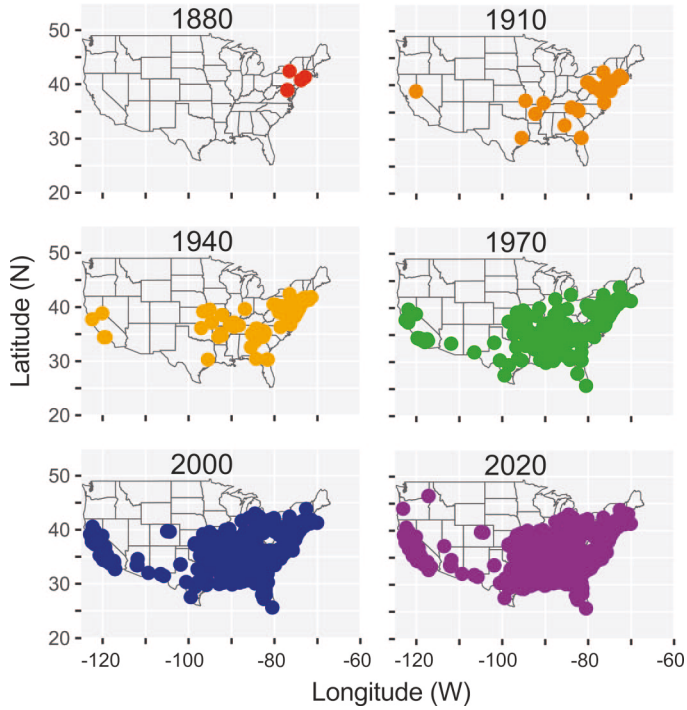

FIG. 1. Maps of six time slices showing the invasion history of *Lonicera japonica* in the USA based on herbarium records. An animated version can be found at <https://github.com/barrettlab/2021-Genomics-bootcamp/wiki/2022-Biol-320-Lonicera-japonica-invasion-history-animation>.

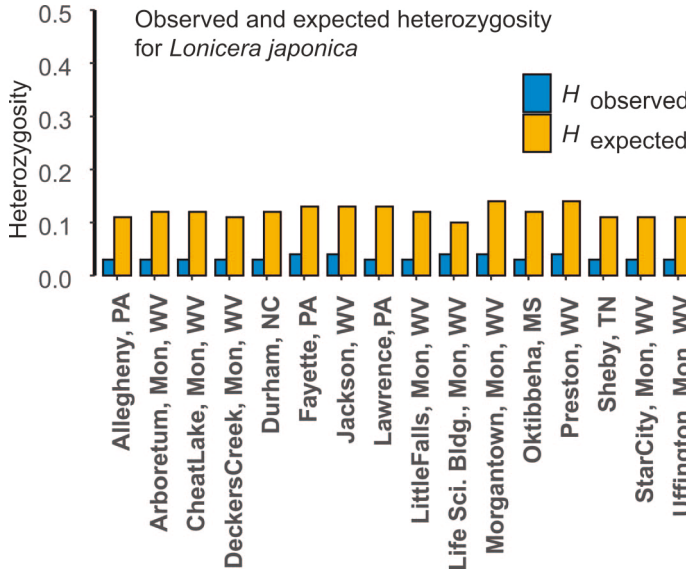

FIG. 2. Estimates of observed (*Ho*, blue) and expected heterozygosity (*He*, orange) among sampling localities.

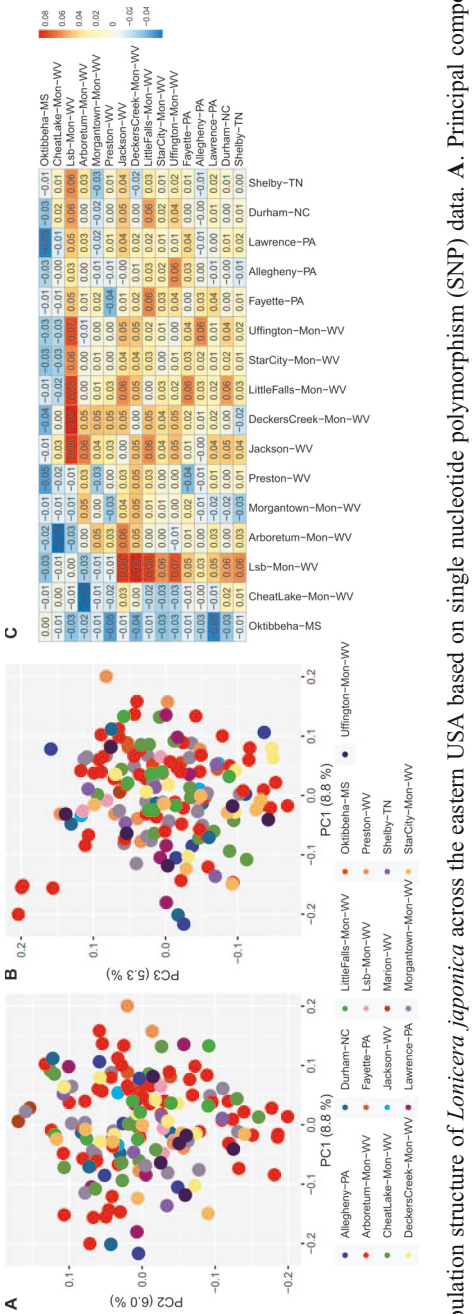

FIG. 3. Population structure of *Lonicera japonica* across the eastern USA based on single nucleotide polymorphism (SNP) data. **A**. Principal components analysis (PCA) of 1,571 linkage disequilibrium-thinned SNPs, showing PCA axes 1 and 2, and **B**. Axes 3 and 4. **C**. Pairwise *Fst* estimates among sampling localities.

clustering among sampling localities (Fig. 3A, B). Mean differentiation across localities was relatively low (overall $Fst = 0.0347$), and most pairwise comparisons of *Fst* between sampling localities ranged from 0–0.09, suggesting an overall lack of differentiation among populations (Fig. 3C). Discriminant analysis of principal components revealed an optimal number of five genomic clusters (k = 5, optimal principal components retained = 80, discriminant functions retained = 3, BIC score = 624.98; Fig. 4A–C). Discriminant Axis 1 differentiated Cluster 5 from the remaining Clusters (Fig. 3A, B), and to a lesser extent differentiated Clusters 2 and 3. Discriminant Axis 2 further differentiated Cluster 2, 3, and 5, while Discriminant Axis 3 differentiated Clusters 1 and 4 (Fig. 3C). Plotting of ancestry coefficients from the DAPC revealed an overall pattern of population admixture, with all localities except for Fayette, PA composed of $\geq$ 2 genomic clusters (Fig. 3D). Samples from the Arboretum locality (Monongalia County, WV) were represented by all five clusters, whereas representatives of four clusters were observed in Morgantown (Monongalia, WV), Cheat Lake (Monongalia, WV), Shelby (TN), Little Falls (Monongalia, WV), Star City (Monongalia, WV), and Uffington (Monongalia, WV); it should be noted that samples sizes varied widely from each locality. Genomic Cluster 5 was only represented by a few individuals from the Arboretum locality and was not sampled elsewhere in this study. By contrast, analysis with STRUCTURE identified the optimal k-value to be 3 (delta-k = 3.529, log-likelihood = 28442.18, standard deviation = 40.61), followed by k = 5 (delta-k = 1.155, log-likelihood = 27826.53, standard deviation = 643.8; Supplemental Material, File S3). The overall results were similar to those from the DAPC, with little evidence for population structure.

Network analysis of multilocus genotypes reveal a similar overall pattern to the DAPC, showing at least five genotype clusters, with no clear pattern of geographic structuring among them (Fig. 3E). Hierarchical clustering of Nei's genetic distance among localities further supports an overall lack of population structure (Fig. 3F). Of all sampling localities, the Arboretum and Morgantown (Monongalia, WV) were most similar, followed by Preston (WV), Cheat Lake (Monongalia, WV), Decker's Creek (Monongalia, WV), and Jackson (WV). A second cluster comprised

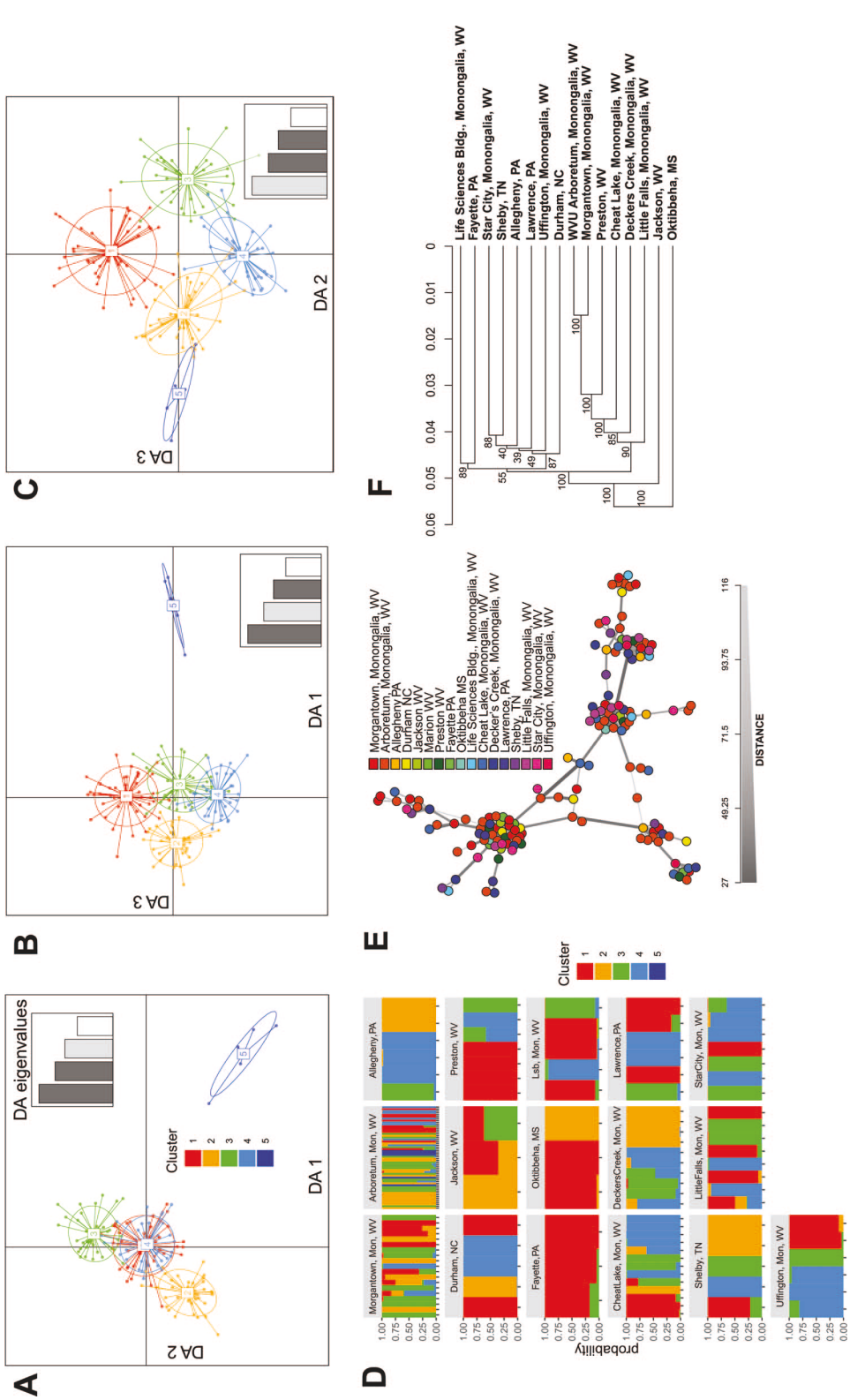

FIG. 4. Additional analyses of population structure for *Lonicera japonica* in the eastern USA. **A**–**C**. Discriminant analysis of principal components (DAPC) for the optimal value of k = 5. **A**. Axes 1 and 2, **B**. Axes 1 and 3, **C**. Axes 2 and 3. **D**. Ancestry coefficient plot of the five ancestral genomic population clusters by sampling locality. **E**. Multilocus genotype network, with colors in legend indicating sampling locality (origin) of each sample. **F**. Dendrogram based on Nei's genetic distance depicting genetic distance among sampling localities.

three localities from Pennsylvania (Allegheny, Lawrence, and Fayette), Shelby (TN), and two localities from Monongalia, WV (Uffington and Life Sciences Building). The most divergent to all other localities was Oktibbeha (MS).

Discussion. We conducted an analysis of invasion history, genetic variation, and population structure for one of the most problematic, weedy vines in eastern North America, *Lonicera japonica*. We reconstructed the invasion history of this species in the USA using digitized herbarium records and showed the rapid spread across the USA in the early 1900s, including both regional spread and long-distance dispersal to the western USA. We applied a cost-effective method for SNP genotyping (MIG-seq), leveraging a recently published chromosome-level genome sequence to quantify patterns of genomic variation. Our analyses revealed an overall lack of population structure and high inbreeding across the eastern USA, corroborating earlier studies based on allozymes, and in line with a species that was deliberately and ubiquitously introduced for erosion control and as an ornamental. Lastly, we discuss how we used plant invasion genomics as an accessible tool for integrating research and undergraduate education.

INVASION HISTORY OF *L. JAPONICA* IN THE USA. *Lonicera japonica* is thought to have been first introduced to the northeastern USA in 1862 (as the cultivar "Hall's honeysuckle," *L. japonica* var. *halliana*), but had likely arrived in the USA previously, as the earliest herbarium record of this species is from Kentucky in 1846 (Pelczar 1995, Schierenbeck 2004). Other records indicate that the species was present as far south as Virginia, Georgia, and Florida by 1900 (Leatherman 1955, Schierenbeck *et al*. 1995). Our analysis of digitized herbarium records corroborates these observations: by 1910, *L. japonica* was already widespread across the Northeast and Mid-Atlantic states, and had been collected in Georgia, Florida, the Carolinas, Missouri, Arkansas, and Texas, with a single record in California (Fig. 1). By 1970, it had become established in nearly all USA states east of the Mississippi River, and was widespread throughout the eastern regions of Texas, Oklahoma, and Kansas. By 2000 it was widespread throughout nearly all of California at lower elevations, in southern Arizona and New Mexico, and in northeastern Colorado. Its continued expansion is evidenced by more recent collections in Oregon, Utah, and Idaho as of 2020.

Environmental constraints on the continued expansion of this species are believed to largely consist of soil characteristics (preferring well-drained, acidic soils), minimum winter temperatures (ice/frost damage), drought, and soil temperatures required for seed stratification (Leatherman 1955, Schierenbeck 2004). Interestingly, the earliest collections in the northeastern USA appeared close to the northern edge of the distribution in North America, although the species is now present in southern Maine, northern New York State, southern Ontario (Canada), northern Wisconsin, Michigan's Upper Peninsula, and northwestern Washington state (EDDMapS 2023). Continued northern and western expansion may be driven in part by milder winters and changing precipitation patterns due to climate change, but there is also evidence of adaptive evolution to withstand more extreme cold (Evans *et al*. 2013, Kilkenny and Galloway 2016). In common garden experiments, plants from the northern and western invasion fronts were less susceptible to cold than were plants from older, more established "core" regions of the invasive range in the USA, suggesting post-establishment selection for cold tolerance along the invasion front (Kilkenny and Galloway 2016). In the eastern USA, *L. japonica* is predominantly found at lower elevations, but a similar adaptive scenario may be relevant to expansion in higher elevations (*e.g.,* in Appalachia) as is the case for northward expansions (Strasbaugh and Core 1977, Hardt 1986, Pelczar 1995).

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *L. JAPONICA* IN THE USA. Analysis of 1,571 filtered SNPs and sampling of 166 individuals from 16 localities across the eastern USA for *L. japonica* revealed low overall genetic diversity and a general lack of population structure. Our overall estimate of population subdivision ($Fst = 0.0347$) was similar to that of Schierenbeck *et al*. (1995), who estimated $Gst = 0.092$ for localities sampled across eastern Georgia and western South Carolina based on allozyme data. Similarly, we found relatively high estimates of inbreeding coefficients (*Fis* overall = 0.7437), whereas Schierenbeck *et al*. (1995) calculated $Fis = 0.118$ based on allozyme data. While these estimates are notably different, they both indicate some level of apparent inbreeding within populations, possibly driven in part by clonal propagation of this vining species, but also

possibly driven by short dispersal distances for pollen (*i.e.,* within patches). Possible explanations for these different *Fis* estimates may lie in differences in resolution between SNP and allozyme markers, our expanded sampling of populations across a broader geographic scale, temporal factors associated with ongoing neutral or adaptive evolution in this species (*i.e.,* sampling in the 1990s *vs*. 2020s), or some form of bias in either SNP-based or allozyme-based estimates. While we found no evidence for 100% identical clones at any of the sampling sites (Fig. 1E), we did find evidence of significant linkage disequilibrium at several sites (Table 1, based on significant values for *Ia* and *r-barD* after clone correction).

Our estimates of expected heterozygosity are also similar to Schierenbeck *et al*. (1995), with mean $He = 0.1204$ for SNP data *vs*. mean $He = 0.189$ for allozyme data, suggesting low overall levels of genetic diversity. Other representations of population structure further demonstrate a lack of distinctness among localities across the broader eastern-USA invasive range for this species (PCA, DAPC, STRUCTURE, multilocus genotype network analysis, hierarchical cluster analysis; Fig. 4). There is no discernable pattern of geographic differentiation evident from our analyses, suggesting a highly admixed gene pool for this species in North America. It must be noted that our main sampling focus was in northern West Virginia (mid-latitude), and thus our interpretation of overall patterns of variation may have been influenced by this. However, this does not appear to be the case, as sampling localities in WV are virtually indistinguishable from those more broadly sampled in the eastern USA (Fig. 4 D–F).

Many factors may have contributed to the patterns observed in this and earlier studies of genetic variation in *L. japonica*. First, this is an obligately outcrossing, perennial species, pollinated by a variety of animals including birds and both diurnal and nocturnal insects (Leatherman 1955, Miyake and Yahara 1998). Further, seeds are dispersed locally or perhaps more broadly by mammals and birds (*e.g.,* White and Styles 1992). Both the pollination and seed dispersal syndromes of this species would be expected to lead to frequent local or regional dispersal, effectively facilitating admixture (*e.g.,* Barriball *et al*. 2015). More importantly, however, is the way in which *L. japonica* was likely spread across the USA, both historically and contemporarily. Repeated anthropogenic dispersal by deliberate planting for erosion control and ornamental purposes, followed by local escapes from cultivation and subsequent spread may be a stronger factor in determining the current distribution of genetic variation in this species than wildlife-mediated dispersal (*e.g.,* Brusa and Holzapfel 2018, Alvarado-Serrano *et al*. 2019). Thus, it is not surprising that *L. japonica* would exhibit low levels of population structure in the eastern USA, especially given its long history as an invasive species in the USA and the fact that it is still sold in garden stores (Schierenbeck 2004; C. Barrett *personal observation*). By comparison, the few studies on population genetics of *L. japonica* in the native range suggest relatively higher levels of population structure (*e.g.,* Fu *et al*. 2013, He, Zhang *et al*. 2017, He, Qian *et al*. 2017), but these studies likely included multiple, possibly divergent varieties that may not be represented in the USA. Certainly, future research on population genomics of *L. japonica* should seek to sample representatives across the entire spectrum of variation in the native and invasive ranges (the former including representatives of all known varieties, and the latter on multiple continents), to compare patterns of genetic variability and trace the origins of invasive populations.

Traditional theory of invasion genetics centered around the expectation of single introductions, drastic genetic bottlenecks upon establishment representing a fraction of the diversity from the native range, and subsequent spread (*e.g.,* see Barrett and Husband 1990, Novak and Mack 2005, Dlugosch and Parker 2008, Barrett 2015). Yet, as genomic methods enable a rapid increase in invasion studies, the patterns emerging are not so simple, and the aforementioned scenario seems to be the exception rather than the rule (*e.g.,* Sakai *et al*. 2001, Lee 2002, Frankham 2005, Dlugosch and Parker 2008, Sutherland *et al*. 2021). For example, several studies have concluded that invasions are often repeated events, with multiple introductions, subsequent establishments, spread from points of introduction, secondary contact, and possibly hybridization with native or other invasive species (Ellstrand and Schierenbeck 2000, Blair and Hufbauer 2010). Admixture after multiple invasions may provide genetic variation for rapid adaptation to conditions in the invasive range, bringing together novel allelic combinations that otherwise would have remained geographically isolated in the native range (Dlugosch *et al*. 2015).

Because we were unable to include sampling from the native range, it is currently not possible to assess whether multiple introductions from different source populations have occurred for *L. japonica* in North America. The overall pattern of admixture and lack of population structure observed, which was likely facilitated and exacerbated by deliberate, human-aided dispersal over two centuries (Figs. 3, 4), may be compatible with one or multiple introductions. The low overall genetic diversity of *L. japonica* in the current study (USA only) may indicate an initial introduction from a single source (possibly "Hall's honeysuckle") and subsequent, rapid dispersal. Or, if dispersal and gene flow were and continue to be frequent enough, it is possible that the signal of distinct introductions from genetically distinct source populations has essentially been homogenized. Regardless, sampling from across the native range will be critical in future investigations.

Lonicera japonica represents an apt case study in global patterns of rapid evolution, as it is invasive on all continents aside from Antarctica (Schierenbeck 2004). Spatiotemporal comparisons of patterns of invasion history and genomic variation (*e.g.,* using material sampled from herbarium specimens) will be extremely powerful in elucidating the environmental and genomic factors associated with rapid, post-invasion evolution (*e.g.,* Kreiner *et al.* 2022). Future studies should emphasize collecting densely sampled SNP data from populations in the native and invasive ranges (on a global scale) to identify: 1) fundamental differences in population structure and genetic diversity in the native *vs*. invasive ranges, 2) spatiotemporal patterns of variation linked to invasion routes and invasion history, and 3) evidence for adaptive variation linked to climate, soils, pathogens (or a lack thereof), and other environmental factors post-invasion.

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