Biol Invasions (2015) 17:2387–2402
DOI 10.1007/s10530-015-0882-7

ORIGINAL PAPER

Inferring invasion patterns of *Lonicera maackii* (Rupr) Herder (Caprifoliaceae) from the genetic structure of 41 naturalized populations in a recently invaded area

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Received: 7 April 2014 / Accepted: 16 March 2015 / Published online: 26 March 2015

Abstract We studied the genetic structure of populations of the invasive shrub Amur honeysuckle (*Lonicera maackii*) in 41 woodlots in an agricultural landscape in a recently invaded area in southwest Ohio. Using six polymorphic microsatellite loci, we found high allelic diversity and high heterozygosity in all populations and low to moderate levels of genetic differentiation among populations. We also found significant positive correlations between geographic distance and (1) Nei's genetic distance and (2) the coefficient of genetic differentiation ($F_{ST}/(1 - F_{ST})$) among all possible pairs of populations. These relationships were stronger when we tested the correlations of each of five putative source populations against the remaining non-source populations; and were especially strong when only the populations located within 30 km of each putative source were included in the analyses. Genetic analysis also revealed the existence of four distinct clusters that were not equally distributed across the study area, with high levels of admixture within populations. In particular, our findings indicate that the individuals present in six newly established populations in Darke county are made up of individuals belonging to multiple clusters, suggesting that these new populations were colonized from multiple nearby source populations. From these findings, we infer that the genetic structure of these *L. maackii* populations is determined by a combination of long-distance dispersal events and shorter distance diffusion from neighboring uninvaded woodlots, as well a history of multiple introductions into Ohio, and a predominantly outcrossing mating system.

Keywords Long-distance dispersal · Diffusion · Genetic structure · Genetic admixture · Amur honeysuckle

Introduction

It is well known that genetic variation in plant populations is not evenly distributed across a landscape (Loveless and Hamrick 1984; Schnabel and Hamrick 1995; Holderegger et al. 2010; Dixon et al. 2011). Many studies have shown that genes and genotypes tend to be geographically clumped, with marked genetic differences occurring over a short distance, thus resulting in a non-random distribution of

Electronic supplementary material The online version of this article (doi:10.1007/s10530-015-0882-7) contains supplementary material, which is available to authorized users.

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genetic variation (Nevo et al. 1981, 1983, Holderegger et al. 2010). Two related factors are critical in shaping the genetic composition of plant populations; the mating system and the level of gene flow within and between populations (Loveless and Hamrick 1984; Slatkin 1985, 1987; Dixon et al. 2011; Freeland et al. 2012; Magalhaes et al. 2011).

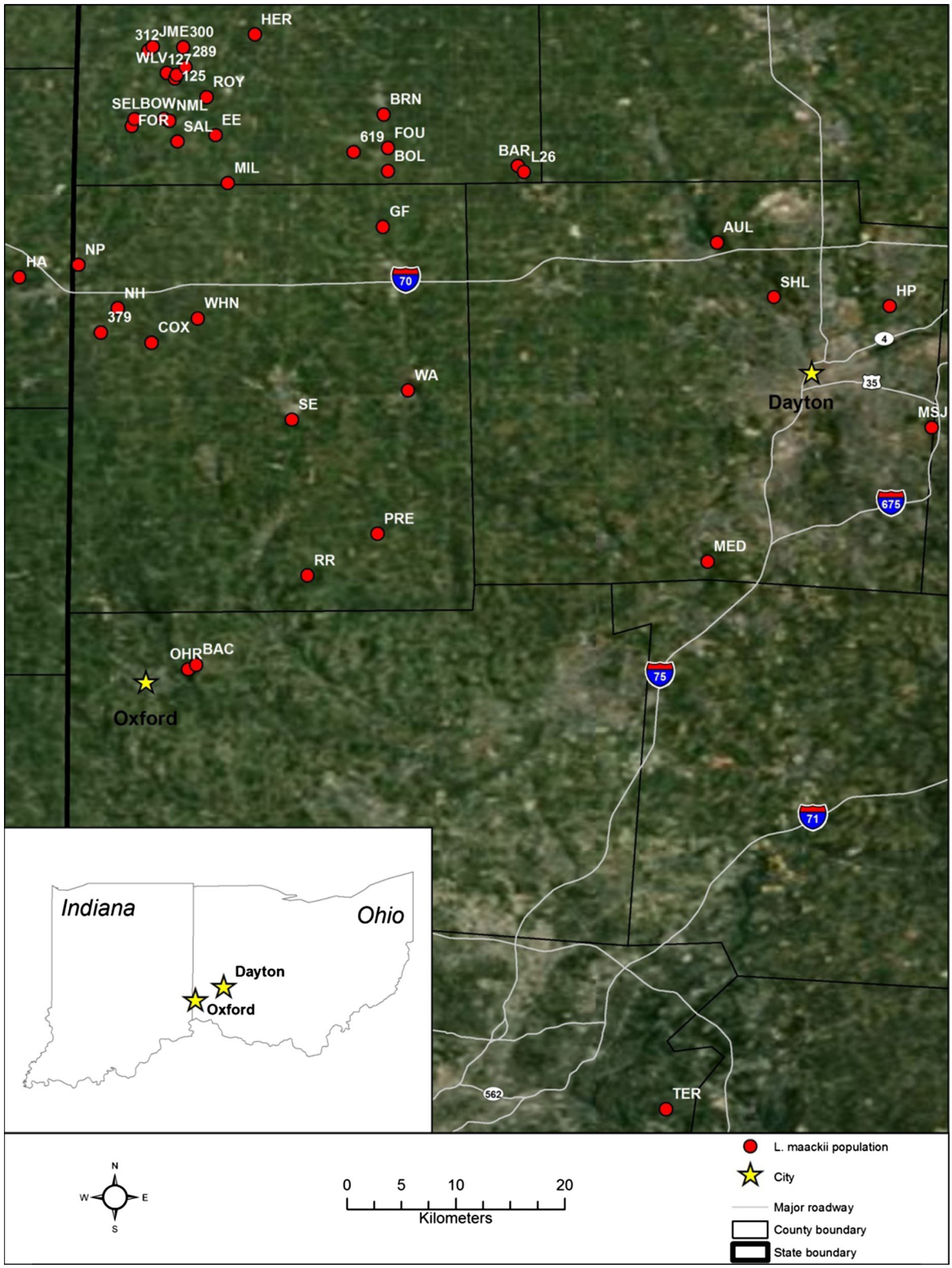
The role of mating system and gene flow on genetic diversity and differentiation among populations is also evident in invasive plants in their new geographic range. In general, predominantly outcrossing invasive species like *Daucus carota* (Rong et al. 2010, 2013), *Ambrosia artemissifolia* (Chun et al. 2010), and *Carduus acanthoides* (Mandák et al. 2009) have high numbers of alleles in their populations and low levels of genetic differentiation among populations. In contrast, predominantly selfing invasives such as *Alliaria petiolata* (Durka et al. 2005) show low allelic diversity within populations and higher levels of genetic differentiation among populations. However, the number of introductions responsible for the establishment of plant invasions also plays an important role in determining the genetic structure, where genetic diversity increases as the number of introductions increases (Genton et al. 2005; Marrs et al. 2008; Chun et al. 2010; Pairon et al. 2010). Moreover, the levels of genetic differentiation among populations can vary according to the origins of the populations (Berthouly-Salazar et al. 2013; Raymond et al. 2013).

Lonicera maackii, Amur honeysuckle, is an invasive woody shrub that is native to eastern Asia and was introduced to North America primarily for soil stabilization (Luken and Thieret 1996). *Lonicera maackii* is a predominantly outcrossing species (Barriball et al. 2014), and seed dispersal by birds (Bartuszevige and Gorchov 2006) and white-tailed deer (Castellano and Gorchov 2013) are the main mechanisms for dissemination and range expansion. *Lonicera maackii* can escape from cultivation and invade multiple habitat types, including old fields and forest understory and edges (Luken and Thieret 1996; Bartuszevige et al. 2006). Once established, *L. maackii* can form dense stands with plants as tall as 6 m (Luken and Thieret 1996; Deering and Vankat 1999) and likely out-competes its neighbors by expanding its leaves earlier in the spring than native plants and retaining its leaves well into the fall (Trisel and Gorchov 1994). As result, *L. maackii* causes a decline in the abundance of native annuals that are shade intolerant or which photosynthesize in the early spring (Gould and Gorchov 2000), reduces the growth and reproduction of perennial herbs (Miller and Gorchov 2004, McKinney and Goodell 2010, 2011), and affects forest succession (Hartman and McCarthy 2008; Kuebbing et al. 2013) and ecosystem functions (Arthur et al. 2012; McNeish et al. 2012; McEwan et al. 2012; Poulette and Arthur 2012). *Lonicera maackii* is invading in several areas of North America, and we focused on one of these, in southwest Ohio (Fig. 1), to investigate colonization dynamics as an invasion advances into new areas.

Fig. 1 Map of the distribution of the 41 populations of ▶ *Lonicera maackii* included in this study, located throughout southwest Ohio and adjacent Indiana

The invasion history for *Lonicera maackii* in Ohio has been described from herbarium specimens of naturalized shrubs (Trisel 1997). The earliest record in Ohio is from 1952 in Hamilton county, one of the earliest records of naturalized populations of this plant in the United States (Braun 1961). In Butler county, the earliest record is from the city of Oxford in 1962; Hutchinson and Vankat (1998) report *L. maackii* was planted in Oxford around 1960, where it subsequently naturalized and spread to the north. In Montgomery and Miami counties, the earliest specimens are from 1977. The earliest specimens for Warren and Preble counties were in 1988 and 1993, respectively. In Wayne county, Indiana, the earliest record is from 1964. The earliest specimen from Darke county was collected in 1995 (M. Vincent, pers. comm.) and by 2002, several woodlots in southern Darke county had been invaded (Bartuszevige et al. 2006). Even though most counties in southwest Ohio have well-established populations of *L. maackii*, Darke county is of particular interest because the invasion in the southwest portion of the county is ongoing, and many woodlots remain uninvaded or were only recently invaded. Therefore, this area provides an opportunity to examine the range expansion of *L. maackii* into newly occupied areas. This study could serve as a model for the mechanism of range expansion of other invasive understory shrubs with similar modes of seed dispersal such as *Elaeagnus umbellata* (Autumn olive), and *Rhamnus cathartica* (Common buckthorn).

Other authors have used genetic variation and differentiation among populations to infer demographic processes such as extinction and colonization rates


HER
312
JME300
289
WLV
127
125
ROY
SELBOW
NML
FOR
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BRN
619
FOU
BOL
BAR
L26
MIL
GF
AUL
NP
HA
70
SHL
HP
NH
379
WHN
COX
4
WA
35
Dayton
SE
MSJ
675
PRE
MED
RR
OHR
BAC
75
Oxford
71
Indiana
Ohio
Dayton
Oxford
562
TER
N
W
E
S
0
5
10
20
Kilometers
L. maackii population
City
Major roadway
County boundary
State boundary

and seed dispersal (DeWoody et al. 2004; Hu et al. 2010). For example, neutral genetic markers have been used to determine the most likely source population for newly established individuals (Cain et al. 2000; DeWoody et al. 2004; Hu et al. 2010). In turn, this information can be used to estimate dispersal distance and ultimately, patterns of dispersal and colonization. The patterns of dispersal (gene flow) and the number and source of individuals establishing a new population will determine the level of genetic differentiation among populations at the landscape level (Whitlock and McCauley 1990; Hanski 1998). Therefore, genetic analysis can also be used to elucidate whether invasions proceed along expanding fronts or through long-distance dispersal events followed by local expansion (Auld and Coote 1980; Gorchov et al. 2014a).

Bartuszevige and collaborators (Bartuszevige et al. 2006) examined woodlots in Darke county, Ohio and found that *Lonicera maackii* was more likely to have colonized woodlots that were close to towns. Landscape features, such as abundance of edge habitat around woodlots or proximity to towns, were found to be more important than community features, such as light availability, in determining the density of *L. maackii* (Bartuszevige et al. 2006). Based on these findings, Bartuszevige et al. (2006) inferred that *L. maackii* populations were initiated by multiple independent dispersal events from woodlots near towns. Over a more extensive area Gorchov et al. (2014b) reported similar findings, where woodlots were susceptible to invasion by *L. maackii* regardless of their age or other characteristics, but the extent of cropland around woodlots impeded invasion. Preliminary analyses performed by McNutt (2010) suggest that *L. maackii* is most likely spreading through the establishment of focal populations by long-distance dispersal events that are followed by outward expansion. Examination of age structures (Gorchov et al. 2014a) further supports this model of stratified diffusion.

Here, we studied the levels of genetic diversity and differentiation among 41 naturalized populations of the invasive shrub *Lonicera maackii* growing throughout southwest Ohio to determine the pattern of range expansion. In particular, we tested two main hypotheses: (1) *L. maackii* populations were initiated by multiple independent dispersal events from different sources, and (2) a combination of long-distance dispersal events followed by expansion to nearby woodlots is the mechanism for range expansion of *L. maackii*. Additionally, we intended to use this information to determine the role of potential source populations in the ongoing invasion of *L. maackii* as it colonizes previously unoccupied woodlots in Darke county, Ohio.

Materials and methods

Study area

This study was conducted in southwest Ohio, USA. It includes 40 populations of *Lonicera maackii* located in six counties (Fig. 1). Hamilton, Butler, Preble and Darke Counties are located along Ohio's border with Indiana. Montgomery county is located east of Preble county. In addition, we included one population from Wayne county, Indiana, a few miles west of the Ohio border. Agricultural fields dominate the landscape in most of the study area, with patches of forest (woodlots) scattered among the fields. Woodlots differ not only in size but also in density of *L. maackii*. In large portions of Darke county, many woodlots still remain free of *L. maackii* or have only recently been invaded (Gorchov et al. 2014a), indicating that there is an active expansion of the species range in this area.

Sample collection

In order to determine the genetic structure of *Lonicera maackii* in the study area, leaf tissue was collected from at least 30 individuals in each population. However, a smaller number of plants were sampled from recently colonized sites in Darke county and within areas where *L. maackii* populations were being eradicated. In general, *L. maackii* shrubs initiate flowers and seed after 6 years of growth (Gorchov et al. 2014a). We targeted large reproductive plants in all plots; but in recently invaded plots we sampled as many individuals as possible, often including non-reproductive shrubs. Moreover, some plots only had a handful of reproductive individuals. To reduce the chances of sampling closely related individuals within a woodlot, leaf tissue was taken from plants located at least 5 m apart. Leaf samples were stored in a −40 °C freezer until DNA extraction. Total genomic DNA was extracted from leaves using a modification of the

CTAB protocol described by Cullings (1992) and Doyle and Doyle (1987).

In addition to leaf tissue collection, Gorchov et al. (2014a) determined the ages of the oldest individuals of *L. maackii* in several of the sample populations. Annual rings were counted on stem segments of shrubs from a subsample of 27 populations to determine their age structures, and the ages of two additional populations were determined on the basis of historical records. We considered the ages of the oldest individuals as an indicator of age of the invasion, that is, the time from the establishment of the first shrubs in the woodlot to the time when the samples were taken. Eleven populations were identified as old populations based on ring counts or historical records (>16 years), and 13 as young populations (<12 years). The remaining populations were considered moderate (12–16 years).

Microsatellite analysis

Six microsatellite loci were used to study the genetic diversity of *Lonicera maackii* (Table 1). These polymorphic loci were previously developed for specific use with *L. maackii* (Rocha et al. 2014). The forward primers are labeled with WellRED fluorescent dyes (D3 or D4, Integrated DNA Technologies). Touchdown PCR reactions were performed using a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) in a 20 μL solution containing 60–80 mg of genomic DNA, 1X Tris buffer (pH 8.0) (IDAHO Technology Inc., Salt Lake City, Utah), 1.625 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM of each primer, and 1 unit of recombinant Taq polymerase (Fermentas, Vilnius, Lithuania). Genotyping of the PCR product was conducted through capillary electrophoresis in an automated genetic DNA analysis system (CEQ 8800, Beckman Coulter, Fullerton, CA, USA). Four microliters of PCR product were mixed with 28 and 0.4 μL of formamide and 400 bp DNA size standard (Genolab), respectively, preceding the DNA analysis. Fragments were identified on the basis of their size and according to their mobility in relation to the size standard.

Data analysis

Genetic analysis was conducted using GenAlEx (version 6.4, Peakall and Smouse 2006) to determine the levels of genetic variation within and among the 41 populations of *L. maackii*. This program was used to calculate common indicators of genetic diversity, such as average number of alleles per locus, effective number of alleles, and observed and unbiased expected heterozygosity. In addition, this program calculated Wright's F-statistics. The program GENEPOP

Table 1 Oligonucleotide primer sequences, repeat motifs, number of alleles, allele size range, and annealing temperatures for the six microsatellite loci used in this study (after Rocha et al. 2014)

Locus	Repeated motif	5′-3′ Sequence	No. of alleles	Allele size range	Initial annealing temperature (°C)
Di3F	CA9	AAAAGGCAAAGAAGCTCTTGGCA	7	216–228	62
Di3R		AGAAAAGAAGTCAGACTCTGCA			
Di4F	CT19	CTCATTCAGTCAAGTCCAAGT	13	124–152	50
Di4R		CGATGCTACATCATAATTAACAG			
Di19F	CT12	CGTGTTCCCCTTCTCTCACT	20	232–272	62
Di19R		CGGGGCTGCTTATCTTCTTC			
Tet21F	GTAT6.GTAT7	GCCTCCACCGATCTACTTCA	17	137–201	63
Tet21R		TCGGACGGTCGTTATGTGTA			
Tet22F	GTAT16	CATGAAGCCATTCGAAATCA	18	148–220	63
Tet22R		AACCACTCCATTTCTGTGACG			
Tri8F	GAA15	TCAAACGAGCTCCTAGATTGTAA	15	133–178	62
Tri8R		GTTAGCGTGTTGCGTTCACT			

A touch-down method for PCR is used, with the annealing temperature eventually decreasing (in increments of 1°) to 5° lower than the initial temperature

(Version 4.3, Rousset 2008) was used to determine if the populations were in Hardy–Weinberg and to test for linkage disequilibrium among loci. The program GenAlEx was also used to conduct an Analysis of Molecular Variance (AMOVA) to estimate the distribution of genetic diversity among populations and to determine levels of population differentiation. Two separate GenAlEx analyses were also conducted considering only the 11 populations grouped as old (>16 years) and the 13 populations grouped as young (<12 years).

In order to determine if the level of genetic differentiation among all 41 populations could be explained on the basis of the geographic distance that separates them (isolation by distance), we also used GenAlEx to calculate a Mantel correlation between geographic distance (in kilometers, km) and Nei's (1972) unbiased genetic distance among all possible population pairs. Another Mantel correlation was used to examine the relationship among all possible population pairs between the natural log of geographic distance and the coefficient of genetic differentiation ($F_{ST}/(1 - F_{ST})$), as proposed by Rousset (1997). These correlations provided two independent estimates of the effect of geographic distance on genetic differentiation.

In order to explore whether each of five potential source populations served as a source for the other populations, we carried out five additional tests of geographic distance on genetic differentiation. Three large, older populations were used as potential sources of invasion based on historical accounts: Terrace Park (TER) from Cincinnati, Hamilton county (Ohio), Mount Saint John (MSJ) from Dayton, Montgomery county (Ohio), and Hayes Arboretum (HA) from Richmond, Wayne county (Indiana). A fourth potential source old population, JME, in western Darke county (Ohio) was selected based on McNutt's (2010) finding that it was genetically distinct from nearby populations. A fifth old population, HER, was later selected based on its genetic distinctiveness, as revealed by the Structure analysis (see below). For each of these putative source populations, we calculated both the geographic distance (km) and Nei's (1972) unbiased genetic distance with every non-source population, then calculated the Mantel correlation between these measures.

Genetic structure was analyzed with the use of Bayesian model-based clustering with the program Structure (version 2.3.4, Pritchard et al. 2000; Hubisz et al. 2009) to determine if genetic similarity among populations could be explained by factors other than geographic distance. 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 20,000, followed by 100,000 MCMC repetitions. We found that this length of the burn-in period was sufficient to produce approximate stationarity (convergence) of all parameters in the output among 20 runs (CV < 0.08 %). A graphic representation of the results generated by Structure was presented using the program Distruct (version 1.1, Rosenberg 2004). Options were selected to allow admixture, assume independence among loci, and ignore population affiliations when defining clusters. In order 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 in order to obtain ΔK, 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 ΔK can be used as an indicator of the number of ancestral population clusters in the area. The program Structure Harvester v6.0 (Earl and vonHoldt 2012) was used for calculating parameters of Evanno et al. (2005). Following the Structure analysis, a map was made with ArcMap (version 10.0, ESRI 1999–2010), displaying populations represented as pie charts, with percentages representing the likelihood of cluster assignments for each population.

We used the program GeneClass2 (Baudouin et al. 2004; Piry et al. 2004) to identify the potential sources of *Lonicera maackii* plants found in the six most recently established populations in Darke county; namely, BRN, 619, FOU, BOL, BAR, and L26. GeneClass2 uses a Bayesian method based on multilocus genotypes to identify individuals that are immigrants to each population following the methodology proposed by Rannala and Mountain (1997) (also see Wilson and Rannala 2003). The program uses several Monte Carlo resampling algorithms to compute, and estimates, for each test individual, the probability of belonging to each potential source (reference) population or to be a resident (i.e., not a first-generation migrant) in the population where it was sampled.

Results

A total of 927 individuals from 41 populations of *Lonicera maackii* were analyzed in this study. A total of 93 alleles were found across the six loci examined. High levels of allelic diversity were found across all populations, with the actual number of alleles (N_a) and effective alleles (N_e) per locus equaling 8.13 and 4.79. In addition, the results (Table 2) also revealed high levels of heterozygosity across all loci and all populations. Our analyses revealed significant deviations from HWE in 28 of the 41 populations for at least one locus after Bonferroni correction for multiple tests ($\alpha = 0.05$) (Table S1). Given that these 28 populations were recently established and most likely received seeds from multiple nearby invaded woodlots, it is reasonable to expect that they will not be in equilibrium. Moreover, we also found linkage disequilibrium between loci Di3 and Tetra 21 and between loci Tetra 21 and Tetra 22, but it was restricted to two different populations for each pair of loci. It is difficult to make inferences about linkage disequilibrium given the deviations from HWE observed in these populations. We also found low to moderate levels of genetic differentiation among populations, with low inbreeding, and moderate gene flow (Table 3). An Analysis of Molecular Variance (AMOVA) showed that 90 % of the variance is occurring within populations, with 10 % of the variance attributed to the differences among populations.

The two separate GenAlEx runs for old and young populations revealed old populations (>16 years) have higher allelic diversity than young populations ($N_{a(old)} = 24.29 \pm 0.64$ versus $N_{a(young)} = 18.14 \pm 0.85$, Mann–Whitney $W = 165.5$, $p = 0.021$). Our results also showed that the younger populations have slightly lower levels of inbreeding than older populations ($F_{is} = 0.015 \pm 0.047$ and 0.057 ± 0.021, respectively) and higher levels of heterozygosity (Ho = 0.75 and 0.71, respectively) and gene flow. However, observed heterozygosity did not differ significantly between old and young populations (Mann–Whitney $W = 138$, $p = 0.341$). Additionally, we found that the level of genetic differentiation among populations was dependent on population age. Analyses of Molecular Variance (AMOVA) showed that 12 % of the genetic variation was found among populations when we considered only old populations. In contrast, just 7 % of the variation could be

Table 2 Population labels, age of populations, sample size (N), average number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o) and unbiased effective heterozygosity (H_e) for each of the 40 populations from southwest Ohio and one population from Indiana included in this study

Pop	Age	N	Na	Ne	Ho	He
125	Moderate	32	10.17	5.49	0.63	0.77
127	Old	34	8.83	5.02	0.66	0.76
289	Young	18	9.00	5.32	0.74	0.79
300	Moderate	24	7.00	3.43	0.54	0.70
312	Young	39	10.50	5.16	0.66	0.79
379	nd	37	8.17	3.99	0.77	0.75
619	Young	25	9.83	6.04	0.73	0.79
AUL	nd	20	9.50	5.58	0.59	0.82
BAC	nd	18	8.50	5.33	0.71	0.79
BAR	Young	25	10.17	5.63	0.72	0.82
BOL	Old	24	8.50	5.05	0.85	0.81
BOW	Old	36	8.33	4.71	0.74	0.78
BRN	Young	30	9.67	4.72	0.71	0.77
COX	nd	18	7.33	4.58	0.80	0.78
EE	Young	8	4.50	3.29	0.79	0.72
FORE	Young	12	6.33	4.00	0.65	0.77
FOU	Young	10	8.17	5.61	0.75	0.83
GF	nd	20	7.67	3.66	0.68	0.69
HA	Old	31	7.00	4.36	0.75	0.72
HER	Old	22	5.50	3.38	0.68	0.71
HP	nd	19	8.50	5.34	0.59	0.82
JME	Old	27	7.50	3.85	0.72	0.73
L-26	Young	23	8.50	5.10	0.78	0.79
MED	nd	19	8.67	5.20	0.61	0.80
MILL	Young	14	6.67	4.42	0.82	0.76
MSJ	Old	20	9.33	5.68	0.73	0.82
NEWJ	Old	19	7.67	4.52	0.74	0.78
NH	nd	12	7.67	4.73	0.55	0.78
NMILLS	Young	8	5.50	4.06	0.85	0.78
NP	nd	18	6.00	3.90	0.65	0.72
OHR	Old	29	8.83	5.48	0.66	0.82
PRE	nd	18	8.50	4.79	0.75	0.75
ROY	Young	23	9.33	4.97	0.71	0.79
RR	Young	21	9.50	5.58	0.79	0.83
SALL	Old	27	7.33	4.62	0.64	0.79
SE	nd	17	8.17	5.15	0.81	0.82
SELL	Moderate	26	7.50	4.72	0.75	0.80
SHL	nd	17	7.83	4.80	0.67	0.74
TER	nd	21	9.33	4.97	0.72	0.79
WA	nd	23	8.33	5.70	0.80	0.82

Table 2 continued

Pop	Age	N	Na	Ne	Ho	He
WHIN	nd	22	7.83	4.51	0.67	0.76
WOLV	Moderate	21	8.50	4.98	0.72	0.79
Mean		927	8.13	4.79	0.71	0.78

nd Population for which age was not determined

attributed to the differences among populations among the 13 more recently established populations.

We found a significant Mantel correlation ($r = 0.23$, $p = 0.01$) between geographic and genetic distance among all possible population pairs (Figure S1). Population pairs averaged a Nei's unbiased genetic distance of 0.24 ± 0.01 and a geographic distance of 30.03 ± 0.73 km. The second Mantel correlation, testing isolation by distance using the coefficient of genetic differentiation (Figure S1), was also significant ($r = 0.14$, $p = 0.04$). For three of the five follow-up analyses, in which each of the putative source populations was compared to all of the non-source populations, we found significant positive relationships between genetic and geographic distances (Table 4, Figure S2). For the remaining two putative source populations, HA and HER, correlations between genetic and geographic distances with the other 37 populations were not significant. However, further analyses with geographic distance limited to 30 km from each of these sources did show significant positive correlations between genetic and geographic distance. The source populations in Darke county (JME and HER) also showed stronger relationships with geographic distance limited to 30 km.

The genetic structure of these 41 populations of *L. maackii* is best described by four clusters ($K = 4$), using the program Structure following the methodology proposed by Evanno et al. (2005) (Fig. 2). These four clusters were still supported when we ran only the 11 old populations (>16 years) in Structure (Fig. 2). Both a bar graph of the populations arranged east to west (Fig. 3) and the geographic representation of these data (Fig. 4) show that the likelihood of belonging to each cluster is not equally distributed across the study area; instead, different clusters tend to be most likely in different parts of the study area. The blue cluster represents a group associated with one woodlot (JME), and is the cluster that dominates in the southwest portion of recently invaded Darke county. Meanwhile, the green cluster is more abundant in the sample population from Indiana (HA) and adjacent Preble county. The yellow cluster is dominant in Hamilton county (TER), where naturalized *L. maackii* was first observed, and in several populations north of TER present in Butler, southern Preble, and Montgomery counties. The population from Dayton (MSJ) is most likely included in the same cluster as TER.

Table 3 A Mean number of alleles per locus (N_a), mean effective number of alleles per locus (N_e), mean observed heterozygosity (H_o) and mean unbiased effective heterozygosity (H_e) for 13 young, 4 intermediate (moderate) and 11 old populations of *Lonicera maackii*. B Mean levels of inbreeding (F_{IS}), genetic differentiation (F_{ST}) and gene flow (Nm) for young populations, old populations and for all 41 populations of *Lonicera maackii*

	Mean number of alleles per locus (Na)	Mean effective number of alleles per locus (Ne)	Mean observed heterozygosity (Ho)	Mean expect heterozygosity (He)
A				
Age of population				
Old	7.88 ± 0.25	4.67 ± 0.16	0.72 ± 0.02	0.77 ± 0.01
Moderate	8.29 ± 0.28	4.66 ± 0.16	0.66 ± 0.02	0.77 ± 0.01
Young	8.28 ± 0.34	4.92 ± 0.22	0.75 ± 0.02	0.79 ± 0.01
All populations	8.14 ± 0.17	4.78 ± 0.12	0.72 ± 0.01	0.78 ± 0.01
		F_{IS}	F_{ST}	Nm
B				
All 42 pops		0.057 (0.026)	0.083 (0.006)	2.846 (0.222)
Old populations (n = 11)		0.057 (0.021)	0.084 (0.007)	2.834 (0.270)
Young populations (n = 13)		0.015 (0.047)	0.069 (0.008)	3.520 (0.305)

Numbers in parentheses indicate standard error

Table 4 Mantel correlations between genetic and geographic distance for each of the five potential *Lonicera maackii* source populations

Source population	Mantel correlation coefficient (p value)	
	All populations	30 km limit
TER	0.512 (0.006)	–
MSJ	0.494 (0.001)	–
JME	0.332 (0.034)	0.431 (0.020)
HA	−0.056 (0.728)	0.377 (0.044)
HER	0.250 (0.11)	0.510 (0.005)

Numbers in parentheses indicate p values

The first correlation involves all possible population pairs for each of the five source populations. The second correlation includes only those populations located <30 km from each source population. This second analysis was not conducted for TER and MSJ because less than four populations within 30 km were sampled

Finally, an additional red cluster grouping (HER) was identified in the most northern sample populations, east of the blue cluster in Darke county.

For each of the four clusters defined by Structure, we selected one old population close to the newly invaded areas in Darke county to serve as representative of each cluster. These four populations; namely, HA, JME, HER and SE were used as potential source population in order to identify the most likely origin of all individuals (immigrants) to each of six newly established population (BRN, 619, FOU, BOL, BAR, and L26). Using GeneClass2, we determined which source population is the most likely source for each individual present in the six populations tested. Assignment of each individual to a source is based on the highest likelihood of belonging to that source and the lowest likelihood ratio. Our findings indicate

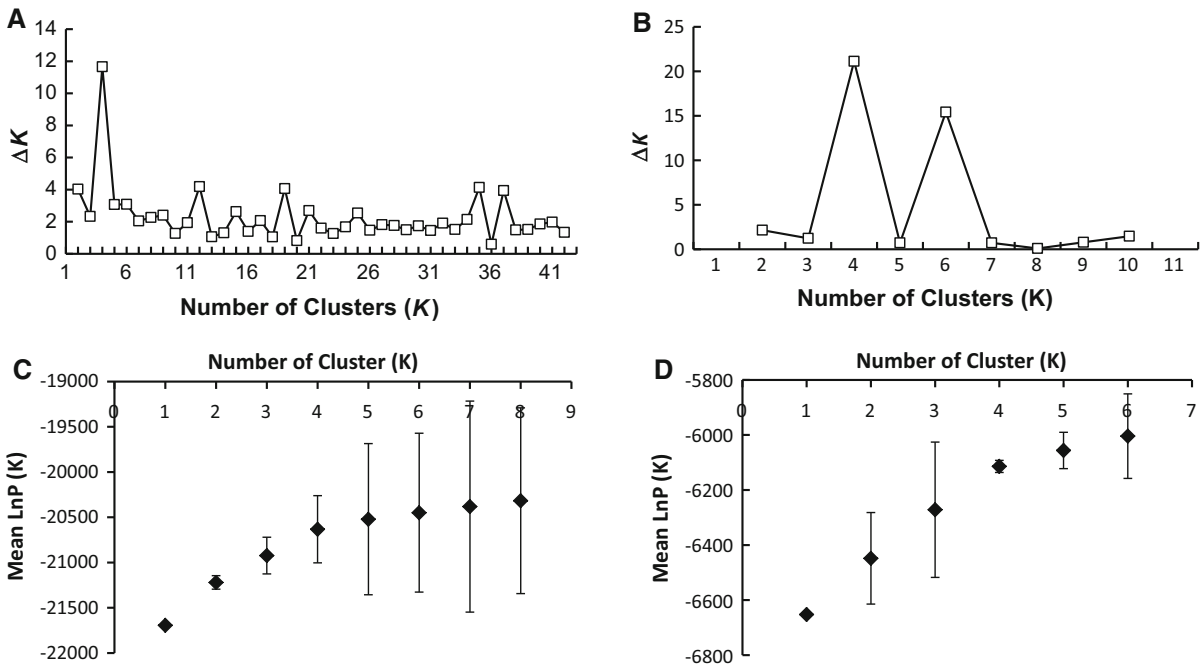


Fig. 2 Determination of the number of clusters present in a sample of populations of *Lonicera maackii* and mean log likelihood values (±variance) for each value of K as estimated by the programs Structure (version 2.3.4, Pritchard et al. 2000; Hubisz et al. 2009) and Structure Harvester (Earl and vonHoldt 2012). **a** Number of clusters present in the sample of 41 populations of *Lonicera maackii*. **b** Determination of the number of clusters present in a sample of 11 old (>16 years) populations of *Lonicera maackii*. **c**, **d** Mean log likelihood values (±variance) for each value of K for all 41 populations (**c**) and for the sample of 11 old populations (**d**). ΔK 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). The number of clusters corresponds to the modal value, which in this case in both cases corresponds to $K = 4$

that the individuals present in the six newly established populations are made up of individuals belonging to multiple sources (Fig. 5). This indicates that these new populations are likely to be colonized from multiple nearby source populations.

Discussion

Our finding of high genetic diversity in this set of populations of invasive *Lonicera maackii* supports the findings of McNutt (2010), who reported high levels of genetic diversity in 17 populations of *L. maackii* in southwest Ohio. In that study, a total of 58 different alleles were found across five microsatellite loci. We found a total of 93 alleles across the six loci we examined in 41 populations. We also found that old populations (>16 years) have higher allelic diversity than young populations (Table 3) suggesting that, once established, populations continue to receive propagules with new alleles. Although the allelic diversity was significantly different between the old and young populations, there were no significant differences in observed heterozygosity. The high levels of allelic diversity and heterozygosity that we found in all examined populations indicate that there is little inbreeding, supporting the hypothesis that this species is a predominant outcrosser (Goodell and Iler 2007; Barriball et al. 2014). Other predominantly outcrossing invasive species also display high allelic diversity and heterozygosity (Marrs et al. 2008; Mandák et al. 2009). For example, *Carduus acanthoides* (Mandák et al. 2009) has a high level of outcrossing, which results in high levels of heterozygosity. In addition, *C. acanthoides* is also capable of dispersing seeds long distances (Feldman and Lewis 1990), which facilitates gene flow among populations and may contribute to the high allelic diversity present in its populations.

Further analysis of the genetic structure using Bayesian model-based clustering supports the existence of more than one genetically distinct group of *Lonicera maackii* populations in southwest Ohio. McNutt (2010) proposed the existence of nine distinct clusters ($K = 9$) among the 17 populations that she examined; however, further analysis to determine the number of clusters using the methodology proposed by Evanno et al. (2005) supports the existence of only three well-defined clusters ($K = 3$) among those 17 populations. The present analysis combines the 17 populations from McNutt's study with 24 additional populations, and our Structure analysis revealed the existence of four distinct clusters ($K = 4$), adding only one new cluster. Despite the difference in the number of clusters, there are similarities between our analysis and that of McNutt (2010), as both show the existence of a cluster that is more common in the southern part of the study area which includes some of the oldest populations in Ohio. In particular, this cluster includes one population in Hamilton county (TER) with extremely large individuals (Thoelmeier and Luken 1996) that suggests it is one of the oldest naturalized

Fig. 4 Geographic distribution of the cluster assignments (▶) ($K = 4$) for each sample population of *Lonicera maackii*. *Pie graphs* display the likelihoods of belonging to each of the four clusters. The number of clusters was determined as proposed by Evanno et al. (2005) and estimated from the program Structure (Hubisz et al. 2009)

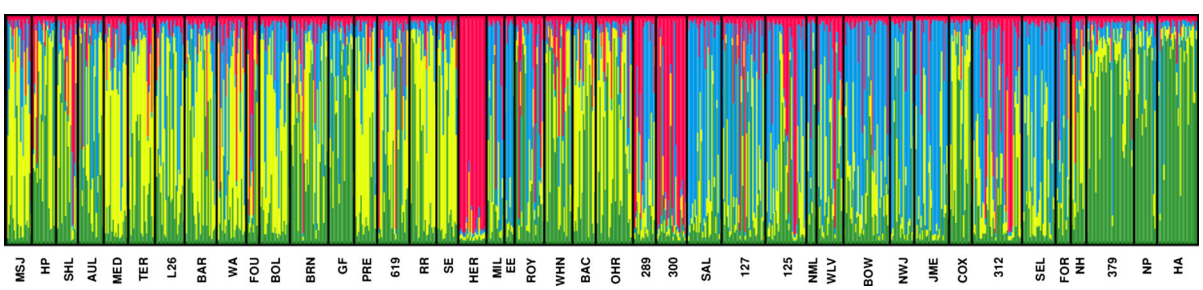
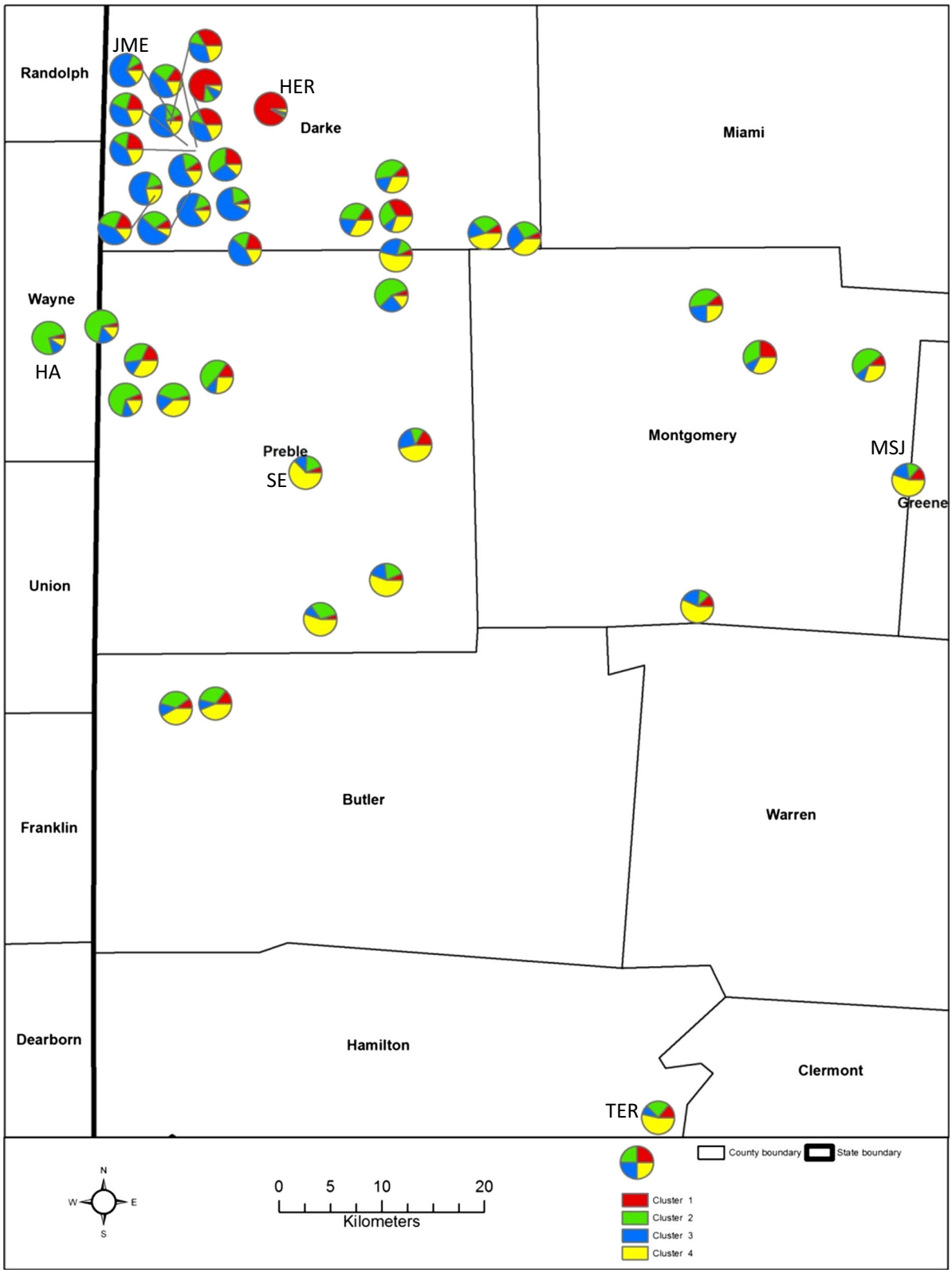


Fig. 3 Structure (Hubisz et al. 2009) estimates of the genetic structure of 41 populations of *Lonicera maackii*. These locations are organized from east to west (MSJ to HA). *Colors* represent the four population clusters (K), as determined by Evanno et al. (2005) and each colored line represents a single individual with the percent of its genome partitioned into four colored segments. The length of the *color* represents the individual's estimated membership for each of the four clusters. Numbers along the *x-axis* represent the different populations, with thin *black lines* separating each population. Populations NWJ and JME correspond to two samples taken from the same woodlot


JME
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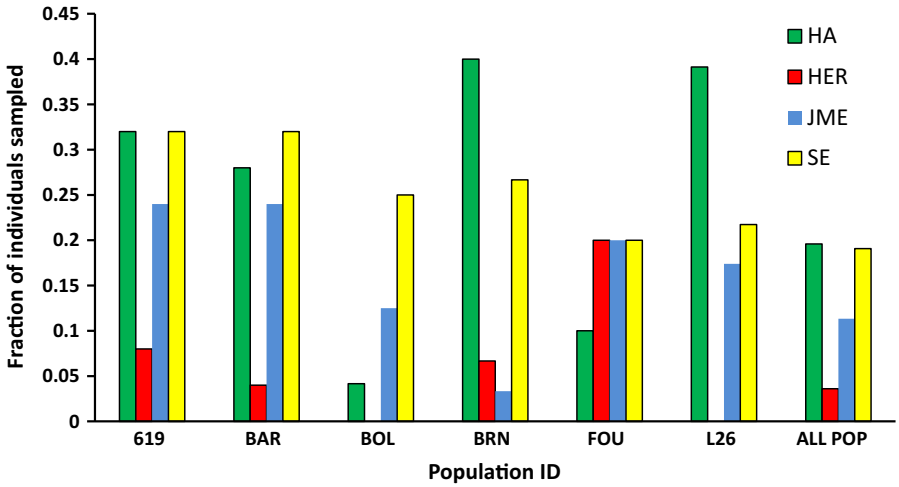

Fig. 5 Proportion of individuals in the sample taken from six recently invaded woodlots that were assigned to each of the four source populations defined using the program Structure. Assignment of individuals sampled in each woodlot was conducted using the program Geneclass2. The program uses Bayesian method based on multilocus genotypes to identify individuals that are immigrants to each population following the methodology proposed by Rannala and Mountain (1997)

population of *Lonicera maackii* established in Ohio, and old populations from Butler, Preble, and Montgomery Counties (yellow cluster, Fig. 4). This cluster (yellow) dominates across a wide geographic range, probably reflecting its long history in the area. Two clusters (green, blue) are more common in the western portion of the study area and each of them includes one of the potential source populations (HA, JME) proposed by McNutt (2010). The fourth cluster identified (red) is new with the populations we added to the study, and is most abundant in the northern part of our study area and dominant in two older populations (Fig. 4).

Our results showed that a large amount of genetic diversity exists within populations of *L. maackii* and significant genetic admixture of the four distinct clusters is observed in most populations. This suggests that each woodlot experiences multiple colonization events and that the colonizing material may originate from genetically distinct nearby woodlots. High allelic diversity can be a result of multiple introductions (Dlugosch and Parker 2008), and high quantities of heterozygotic individuals within a population will decrease the chances for inbred individuals and exposed deleterious mutations (Charlesworth and Charlesworth 1999). The F_{IS} values (Table 3) suggest that populations of *L. maackii* in southwest Ohio do experience some inbreeding; however the majority of individuals are heterozygous. Barriball et al. (2014) found that despite the high outcrossing rate observed in one population of *L. maackii* ($t_m = 0.97$) in Ohio, there was also a significant incidence of biparental inbreeding ($t_m - t_s = 0.12$). If similar levels of biparental inbreeding are typical across the range of this species, it may explain the reduction in observed heterozygosity with respect to Hardy–Weinberg expectations.

Relatively low levels of genetic differentiation among populations is expected for perennial plants with a predominantly outcrossing breeding system and animal-dispersed seeds (Hamrick and Godt 1990). Bartuszevige and Gorchov (2006) reported that at least five bird species disperse *Lonicera maackii* seeds in the study area, with American robins (*Turdus migratorius*) and European starlings (*Sturnus vulgaris*) being the most important due to their abundance. They also reported that the American robin in particular has a preference for edge-site foraging and, in turn, is projected to disperse seeds preferentially along wooded corridors and woodlot edges. White-tailed deer also disperse viable seeds of *L. maackii* (Castellano and Gorchov 2013, P.W. Guiden unpubl.). At the level of entire invasions, long-distance dispersal has been argued to foster high genetic diversity and maximize evolutionary potential during rapid geographic range expansion (Berthouly-

Salazar et al. (2013) and Raymond et al. (2013). However, long distance dispersal can have a homogenizing effect on diversity among populations. McNutt (2010) suggested that long distance dispersal could explain the low levels of genetic differentiation and high levels of genetic diversity observed in *L. maackii* populations. Such a pattern of seed dispersal may generate new populations founded by seeds from multiple sources, resulting in the establishment of populations with high levels of genetic diversity and showing relatively low divergence from other nearby populations. Evidence for this pattern of colonization comes from population BRN, which had only two shrubs that had reached reproductive age, but where 18 of 28 younger, non-reproductive individuals had alleles that were different from those oldest two shrubs. Furthermore, once established, populations in different neighbouring woodlots may experience a high level of genetic exchange, preventing genetic differentiation among nearby woodlots.

Additional evidence for colonization from multiple sources comes from our finding that genetic differentiation was weaker among young populations than among old populations. Since the young populations are too young to have contributed propagules to each other, we interpret their genetic similarity to multiple colonization from seeds from more distinctive older populations, some of which are close and some more distant. This long-distance dispersal would counterbalance the cumulative effects of potential bottlenecks caused by sequential founding events occurring at the edge of an expansion front. However, the pattern of greater genetic differentiation among old populations could also be due to the greater geographic span of the sampled old populations, whereas our young populations were all sampled from a smaller region in southwest Darke county.

This analysis of genetic structure indicates that individuals sampled from recently established populations in southwestern Darke county have genotypes that resemble more than one of the identified genetic clusters (Figs. 3, 4) and each of these populations is comprised of a near equal mixture of all four putative source populations (Fig. 5), each of which is primarily comprised of one of the four clusters. This finding on young populations of *L. maackii* contrasts with our observations for old populations that are dominated by individuals with a higher likelihood of belonging to a single cluster. These patterns provide further evidence for our inference that stratified dispersal has shaped this invasion. In our view, new populations are founded by long-distance dispersal events from multiple sources, giving rise to populations that are a mixture of individuals from different genetic clusters. Subsequently, these heterogeneous populations serve as propagule sources for the establishment of new populations in nearby woodlots. This interpretation is consistent with age structures in the youngest part of this invasion in Darke county (Gorchov et al. 2014a) and in the patterns of dispersal reported for *Brachypodium sylvaticum* at the local geographical scale (Ramakrishnan et al. 2010).

These inferences for how the genetic structure of *L. maackii* populations has been shaped by dispersal follow Chun et al.'s (2010) explanation for the low levels of genetic differentiation among invasive ragweed (*Ambrosia artemisiifolia*) populations in France. They argued that diverse invasive populations result from extensive gene flow that promoted genetic admixture among distinct source populations introduced from North America. Admixture of genetically distinct clusters may result in increases in fitness and thus invasion success of *L. maackii*, as has been argued for other invasive species (Facon et al. 2008; Keller and Taylor 2010). For *L. maackii*, high genetic diversity can be promoted not only by dispersal and admixture, but also by the history of multiple introductions of *L. maackii* into the United States (Luken and Thieret 1996), which is consistent with the patterns described for other species (Tarin et al. 2013).

Despite overall moderate levels of genetic differentiation, the existence of a significant correlation between genetic and geographic distance among populations may also be used to elucidate the patterns of colonization for *L. maackii* in southwest Ohio. Considering the size of the study area and the proposed existence of four distinct genetic clusters that are unevenly distributed across the landscape, we found a significant correlation between geographic distance and two indicators of genetic differentiation (isolation by distance). These findings suggest that colonization may be occurring through local expansion to nearby woodlots. However, geographic distance only explains a fraction of the levels of genetic differences among these populations. Still, we found stronger correlations when we examined the correlations between genetic and geographic distance for each of the proposed source populations and the other non-

source populations (Table 4). This suggests that woodlots nearest to the source populations are being colonized primarily through small-scale advancements. In this case, the oldest and most established populations would be serving as seed sources in the surrounding area. The yellow cluster is most likely the oldest, as it is present in the south and in Hamilton county, where naturalized populations of *L. maackii* were first observed in Ohio (Braun 1961). It is also the cluster with the widest geographic range, probably because it was able to establish over long distances before its range was disrupted by a separate introduction. The green cluster may correspond to an early introduction of *L. maackii* at the Hayes Arboretum (HA) in Indiana (HA), and appears to have contributed strongly to other populations only to about 30 km (Table 4; Fig. 4).

We propose that the presence of high levels of genetic diversity and moderate levels of differentiation among populations of *L. maackii* are due to the history of multiple introductions into Ohio, long-distance dispersal, and a predominantly outcrossing mating system. Long-distance dispersal events mediated by animal seed dispersal have allowed *L. maackii* to maintain high levels of genetic diversity as it colonizes new areas, while maintaining only a moderate amount of genetic differentiation. In addition to long-distance dispersal events, established populations of *L. maackii* are expanding locally, resulting in colonization of uninvaded woodlots near older populations. Older source population have a high amount of genetic diversity, serving as genetic templates for successful new establishments of *L. maackii*s, with younger populations displaying low inbreeding and high heterozygosity. Additionally, a high outcrossing rate is a key factor contributing to the high percentage of heterozygotes maintained in populations of *L. maackii*.

Our findings contribute to elucidate a fundamental question in invasion biology: how do invasive species expand their range within their introduced habitat? We found that both long and short distance dispersal play important roles in the invasive behavior of *Lonicera maackii*. Long-distance dispersal results in greater spatial spread of *L. maackii*, consequently establishing multiple new populations that eventually serve as seed sources for further range expansion. Short-distance dispersal from newly established populations facilitates the colonization of neighboring woodlots. Our observations of increasing genetic differentiation between putative source populations and sink populations as geographical distances between them increase (isolation by distance) supports the colonization of neighboring woodlots as a mechanism of range expansion. Similarly, the high level of admixture found within populations, especially among newly established populations supports the occurrence of extensive long distance dispersal. Overall, we found that *L. maackii* follows the model of *stratified diffusion* proposed by Hengeveld (1989); that is, a combination of neighboring spread and long distance dispersal. The consequences of both modes of dispersal for the adequate management of invasive plants are discussed by Mack (1985), Moody and Mack (1988) and Gorchov et al. (2014a).

Acknowledgments The authors would like to thank the property owners who allowed access to their lands for sample collection. Thank you to S. Castellano, M. Monfredi and M. Rocha for assisting with sample collection and field work. This study was funded by grants to Kelly Barriball from the Kent State Graduate Student Senate and the Art and Margaret Herrick Aquatic Ecology Research Fund, and the United States Department of Agriculture, Cooperative State Research Education and Extension Service, National Research Initiative Competitive Grant, # 2007-35320-18349 to David Gorchov, Mary Henry, and Oscar Rocha.

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