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Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia

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Abstract

Admixture between differentiated populations is considered to be a powerful mechanism stimulating the invasive success of some introduced species. It is generally facilitated through multiple introductions; however, the importance of admixture prior to introduction has rarely been considered. We assess the likelihood that the invasive *Ambrosia artemisiifolia* populations of Europe and Australia developed through multiple introductions or were sourced from a historical admixture zone within native North America. To do this, we combine large genomic and sampling data sets analysed with approximate Bayesian computation and random forest scenario evaluation to compare single and multiple invasion scenarios with pre- and postintroduction admixture simultaneously. We show the historical admixture zone within native North America originated before global invasion of this weed and could act as a potential source of introduced populations. We provide evidence supporting the hypothesis that the invasive populations established through multiple introductions from the native range into Europe and subsequent bridgehead invasion into Australia. We discuss the evolutionary mechanisms that could promote invasiveness and evolutionary potential of alien species from bridgehead invasions and admixed source populations.

KEYWORDS
admixture, *Ambrosia artemisiifolia*, approximate Bayesian computation (ABC), bridgehead invasion, introduction history, random forests

1 | INTRODUCTION

Globalization has been accompanied by worldwide flourishing of alien species. Non-natives can have disruptive and disastrous ecological consequences by out-competing their indigenous counterparts and affecting invaded ecosystems. During an invasion process, several demographic events can lead to changes in genetic variation, and thereby influence the evolutionary potential and invasiveness of alien species (Estoup et al., 2016; Facon et al., 2006; Lee, 2002; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008; Rius & Darling, 2014). Within small founding populations and on the invasion front, genetic drift can lead to reduced genetic diversity, potentially impacting additive genetic variation (Bock et al., 2015; Dlugosch & Parker, 2008; Excoffier & Ray, 2008; Peischl, Dupanloup, Kirkpatrick, & Excoffier, 2013; Wright, 1931). Such bottlenecks can also result in increased genetic load due to the reduced efficacy of selection (Blackburn, Lockwood, & Cassey, 2015; Frankham, 1995). An apparent contradiction between the expected negative effects of

introduction on genetic variation and fitness, and the invasion success of some species, has been termed the "genetic paradox of invasion" (Frankham, 1995; Allendorf & Lundquist, 2003; for review see Estoup et al. (2016)).

One potential resolution to the genetic paradox of invasion for some invaders may be admixture: the mixing of genotypes from differentiated genetic backgrounds (Dlugosch & Parker, 2008; Prentis et al., 2008; Rius & Darling, 2014; Roman & Darling, 2007). Such admixture can arise within the native range prior to colonization (Keller, Fields, Berardi, & Taylor, 2014; Martin et al., 2014), or as a more frequently described consequence of multiple introductions (Dlugosch & Parker, 2008; Uller & Leimu, 2011). Admixed genotypes in an introduced range could subsequently act as bridgehead—a successful invasion acting as a source for further introductions (Lombaert et al., 2011). Depending on the influx of genetic material, beneficial outcomes of intraspecific hybridization include higher genetic variance within populations, heterosis, as well as novel and/or transgressive phenotypes (Bock et al., 2015; Rius & Darling, 2014; Verhoeven, Macel, Wolfe, & Biere, 2011). Disentangling the demographic history of an invader and identifying the putative source populations provide valuable information about the amount and type of genetic variation present in the introductions, which can have significant consequences on the success of invasion (Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015; Dlugosch & Parker, 2008; Estoup et al., 2016). Knowledge of these processes is key for studies concerned with understanding how and whether alien populations have adapted since their introduction and the role of pre-adaptation to invasion success (Barrett, 2015; Estoup et al., 2016; Facon et al., 2006; Rius & Darling, 2014). The potential role of admixture to invasion success, be it via admixture in the native range, through multiple introductions, or through bridgehead introductions, illustrates the importance of unravelling introduction history. Understanding introduction history requires broad sampling of the native range (Bossdorf et al., 2005; Cristescu, 2015; Dlugosch & Parker, 2008) along with a comparison with the introduced areas (Dlugosch & Parker, 2008). However, sampling limitations have often hampered reconstructions of evolutionary changes that accompany invasion (Cristescu, 2015; Lombaert et al., 2011).

The invasive annual weed *Ambrosia artemisiifolia* L. (common ragweed, Asteraceae) is native to North America and introduced to Europe, South America, Australia and Asia where it is considered as an agricultural pest (Oswalt & Marshall, 2008). In Europe, the first known introduction of *A. artemisiifolia* was in France around 1850 and most likely originated from contamination of imported seeds from North America. Later major introductions have been tied to imports during the two World Wars (Chauvel, Dessaint, Cardinal-Legrand, & Bretagnolle, 2006). Given the early expansion of this weed in Europe, introduced European populations could have acted as a bridgehead for subsequent introductions elsewhere. This pathway has, however, not been tested within this species. In Australia, *A. artemisiifolia* was first observed at the beginning of the 20th century, and has become increasingly abundant in southeastern Queensland and New South Wales since around 1950 (Palmer & McFadyen, 2012). The population structure of this weed within the introduced European range has previously been described and accredited to multiple introductions from various source populations originating from North America (Chun, Fumanal, Laitung, & Bretagnolle, 2010; Gaudeul, Giraud, Kiss, & Shykoff, 2011; Genton, Shykoff, & Giraud, 2005). Recently, Martin et al. (2014) suggested that these signatures could be due to pre- rather than postintroduction admixture. These authors observed a shift in genetic structure in a population genetic analysis of historic and contemporary samples of this species from across the native North American range. This pre-introduction shift in the native range was attributed to human-mediated admixture of western and eastern native genotypes (Martin et al., 2014). As admixture in the native range seems to have been associated with land use change and human-mediated dispersal (Martin et al., 2014), this raises the possibility that pre-adaptation to anthropogenic-disturbed environments (Hufbauer et al., 2012) in North America facilitated its introduction and spread elsewhere.

In this study, we aimed to assess the contribution of historical admixture, multiple introductions and bridgehead invasion to the successful introductions of *A. artemisiifolia* into Europe and Australia. We reconstructed the introduction history of *A. artemisiifolia* within Europe and Australia using approximate Bayesian computation (ABC) based on random forest (RF) algorithms, using 1022 SNP loci identified in 466 samples collected at 85 locations from across the entire native range of North America, and much of the European and Australian introduced ranges. ABC-RF analyses are superior to other approaches because they have the capacity to differentiate among competing complex introduction scenarios (Fraimout et al., 2017; Pudlo et al., 2016) while using the increased resolution of genomic data sets (e.g., Momigliano et al., 2017). This approach might be particularly informative in cases like ragweed where traditional approaches with few markers may be unable to identify source populations especially given complex introduction histories and weak population structure in the native range. We complemented this analysis with an assessment of genetic population structure (1,022 SNPs) and diversity (10,100 SNPs) within the native and introduced ranges. We provide evidence that the invasive European population likely established through multiple introductions from two major genetic clusters that we identified, while Australian populations appear to have been sourced from a subsequent bridgehead invasion from the European introduction.

2 | MATERIALS AND METHODS

2.1 | Study species

Ambrosia artemisiifolia is a monoecious annual commonly found in disturbed habitats (Oswalt & Marshall, 2008). A full-grown *A. artemisiifolia* plant can produce large quantities of seed that can stay dormant for many years (Bassett & Crompton, 1975). This species is an agricultural pest and has significant effects on crop productivity with competition experiments showing yield losses in corn and soya bean reaching 70% (Brandes & Nitzsche, 2006; Weaver, 2001). Its

wind-spread pollen is a leading cause of hay fever worldwide (Laaidi, Laaidi, Besancenot, & Thibaunod, 2003). Rising CO_2 levels and ongoing climate change are predicted to stimulate *A. artemisiifolia* growth and pollen production, increasing the impact on public health (Emberlin, 1994; Ziska & Caulfield, 2000).

2.2 | DNA extraction and sequence filtering

We collected leaf samples between 2008 and 2014 from three continents: the native range of North America (230 individuals, 42 sampling locations) and introduced ranges of Europe (195 individuals, 36 sampling locations) and Australia (41 individuals, seven sampling locations; Figure 1, Table S1). At each sampling locations, we randomly selected up to seven plants growing at least 1 m apart to reduce the chance of the plants being close relatives due to local pollen and seed dispersal. For each plant, we placed two green leaves into paper envelopes, which were then stored at room temperature in a sealed plastic bag containing silica gel. We extracted genomic DNA (gDNA) from 20 to 30 mg dried leaf tissue for 374 samples using the NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) and an additional 92 samples using the DNAeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). We assessed DNA quantity (>8.5 ng/μl) using a QuBit broad-sensitivity DNA quantification system (Invitrogen, Carlsbad, CA, USA).

2.3 | Genotype-by-sequencing and SNP calling

We performed genotype-by-sequencing library construction with single restriction enzyme *Pst-1* using an amended version of the Elshire et al., 2011 protocol (see Appendix S1). We explored quality statistics of the raw reads using FastQC (http://hannonlab.cshl.edu/fastx_toolkit). We demultiplexed reads using STACKS process_radtags (Catchen, Amores, Hohenlohe, Cresko, & Postlethwait, 2011). After removing adapter sequences, we trimmed reads using Sickle (Joshi & Fass, 2011) with a Q-score of ≥20 and read length of ≥50 base pair. We filtered reads with FASTQ quality filter (http://hannonlab.cshl.edu/fastx_toolkit), allowing for Q-score of 20 or higher for ≥90% of the reads. We aligned filtered reads to a draft reference genome using the Burrows-Wheeler Aligner (Li & Durbin, 2009). The unpublished draft genome was derived from a single diploid individual from the northwest part of the native range (location A19, see supporting information for the location information). This same population was included in our current study. The species is diploid, with a gametic chromosome number of 18 (http://www.tropicos.org/Project/IPCN) and a genome size of ~1,135 Mbp (Kubešová, Moravcova, Suda, Jarošík, & Pyšek, 2010). Multiple whole-genome shotgun libraries were sequenced at 110× coverage using 100-bp paired-end reads, and two Dovetail Chicago libraries were sequenced (14.5× coverage) to create

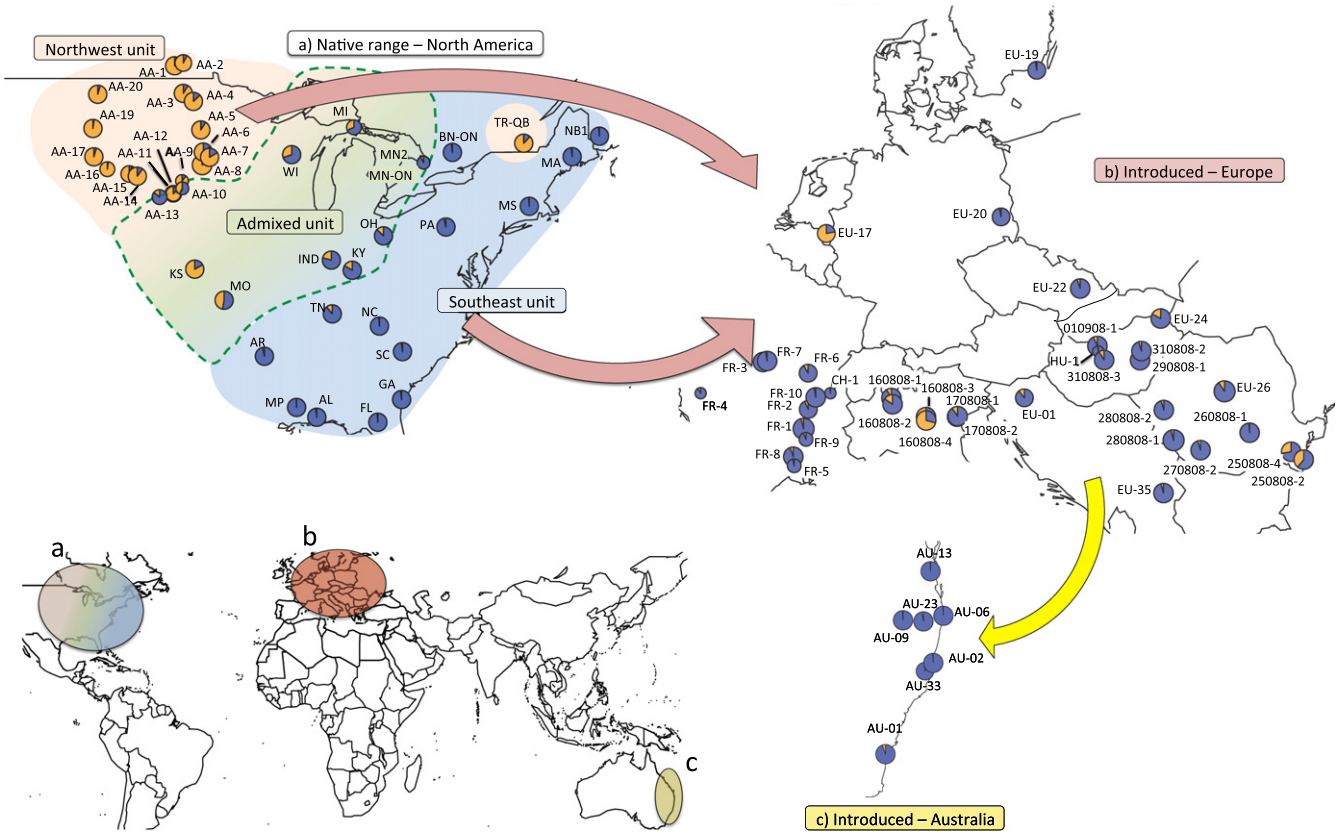

FIGURE 1 Pie charts of STRUCTURE assignment for $K = 2$ (Figure 5) for *Ambrosia artemisiifolia* sampling locations in North America (a), Europe (b) and Australia (c) with invasion routes as inferred from ABC-RF. Assignment to genetic units for ABC-RF is shown in (a). Pie sizes are proportional to number of samples per sampling location. Geographic distances are not to scale [Colour figure can be viewed at wileyonlinelibrary.com]

the draft genome assembly used in this study (Putnam et al., 2016). All sequencing was conducted on the Illumina HiSeq2000. The genome was assembled using MERACULOUS (Chapman et al., 2011) and HiRise for scaffolding (Putnam et al., 2016). The scaffold N50 is 522 Kb in 807 scaffolds, while the N90 is 88 Kb in 3,190 scaffolds. The total scaffold number is 16,702, with a total length of 1,420 Mbp, and with called bases over 893 Mbp (37% Ns).

Local realignment around indels was implemented with Picard (http://picard.sourceforge.net) and GATK (McKenna et al., 2010). We called variants with GATK UnifiedGenotyper at the quality threshold of a Q-score ≥50 and used GATK hard filtering of variants; a minimum quality by depth of 2, a maximum Fisher-Strand bias of 60.0, minimum mapping quality rank sum test of −12.5, minimum root mean square mapping quality of 40.0 and a minimum read position rank sum test of −8.0. We subsequently filtered variant calls using VCFtools (Danecek et al., 2011) and custom scripts on a genotype and variant quality of ≥20, depth of 5–240 and a minor allele frequency of 0.05. Finally, we removed 50 loci displaying heterozygosity frequencies of >0.7 to remove potential paralogues. This latter filtering step is lenient in regards to heterozygosity frequency expectations under Hardy–Weinberg equilibrium (with a maximum heterozygous genotype frequency of 0.5 for biallelic loci) and had no considerable effect on F_{IS} values (results not shown). We identified a total of 10,100 polymorphic SNPs with a call rate of 50% or more. We then selected a total of 1,022 unlinked biallelic SNPs by shuffling the full SNP table and randomly drawing from each contig as STRUCTURE requires the use of unlinked loci for clustering (Pritchard, Stephens, & Donnelly, 2000). To streamline between interdependent methods, we used this unlinked SNP set to select and subset genetic units (STRUCTURE, PCA, pairwise F_{ST} analyses) and to reconstruct invasion history using ABC.

2.4 | Genetic diversity within native and introduced ranges

All analyses were conducted in R v3.2.3 (R Development Core Team, 2014) unless stated otherwise. We calculated genetic diversity indices (allelic richness (A_R), gene diversity (H_S), observed heterozygosity (H_O) and inbreeding coefficients (F_{IS})) for each sampling location using the HIERFSTAT package (Goudet, 2005). We calculated means and confidence intervals for these indices over all 10,100 loci, excluding loci with <4 individuals and excluding sampling locations with >90% missing values over all loci (9 loci excluded). We summarized A_R, H_S and H_O by averaging across populations within each geographic range (i.e., North America, Europe and Australia) and within the genetic units defined for ABC analyses (described below). We tested for differences among the geographic ranges in allelic richness and F_{IS} using means for each sampling location by implementing the Kruskal–Wallis test and post hoc Nemenyi test for pairwise multiple comparisons (PMCMR package, Pohlert, 2014).

2.5 | Genetic differentiation

To identify population genetic differentiation, we estimated Weir and Cockerham's (1984) pairwise F_{ST} between sampling locations and between genetic units defined for ABC (described below) over the 1,022 unlinked SNP data set using the DIVERSITY package (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). To test for genetic dependence (relatedness) of sampling locations caused by isolation by distance (IBD) within each geographic range and clusters defined for ABC analysis (described below) including all sampling locations, we tested associations between $F_{ST}/(1 - F_{ST})$ and log-transformed geographic distances within sampling locations using a Mantel test with 1,000 replicates using the ecodist package (Goslee & Urban, 2007).

2.6 | Genetic clustering

We inferred population genetic structure with STRUCTURE v2.3.4, a Bayesian clustering method that allocates individuals into clusters on the basis of their genotypes (Pritchard et al., 2000). We ran STRUCTURE on 1,022 unlinked SNPs of 466 individuals from 85 sampling locations. Additionally, we ran analysis on subsets of the data to explore subclustering within each continent. We performed the analysis using the admixture model, correlated allele frequencies, no location prior, an uniform alpha individually defined for each population (Wang, 2017) for the number of clusters (K) ranging from 1 to 10, with 20 independent runs per K. Each run comprised of a burn-in of 200,000 followed by 1,000,000 iterations. STRUCTUREHARVESTER v0.6.94 (Earl & vonHoldt, 2011) was used to format the STRUCTURE output. We used log probability and delta K statistic to determine the uppermost clustering level (Evanno, Regnaut, & Goudet, 2005). Nevertheless, we present STRUCTURE results for K from 2 to 8 in order to evaluate differentiation at higher levels of K in the supporting information. We processed the 20 runs using the Greedy algorithm in CLUMPP v1.1.2 (Jakobsson & Rosenberg, 2007) testing 1,000 random input order repeats per K. We found no evidence for multimodality for the most likely K (2) in the global data set as tested using CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). Finally, we visualized data with DISTRUCT v1.1 (Rosenberg, 2003). To further explore relationships between native and introduced sampling locations, we summarized genetic differentiation using a principal components analysis (PCA) within the ADEGENET package (Jombart, 2008). We applied this method on population means for the complete data set to examine genetic variation both within and between continents. Missing values were substituted with mean allele frequencies for the PCA (Jombart, 2008).

2.7 | Outline of ABC-RF approach

We applied approximate Bayesian computation (ABC; Beaumont, Zhang, & Balding, 2002) using DIYABC v2.1.0 (Cornuet et al., 2008, 2014) to simulate provenance from nonadmixed versus admixed source populations, as well as postintroduction admixture during

multiple introductions for both the introduced ranges of Europe and Australia and bridgehead introduction from Europe into Australia. Briefly, within ABC posterior probabilities of different invasion scenarios are obtained by comparing the observed data set to a large number of simulated data sets defined under invasion models given a set of demographic and historical parameters (Cornuet et al., 2008). This approach has been shown to be successful in inferring demographic history in systems that have undergone multiple introductions, bottlenecks and/or admixtures (Estoup & Guillemaud, 2010). We selected among simulated introduction scenarios using ABC-RF (Pudlo et al., 2016), which is a novel approach based on random forest machine learning algorithms. This tool has been shown to outperform existing ABC model selection techniques in precision, computation time and robustness (Marin, Raynal, Pudlo, Ribatet, & Robert, 2016; Pudlo et al., 2016). A large set of summary statistics can be used, as RF is robust against common issues encountered in other methods related to the "curse of dimensionality" (Pudlo et al., 2016), such as collinearity (Blum, Nunes, Prangle, & Sisson, 2013; Estoup & Guillemaud, 2010) and "noise" in the data (Marin, Pillai, Robert, & Rousseau, 2014; Marin et al., 2016). Finally, RF can provide more accurate results using only a low number of simulated data sets per scenario (10^3–10^4, Marin et al., 2016; Pudlo et al., 2016; Fraimout et al., 2017) compared to traditional model evaluation approaches (10^5–10^6, Bertorelle, Benazzo, & Mona, 2010). This last advantage become evident in the current study, as CPU time required for the large data set (1,022 SNPs and 466 individuals, simulating 10^4 data sets per scenario) amounted to 420 hr for the main data sets only. Together with replicate simulations required for reduced data sets, such analyses would not be feasible using traditional methods (Fraimout et al., 2017; Momigliano et al., 2017; Pudlo et al., 2016).

The number of scenarios to be compared within the ABC framework increases drastically with the number of potential source and target population units included in the analysis (Estoup & Guillemaud, 2010; Lombaert et al., 2014). To reduce the complexity of the invasion scenarios, we pooled sampling locations into units showing genetic similarity. For the native range, we defined genetic clusters based on global STRUCTURE assignment scores at $K = 2$, the uppermost clustering level (see section 3). As the central area in the native range constituted of admixed populations according to global STRUCTURE results (see section 3), we were interested in the possibility that this admixed native source had contributed to the introductions. Consequently, we defined three prior genetic units in the native range: southeast (SE), northwest (NW) and admixed (i.e., pre-introduction admixture, AD). Genetic units SE and NW consisted of sampling locations with average proportion of membership to the first STRUCTURE cluster (Q) below 0.15 and above 0.85, respectively, and the admixed unit consisted of sampling locations with $0.15 < Q < 0.85$ (averaged over all individuals within a sampling location). We assumed the native admixed genetic unit did not falsely result from IBD in the native range, as IBD was not significant within the native range as a whole (Table 1) or within the NW and SE cluster. According to the global STRUCTURE results, we pooled European and Australian samples to a single genetic unit within each range (see section 3). Pooling diverged sampling locations as a single unit can alter conclusions drawn from ABC (Lombaert et al., 2014) and introduce the Wahlund effect. Accordingly, we evaluated robustness of the scenario choices by building reduced data sets that excluded divergent sampling locations (Appendix S2 and Table S2).

2.8 | Reconstruction of demographic histories

To reconstruct the introduction history of *A. artemisiifoliia* from its native North American range into either Europe or Australia, we considered introduction scenarios for each range separately with initial, possibly bottlenecked, introductions from the SE, NE or AD genetic units. In the face of repeated introductions, as is prevalent in *A. artemisiifolia* (Chauvel et al., 2006), we improved the first three single-introduction scenarios from different native units by including a secondary introduction from native sources with a second possible bottleneck, leading to a total of 12 scenarios (Figure 2). As Australian introduction is more recent than European introduction, we included a scenario stipulating a bridgehead invasion (Lombaert et al., 2010) —a successful invasion (here Europe) acting as a source for further introductions (here Australia; Figure 3). To reduce the number of scenarios to be compared, we added a bridgehead invasion to the most likely European introduction scenarios according to ABC-RF and tested this against the most likely Australian introduction scenario from the native range (see section 3).

We set uniform priors on all model parameters (Table S3) with the exception of timing of admixture in the native range. As native admixture increased rapidly in more recent years following deforestation and intensification of agriculture (Martin, Olsen, Samaniego, Zimmer, & Gilbert, 2016; Martin et al., 2014), prior sampling followed a log-uniform distribution to favour lower values. We specified prior lower bounds for ancestral divergence and upper bounds for the admixture in the native range based on pollen records, which are consistent with southeast and northwest native genetic units prior to 500 years before present (Williams, Shuman, Webb, Bartlein, & Leduc, 2004). We set parameter prior upper bounds for the timing of the initial introduction several years before the first known occurrence of *A. artemisiifolia* within the introduced ranges (Europe: 180 years before present (ybp); Australia: 120 ybp), with a lower bound set before the onset of known major secondary introductions (Europe & Australia: 100 ybp)(Chauvel et al., 2006; Palmer & McFadyen, 2012). We set the upper prior bound of secondary introductions equal to that of the initial introduction—180 and 120 ybp for Europe and Australia, respectively—with the condition that initial introduction always occurred prior to secondary introduction. This prior would allow a secondary introduction to practically coincide with or be temporally separated from the primary introduction. For Europe, we set the lower prior bound of subsequent introduction events to the end of the last major introduction during the Second World War (60 ybp, Chauvel et al., 2006). For Australia, this prior was set to the last described population increase and spread (50 ybp, Palmer & McFadyen, 2012). Bottleneck priors were set so as to

Range	Summary statistics			F_{ST}	IBD	
	A_R	H_S	H_O	Mean	*r*	*p*
Native, full	1.256 (0.007)	0.262 (0.002)	0.213 (0.004)	0.052	.017	.770
Native, NE	1.243 (0.006)	0.252 (0.003)	0.176 (0.002)	0.032	.137	.253
Native, AD	1.270 (0.018)	0.273 (0.009)	0.243 (0.015)	0.033	**.253**	**.028**
Native, SE	1.264 (0.009)	0.267 (0.001)	0.243 (0.002)	0.066	.012	.929
Introduced, Europe	1.247 (0.006)	0.254 (0.002)	0.199 (0.002)	0.059	.023	.683
Introduced, Australia	1.230 (0.014)	0.235 (0.002)	0.196 (0.002)	0.116	.072	.838

Abbreviations represent allelic richness (A_R); gene diversity (H_S); observed heterozygosity (H_O, bold values are significantly (α = .05) different from H_S); and inbreeding coefficient (F_{IS}, bold values indicate significant (α = .05) departure from zero).

TABLE 1 Population diversity statistics for *Ambrosia artemisiifolia* within native, introduced European and Australian ranges and genetic units defined for ABC analyses based on full data sets, averaged over sampling locations (±95% confidence)

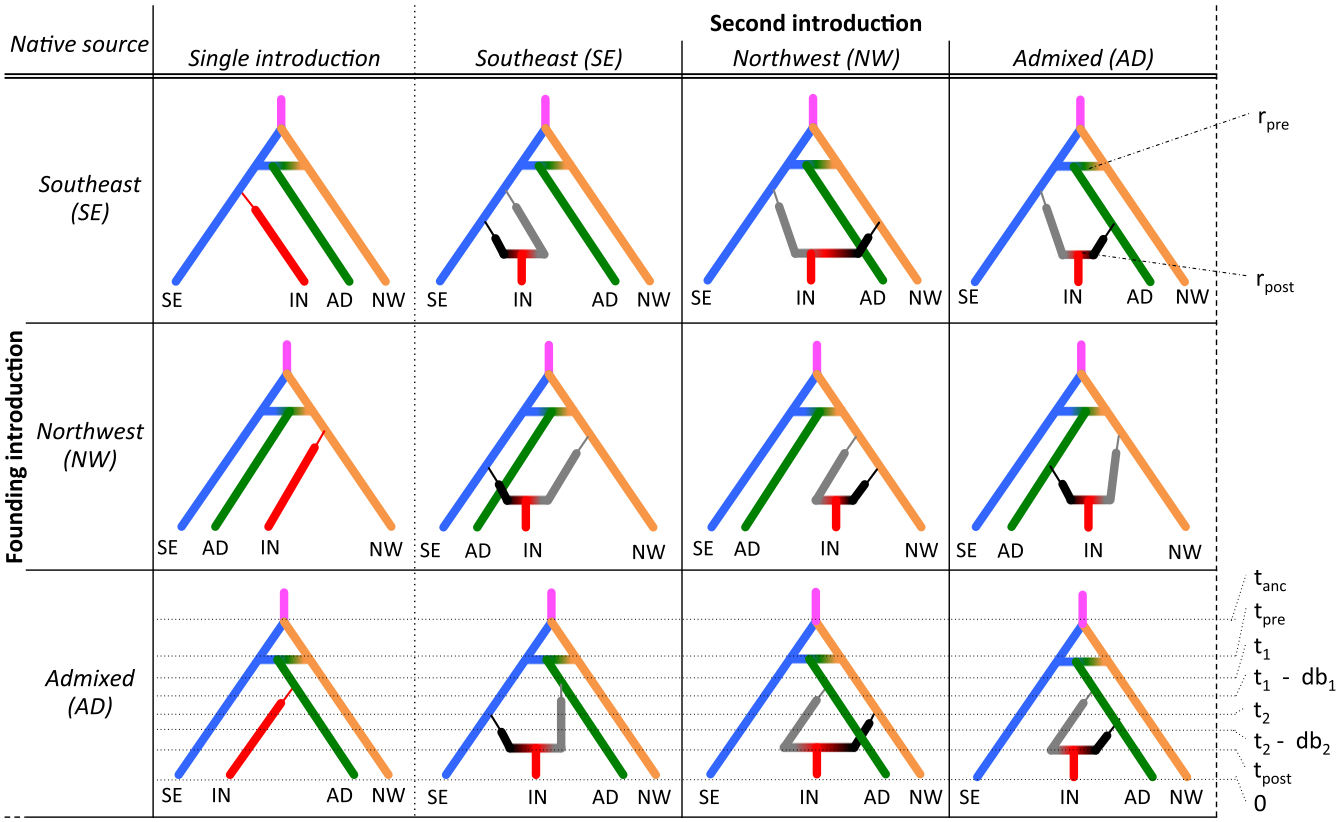

FIGURE 2 Graphical illustration of *Ambrosia artemisiifolia* introduction scenarios from divergent native North American genetic units (SE, NW and AD) during initial (rows) and secondary (columns) introduction, tested using ABC-RF for Europe and Australia introduced ranges (IN) independently (Table 3). Thin lines indicate bottlenecks of duration *dbi* with effective population sizes of *Nbi*. For parameters descriptions and priors see Table S3. Time is not to scale [Colour figure can be viewed at wileyonlinelibrary.com]

simulate no bottleneck (0 years) to a severe bottleneck (Europe: 0–60 years; Australia: 0–50 years). These priors are bound by the last known population increase as described above. We adjusted lower and upper limits of all other priors by evaluating posterior distributions of preliminary simulated data sets, setting the prior distribution as wide as possible but within biological reason (Bertorelle et al., 2010). We assumed no migration between any of the genetic units. We conducted analyses on the 1,022 unlinked SNP data set, specifying a MAF criterion of 0.05 for all simulations. We included all possible summary statistics (Table S4) provided by DIYABC and ran 10,000 simulations for each scenario.

We evaluated model fit and posterior distributions of ABC simulations using the random forest (RF) approach implemented in the ABCRF package v1.3 in R (Marin et al., 2016; Pudlo et al., 2016). For each ABC analysis, we grew a classification forest of 1,000 trees based on all simulated data sets. The ABCRF package estimates a prior error (measure of classification of votes to the wrong scenario) for each analysis using the out-of-bag-errors (Appendix S2). Then, the

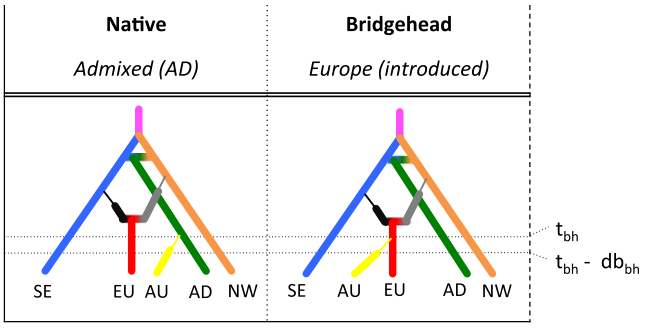

FIGURE 3 Graphical illustration of introduction scenarios of *Ambrosia artemisiifolia* to Australia from the native American admixed zone (AD), as well as and a bridgehead introduction from Europe are based on the European and Australian introduction scenario receiving most votes (Table 3). Nonbridgehead parameters are as in Figure 2 [Colour figure can be viewed at wileyonlinelibrary.com]

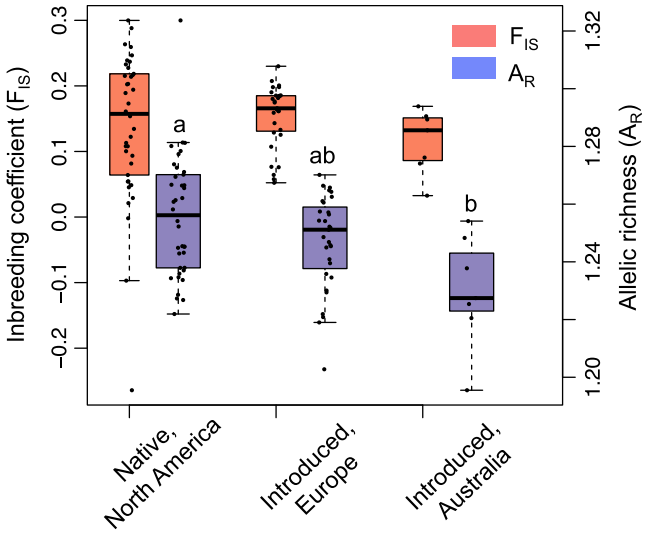

FIGURE 4 Inbreeding coefficients (FIS, red) and allelic richness (AR, blue) for 85 *Ambrosia artemisiifolia* sampling locations at the native North American and introduced European and Australian ranges. No significant differences between ranges were identified in FIS. For AR, letters depict significant differences between ranges (α = .05) [Colour figure can be viewed at wileyonlinelibrary.com]

scenario with the highest classification vote is selected as the most likely scenario for which a posterior probability is calculated. To evaluate the global performance of our ABC scenario choice, we performed posterior model checking in DIYABC (Appendix S2). Finally, we inferred posterior distribution values of parameters of the selected scenario under a regression by random forest methodology (Marin et al., 2016), with a classification forest of 1,000 trees (see Appendix S2 for additional ABC-RF description and evaluation).

3 | RESULTS

3.1 | Genetic diversity within sampling locations

We found homozygous excess in a large number of geographic sampling locations within the native and two introduced ranges of *A. artemisiifolia* (Figure 4, Table S1, Fig. S1). Within the native range, homozygous excess was higher in northwestern sampling locations compared to southeastern locations (latitude Spearman's rho = 0.693, $p < .001$; longitude Spearman's rho = 0.509, $p = .001$). Sampling location allelic richness was significantly reduced in the introduced Australian range compared to the native range (Kruskal–Wallis $\chi^2 = 8.998$, $df = 2$, $p = .011$), but no significant difference was present between the European range and the native range ($p = .275$), or both introduced ranges ($p = .124$, Figure 4).

3.2 | Population structuring and differentiation

We found low genetic structure within the native and two introduced ranges of *A. artemisiifolia*, with a uppermost clustering level at $K = 2$ in STRUCTURE (Figure 5; Fig. S2) according to the ΔK method (Evanno et al., 2005). This analysis clearly identifies a southeastern and northwestern genetic unit in the native range, with most individuals within these units having assignment of $Q > 0.85$ to their respective cluster. We refer to these genetic units as the southeastern and northwestern genetic units throughout. Individuals from 11 sampling locations geographically located between these two main genetic units showed mixed assignment to either cluster ($0.151 < Q < 0.858$). We refer to this unit as the admixed genetic unit (Figure 1). Some sampling locations (7/36) within the introduced range of Europe showed intermediate cluster assignments, whereas all introduced Australian sampling locations showed assignment similar to southeastern native sampling locations. This assignment of Australian sampling locations and southeastern North American sampling locations to the same cluster was evident to some extent at higher K values (Fig. S2). The PCA results reflected the patterns identified in STRUCTURE in that Europe showed most overlap with the admixed and southeast unit, whereas Australia showed more extreme differentiation from the native range but some overlap with the southeast unit (Fig. S3).

To reveal subpopulation structure within each range, we conducted the structure analysis separately for North America, Europe and Australia (Fig. S2). For North America, the uppermost clustering level was two, although we found higher clustering emerging in the European subset at $K = 3$ and $K = 6$ (represented by peaks in ΔK), which could be ascribed to a few distinct sampling locations in Romania, Italy, the Netherlands and Germany. A peak in ΔK appeared in the Australian subset at $K = 7$.

We found low genetic differentiation within the native and two introduced ranges (Table 1; Table S5). Genetic differentiation was highest in the introduced Australian range ($F_{ST} = 0.116$), which was also reflected by a widespread of Australian individuals in the PCA (Fig. S3), and a high number of pairwise $F_{ST} > 0.1$ within this range (Table S5). We found no evidence of IBD within the native range ($r = .017$, $p = .770$) as a whole, although IBD was present within the admixed genetic unit ($r = .253$, $p = .028$). This would be expected if the amount of admixture between the clusters was a function of

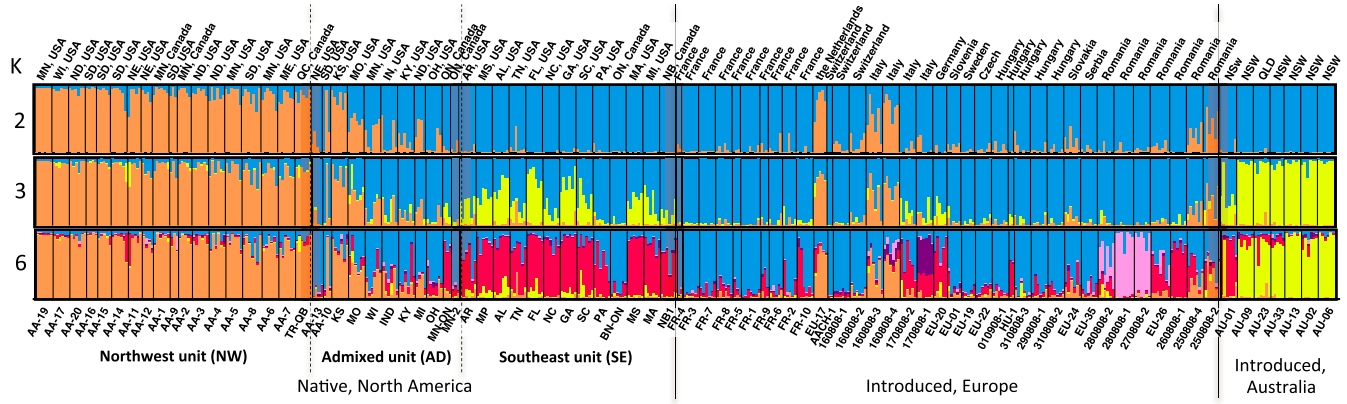

FIGURE 5 STRUCTURE output for K of 2, 3 and 6 for 20 replicate runs each on 1,022 randomly selected unlinked SNPs within 466 *Ambrosia artemisiifolia* samples summarized with CLUMPP and visualized with Distruct. For full overview of STRUCTURE runs see Fig. S2 [Colour figure can be viewed at wileyonlinelibrary.com]

geographic distance to the SE or NW cluster. No such pattern was apparent within the introduced ranges (Europe: r = .023, p = .683; Australia: r = .072, p = .838) or within the main native genetic units (northwestern: r = .137, p = .253; southeastern: r = .012, p = .929; Table 1). Differentiation between North American genetic units was low for all pairwise comparisons (F_{ST} = 0.013–0.034; Table 2). Europe was not highly differentiated from the native units (F_{ST}=0.004–0.021), although Australia showed higher differentiation from the native units (F_{ST} = 0.028–0.052)(Table 2).

3.3 | Invasion history of *Ambrosia artemisiifolia* using ABC-RF

The European invasion was characterized by multiple introductions (concurrent or temporally separated) from the southeast and northwest native North American range as revealed by ABC-RF model selection. The invasion scenario receiving most votes (23.4%) described an initial introduction sourced from the NW and a secondary introduction from the SE genetic units (posterior probability p = .501, Table 3). Overall, European invasion scenarios including an introduction of the native admixed source (6/12 scenarios) received 19.9% (Table 3) of the votes opposed to 80.1% (Table S6) of the votes received by scenarios not including an admixed source, suggesting pre-introduction admixture did not play a large role in shaping the European invasion. The admixed region of the native range contributed to a large extent to the Australian populations, where the single-introduction scenario received the most ABC-RF votes (44.2%, p = .599, Table 3). With the addition of a bridgehead into Australia however, the bridgehead introduction was found to be most likely (92.6% of votes, p = .998, Table 3). This scenario including a bridgehead introduction summarizes the "best" introduction scenarios from preceding analyses and was used for posterior parameter inferences. ABC-RF inferences were robust to data subsetting (Table S6).

Most posterior parameter estimates for the scenario receiving most votes were quite wide and close to the set prior boundaries (Table S7)—a common finding in ABC (Csilléry, Blum, Gaggiotti, & François, 2010). Nevertheless, some important inferences could be made from a select few posterior parameter distributions. First, the formation of the native admixture zone was predicted to be around 218 years ago (median, Table S7), well before the first reported introduction of *Ambrosia artemisiifolia* into Europe, suggesting this zone could have contributed to invasive populations worldwide. Moreover, the relatively narrow posterior parameter distribution of the Australian introduction from the bridgehead Europe suggests this single introduction was followed by long bottleneck period (d_{bh}) of at least 15 years (2.5% quantile) with a relatively small founding population (Nb_5) of maximum 564 individuals (97.5% quantile). Conversely, bottleneck size (Nb_6 & Nb_7) and duration (db_1 & db_2) for the European introduction from the native North America were

TABLE 2 Pairwise F_{ST} for genetic units defined for ABC analyses based on full data sets

	Native, northwest	Native, admixed	Native, southeast	Introduced, Europe	Introduced, Australia
Native, NE		0.016	0.034	0.021	0.052
Native, AD	0.016		0.013	0.004	0.038
Native, SE	0.034	0.013		0.012	0.028
Introduced, Europe	0.021	0.004	0.012		0.034
Introduced, Australia	0.052	0.038	0.028	0.034	

TABLE 3 Summarized results of ABC-RF model selection for introduction scenarios of *Ambrosia artemisiifolia* from divergent native sources of North America (northwest NW, admixed AD and southeast SE) during single or multiple introductions into Europe (a) and Australia (b) (Figure 2) and single introduction from native AD and bridgehead Europe (EU) into Australia (Figure 3)

Analysis	Number of scenarios	Number of summary statistics	Best scenario	Number of RF votes (/1,000)	Prior error (%)	RF posterior probability
(a) European introduction from NW, AD and/or SE	12	112	Multiple introductions from SE & NW	234	44.01	0.501
(b) Australian introduction from NW, AD and/or SE	12	112	Single introduction from AD	442	47.30	0.599
(c) Australia single introduction from AD or EU	2	220	Single introduction from EU	926	0.10	0.998

Scenarios in c are based on the European and Australian introduction scenario receiving most votes (a&b). Analyses consisted of a number of competing scenarios, summarized with all summary statistics available in DIYABC, for which a prior error rate was computed. The best scenario was selected based on a number of random forest (RF) votes, where the RF posterior probability gives the degree of confidence in this selected scenario.

approaching set prior boundaries and therefore not conclusive. Finally, this bridgehead scenario shows narrow posterior distributions of admixture rates, suggesting a median 0.76 contribution of SE to the admixed native source AD (r_{pre}) and 0.74 contribution ($1 - r_{post}$) of a founder population derived from the SE unit to Europe and subsequently Australia (Table S7).

4 | DISCUSSION

We compared the contribution of pre-introduction admixture, multiple introductions and bridgehead invasion of the global weed *A. artemisiifolia* within two of the introduced ranges. ABC simulations indicated admixture following multiple introductions from distinct genetic clusters found in North America played a significant role to the successful European invasion of this weed, subsequently acting as a bridgehead for the Australian introduction. Our study emphasizes the need for careful examination of the population structure and demographic history of invasive species, as introduction pathways–such as bridgehead introduction–may affect evolutionary processes in the introduced ranges. This type of human-assisted spread may become increasingly more common, particularly for species like *A. artemisiifolia*, which is frequently associated with human-modified habitats and intensive agriculture.

4.1 | Genetic diversity and differentiation in native and introduced ranges

The introduction history of common ragweed in Australia has not yet been the subject of much investigation. Although common ragweed has persisted on this continent for over 100 years, it has not become as geographically widespread as its European counterpart. We found lower allelic richness in Australia compared to the native range, while no significant reduction in allelic richness was found in Europe. In addition, Australian sampling locations were more differentiated from each other and from the native and European ranges compared to the other ranges. This result suggests less frequent and/or more bottlenecked Australian introduction(s), as is confirmed by ABC-RF parameter estimation, showing a single Australian introduction followed by a long bottleneck of >15 years. This corresponds to the known colonization history of *A. artemisiifolia*, as repeated introductions of this invader within Europe commenced during the 19th century (Chauvel et al., 2006), whereas the Australian introduction was more recent (Palmer & McFadyen, 2012). Moreover, Australia introduced strict quarantine laws (c. 1908, Quarantine Act, 1908) potentially limiting the frequency of introduction and size of founder populations.

Our results show that a large number of sampling locations were heterozygote deficient, which is consistent with previous studies of *A. artemisiifolia* genetic diversity (Chun, Le Corre, & Bretagnolle, 2011; Chun et al., 2010; Gaudeul et al., 2011; Gladieux et al., 2010; Martin et al., 2014). Northwestern locations within the native range showed higher heterozygote deficiency compared to southeastern locations. A similar longitudinal pattern in *A. artemisiifolia* observed by Martin et al. (2014) has been interpreted by the authors to be a result of higher rates of selfing and/or biparental inbreeding due to local pollen and seed dispersal in the smaller, low-density, isolated western populations opposed to outbreeding in larger, high density, interconnected eastern populations. A shift in mating system could arise in low-density conditions or during colonization (Barrett, Colautti, & Eckert, 2008) and might explain the geographic pattern in F_{IS}. However, *A. artemisiifolia* in Ontario exhibit high levels of outcrossing and a sporophytic self-incompatibility system (Friedman & Barrett, 2008) and no evolutionary shift towards selfing has been found in the introduced range of China (Li, Liao, Wolfe, & Zhang, 2012). The Wahlund effect resulting from local substructuring could

be a cause for the observed heterozygote deficiency. However, pollen dispersal within this species is likely high as it is wind-pollinated (Martin, Chamecki, Brush, Meneveau, & Parlange, 2009) and so substructuring might be expected to be limited within each sampling location (Genton et al., 2005; Martin et al., 2014). These contrasting observations remain curious, and geographic variation in selfing and biparental inbreeding has yet to be investigated in this species.

4.2 | Pre- versus postintroduction admixture during invasion

We found some mixed assignment of European individuals in STRUCTURE and PCA and population allelic richness equal to the native range. This complex European genetic structure has been previously observed and was accredited to multiple introductions from various source populations and postintroduction admixture (Chun et al., 2010; Gaudeul et al., 2011; Genton et al., 2005), which is a common conclusion for invasive species with admixed genetic backgrounds (Cristescu, 2015; Dlugosch & Parker, 2008; Facon, Pointier, Jarne, Sarda, & David, 2008; Uller & Leimu, 2011). Conversely, a human-mediated intraspecific hybrid zone within native North America has been proposed as a possible introduction source (Martin et al., 2014).

According to ABC-RF posterior parameter analyses, the pre-admixed native zone originated well before the first reported introductions (>180 years ago), suggesting this zone could have been an introduction source for the global *A. artemisiifolia* invasions. However, ABC-RF analysis encompassing diverse introduction scenarios supports the establishment of European populations through multiple introductions of the two nonadmixed native units, with less likely contribution of native pre-introduction admixture. Although the analysis without a bridgehead scenario suggested Australia originated from the native admixture zone, further investigation indicated the invaded European range instead acted as a bridgehead for the single introduction into Australia. Our results underline the importance of testing combinations of contrasting introduction scenarios simultaneously.

Using simplified versions of the true demographic histories is a widely recognized limitation of ABC inference (e.g., Csilléry et al., 2010) and could possibly contribute to the relative low posterior probabilities (0.501–0.599) found for scenarios depicting introduction from the native range as only source (i.e., the nonbridgehead scenarios). Similar posterior probabilities have been reported in other studies, with contrasting interpretations by the authors (Fraimout et al., 2017; Momigliano et al., 2017; Pudlo et al., 2016).

In our study, we grouped sampling locations based on relatively homogeneous genetic clusters identified by STRUCTURE (NE mean F_{ST} = 0.032; SE mean F_{ST} = 0.066) with no evidence of isolation by distance within these two genetic clusters. Although it is possible more complex cluster schemes could support different introduction histories, our results were consistent among stringently filtered subsets where divergent populations within each cluster were excluded. Moreover, the analysis was powerful in distinguishing between single versus multiple introductions and pre- versus postintroduction admixture or bridgehead invasion, but we cannot exclude the existence of additional introductions from other sources. For example, introductions might have been sourced from native locations that were not captured in our broad sampling of the North American range (e.g., south Florida). In addition, the Australian introduction, which occurred more recently than the European introduction, may have had contributions from another yet unsampled introduction such as Asia, South America or eastern Europe. Other possibilities include re-introductions from Europe or anywhere else in the world back into the native North America. Given the very short time since the initial invasions, we find it unlikely that a large number of non-native individuals could have had a significant impact on the well-established native populations. Moreover, we are limited in the complexity of the models and could not explore the myriad of possibilities imaginable through ABC simulations.

The discovery of multiple introductions and admixture or bridgehead invasions to the non-native European and Australian populations does not confirm the causality of these processes in driving successful invasion in *A. artemisiifolia*. Patterns discovered in this study might simply reflect historic trade routes and admixture may not have contributed to the invasiveness of this species. However, our finding of admixture as a predominant feature in Europe through multiple introductions and in Australia through a bridgehead introduction raises the question of fitness benefits of hybridization to a successful invasion. Moreover, given the global nature of the invasion, the native admixture zone should be considered as potential source for nonsampled invasive introductions in Asia and elsewhere. Although the beneficial consequences of pre- and postintroduction admixture are similar (described below), the role of pre-admixed source populations as a pathway to invasion has been given less attention in the literature than multiple introductions and admixture postinvasion (Keller & Taylor, 2010; Keller et al., 2014; Rius & Darling, 2014).

The immediate fitness benefits of admixture are most widely established to be a consequence of heterosis, stimulating invasiveness through increased growth and vigour, which may in turn "catapult" populations past the lag phase of colonization (Drake, 2006; Facon, Jarne, Pointier, & David, 2005; Keller et al., 2014; Wagner, Ochocki, Crawford, Compagnoni, & Miller, 2016). Through this process, admixed populations could outperform single-source invasions, as has been shown in an experimental study on the beetle *Callosobruchis maculatus* (Wagner et al., 2016). Within an experimental study of *A. artemisiifolia*, certain native crosses displayed heterosis whereas other crosses between native populations had no fitness impacts or lead to mild outbreeding depression (Hahn & Rieseberg, 2016). By contrast, no heterosis was found in crosses among the European populations. This heterosis experiment aligns with the genomic analysis presented in the current study, as crosses between nonadmixed native genetic clusters (here northwest X southeast) were more likely to display heterosis as opposed to crosses to the historically admixed populations or between European populations. In addition, populations from the northwestern cluster in the native

range consistently underperformed in traits associated with invasive success compared to introduced European populations in common gardens under a range of environments (Hodgins & Rieseberg, 2011).

Admixture could also facilitate adaptation through increased genetic variation (Anderson & Stebbins, 1954) and creation of novel or transgressive phenotypes (Rieseberg, Archer, & Wayne, 1999; Stebbins, 1969). Patterns of genetic differentiation of traits, such as latitudinal variation in flowering time, have evolved repeatedly in *A. artemisiifolia* following introduction (Chun et al., 2011; Hodgins & Rieseberg, 2011; Li, She, Zhang, & Liao, 2014) and are consistent with rapid local adaptation within the introduced ranges. In Europe in particular, no evidence of substantial bottlenecks limiting genetic variation are evident. This raises possibility that the influx of genetic material during introduction fuelled rapid local adaptation perhaps enhancing the rate of spread of the invader in Europe. Even in Australia, where allelic richness is significantly reduced relative to the native range, the ABC analysis is consistent with a European source population, which was likely admixed.

4.3 | Future directions

Mechanisms that could promote invasiveness through bridgehead invasions could also play an important role in the success of pre-introduction admixture. We recommend that admixed source populations should be considered as a factor potentially impacting invasiveness both for initial and subsequent colonizations. In addition, this study underlines the necessity of more intricate testing of introduction scenarios (Cristescu, 2015), as classical population approaches might prove to be misleading (Falush, van Dorp, & Lawson, 2016; Lombaert et al., 2014). For example, the role of the European invasion as a source of the Australian introduction would not have been considered without the ABC analysis. Identifying the specific source populations of introduced species might play a role in predicting its invasive success and could mean management effort should be focused on preventing introductions from admixed or multiple divergent source populations.

Better understanding of the importance of admixture to invasion requires: (i) an accurate reconstruction of invasion routes (Cristescu, 2015); (ii) examination of admixture costs and benefits within native and invasive populations; and (iii) fitness assessments of admixed versus nonadmixed genotypes in the invasive range(s) over multiple generations (Cristescu, 2015). As our data elucidate the first of the three requirements, future studies need to further explore the costs and consequences of admixture for invasion using genomic and experimental data in this highly diverse and widespread invader.

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DATA ACCESSIBILITY

Sequence data are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive under Bioproject PRJNA374597.

AUTHOR CONTRIBUTIONS

L.B. and K.H. conceived the project with input from E.L., B.G. and L.R. K.H., L.B. and K.N. collected samples, K.N. performed molecular laboratory work. L.B. analysed data and drafted manuscript. All authors contributed to and approved the final manuscript.

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