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The rapid spread of *Leptoglossus occidentalis* in Europe: a bridgehead invasion

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Abstract
Retracing the routes of invasions and determining the origins of invading species is often critical in understanding biological invasions. The Western conifer seed bug, *Leptoglossus occidentalis*, an insect native of western North America, was first accidentally introduced to eastern North America and then to Europe. The colonization of the entire European continent occurred in ca. 10–15 years, probably promoted by independent introductions in different parts of Europe. A multi-marker approach (mtDNA and microsatellites) combined with approximate Bayesian computation analyses was used to track the origin of European populations and to determine whether this rapid invasion was caused by multiple introductions. Our results show that at least two independent introductions of *L. occidentalis* have occurred in Europe. Moreover, the analyses showed a stronger genetic similarity of European invasive populations with the eastern North American populations than with those of the native range, suggesting that invasive North American population acted as a bridgehead for European invasion. The results also revealed that natural dispersal as well as human-mediated transportations as hitchhikers probably enhanced the rapid spread of this invasive pest across Europe. This study illustrates the complexity of a rapid invasion and confirms that bridgehead and multiple introductions have serious implications for the success of invasion.

Keywords Approximate Bayesian computation · Microsatellite · Mitochondrial DNA · Multiple introductions · Source population · Western conifer seed bug

Key message

- The colonization of Europe by the Western conifer seed bug, *Leptoglossus occidentalis*, a serious pest of conifer seeds, occurred in less than 15 years
- The combination of traditional population genetic analyses and approximate Bayesian computation analyses allowed reconstructing the invasion scenario and tracking the origin of European populations
- The European invasion likely results from multiple independent introductions originating from eastern North America, the first invaded area, suggesting a bridgehead invasion scenario
- The data confirm the complexity of this invasion process and provides useful data for management of this seed pest

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Introduction

The increased intercontinental movements of goods and people over the recent decades have led to a steep increase of introductions of alien species beyond their native ranges (Seebens et al. 2017; Westphal et al. 2008). This is especially true for terrestrial invertebrates and more particularly for insects (Gandhi and Herms 2010; Roques 2010). Among successful invaders, many are responsible for severe economic, ecological and public health damage (Aukema et al. 2011; Juliano and Lounibos 2005; Kenis and Branco 2010; Kenis et al. 2017). Due to the potential threat that alien insects represent, retracing the routes of invasions and determining the source of introduced populations is an important step to establish suitable management programs such as the development of biological control solutions, or the development of strategies for understanding invasion pathways and preventing new accidental introductions from the identified source population (Estoup and Guillemaud 2010).

The Western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae), is a good example of successful invaders. This polyphagous species is considered as a major pest of conifer seeds in commercial seed orchards (Lesieur et al. 2014b; Strong 2016) but may also strongly affect the potential of regeneration in natural stands (Lesieur et al. 2014b) as well as the production of edible seeds (Bracalini et al. 2013; Farinha et al. 2017). Its native range covers western North America (wNA), where it is widely distributed from British Columbia to Mexico and from the Pacific coast to Colorado (Koerber 1963). In the 1950s, the species was discovered outside its native range in eastern North America (eNA) with a first record in Iowa (Schaffner 1967). Since then, its eastern invasion has been extensively documented, and the species was reported to reach the Atlantic Coast in the 1990s (Gall 1992; McPherson et al. 1990; Ridge-O'Connor 2001) and spread as far east as Nova Scotia in 2008 (Scudder 2008). In Europe, the species was first reported in northern Italy in 1999 (Taylor et al. 2001). It was then observed to colonize the whole European continent in less than 15 years (Dusoulier et al. 2007; Fent and Kment 2011; Gapon 2012; Malumphy et al. 2008). Moreover, recent observations in Eastern Asia (Ahn et al. 2013; Ishikawa and Kikuhara 2009; Zhu 2010), northern Africa (Ben Jamaa et al. 2013; Gapon 2015), Asia Minor (Van der Heyden 2018) and South America (Faúndez and Rocca 2017) confirmed that *L. occidentalis* has become a highly successful worldwide invader.

The colonization of Europe occurred within a very short time frame (ca. 10–15 years) as was the case for many alien insect species having arrived in Europe in the recent past (Roques et al. 2016). This rapid invasion could have multiple explanations. In addition to the first Italian report, several independent introductions were suspected because of spatially disconnected first records in Spain (Pérez Valcárcel and Prieto Piloña 2010; Ribes and Escolà 2005), France (Dusoulier et al. 2007), Belgium (Aukema and Libeer 2007) and Great Britain (Malumphy et al. 2008). Observations near important harbor areas (e.g., Venice, Barcelona, Le Havre, Ostend or Weymouth) suggested that the propagules could have been transported by ships as hitchhikers in containers (Dusoulier et al. 2007). *Leptoglossus occidentalis* is known to aggregate for overwintering in many different kinds of sites such as under loose bark, in holes of dead trunks, in birds' nests, but also within man-made habitats such as buildings and containers (Blatt 1994). Interceptions of adults in containers transporting timber logs and wood panels from eastern North America suggested that timber trade may be its primary introduction pathway (Dusoulier et al. 2007; Malumphy et al. 2008). Moreover, these interceptions also suggested that some of the European populations may originate from eastern North America, corresponding to a bridgehead invasion scenario. A bridgehead invasion is considered when a primary invasive population serves as a source for subsequent invasions (Lombaert et al. 2010). Afterward, individuals (eggs, nymphs or adults) may have spread as hitchhikers within the invaded areas along with the trade of their host plants, for example with commercial Christmas trees or other ornamental trees (Gall 1992; Gapon 2012). Furthermore, the strong flight capacities of adults (Lesieur 2014; Malumphy et al. 2008; Ridge-O'Connor 2001) could have enhanced the rapid dispersal of the species across the European continent. We therefore tested the hypothesis that multiple introductions of *L. occidentalis* from North America combined with human-mediated and natural dispersal within the continent were crucial for its invasion.

Molecular genetics approaches have been widely applied to reconstruct such invasion histories and colonization routes (Estoup and Guillemaud 2010; Kirk et al. 2013). However, the stochasticity of demographic and genetic events associated with biological invasions (such as founder events, genetic admixture, etc.) may produce complex genetic signals (Dlugosch and Parker 2008; Guillemaud et al. 2010; Rius and Darling 2014). Therefore, retracing the invasion routes may be challenging.

To reconstruct the invasion history of *L. occidentalis*, we compared the genetic structure and diversity in the native range with those observed in the invaded areas of both eNA and Europe. Furthermore, we aimed to identify the most likely source(s) for the European populations and to determine whether the European invasion proceeded from one or multiple introduction events. For this purpose, we used a

multi-marker strategy, combining data from microsatellites markers and sequences of mitochondrial cytochrome *b* gene, obtained from individuals collected in North America and Europe. We also combined traditional population genetic analyses and approximate Bayesian computation (ABC) analyses (Beaumont et al. 2002) for deciphering the European invasion routes of *L. occidentalis*.

Materials and methods

Collection sites, sampling and DNA extraction

The sampling of *L. occidentalis* individuals spanned native range and invaded areas in North America and Europe. This species shows a chaotic population dynamics (i.e., high annual population fluctuations) both in native area (W. Strong, comm. pers.) and invaded areas (Lesieur et al. 2014a, b; Tamburini et al. 2012); therefore, the sampling resulted from available sites and opportunistic collections, especially in eastern North America. The sampling largely spanned the native range with 16 sites in western North America (Fig. 1 and Supplementary material, Table S1). Invaded areas have been sampled as follows: (1) the first invaded area, eastern North America (seven sites) and (2) the European invaded area (34 sites distributed across the continent). In total, 656 *L. occidentalis* specimens were collected in seed orchards, ornamental trees and natural conifer stands as well as in buildings where adults seek shelter to overwinter in the fall. All samples were stored in 96% ethanol at − 20 °C. DNA was obtained from muscle tissue of the hind femur using NucleoSpin® Tissue XS kit (Macherey-Nagel, Germany) following the manufacturer's instructions. DNA was eluted in 30 µl of the elution solution and stored at − 20 °C. The concentration of the individual eluted DNA was about 20 ng/µl.

Molecular analyses of mitochondrial DNA

DNA protocols

Mitochondrial cytochrome *b* gene (Cyt*b*) was amplified in 254 individuals via PCR using the general insect primer pair CP1 (Harry et al. 1998) and CB2 (Jermiin and Crozier 1994). All PCR products were purified using the NucleoSpin® Extract II kit (Macherey-Nagel, Germany) and directly sequenced with the amplification primers. Sequencing was performed using the ABI Prism® BigDye v3.1 Cycle Terminator Sequencing Kit (Applied Biosystems, USA) and carried out with an ABI 3500 Genetic Analyzer (Applied Biosystems, USA). All sequences were obtained in the forward and reverse directions, assembled into consensus contigs using CodonCode Aligner (www.codoncode.com) and

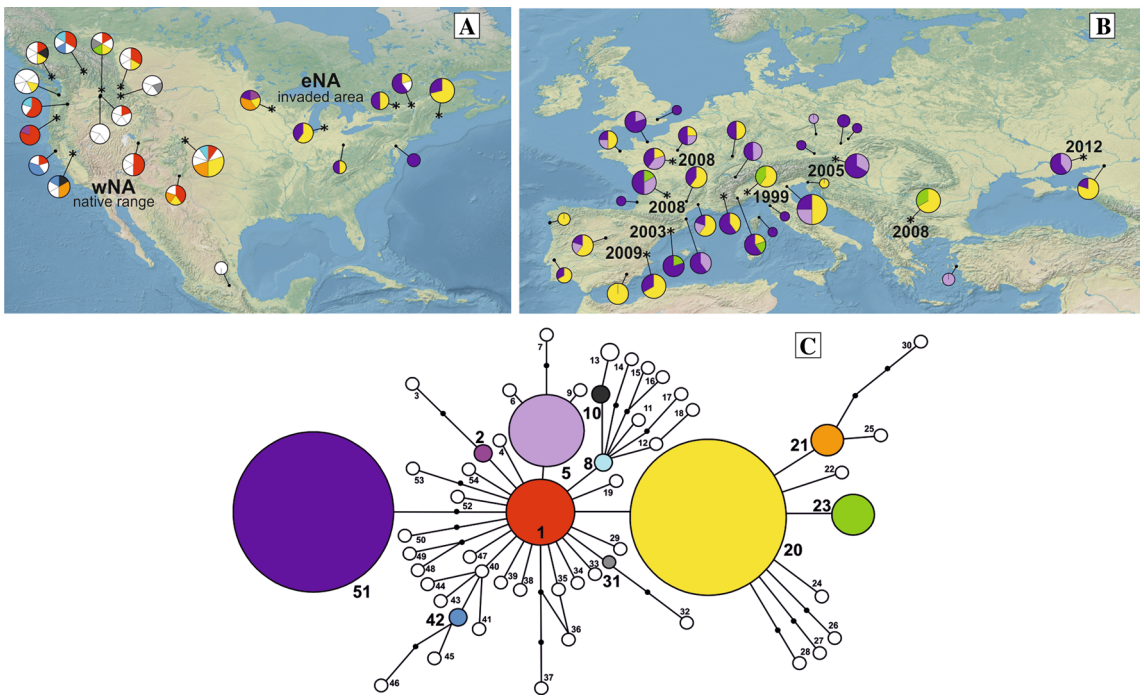

Fig. 1 Geographic location of *Leptoglossus occidentalis* samples and distribution of the *COI* mitochondrial haplotypes in **a** North America and **b** Europe. **c** *COI* mitochondrial haplotype network. Single haplotype or haplotype found in a single site is represented in white. Asterisks represent the sampling sites where both mtDNA and microsatellites studies were carried out. Dates correspond to the first observations of the species in the sampling sites

then aligned using CLUSTAL W (Thompson et al. 1994) implemented in CodonCode.

Data analyses

Analyses were performed for all populations for which at least three individuals had been sequenced. Gene diversity H_d was calculated using Arlequin v 3.11 (Excoffier et al. 2005). Allelic richness r was computed using the rarefaction method proposed by Petit et al. (1998) with Contrib (http://www.pierroton.inra.fr/genetics/labo/Software/Contrib). Statistical parsimony network was computed with TCS v 1.21 (Clement et al. 2000). To solve the few network ambiguities that occurred, we used topological, geographic and frequency criteria (Crandall and Templeton 1993). To better characterize the native range, occurrence of a significant geographic structure was assessed by testing whether G_{ST} (coefficient of genetic variation over all populations) was significantly smaller than N_{ST} (equivalent coefficient taking into account the similarities between haplotypes) by the use of 1000 permutations in the program Permut (Pons and Petit 1996).

Molecular analyses of microsatellite markers

DNA protocols

Eleven microsatellite markers developed by Lesieur et al. (2014a) were used to genotype a subsample of 506 individuals (Supplementary material Table S1). PCR amplifications were performed following the protocol described in Lesieur et al. (2014a). PCR products were run in an ABI 3500 Genetic Analyzer using the size standard GeneScan™-600 LIZ® (Applied Biosystems, USA). Alleles were scored with GeneMapper® v 4.1 (Applied Biosystems, USA).

Data analyses

Observed and expected heterozygosity (*Ho* and *He*) and allelic richness obtained with the rarefaction method (*AR*) were estimated using FSTAT 2.9.3.2 (Goudet 2002). We also calculated inbreeding coefficients (F_{IS}) with Genepop 4.2.1 (Rousset 2008). Deviation from Hardy–Weinberg equilibrium and linkage equilibrium between pairs of loci were tested with Genepop. Sequential Bonferroni corrections (Rice 1989) for multiple comparisons were applied for both tests.

FreeNA (Chapuis and Estoup 2007) was used to estimate the null allele frequencies for each locus in each population according to the Expectation Maximization algorithm described by (Dempster et al. 1977). The FreeNA software was also used to calculate F_{ST} values using the excluding null allele correction method. Genotypic pairwise differentiation was tested using Fisher's exact tests implemented in Genepop. We used the sequential Bonferroni correction to correct for multiple comparisons. Pairwise Cavalli-Sforza and Edwards' chord distance measures (Cavalli-Sforza and Edward 1967) using the genotype data set corrected for null alleles were calculated in Population 1.2.32 software (Langella 1999). The resulting distance matrix was used to build a population-based neighbor-joining (NJ) tree. The robustness of the nodes was evaluated by carrying out 1000 bootstrap replicates over loci. The NJ tree was visualized with TreeView (Page 1996).

To explore the population structure within the whole data set, we used the Bayesian clustering approach implemented in Structure 2.3.4 (Pritchard et al. 2000). An admixture model with correlated allele frequencies and sampling location as prior was used. In situations of low levels of genetic divergence or a limited number of loci, this model allows a more accurate detection of genetic structure (Hubisz et al. 2009). The burn-in period of each run was set to 200,000 followed by 1,000,000 MCMC iterations. We performed 20 independent runs for each value of K ranging from 1 to 10. We assessed the uppermost level of population structure by using the ΔK method (Evanno et al. 2005) implemented in Structure Harvester (Earl and Vonholdt 2012). The graphical display of genetic structure was produced with Distruct (Rosenberg 2004).

Inferring invasion scenarios using mitochondrial and microsatellite data

An approximate Bayesian computation (ABC) approach was performed using the software DIYABC 2.0 (Cornuet et al. 2014) with both mitochondrial and microsatellite markers to test for possible introduction routes of *L. occidentalis* into Europe.

For sake of simplicity and based on the low genetic structure observed across the native range (see Results), only one population was considered to be representative of this area (i.e., Missoula, due to its low differentiation with the other wNA samples). Sequential ABC analyses of invasion scenarios were performed taking into account the different observations in Europe in their order of first observation date. In the case of *L. occidentalis*, dates of first sighting and date of introduction are likely to be highly correlated (Schaffner 1967). The sampling in eNA gave us a limited representation of the genetic diversity in this invaded area (only two samples for which the number of *L. occidentalis* individuals genotyped with the microsatellite markers was higher than five), and thus, some invasive source populations could have been missed. Consequently, an unsampled invasive eNA source population was included as a putative origin of European populations. In the scenarios analyzed, the sampled and unsampled eNA populations recently diverged

from a common ancestral population. A visual support of the tested scenarios for every analysis is provided in Supplementary material Fig. S1.

Northern Italy being the first place where *L. occidentalis* was observed in Europe, consequently, the origin of the Italian sample was first examined. The native wNA population, an unsampled eNA invasive population or an admixture between them were considered as potential sources, thereby defining three scenarios (Supplementary material, Fig. S1a). Each subsequent analysis took into account the scenario that was selected in the previous analysis. Therefore, in the second analysis, to decipher the origin of Barcelona's population that corresponded to the second suspected introduction area in Europe, there were three potential source populations—the native wNA, an unsampled eNA invasive population, the Italian outbreak—and the various admixed populations between these sources. Consequently, six scenarios were compared (Supplementary material, Fig. S1b). In the same way, the following analysis (origin of Vienna outbreak) included ten competing scenarios (Supplementary material, Fig. S1c). The last analyses dealt with invasion histories of the French, Valencian and Bulgarian populations, each analysis being constructed in the same way. In order to reduce the number of competing scenarios and based on Structure results and the NJ tree, the wNA sample was not considered as a source of invading European populations. Regarding the date of first detection of the French, Valencian and Bulgarian populations (each of them comprised between 2007 and 2009) (Table 1), we hypothesized that one could not be a potential source of the others. The idea behind this hypothesis is that a very recently founded population may not be large enough to serve as a source for new introductions. Therefore, ten competing scenarios were finally tested in each analysis (Supplementary material, Fig. S1d to Fig. S1h).

The ABC analyses were performed using parameter values drawn from the prior distributions described in Table 1. Further details of the model specifications, DIYABC run parameters and the estimates of the posterior probabilities are presented in Supporting material (Appendix 1).

Table 1 Prior distribution of parameters used for modeling the different scenarios of the European invasion of *Leptoglossus occidentalis*

Parameters	Interpretation	Distribution	
N_i	Effective population size	Log-uniform	[1000; 100000]
t_{Uns}	Time to introduction event based on first observation in eNA (Schaffner 1967)	Uniform	[77; 82]
t_{Pitt}	Time to introduction event for Pittston (eNA) based on first observation (Ridge-O'Connor 2001)	Uniform	[25; 30]
t_{Ales}	Time to introduction event for Alessandria (northern Italy) based on first observation (Taylor et al. 2001)	Uniform	[18; 23]
t_{Barc}	Time to introduction event for Barcelona (Spain) based on first observation (Ribes & Escolà 2005)	Uniform	[12; 17]
t_{Vien}	Time to introduction event for Vienna (Austria) based on first observation (Rabitsch and Heiss 2005)	Uniform	[9; 14]
t_{Sepo}	Time to introduction event for Serre-Ponçon (France) based on first observation (Dusoulier et al. 2007)	Uniform	[6; 11]
t_{Yvoy}	Time to introduction event for Yvoy le Marron (France) based on first observation (A. Roques comm. pers.)	Uniform	[4; 9]
t_{Lave}	Time to introduction event for Lavercantière (France) based on first observation (A. Roques comm. pers.)	Uniform	[4; 9]
t_{Kyou}	Time to introduction event for Kyoustendill (Bulgaria) based on first observation (Simov 2008)	Uniform	[4; 9]
t_{Vale}	Time to introduction event for Valencia (Spain) based on first observation (Pérez Valcárcel & Prieto Piloña 2010)	Uniform	[4; 9]
db_i	Duration of bottleneck	Uniform	[0; 10]
Nb_i	Effective number of founders during an introduction step	Log-uniform	[2; 1000]
ar	Admixture rate for scenarios with admixture	Uniform	[0.1; 0.9]
Mean μseq	Mean mutation rate for mitochondrial marker	Uniform	$[10^{-8}; 10^{-6}]$
Mean μmic	Mean mutation rate for microsatellite markers	Uniform	$[10^{-5}; 10^{-3}]$
Mean P	Mean parameter of the geometric distribution	Uniform	[0.1; 0.3]
Mean μ_{SNI}	Mean single nucleotide insertion/deletion rate	Uniform	$[10^{-8}; 10^{-4}]$

Time parameters (including duration of bottleneck) are translated into numbers of generations assuming 1.5 generation per year. The following conditions were used in the different analyses $t_{\text{Uns}} > t_{\text{Pitt}} > t_{\text{Ales}} > t_{\text{Barc}} \geq t_{\text{Vien}} \geq t_{\text{Sepo}}$; t_{Yvoy}; t_{Lave}; t_{Kyou} and t_{Vale}. A generalized stepwise mutation model (GSM) was used for microsatellites with a mean mutation rate (mean μmic), a mean parameter of the geometric distribution (mean P) of the length in number of repeats of mutation events. Each locus had a possible range of 40 contiguous allelic states and the mean insertion or deletion of single nucleotide mean μ_{SNI}. Mitochondrial marker was assumed to follow a Kimura-2-parameters model with a mean mutation rate (mean μi). The fixed boundaries of each prior are shown within brackets

Results

Mitochondrial DNA results

MtDNA from 254 individuals of *L. occidentalis* from the 57 North American and European population samples was amplified and sequenced. The final alignment of the Cyt*b* sequences comprised 662 bp. Fifty four different haplotypes were identified (Fig. 1) and named H1 to H54. They are available from GenBank under accession numbers MG251982 to MG252035. No insertion or deletion was present and all the haplotypes gave clear, unambiguous sequence chromatograms, and no indication of pseudogenes was observed.

In wNA, the maximum divergence between haplotypes was nine mutation steps while haplotypes found within eNA and Europe differed by five and four mutation steps, respectively. The geographic distribution of the haplotypes is shown in Fig. 1. Haplotype diversity was higher in wNA than in the two other regions (Fig. 1 and Supplementary material Table S1). Among the 51 haplotypes, we found 48 haplotypes in the native range (wNA), 44 were exclusively present in this area, with H1 as the most common haplotype. The G_{st} value (0.039) did not differ significantly from the N_{st} value (0.035), indicating a lack of phylogeographic structure existing in haplotype distribution in wNA. Only five haplotypes were found in eNA and four in Europe, these invaded areas sharing two haplotypes (H20 and H51), which were the most common ones in both regions. Only the haplotype (H20) was shared by the native wNA range and the two invaded areas of eNA and Europe. Europe also showed one private haplotype (H5) while the haplotype H23, observed in five European sites, was only observed once in the native wNA.

Microsatellite results

After sequential Bonferroni corrections, seven cases of significant linkage disequilibrium were found in the 1045 pairwise tests carried out. However, a given pair of loci was never in significant linkage disequilibrium in more than two samples. The 11 microsatellite markers were thus considered independent.

A total of 242 alleles were found, of which 130 were observed exclusively in wNA, whereas five were only noticed in invasive populations (four in eNA and one in Europe). Allelic richness was larger in samples from wNA than in invasive samples, and eNA samples showed a higher allelic richness than European ones (Supplementary material Table S1). Expected heterozygosity ranged from 0.628 to 0.671 for wNA samples and from 0.487 to 0.623 for invasive populations (Supplementary material, Table S1). Positive F_{is} values and significant heterozygote deficiencies were observed in all populations (Supplementary material, Table S1). Moreover, four microsatellite loci (Lep04, Lep05, Lep31, Lep36) had a mean estimated proportion of null alleles above 8% while the others never exceeded 5%. Therefore, all data analyses were repeated without these markers. Significant heterozygote deficiencies were still observed in all native samples but only in two invasive samples (Serre-Ponçon and Vienna) after these loci had been removed.

The pairwise F_{ST} values, estimated using the excluding null allele correction method (Supplementary material, Table S2), were very close to the ones obtained when using the conventional method. In wNA, despite locations relatively distant from each other (up to 1800 km), pairwise F_{ST} estimates were low, ranging from -0.003 to 0.025. Pairwise genetic differentiation between eNA and wNA samples was larger with F_{ST} comprised between 0.027 and 0.082. European samples showed a higher level of genetic differentiation with North American samples. Considering all North American populations, each European sample has the lowest F_{ST} values with eNA populations and particularly with Pittston (F_{ST} comprised between 0.029 and 0.065). Overall, the highest F_{ST} value was observed between the two Spanish samples, Barcelona and Valencia (0.141).

The NJ tree constructed from Cavalli-Sforza and Edward's chord distances showed a split between two groups: (1) the wNA populations and (2) all invasive populations (Supplementary material, Fig. S1). Limitation of the analysis of genetic differentiation to the seven loci with a low proportion of null alleles produced qualitatively similar results (data not shown).

When considering the whole data set, results of the Structure clustering suggest a number of clusters of $K = 2$ in all runs, corresponding to a clear wNA cluster and a clear European one (Fig. 2). Samples from eNA were intermediate between both clusters, with a larger contribution of the European cluster (the proportion of membership for each eNA sample was comprised between 0.55 and 0.93; Fig. 2). When only considering the invaded areas (eNA and Europe), the ΔK method suggested that the uppermost level of population structure was $K = 2$, one cluster formed by the eastern European samples while the other one grouped eNA, Spanish and Italian samples. French population showed various sign of admixture between the two clusters. Increasing the number of clusters to $K = 3$, population of Barcelona was clearly differentiated from the rest forming a homogeneous and distinct cluster. Interestingly, when assuming $K = 4$, Structure suggested that individuals from Valencia formed a new cluster. Restriction of the Bayesian clustering analysis to the seven loci with low proportion of null alleles had no qualitative effect on the results obtained, and the use of other

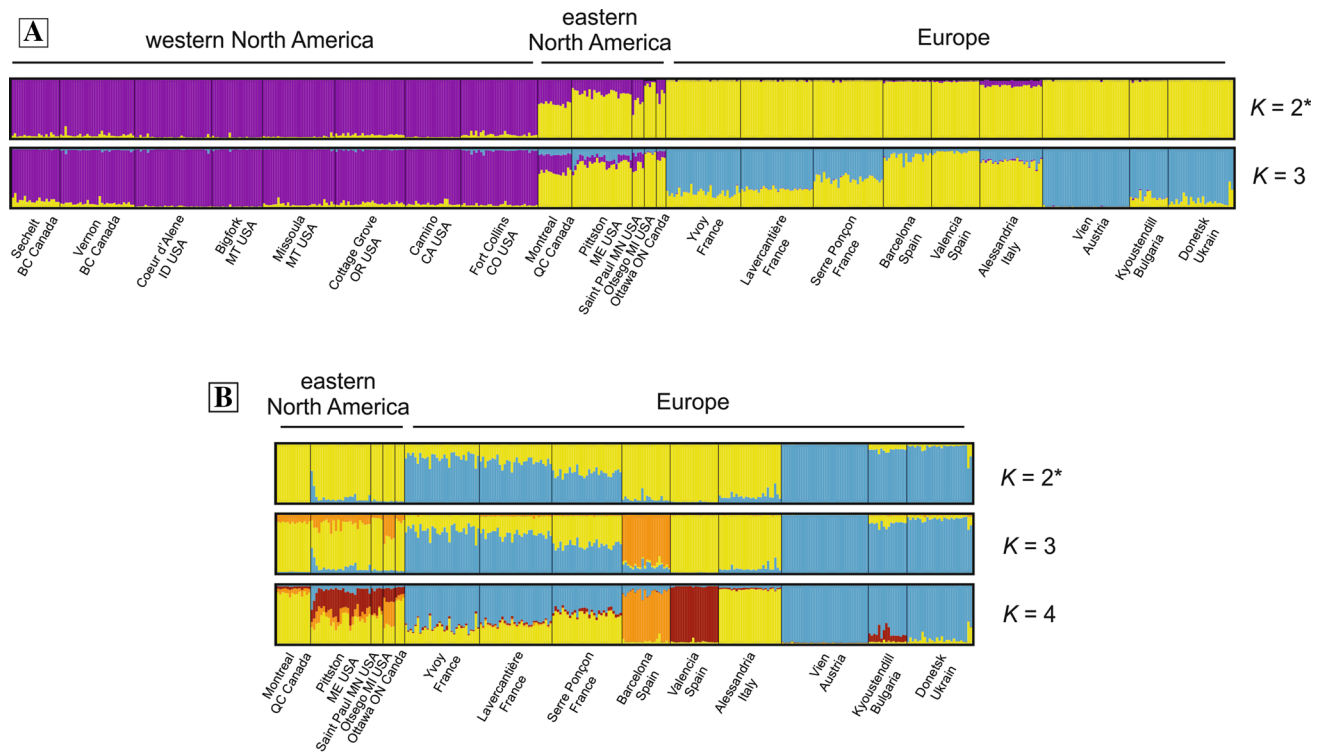

Fig. 2 Graphical representation of population genetic structure estimated by the Bayesian clustering approach implemented in Structure software. Regions are indicated above the plots, whereas sampling localities and countries are indicated below. Each individual is represented by a vertical line, and the proportion of each color corresponds to the percentage of coancestry in each genetic cluster. **a** Assignment of the 506 individuals (whole data set) to $K = 2$ and $K = 3$. **b** Assignment of the invasive populations (288 individuals) to $K = 2$, $K = 3$ and $K = 4$. *Indicates optimal number of clusters estimated with the ΔK method of Evanno et al. (2005)

Structure models (with or without correlated allele frequencies or sampling location information) gave similar results (data not shown).

Inferring invasion scenarios using mitochondrial and microsatellite data

The two first ABC analyses which took into account the outbreaks of northern Italy and Barcelona clearly indicated that these populations originated from two independent introductions from eNA (Table 2; Supplementary material Fig. S2a and Fig. S2b). The choice of the scenario involving an unsampled invasive population in eNA was supported for both analyses by high posterior probabilities (0.79 and 0.84, respectively), moderate type I errors and low type II errors and substantial BF values (Table 2).

The subsequent results were less clear-cut. However, scenarios involving wNA as a source were never selected. Indeed, when testing the origin of the Vienna sample, the selected scenario (an origin from an unsampled invasive eNA population) showed a weak posterior probability (0.31) and 95% confidence interval overlapping with four different scenarios (Table 2, Supplementary material Fig. S2c). This was true even when the analysis was repeated with the competing scenarios displaying an overlap only. This made it impossible to firmly distinguish between the different scenarios. However, on the basis of the most likely scenario, we chose to perform the subsequent analyses considering the unsampled eNA population as the origin of population of Vienna. Model checking analysis indicated that the data simulated under the selected model and posteriors fitted rather well the observed genetic data (Supplementary material Table S3).

When considering French samples, the most likely scenario for the population of Serre-Ponçon was an admixture from Italian and eastern European populations while those of Yvoy-le-Marron and Lavercantière corresponded to an admixture between individuals from the unsampled eNA population and the eastern European cluster (Table 2, Supplementary material Fig. S2d to Fig. S2f). For Yvoy-le-Marron and Lavercantière samples, all previous analyses showed that these two populations are genetically close and thus may correspond to two replicates of the same cluster. Although the type I errors were high, the same result was obtained for the two populations and gives consistency to the selected scenario. Again, model checking analyses suggested that

Table 2 Most likely scenarios and confidence in scenario choice obtained from the sequential ABC analyses attempting to decipher the European invasion of *Leptoglossus occidentalis*

Population considered	Number of competing scenarios	Selected scenarios	Posterior probability [95% CI]	Error of scenario choice		Bayes factor (selected scenario vs. second one)
				Type I	Type II	
Alessandria	3	Introduction from an unsampled eNA population	0.79 [0.74;0.85]	0.21	0.15	3.76
Barcelona	6	Introduction from an unsampled eNA population	0.84 [0.79;0.88]	0.51	0.09	9.33
Vienna	10	Introduction from an unsampled eNA population[†]	0.31 [0.20;0.42]*	0.63	0.06	1.08
Serre-Ponçon	10	Admixture: Alessandria + Vienna	0.55 [0.45;0.65]	0.60	0.06	3.24
Yvoy	10	Admixture: unsampled eNA population + Vienna	0.66 [0.57;0.74]	0.73	0.05	2.11
Lavercantière	10	Admixture: unsampled eNA population + Vienna	0.68 [0.53;0.82]*	0.82	0.05	1.44
Valencia	10	Admixture: unsampled eNA population + Alessandria	0.60 [0.50;0.70]	0.75	0.04	1.67
Kyoustendill	10	Admixture: Alessandria + Vienna[†]	0.52 [0.35;0.68]*	0.60	0.05	1.08

[†]Selected scenarios but with a 95% confidence interval overlap even when the analysis was repeated only with the competing scenarios displaying an overlap

*New posterior probabilities of scenarios after a second ABC analysis due to 95% confidence interval overlap. Type I error corresponds to the proportion of simulations in which the true scenario is not selected. Type II error is the proportion of simulations in which the scenario considered is selected but is not the true one. A Bayes factor of 3–10 is taken as substantial support, greater than 10 as strong support

the data simulated under the chosen model and posteriors fitted rather well the observed genetic data (Supplementary material Table S3).

An admixture between the unsampled eNA population and individuals from northern Italy was selected when considering the Valencian population, and no 95% confidence interval overlap was observed (Table 2, Supplementary material Fig. S2g). For the Bulgarian sample, it was impossible to distinguish between two scenarios (scenarios displaying a confidence interval overlap even after a second analysis). Both scenarios indicated an Austrian origin admixed with either the unsampled eNA or Italian population (Table 2, Supplementary material Fig. S2g). However, the scenario involving an admixture between the Austrian population and individuals from northern Italy was the scenario with the highest probability. For each selected scenario involving an admixture, the posterior distribution of admixture rates was estimated and reported in Supplementary material Table S3.

Discussion

The results reported here confirmed that the European invasion of the Western conifer seed bug, *Leptoglossus occidentalis*, originated from eNA, an area primarily invaded from the native range of wNA. Both mitochondrial and microsatellite data supported an origin from eNA. Furthermore, multiple independent introductions in Europe from eNA were highlighted; a first one in northern Italy and a second one in the area of Barcelona (Spain). The results of ABC analysis for these two populations were supported by high posterior probabilities and the 95% CI of the most likely scenario never overlapped with those of other competing scenarios. The ABC analyses also pointed out possible additional introductions from eNA and possible admixture with already established European populations. These results indicate that the eNA acted as a bridgehead for the European invasion. Although the significance of the bridgehead effect was only recently formalized based on the worldwide invasive Harlequin ladybird, *Harmonia axyridis* (Lombaert et al. 2010), it is potentially a rather common phenomenon observed in different groups of animals and plants (Boheemen et al. 2017; Garnas et al. 2016). This scenario is considered as evolutionarily parsimonious because it requires a single evolutionary shift in the bridgehead population against multiple changes in case of introduced populations becoming invasive independently (Lombaert et al. 2010).

The present data are in line with previous suspicions of multiple introductions to Europe from eNA. Multiple introductions were further illustrated by two interceptions of *L. occidentalis* in timber shipments from the USA to Europe (Dusoulier et al. 2007; Malumphy et al. 2008) with a Pennsylvanian origin for the French interception (J. C. Streito, comm. pers.). The results also shed light on secondary spread of the pest within Europe. This is undoubtedly

promoted by the hitchhiking habits of the *L. occidentalis*, allowing long-distance human-mediated transportations, especially large aggregations of the bugs within commercial shipping containers and other man-made structures. As observed for several other insect pests (Boissin et al. 2012; Javal et al. 2017; Lombaert et al. 2014), the European invasion of *L. occidentalis* is a complex scenario involving several independent introductions combined with the spread of individuals from established populations. However, there are still some obscure issues. The clustering pattern observed in Vienna and Valencia samples, for instance, could be the result of stochastic processes involving genetic drift rather than a separate introduction from eNA. Moreover, the ABC results were ambiguous and, for some samples, no competing scenario could be clearly selected. However, the scenarios involving an origin from wNA were never selected. Even if the ABC method is a powerful tool to reconstruct the invasion history of invasive species, it might also present some limitations. The rapid invasion of Europe by *L. occidentalis*, the intensity of trade and travel, the affinity of the species to man-made structures and its strong flight capability suggest the potential for highly complex invasion scenarios. Consequently, we cannot exclude that more complex scenarios than those formulated in this study exist and were not tested here. Moreover, DIYABC software assumes that there is no recurrent migration between the populations (Cornuet et al. 2014). The sampled European localities are not geographically disjunct; accordingly, migration between populations could be an important parameter, as suggested by the weak geographic structure in the native range. This highlights the challenge that tracing the invasion at a fine scale represents, especially for invasive species showing high dispersal capacities such as *L. occidentalis*.

Europe was, and perhaps is still, subject to a high propagule pressure—i.e., number of introduction events and the number of individuals in each introduction—as suggested by historical data (Dusoulier et al. 2007; Malumphy et al. 2008) and the present results. It is now well recognized that increasing the propagule pressure is likely to raise the probability of a successful establishment and thus a subsequent invasion (Simberloff 2009).

The European expansion of *L. occidentalis* was enhanced by human activity but environmental factors and the biological traits of the species have probably contributed to its invasive success. Most parts of Europe constitute a suitable habitat for the species (Zhu et al. 2013), and depending on locality, up to four generations per year were estimated (Barta 2016). Moreover, the high fecundity of *L. occidentalis* (Barta2016; Bates and Borden 2005), its strong flight capability and its capacity to exploit most native European conifers (Lesieur et al. 2014b; Tamburini et al. 2012) have contributed to the invasion success of this species. For instance, the flight capability of the adults would enable the bug to overcome any patchiness in the distribution of hosts. Equally, trees outside forests have an important role in the spread of invasive species (Paap et al. 2017; Rossi et al. 2016a). The black pine, *Pinus nigra*, one of the *L. occidentalis'* preferred hosts (Lesieur et al. 2014b), has been widely used for large-scale afforestation and ornamental plantations throughout France (Rossi et al. 2016a, b). These ornamental trees associated with built-up areas may provide relay points enhancing its spread. Additionally, all the available data indicate that *L. occidentalis* is poorly regulated by European predators and parasitoids, allowing a rapid population growth (Niccoli et al. 2009; Binazzi et al. 2013).

Most of the alien insects that have established in Europe since the 1990s appear to have spread faster than prior incursions; this coincides with both an increase in trading activities and the political changes including the dismantling of custom checkpoints with an enlarged European Union (Roques et al. 2016). Thus far, the routes of invasion and the ways of spread within the invaded areas have been retraced for a limited number of these alien insect species; for instance, the harlequin ladybird, *Harmonia axyridis* (Lombaert et al. 2014) or the Asian long-horned beetle, *Anoplophora glabripennis* (Javal et al. 2017). These case studies pointed out that the processes of invasion as well as their further spread could be highly complex, involving multiple introductions from different sources, human-mediated spread and natural dispersal. The European invasion of *L. occidentalis* provides an additional case study and confirms (1) the high complexity of some invasions and (2) that bridgehead effects may be more frequent than initially considered in invasion processes. In light of these results, limiting the spread to new areas such as South Africa and New-Zealand which appear suitable for the species (Zhu et al. 2013) could be a difficult challenge, because of the tendency of the species to travel as a hitchhiker. The European invasion of *L. occidentalis* (i.e., multiple introductions combined with the secondary spread) highlights the fact that effort should be made at preventing new introductions at points of entry and limiting human-mediated spread of the established populations. In this context, Europe can no longer be ignored as a source for subsequent invasions and raises the prospect of European populations acting as a bridgehead for future invasions.

Author contributions statement

VL, AR, MAAR conceived and designed the experiments. VL, WS, AR performed the sampling. VL, BC performed the experiments. VL, EL, TG analyzed the data. VL, EL, TG, WS, AR, MAAR wrote the paper.

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Compliance with Ethical Standards

Conflict of interest The authors state that there is no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Likewise, collection on public lands was conducted in compliance with existing regulations for insects defined as non-commercial, as determined by local offices. Furthermore, for sampling carried out on private lands, we had permission from the owners. Additionally, these field studies did not involve endangered or protected species.

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