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ORIGINAL ARTICLE

Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, *Diabrotica virgifera virgifera*

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Abstract

Invasive species provide opportunities for investigating evolutionary aspects of colonization processes, including initial foundations of populations and geographic expansion. Using microsatellite markers and historical information, we characterized the genetic patterns of the invasion of the western corn rootworm (WCR), a pest of corn crops, in its largest area of expansion in Europe: Central and South-Eastern (CSE) Europe. We found that the invaded area probably corresponds to a single expanding population resulting from a single introduction of WCR and that gene flow is geographically limited within the population. In contrast to what is expected in classical colonization processes, an increase in genetic variation was observed from the center to the edge of the outbreak. Control measures against WCR at the center of the outbreak may have decreased effective population size in this area which could explain this observed pattern of genetic variation. We also found that small remote outbreaks in southern Germany and north-eastern Italy most likely originated from long-distance dispersal events from CSE Europe. We conclude that the large European outbreak is expanding by stratified dispersal, involving both continuous diffusion and discontinuous long-distance dispersal. This latter mode of dispersal may accelerate the expansion of WCR in Europe in the future.

Introduction

Biological invasions may cause ecological (reviewed in Olden et al. 2004), health (e.g. Ruiz et al. 2000) and economic problems (Pimentel et al. 2001). In addition to these practical considerations, biological invasions provide opportunities to investigate various aspects of the colonization process, including initial foundations of populations and patterns of geographic expansion into new areas. At the time of introduction, the number of founders affects the chances of a population becoming established, through demographic (e.g. Allee effect, Drake and Lodge 2006) or genetic effects (e.g. inbreeding depression, Elam et al. 2007). After establishment, dispersal is crucial, because it determines the rate of spatial expansion and influences the genetic structure of the expanding populations and, hence, their adaptation to new environments (Sax et al. 2005). Dispersal may be required to bring novel combinations into marginal habitats which can present an adaptive challenge. However, gene flow may limit expansion, because genetic homogenization may limit adaptation at the margins of the spatial distribution (Kirkpatrick and Barton 1997).

Molecular genetic markers can be used to determine population genetic structure, making it possible to reconstruct the introduction and expansion patterns of invasive populations, thereby providing insight into aspects of introduction and spatial expansion (e.g. Estoup et al.

2004 for the cane toad; Williams et al. 2007 for the Brazilian peppertree). Such knowledge may have major implications for the management of invasive pests. For instance, the eradication of newly founded populations at the colonization front of an expanding outbreak can slow the expansion of invasive species, as shown for some invasive noxious weeds (e.g. Moody and Mack 1988) and insects (e.g. Johnson et al. 2006).

The univoltine western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is native to North America and is one of the most destructive pests of cultivated corn, *Zea mays* L. This insect rapidly expanded its range in North America during the last century (Gray et al. 2009) and was recently introduced into Europe, where it was first observed near Belgrade, Serbia, in 1992. An international network has since been set up and provides an annually updated, detailed description of the distribution and expansion of WCR in Europe (Kiss et al. 2005). After its introduction, WCR rapidly spread throughout Central and South-Eastern (CSE) Europe, extending its range by up to 100 km per year (Baufeld and Enzian 2001). The continuously expanding CSE European outbreak now extends from Austria to Ukraine and from southern Poland to northern Bulgaria. A number of isolated outbreaks have been detected almost every year since 1998, in various countries including Italy, France, Switzerland, Belgium, the United Kingdom, the Netherlands and Germany (Anonymous 2007; Edwards and Kiss 2007). Recent population genetic studies have provided evidence for repeated transatlantic introductions of this insect, accounting for the initiation of at least some of these European outbreaks, including that in CSE Europe (Miller et al. 2005; Ciosi et al. 2008). However, the source populations have yet to be identified for some outbreaks.

Most WCR movements are probably local (Spencer et al. 2005). However, several studies have reported the dispersal of WCR over large distances, partly through active long-distance flights, but largely through passive transport by wind, thunderstorms and human-mediated transportation (Coats et al. 1986; Grant and Seevers 1989; Spencer et al. 2005; Gray et al. 2009). The history of WCR range expansion in the US and Europe is also consistent with the long-distance mobility of WCR (Gray et al. 2009). Thus, both local diffusion and long-distance dispersal have probably played a role in the expansion of WCR in CSE Europe. The isolated outbreak of Friuli (north-eastern Italy) first detected in 2003 is currently the only outbreak for which long-distance dispersal of WCR from the large CSE European expanding outbreak has been demonstrated (Miller et al. 2005; Ciosi et al. 2008). However, more recent outbreaks detected in southern Germany in 2007 – near Passau in Bavaria in south-eastern Germany and near Frickingen in Baden-Württemberg in south-western Germany – have yet to be analyzed and may also result from the establishment of remote colonies originating from CSE Europe.

We examined here the spatial genetic structure and characterized the expansion process of WCR within its largest area of expansion in Europe: the CSE European outbreak. We combined a specific transect sampling scheme along the WCR expansion with analyses of microsatellite data and historical information concerning WCR expansion. We addressed the following specific questions: (i) Does the genetic evidence suggest single or multiple introductions of WCR in CSE Europe? (ii) Does it support successive founder events during expansion? (iii) Does it indicate stratified dispersal, involving both local movements and long-distance dispersal? (iv) Do the recent outbreaks in southern Germany constitute examples of long-distance dispersal events?

Materials and methods

Sample collection

We collected WCR samples in CSE Europe, at 19 sites in five countries, in 2003 (see details in Fig. 1 and Table S1). Samples were collected along two almost linear transects running from the area in which WCR was first detected (near Belgrade Airport in Serbia) to the expansion front in 2003. These transects are referred to hereafter as the 'western' and 'eastern' transects. The western transect consisted of nine samples taken along a transect running from Belgrade to the eastern end of Austria. The eastern transect consisted of 10 samples taken along a transect running from Belgrade to eastern Hungary. The sample from Stara-Pazova in Serbia is common to both transects. Careful monitoring of the CSE European outbreak made it possible to assign a precise year of first observation to each sample (Kiss et al. 2005; I. Hatala-Zsellér, personal communication, Csongrád County Plant and Soil Protection Service, Hódmezövásárhely, Hungary; I. Sivcev, personal communication, Institute for Plant Protection and Environment, Zemun, Serbia; I. Grozea, personal communication, Banat University, Timisoara, Romania). For each transect, the sampling sites were carefully chosen so as to obtain an even distribution of the years of first observation between 1992 (first observation of WCR in Europe) and 2002 (last year before sampling). One sample from the western part of the invaded area infested in 1999 (the Turanovac sample) was added to the two transects. At each sampling site, we sampled 30–50 adult beetles from maize fields (typically, we could find from less than 1 to 10 adult beetles per maize plant in CSE Europe). A sampling site was defined as a collection area

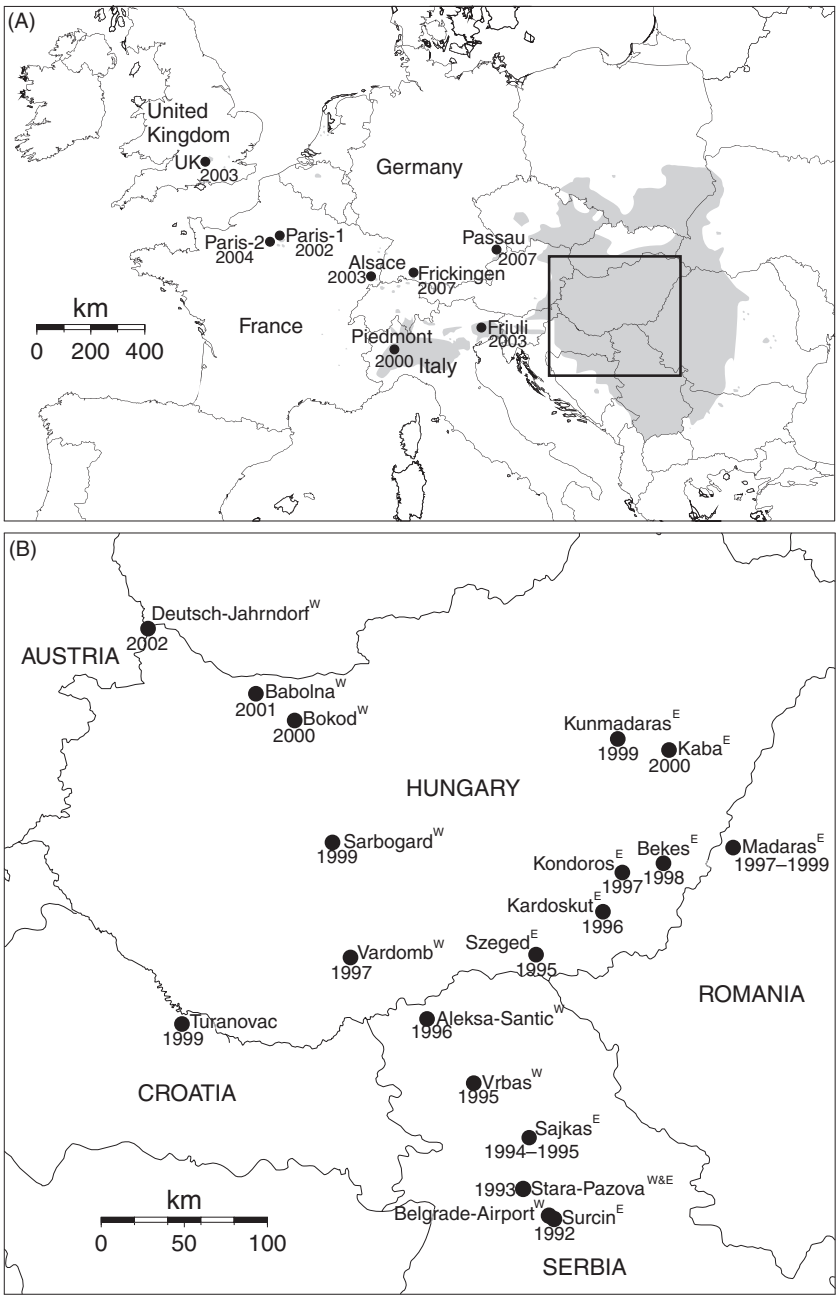

Figure 1 Location of sampling sites and geographic distribution of the western corn rootworm (WCR) in 2007, together with year of first observation: (A) In Europe; distribution area, shown in gray, is defined as areas in which WCR has been observed for at least 1 year (Edwards and Kiss 2007). (B) In the Central and South-Eastern European area; ^{W}: sample from the western sampling transect. ^{E}: sample from the eastern sampling transect. The names of the countries in which insects were collected are shown in capital letters.

of less than 100 m × 100 m. In most cases, sampling was carried out with an aspirator device or a funnel with an attached gauze bag, into which beetles where shaken from the plants. Sex pheromone-baited transparent sticky traps (PAL, Csalomon® family of traps, Hungarian Academy of Sciences, Budapest, Hungary) were used in areas infested in 2001 and 2002 because WCR population densities were low in these areas.

Samples from two outbreaks detected in 2007 in southern Germany near Passau in Bavaria and near Frickingen in Baden-Württemberg were included (Table S1 and Fig. 1), to test the hypothesis of recent long-distance

dispersal events from the CSE European area. The Passau and Frickingen samples were collected with the sex pheromone-baited transparent sticky traps (PAL) used for WCR monitoring in Europe (Kiss et al. 2005). For identification of the source populations of the CSE European samples and the two German samples, we also included a substantial number of European and American samples in our analyses. Samples of WCR from most other European outbreaks were collected from six sites in three countries (see details in Table S1 and Fig. 1) between 2003 and 2005. The European samples from Friuli in north-eastern Italy, Piedmont in north-western Italy, Paris-1, Paris-2, Alsace in eastern France and the UK studied here were those previously analyzed by Ciosi et al. (2008). We thus sampled every outbreak detected in Western Europe up to 2007, with the exception of four locations at which beetles were no longer detected after 2003 – Venice, in north-eastern Italy (outbreak detected in 1998), Belgium and the Netherlands (outbreaks detected in 2003) – or at which too few beetles were observed: the Lahr airport site in south-western Germany (outbreak detected in 2007). We added samples from five North American locations (in Mexico, Arizona, Texas, Illinois, and Pennsylvania), previously analyzed by Kim and Sappington (2005a) and Ciosi et al. (2008). These five samples were representative of the principal structured population entities of WCR in its native continent (for details see Ciosi et al. 2008).

DNA extraction and microsatellite analyses

All WCR samples were stored in absolute ethanol until DNA extraction. Template material for the polymerase chain reaction (PCR) amplification of microsatellites was obtained with three different protocols. (i) For the Deutsch-Jahrndorf sample, DNA was extracted from the thorax and abdomen of each specimen with the AquaPure genomic DNA kit (Bio-Rad, Hercules, CA). (ii) For the Frickingen and Passau samples, we used the thorax or half the body, cut lengthwise, with the DNeasy tissue kit (Qiagen, Hilden, Germany). (iii) For all other insects, the 'salting out' rapid extraction protocol of Sunnucks and Hales (1996) was used to extract DNA from the head of each individual. Individuals were washed at least three times in 0.065% NaCl before each protocol, to remove ethanol from the tissues. For the first two extraction protocols, the body part used was first dissected out, placed in a 1.5 mL microcentrifuge tube, frozen in liquid nitrogen and pulverized with a micropestle. DNA was extracted from the pulverized material. Six dinucleotide (DVV-D2, DVV-D4, DVV-D11, DVV-D5, DVV-D8, DVV-D9) and two trinucleotide (DVV-T2 and DVV-ET1) microsatellite loci (Kim and Sappington 2005b; Miller et al. 2005) were amplified in two separate multiplex PCR, and were analyzed as described by Miller et al. (2007).

Genetic variation within WCR samples

Genetic variation within samples was assessed by determining the mean number of alleles per locus (A), the mean expected heterozygosity (H) (Nei 1987) and the mean variance of absolute allelic size (V). The variables A and H were calculated with GENECLASS 2 ver. 2.0.g (Piry et al. 2004) and V was calculated with DIYABC v.0.7.1 (Cornuet et al. 2008). We also calculated the coefficient of inbreeding F_{IS} with GENEPOP ON THE WEB (Raymond and Rousset 1995b). For comparisons of A values between population samples, we estimated allelic richness (AR) on the basis of minimum sample size, using the rarefaction method (Petit et al. 1998) implemented in FSTAT 2.9.3 (Goudet 2001). The significance of differences in AR, H and V between samples was assessed with the nonparametric Friedman and Wilcoxon sign rank tests (with locus as a repetition unit). Deviation from Hardy–Weinberg equilibrium was assessed with the probability test approach, using GENEPOP ON THE WEB.

Genetic variation between WCR samples

Exact tests of population genic differentiation (Raymond and Rousset 1995a) were carried out with GENEPOP ON THE WEB. As pairwise differentiation tests involve non orthogonal and multiple comparisons, we corrected significance levels with Benjamini and Hochberg's (1995) false discovery rate procedure when necessary. GENEPOP ON THE WEB was also used to calculate Weir and Cockerham's (1984) estimator of pairwise F_{ST}, a statistic summarizing genetic differentiation between pairs of populations.

Clustering analysis of WCR population genetic structure in CSE Europe

Various Bayesian methods of clustering of individuals based on their microsatellite genotypes are currently available to infer population genetic structure in the invaded area of CSE Europe. Because there is little agreement about which method may be most appropriate, we used several of these methods to verify the consistency of the results obtained here.

1) The first approach is implemented in STRUCTURE software (Pritchard et al. 2000). The microsatellite data were converted from GENEPOP to STRUCTURE format with CREATE software v.1.1 (Coombs et al. 2008). We checked for repeatability of the results and convergence of the Markov chain Monte Carlo (MCMC) by carrying out a

series of 10 replicate runs for each prior value of the number (K) of clusters, set between 1 and 19 (the actual number of samples). Each run consisted of a burn-in of 2×10^4 iterations, followed by 10^5 iterations. Two types of inference were performed, using the admixture model of ancestry together with the correlated allele frequencies model (Falush et al. 2003), with and without the use of sampling location as prior information (Hubisz et al. 2009). Default values were maintained for all other parameters. K was estimated at the value with the highest likelihood for the data $P(X|K)$ and with the ΔK statistics of Evanno et al. (2005), which is based on the rate of change in the log-likelihood between successive K values. The results obtained in each analysis were confirmed by performing longer runs with K sets between 1 and 5, with 10 replicate runs at each K, 2×10^5 burn-in iterations, and 10^6 iterations. The results of STRUCTURE analyses were then visualized in DISTRUCT v.1.0 (Rosenberg 2004).

2) In a second approach, we included the spatial coordinates of individuals as prior information using GENELAND v.3.1.4 (Guillot et al. 2005a,b). We performed 10 replicated analyses to check for convergence, allowing K to vary from 1 to 19 clusters and using the following parameters: 10^6 MCMC iterations, maximum rate of Poisson process fixed at 50, maximum number of nuclei in the Poisson-Voronoi tessellation fixed at 450, and an uncertainty associated with the spatial coordinates of 3 km. We used the Dirichlet model of allelic frequencies, as this model has been shown to perform better than the alternative model (Guillot et al. 2005a). We inferred the number of clusters (K) from the modal value of K for the 10 runs.

3) Finally, we used an approach implemented in BAPS 5.2 software (Corander et al. 2003, 2004). We estimated K in four different ways, clustering individuals or groups of individuals (samples), and with and without the use of their spatial coordinates as prior information. In each case, we conducted a series of five replicate runs with the *a priori* upper limit for the number of clusters set at 19 (the actual number of samples) for each run.

Geographic and temporal analyses of genetic variation

Each sample of WCR from CSE Europe can be characterized in terms of its *x-y* spatial coordinates, its angular coordinate (see below) and the year of first observation of WCR at the sampling site. As the expansion of WCR in CSE Europe is largely radial, the year of first observation provides an indication of the relative location of the sample in the colonization process. The Madaras sample was not included in the temporal analysis because we considered its year of first observation to be too imprecise. The spatial and temporal features shaping the population genetic structure within the CSE European zone of expansion were investigated with the following procedures:

1) Genetic isolation by geographic distance was investigated using Rousset's (1997) regression-based framework, by analyzing the correlation between $F_{ST}/(1 - F_{ST})$ values and the natural logarithm of geographic distance between samples.

2) Genetic isolation by temporal distance was investigated by analyzing the correlation between F_{ST} and the difference in year of first observation ($\Delta_1{}^{st}{}_{obs}$) between samples. Note that in each transect, $\Delta_1{}^{st}{}_{obs}$ and the geographic distance between samples were strongly correlated (Spearman's $r = 0.97$ and 0.86, and Mantel tests $P = 1 \times 10^{-5}$ and 4×10^{-5} for the western and the eastern transect, respectively). Genetic isolation by geographic and temporal distance were investigated considering each transect separately.

3) Spatial expansions into new territories may lead to the spread of rare alleles over large geographical areas where they can reach high frequencies (Edmonds et al. 2004; Klopfstein et al. 2006). This phenomenon, called 'gene surfing' (Klopfstein et al. 2006), is caused by genetic drift that prevails over other evolutionary forces at the front of expansion. Empirical (Hallatschek et al. 2007) and simulation (Excoffier and Ray 2008) studies on spatial genetic patterns for a single locus, have recently demonstrated that gene surfing can cause the emergence of genetically homogeneous sectors and thus of a between-sector genetic differentiation. We addressed this issue in the CSE area of WCR expansion, by investigating the genetic isolation by angular distance expected when considering several loci. We attributed to each sample an angle between the line of latitude passing through the origin of the outbreak (Belgrade, Serbia) and the line connecting Belgrade to each sample (Table S1). The pattern of genetic isolation by angular distance was investigated by analyzing the correlation between F_{ST} and differences in angle measured for all pairs of samples. The Belgrade-Airport, Surcin and Stara-Pazova samples were all located geographically close to Belgrade, and their angular coordinates were therefore uncertain. We thus removed these three samples from the genetic isolation by angular distance analysis. Correlations between distance matrices were analyzed with the Mantel test implemented in GENEPOP ON THE WEB.

4) Correlations between genetic variation within samples (AR, H and V) and the year of first observation or geographic distance to the center of the outbreak were assessed by Spearman's rank correlation tests performed with the *spearman.test* function available in the *pspearman* library of R (R Development CoreTeam 2008), with the aim of detecting possible decreases in genetic variation during the expansion process.

Source populations of the Frickingen, Passau and Friuli outbreaks

We calculated the mean multilocus individual assignment likelihood of each outbreak sample *i*, – Frickingen and Passau from southern Germany, and Friuli from north-eastern Italy – to each sample of 31 possible source populations *s,* as shown in Table 1 (hereafter denoted $L_{i\to s}$; see Pascual et al. 2007; and Ciosi et al. 2008) with GENECLASS 2 ver. 2.0.g (Piry et al. 2004). For each outbreak considered, the most probable source population was identified as that with both the highest $L_{i\to s}$ value and the lowest F_{ST}-value with the outbreak considered. No ad hoc statistical test has yet been described for the comparison of mean individual assignment likelihoods or F_{ST}. Moreover, non parametric tests, such as the Friedman analysis of variance by rank or pairwise Wilcoxon signed rank test, using locus as a repetition unit, are not sufficiently powerful for such comparisons, due to the limited number of loci and the large inter-locus variance of the statistics used.

We then documented the effect of introduction events on genetic variation within populations, by comparing the Frickingen, Passau and Friuli outbreaks with their identified source populations.

Determination of the number of introductions into CSE Europe

The following procedures were used to detect multiple introductions in the CSE European outbreak. We used three procedures based on different assumptions to compensate for a potentially low statistical power and then to avoid false negatives. The first method (1) does not include statistical tests and is sample-centered, the second one (2) includes statistical tests and is individual-centered and the third one (3) is focused on cotemporary gene flow and detection of first generation immigrants.

1) The most probable source population of each CSE European sample was identified by the procedure described above for the Frickingen, Passau and Friuli outbreaks. In this analysis, potential sources were populations with a first observation year ≤2003 (i.e. the year of collection of CSE European samples). The Friuli outbreak was excluded as a potential source population from the analysis because it is known to have originated in CSE Europe (Miller et al. 2005; Ciosi et al. 2008), and thus most CSE European samples are expected to wrongly point to Friuli as its most probable source. Most of the non CSE European outbreaks have been observed for the first time after the occurrence of WCR at CSE European sample locations and could thus not be the source of primary introductions in CSE Europe. In this assignment analysis potential sources have thus been considered in two ways: (i) North American populations could be the source of multiple introductions in CSE Europe (either primary or secondary) and (ii) European outbreaks could be the source of secondary introductions in CSE European locations where WCR had already been observed.

2) Ciosi et al. (2008) suggested that the northern USA (represented by the Illinois and Pennsylvania samples) is the sole source population for the CSE European outbreak. We tested this hypothesis for the large number of individuals sampled at various sites within the CSE European area, by calculating the probability of excluding Illinois and Pennsylvania as source populations for each CSE European individual, using GENECLASS 2 ver. 2.0.g. Probabilities were calculated by using the assignment likelihood calculation of Rannala and Mountain (1997), with resampling based on the simulation algorithm of Paetkau et al. (2004). Ten thousand individuals were simulated per population and a threshold of 0.05 was used to exclude 'northern USA' as the source population of any target individual.

3) Multiple introductions at a single site are expected to leave a genetic signature only if the individuals introduced originate from sources that are genetically differentiated from the outbreak considered. Given the number of loci used, only first-generation immigrants would be detectable (see Rannala and Mountain 1997 for a discussion on the power of statistical tests of assignment). We used GENECLASS 2 ver. 2.0.g (Piry et al. 2004) to detect first-generation immigrants. Given that CSE European individuals were sampled in 2003, this test is specific to introductions in 2002–2003. A thousand individuals were simulated per population, with the algorithm of Paetkau et al. (2004) and the assignment likelihood calculation of Rannala and Mountain (1997) was used. The statistic used to identify first-generation immigrants was the L_{home}/L_{max} ratio, where L_{home} is the assignment likelihood of an individual to the population from which it was sampled and L_{max} is the maximum assignment likelihood for this individual over all populations considered. As explained above populations considered as potential sources were those with a first observation year ≤2003, including the CSE European outbreak itself, with the exception of Friuli which was excluded from the analysis. We considered an individual to be a first-generation immigrant if the probability of the corresponding L_{home}/L_{max} ratio was below alpha = 0.05.

Results

Genetic variation within WCR samples

The dataset of CSE European samples showed moderate polymorphism, with a mean of 4.625 (SD = 2.973) alleles

Table 1. Pairwise estimates of F_{ST} (Weir and Cockerham 1984) and mean individual assignment likelihood ($L_{i\to s}$) of the Passau, Frickingen and Friuli samples for each potential source population.

Geographic area	Potential source population	Location	1st obs.	Passau, Germany		Frickingen, Germany		Friuli, Italy	
				$L_{i\to s}$	F_{ST}	$L_{i\to s}$	F_{ST}	$L_{i\to s}$	F_{ST}
North American range	Mexico	Durango, Mexico	<1940	15.989	0.284	16.604	0.229	16.664	0.319
	Arizona	Willcox, Arizona	<1974	15.229	0.213	15.559	0.164	18.62	0.276
	Texas	New Deal, Texas	<1980	9.135	0.177	9.427	0.132	10.048	0.226
	Illinois	Champaign, Illinois	<1974	7.504	0.158	7.848	0.115	9.175	0.229
	Pennsylvania	Bellefonte, Pennsylvania	<1985	7.669	0.170	7.734	0.116	8.153	0.218
Western European isolated outbreaks	Piedmont	Oleggio, Italy	2000	15.092	0.345	13.652	0.242	15.354	0.357
	Paris-1	Roissy Airport, France	2002	11.379	0.299	11.775	0.241	14.494	0.439
	UK	Slough, United Kingdom	2003	8.174	0.191	8.877	0.134	11.301	0.285
	Friuli	Buttrio, Italy	2003	11.052	0.239	11.466	0.168	–	–
	Alsace	Mulhouse Airport, France	2003	7.881	0.176	8.678	0.106	9.44	0.267
	Paris-2	Pierrelaye, France	2004	11.350	0.220	12.054	0.177	12.777	0.278
	Passau	Passau, Germany	2007	3.378	–	5.438	0.063	5.891	0.239
	Frickingen	Frickingen, Germany	2007	**4.100**	0.063	4.270	–	5.144	0.168
Central and South Eastern European area of Expansion	Belgrade-Airport^{W}	Belgrade Airport, Serbia	1992	5.087	0.104	5.626	0.061	4.863	**0.116**
	Surcin^{E}	Surcin, Serbia	1992	4.328	0.045	4.98	0.027	5.209	0.153
	Stara-Pazova$^{W\&E}$	Stara Pazova, Serbia	1993	4.519	0.066	4.883	0.024	4.696	**0.116**
	Vrbas^{W}	Vrbas, Serbia	1995	4.184	0.051	**4.537**	0.004$^{\Phi}$	4.934	0.143
	Aleksa-Santic^{W}	Aleksa Santic, Serbia	1996	4.263	0.053	4.613	0.006	5.025	0.147
	Vardomb^{W}	Vardomb, Hungary	1997	4.120	**0.039**	4.619	0.006	5.153	0.146
	Sarbogard^{W}	Sarbogard, Hungary	1999	4.146	0.051	4.576	0.012$^{\Phi}$	4.806	0.136
	Bokod^{W}	Bokod, Hungary	2000	4.241	0.051	4.548	**0.002$^{\Phi}$**	5.32	0.155
	Babolna^{W}	Babolna, Hungary	2001	4.299	0.055	4.626	0.008$^{\Phi}$	5.267	0.167
	Deutsch-Jahrndorf^{W}	Deutsch Jahrndorf, Austria	2002	4.287	0.051	4.609	0.004$^{\Phi}$	5.184	0.159
	Sajkas^{E}	Sajkas, Serbia	1994–1995	4.286	0.057	4.705	0.015	4.957	0.145
	Szeged^{E}	Szeged, Hungary	1995	4.327	0.056	4.655	0.009$^{\Phi}$	5.118	0.157
	Kardoskut^{E}	Kardoskut, Hungary	1996	4.276	0.045	4.906	0.020	5.066	0.149
	Kondoros^{E}	Kondoros, Hungary	1997	4.432	0.060	4.869	0.016	5.353	0.156
	Bekes^{E}	Bekes, Hungary	1998	4.829	0.087	5.214	0.043	5.245	0.158
	Kunmadaras^{E}	Kunmadaras, Hungary	1999	4.667	0.077	4.989	0.027	5.38	0.153
	Madaras^{E}	Madaras, Romania	1997–1999	4.474	0.063	4.770	0.013	5.329	0.153
	Kaba^{E}	Kaba, Hungary	2000	4.729	0.068	5.299	0.047	5.267	0.164
	Turanovac	Turanovac, Croatia	1999	4.182	0.042	4.759	0.017	**4.64**	0.125
Most likely source population				Vardomb^{W}		Vrbas^{W}	Bokod^{W}	Turanovac	Belgrade-Airport^{W} or Stara-Pazova$^{W\&E}$

$-\log_{10}$ values of the $L_{i\to s}$ are indicated. Highest $L_{i\to s}$ and minimum F_{ST} are indicated in bold typeface. The most likely source population for each sample is indicated in the last row.

1st obs: first observation year.

$^{\Phi}$: Nonsignificant pairwise differentiation exact tests.

^{W}: Sample from the western sampling transect.

^{E}: Sample from the eastern sampling transect.

per locus over all samples. The mean number of alleles was homogeneous over CSE Europe, varying from 3 (SD = 1.195) (*AR* = 2.996, SD = 1.192) in Turanovac to 3.625 (SD = 1.685) (*AR* = 3.307, SD = 1.411) in Kaba (Table S2). Mean expected heterozygosity ranges from 0.442 in Sarbogard to 0.506 in Kunmadaras (Table S2). The mean variance of absolute allelic size varied from 10.366 (SD = 14.685) at Belgrade-Airport to 14.238 (SD = 20.929) in Vardomb (Table S2). No significant differences between samples were detected for any of the statistics summarizing genetic variation within population samples (Friedman's test by rank performed over loci, χ^2 = 23.17, 19.4 and 24.60, d.f. = 18, P = 0.184, 0.368 and 0.136 for *AR*, *H* and *V*, respectively).

We found significantly fewer alleles (−55%) and a lower heterozygosity (−26%) in CSE Europe (complete data set) than in Pennsylvania, the putative source population of this outbreak in north-eastern America (Wilcoxon's signed rank tests, P = 0.018 and 0.036 for *AR* and *H*, respectively). The mean variance of absolute allelic size (*V*) did not differ significantly between CSE Europe (complete data set) and Pennsylvania (Table S2; Wilcoxon's signed rank test, P = 0.484).

Genetic variation between CSE European WCR samples

Most pairwise comparisons (79% before and 87% after correction for multiple comparisons) revealed no significant genetic differentiation (Table S3) and pairwise F_{ST}-values were low (mean = 0.004, SD = 0.01). The sample collected from the site of the first observation within the area studied (i.e. the Belgrade-Airport sample) displayed significant genetic differentiation from all other samples except the Surcin, Stara-Pazova, Vrbas, and Kardoskut samples (Table S3). The three north-eastern samples, Kunmadaras, Kaba, and Bekes, were genetically differentiated from two central samples (Vrbas and Sajkas) and three western samples (Bokod, Sarbogard, and Vardomb) (Table S3).

Clustering analysis of WCR population genetic structure in CSE Europe

Bayesian clustering analyses performed with Structure provided consistent results over the 10 runs tested for each K and over the two models tested, irrespective of the length of the runs. The natural logarithm of the likelihood of the data $\ln P(X|K)$ slightly increased from K = 1 to K = 2, for which it was maximal (Fig. S1A,S1B). Using the ΔK statistics led to the same estimation of K = 2 (data not shown). The following analyses of model parameters at K = 2 indicated an absence of population structure in the area of expansion in CSE Europe (Pritchard et al. 2007, 2009). For example, in both analyses, all individuals were admixed and the individual proportions of ancestry from each cluster (Q values) were randomly distributed between samples. In the analysis in which sampling location was not used as prior information, Q values were roughly symmetric ($\sim$ 1/2 in each cluster). Moreover, in the analysis in which sampling location was used as prior information, at K = 2, 99% of individuals were assigned to a single cluster with a mean Q = 0.89 (SD = 0.11) (Fig. S2) and the value of r, which parameterizes the amount of information carried by the locations, was much greater than 1. This is also consistent with an absence of population structure (Pritchard et al. 2009).

Geneland and Baps confirmed the absence of genetic structure throughout the expansion area in CSE Europe, with all individuals belonging to a single cluster. Posterior distributions of the estimated number of clusters (K) across the 10 replicates performed in Geneland displayed a clear mode at K = 1 (Fig. S1B) and a probability of 1 for K = 1 was obtained for all calculation in Baps.

Geographic and temporal analyses of genetic variation

Weak but significant genetic isolation by geographic distance was detected only in the eastern transect [Fig. 2A; Mantel test on the correlation between $F_{ST}/(1 - F_{ST})$ and the natural logarithm of geographic distance between samples, P = 0.047, slope = 0.002 for the eastern transect and P = 0.115, slope = 0.005 for the western transect]. When combining the probabilities obtained for the western and eastern transects by Fisher's method (Sokal and Rolf 1995, p. 794–797), we detected an overall significant genetic isolation by geographic distance (P = 0.034).

Weak but significant genetic isolation by temporal distance was observed in both transects (Fig. 2B; Mantel tests on the correlation between F_{ST} and $\Delta_1^{st}{}_{obs}$, P = 0.022 and 0.020, and slopes ≤0.002 for the western and the eastern transect, respectively). After combining the two transect probabilities by Fisher's method, overall genetic isolation by temporal distance was highly significant (P = 0.004).

Finally, a highly significant but weak correlation between F_{ST} and the differences of angle measured for all pairs of samples was detected (Fig. 2C; Mantel test, $P = 7.6 \times 10^{-3}$; slope $= 1.3 \times 10^{-4}$).

No significant correlation between the allelic richness of the samples (*AR*) and the year of first observation or geographic distance from Belgrade was detected in either of the transects (Spearman's $r \leq 0.15$ and $P \geq 0.71$) (Fig. 3A,B). Allele size variance (*V*) displayed a positive correlation of marginal significance with the year of first observation and with geographic distance from Belgrade,

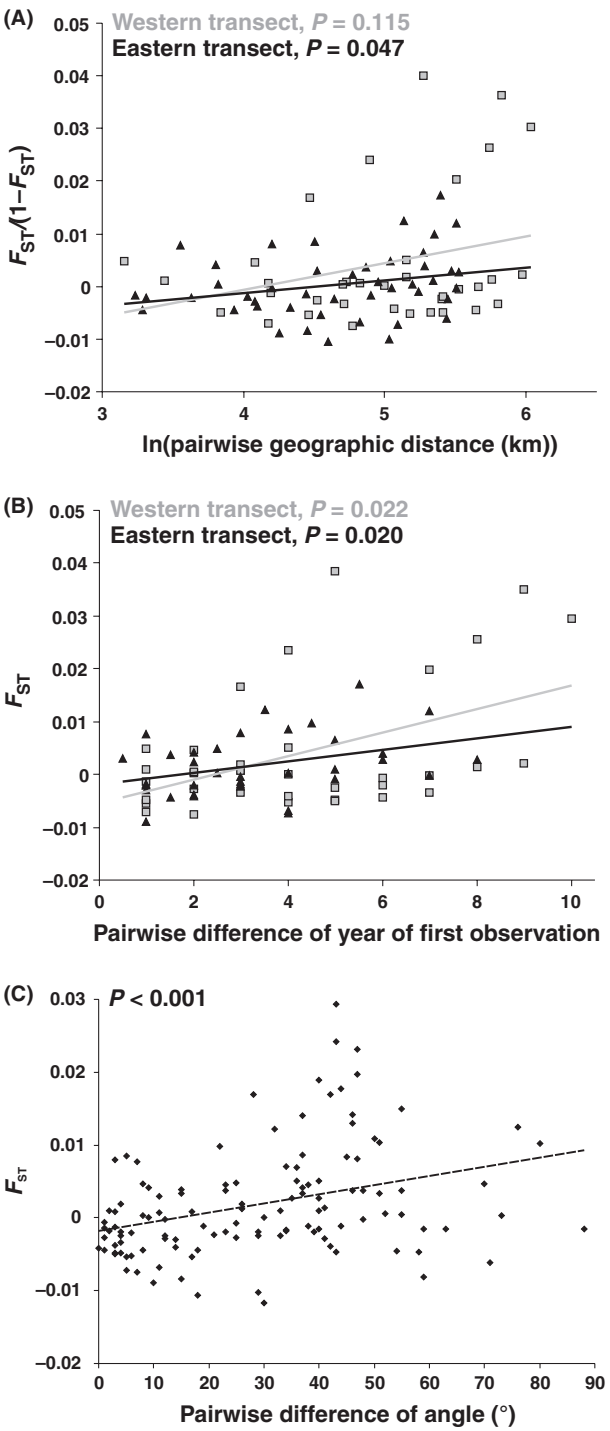

Figure 2 Patterns of genetic isolation by geographic (panel A), temporal (panel B) and angular distance (panel C) among Central and South-Eastern (CSE) European sample sites of the western corn rootworm. Linear regression lines and *P*-values for Mantel tests analyzing the correlation between genetic and geographic, temporal or angular distance are shown. In panels A and B, gray and black items correspond to the western and the eastern transects, respectively.

in the western transect (Spearman's $r = 0.65$ and $P = 0.07$ for both tests), but not in the eastern transect (Spearman's $r \leq 0.33$ and $P \geq 0.35$) (Fig. 3C,D). Furthermore, heterozygosity (H) was positively correlated with the year of first observation and with the geographic distance from Belgrade in the eastern transect (Spearman's $r \geq 0.8$ and $P = 0.01$ for both tests), but not in the western transect (Spearman's $r = 0.09$ and $P = 0.84$ for both tests) (Fig. 3E,F). When combining the probabilities obtained for the two transects by Fisher's method, H displayed a positive correlation of marginal significance with the year of first observation ($P = 0.06$) and a significant positive correlation with geographical distance ($P = 0.03$). When probabilities were combined, correlations based on AR and V remained non significant ($P > 0.11$).

Source populations of the Frickingen, Passau and Friuli outbreaks

Comparisons between the Frickingen and Passau samples and all potential source populations in Europe and North America gave small to large F_{ST} estimates, ranging from 0.002 between Frickingen and Bokod to 0.345 between Passau and Piedmont (Table 1). All pairwise genetic differentiations tests gave significant results, with the exception of the six comparisons of Frickingen with six sites sampled in the CSE European area: Deutsch-Jahrndorf, Babolna, Bokod, Sarbogard and Vrbas, all from the western transect, and Szeged (Table 1).

The CSE European area was identified as the most probable source population for the Frickingen outbreak with the highest $L_{i\to s}$ and the minimum F_{ST}-values obtained for Vrbas and Bokod, respectively (Table 1). More generally, $L_{i\to s}$ and F_{ST}-values indicated that the western part of the CSE European area of expansion was the most probable source region for the Frickingen outbreak.

For the Passau outbreak, the highest $L_{i\to s}$ value was obtained for the Frickingen sample (Table 1). By contrast, the minimum F_{ST}-value was obtained for a CSE European sample, Vardomb, identifying this sample site as the potential source of the Passau outbreak (Table 1). We therefore cannot unambiguously identify a single source population for the Passau outbreak. As the Frickingen and many CSE European samples are genetically similar (Table 1), similar F_{ST} and $L_{i\to s}$ values were obtained when assigning Passau to Frickingen or CSE European samples (e.g. Vardomb and Sarbogard). The source of the Passau outbreak may therefore be either the area of expansion in CSE Europe or the Frickingen outbreak.

Finally, both the $L_{i\to s}$ and F_{ST} values identify the area of expansion in CSE Europe as the most probable source

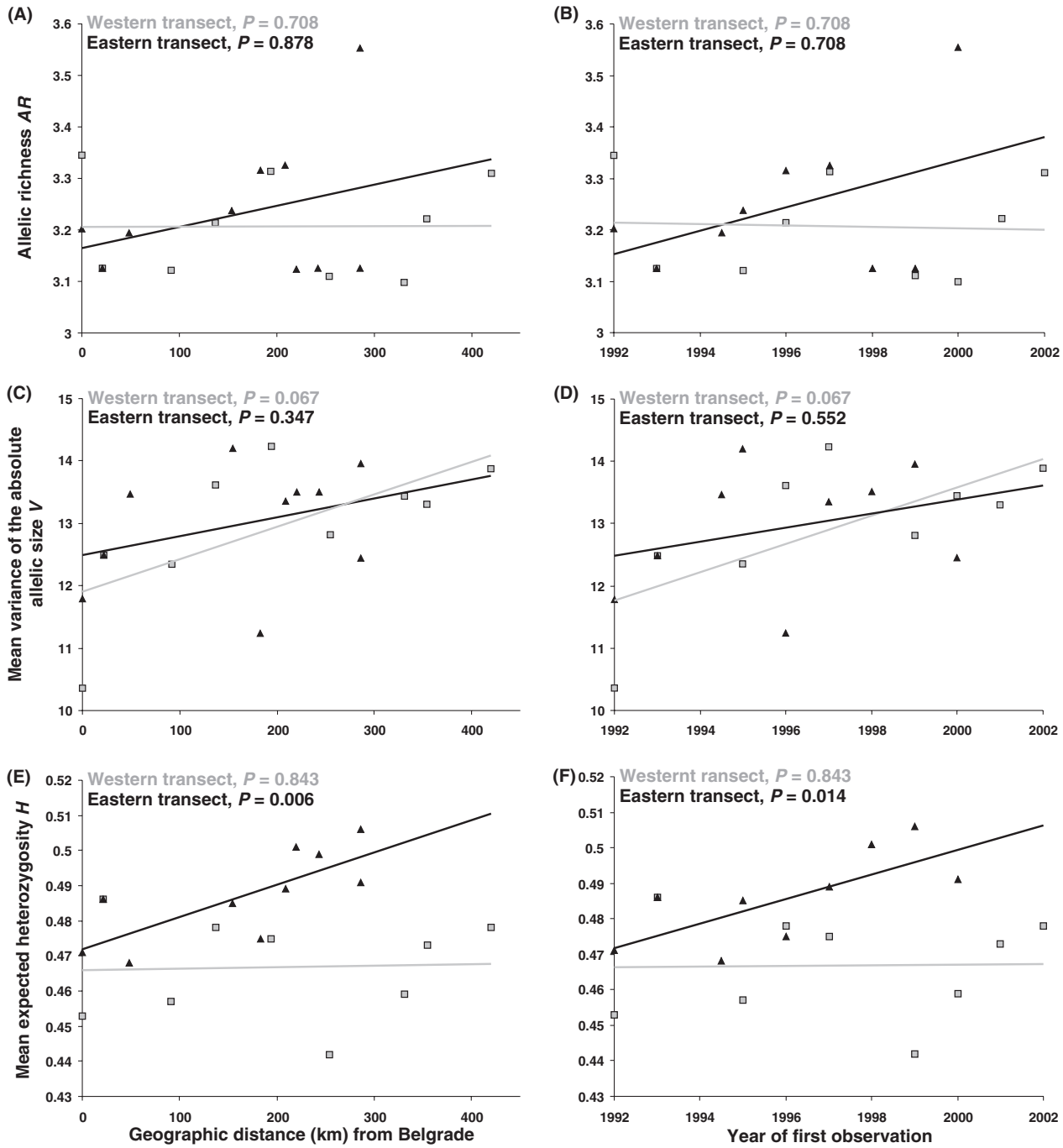

Figure 3 Correlations between genetic variation parameters within sampled sites [allelic richness *AR* based on minimum sample size (*N* = 28 in Belgrade-Airport and Kaba for loci DVV-D11 and DVV-ET1 respectively), using the rarefaction method (panels A and B), mean variance of the absolute allelic size *V* (panels C and D) and mean expected heterozygosity *H* (panels E and F)] and their geographic distance to the center of the Central and South-Eastern European area of expansion (panels A, C and E) or year of first observation (panels B, D and F). Linear regression lines and *P*-values for Spearman's rank correlation tests are shown. Gray and black items correspond to the western and eastern sampling transects, respectively.

of the Friuli outbreak. More precisely $L_{i\rightarrow s}$ values identified Turanovac and F_{ST} values identified Belgrade-Airport or Stara-Pazova, thus indicating that the center and western part of the CSE European area of expansion were the most likely source regions for the Friuli outbreak (Table 1).

Building on previous results, it is now possible to document the effect of introduction events on genetic variation within populations, by comparing the Frickingen, Passau and Friuli outbreaks with their identified source populations. For simplicity, data for the Passau, Friuli and Frickingen outbreaks were first compared with the complete CSE European data set. The Passau and Friuli outbreaks were less variable than their putative sources in CSE Europe, whereas the Frickingen outbreak was not. Allelic richness (*AR*) was 16.5% and 44.3% lower in the Passau and Friuli samples, respectively, than in CSE Europe (Wilcoxon's sign rank tests, $P = 0.018$ for both tests). Heterozygosity (*H*) was 26.7% and 38.66% lower in the Passau and Friuli samples, respectively, than in CSE Europe (Wilcoxon's sign rank tests, $P = 0.018$ for both tests). By contrast, *AR* and *H* were similar in Frickingen and in CSE Europe (Wilcoxon's sign rank tests, $P = 0.063$ for *AR* and 0.176 for *H*). The mean variance of absolute allelic size (*V*) was similar for the Frickingen, Passau and Friuli samples and for CSE Europe (Wilcoxon's sign rank tests, $P > 0.128$ for each test). Genetic variation within the Passau outbreak did not differ significantly from that in its alternative putative source population, Frickingen (Wilcoxon's signed rank tests on *AR*, *H* and *V*, $P > 0.09$ for each test).

Determination of the number of introductions into CSE Europe

The three procedures used to detect multiple introductions in the CSE European outbreak gave similar results:

1) Highest mean multilocus individual assignment likelihood of each sample *i* to each possible source population *s* ($L_{i\to s}$) clearly identified the northern USA (represented by the Illinois and Pennsylvania samples) as the most probable source of the 19 sampling sites in CSE Europe (Table 2). The highest $L_{i\to s}$ obtained between each CSE European sample and the northern USA samples was substantially higher than the second highest $L_{i\to s}$ (with a difference of 0.42 to 1.64 $\log_{10}$ ($L_{i\to s}$) units). Similar results were obtained with F_{ST}-values for nine sampling sites in CSE Europe. F_{ST}-values identified Alsace as the probable source for the other 10 sites (Table 2).

2) Northern USA (represented by the Illinois and Pennsylvania samples) was excluded as a possible source population ($P < 0.05$) for only six individuals from CSE Europe (i.e. only 0.8% of the total of 706 individuals). Setting the threshold of this analysis to 0.05, we expected a mean of $0.05 \times 706 = 35.3$ type I errors in the data set and thus considered the six individuals excluded to be negligible.

3) With an alpha of 0.05, none of the 706 individuals sampled in CSE Europe was identified as a first-generation immigrant originating from a genetically differentiated population ($P > 0.06$). With an alpha of 0.2, 13 individuals were identified as first generation immigrants coming from the CSE European outbreak itself. These 13 individuals were not spatially concentrated. For the other 693 individuals, the probability of the corresponding L_{home}/L_{max} ratio was >0.55.

We thus obtained no evidence of multiple introductions from genetically differentiated source populations at different sites within the CSE European area. Moreover, when Belgrade was considered as a possible source, the $L_{i\to s}$ and F_{ST}-values identified the Belgrade area as the most likely source population for all other CSE European samples (Table 2). Thus, we found no evidence of multiple introductions from the same source population at different sites within the CSE European area.

Discussion

By combining a specific sampling scheme along the WCR expansion, microsatellite data and historical information, we characterized the invasion dynamics of WCR in its largest area of expansion in Europe, that of the Central and South-Eastern European outbreak. Within this area, we detected only one genetically homogeneous cluster based on Bayesian analyses, weak genetic differentiation between pairs of samples and weak but statistically significant patterns of genetic isolation by geographic distance and temporal distance. Unexpectedly, it seems that we have highlighted a very small increase in genetic variation from the center to the edge of the outbreak. Finally, we showed that three small geographically distant outbreaks (two in southern Germany and one in north-eastern Italy) most likely originated from CSE Europe. We will present the evidence to suggest that the CSE European outbreak (i) originated from a single introduction, (ii) is expanding through both continuous diffusion and discontinuous long-distance dispersal, and thus through stratified dispersal (Shigesada et al. 1995), and (iii) that human efforts to control WCR may account for the slight increase in genetic variation in the direction of expansion.

A single origin for the CSE European outbreak

Invading populations may result from one or more introduction events. In the case of repeated introductions from genetically differentiated source populations into different locations of a new area, we expect to observe, at least transiently, a mosaic of genetically differentiated patches within the area of expansion (e.g. Genton et al. 2005 for the common ragweed; Voisin et al. 2005 for a brown

Table 2. Weir & Cockerham's estimator of pairwise F_{ST} (1984) and mean individual assignment likelihood ($L_{i\to s}$) of each Central and South-Eastern European (CSE) sample of the western corn rootworm to each potential source population and to both samples from Belgrade area.

	Potential source populations										
	North America					Europe				Belgrade area	
Sample names	Mexico	Arizona	Texas	Illinois	Pennsylvania	Piedmont, Italy	Paris-1, France	Alsace, France	UK	Belgrade-Airport	Surcin
Belgrade-Airport[W]	16.391 (0.224)	16.657 (0.167)	8.959 (0.103)	8.270 (0.109)	**7.589 (0.095)**	12.925 (0.197)	11.021 (0.257)	9.002 (0.118)	8.572 (0.126)	–	5.201 (0.014)
Surcin[E]	16.555 (0.213)	16.894 (0.159)	9.188 (0.109)	8.386 (0.106)	**7.991 (0.104)**	15.022 (0.236)	11.499 (0.234)	8.893 (0.112)	8.951 (0.124)	5.346 (0.014)	–
Stara-Pazova[W&E]	17.323 (0.204)	17.096 (0.148)	9.236 (0.098)	8.495 (0.098)	**8.221** (0.095)	14.941 (0.217)	11.942 (0.228)	8.943 **(0.093)**	9.302 (0.120)	5.308 (0.005)	5.110 (–0.002)
Vrbas[W]	16.190 (0.214)	15.511 (0.142)	8.897 (0.101)	7.827 (0.093)	**7.610** (0.091)	13.296 (0.204)	10.900 (0.217)	8.364 **(0.085)**	8.628 (0.109)	5.234 (0.017)	4.964 (0.006)
Aleksa-Santic[W]	17.251 (0.210)	16.314 (0.144)	9.065 (0.100)	8.026 (0.092)	**7.879** (0.092)	14.543 (0.219)	11.352 (0.209)	8.842 **(0.087)**	8.888 (0.109)	5.442 (0.024)	5.124 (0.002)
Vardomb[W]	16.893 (0.205)	16.245 (0.145)	9.315 (0.108)	**8.186** (0.095)	8.200 (0.100)	15.358 (0.236)	11.602 (0.213)	8.810 **(0.080)**	9.034 (0.116)	5.772 (0.039)	5.337 (0.007)
Sarbogard[W]	16.739 (0.234)	16.112 (0.167)	9.158 (0.119)	8.285 (0.115)	**8.000** (0.113)	14.279 (0.227)	11.510 (0.240)	8.424 **(0.098)**	9.035 (0.132)	5.246 (0.020)	4.887 (0.005)
Bokod[W]	16.198 (0.209)	15.077 (0.142)	9.003 (0.105)	7.887 (0.096)	**7.553** (0.097)	13.531 (0.225)	10.895 (0.216)	8.404 **(0.087)**	8.850 (0.118)	5.346 (0.026)	5.005 (0.005)
Babolna[W]	16.727 (0.209)	16.232 (0.148)	9.284 (0.107)	8.278 (0.098)	**8.194** (0.104)	14.799 (0.231)	11.384 (0.208)	8.671 **(0.089)**	9.182 (0.114)	5.772 (0.035)	5.126 (0.000)
Deutsch-Jahrndorf[W]	15.829 (0.196)	15.371 (0.135)	8.984 (0.098)	7.967 (0.087)	**7.882** (0.092)	14.265 (0.221)	10.967 (0.203)	8.607 **(0.082)**	9.059 (0.107)	5.689 (0.030)	5.164 (–0.001)
Sajkas[E]	17.029 (0.221)	16.423 (0.158)	9.224 (0.115)	8.261 **(0.110)**	**8.220** (0.112)	15.338 (0.246)	11.875 (0.235)	8.844 (0.111)	9.471 (0.136)	5.530 (0.029)	5.009 (0.000)
Szeged[E]	16.563 (0.198)	15.979 (0.138)	9.147 (0.093)	8.196 (0.082)	**7.887** (0.083)	14.454 (0.209)	11.416 (0.198)	8.935 **(0.082)**	9.044 (0.100)	5.669 (0.028)	5.208 (–0.002)
Kardoskut[E]	16.252 (0.200)	16.406 (0.143)	9.124 (0.101)	8.338 (0.099)	**7.868 (0.097)**	14.697 (0.228)	11.757 (0.235)	8.997 (0.105)	9.036 (0.117)	5.375 (0.015)	5.043 (–0.007)
Kondoros[E]	17.366 (0.204)	16.748 (0.139)	9.273 (0.091)	8.513 (0.088)	**8.210 (0.081)**	15.026 (0.198)	11.865 (0.206)	9.459 (0.096)	9.124 (0.097)	5.783 (0.019)	5.415 (–0.001)
Bekes[E]	16.498 (0.192)	16.606 (0.137)	9.009 (0.087)	8.434 **(0.082)**	**8.025** (0.086)	15.058 (0.220)	11.453 (0.208)	9.487 (0.100)	9.254 (0.106)	5.640 (0.024)	5.271 (0.004)
Kunmadaras[E]	16.467 (0.184)	16.254 (0.131)	9.163 (0.083)	8.404 (0.077)	**8.025 (0.077)**	14.663 (0.203)	11.535 (0.201)	9.683 (0.093)	9.158 (0.095)	5.714 (0.017)	5.354 (0.000)
Madaras[E]	16.671 (0.192)	16.626 (0.137)	9.319 (0.091)	8.462 (0.084)	**8.181 (0.083)**	14.914 (0.207)	11.572 (0.202)	9.355 (0.086)	9.117 (0.098)	5.714 (0.018)	5.271 (–0.006)
Kaba[E]	16.516 (0.196)	17.341 (0.148)	9.307 (0.104)	8.777 **(0.098)**	**8.142** (0.100)	16.312 (0.249)	11.768 (0.220)	9.559 (0.109)	9.566 (0.123)	5.836 (0.036)	5.421 (0.003)
Turanovac	16.598 (0.189)	16.729 (0.136)	9.233 (0.097)	8.294 (0.090)	**7.912** (0.094)	14.955 (0.225)	12.011 (0.215)	8.700 **(0.084)**	9.285 (0.112)	5.520 (0.023)	5.055 (0.000)

Potential sources were populations with a first observation year ≤2003 (i.e. the year of collection of CSE European samples). The Friuli outbreak was excluded from the analysis because it is known to have originated in CSE Europe, and thus most CSE European samples are expected to wrongly point to Friuli as its most probable source. $-\log_{10}$ of the $L_{i\to s}$ are indicated and F_{ST} are in parentheses. For each CSE European sample the maximum $L_{i\to s}$ and minimum F_{ST} with North American and European samples are indicated in bold typeface.

–: not suitable.

[W]: Sample from the western sampling transect.

[E]: Sample from the eastern sampling transect.

alga). No such evidence of multiple introductions from genetically differentiated source populations at different sites was observed in the large area of the CSE European outbreak of WCR. Indeed, measurements of interpopulation genetic variation and spatial Bayesian clustering indicated little or no genetic structure in the CSE Europe area of expansion of WCR. This genetic homogeneity is consistent with the homogeneity in susceptibility and resistance to insecticides reported in CSE Europe (Ciosi et al. 2009).

The highest $L_{i\to s}$ values identified the northern USA population as the source of all CSE European samples. However, the minimum F_{ST} values identified Alsace (France) as a possible source of some CSE European samples. This result is not particularly surprising given the substantial genetic similarity between the Alsace and northern USA populations (Ciosi et al. 2008). However, we consider that the Alsace population is not a likely source population for CSE European sites because (i) the Alsace population was first observed in 2003 (the year of CSE Europe sampling), (ii) population density was very low in Alsace at this time (we analyzed all nine beetles observed), (iii) $L_{i\to s}$ values clearly identify the northern USA population as the source population while F_{ST} do not clearly identify Alsace and (iv) F_{ST} estimate is not corrected to reduce the influence of bottleneck during introduction (Gaggiotti and Excoffier 2000), in contrast to $L_{i\to s}$ (Pascual et al. 2007). Moreover, the hypothesis of a common spatial origin (in northern USA) of individuals sampled in CSE Europe could not be rejected for 700 of the 706 individuals studied. Multiple introductions from the same source population or from genetically similar populations would be expected to be genetically equivalent to a single introduction of a large number of individuals (Roman and Darling 2007). However, this would probably not be the case if the multiple introductions occurred at different sites within the invaded area. The hypothesis of multiple introductions from the same or from genetically similar source populations at a single location cannot therefore be rejected for CSE Europe, but we found no evidence of multiple introductions into different locations within the CSE European area. Based on the parsimony principle, this outbreak therefore probably corresponds to a single expanding population originating from a single introduction in the Belgrade area.

Expansion process in CSE Europe

The expansion process should leave specific genetic signatures in invading populations. The simplest expansion model for invading species, the 'wave of advance' model (Fisher 1937), considers dispersal to be a random diffusion process. Under this model, we expect a pattern of genetic isolation by geographic distance, the intensity of which depends on the balance between gene flow and drift (Slatkin 1993). We detected such a pattern of genetic isolation by geographic distance in the CSE European outbreak of WCR, indicating greater gene flow between geographically close than between geographically distant locations, and thus that the dispersal of WCR is spatially limited.

Theoretical studies have shown that founder events in populations located at the edge of the expansion may have two consequences: (i) substantial genetic differentiation between the center and the periphery of a colonized area (Le Corre and Kremer 1998; Excoffier and Ray 2008), and (ii) a decrease in genetic variation in the direction of colonization (Le Corre and Kremer 1998; Hallatschek and Nelson 2008). A decrease in genetic variation in the direction of colonization has been documented for many organisms (e.g. Prugnolle et al. 2005 for humans; Williams et al. 2007 for Brazilian peppertrees). In CSE Europe, we found population genetic differentiation to be weak and we observed no decrease in genetic variation in the direction of colonization. This suggests an absence of successive major founder events at the front of the invaded area during the expansion process or that the effect of dispersal outweighed the effect of genetic drift related to the founder events. Theoretical and simulation studies have shown that a combination of short- and long-distance dispersal (i.e. stratified dispersal), during geographic expansion may maintain genetic diversity in expanding populations (Ibrahim et al. 1996; Le Corre and Kremer 1998; Davies et al. 2004; Bialozyt et al. 2006). However this dispersal model does not account for the small increase in genetic variation along the axis of colonization suggested by the weak but significant positive correlation of heterozygosity (H) with the year of first observation and with the geographic distance from Belgrade in the eastern transect. We argue that human activities, including control measures against WCR in particular, could be responsible for the observed pattern (no decrease in genetic variation and even a small increase in the direction of colonization). Significant damage to crops in Serbia and southern Hungary, has led to the establishment of integrated pest management (IPM), including crop rotation, which may have resulted in a decrease in WCR population density. Such a decrease has been documented in Serbia and southern Hungary following the establishment of IPM (Ripka and Princzinger 2001; Sivcev and Stankovic 2004; Boriani et al. 2006; Sivcev et al. 2009). In 2003, the year of sampling, IPM against WCR had been used in Serbia for at least 5 years (Sivcev and Stankovic 2004; Sivcev et al. 2009) and in southern Hungary for 2 years (Ripka and Princzinger 2001). Few or no WCR control methods were implemented in central and

northern Hungary, and in the sampled areas of Austria and Romania (Kiss et al. 2005). WCR populations may thus have underwent a decrease in size in Serbia and in southern Hungary, with this decrease more persistent in Serbia, but no such decrease in population size was observed further north. These demographic bottlenecks were probably associated with genetic bottlenecks, which lasted longer in Serbia than in southern Hungary. The erosion of the genetic variation induced by these genetic bottlenecks should thus have been stronger in Serbia than in southern Hungary and absent in northern Hungary. This may explain the pattern of absence of decrease and even of small increase in genetic variation from Serbia to northern Hungary suggested by our results. This hypothesis is consistent with the significant genetic differentiation observed between the sample collected at the location at which WCR was first sighted in the study area (i.e. the Belgrade-Airport sample) and almost all other samples. Differences in population densities due to geographic and temporal heterogeneities in control measures may frequently occur in a number of pest species. It is therefore possible that such a pattern of absence of decrease and small increase in genetic variation during colonization may be found in other invading pests for which control strategies are used, above a certain density threshold.

We observed a pattern of genetic isolation by angular distance together with a significant east-west genetic differentiation in CSE Europe, suggesting that the expanding population is divided into genetically differentiated sectors. The fragmentation of a colonized region into genetically differentiated sectors and the occurrence of allele frequency clines parallel to the colonization front have recently been attributed to a phenomenon called 'gene surfing' in theoretical studies (Edmonds et al. 2004; Klopfstein et al. 2006). During 'gene surfing', rare alleles or mutations may invade parts of the space not yet colonized due to genetic drift at the edge of an expanding population (Hallatschek et al. 2007; Excoffier and Ray 2008). However, in our case, we observed no evidence of genetic drift at the edge of the expansion (weak population genetic differentiation and no loss of diversity in the direction of colonization.). An anisotropic dispersal of WCR with effective dispersal more frequent in the direction of expansion due to weak competition beyond the front might also account for the observed pattern. Computer simulation-based studies are needed to test this hypothesis.

WCR is expanding in CSE Europe through stratified dispersal

The Frickingen outbreak in south-eastern Germany resulted from an introduction from the western part of the CSE European WCR area. Similarly, the Friuli outbreak resulted from an introduction of WCR from the center and/or the western part of the CSE European area. Finally, Frickingen and the western part of the CSE European outbreak can both be sources of the Passau population in south-western Germany. These geographically isolated outbreaks thus probably result from long-distance dispersal events from the continuously growing CSE European area, with different degrees of genetic diversity loss. Therefore, WCR appears to expand in CSE Europe through both local continuous dispersal and discontinuous long-distance dispersal. This corresponds to a stratified dispersal (Shigesada et al. 1995) process, in which satellite colonies are founded outside the main expanding population. These colonies eventually merge with the main expanding population and contribute to advance the front (Shigesada et al. 1995).

Large distances (>300 km) separate the Friuli and Frickingen outbreaks from their source populations, and the Alps stand between the CSE European area of expansion and both these isolated outbreaks. Moreover, the CSE European outbreak was not the geographically closest population to Friuli (when sampling was performed) and to Frickingen. When they were first observed, these outbreaks were closer to NW Italy and Alsace respectively. Human activities may therefore be the chief means of long-distance WCR dispersal in Europe, although north American studies have also suggested a major role for wind in the long-distance dispersal of WCR (Onstad et al. 1999; Isard et al. 2004). Whatever the means of transport, stratified dispersal seems to have a major impact on the geographic expansion of a species. The growth of satellite colonies and their coalescence with the main expanding population may lead to greater rates of geographic expansion than observed in cases of expansion without long-distance dispersal (Shigesada et al. 1995). Moreover, the longer the length of the expansion front, the more frequent long-distance dispersal events are likely to be. Therefore stratified dispersal may also result in an acceleration of the rate of expansion as the length of the front increases whereas this rate remains constant in a continuous diffusion (Shigesada et al. 1995). Metcalf (1983) reported such an acceleration of the rate of geographic expansion of WCR in the US Corn Belt and Gray et al. (2009) concluded that it probably resulted from stratified dispersal. A similar acceleration of the rate of expansion of the CSE European population may therefore be expected in the near future.

Conclusion

Genetic evidence suggest that a single introduction is responsible for the foundation of the Central and South-Eastern European outbreak of the western corn rootworm

and that this invasive population is expanding through stratified dispersal. Combined with historical documentation of population size and of the establishment of management strategies, our results also suggest that control measures against the western corn rootworm are probably responsible for genetic bottlenecks at the center of the outbreak. Thus, human activities probably affect the population dynamics of the pest, fortuitously increasing its capacity to disperse or deliberately decreasing pest population densities, thereby globally affecting the population structure of the western corn rootworm.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Estimated number of populations from STRUCTURE (A) and GENELAND (B) analyses. (A) Mean (±SD) natural logarithm of the likelihood of the data [LnP($X|K$)] over 10 STRUCTURE replicated runs for each value of the putative number of clusters (K). Black triangles and the solid line show the results of the analysis, performed with STRUCTUREV.2.2, that did not use the sampling locations as prior information. Open squares and the dashed line show the results of the analysis, performed wit STRUCTUREV.2.3.1, that used the sampling locations as prior information. (B) Posterior density distribution of the number of clusters (K) estimated from the highest-probability GENELAND run (among ten).

Figure S2. Estimated population structure from both STRUCTURE analyses for $K = 2$ to $K = 5$. Each individual is represented by a thin horizontal line divided into K coloured segments that represent the individual's estimated membership fractions in K clusters. Black lines separate individuals from different sample localities. Each plot, produced with DISTRUCT (Rosenberg 2004), is based on the highest-probability run (among ten) at that value of K. ^{W}: sample from the western sampling transect. ^{E}: sample from the eastern sampling transect.

Table S1. Description of the western corn rootworm samples used in this study.

Table S2. Statistics summarizing genetic variation within each western corn rootworm sampled population in America and Europe.

Table S3. Weir & Cockerham's estimator of pairwise F_{ST} (1984) between western corn rootworm sampled populations in the Central and South-Eastern European expanding area.

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