Secondary Contact and Admixture between Independently Invading Populations of the Western Corn Rootworm, *Diabrotica virgifera virgifera* in Europe

Gérald Bermond1,2,3*, Marc Ciosi4,5, Eric Lombert1,2,3, Aurélie Blin1,2,3, Marco Boriani6, Lorenzo Furlan7, Stefan Toepfer8, Thomas Guillemaud1,2,3

1 INRA, UMR 1355 Institute Sophia Agrobiotech, Equipe “Biologie des Populations Introduites”, Sophia Antipolis, France, 2 Université de Nice Sophia Antipolis, UMR Institute Sophia Agrobiotech, Equipe “Biologie des Populations Introduites”, Sophia Antipolis, France, 3 CNRS, UMR 7254 Institute Sophia Agrobiotech, Equipe “Biologie des Populations Introduites”, Sophia Antipolis, France, 4 INRA, UMR 7254 Institute Sophia Agrobiotech, Equipe “Biologie des Populations Introduites”, Sophia Antipolis, France, 5 MBB Unit, International Centre of Insect Physiology and Ecology, Nairobi, Kenya, 6 Servizio fitosanitario regionale, Regione Lombardia, Milano, Italy, 7 Veneto Agricoltura, Legnaro, Italy, 8 CABI Europe, Switzerland, c/o Plant Protection Directorate, Hodmezovasarhely, Hungary

Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is one of the most destructive pests of corn in North America and is currently invading Europe. The two major invasive outbreaks of rootworm in Europe have occurred, in North-West Italy and in Central and South-Eastern Europe. These two outbreaks originated from independent introductions from North America. Secondary contact probably occurred in North Italy between these two outbreaks, in 2008. We used 13 microsatellite markers to conduct a population genetics study, to demonstrate that this geographic contact resulted in a zone of admixture in the Italian region of Veneto. We show that i) genetic variation is greater in the contact zone than in the parental outbreaks; ii) several signs of admixture were detected in some Venetian samples, in a Bayesian analysis of the population structure and in an approximate Bayesian computation analysis of historical scenarios and, finally, iii) allelic frequency clines were observed at microsatellite loci. The contact between the invasive outbreaks in North-West Italy and Central and South-Eastern Europe resulted in a zone of admixture, with particular characteristics. The evolutionary implications of the existence of a zone of admixture in Northern Italy and their possible impact on the invasion success of the western corn rootworm are discussed.

Introduction

In the early stages of biological invasions, the genetic diversity of populations may be reduced by bottlenecks [1,2]. This may hinder adaptation to new environmental conditions. However, multiple introductions into the same area followed by admixture may increase genetic variability, offsetting the effect of genetic bottlenecks associated with each introduction [3,4]. If the various sources are genetically differentiated, this process results in the conversion of interpopulation genetic variation into intrapopulation genetic variation (as reported for the Cuban lizard in Florida, [5]). Conversely, intrapopulation variation may be converted into interpopulation variation if multiple introductions from a single source occur in geographically disconnected areas (e.g., [6]). Secondary contact and admixture between such independently introduced populations can eventually lead to restoration of the genetic variation found in the source population.

Admixture may thus have a positive impact on invasion, through the generation of novel genotypes [3], an increase in intrapopulation genetic variation [5] and heterosis [7-9], in which admixed individual fitness exceeds the fitness of the parental populations. Finally, deleterious mutations may be purged from introduced populations during bottlenecks [10,11]. This may lead to an unusual phenomenon in which multiple introductions followed by admixture may result not only in the restoration of genetic variation, but also in the fitness of the population exceeding that of the source population.

The invasion of Europe by the western corn rootworm (WCR, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae)) has involved multiple introductions into several disconnected geographic areas from a single source population in the northern USA [6,12]. The WCR is native to Central America [13,14], probably evolved with corn in Mexico and reached the South Western USA about 3000 years ago, together with its host plant, corn [15]. During the second half of the 20th century, the WCR has rapidly expanded its range across areas of corn production in the American Mid West (see [16] for a review), finally reaching the north-eastern coast of the USA in the late 1980s (see [17] for a review). In the late 20th century and the early 2000s, WCR was introduced into Europe repeatedly from the Northern USA [6,12]. It first observed near Belgrade Airport in Serbia, in 1992. An international monitoring...
network has since monitored the distribution and of the WCR and its annual expansion in Europe [18]. There are two types of infested area: i) geographic areas of continuous WCR range expansion in Central and South-Eastern Europe (CSE Europe), North-West Italy (NW Italy) and Bavaria in Southern Germany, corresponding to “invasive” outbreaks and ii) many small disconnected outbreaks that have not persisted over time or expanded geographically, such as area of the North-East Italian (NE Italy) outbreak, which originated from CSE Europe [6,12,19]. During the multiple introductions of WCR in Europe from North America [12] strong bottlenecks lead to the formation of several genetically differentiated outbreaks [6]. For example Giosi et al. [6] found a $F_{CT}$ of 0.25 between CSE Europe and NW Italy. The largest outbreak, in CSE Europe, currently covers 16 countries extending from Austria to Ukraine and Southern Poland to Northern Greece. There has also been a westward expansion into eastern parts of Italy. In parallel, the NW Italian invasive outbreak has progressed eastwards. Since 2008, the NW and NE Italian and CSE European outbreak populations have been in close geographic proximity or even in contact, as in the Italian region of Veneto [19–21]. The current geographic distribution of WCR is now continuous in Northern Italy [21,22] (Figure 1). Because the various outbreaks were strongly genetically differentiated, there is probably currently an admixture Northern Italy between the NW Italian, NE Italian and CSE European invasive outbreaks. This situation provides us with an ideal opportunity to follow an evolutionary process in real time in nature. The aim of this study was to identify, document and characterize the putative admixture in Northern Italy, following contact between the WCR outbreak populations of NW and NE Italy and that of CSE Europe.

Materials and Methods

Sample Collection

No permission is required to collect samples of this species. WCR has no value and is a pest species whose populations are controlled by insecticide treatments or plant rotation wherever they occur. Samples were collected on a west-east transect crossing the outbreak areas of NW Italy, NE Italy and CSE Europe (Figure 1 and Table 1). Adult beetles were caught with sticky traps at the Storo site (NW Italian outbreak) or by hand (net or funnel bound with a muslin bag) at all other sites. The WCR samples used in this study were collected during summer, from June to August, at 12 sites in Italy, Hungary and Serbia (see details in Table 1). At each of the study sites, all the individuals collected were obtained from a single corn field. We distinguished between two types of populations: the parental populations, corresponding to the invasive outbreaks of NW Italy, NE Italy and CSE Europe and the potentially admixed populations in the contact zone between the parental invasive outbreak populations.

Additional samples (Figure 1, Table 1) collected in 2010 in the outbreaks of NW Italy, NE Italy and CSE Europe and in 2003 in Northern USA (the native area) were only used for the temporal analysis (see Results section, Temporal and spatial analyses). For the Northern USA sample, DNA was extracted from individuals with the BioRad Aqua Pure isolation kit (BioRad, Hercules, CA) according to the manufacturer’s instructions, whereas, for other samples, DNA was extracted with the DNeasy tissue kit (Qiagen, Hilden, Germany), as explained above.

DNA Extraction and Microsatellite Analyses

All WCR samples were stored in 90–96% ethanol until DNA extraction. Template material for the polymerase chain reaction (PCR) amplification of microsatellite loci was obtained with two different protocols. For the NE Italian sample, the ‘salting out’ protocol of Sunnucks and Hales [23] was used for the rapid extraction of DNA from the head of each individual. For the other samples, we extracted DNA from the thorax or half the body, cut lengthwise, with the DNeasy tissue kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions, with an elution volume of 100 µl. Individuals were washed at least three times in 0.065% NaCl before extraction, to remove ethanol from the tissues.
Table 1. Description of the within-population genetic variation of western corn rootworm samples from Northern Italy and CSE Europe.

| Population type | Sample | Origin (Region (Country)) | Year of 1st observation | N | Sampling date | Distance to Oleggio (km) | A (DC) | A (AR) | He | F<sub>e</sub> | p-HW |
|-----------------|--------|---------------------------|-------------------------|---|---------------|--------------------------|--------|--------|----|---------|------|
| Parental        | Oleggio | NW Italy Piedmont (Italy) | 2000                    | 40 | 2007          | 0                        | 3.77 (2.42) | 2.70 (1.49) | 0.41 | 0.04    | 0.40 |
|                 | Olcenengo | NW Italy Piedmont (Italy) | 2000                    | 30 | 2010          | –                        | 3.39 (2.66) | 2.66 (1.60) | 0.41 | 0.10    | 0.39 |
|                 | Fontanella | NW Italy Lombardia (Italy) | 2000                    | 33 | 2007          | 92                        | 2.77 (1.79) | 2.36 (1.32) | 0.37 | 0.06    | 0.51 |
|                 | Castenagno | NW Italy Lombardia (Italy) | 2000                    | 30 | 2010          | –                        | 3.00 (1.92) | 2.43 (1.32) | 0.37 | –0.06   | 0.56 |
|                 | Storo    | NW Italy Trentino (Italy) | 2000                    | 90 | 2007          | 151                      | 3.39 (1.85) | 2.38 (1.24) | 0.39 | 0.02    | 0.66 |
| Potentially admixed | Borso del Grappa | – Veneto (Italy) | 2002                    | 20 | 2009          | 240                      | 3.15 (1.91) | 2.85 (1.64) | 0.41 | 0.04    | 0.66 |
|                 | Conselve | – Veneto (Italy) | 2002                    | 20 | 2009          | 247                      | 3.00 (1.83) | 2.60 (1.48) | 0.39 | –0.06   | 0.26 |
|                 | Piave di Sacco | – Veneto (Italy) | 2002                    | 11 | 2009          | 265                      | 3.00 (2.04) | 2.85 (1.79) | 0.46 | 0.17    | 0.39 |
|                 | Scorze | – Veneto (Italy) | 2002                    | 10 | 2009          | 278                      | 3.15 (1.68) | 3.08 (1.56) | 0.53 | 0.00    | 1    |
|                 | San Donà di Piave | – Veneto (Italy) | 2002                    | 9  | 2009          | 305                      | 3.39 (1.85) | 3.37 (1.83) | 0.54 | 0.00    | 0.33 |
|                 | Summaga | – Veneto (Italy) | 2002                    | 17 | 2009          | 327                      | 3.23 (1.64) | 2.94 (1.38) | 0.48 | 0.04    | 0.46 |
| Parental        | Buttrio<sup>5</sup> | NE Italy Friuli (Italy) | 2003                    | 27 | 2003          | –                        | 1.77 (0.73) | 1.72 (0.64) | 0.29 | 0.03    | 0.58 |
|                 | Premariacco | NE Italy Friuli (Italy) | 2003                    | 29 | 2010          | –                        | 2.70 (1.25) | 2.22 (0.81) | 0.34 | 0.08    | 0.15 |
|                 | Pince | CSE Europe Prekmurje (Slovenia) | 2003 | 27 | 2010 | – | 2.77 (1.24) | 2.63 (1.15) | 0.44 | 0.08 | 0.06 |
|                 | Szekszard | CSE Europe Tolna (Hungary) | 1992 | 39 | 2007 | 780 | 2.77 (1.34) | 2.63 (1.11) | 0.46 | –0.05 | 0.58 |
|                 | Csepaja | CSE Europe Voivodine (Serbia) | 1992 | 30 | 2007 | 940 | 2.92 (1.50) | 2.69 (1.15) | 0.46 | 0.00 | 0.59 |
| Native          | Bellefonte | Northern USA Pennsylvania (USA) | 1985 | 42 | 2003 | – | 6.10 (4.41) | 4.23 (2.16) | 0.61 | 0.05 | 0.20 |

Note: NW: North-West. NE: North-East. CSE: Central and South-Eastern. Buttrio<sup>5</sup>: this sample was named Friuli in Ciosi et al. [8] and NE Italy in Miller et al. [12]. Distances to Oleggio are provided only for populations included in the spatial analysis. N: sample size. A: mean number of alleles per locus. A was determined by direct counts (DC) and allelic richness (AR) analysis. AR is based on the smallest sample size (N = 8 for several loci of the sample San Donà di Piave). Standard deviations between loci are shown in parentheses. H: mean expected heterozygosity [27]. p-HW: p-values for the exact test of deviation from HW equilibrium.

doi:10.1371/journal.pone.0050129.t001
We amplified 13 WCR microsatellite loci (including di and tri-nucleotides [6,24]) in three separate multiplex PCR performed in a PTC-225 MJ Research thermocycler. The first reaction amplified the DVV-D2, DVV-D4, DVV-D11, and DVV-T2 microsatellites, the second amplified DVV-D5, DVV-D8, DVV-D9 and DVV-ET1, and the third amplified Dba01, Dba05, DVV-T3, DVV-D12 and Dba07.

The thermal cycling conditions were the same for all three reactions and were as described by Miller et al. [25]. Forward primers were 5’-labeled with a fluorescent dye for detection of the PCR products on an Applied Biosystems 3130xl Genetic Analyzer. Signal strength was rendered equivalent for different markers, by mixing labeled and unlabeled forward primers in the proportions (labeled/unlabeled) described by Miller et al. [25] for the first two sets of markers and in the following proportions for the third set: Dba01 2:1; Dba05 1:1; DVV-T3 1:0; DVV-D12 1:0 and Dba07 1:0. The primers used for DVV-D12 amplified were modified from those originally described by Kim et al. [26] (primers used here: F: 5’-GATTCTGAGTAAAGAGGAAACG-3’; R: 5’-CACACGCTTTCTGTAAATCATCTAC-3’). This decreased the frequency of null allele detection at this locus to a negligible level. All three multiplex reactions were analyzed as described by Miller et al. [25]. All individuals were unambiguously assigned to a diploid multilocus genotype (two peaks per individual at the maximum).

Classical Statistical Genetics Analysis

Genetic variation within samples was evaluated by determining the mean number of alleles per locus (A) and mean expected heterozygosity (He) [27]. A and He were calculated with GENECLASS version 2.0.h [28]. We also calculated Fis with GENEPOP ver. 4.0.1 [29,30]. We compared A values between population samples by estimating allelic richness (AR) based on the smallest sample size, by the rarefaction method [31] implemented in Fstat version 2.9.3 [32]. The various loci are the independent statistical units because they have their own coalescence story. Hence, the differences in AR and He between samples were assessed in a one-sided Wilcoxon signed rank test (with greater genetic variation in the supposed admixed populations in the contact zone between the parental invasive outbreaks as an alternative hypothesis) with locus as a repetition unit. The significance of the differences between the contact zone (Veneto) and the parental outbreak areas (NW Italy, NE Italy and CSE Europe) was then assessed by combining the probabilities obtained for each sample from Veneto by Fisher’s method [33]. We also used the permutation procedure implemented in Fstat version 2.9.3 to test homogeneity of allelic richness and heterozygosity among samples [32]. We tested for deviation from Hardy-Weinberg equilibrium with the probability test approach implemented in GENEPOP version 4.0.1 [29,30]. Genetic variation between samples was assessed by calculating Weir and Cockerham’s [34] pairwise FST and by assessing genic differentiation between pairs of samples [35] with GENEPOP version 4.0.1 [29,30]. When multiple tests were performed to test the same hypothesis, significance levels were lowered according to the Benjamini and Hochberg procedure [36].

Bayesian Analysis of the Population Structure of WCR

We inferred the genetic structure of the WCR in Northern Italy, using the Bayesian method implemented in STRUCTURE version 2.3.3 [37]. We performed 20 runs for each value of the number of clusters (K), defined as lying between 1 and the total number of sampling sites. Each run consisted of a burn-in of 5×10^5 iterations, followed by 10^5 iterations. We used the admixture model of ancestry together with the correlated allele frequencies model [38], with and without the use of sampling location as prior information [39]. Default values were maintained for all other parameters. K was estimated with Evanno’s ΔK statistic [40], which is based on the rate of change in log-likelihood between successive values of K and the variability of log-likelihood between different runs. The most likely run was then represented with DISTSTRUCT version 1.0 [41], see Figure 3).

Genetic differentiation was found between two samples collected from NE Italy in 2003 and 2010 (see details in Results, section Temporal and spatial analyses and Figure 1). We therefore removed the Buttrio (NE Italy) site from the Bayesian analysis and analyses of geographic population structure. However, as the NE Italian outbreak population may be a source of the Veneto populations, we considered the NE Italian sample from 2003 in the Approximate Bayesian Computation (ABC) analysis.

Geographic Analyses and Estimation of Admixture Rate

We tested the effect of geographic distance to Oleggio (the sampling site at the extreme west of the studied area, defined here as the geographic reference point) on microsatellite allelic frequencies. We defined a geographic axis passing through the various sampling sites and we then projected each sampled population orthogonally on this axis (Figure 1). The projected coordinates were then used to calculate the distance between each sample and the Oleggio sample. For each locus, the effect of this distance on allelic frequencies was assessed with a generalized linear model in SAS version 9.1.3 [42]. A multinomial distribution was chosen for the residual error and a cumulative logit function was used as the link. In cases of overdispersion an F test was carried out rather than a χ² test to evaluate the effect of distance [43].

The admixture rate was estimated (i) as described by Choisy et al. [44], with Oleggio and Crepaja as representative samples of the parental populations (these sites are the closest to the sites at which WCR was first observed in each parental outbreak), or (ii) with the coefficient of coancestry Q calculated with STRUCTURE version 2.3.3 [37,38].

ABC Analysis of Historical Scenarios of WCR Invasion in Northern Italy

For each sampling site within the contact zone (i.e. for each of the six Venetian target samples), we conducted six independent ABC analyses comparing various historical scenarios (Figure S1, Supporting information) differing in terms of the source of the population at the Venetian site concerned. Each parental outbreak population (CSE Europe, NE and NW Italy) was represented by the sample obtained from the site closest to the location at which the first observation for the outbreak concerned was reported (Crepaja, Buttrio and Oleggio for the CSE Europe, NE and NW Italy outbreaks, respectively). We thus considered a total of nine scenarios for each Veneto site, according to the origin of the population: (i) NW Italy outbreak (represented by Oleggio), (ii) NE Italy outbreak (represented by Buttrio), (iii) CSE Europe outbreak (represented by Crepaja), (iv, v, vi) all three possible scenarios of a single admixture between NW Italy, NE Italy and CSE Europe outbreak populations (vii, viii and ix) all three possible scenarios of a double admixture between NW Italy, NE Italy and CSE Europe outbreak populations. The history of the putative source populations was defined in accordance with published reports [6,12]. The NW Italy and CSE Europe populations were considered to have originated independently from North America, whereas the NE Italy population was considered to be derived from the CSE Europe population.
ABC analyses [45] were performed with DIYABC version 1.0.4.40 [46], with parameter values drawn from the prior distributions described hereafter and obtained by simulating 10^6 microsatellite datasets for each competing scenario. Simulated and observed datasets were summarized with summary statistics, which were then used to calculate Euclidean distances between the simulated and observed datasets. We then estimated the posterior probabilities of the competing scenarios by polychotomous logistic regression [47] on the 1% of the simulated datasets closest to the observed dataset, after reducing the parameter space by a linear discriminant analysis (LDA) approach [48]. In cases of an overlap between the confidence intervals of the two largest posterior probabilities (each corresponding to a particular historical scenario), we repeated the ABC analyses with the two competing scenarios only.

We used the summary statistics describing genetic variation within and between populations generally used for approximate Bayesian computation analyses [47,49–51]. For each population and each population pair, we used the mean number of alleles per locus, mean heterozygosity, the mean ratio of the number of alleles to the range of allelic size, the FST between pairs of populations and mean individual assignment log-likelihoods of individuals from population j being assigned to population i and the maximum likelihood estimates for admixture proportions.

The prior distributions of the historical, demographic and mutational parameters used in the ABC analysis were as follows: N_i, the effective population size of the WCR American (USA) or European (CSE Europe, NW Italy, NE Italy and Veneto) source populations and N_E, the effective size of the ghost population (an unsampled population called the “ghost population” was included in the analysis for “double admixture” scenarios between the NE and NW Italy and CSE outbreaks; this “ghost population” is the result of a single admixture between two of the three putative source populations, depending on the scenario), were drawn from a uniform distribution bounded by 1000 and 200000 (Uniform[1000; 200000]); N_P and N_E, the effective number of founders of the European populations, were drawn from a Uniform[1; 100] distribution; the bottleneck duration of population i, BD_i, was drawn from a Uniform[1;5] population. Each introduced population i (i = 1, 2, 3, 4 for Veneto (the contact zone in Northern Italy), NE Italy, NW Italy and CSE Europe, respectively) was founded by individuals originating from its source population i and (t_i, for the ghost population) generations before the present (i.e. 2010, year of the study). As WCR is univoltine, t_i and t_G are also the number of years before the present, t_1, t_2, t_3, t_4, and t_G were drawn from between one and five generations (or years) before the date of the first observation (see Table 1) and were thus drawn from Uniform[9;13], [8;12], [11;15] and [19;23] distributions, respectively. In all double admixture scenarios, we fixed t_1<=t_2. We used a generalized stepwise mutation model (GSM) [32]. A mean mutation rate across loci, μ, was first drawn from a Uniform [10^-3; 3x10^-4] distribution, and single locus mutation rates, μ_i, were then drawn from gamma distributions with a mean of μ and a shape parameter of 2 (rate = 2/μ). For each locus, the coefficient P of the geometric distribution of repeat units by which a new mutant allele differs from its ancestor was drawn from an exponential distribution with a mean of 0.22.

We evaluated the ability of ABC to select the true scenario correctly, by analyzing test datasets simulated from known competing scenarios. For each scenario, one hundred such datasets were simulated with parameter values drawn from the same probability distributions as the priors. The posterior probabilities of each competing scenario were estimated for each simulated test dataset, with the same ABC procedure as described above, and were used to calculate type I and II errors in the selection of scenarios. Type I error is the proportion of simulations in which the scenario considered is excluded but is actually the true one. Type II error is the proportion of simulations in which the scenario considered is selected but is not the true one. Small Type 2 errors provide good confidence in the results even if the Type 1 errors are large.

**Results**

**Temporal and Spatial Analyses**

The main body of the present study concerns spatial analyses of WCR genetic variation. When the samples were not collected within the same year as in our case, a procedure must guarantee that no temporal confounding effect occurs. Such a temporal effect was hence tested using samples collected within the same area at various dates (Lombardy, Piedmont, Friuli and CSE Europe). Locations showing a significant temporal effect were removed from the spatial analysis.

No temporal differentiation was found between samples collected in the NW Italy outbreak in Piedmont (Oleggio, 2007, and Olenenego, 2010), in Lombardia (Fontanella, 2007, and Castegnato, 2010) and in the CSE Europe outbreak (Hungary (Tolna) and Serbia (Voovodine) in 2007 and Slovenia (Prekmurje) 2010; p>0.08 for all pairwise comparisons considered). These locations were thus kept and the 2007 samples were arbitrarily chosen for subsequent spatial analyses. A significant temporal differentiation was found in Friuli (Buttiro, 2003, and Premariacco, 2010; p = 0.001). Friuli was thus not considered for the spatial analyses.

The Friuli sample of Buttiro was sampled in 2003, i.e. before the contact between the NW, NE Italian and CSE European outbreaks in 2008. Hence, it properly describes the north eastern Italian outbreak before any contact between the various outbreaks.

Buttiro was therefore used in the ABC historical scenario comparison.

**Genetic Variation within and between Populations**

Overall, the European WCR populations displayed moderate polymorphism, with 5.31 (SD = 3.71) alleles per locus over all samples. Within samples, the mean number of alleles was between 1.77 (SD = 0.73) for Buttiro in NE Italy and 3.77 (SD = 2.43) in Oleggio in NW Italy (Table 1). Mean expected heterozygosity (He) was low to moderate and varied from 0.29 for Buttrio in NE Italy to 0.54 for San Donà di Piave in Veneto (Table 1). Fts estimates were low and no significant deviation from Hardy-Weinberg equilibrium was observed (Table 1). Samples from Veneto contained a significantly larger number of alleles than parental samples (Fisher’s method for the combination of probabilities, \( \chi^2 = 121.28; df = 36; p<10^{-3} \); \( \chi^2 = 66.31; df = 12; p<10^{-3} \); \( \chi^2 = 61.24; df = 24; p<10^{-3} \) for the comparisons “Veneto/NW Italy, Veneto/NE Italy and Veneto/CSE Europe”, respectively; Figure 2) and heterozygosity was significantly greater for samples from Veneto than for all other samples (Fisher’s method for the combination of probabilities, \( \chi^2 = 110.97; df = 36; p<10^{-3} \); \( \chi^2 = 60.22; df = 12; p<10^{-5} \); \( \chi^2 = 40.81; df = 24; p<0.05 \) for the comparisons “Veneto/NW Italy, Veneto/NE Italy and Veneto/CSE Europe”, respectively; Figure 2). Permutation tests performed with Fststat revealed a significantly larger allelic richness (one sided-test, \( p = 4x10^{-3} \)) and a marginally significantly larger heterozygosity (one sided-test, \( p = 0.062 \)) in Veneto compared to parental samples. Most pairwise comparisons of samples (92%) showed significant genetic differentiation (Table 2). Veneto samples generally displayed significant genetic differentiation from...
Bayesian Analysis of the Structure of WCR Samples in Italy and CSE Europe

The number of genetic clusters ($K$) was estimated at $K=2$, whatever the model used. The analysis of coancestry coefficients ($Q$) (for the model with the use of sampling location as prior information) (Figure 3) indicated that (i) the vast majority of individuals from the west of the transect belonged to a single cluster (with $Q>0.86$ for 161 of 163 individuals) (ii) individuals from the east belonged to another cluster (with $Q<0.07$ for all individuals) and that (iii) a large number of individuals from Scorze`, San Dona` di Piave, Borso del Grappa and Piove di Sacco are admixed between both clusters (with $Q$ between 0.20 and 0.80). One individual from Summaga, a population belonging to the eastern genetic cluster, was assigned to the western genetic cluster ($Q=0.97$).

Detection of Clines of Allelic Frequencies among WCR Samples in Italy and CSE Europe

A significant effect of geographic distance on allelic frequencies was found for six of the 13 loci studied ($p<0.0033$ for each of these 6 loci; see Figure 4 for example). Distance had a significant effect over all loci (Fisher’s method for the combination of probabilities, $\chi^2 = 113.62$, df = 26, $p<10^{-6}$). The admixture rates obtained by the two different methods (the method of Choisy et al. [44] and the coancestry coefficient from STRUCTURE) were very similar and closely matched a sigmoid logit model ($R^2 = 0.90$ for both methods). Figure 4 shows that the center of the cline is located about 310 km from Oleggio, in Veneto, and that this cline is about 100 km wide.

The effect of the geographic distance on the $F_{ST}/(1-F_{ST})$ between each sample and Oleggio, the sample located in the extreme west of the studied area was significant (Spearman rank-order correlation test, $p<10^{-5}$, Figure 4). Genetic differentiations compared to Oleggio sample are very low for western samples (NW Italy outbreak) and substantial for eastern samples, with a $F_{ST}$ of about 0.25 in CSE Europe outbreak. A sharp discontinuity of $F_{ST}/(1-F_{ST})$ is observed in the contact zone, i.e. in Veneto, where values are intermediate.

ABC Analysis of Historical Scenarios of WCR Invasion in Northern Italy

The ABC procedure was used to calculate the posterior probabilities of the evolutionary scenarios describing the origin of each population sampled in Veneto. According to the highest probabilities with non overlapping confidence intervals, four of the six Veneto samples probably originate from admixture events: samples from Scorze` and San Dona di Piave probably originate from admixture between the outbreak populations of NW Italy and CSE Europe (Table 3); samples from Borso del Grappa and Summaga probably result from double admixture between NW Italy, NE Italy and CSE Europe outbreak populations; samples from Conselve and Piove di Sacco have a simple origin in NW Italy.

The largest posterior probabilities were only moderate (Table 3) and some type I errors were large (four
Table 2. Pairwise $F_{ST}$ estimates [34] between Northern Italian and Central and South-Eastern European samples of the western corn rootworm.

| Population          | North-West Italy | Contact zone       | North-East Italy | Central and South-Eastern Europe |
|---------------------|------------------|--------------------|------------------|----------------------------------|
| Sample site         | Oleggio          | Fontanella         | Borso del Grappa | Conselve                         |
| Fontanella          | 0.00             |                    |                  |                                  |
| Storo               | 0.01             | 0.01               |                  |                                  |
| Borso del Grappa    | 0.03             | 0.02               | 0.02             |                                  |
| Conselve            | 0.03             | 0.02               | 0.01             | 0.04                             |
| Piove di Sacco      | 0.02             | 0.02               | 0.01             | 0.03                             |
| Scorzè              | 0.11             | 0.12               | 0.12             | 0.06                             |
| San Donà di Piave   | 0.08             | 0.10               | 0.10             | 0.06                             |
| Summaga             | 0.24             | 0.27               | 0.28             | 0.20                             |
| Buttrio             | 0.38             | 0.42               | 0.40             | 0.36                             |
| Székszard           | 0.25             | 0.27               | 0.28             | 0.19                             |
| Crepaja             | 0.25             | 0.26               | 0.27             | 0.17                             |

Note: Significant pairwise differentiation tests after correcting for the false-positive rate by the procedure of Benjamini and Hochberg [36] are shown in bold typeface. From left to right, samples are ordered from west to east, from Italy to Serbia.

doi:10.1371/journal.pone.0050129.t002
of the six values are between 0.43 and 0.67). However, as explained in the material and method, low type 2 errors (<0.09, Table 3) suggest that we can have a high degree of confidence in the choice of scenario.

Discussion

Since the first observation of the CSE European outbreak of WCR near Belgrade in 1992, a monitoring network has followed the progression of this pest in Europe [17,18,22]. No contact between the NW Italian, the NE Italian and CSE European outbreak populations was detected until 2008, since when the distribution of WCR in Northern Italy has been continuous, extending from NW Italy to CSE Europe [20,21]. Population genetic analysis of the WCR collected in the zone of secondary contact between the NW Italian and CSE outbreak populations revealed a high degree of genetic heterogeneity, determined principally by geography. Our results confirm show the existence of a zone of admixture in Northern Italy, with the occurrence of admixture between highly differentiated populations.
Table 3. ABC analysis of the nine historical scenarios describing the history of each population sampled in the secondary contact zone (Veneto).

| Historical scenarios | Without admixture | Single admixture | Double admixture | Errors |
|----------------------|-------------------|------------------|------------------|--------|
|                      | NW Italy | NE Italy | CSE Europe | NW Italy/NE Italy | NE Italy/CSE Europe | NW Italy/CSE Europe | (NW Italy/NE Italy)/CSE Europe | (CSE Europe/NE Italy)/NW Italy | Type I | Type II (min–max) |
| Borso del Grappa     | 0.19 [0.18;0.21] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.17 [0.16;0.18] | 0.0 [0.0;0.0] | 0.31 [0.30;0.33] | 0.02 [0.02;0.02] | 0.29 [0.28;0.31] | 0.01 [0.01;0.01] | 0.66 | 0.08 (0.0–0.23) |
| Conselve             | 0.99 [0.98;0.99] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.01 [0.0;0.0] | 0.0 [0.0;0.0] | 0.10 | 0.04 (0.0–0.12) |
| Piove di Sacco       | 0.27 [0.21;0.32] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.18 [0.14;0.21] | 0.0 [0.0;0.0] | 0.22 [0.18;0.26] | 0.0 [0.0;0.0] | 0.33 [0.28;0.37] | 0.0 [0.0;0.0] | 0.06 | 0.05 (0.0–0.14) |
| Scorze               | 0.0 [0.0;0.0]   | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.07 [0.06;0.09] | 0.0 [0.0;0.0] | 0.54 [0.49;0.59] | 0.10 [0.08;0.12] | 0.26 [0.22;0.30] | 0.03 [0.02;0.03] | 0.58 | 0.09 (0.0–0.23) |
| San Donà di Piave    | 0.01 [0.00;0.01] | 0.0 [0.0;0.0] | 0.0 [0.0;0.0] | 0.17 [0.14;0.20] | 0.0 [0.0;0.0] | 0.41 [0.37;0.46] | 0.07 [0.06;0.09] | 0.31 [0.26;0.34] | 0.03 [0.02;0.04] | 0.43 | 0.07 (0.02–0.30) |
| Summaga              | 0.0 [0.0;0.0]   | 0.0 [0.0;0.0] | 0.03 [0.02;0.04] | 0.02 [0.01;0.02] | 0.01 [0.0;0.01] | 0.13 [0.10;0.15] | 0.61 [0.57;0.66] | 0.11 [0.09;0.14] | 0.09 [0.07;0.11] | 0.67 | 0.06 (0.01–0.15) |

Posterior probabilities are given, with their confidence intervals and associated type I and type II errors.

Note: 95% confidence intervals are indicated in brackets. Values in parentheses are the new posterior probabilities of scenarios re-analyzed in ABC due to the overlap between confidence intervals. The highest probability values are shown in bold typeface and indicate the best scenario. NW: North-West. NE: North-East.

CSE: Central and South-Eastern.

doi:10.1371/journal.pone.0050129.t003
A Contact and Zone of Admixture in Northern Italy, in the Veneto Region

The current contact zone between the different European outbreak populations (NW Italy, NE Italy and CSE Europe) is located in Veneto. This zone has been intensively monitored with pheromone traps since the first captures of WCR in the Venetian region in 1998, leading to the implementation of an eradication program. Since 2008, the geographic distribution of WCR has been continuous in Northern Italy [21,22] due to contact between the outbreak populations of NW and NE Italy and CSE Europe [19–21].

Our analysis of the population genetic structure of WCR in Northern Italy and CSE Europe showed that the WCR populations sampled in Veneto resulted mostly from contact and admixture between the NW Italian and CSE European outbreak populations. The admixture analysis, the Bayesian analysis of population genetic structure and the ABC analysis of scenario choice all indicate that the Veneto region is a zone of admixture, containing admixed individuals. The genomes of individuals sampled in Veneto could be attributed to both the genetically different clusters of NW Italy and CSE Europe, with various levels of admixture. Moreover, the rate of admixture between the NW Italian, NE Italian and CSE Europe outbreaks varied evenly across a gradient from west to east. 

According to the ABC analysis, most samples from the western part of Veneto have a simple NW Italian origin, whereas all the other sample populations result from single or double admixture events between the three parental outbreak populations (NE and NW Italy and CSE Europe). Double admixture between individuals originating from distant populations (here, the three parental populations) accounted for the origin of the most westerly sample from Veneto (Borso del Grappa). This implies that individuals from the eastern outbreak populations (the NE Italy and CSE Europe outbreaks) migrated to the western part of Veneto, suggesting that long-distance dispersal occurs. Long-distance dispersal is also suggested by the detection of an individual genetically assigned to the western genetic cluster in Summag, a population from the eastern genetic cluster. Recent studies have suggested that long-distance dispersal is common in WCR and that invasive populations of WCR are expanding through stratified dispersal [17,53].

The occurrence of a cline in the Veneto region in 2009, even with such a large width (about 100 km), is consistent with the short period of time between secondary contact (2008) and sampling (2009). The WCR has a considerable capacity for dispersal, as shown by the rapid rate of expansion of the CSE Europe outbreak population (60–100 km (60–100 km per year [54])). A long period between sampling and contact would therefore probably have led to the homogenization of microsatellite frequencies over space [55].

A thorough clinal analysis of WCR in Veneto is required, to estimate dispersal parameters (e.g., [56]). Under the effect of dispersal alone, allelic frequencies tend to become homogeneous over the zone of admixture, so the slope of the cline depends on the strength of dispersal and contact time (e.g., [55]). The speed at which the slope decreases over time is thus a direct function of the dispersal intensity [57]. Temporal analysis of the North Italian zone of admixture should therefore provide an estimate of the dispersal capacities of WCR, as recently reported for Biston betularia by Saccheri et al. [58].

It is noteworthy that biological invasions may provide numerous opportunities to estimate dispersal parameters using such analysis of frequency cline. It is now admitted that multiple invasions are frequent [59,60]. This can lead to situations, as in WCR, in which recently introduced populations display large neutral genetic differentiation and will eventually merge during their geographical expansion. This evolutionary scenario, that allows estimation of dispersal through clinal analysis, probably occurred in the case of the green crab Carcinus maenas in the north Eastern American coast [60,61]. It also probably occurred in the plant pathogenic fungus Mycosphaerella fijiensis in Africa [62], and is currently occurring in the Asian ladybeetle Harmonia axyridis in North America [51,63]. Even though biological situations allowing such dispersal estimation are abundant, the literature provides no example of such studies to our knowledge. As discussed by Ciosi et al. [6] this lack may result from a simple difficulty: The rapid spatial spreading, the late observation and the late sampling of the invasive populations likely result in the observation of a single genetically homogenized population, with no observable frequency clines.

In cases of selection acting against hybrids, the clines may remain stable over time. The sampling of WCR in Northern Italy in consecutive years, followed by a temporal analysis of cline shape (slope and width), may thus provide information about the balance between dispersal and selection [64–66] in this pest species.

Evolutionary Implications of the Existence of a WCR Admixture Zone in Northern Italy

We found that genetic variation was greater in the zone of admixture than in the parental outbreak areas. Moreover, the genetic variation within the zone of admixture is approaching that of the Northern USA source population and thus displays substantial restoration of the genetic variation lost during the introduction and establishment of the invasive outbreaks. Indeed, the mean allelic richness and the mean heterozygosity of the zone of admixture represent 70% and 77% of that of the Northern USA source population, respectively, whereas the NW Italian, the NE Italian and CSE Europe outbreaks display a mean of 59, 41 and 63%, of the allelic richness and 64, 47 and 76% of the heterozygosity found in the Northern USA, respectively (see more details in the Figure S2). This study and previous population genetics studies of European populations of WCR provide information about the way in which this increase occurred, because it has been possible to observe (i) a loss of genetic variation during the founding of most of independently introduced populations [6,12], and (ii) admixture between the outbreak populations leading to a partial restoration of genetic variation (this study). An increase in neutral genetic variation was observed, but our results provide no information about differences in the phenotypic variability and fitness of WCR from the zone of admixture and from the parental areas. Recent invasion studies have reported example of a positive effect of admixture on the invasive capacity of animals such as the Asian ladybird Harmonia axyridis [67] and the freshwater snail Melanoides tuberculata [8], and of plants [3], such as Silene vulgaris [68]. In WCR, further quantitative genetics studies of life history traits (such as fertility, longevity and dispersal) are required to determine whether admixture has been and is currently an advantage for the invasion of Europe by this species.

Supporting Information

Figure S1 Graphical representation of the competing scenarios used for the ABC analyses on our European dataset. Scenarios (i), (ii) and (iii) represent the “simple origin” scenarios, scenarios (iv), (v) and (vi) are “single admixture” scenarios and (vii), (viii) and (ix) are “double admixture” scenarios. Historical and demographic parameters were identical for all
introduction models, Time 0 is the present and represents the year of the study (2010). The Veneto population was founded \( t_2 \) generations before the present, had an effective number of founders \( N_{F_{ Veneto}} \) with the population remaining at this size for \( BD_2 \) generations (bottleneck duration) and then reached a larger stable effective population size \( N_{S_{ Veneto}} \). The putative source populations, the Central and South-Eastern European (CSE Europe) and North-West (NW) Italian outbreak populations, diverged from the USA population \( t_4 \) and \( t_5 \) generations ago with an effective number of founders \( N_{F_{CSE}} \) and \( N_{F_{NWIta}} \), bottleneck durations \( BD_4 \) and \( BD_5 \) and stable effective population sizes \( N_{S_{CSE}} \) and \( N_{S_{NWIta}} \), respectively. The North-East (NE) Italian population was founded \( t_2 \) generations ago from the CSE European population, with an effective number of founders \( N_{F_{NEItaly}} \), a bottleneck duration \( BD_2 \) and an effective population size \( N_{S_{NEItaly}} \). When admixture occurs, the admixture rates \( ar \) and \( 1-ar \) are the genetic contribution of each of the source populations to the origin of the Veneto population. An unsampled population called the “Ghost population” was included into the analysis to allow “double admixture” scenarios (scenario (vii), (viii) and (ix)) between NE and NW Italy and CSE outbreak populations. This “Ghost population” is the result of a single admixture between two of the three putative source populations (the populations involved depend on the scenario), \( t_G \) generations ago (\( t_G > t_2 \) and \( t_G < t_2 \)), with an effective number of founders \( N_{F_{GhostPopulation}} \), a bottleneck duration \( BD_G \) and an effective population size \( N_{S_{GhostPopulation}} \). The rates of admixture corresponding to the “Ghost population” are \( ar_G \) and \( 1-ar_G \). For all models, populations were assumed to be isolated from each other, with no exchange of migrants.

References

1. Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molodky J, et al. (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305–332.
2. Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that Drive Evolutionary Change: insights from species introductions and invasions. In: Sax DF, Stachowicz JJ, Gaines SD, editors. Species Invasions: Insights into Ecology, Evolution and Biogeography. Sunderland, MA USA: Sinauer Associates Inc. 229-257.
3. Elstrand NC, Schiereneck KA (2008) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America 97: 7043–7050.
4. Facon B, Pointier JP, Jarne P, Sarda V, David P (2000) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Current Biology 10: 363–367.
5. Kolbe JJ, Gisler RE, Schetino LRG, Lara AC, Larson A, et al. (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177–181.
6. Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A, et al. (2008) Invasion of Europe by the western corn rootworm, Diabrotica virgifera virgifera: multiple transatlantic introductions with various reductions of genetic diversity. Molecular Ecology 17: 3614–3627.
7. Cheres MT, Miller JP, Crane JR, Knapp SJ (2000) Genetic distance as a predictor of heterosis and hybrid performance within and between heterotic groups in sunflower. Theoretical and Applied Genetics 100: 809-894.
8. Facon B, Pointier JP, Jarne P, David P (2003) Hybridization and invasiveness in the freshwater snail Melanoides tuberculata: hybrid vigour is more important than increase in genetic variance. Journal of Evolutionary Biology 16: 524–533.
9. Reif JC, Melchinger AE, Xia XC, Warburton ML, Housington DA, et al. (2003) Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Science 43: 1275–1282.
10. Facon B, Huflauser RA, Tayeh A, Loiseau A, Lambart E, et al. (2011) Inbreeding Depression Is Purged in the Invasive Insect Harmonia axyridis. Current Biology 21: 424–427.
11. Pajol B, Zhou SR, Vilas JA, Pannell JR (2009) Reduced inbreeding depression after species range expansion. Proceedings of the National Academy of Sciences of the United States of America 106: 13379–13383.
12. Miller N, Estoup A, Toepfer S, Bourguet D, Lapchin L, et al. (2005) Multiple transatlantic introductions of the western corn rootworm. Science 310: 992.
13. Branson TF, Krysan JL (1981) Feeding and oviposition behavior and life cycle strategies of Diabrotica: an evolutionary view with implications for pest management. Environmental Entomology 10: 826–831.
14. Smith RF (1966) Distributional patterns of selected western North American insects: the distribution of diabroticites in western North America. Bulletin of Entomological Society of America 12: 108–110.
15. Krysan JL, Smith RF (1987) Systematics of the virgifera species group of Diabrotica (Coleoptera: Chrysomelidae: Galerucinae). Entomology 5: 375–404.
16. Chiang HC (1973) Bioinomics of the northern and western corn rootworms. Annual Review of Entomology 18: 47–72.
17. Gray ME, Sappington TW, Miller NJ, Moeser J, Behn MO (2009) Adaptation and successfulness of Western Corn Rootworm: Intensifying Research on a Worsening Pest. Annual Review of Entomology 54: 303–321.
18. Kiss J, Edwards CR, Berger HK, Cate P, Cane M, et al. (2005) Monitoring of western corn rootworm (Diabrotica virgifera virgifera LeConte) in Europe 1992–2003. Western Corn Rootworm: Ecology and Management: 29–39.
19. De Luigi V, Furlan L, Palmieri S, Vettorazzo M, Zanini G, et al. (2011) Results of WCR monitoring plans and evaluation of an eradication programme using GIS and Indicator Kriging. Journal of Applied Entomology 135: 30–46.
20. Edwards CR, Kiss J (2008) New WCR 2007 General Spread Map for Europe. http://extensionentmpurdueedu/wcr/.
21. Edwards CR, Kiss J (2009) New WCR 2008 General Spread Map for Europe. IWGO Newsletter 29.
22. Edwards CR, Kiss J (2010) New WCR 2009 General Spread Map for Europe. IWGO Newsletter 30.
23. Summucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution 13: 510–524.
24. Kim KS, Stolz U, Miller NJ, Waits ER, Guillenaud T, et al. (2008) A core set of microsatellite markers for Western corn rootworm (Coleoptera: Chrysomelidae) population genetics studies. Environ Entomol 37: 293–300.
25. Miller NJ, Ciosi M, Sappington TW, Ratchiffe ST, Spencer JL, et al. (2007) Genome scan of Diabrotica virgifera virgifera for genetic variation associated with crop rotation tolerance. Journal of Applied Entomology 131: 378–385.
26. Kim KS, Sappington TW (2005) Polymorphic microsatellite loci from the western corn rootworm (Insecta: Coleoptera: Chrysomelidae) and cross-amplification with other Diabrotica spp. Molecular Ecology Notes 5: 115–117.
27. Nei M (1987) Molecular Evolutionary Genetics. New York: Columbia University Press.
28. Pay S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, et al. (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95: 536–539.
29. Raymond M, Rouset F (1995) Genepop (version 1.2), a population genetics software for exact tests and ecumennism. Journal of Heredity 86: 248–249.
30. Rouset F (2008) GENEPOP 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8: 103–106.
31. Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of microsatellite markers. Conservation Biology 12: 844–855.
32. Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.5). Updated from Goudet (1995).
33. Sokal RR, Rolf FF (1995) Biometry. The Principles and Practice of Statistics in Biological Research. New York: W.H. Freeman and Company.
34. Weir BS, Cockerham CC (1984) Estimating $\theta$ and $\theta_W$. Genetics 108: 19–30.
35. Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49: 1385–1392.
36. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological 57: 289–300.
37. Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–955.
38. Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Loci linked and correlated allele frequencies. Genetics 164: 1567–1585.
39. Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9: 1322–1332.
40. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.
41. Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4: 361–370.
42. SAS Institute Inc. (2008) SAS. 9.1.3 ed. Cary, NS, USA. pp. Statistical Analysis.
43. Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137–138.
44. Choisy M, Franck P, Cornuet JM (2004) Estimating admixture proportions with Approximate Bayesian computation. Bioinformatics 20: 2713–2719.
45. Beaumont MA, Zhang WY, Balding DJ (2002) Approximate Bayesian computation in population genetics. Genetics 162: 2025–2035.
46. Cornuet JM, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). Bmc Bioinformatics 11.
47. Cornuet JM, Santos F, Beaumont MA, Robert CP, Marin JM, et al. (2008) Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. Bioinformatics 24: 2713–2719.
48. Estoup A, Lombaert E, Marin JM, Guillemaud T, Puello P, et al. (2012) Estimation of demo-genetic model probabilities with Approximate Bayesian Computation using linear discriminant analysis on summary statistics. Molecular Ecology Resources 12: 846–855.
49. Guillemaud T, Beaumont MA, Ciosi M, Cornuet JM, Estoup A (2010) Inference introgression routes of invasive species using approximate Bayesian computation on microsatellite data. Heredity 104: 88–99.
50. Guillemaud T, Beaumont MA, Ciosi M, Cornuet JM, Estoup A (2010) Inference introgression routes of invasive species using approximate Bayesian computation on microsatellite data. Heredity 104: 88–99.
51. Lombaert E, Guillemaud T, Cornuet JM, Malassa T, Facon B, et al. (2010) Bridgehead Effect in the Worldwide Invasion of the Biocontrol Harlequin Ladybird. PLoS ONE 5.
52. Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Molecular Ecology 11: 1591–1604.
53. Ciosi M, Miller NJ, Toepfer S, Estoup A, Guillemaud T (2011) Stratified dispersal and increasing genetic variation during the invasion of Central Europe by the western corn rootworm, Diabrotica virgifera virgifera. Evolutionary Applications 4: 54–70.
54. Baufeld P, Enzian S (2001) Simulations model for spreading scenarios of western corn rootworm (Diabrotica virgifera virgifera) in case of Germany. IWGO Newsletter 22: 14–15.
55. Endler JA (1977) Geographic Variation, Speciation, and Clines. Monographs in population biology. New Jersey: Princeton University Press. 30–96.
56. Barton NH, Gale KS (1993) Genetic analysis of hybrid zone. In: Harrison RG, editor. Hybrid zones and the evolutionary process. New York: Oxford University Press. 13–45.
57. Gay L, Crochet PA, Bell DA, Lenormand T (2008) Comparing Clines on Molecular and Phenotypic Traits in Hybrid Zones: a Window on Tension Zone Models. Evolution 62: 2789–2906.
58. Saccheri IJ, Rouset F, Watts PC, Brakefield PM, Cook LM (2008) Selection and gene flow on a diminishing cline of melanic peppered moths. Proceedings of the National Academy of Sciences of the United States of America 105: 16212–16217.
59. Boudzor O, Augé H, Lafuma I, Rogers WE, Stiermann E, et al. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144: 1–11.
60. Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends in Ecology & Evolution 22: 454–464.
61. Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader’s range. Proceedings of the Royal Society B-Biological Sciences 273: 2453–2459.
62. Rieux A, Halkett F, de Bellaire LD, Zapater MF, Rouset F, et al. (2011) Inferences on pathogenic fungus population structures from microsatellite data: new insights from spatial genetics approaches. Molecular Ecology 20: 1661–1674.
63. Lombaert E, Guillemaud T, Thomas CE, Handley JL, Li J, et al. (2011) Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird Harmonia axyridis. Molecular Ecology 20: 4654–4670.
64. Barton NH, Hewitt GM (1985) Analysis of Hybrid Zones. Annual Review of Ecology and Systematics 16: 113–146.
65. Mallet J, Barton N (1989) Inference From Clines Stabilized by Frequency-Dependent Selection. Genetics 122: 967–976.
66. Slatkin M (1973) Gene flow and selection in a cline. Genetics 73: 733–756.
67. Turgeon J, Tsuy, A, Facon B, Lombaert E, De Clercq P, et al. (2011) Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (Harmonia axyridis) involved in the European invasion. Journal of Evolutionary Biology 24: 1044–1052.
68. Keller SR, Taylor DR (2010) Genomic admixture increases fitness during a biological invasion. Journal of Evolutionary Biology 23: 1720–1731.