The fast invasion of Europe by the box tree moth: an additional example coupling multiple introduction events, bridgehead effects and admixture events

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Abstract Identifying the invasion routes of non-native species is crucial to understanding invasions and customizing management strategies. The box tree moth, *Cydalima perspectalis*, is native to Asia and was recently accidentally introduced into Europe as a result of the ornamental plant trade. Over the last 15 years, it has spread across the continent and has reached the Caucasus and Iran. It is threatening *Buxus* trees in both urban areas and forests. To investigate the species’ invasion routes, native and invasive box tree moth populations were sampled, and moth’s genetic diversity and structure were compared using microsatellite markers. Our approximate Bayesian computation analyses strongly suggest that invasion pathways were complex. Primary introductions originating from eastern China probably occurred independently twice in Germany and once in the Netherlands. There were also possibly bridgehead effects, where at least three invasive populations may have served as sources for other invasive populations within Europe, with indication of admixture between the two primary invasive populations. The bridgehead populations were likely those in the countries that play a major role in the ornamental plant trade in Europe, notably Germany, the Netherlands, and Italy. All these invasion processes likely facilitated its fast expansion across Europe and illustrate the role played by the ornamental plant trade not only in the moth’s introduction from China but also in the species’ spread across Europe, leading to an invasion with a complex pattern.

Keywords *Cydalima perspectalis* · Approximate Bayesian computation · Random forest method · Invasion pathways · Human-mediated dispersal · Ornamental plant trade
Introduction

It is well known that human activities result in the transportation and introduction of non-native species across the world (Blackburn et al. 2011; Essl et al. 2015; Gippet et al. 2019). Insects represent a large share of these new introductions and the rate at which such insect species are being introduced is still climbing (Seebens et al. 2017; Bonnamour et al. 2021). In Europe, most of the non-native insects that have entered and established themselves since the 1990s are spreading faster than ever across the continent (Roques et al. 2016). The liberalization of commercial policies and travel in Europe, and the subsequent dismantling of border checkpoints within the European Union (EU), have facilitated trade, especially that of ornamental plants. These changes may have also simultaneously contributed to the dispersal of non-native species across Europe (Roques et al. 2016). Understanding the invasion pathways of non-native insect species is a crucial part of identifying the factors driving invasions (Allendorf and Lundquist 2003; Bock et al. 2015). The knowledge gathered will help identifying trends on invasion pathways underlying faster spread observed for insects introduced accidentally (Sakai et al. 2001; Essl et al. 2015; Roques et al. 2016; Fahrner and Aukema 2018).

Human-mediated invasions often involve complex pathways (Garnas et al. 2016; Meurisse et al. 2019). To better understand invasion pathways, it is important to characterize the biology and ecology of non-native species, to consider when they first appeared in their invasive ranges, and to examine any customs records of their interception (Bertelsmeier et al. 2018), including the commercial products in which they occurred (Essl et al. 2015; Brockerhoff and Liebhold 2017). However, these data can be misleading, sparse, incomplete, or absent, making it difficult to trace invasion histories. In this context, molecular resources can help fill in the gaps (Estoup & Guillemaud, 2010; Lawson et al. 2011). Microsatellite markers can reveal whether the invasion process was characterized by certain common phenomena, such as bottlenecks, multiple introductions, or admixture events (Estoup and Guillemaud 2010; Lawson Handley et al. 2011; Bock et al. 2015; Dlugosch et al. 2015). Microsatellite markers can also highlight bridgehead effects, which occur when a primary invasive population serves as the source for secondary invasive populations (Lombaert et al. 2010; Lawson Handley et al. 2011). That said, it is not always straightforward to infer invasion routes using molecular data because appropriate analytical methods are often lacking to obtain detailed information (i.e., population source, number of independent introductions), as shown in previous studies (Guillemaud et al. 2010; Cristescu 2015). Luckily, approximate Bayesian computation (ABC) is an effective method for dealing with complex scenarios. It is a powerful tool that combines the use of simulated datasets and summary statistics (Beaumont et al. 2002; Csilléry et al. 2010; Bertorelle et al. 2010; Beaumont 2010), and it is a particularly good analytical fit for research on non-native species (e.g., Lombaert et al. 2010; van Boheemen et al. 2017; Fraimout et al. 2017; Lippens et al. 2017; Lesieur et al. 2019). Indeed, in the case of recently established invasive populations, geographical and historical information can be used to define and assess hypothetical invasion scenarios in order to infer introduction route(s) (Estoup and Guillemaud, 2010). Furthermore, ABC can be used to analyze additional datasets and to estimate demographic, historical, and genetic parameters (Guillemaud et al. 2010). This method was used to investigate the possible invasion routes of certain non-native insects (e.g., Ryan et al. 2019; Mutitu et al. 2020), including species that were observed spreading quickly across Europe (Roques et al. 2016) like the Harlequin ladybird, Harmonia axyridis (Lombaert et al. 2010, 2014a, b), the spotted wing drosophila, Drosophila suzukii (Fraimout et al. 2017), and the western conifer seed bug, Leptoglosus occidentalis (Lesieur et al. 2019). In these three cases, researchers observed that invasion dynamics and dispersal processes were complex, involving multiple introductions, admixture events, and bridgehead effects. However, such studies remain scarce, especially for species associated with the ornamental plant trade, which is one of the current major pathways for the introduction of non-native insects (Kenis et al. 2007; Roques 2010; Liebhold et al. 2016; Eschen et al. 2017).

The box tree moth (BTM; Cydalima perspectalis (Walker), Lepidoptera: Crambidae) is native to China, Japan, and Korea (Maruyama and Shinjaki 1987; Xiao et al. 2011; Kim and Park 2013). It is a multivoltine species that produces two to four generations per year in its invaded range (Nacarro et al. 2014; Göttig and Herz 2017). In the invaded range, damage
has been recorded only on *Buxus* species (Wan et al. 2014; Matošević et al. 2017; Ferracini et al. 2022). In its native range, the insect has been reported to develop on several *Buxus* species, and three other host plants (*Ilex purpurea, Euonymus alatus* and *E. japonicus*) (Wan et al. 2014). However, the use of these three plants as host by BTM has recently been debated. Several performance experiments done with invasive populations showed that BTM larvae were only able to develop on *Buxus* species, suggesting that the moth is an herbivorous specialist of this plant genus (Brua 2014; Matošević et al. 2017; Ferracini et al. 2022). In Asia, approximately 40 native species of *Buxus* occur, among which at least 15 species are found in China (Kohler and Brückner 1989; Fang et al. 2011). In contrast, only up to 4 species, including the widespread *B. sempervirens*, are naturally present in Europe, the Caucasus and Iran (Di Domenico et al. 2012; Mitchell et al. 2018). These plants usually consist of shrubs that are naturally found in forest stands. Additionally, their slow growth pattern has developed gardeners’ interest leading to a popular use as ornamental trees in urban environments.

BTM was accidentally introduced into Europe from Asia, likely as a result of the ornamental plant trade (Kenis et al. 2013). The species was first detected in 2007 in the German cities of Weil am Rhein and Kehl (state of Baden-Württemberg) (Krüger 2008). That same year, invasive populations also appeared in nearby cities in Switzerland (Leuthardt et al. 2010), as well as in plant nurseries in the Netherlands (Van der Straten and Muus 2010). Then, between 2007 and 2010, BTM was observed in several countries of western Europe, namely France (Feldtrauer et al. 2009), Belgium (Casteels et al. 2011), Italy (Bella 2013), and the United Kingdom (Salisbury et al. 2012). In the years that followed, the moth appeared in central and southeastern Europe (e.g., Croatia [Koren & Crne, 2012]; Hungary [Sáfián & Horváth, 2011]; and Greece [Strachinis et al. 2015]) as well as in Turkey [Hizal et al. 2012]; southern Russia [Gninenko et al. 2014]; Georgia [Matsiakh et al. 2018] and Iran (Ahangaran 2016). At present, BTM has invaded more than 30 Eurasian countries (Bras et al. 2019), where it is threatening several *Buxus* species (Mitchell et al. 2018) both in urban and forest stands (Ferracini et al. 2022). It has been observed for the first time in 2018 in Canada (Frank 2019) and was also intercepted several time in the USA on nursery plants shipped from Canada in 2020 and 2021 (USDA-APHIS 2021).

In Europe, *Buxus* trees are widely used as ornamental plants, especially *B. sempervirens*, in public and private gardens, which results in their strong commercial trade (Kenis et al. 2018; Matošević, 2013; EPPO, 2012). It has been hypothesized that the *Buxus* tree trade between China and Europe led to the insect’s arrival in Europe (Leuthardt et al. 2010; Casteels et al. 2011; Nacambo et al. 2014). Results from a study analyzing DNA sequences from native and invasive BTM populations suggest that the moth may have been introduced from eastern China on several occasions (Bras et al. 2019). Further evidence for the occurrence of multiple introductions is enhanced by the geographical disparity in the records of the moth’s first appearance in 2007. Indeed, recently introduced populations were found simultaneously in Germany, the Netherlands, and Switzerland (Krüger 2008; Leuthardt et al. 2010; Van der Straten and Muus 2010). Moreover, the moth’s population genetic structure in Europe (Bras et al. 2019) suggests that the species may have spread across the continent as a result of the ornamental plant trade among European countries (Kenis et al. 2013; Matošević, 2013). Indeed, several lines of evidence indicate that humans may have facilitated BTM’s dispersal: (1) the species was intercepted in the Netherlands (EPPO, 2012), a major player in the horticulture industry (Eschen et al. 2017); (2) it was first observed in plant nurseries (Van der Straten and Muus 2010; Casteels et al. 2011; Salisbury et al. 2012); and (3) these initial records were associated with extremely disparate locations. However, because detailed data on the trade of specific plant genera are unavailable for many countries (Eschen et al. 2017), it has been challenging to identify the import/export routes of *Buxus* trees in Europe. In general, it has been difficult to trace both the pathways making up the ornamental plant trade into Europe (Dehnen-Schmutz et al. 2010) and the invasion routes of the pests introduced as a result, especially those of species not included in the EPPO alert list, such as BTM (Strachinis et al. 2015).

In this study, we aimed to characterize BTM’s invasion routes into and across Europe. We assessed the evidence for the existence of multiple introductions, namely those hypothesized by Bras et al. (2019), as well as for bridgehead effects and admixture events, given that all these processes could have contributed
to the species’ successful introduction and fast expansion. Because historical records of documented introductions are scarce, we employed genetic methods (as recommended by Estoup & Guillemaud, 2010). First, we sampled and genotyped individuals from BTM’s native and invaded ranges. Second, we used ABC to infer the species’ invasion routes across Europe.

Materials and methods

Sampling

Individual box tree moths were collected at 37 locations in the species’ native and invaded ranges between 2012 and 2017 (Table 1). We preferentially sampled larvae and pupae, which were collected from different Boxus trees to minimize the likelihood of sampling closely related individuals (i.e. from the same mating pair). When this was not possible, we used pheromone traps to collect adults. We tried to obtain at least 20 specimens per location, but samples sizes were smaller at 9 sites (Table 1). All specimens were stored in 96% alcohol at -21 °C to preserve their DNA.

In BTM’s native range, 13 populations were successfully sampled. The sampling effort covered most of BTM’s putative range (Supplementary Material Figure S1). But no moths could be found in Japan or in China’s southern provinces of Guangdong, Guangxi, and Fujian despite the species having been recorded there (Kawazu et al. 2007; Bras et al. 2019). In other parts of China and in South Korea, larvae and pupae were mostly collected in urban areas where Boxus trees (mostly B. microphylla) have been planted for ornamental purposes. One exception occurred in Fuyang (N-FU, Zhejiang province, China; see Table 1 for population codes), where moths were obtained from Boxus trees planted in a sentinel plant nursery established on the border between farmland and a natural forest (Kenis et al. 2018).

In BTM’s invaded range, samples were obtained from 13 countries in Europe and Eastern Black Sea region. A total of 24 populations were sampled (Table 1; Supplementary Material Figure S2). We sampled at locations where the moth was first observed in Europe in 2007, namely Weil Am Rhein (I-WAR) and Kehl (I-KE) in Germany and Giessen (I-GI) in the Netherlands. Only larvae and pupae were collected, except at Legnaro (I-LE), Italy, where adults were caught using pheromone traps. All the samples were obtained from man-made habitats, with the exception of those for the Bzyb Valley (I-BV) and Mitrala Park (I-MNP) in Georgia and Solokhail (I-SO) in Russia, where insects were collected in natural forests of Boxus colchica.

Genotyping

All the specimens were dissected prior to DNA extraction. Using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), DNA was extracted from either the thoracic muscles, in the case of the larvae and pupae, or a leg, in the case of the adults. All vouchers are stored at −21 °C at the URZF INRAE laboratory in Orléans, France. The DNA was diluted with ultrapure water when its concentration exceeded 50 ng/µL. Fifteen microsatellite markers developed by Bras et al. (2018) were used to genotype individuals from both the native and invaded ranges with the same multiplex PCR sets. PCR amplification and allele scoring were performed as described in Bras et al. (2018). In total, we genotyped 303 individuals from the native range and 593 individuals from the invaded range.

Genetic analyses: genetic diversity and population structure

In a first step, we assessed the genetic diversity and the relationship between populations both from the native and invaded ranges. The software Arlequin v. 3.5 (Excoffier and Lischer 2010) was used to calculate mean allele number (An), observed heterozygosity (Ho), expected heterozygosity (He), and to test for significant deviation from Hardy–Weinberg proportions (HW), and linkage disequilibrium (LD). To deal with multiple pairwise comparisons between loci, a sequential Bonferroni correction (Rice 1989) was applied to the results for both HW and LD. Null alleles at each locus were estimated using FreeNA (Chapuis and Estoup 2007). Allelic richness (AR) for each population was calculated based on the minimum sample size of 14 individuals using FSTAT v. 2.9.3 (Goudet 1995). FreeNA software was also used to calculate pairwise FST values using the excluding null allele correction method. We reconstructed a population-level neighbor-joining tree using
Table 1: Sampling information and genetic diversity of the native and invasive box tree moth (Cydalima perspectalis) populations

| Pop code | Country | Province | Population | GPS coordinates | Sampling year | First obs | N | AR | Ho | He |
|----------|---------|----------|------------|----------------|--------------|-----------|---|----|----|----|
| N-HU     | China   | An      | Hu He      | 33°57.216'N; 116°44.537'E | 2017          | –         | 30 | 3.57 | 4.46 | 0.56 |
| N-BE     | Beijing | Beijing  | Beijing    | 39°57.424'N; 116°17.407'E | 2010–2017     | –         | 20 | 4.32 | 5.39 | 0.51 |
| N-GU     | Gucheng | Gucheng  | Gucheng    | 26°27.590'N; 116°40.957'E | 2017          | –         | 18 | 4.10 | 4.35 | 0.51 |
| N-NA     | Nanjing | Nanjing  | Nanjing    | 25°27.464'N; 114°55.521'E | 2017          | –         | 24 | 4.84 | 5.13 | 0.48 |
| N-SHE    | Shenyang | Shenyang | Shenyang   | 32°57.277'N; 118°47.514'E | 2007          | –         | 24 | 4.35 | 4.73 | 0.43 |
| N-TA     | Tianan  | Tianan   | Tianan     | 36°11.183'N; 121°51.280'E | 2012–2017     | –         | 24 | 3.85 | 4.57 | 0.49 |
| N-SHA    | Shanghai | Shanghai | Shanghai   | 31°13.817'N; 121°52.451'E | 2010          | –         | 30 | 4.02 | 5.08 | 0.47 |
| N-CH     | Sichuan | Sichuan  | Sichuan    | 29°51.168'N; 118°32.481'E | 2016          | –         | 24 | 4.20 | 5.00 | 0.47 |
| N-NJ     | Nanjing | Nanjing  | Nanjing    | 31°25.930'N; 119°37.514'E | 2017          | –         | 20 | 4.16 | 4.92 | 0.51 |
| N-XI     | Xian    | Xian     | Xian       | 38°57.216'N; 116°32.451'E | 2016          | –         | 20 | 4.16 | 4.92 | 0.51 |
| N-SH     | Shenyang | Shenyang | Shenyang   | 32°57.464'N; 114°55.521'E | 2017          | –         | 20 | 4.16 | 4.92 | 0.51 |
| N-ZH     | Zhejiang | Zhejiang | Zhejiang   | 29°51.168'N; 118°32.451'E | 2016          | –         | 20 | 4.16 | 4.92 | 0.51 |
| I-GH     | Belgium | Flanders | Ghent      | 51°2.233'N; 4°19.525'E | 2015          | 10        | 2.86 | 3.31 | 0.41 |
| I-ZA     | Croatia | Croatia  | Zagreb     | 49°12.413'N; 16°31.925'E | 2014          | 10        | 2.86 | 3.31 | 0.41 |
| I-EC     | Czech Republic | Brno | 46°52.453'N; 19°31.925'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
| I-WR     | Germany | Germany  | Weimar     | 50°32.413'N; 11°31.925'E | 2017          | 10        | 2.86 | 3.31 | 0.41 |
| I-RH     | Netherlands | Groningen | 51°51.875'N; 10°7.527'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
| I-KA     | Greece  | Greece   | Katerini   | 40°16.423'N; 22°30.948'E | 2008          | 10        | 2.86 | 3.31 | 0.41 |
| I-BA     | Germany | Belgium  | Kehl       | 45°34.237'N; 11°30.948'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
| I-LE     | Italy   | Italy    | Legnano    | 46°34.251'N; 11°30.948'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
| I-NO     | Netherlands | Brussels | 51°51.875'N; 10°7.527'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
| I-NL     | Netherlands | Groningen | 51°51.875'N; 10°7.527'E | 2016          | 10        | 2.86 | 3.31 | 0.41 |
Population v. 1.2.32 (http://bioinformatics.org/~tryphon/populations/) and Cavalli-Sforza and Edwards chord distances. A discriminant analysis of principal components (DACP) (Jombart et al. 2010) was employed to preliminarily explore the genetic structure of the dataset; the adegenet package (Jombart 2008) implemented in R software (R Development Core Team 2008) was used.

To further explore the dataset’s genetic structure and to identify its potential number of genetic clusters, we used a Bayesian clustering approach implemented in STRUCTURE (Pritchard et al. 2000). Three independent STRUCTURE analyses were performed using (i) the data from all the specimens; (ii) the data from the native populations (eastern Asia); and (iii) the data from the invaded populations (Europe, Eastern Black Sea region). Parameter values were estimated using an admixture model where allele frequencies were correlated among populations. For each analysis, 15 replicate runs were performed for each value of cluster number (K). Every run comprised a burn-in period of 200,000 MCMC iterations that was followed by 1,000,000 iterations. For the full dataset and the dataset for the invasive populations, K was set to range between 1 and 15. For the dataset for the native populations, K was set to range between 1 and 10. The uppermost level of population structure was estimated for each analysis using the ΔK method (Evanno et al. 2005), which was implemented in STRUCTURE Harvester (Earl and vonHoldt 2011). For each K, the mode among runs was checked using the Greedy algorithm in Clumpp (Jakobsson and Rosenberg 2007) via the online pipeline CLUMPAK (Kopelman et al. 2015). Genetic structure was visualized using Distruct v. 1.1 (Rosenberg 2004).

Inferring invasion scenarios with approximate Bayesian computation

We used ABC (Beaumont et al. 2002) to infer BTM’s invasion routes into and across Europe. As ABC scenarios can be complex and thus difficult to assess (Estoup et al. 2012), we only used a selected number of native and invasive populations in our ABC analyses. More specifically, we focused on the populations that corresponded to the genetic clusters identified with STRUCTURE, as per Lombaert et al. (2014a, b).

The STRUCTURE analysis of the native populations revealed that each genetic cluster was...
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discriminate among scenarios more efficiently than traditional ABC methods, especially when complex invasion pathways are involved (see Fraimout et al. 2017). The ABC-RF method is a nonparametric, machine-learning tool which carries out the classification of hundreds of bootstrapped decision trees using summary statistics as predictor variables. It extracts the maximum of information from the entire set of summary statistics, avoiding choosing arbitrary a subset as required in other classical ABC methods. A “prior error rate” (PER) can also be estimated using the simulations that were not employed to build the trees. PER provides a direct means of cross validation. With DIYABC, we generated 10,000 microsatellite datasets per scenario, which we then used to grow a classification forest of 1000 trees. The scenario with the greatest number of votes was defined as being the most likely. When the number of votes was similar among several scenarios (i.e., scenarios differed by fewer than 50 votes), the two scenarios with the greatest numbers of votes were repeated using the ABC-RF method to verify the accuracy of scenario selection. We used the abcrf R package (Pudlo et al. 2016) to perform all the ABC-RF analyses. Finally, we inferred posterior probabilities (PPs) for the final scenario in the final analysis by performing Random Forest regression (Raynal et al. 2019) on classification forests of 1000 trees. Simultaneously, model checking was run with DIYABC to evaluate the fit between the most likely scenario and the observed dataset.

Table 2 Description of the scenario selection process used to infer the invasion routes of the box tree moth (Cydalima perspectalis) and results of each ABC analysis

| Scenarios compared | Population(s) considered | Potential source N-US; N-SE; N-SHA; N-LIJ | Admixture events | Scenario selected | NSS | PER (%) | PP | Number of votes |
|--------------------|--------------------------|------------------------------------------|-----------------|------------------|-----|---------|----|----------------|
| 4                  | I-WAR                    | N-US; N-SE; N-SHA; N-LIJ                 | –               | N-SHA is source  | 76  | 7.69    | 0.72 | 770            |
| 4                  | I-KE                     | N-US; N-SE; N-SHA; N-LIJ                 | –               | N-SHA is source  | 76  | 7.65    | 0.80 | 655            |
| 4                  | I-GI                     | N-US; N-SE; N-SHA; N-LIJ                 | –               | N-SHA is source  | 76  | 7.64    | 0.83 | 653            |
| 10                 | I-WAR; I-KE; I-GI        | N-SHA; I-WAR; I-KE; I-GI                 | –               | 3 independent introductions from N-SHA | 76  | 41.37   | 0.47 | 309            |
| 22                 | I-LU                     | N-US; N-SE; N-SHA; N-LIJ; I-LU; I-WAR; I-KE; I-LI | Native x Invasive; Invasive x Invasive | Admixture between I-WAR and I-LU | 301 | 29.46   | 0.47 | 222            |
| 30                 | I-BU                     | N-US; N-SE; N-SHA; N-LIJ; I-WAR; I-KE; I-GI; I-LU | Native x Invasive; Invasive x Invasive | Admixture between I-WAR and I-GI | 424 | 35.83   | 0.52 | 157            |
| 36                 | I-SO                     | N-US; N-SE; N-SHA; N-LIJ; I-WAR; I-KE; I-GI; I-LU; I-BU | Native x Invasive; Invasive x Invasive | Admixture between I-GI and I-LU | 576 | 37.59   | 0.49 | 250            |
| 36                 | I-BV                     | N-US; N-SE; N-SHA; N-LIJ; I-WAR; I-KE; I-GI; I-LU; I-BU | Native x Invasive; Invasive x Invasive | I-LU is source | 576 | 29.74   | 0.53 | 234            |

For each ABC analysis, we have indicated the number of scenarios compared; the invasive population under consideration; the potential source populations tested; signs of admixture events; the scenario selected based on the Random Forest (RF) method; the number of summary statistics used (NSS); the prior error rate (PER); the posterior probability (PP) estimated by the RF method; and the number of RF votes for 1000 simulated trees.
Table 3  Historical, demographic, and genetic parameters used to assess box tree moth (Cydalima perspectalis) invasion scenarios via ABC analysis

| Parameter                                                                 | Abbreviation | Prior distribution               |
|-------------------------------------------------------------------------|--------------|----------------------------------|
| Stable effective population size                                        | \( N_i \)    | Log-uniform [100; 100000]        |
| Effective population size at founding                                    | \( N_{inf_i} \) | Log-uniform [2; 100]             |
| Bottleneck duration                                                      | \( D_{Bi} \)  | Uniform [0; 21]                  |
| Introduction year: 2007                                                  | \( t_{WAR} \), \( t_{KE} \), \( t_{GI} \) | Uniform [30; 51]                |
| Introduction year: 2010                                                  | \( t_{LU} \)  | Uniform [21; 42]                 |
| Introduction year: 2011                                                  | \( t_{BU} \)  | Uniform [18; 39]                 |
| Introduction year: 2012                                                  | \( t_{SO} \)  | Uniform [15; 36]                 |
| Introduction year: 2015                                                  | \( t_{BV} \)  | Uniform [6; 27]                  |
| Time to merge event between N-SHA and I-WAR                              | \( t_{GH-W} \) | Uniform [30; 1000]               |
| Time to merge event between N-SHA and I-KE                               | \( t_{GH-K} \) | Uniform [30; 1000]               |
| Time to merge event between N-SHA and I-GI                               | \( t_{GH-G} \) | Uniform [30; 1000]               |
| Time since population divergence                                        | \( t_{N-SHA} \), \( t_{N-SE} \), \( t_{N-SHA} \), \( t_{N-LIJ} \) | Uniform [100; 50000]            |
| Demographic event in the native range                                   | \( N_{sup} \) | Uniform [100; 100000]            |
| Admixture coefficient                                                   | \( ra_{LU} \), \( ra_{BU} \), \( ra_{SO} \) | Uniform [0.1; 0.9]               |
| Mutation parameters for the microsatellite markers                       | \( \mu \)     | Uniform [1E-5; 1E-3]             |
|                                                                        | \( P \)       | Uniform [1E-1; 3E-1]             |
|                                                                        | \( SNI \)     | Log-uniform [1E-8; 1E-5]         |

The parameters \( N_{inf_i} \) and \( D_{Bi} \) are associated with the invasive populations. Times (t) are expressed in numbers of past generations with a year being equal to three generations. The microsatellite loci were assumed to follow a generalized stepwise mutation model and were characterized using three parameters: the mutation rate (\( \mu \)), the mean of the geometric distribution of mutation length (\( P \)), and the mean mutation rate for a single nucleotide insertion (\( SNI \)). Note that for \([x; y]\), x and y are the bounds of the prior distributions. Assumptions: \( N_{sup} > N_{N-CUS} \), \( t_{N-SHA} > t_{I-GH-W} \), \( t_{N-SHA} > t_{I-GH-K} \), \( t_{N-SHA} > t_{I-GH-G} \), \( t_{I-GH-W} > t_{I-WAR} \), \( t_{I-GH-K} > t_{I-KE} \), \( t_{I-GH-G} > t_{I-GI} \), \( t_{I-GI} > t_{I-LU} \), \( t_{I-WAR} > t_{I-LU} \), \( t_{I-WAR} > t_{I-BU} \), \( t_{I-GI} > t_{I-LU} \), \( t_{I-GI} > t_{I-LU} \), \( t_{I-GI} > t_{I-LU} \), and \( t_{I-LU} > t_{I-BV} \).

Results

Genetic diversity of the box tree moth

Overall, we genotyped 896 individuals from 37 sampling sites. Genotyping success was high (98.85%), and there was a mean of 8.07 alleles per locus. In this full dataset, six cases of linkage disequilibrium were found after Bonferroni correction. A given pair of loci never displayed significant linkage disequilibrium in more than one population. The fifteen microsatellite markers were thus considered to be independent.

After Bonferroni correction, three populations in the native range (N-BE, N-XI, N-HU; Table 1) and 12 populations in the invaded range (I-KE, I-GI, I-RH, I-LU, I-LE, I-BR, I-KA, I-NI, I-PA, I-LR, I-SA and I-CV; Table 1) had at least one locus that deviated significantly from Hardy–Weinberg proportions. When looking at Hardy–Weinberg proportions for each locus, only the locus BTM22 departed significantly from Hardy–Weinberg equilibrium in 11 populations (1 native population and 10 invasive populations). It also had a high frequency of null alleles (21%). For the other loci, mean frequencies of null alleles were less than or equal to 10%. We thus performed the analyses with and without BTM22 to determine whether inclusion of the locus introduced bias. Since the results were similar in both cases, we have reported the findings obtained with the full, fifteen-microsatellite dataset.

Mean allelic richness was higher in the native populations (4.23) than in the invasive populations (2.96) (Table 1). For the native populations, the ranges of observed heterozygosity (\( Ho \)) and expected heterozygosity (\( He \)) were 0.43–0.56 and 0.50–0.57, respectively. \( Ho \) and \( He \) were lower in the invasive populations, and their ranges were 0.33–0.48 and 0.28–0.49, respectively. For the full dataset, the range for pairwise \( F_{ST} \) was 0.01–0.38; it was smaller for native (0.01–0.14) than for invasive populations (0.01–0.38).
Among the native populations, the south Korean population N-SE had the highest $F_{ST}$ values (0.06–0.14) followed by the southern Chinese populations N-GU (0.06–0.12) and N-LI (0.05–0.13). Compared to the invaded range, $F_{ST}$ values in Asia were increasing when geographic distances between populations were larger (Suppl. Mat. Table S1).

**Population structure**

In the unrooted neighbor-joining tree, the invasive and native populations formed distinct groups (Suppl. Mat. Figure S4). The structure of this tree was consistent with the geographical distribution of the native populations. For the invasive populations, populations at the sites where the moth was first observed in 2007 (Weil Am Rhein, I-WAR; Kehl, I-KE; Giessen, I-GI) occurred in separate groups, and most of the invasive populations were part of the Giessen (I-GI) group. However, the low bootstrap values at most nodes made it difficult to draw any robust conclusions about the relationships between groups. The DAPC analysis yielded the same pattern, except that Katerini (I-KA) did not group with the other invasive populations (Suppl. Mat. Figure S5).

The STRUCTURE analysis results were consistent with those from the neighbor-joining tree, the DAPC analysis, and the F-statistics analyses (Fig. 1). At $K=2$ (selected via the $\Delta K$ method), all the native populations as well as the invasive populations of Weil Am Rhein (I-WAR), Kehl (I-KE), and Delémont (I-DE) belonged to the same genetic cluster. The second genetic cluster included all the other invasive populations, with the exception of Budapest (I-BU) and Paris (I-PA). These latter two populations showed signs of having arisen from admixture events between the two genetic clusters. At $K=3$, the native populations formed a distinct group from the invasive populations.

The analysis focusing on the native populations found evidence of genetic structure (Fig. 1, Suppl. Mat. Figure S6). The uppermost level of structure was $K=2$. South Korea and southwestern China were clearly differentiated from the other native populations. Populations in northern and eastern China were grouped together, and individuals from Xinyang (N-XI) and Lishui (N-LIS) were assigned to the first genetic cluster. At $K=3$, the grouping pattern was consistent with population geography. Populations from South Korea and southwestern China formed two distinct genetic clusters, while populations from northern and eastern China grouped together and were mixed in an unclear pattern.

In the analysis focusing on the invasive populations, $K=2$ was the optimal level of structure (Fig. 1, Suppl. Mat. Figure S6). The populations of Weil Am Rhein (I-WAR) and Kehl (I-KE) (Germany), Delémont (I-DE) (Switzerland), and Katerini (I-KA) (Greece) were clustered together. The second cluster contained all the other invasive populations. The Budapest (I-BU) population and three populations in France (Creuzier-le-vieux, I-CV; Paris, I-PA; and Nice, I-NI) displayed signs of having arisen from admixture events between the two genetic clusters. At $K=7$, Weil Am Rhein (I-WAR), Kehl (I-KE) and Delémont (I-DE) remained grouped together, while Katerini (I-KA) and three other invasive populations (Rherderbrug, Netherlands, I-RH; Zagreb, Croatia, I-ZA; and Paris, France, I-PA) formed individual clusters. The genetic pattern of the other invasive populations was less clear.

Assessing scenarios for the box tree moth’s invasion of Europe

The first set of ABC analyses examined the populations at the locations where BTM was first observed in Europe: Weil Am Rhein (I-WAR) and Kehl (I-KE) in Germany and Giessen (I-GI) in the Netherlands. First, we found that, in each of the ABC analyses, the most likely scenario was that eastern China served as the source for these populations (PER: ~7.6% and PP ranging from 0.72 to 0.83; Table 2, Fig. 2a). Second, when we inferred the relationships between these three invasive populations, the most likely scenario was that there were three independent introductions of the moth from eastern China into Germany (I-WAR; I-KE) and the Netherlands (I-GE) (PER: 41.37% and PP: 0.47). Consequently, in subsequent analyses, we treated eastern China as the source of the Weil Am Rhein (I-WAR) and Kehl (I-KE) populations in Germany and the Giessen (I-GI) population in the Netherlands.

The second set of ABC analyses focused on the invasive populations established after 2007, those in Lucca, Italy (I-LU), Budapest, Hungary (I-BU), Solokhual, Russia (I-SO), and the Bzyb Valley,
Georgia (I-BV) (Fig. 2B). The best scenario for Lucca (I-LU) was that population arose from admixture between the Weil Am Rhein (I-WAR) and Giessen (I-GI) populations (PER: 29.46% and PP: 0.47; Table 2). That said, there was a difference of less than 50 votes between this scenario and a scenario in which only the Giessen (I-GI) population served as its source. We therefore carried out an ABC-RF analysis using just these two scenarios, and the results supported the admixture event between...
Weil Am Rhein (I-WAR) and Giessen (I-GI) populations as the most likely scenario (PER: 9.13% and PP: 0.58). Consequently, this scenario was used in subsequent analyses. For Budapest (I-BU), the most likely scenario was that the population had arisen also from admixture between Weil Am Rhein (I-WAR) population and the Giessen (I-GI) population (PER: 33.56% and PP: 0.52). However, there was a difference of less than 50 votes between this latter scenario and two other scenarios: admixture occurred between the Shanghai (N-SHA) and Giessen (I-GI) populations or admixture occurred between the Shanghai (N-SHA) and Lucca (I-LU) populations. We used the same approach as with Lucca (I-LU), and the most likely scenario remained the occurrence of an admixture event between Weil Am Rhein (I-WAR) and Giessen (I-GI) (PER: 16.37% and PP: 0.62). Consequently, we used this scenario in subsequent analyses. Finally, we found an admixture event between Giessen (I-GI) and Lucca (I-LU) populations as the best scenario for Solokhaul (I-SO) population (PER: 34.98% and PP: 0.48%) whereas considering the alternative Bzyb Valley (I-BV) population, the scenario in which the Lucca (I-LU) population served as source was selected (PER: 27.21% and PP: 0.53; Fig. 2b). None of the ABC analyses resulted in highly probable scenarios where South Korea, southern China, or an unsampled population served as a source.

The posterior model checking performed on the final scenario of Solokhaul (I-SO) population and the alternative population, Bzyb Valley (I-BV), indicated that the selected model and posteriors fitted the observed genetic data (Figure S7). As the PER and PP values were better for the alternative Bzyb Valley (I-BV) population, we chose it to infer the posterior

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Fig. 2 Box tree moth (*Cydalima perspectalis*) invasion scenarios revealed by the ABC analyses. (A) Introduction pathways from Asia to Europe. (B) Dispersal pathways across the invaded range. Note: These figures depict the most likely invasion scenarios arising from the analyses. In Europe, countries where the box tree moth has been observed are colored in red. In Asia, the most likely source area of the box tree moth revealed by our results is colored in blue. The yellow stars indicate the first places where *C. perspectalis* was detected—in Germany and in the Netherlands in 2007 (Krüger 2008; Van der Straten and Muus 2010). The arrows indicate the most probable invasion pathways.
estimations of model parameters on the final scenario (Table S2).

Discussion

The observed genetic diversity and ABC results strongly support that the box tree moth (BTM) dispersed along complex invasion pathways as previously suggested by mtDNA diversity (Bras et al. 2019). We confirmed that C. perspectalis was introduced from Eastern China to Europe with at least three independent introductions in Germany and the Netherlands, two leader countries in ornamental plant trade. ABC simulations emphasize the likely role of both bridgehead, with at least three invasive populations serving as a source of colonists for remote new territories, and admixture events in the moth’s spread across the European invaded range. Our findings emphasize the role played by the ornamental plant trade, which has been an important factor promoting the establishment and spread of non-native phytophagous insects (Eschen et al. 2017; Kenis et al. 2007; Roques, 2010). It certainly appears to have contributed to BTM’s introduction and dispersal across Europe, The Caucasus and Iran.

New insights into the box tree moth’s introduction into Europe from China

Asia is one of the major sources of the non-native insects that have been introduced into Europe (Roques 2010). To help prevent new introductions, it is important to pinpoint the source population of such species within their native ranges (Essl et al. 2015). Using mtDNA markers, Bras et al. (2019) arrived at the conclusion that eastern China could be the source of invasive BTM populations in Europe. Here, the Bayesian analysis of microsatellite markers yielded strong support for this hypothesis. Furthermore, the ABC analyses clearly excluded the possibility that South Korea, southwestern China, or an unsampled population served as a source for the invasive European populations that we sampled. These results are consistent with the large volumes of Buxus trees that have been imported from China to Europe between 2006 and 2010 (EPPO, 2012), and they fit with the fivefold increase of the European imports of plants from China over the period 2005–2015 (Eschen et al. 2015). Furthermore, the coastal economies in China has developed rapidly over the years with Eastern China’s international trade accounted for example for 86.5% of the national total in 2013 (Wan and Yang 2016). It is also thought that eastern China has been the source of several other non-native insects introduced to Europe during the last decade (Roques et al. 2020), such as the yellow-legged hornet, Vespa velutina (Arca et al. 2015) and the spotted wing drosophila, Drosophila suzukii (Fraitout et al. 2017).

Given the moth’s simultaneous appearance in 2007 in three different countries—Germany, the Netherlands, and Switzerland (Krüger 2008; Leuthardt et al. 2010; Van der Straten and Muus 2010)—and its patterns of mtDNA diversity (Bras et al. 2019), it seems likely that the insect was introduced multiple times from China. Our ABC analyses pointed out that BTM was likely independently introduced from eastern China into Germany on two occasions, corresponding to the two first records of its presence in Europe (Krüger 2008), and on one occasion in the Netherlands. Netherlands and Germany’s importation from international countries represents the greatest volume of ornamental plants among EU countries (Eschen et al. 2017) which is supporting such invasive routes. More particularly, since the 2000s, imports from China to the Netherlands represent an important part of such commerce (van Valkenburg et al. 2014), with more than a million of Buxus trees being imported between 2006 and 2010 (EPPO, 2012). However, the prior error rate and posterior probabilities were moderate in our ABC analysis considering the three invasive population together. Statistical support for a scenario does not necessarily mean that it is true (Csilléry et al. 2010), which means other scenarios could also explain the data. In our case, the three initial invasive populations likely originated from the same source area in China and might have been simultaneously introduced within a short period of time. The estimated values of the time since introduction (t_i) for these populations were alike and their first observation records were no more than a year apart, thus supporting this hypothesis. As indicated by Benazzo et al. (2015), repeated introductions from the same source may lead to difficulties to discriminate correctly the number of independent introduction events. Thus, a scenario in which one of the initial invasive populations serving as a source for another invasive population—from Weil Am Rhein to...
Giessen or the other way around—cannot totally be excluded.

Non-native species can more easily become established when they are introduced on multiple occasions directly from their native range (Blackburn et al. 2015; Cristescu 2015), which could have favored BTM’s invasion of Europe. Indeed, propagule pressure plays a crucial role in increasing the probability of establishment of non-native species (Allendorf and Lundquist 2003; Lockwood et al. 2005; Kaňuch et al. 2021). Our ABC analyses suggest that the bottleneck severity was moderate among the initial invasive populations, especially in Weil am Rhein (I-WAR), and Giessen (I-GI). A large number of individuals could have been introduced into Europe from China, which could explain the elevated haplotype diversity observed by Bras et al. (2019) across the moth’s invaded range compared to the diversity in its native range. However, we cannot rule out the possibility that there have been recurrent introductions of the moth from China at the first European observation locations in 2007, given that the importation of Buxus trees has been continuous (EPPO, 2012). Unfortunately, ABC analyses cannot distinguish among such hypotheses because the genetic pattern associated with continual invasions may be genetically similar to that associated with a single introduction event with a large propagule size (Guillemaud et al. 2010).

Admixture events and bridgehead effects were likely involved in the fast spread of the box tree moth across Europe

Most of the non-native insects introduced into Europe over the past two decades—such as the spotted wing drosophila (Drosophila suzukii), the western conifer seed bug (Leptoglossus occidentalis), and the box tree moth (C. perspectalis)—appear to be spreading at unprecedented rates (Roques et al. 2016). The dispersal of both D. suzukii and L. occidentalis are the result of complex invasion dynamics, involving multiple introduction events, bridgehead effects and admixture events (Fraimout et al. 2017; Lesieur et al. 2019). Such genetic patterns may result from human-mediated dispersal, which facilitates the rapid spread of non-native species within the invaded range (Garnas et al. 2016; Bertelsmeier and Keller 2018). Thus, the ever-increasing trade of ornamental plants among European countries (Cadic and Widehem 2006; Dehnen-Schmutz et al. 2010) may have played a crucial role in BTM’s fast range expansion as its flight capacities cannot explain the species’ spread across Europe, the Caucasus and up to Iran in less than ten years (Van der Straten and Muus 2010; Bras 2018).

Our ABC analyses suggest that bridgehead effects coupled with admixture events have been involved in BTM’s dispersal in Europe. Indeed, they brought to light that the invasive populations in Weil Am Rhein (I-WAR, Germany), and in Giessen (I-GI, the Netherlands), have probably both served as source for the populations in Lucca (I-LU, Italy), and in Budapest (I-BU, Hungary), resulting in admixed populations. Our analyses also confirmed that the BTM population of southern Russia had an Italian origin, as suspected by Gninenko et al. (2014). Indeed, the moth was first observed on Buxus trees imported from Italy for the Olympic Village of the 2012 Games in Sochi. All the invasive populations identified as sources in our ABC analyses were present either in areas known as main centers for nurseries and trade in arboricultural products (i.e. Giessen, Lucca; Van der Straten and Muus 2010; Bella 2013) or port (i.e. Weil Am Rhein; Castteels et al. 2011) highlighting the role of human activities through the ornamental plant trade in BTM’s dispersal in Europe. The three separate introductions of BTM into Europe combined with the expanding trade in ornamental plants among European countries (Cadic and Widehem 2006; Dehnen-Schmutz et al. 2010) could have favored admixture between invasive populations. Intraspecific genetic mixing is thought to enhance invasion success (Rius and Darling 2014; Bock et al. 2015), but the role that it played in Europe’s invasion by BTM remains unclear. In fact, the STRUCTURE analysis of the East Asian populations also indicated signs of admixture among eastern China populations. Because our samplings in the native range essentially proceeded from cities where planted Buxus trees could have been imported from various nurseries in China, we cannot exclude the coexistence in such man-made habitats of moth populations from different geographic origins, and their possible in situ hybridization. Moreover, the change of habitat from the forest to the city, and the subsequent selective pressures within these human-modified habitats may then have led to preselect populations adapted to human habitats. Such a two-step process with populations moving from natural habitats to human-disturbed habitats has already been shown to
favor invasion success in some other invasive species such as the little fire ant, *Wasmannia auropunctata* (Foucaud et al. 2013). In the case of BTM, we can then hypothesize that both admixture and pre-adaptation to human-modified habitats in native populations occurring in man-made habitats might have also favored BTM’s invasion success in similar habitats in Europe as the insect was firstly observed in cities (Krüger 2008; Leuthardt et al. 2010; Van der Straten and Muus 2010).

BTM’s rapid dispersal from western Europe up to the Caucasus region seems likely to have been triggered by a combination of the three genetic processes we have highlighted through our ABC analyses: e.g., (i) multiple introductions, bridgehead effects, admixture events, (ii) the species’ biological traits, such as its strong flight capabilities (Bras 2018), and (iii) the widespread availability of the host plant (Nacambo et al. 2014; Thammina et al. 2016; Mitchell et al. 2018). They may also favor the moth’s establishment and spread in North America.

Tracing the dispersal routes of the invasive box tree moth

Our ABC results revealed the complex invasion pathways followed by BTM. They also underscored the difficulties inherent in clearly defining such routes when invasive scenarios are based on sparse information, the invasion process is complex, and range expansion is rapid. Our analyses strongly suggest that BTM was independently introduced at least three times from the same source population in Asia. However, BTM invasive routes among Europe were harder to identify. Despite the robustness of the ABC-RF method, compared to more classical ABC analysis (Pudlo et al. 2016; Fraimout et al. 2017), determining whether several introductions resulted from the same source can be challenging, especially when the species in question is spreading rapidly (Benazzo et al. 2015). The short bottleneck period (identified by the posterior estimations) together with a late sampling of the invasive populations may have led to the formation of several few genetically differentiated outbreaks. This variability coupled with an identical population source in China could partly explain the moderate posterior probability and prior error rate values obtained for the most probable scenarios that we identified in Europe (Bermond et al. 2012; Benazzo et al. 2015; Hargrove et al. 2017). Moreover, DIYABC assumes that no recurrent migration has occurred between populations (Cornuet et al. 2014). However, some of the invasive populations that we examined were likely geographically connected, notably the Weil Am Rhein (I-WAR) and Kehl (I-KE) populations in Germany. Indeed, both were sampled several years after the moth’s initial appearance in 2007. These factors, combined with BTM’s strong flight capacities (Bras 2018), suggest that migration among populations could have contributed to population mixing which may have reduce the likelihood of accurate source assignment. Moreover, signs of admixture among eastern China populations could also have led to a first genetic blur of the boundaries between populations (Hamelin and Roe 2020).

Our study provides a new example of a complex invasion process involving multiple introduction events, bridgehead effects, and admixture events. The invasive scenarios that we identified for BTM are consistent with what is known about ornamental plant trade dynamics in Europe (Dehnen-Schmutz et al. 2010). Complex invasion processes mediated by human activities seem to be the rule rather than the exception for the rapidly dispersing of non-native insects introduced over recent decades into Europe (Fraimout et al. 2017; Lesieur et al. 2019; Lombaert et al. 2014a, b; Sherpa et al. 2019). The recent establishment of BTM in North America (Frank 2019; USDA-APHIS 2021) demonstrates the need of assessing invasion pathways of such species in order to develop appropriate management strategies to avoid further introductions.

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**Data availability** The GenBank accession numbers for the fifteen microsatellite loci used in this study range from MH151896 to MH151910. Microsatellite genotypes are accessible by using the following https://doi.org/10.5281/zenodo.5516932.

**Code availability** Not applicable.

**Declarations**

**Conflicts of interest** All authors declare no conflicts of interests.

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