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Four times out of Europe: Serial invasions of the winter moth, *Operophtera brumata*, to North America

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Abstract

Reconstructing the geographic origins of non-native species is important for studying the factors that influence invasion success, however; these analyses can be constrained by the amount of diversity present in the native and invaded regions, and by changes in the genetic background of the invading population following bottlenecks and/or hybridization events. Here we explore the geographical origins of the invasive winter moth (*Operopthera brumata* L.) that has caused widespread defoliation to forests, orchards, and crops in Nova Scotia, British Columbia, Oregon, and the northeastern United States. It is not known whether these represent independent introductions to North America, or a "stepping stone" spread among regions. Using a combination of Bayesian assignment and approximate Bayesian computation methods, we analysed a population genetic data set of 24 microsatellite loci. We estimate that winter moth was introduced to North America on at least four occasions, with the Nova Scotian and British Columbian populations probably being introduced from France and Sweden, respectively; the Oregonian population probably being introduced from either the British Isles or northern Fennoscandia; and the population in the northeastern United States probably being introduced from somewhere in Central Europe. We discuss the impact of genetic bottlenecks on analyses meant to determine region of origin.

KEYWORDS
approximate Bayesian computation, biosecurity, invasion routes, microsatellites invasive species, *Operopthera brumata*, population genetics

1 | INTRODUCTION

In light of the unprecedented number of introductions of non-native species (Mack et al., 2000), one of the most pressing research needs for evolutionary biologists and ecologists is to identify the factors that influence the establishment of species that have negative ecological and economic impacts (Suarez & Tsutsui, 2008). Multiple introductions (Dlugosch & Parker, 2008), including cryptic ones (Roman, 2006), are thought to play an important role in providing the diversity required to overcome genetic bottlenecks associated with the establishment of populations in novel ecosystems (Darling et al., 2008; Facon et al., 2008). However, when multiple geographically disjunct populations of an invasive species become established, it is often unclear whether the species is a serial invader (i.e., each population was introduced independently) or whether the separate populations represent establishment from within the invasive regions under a "stepping stone" model (see Cerwenka et al., 2014; Lombaert et al., 2010; Oficialdegui et al., 2019; Tonione et al., 2011 for examples). Identifying which mode of introduction occurred (serial invader or stepping stone) is therefore necessary for the study of genetic and ecological factors that drive invasion success as independent populations are required for robust hypothesis-based testing (Kang et al., 2007) – information that is also crucial for focusing management efforts (Floerl et al., 2009).

When reconstructing regions of origins, and determining the numbers of introductions of a focal organism, historical records

should be referenced and robust genetic analyses performed (e.g., Lynch & Saltonstall, 2002; Schwenk et al., 2008). Unfortunately, historical records may not exist for all introduced species, and it is not uncommon for an introduced species to go unnoticed for long periods of time before becoming an invasive pest. Genetic analyses have the ability to independently reconstruct regions of origins, and in some instances provide estimates for the times of introductions (Auger-Rozenberg et al., 2012; Barker et al., 2017; Javal et al., 2019; Lesieur et al., 2019; Lombaert et al., 2010; Oficialdegui et al., 2019; Zardus & Hadfield, 2005). However, the power of these analyses is constrained by numerous factors including the underlying genetic structure of the species, the number of generations since the introduction, the effective size of the founding population(s), the strength of the bottleneck the population(s) experienced, and/or the presence of differing selective pressures in the native and introduced regions. As such, one common finding is for introduced populations to be reconstructed as genetically "distinct" from all sampled source populations (e.g., Barker et al., 2017; Wu et al., 2015). This result could be due to the tendency for commonly implemented Bayesian genetic clustering algorithms to over-split populations (Frantz et al., 2009) or an artefact generated during the interpretation of results (Lawson et al., 2018).

Here, we explore the invasion history of the economically damaging defoliator the winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae). In its native distribution across Europe, North Africa, and western Asia, winter moth defoliates a wide range of tree and shrub species (Ferguson, 1978). Populations of winter moth in Europe have been used as a model for the study of population ecology (Varley et al., 1973), and this species has been critically important for understanding the importance of spatial-synchrony (Jepsen et al., 2009) and synchrony of hatch with host tree bud-burst (Varley & Gradwell, 1960; Visser & Holleman, 2001) – a factor studied in other invasive defoliator populations as well (e.g., Hunter & Elkinton, 2000). The invasion history of winter moth in North America has been well documented, with populations of winter moth first reported in the 1930s in Nova Scotia (Embree, 1967; MacPhee, 1967; MacPhee et al., 1988), the 1950s in Oregon (Kimberling et al., 1986), the 1970s in British Columbia (Gillespie et al., 1978), and in the 1990s in coastal regions of northeastern United States (Elkinton et al., 2010, 2014). These populations are thought to have been introduced by the movement of infected nursery stocks (Ferguson, 1978). However, where in Eurasia these populations were introduced from, and whether these represent a single introduction that was then spread across North America, or multiple introductions (or some combination of these) is unclear. A genetic examination of the invasion history of winter moth in North America was previously conducted, but was unable to discern these patterns due to low levels of mitochondrial DNA diversity in both introduced and native samples (Gwiazdowski et al., 2013).

To overcome this limitation, we examined the invasion history of winter moth in North America using microsatellite loci amplified from individuals collected across its native and introduced regions. Here, we specifically examine how many times winter moth was introduced to North America, and when possible, determine the specific source location. Lastly, we comment on the effects of genetic bottlenecks on the establishment of invasive winter moth populations.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Winter moth males were collected using pheromone-baited traps (Elkinton et al., 2010, 2011) from locations in Europe, North Africa, and western Asia, as well as from the four regions in North America, where established winter moth populations have been recorded (Nova Scotia, British Columbia, Oregon, and the northeastern United States). In addition, larval individuals and adult females, which are wingless and do not fly, were opportunistically collected (see Table S1 for complete collection information, including life stages, and Figure 1).

After collection, adult moths were placed in glassine envelopes (Uline Corporation) and stored at either −20 or −80°C, and larval caterpillars were placed in 95% ethanol and stored at −20°C. For many of the moths included in our analysis (1361 out of 1839), collection and genotype information have been previously reported by us in studies of winter moth population structure in the native or introduced ranges (Andersen et al., 2017; Andersen, Havill, Broadley, et al., 2019; Andersen, Havill, Mannai, et al., 2019; Andersen et al., 2021; see Table S1).

2.2 | Microsatellite genotyping

Genomic DNA was extracted using the EZNA Tissue DNA extraction kit (Omega Bio-tek), following the manufacturer's protocols. For adult males, prior to extraction, the wings and genitalia were removed and stored as vouchers. For adults and larvae, the remaining body parts were then homogenized, using 3/16" stainless steel beads (GlenMills Inc.) with a FastPrep-24 sample homogenizer (MP Biomedicals). After extraction, 24 microsatellites including 15 dinucleotide, 14 trinucletotide, two tetranucelotide, and one pentanucleotide loci (see Havill et al., 2017 for sequence information and amplification methods) were genotyped at the DNA Analysis Facility on Science Hill at Yale University, using a Thermo Fisher Scientific 3730xl DNA analyser. Fragment lengths were determined using the microsatellite plugin in the software program GENEIOUS v. R11 (https://www.geneious.com) in comparison to the GeneScan 500 LIZ size standard (Thermo Fisher Scientific).

Only individuals from which ≥20 microsatellite loci were successfully amplified were included in the analyses. In addition, since winter moth has been reported to hybridize with Bruce spanworm (*O. bruceata* Hulst) in all of its invaded regions (Andersen, Havill, Broadley, et al., 2019) the data set was further filtered to remove hybrids by comparing assignment probabilities based on 12 microsatellite loci

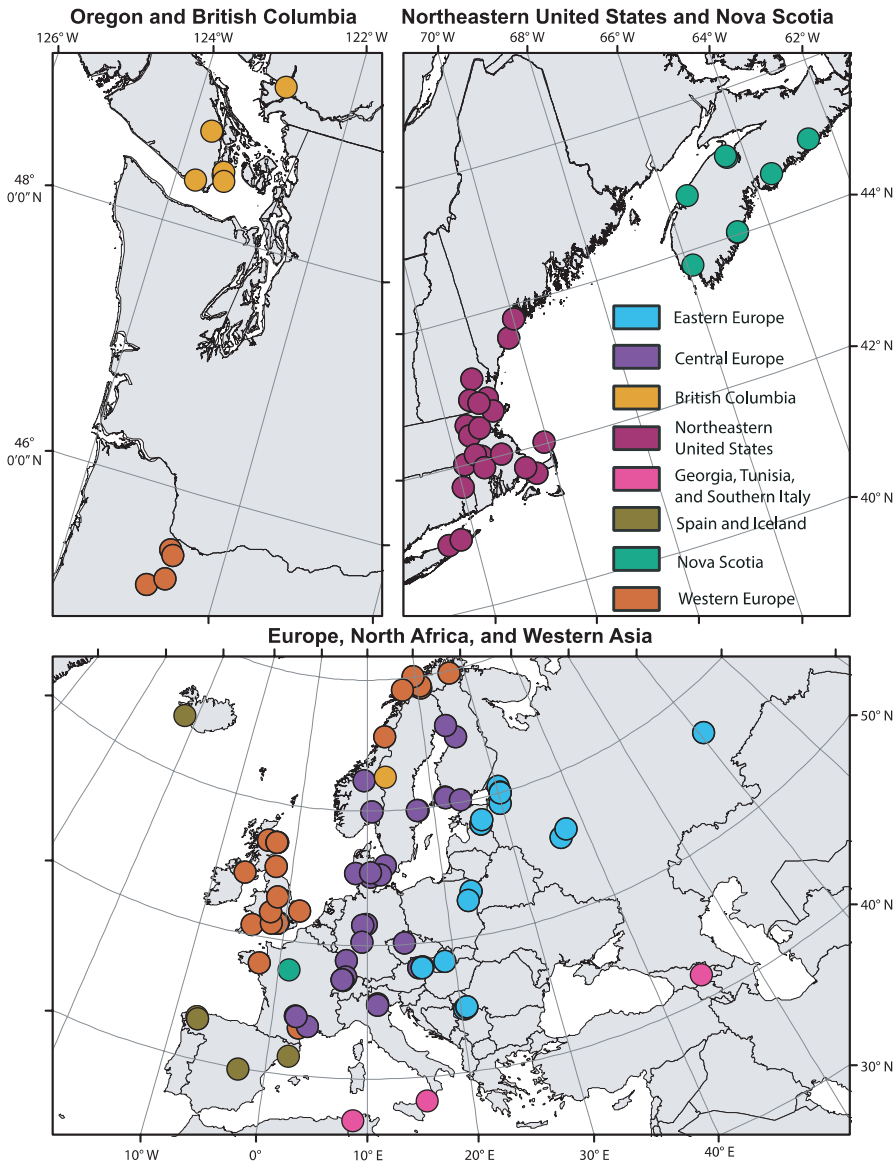

FIGURE 1 Winter moth sample localities in the Pacific Northwest (top left); northeastern United States and Nova Scotia (top right); and in Europe, North Africa, and western Asia. Sample localities are coloured following the results presented in Figure 3

that coamplify in both species (described below). Microsatellite genotypes are provided as a tab-delimited STRUCTURE-formatted supplemental file titled "WinterMothOriginsStructure.txt".

2.3 | Population genetics statistics

For each locality in Europe, North Africa, and western Asia from which ≥10 winter moth individuals were collected, standard population genetic statistics were estimated from the microsatellite genotypes scores, using GENODIVE (Meirmans & Van Tienderen, 2004). In the introduced region, we prioritized sampling a small number of individuals from a large number of locations in each region to obtain a broader representation of genetic diversity (see Suarez & Tsutsui, 2008). Null allele frequencies for each locus were estimated using Dempsters EM method as implemented in GENEPOP (Raymond & Rousset, 1995; Rousset, 2008). In addition, for each invasive winter moth population, we counted the number of alleles found in both the introduced and source populations for each locus.

2.4 | Bayesian sample assignment

To assign individuals to genetic clusters, a two-step approach was taken. First, genotypes for all North American samples were added to the data set presented in Andersen, Havill, Broadley, et al. (2019), and the probability of assignment (*Q*) of sampled individuals to one of two distinct genetic clusters (*K*) representing either pure winter moth or pure Bruce spanworm was calculated using STRUCTURE v.2.3.2 (Falush et al., 2003; Pritchard et al., 2000). These analyses were based on the analysis of 12 microsatellite loci that coamplify between the two species, and ten independent analyses were run using the admixture model, correlated allele frequencies, and default settings, with random starting values, runtimes of 1,000,000

generations, and burnin periods of 100,000 generations. Results were then summarized across runs using CLUMPAK (Kopelman et al., 2015), and hybrid individuals were identified as those receiving scores of $Q < 0.75$ to both the Bruce spanworm and the winter moth genetic clusters. Hybrid individuals were then removed from the data set, and the filtered data set (≥20 loci for each individual) was used to estimate values of Q for all individuals (both native and introduced regions) for values of $K = 2$ through $K = 14$ in STRUCTURE using the run parameters described above. To determine the optimal number of clusters present in the data set, the ΔK statistic (Evanno et al., 2005) was calculated in STRUCTUREHARVESTER (Earl & vonHoldt, 2012), and independent runs were again summarized for major and minor partition schemes using CLUMPAK. Additional analyses also were conducted for a data set that included only the winter moth individuals from North America, with values of Q estimated for each individual to clusters of $K = 2$ through $K = 10$ as described above.

For the full data set that included native and invasive winter moth populations, each value for ΔK with a distinct peak representing a positive rate of change identified using STRUCTUREHARVESTER, the summarized "popfile" of cluster membership coefficients for the major mode calculated in CLUMPAK was used to create a distance matrix using the "dist" function in R v. 4.0.0 (R Core Team, 2020). The resulting matrices were then used to calculate "NeighborNet" networks using SPLITSTREE v.4.14.2 (Huson & Bryant, 2006), and the outputs were examined to identify geographic patterns.

2.5 | Approximate Bayesian computation

To determine whether populations of North American winter moth in distinct geographic regions were the result of a single introduction to Nova Scotia (the first introduced region recorded in North America) that was then subsequently spread to additional locations in North America (i.e., following a stepping stone model), or whether each invasive population represents a novel introduction (i.e., a serial introduction model), or some combination of these, we compared the relatedness of each invasive population to each other and to the "Eastern European", "Central European", and "Western European" winter moth genetic clusters previously reported in Andersen et al. (2017, 2021) using approximate Bayesian computation (ABC), as implemented in the software program DIYABC v.2.1.0 (Cornuet et al., 2008). For these analyses, 30 individuals were selected randomly from each of the three European clusters and from each of the four invasive regions. Ideally, comparisons of all possible scenarios that included representatives from all native clusters and invasive regions would be performed, however; the number of possible scenarios increases at an unmanageable rate with each population added (e.g., there are 10,395 possible "scenarios" in a seven-population analysis). Therefore, we utilized an approach similar to "tournament-ABC" as presented in Stone et al. (2017).

As in Stone et al. (2017), we use a series of hierarchical ABC analyses where subsets of scenarios are first compared in "tournaments" to reduce computational complexity. Here, we first fixed the relationship among the Eastern, Central, and Western European genetic clusters following Andersen et al. (2017), where it was determined that the Central European cluster was probably the result of admixture between the Eastern and Western clusters following the post-glacial recolonization of the European continent after the last glacial maximum. To this topology, we also added an unsampled "ghost" population to represent a possible extra-European origin for each invasive population. Tournament scenarios were then built sequentially, following the documented order of the invasion history (graphical representations of scenarios from each tournament are presented in Figures S1–S4). The first tournament compared four scenarios where the Nova Scotia population could have originated from one of the European clusters or the extra-European "ghost" population. In the second tournament, five scenarios were compared testing the relationship of the Oregon population to each putative source population with the relationship of the Nova Scotia population set based on the "best" scenario from Tournament 1. In the third tournament, six scenarios were compared testing the relationship of the British Columbia population to each putative source population with the relationships of the Oregon and Nova Scotia populations set based on the "best" scenario from Tournament 2. Finally, in the fourth tournament, seven scenarios were compared testing the relationship of the Northeastern United States population to each putative source population with the relationship of the British Columbia, Oregon, and Nova Scotia populations set based on the "best" scenario from Tournament 3.

For each tournament, a reference table of 1,000,000 generations per scenario was generated. Under each scenario we included multiple parameters to allow for changes in population sizes, following splitting/merging events, include genetic bottlenecks during the introduction of each invasive population (modeled following Lombaert et al., 2014), utilize default mutation model parameters, a minimum mean mutation rate of 1×10^{-5}, and the maximum values for the mean and individual locus coefficient P's were both set to 1.0. As per Andersen et al. (2017, 2021), we removed four loci with especially large allelic ranges (02339, 00925, 02191, and 12042) to improve the shape of the cloud of simulated data sets. We calculated three, one sample summary statistics (mean number of alleles, mean genetic diversity, and mean size variance) and three two sample summary statistics (F_{ST}, classification index, and $[d\mu]^2$ distance). For each tournament, the scenario representing the ancestral origin of each invasive population was determined by comparing the results from the Logistic Regression test implemented in DIYABC based on comparisons of 1% of simulated data sets (~10,000) closest to the observed data. Model checking for each tournament was performed by calculating the type I error rate for the "best" scenario from each tournament and type II error rates from all other scenarios in each tournament using the Evaluate Confidence in Scenario Choice command in DIYABC under the Prior Based Error model following the Scenario Specific approach with parameters being drawn from the prior distribution and 500 pseudo-observed test data sets analysed for each scenario under each tournament. Prior distributions for all parameters in all tournaments are presented in Table S2.

3 | RESULTS

3.1 | Microsatellite genotyping

In total, we genotyped 1839 individuals, and after filtering to include only individuals with genotype scores from ≥20 microsatellite loci, 1588 samples were retained for subsequent analyses. When the North American samples were analysed using the 12 microsatellite loci that co-amplify between Bruce spanworm and winter moth, four individuals were classified as being Bruce spanworm and four individuals were identified as probable hybrids. The final data set therefore included 1580 pure winter moth individuals, of which 1543 were adult males, one was an adult female, and 36 were larvae. For the data set that included Bruce spanworm and winter moth individuals, the average probability of assignment score for Bruce spanworm individuals was 0.9878 ± 0.0016, and the average probability of assignment score for winter moth individuals was 0.9935 ± 0.0011. The geographic locations of all included samples are presented in Figure 1.

3.2 | Population genetics statistics

Standard population genetic statistics were calculated for 53 populations from the native distribution of winter moth, as well as from each of the four invasive North American populations (Table S3). In addition, we calculated these same summary statistics for each of the European genetic clusters presented in Figure 1, and compare these results to the diversity observed in the random sub-samples for the DIYABC analyses (Table 1). Populations had on average 7.8 alleles per locus (± 1.71 alleles per locus), with the invasive populations having equal to, or greater allelic diversity than the native populations (average of 8.06 ± 2.05 alleles per locus). The greatest allelic diversity was observed in the population from Pančevo, Serbia (average of 12.04 alleles per locus), and the population with the lowest allelic diversity was Reykjavík, Iceland (average of 3.42 alleles per locus). As per previous winter moth population genetic analyses, all populations showed evidence of deviations from Hardy-Weinberg equilibrium ($p < 0.05$ for all; Table S3). The average amount of missing data per locus was 4.12%, with locus 02191 having the most missing data (15.3%) and locus 32985 having the least (0.4%). The British Columbia population displayed the greatest proportion of alleles shared with its source European lineage (0.54; Table 2), while the Oregon population had the fewest (0.33; Table 3).

3.3 | Bayesian sample assignment

On average, negative log-likelihood scores for the independent STRUCTURE runs increased from $K = 2$ through $K = 13$, before decreasing at $K = 14$ (Figure S5). Based on the ΔK method implemented in STRUCTUREHARVESTER, the optimal partition scheme was determined to

TABLE 1 Genetic summary statistics for winter moth (*Operophtera brumata*) genetic groups (as per Figure 1) including the number of individuals sampled per population (*n*), the average number of alleles per locus (Num), the effective number of alleles per locus per population (Eff_num), the observed heterozygosity (H_o), the expected heterozygosity (H_s), the inbreeding coffecient (G_{IS}), and whether or not the population showed significant deviation from Hardy-Weinberg equilibrium (HWE)

Population clusters	*n*	Num	Eff_num	H_o	H_s	G_{IS}	HWE
Eastern Europe	288	21.3	6.2	0.583	0.738	0.211	0.001
Central Europe	412	21.7	5.1	0.621	0.742	0.163	0.001
Western Europe	422	18.1	4.1	0.547	0.672	0.186	0.001
Spain	37	7.4	3.2	0.500	0.591	0.154	0.001
Tbilisi, Rep. Georgia	28	7.7	3.6	0.519	0.590	0.12	0.001
Reykjavík, Iceland	27	3.4	2.0	0.359	0.414	0.134	0.001
Orti, Italy	26	8.3	4.3	0.592	0.670	0.117	0.001
Mzara Forest, Tunisia	17	4.8	2.8	0.45	0.488	0.078	0.010
Nova Scotia	89	7.4	3.0	0.481	0.610	0.211	0.001
British Columbia	85	10.9	4.1	0.568	0.689	0.175	0.001
Oregon	18	6.0	3.2	0.427	0.656	0.349	0.001
Northeastern United States	124	8.0	3.1	0.522	0.633	0.175	0.001
Subsamples							
Eastern Europe	30	8.2	3.8	0.534	0.661	0.192	0.001
Central Europe	30	9.3	4.6	0.609	0.718	0.152	0.001
Western Europe	30	10.7	5.7	0.568	0.744	0.237	0.001
Nova Scotia	30	5.8	2.9	0.485	0.604	0.198	0.001
British Columbia	30	7.5	3.9	0.559	0.682	0.181	0.001
Northeastern United States	30	1.0	2.9	0.496	0.598	0.171	0.001

Note: Population genetic summary statistics are presented for all sampled populations in Table S2.

TABLE 2 Comparisons of allelic diversity in invasive populations of *Opterophtera brumata* in North America and their Central European source population as estimated using DIYABC

	Central Europe	Nova Scotia		British Columbia		Northeastern U.S.	
Locus	N Alleles	Shared	Private	Shared	Private	Shared	Private
01619	7	3	0	5	0	3	0
02339	14	4	3	7	0	5	0
28247	37	13	0	19	0	15	0
34463	26	14	0	20	0	8	0
07650	28	7	1	11	0	8	0
29309	18	7	0	9	0	8	0
02565	11	5	0	9	0	6	0
32985	7	3	1	4	0	3	1
00952	55	7	2	19	3	10	0
03475	7	3	0	3	0	4	2
12853	10	6	1	6	0	3	0
31399	17	7	0	12	1	5	1
02191	40	9	4	9	5	11	2
00672	25	7	0	13	1	11	1
01585	22	7	0	11	0	10	0
24979	19	5	1	8	0	6	0
12042	59	7	0	24	2	11	0
24011	26	9	0	13	1	8	0
03280	15	7	0	10	0	4	0
18760	11	6	0	7	0	6	0
05159	18	5	0	10	5	9	1
16696	13	4	0	6	1	7	0
03270	10	5	6	7	1	6	4
01762	25	9	1	10	3	10	3
Average ratio		**0.36**		**0.54**		**0.39**	

Note: Statistics include the number of alleles in the source population (N Alleles), the number of alleles shared between each invasive population and the source population (Shared), and the number of alleles found in each invasive population but not in the source (Private). The average ratio of the number of shared alleles in each invasive population compared to the number of alleles in the source population is presented in bold.

be K = 3 (Figure S6). However additional peaks, potentially representing additional partition schemes, were detected at K = 6, K = 8, and K = 13. Individual probabilities of assignment (Q) for major modes for values of K = 3, K = 6, K = 8, and K = 13 (Figure 2) show the clustering of the invasive populations relative to those in the native range. Major modes for values of K = 2 through K = 1, and the number of independent runs associated with each mode are presented in Figure S7. Sequentially, at K = 2, all eastern European samples, the samples from Tunisia and southern Italy, and the samples from the northeastern United States were assigned to one cluster, while all other samples were assigned to a second. At K = 3, the samples from the northeastern United States emerged as a unique cluster. At K = 4, the samples from Nova Scotia emerged as a unique cluster. At K = 5, the samples from Central Europe and British Columbia were separated from those from Western Europe. At K = 6 and K = 7, the samples from remote locations (i.e., Iceland, Tunisia, southern Italy, Georgia) received partial assignment to novel clusters. At K = 8, the samples from British Columbia emerged as a unique cluster. At K = 9, the samples from southern Italy and Georgia emerged as unique clusters. At K = 10, the samples from Iceland emerged as a unique cluster. At K = 11, the samples from northern Fennoscandia emerged as a unique cluster. At K = 12, the samples from Oregon emerged as a unique cluster, and finally at K = 13 and K = 14 no additional biogeographic structuring was observed. A summary of the biogeographic patterns based on the distance matrix analysis of the population membership coefficients (STRUCTURE "popfiles") for K = 6, K = 8, and K = 13 are presented graphically in Figures S8, Figure 3, and Figure S9, respectively.

For the analysis that included only individuals from the introduced populations, the negative log-likelihood scores for the independent STRUCTURE runs increased from K = 2 through K = 5, (Figure S10), and based on the ΔK method implemented in STRUCTUREHARVESTER, the

TABLE 3 Comparisons of allelic diversity in invasive populations of *Opterophtera brumata* in Oregon and its Western European source population as estimated using DIYABC

	Western Europe	Oregon	
Locus	N Alleles	Shared	Private
01619	7	4	0
02339	12	4	0
28247	33	10	0
34463	25	5	0
07650	17	7	0
29309	14	7	0
02565	10	5	0
32985	6	2	3
00952	34	3	4
03475	8	5	2
12853	10	3	4
31399	17	6	0
02191	36	5	7
00672	26	9	0
01585	20	4	0
24979	14	3	0
12042	47	3	2
24011	24	6	0
03280	15	6	1
18760	9	5	0
05159	18	6	0
16696	10	2	1
03270	7	3	1
01762	16	5	0
Average ratio		**0.33**	

Note: Statistics are presented as in Table 2.

optimal partition scheme was determined to be $K = 3$ (Figure S11). At $K = 2$, nearly every individual in the Northeastern United States was assigned to one distinct genetic cluster, while all of the individuals from all of the other invasive locations were assigned to another. At $K = 3$, all of the Nova Scotia individuals emerged as a distinct cluster, and at $K = 4$, the British Columbia and Oregon individuals emerged as distinct clusters at $K = 5$, the British Columbia individuals were assigned to two clusters, and at increasing values of K, the British Columbia and the Oregon samples were increasingly assigned admixed assignment scores (Figure S12).

3.4 | Approximate Bayesian computation

Tournament comparisons of invasion scenarios indicated that each of the four invasive populations in North America were more closely related to populations from Europe than to each other, or to an unsampled extra-European "ghost" population, suggesting four separate introduction events from Europe for winter moth (Figure 4; Table 4). Comparison of the Nova Scotia population to the Western European, Central European, and Eastern European genetic clusters, indicated that it was most likely introduced from Central Europe (Figure S1), and this relationship received high support based on logistic regression analysis ($p = 0.865$). The Oregon population most likely originated from Western Europe (Figure S2), and this relationship received high support based on logistic regression analysis ($p = 0.979$). The British Columbia population most probably represents an additional independent introduction from Central Europe (Figure S3), and this relationship received high support based on the logistic regression analysis ($p = 0.995$). The population in the northeastern United States population was also reconstructed as a third introduction from Central Europe (Figure 4; Figure S4), and this relationship was also highly supported ($p = 0.988$). Posterior estimates for all parameters for the best scenario from each tournament are presented in Table S4. Type I error rates for supported scenarios, as estimated using the Evaluate Confidence in Scenario Choice command in DIYABC, ranged from 0.106 to 0.194, while type II error rates ranged from 0.003 to 0.224 (Table 4).

4 | DISCUSSION

Identifying the number of introductions of an invasive organism is critical for evolutionary and ecological studies of the factors that influence the probability of establishment of invasive species and how non-native species adapt to their introduced environments and ecosystems, (Allendorf & Lundquist, 2003; Dlugosch & Parker, 2008; Lavergne & Molofsky, 2007; Sakai et al., 2001). Unfortunately, reconstructing the invasion histories of non-native organisms can both computationally demanding and/or biologically untenable due to the combined effects of genetic bottlenecks, hybridization, and rapid evolution (Buhk & Thielsch, 2015; Ficetola et al., 2008; McEvoy et al., 2012; Mesgaran et al., 2016; Prentis et al., 2008). Using a combination of Bayesian clustering and approximate Bayesian computation methods, we find that invasive populations of winter moth in Nova Scotia, British Columbia, and the northeastern United States were all introduced separately from Central Europe, and that the invasive population in Oregon was introduced from Western Europe (Figures 3 and 4). Distance analyses of the coefficient of membership assignments from our Bayesian clustering analyses, suggest that the invasive population in Nova Scotia is most closely related to a population of winter moth in Orleans, France, and that the invasive population in British Columbia is most closely related to a population of winter moth in Uggvallen, Sweden (Figure 3), representing potential source localities for both Canadian populations.

The relationships of the American populations were less clear based on distance analyses; however, as the Oregon population was closely related to a large number of populations from the British Isles and northern Fennoscandia, while the northeastern United States population was unrelated to any sampled European population. In a recent study of winter moth in Fennoscandia – where winter moth exists at outbreak densities in much of the

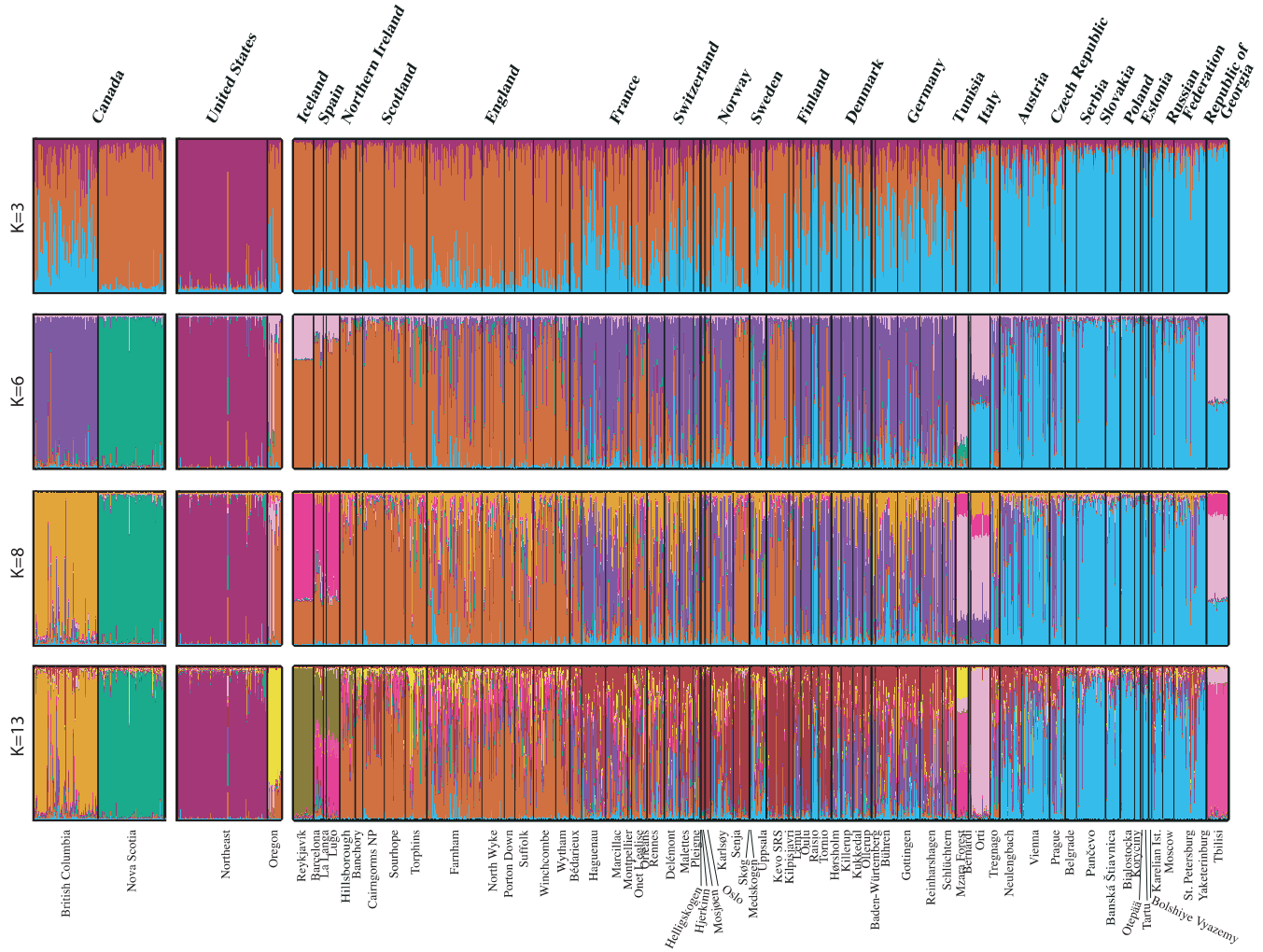

FIGURE 2 Major modes detected using CLUMPAK for supported partitions based on the results presented in Figure S6. Samples are grouped by country, and locality, except in North America, where for clarity they are grouped by geographic region. Thin dark lines are used to differentiate sample localities, and thick dark lines to separate countries. Countries in the native range of winter moth are ordered roughly from west (left) to east (right)

region – we determined that the population there was introduced to the region ~2000 years ago from the British Isles, either via dispersal across the North Sea or by human-mediated dispersal (Andersen et al., 2021). Therefore, without finer-scale genomic analyses (e.g., genotype-by-sequencing), it is unlikely that we will be able to determine which of these two locations (British Isles or northern Fennoscandia) are the source of the Oregon population. The northeastern United States population, in contrast to all other sampled invasive populations, was clearly distinct from other winter moth populations, including the invasive population in Nova Scotia that can be found only a short distance away across the Bay of Fundy. While it is possible that we have yet to sample the source of this invasive population, given our extensive sampling of the native distribution of winter moth, we believe this is unlikely. For example, the only regions that we did not sample from which winter moth has previously been reported are Japan, Taiwan, and the Russian far-east (Troubridge & Fitzpatrick, 1993), and in those locations, records of winter moth are most probably misidentifications of different species that are native to those regions (Nakajima, 1991). In addition, our inclusion of an unsampled "ghost" population in our DIYABC analyses to represent a possible extra-European source was not supported in any tournament ($p \leq 0.01$ in all analyses). Lastly, as part of ongoing efforts to study the evolution of the genus *Operophtera*, we have worked with collaborators in both the Russian far-east and in Japan to collect samples using traps baited with the winter moth sex-pheromone, and based on preliminary DNA-barcoding results all samples that were collected in these regions have been native *Operophtera* species (N. P. Havill, unpublished data). Therefore, we believe that the genetic distinctness of the northeastern United States population could be an effect of drift amplified by a strong genetic bottleneck associated with its introduction and/or possibly driven by local adaptation to its novel environment (similar to that seen by Butin et al., 2005) following its introduction from Central Europe.

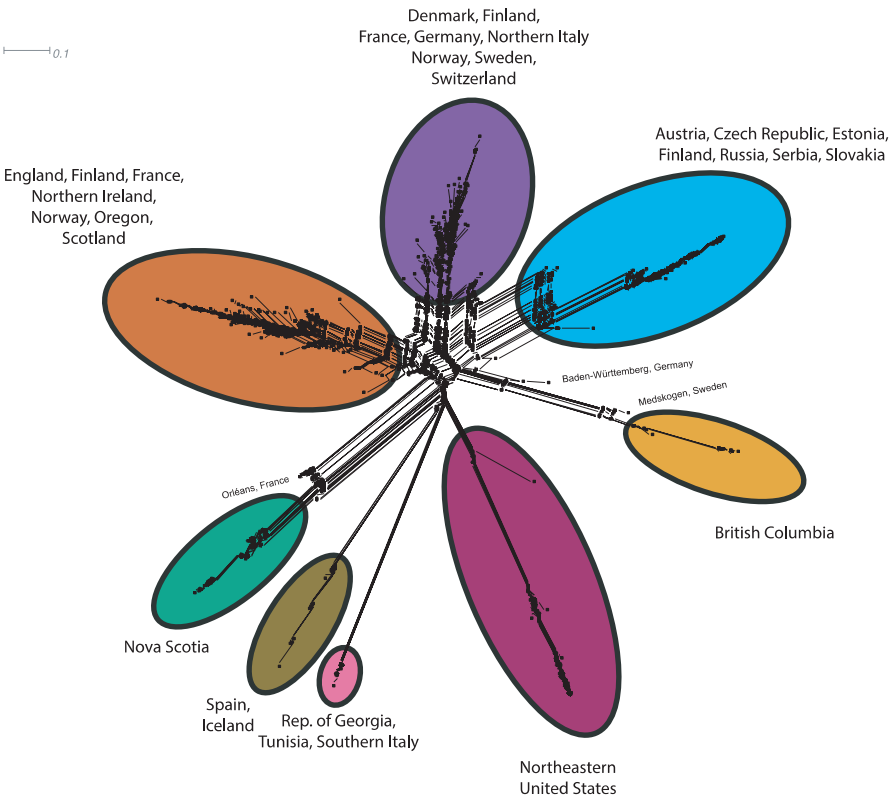

FIGURE 3 NEIGHBORNET analysis of the population coefficient of assignments for the major mode of $K = 8$ as summarized by CLUMPAK. Label names for populations within broader geographic clusters have been removed for clarity

4.1 | Hybrid origins

Hybridization has long been known to play an important role in the evolutionary trajectories of species and populations (Allendorf et al., 2001), and the promotion of the establishment of invasive species (e.g., Allendorf & Lundquist, 2003; Benvenuto et al., 2012; Mesgaran et al., 2016; Sakai et al., 2001). Soon after the discovery of an invasive population of winter moth in the northeastern United States, hybridization between the non-native winter moth and the native Bruce spanworm was demonstrated by sequencing the G6PD nuclear gene (Elkinton et al., 2010, 2014). Subsequent studies have found that multigenerational hybridization is occurring between these two species in the northeastern United States (Havill et al., 2017), and that hybridization between the two species has occurred in all of the locations that winter moth has established (Andersen et al., 2019). In a recent study along a transect running west (primarily Bruce spanworm) to east (primarily winter moth) in Massachusetts with traps spaced approximately 10 km apart, ~1% of surveyed individuals were hybrids though in a distinct hybrid zone between the two species hybridization rates of over 10% were reported at several locations (Griffin et al., 2021). Here we find that that only four individuals among our samples were classified as hybrids, suggesting that while hybridization occurs readily between these two species, genomic introgression has probably not played an important role in the establishment of winter moth populations. It is important to note, however, that this result could be driven by a failure of the loci to amplify in hybrids, as only 12 of our 24 loci have been shown to consistently amplify in Bruce spanworm (Havill et al., 2017), so early generation hybrids would probably have been filtered out by our 20-locus minimum. As such, genomic analyses (such as those described above) could be useful for examining introgression between winter moth and Bruce spanworm populations in North America. It is also worth noting that all three of the invasive populations that originated from Central Europe are themselves the product of ancestral admixture between the Western and Eastern European winter moth lineages, and this ancestral admixture could have increased the likelihood of establishment of these winter moth lineages (see Bennet et al., 2016 for a similar example).

4.2 | Effects of genetic bottlenecks

Genetic bottlenecks play an important role in the establishment of invasive species (Dlugosch & Parker, 2008; Suarez & Tsutsui, 2008), with numerous examples existing of instances where multiple introductions have been important for overcoming propagule pressures (Kolbe et al., 2004; Lavergne & Molofsky, 2007; Simberloff, 2009). There is even an example where bottlenecks may have aided the establishment of an invasive species (Tsutsui et al., 2000). Here, we find evidence for multiple independent introductions of winter moth to North America, and that all four invasive populations experienced bottlenecks resulting in temporary reductions in their effective population sizes (Table S4). However, despite these bottleneck events, contemporary populations in each of the invaded regions display genetic diversity and effective population sizes comparable to their European source populations (Tables 1–3, Table S4). This finding may not be too surprising however, given the large population sizes that winter moth in its invasive region,

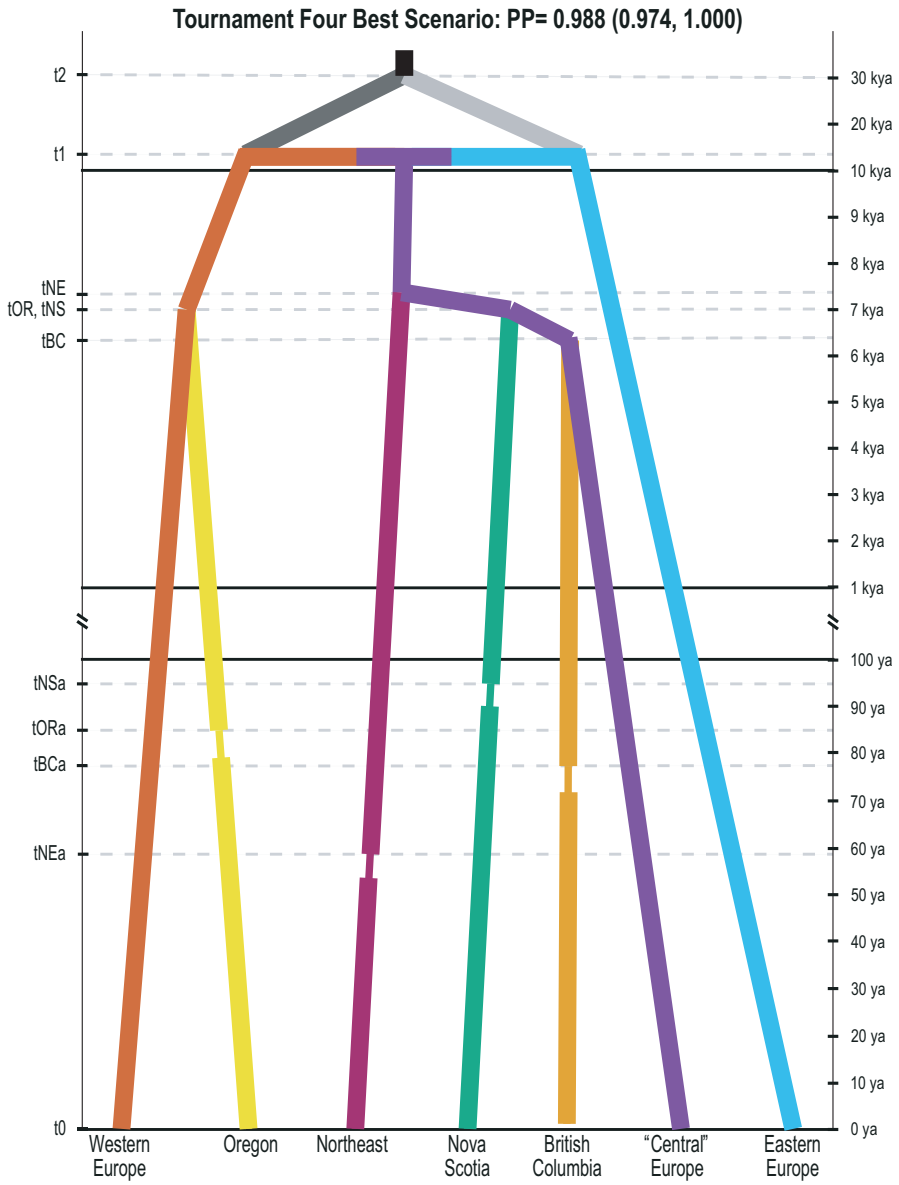

FIGURE 4 DIYABC result from the final tournament that included all four invasive populations. Time along the *y*-axis is drawn using a log10 scale. Values between 100 years ago and 1000 years ago are not shown to make the figure more compact. For each population, changes in colours represent different population size parameters (values shown in Table S3)

coupled with the complex and dynamic nature by which microsatellite regions mutate within lepidopteran genomes (Zhang, 2004) as well as the possible effect of rapid adaptation by the invasive populations to their new environment (John & Stephan, 2020). As such, winter moth represents an ideal system to conduct comparative analyses on factors that influence establishment and ecological impact as each introduced population represents a unique and independent data point.

4.3 | Distance analyses as a complement to ABC analyses

Historically, straightforward phylogenetic methods have been used to reconstruct the origins of invasive populations, particularly asexual organisms (e.g., Havill et al., 2006; Qin & Gullan, 1998). However, for sexual organisms where recombination and larger effective population sizes make the results from phylogenetic inference ambiguous, a popular workflow for determining the origin of an introduced population includes the following steps: (a) to identify distinct genetic clusters either using Bayesian algorithms such as those implemented in STRUCTURE, FASTSTRUCTURE (Raj et al., 2014), and ADMIXTURE (Alexander & Lange, 2011), and/or to use measures of genetic distance (e.g., Latreille et al., 2019; Negawo et al., 2020; Rahi et al., 2020); (b) to create a random subset of equal-numbered individuals from each genetic or geographic cluster; and (c) to compare potential introduction scenarios using approximate Bayesian computation. Unfortunately, methods for the interpretation of "admixed" populations (i.e., populations with mixed probabilities of assignment) are needed as populations with assignment to multiple genetic clusters is a common result (as reviewed in Lawson et al., 2018). We believe that distance-based clustering of the population coefficients of assignment from Bayesian clustering algorithms (such as the STRUCTURE

TABLE 4 DIYABC scenario results for each tournament

Scenario	Posterior probability	Type I error	Type II error
Tournament 1 – Source of the Nova Scotia population			
1 – Western Europe	0.068 (0.000, 0.649)	n/a	0.074
2 – Central Europe	0.865 (0.765, 0.960)	0.123	n/a
3 – Eastern Europe	0.000 (0.000, 0.628)	n/a	0.091
4 – Ghost Population	0.066 (0.000, 0.719)	n/a	0.097
Tournament 2 – Source of the Oregon population			
1 – Western Europe	0.979 (0.969, 0.989)	0.194	n/a
2 – Central Europe	0.017 (0.009, 0.026)	n/a	0.298
3 – Eastern Europe	0.000 (0.000, 0.000)	n/a	0.264
4 – Ghost Population	0.004 (0.001, 0.006)	n/a	0.122
5 – Nova Scotia	0.000 (0.000, 0.000)	n/a	0.224
Tournament 3 – Source of the British Columbia population			
1 – Western Europe	0.001 (0.000, 0.002)	n/a	0.162
2 – Central Europe	0.995 (0.990, 1.000)	0.106	n/a
3 – Eastern Europe	0.000 (0.000, 0.000)	n/a	0.063
4 – Ghost Population	0.003 (0.000, 0.007)	n/a	0.046
5 – Nova Scotia	0.001 (0.000, 0.002)	n/a	0.194
6 – Oregon	0.001 (0.000, 0.001)	n/a	0.100
Tournament 4 – Source of the Northeastern United States population			
1 – Western Europe	0.001 (0.000, 0.979)	n/a	0.101
2 – Central Europe	0.988 (0.974, 1.000)	0.154	n/a
3 – Eastern Europe	0.011 (0.000, 0.984)	n/a	0.081
4 – Ghost Population	0.000 (0.000, 0.979)	n/a	0.000
5 – Nova Scotia	0.000 (0.000, 0.979)	n/a	0.003
6 – Oregon	0.000 (0.000, 0.979)	n/a	0.003
7 – British Columbia	0.000 (0.000, 0.979)	n/a	0.026

"popfiles") presents a rapid and useful approach for the reconstruction of the regions of origins of non-native populations, particularly when populations in the native range are highly admixed and/or have limited genetic diversity, as we saw with populations of winter moth. This approach is particularly attractive in that it is almost instantaneous (that is, after clustering runs have completed). Additionally, the approach removes the need for the investigator to define arbitrary cutoffs for population assignments. For example, in instances when individuals have mixed probabilities of assignment based on Bayesian assignment (e.g., $Q \leq 0.75$ to any one cluster when averaged across STRUCTURE runs), assigning samples or populations to distinct clusters might not be possible visually but is trivial for distance-based clustering algorithms, like the one implemented in R.

4.4 | Conclusions

Here. we find that winter moth is a serial invader of North American forests and orchards, with at least four introductions from Europe. These populations were introduced from a diversity of locations in Western and Central Europe. Given the availability of a sequenced genome (Derks et al., 2015), and its historical use in population ecology (Varley & Gradwell, 1960; Varley et al., 1973), we hope that our work encourages the use of winter moth as a model organism for comparative studies of the genomic factors that influence the establishment of invasive species. Lastly, we hope that our method for the interpretation of STRUCTURE results can provide rapid and accurate inferences into the geographic regions of origins of non-native species.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of the manuscript. J.C.A. and N.P.H. performed genotyping, J.C.A. conducted analyses, A.C. provided laboratory access, and J.S.E. coordinated sample collection and secured funding.

DATA AVAILABILITY STATEMENT

Genotype scores for all samples are provided in the STRUCTURE-formatted supplemental file titled "WinterMothOriginsStructure.txt".

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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