

# 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 invasive species is critical for establishing effective management strategies. Frequently, molecular investigations are undertaken when the source population is not known, however; these analyses are constrained both by the amount of diversity present in the native region 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 (*Operophtera brumata* L.) that has caused widespread defoliation to forests, orchards, and crops in four discrete regions: Nova Scotia, British Columbia, Oregon, and the northeastern United States. It is not known whether these represent independent introductions to North America, or “stepping stone” spread among regions. Using a combination of Bayesian assignment and approximate Bayesian computation methods, we analyzed a population genetic dataset of 24 polymorphic 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 likely being introduced from France and Sweden, respectively; the Oregonian population likely being introduced from either the British Isles or northern Fennoscandia; and the population in the northeastern United States likely being introduced from somewhere in Central Europe. To our surprise, we found that hybridization has not played a large role in the establishment of winter moth populations even though previous reports have documented widespread hybridization between winter moth and a native congener. We discuss the impact of genetic bottlenecks on analyses meant to determine region of origin.

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

**Short Title:** Serial invasions of the European winter moth

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region 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 (*Operophtera brumata* L.) that has caused widespread defoliation to forests, orchards, and crops in four discrete regions: Nova Scotia, British Columbia, Oregon, and the northeastern United States. It is not known whether these represent independent introductions to North America, or “stepping stone” spread among regions. Using a combination of Bayesian assignment and approximate Bayesian computation methods, we analyzed a population genetic dataset of 24 polymorphic 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 likely being introduced from France and Sweden, respectively; the Oregonian population likely being introduced from either the British Isles or northern Fennoscandia; and the population in the northeastern United States likely being introduced from somewhere in Central Europe. To our surprise, we found that hybridization has not played a large role in the establishment of winter moth populations even though previous reports have documented widespread hybridization between winter moth and a native congener. We discuss the impact of genetic bottlenecks on analyses meant to determine region of origin.

**Keywords:** Approximate Bayesian computation, region of origins, invasive species, biosecurity

## Introduction:

In light of the unprecedented number of introductions of nonnative 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, Bagley, Roman, Tepolt, & Geller, 2008; Facon, Pointier, Jarne, Sarda, & David, 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, Alibert, Brandner, Geist, & Schliwen, 2014; Lombaert, Guillemaud, Cornuet, Malausa, Facon, & Estoup, 2010; Oficialdegui et al., 2019; Tonione, Reeder, & Moritz, 2011 for examples). Identifying which mode of introduction occurred (serial invader or stepping stone) is necessary for the study of genetic and ecological factors that drive invasion success as independent populations are necessary for robust hypothesis-based testing (Kang, Buckley, & Lowe, 2007), and this information is also crucial for focusing management efforts (Floerl, Inglis, Dey, & Smith, 2009).

When reconstructing regions of origins, and determining the numbers of introductions of a focal organism, ideally historical records should be observed and robust genetic analyses performed (e.g., Lynch & Saltonstall, 2002; Schwenk, Brede, & Streit, 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, Andonian, Swope, Luster, & Dlugosch, 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 are 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, Cellina, Krier, Schley, & Burke, 2009) or an artifact generated during the interpretation of results (Lawson, Van Dorp, & Falush, 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, Gradwell, & Hassell, 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 (Embree, 1965, 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 1930's in Nova Scotia (Embree, 1967; MacPhee, 1967; MacPhee, Newton, & McRae, 1988), the 1950's in Oregon (Kimberling, Miller, & Penrose, 1986), the 1970's in British Columbia (Gillespie, Finlayson, Tonks, & Ross, 1978), and in the 1990's in coastal regions of northeastern United States (Elkinton et al., 2010; Elkinton, Liebhold, Boettner, & Sremac, 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. Previously, a genetic examination of the invasion history of winter moth in North America was conducted, but was unable to discern these patterns due to low levels of mitochondrial DNA diversity in both introduced and native samples (Gwiazdowski, Elkinton, DeWaard, & Sremac, 2013).

To overcome this limitation, we examine the invasion history of winter moth in North America using polymorphic microsatellite loci amplified from individuals collected across its native and introduced regions. 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 role of hybridization between winter moth and a North American congener the Bruce spanworm (*O. bruceata* Hulst), and the effects of genetic bottlenecks on the establishment of invasive winter moth populations.

## Materials and Methods:

### *Sample collection*

Winter moth males were collected using pheromone-baited traps (Elkinton et al., 2010; Elkinton, Lance, Boettner, Khimian, & Leva, 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, Figure 1). In addition, larval individuals and adult females, which are wingless and do not fly, were opportunistically collected (see supplemental Appendix S1 Table S1 for complete collection information, including life stages).

After collection, adult moths were placed in glassine envelopes (Uline Corporation, USA) and stored at either -20°C 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, collection and genotype information was previously reported (e.g., Andersen, Havill, Caccone, & Elkinton, 2017; Andersen et al., 2019a,b, In Press) (Supplemental Appendix S1 Table S1).

### *Microsatellite genotyping*

Genomic DNA was extracted using the EZNA® Tissue DNA extraction kit (Omega Bio-tek; Norcross, Georgia), following the manufacturer 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.; Clifton, New Jersey) with a FastPrep-24 Sample Homogenizer (MP Biomedicals, Santa Ana, California). After extraction, 24 polymorphic microsatellite loci (Havill et al., 2017) were genotyped at the DNA Analysis Facility on Science Hill at Yale University, using a Thermo Fisher Scientific 3730xl DNA Analyzer. 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; Waltham, Massachusetts).

Only individuals from which [?] 20 microsatellite loci were successfully amplified were included in analyses.

In addition, since winter moth has been reported to hybridize with Bruce spanworm in all of its invaded regions (Andersen et al., 2019a) the dataset was further filtered to remove hybrids by comparing assignment probabilities based on 12 polymorphic loci that co-amplify in both species (described below). Microsatellite genotypes are provided as a tab-delimited Structure-formatted supplemental file titled “WinterMothOriginsStructure.txt”.

### *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).

### *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 dataset presented in Andersen et al. (2019a), 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, Stephens, & Pritchard 2003; Pritchard, Stephens, & Donnelly, 2000). These analyses were based on the analysis of 12 polymorphic microsatellite loci that co-amplify 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 burn-in periods of 100,000 generations. Results were then summarized across runs using Clumpak (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 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 dataset, and the filtered dataset ([?] 20 polymorphic 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 dataset, the [?]  $K$  statistic (Evanno, Regnaut, & Goudet, 2005) was calculated in StructureHarvester (Earl & vonHoldt, 2012), and independent runs were again summarized for major and minor partition schemes using Clumpak.

For each value for [?]  $K$  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.

### *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, In Press) using approximate Bayesian computation (ABC), as implemented in the software program DiyABC v.2.1.0 (Cornuet et al., 2008). For these analyses, thirty individuals were selected randomly from each of the three European clusters and from each of the four invasive regions. Ideally, we would be able to perform comparisons of all possible scenarios that include representatives from all native clusters and invasive regions, however; the number of possible scenarios increases at an unmanageable rate with each taxon added (e.g., there are 10,395 possible “scenarios” in a seven-taxa analysis). Therefore, we utilized an approach similar to “tournament-ABC” 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 likely 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 Supplemental Appendix 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, and included the potential for a genetic bottleneck for each invasive population, default mutation model parameters, the minimum mean mutation rate set to  $1 \times 10^{-5}$ , and maximum values for the mean and individual locus coefficient  $P$ 's were both set to 1.0. As per Andersen et al. (2017, In Press), we removed four loci with especially large allelic ranges (02339, 00925, 02191, and 12042) to improve the shape of the cloud of simulated datasets. 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  $[d_{ij}]^2$  distance). Φορ εαση τουρναμεντ, της σσεναριο ρεπρεσεντινγ της ανεστραλ οριγιν οφ εαση ινασιε ποπυλατιον ως δετερμινεδ βψ ζομπαρινγ της ρεσουλτς φρομ της Λογιστις Ρεγρεσσιοντεστ ιμπλεμεντεδ ιν ΔιψΑΒ<sup>α</sup> βασειδ ον ζομπαρισονς οφ 1% οφ σιμυλατεδ δατασετς ζλοσεστ το της οβσερεδ δατα. Μοδελ ζηεσκινγ φορ εαση τουρναμεντ ως περφορμεδ βψ ζομπαρινγ της ρεσουλτς φρομ α πρινσιπλε ζομπονεντς αναλψσις (Π<sup>α</sup>Α) τηατ ινζλυδεδ της συππορτεδ σσεναριο ας παρτ οφ της Περφορμ Μοδελ ηεσκινγ αναλψσις.

## Ρεσουλτς:

### Μικροσατελλιτε γενοτυπινγ

Αφτερ φιλτερινγ το ινζλυδε ονλψ ινδιδυαλς ωιτη γενοτυπς σεσορες φρομ  $[;]$  20 μικροσατελλιτε λοσι, 1,588 σαμπλες ωερε ρεταινεδ φορ συβσεχεντ αναλψσες. Ωηνεν της Νορτη Αμερισαν σαμπλες ωερε αναλψζεδ υσινγ της 12 πολψμορπηις μικροσατελλιτε λοσι τηατ ζο-αμплиφβ βετωεεν Βρυσε σπανωορμ ανδ ωιντερ μοτη, φοур ινδιδυαλς ωερε ζλασσифιεδ ας βεινγ Βρυσε σπανωορμ ανδ φοур ινδιδυαλς ωερε ιδεντιφιεδ ας προβαβλε ηψβριδς. Τηε φιναλ δατασετ τηερεφορε ινζλυδεδ 1,580 πυρε ωιντερ μοτη ινδιδυαλς.

### Ποπυλατιον γενετις στατιστις

Στανδαρδ ποπυλατιον γενετις στατιστις ωερε ζαλζυλατεδ φορ 53 ποπυλατιονς φρομ της νατιε διστριβυτιον οφ ωιντερ μοτη, ας ωελλ ας φρομ εαση οφ της φοур ινασιε Νορτη Αμερισαν ποπυλατιονς (Συπλεμενταλ Αππενδιξ Σ1 Ταβλε Σ2). Ποπυλατιονς ηαδ ον αεραγε 7.8 αλλελες περ λοσυς ( $\pm 1.71$  alleles per locus), with the invasive populations having equal to, or greater allelic diversity than the native populations (average of  $8.06 \pm 2.05$  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).

### Bayesian sample assignment

On average, negative log-likelihood scores for the independent Structure runs increased from  $K = 2$  through  $K = 13$ , before decreasing at  $K = 14$  (Supplemental Appendix S1 Figure S5). Based on the  $[?]K$  method implemented in StructureHarvester, the optimal partition scheme was determined to be  $K = 3$  (Supplemental Appendix S1 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  $K = 14$  are presented in Supplemental Appendix S1 Figure S7. 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 Supplemental Appendix S1 Figure S8, Figure 3, and Supplemental Appendix S1 Figure S9, respectively.

### *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). 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 (Supplemental Appendix S1 Figure S10a), and this relationship received high support based on logistic regression analysis ( $P = 0.98$ ). The Oregon population most likely originated from Western Europe (Supplemental Appendix S1 Figure S10b), and this relationship received high support based on logistic regression analysis ( $P = 0.96$ ). The British Columbia population most likely represents an additional independent introduction from Central Europe (Supplemental Appendix S1 Figure S10c), and this relationship received high support based on the logistic regression analysis ( $P = 1.00$ ). The population in the northeastern United States population was also reconstructed as a third introduction from Central Europe (Figure 4), and this relationship was also highly supported ( $P = 1.00$ ). Based on examinations of the posterior estimates, each of the invasive populations experienced a strong genetic bottleneck when they diverged from European populations ~100 ya (Supplemental Appendix S1 Table S3). Visualization of the PCAs for the supported scenario from each tournament based on the *Perform Model Checking* analysis in DiyABC, indicated that posterior values from simulated datasets formed a distinct cloud encompassing the sample dataset (Supplemental Appendix S1 Figures S11 through S14).

### **Discussion:**

Reconstructing the invasion pathways for non-native pests represents a critically important step for the prevention of additional introductions (Le Roux & Wicczorek, 2009), and can help the implementation of biosecurity programs for particular pest species and their relatives by identifying the most likely entry routes (e.g., Maxwell, Vettraino, Eschen, & Andjic, 2014). Moreover, reconstruction of the invasion history is crucial for effective biological control, as it enables the identification of regions from which to evaluate natural enemies (e.g., Goolsby et al., 2006). For evolutionary and ecological studies of 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), identifying the number of introductions of an invasive organism is critical (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, Bonin, & Miaud, 2008; McEvoy, Higgs, Coombs, Karcetin, & Starcevich, 2012; Mesgaran et al., 2016; Prentis, Wilson, Dormontt, Richardson, & Lowe, 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 Ugdevallen, 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, with the Oregon population being closely related to a large number of populations from the British Isles and northern Fennoscandia, and the northeastern United States population being unrelated to any sampled European population. In a recent study of winter moth in Fennoscandia, we determined that the population there was introduced to the region ~2,000 ya from the British Isles, either via dispersal across the North Sea or by human-mediated dispersal (Andersen et al. In Press). Therefore, without finer-scale genomic analyses (e.g., genotype-by-sequencing), we will unlikely 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 likely 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 [?] 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 (NPH unpublished data). Therefore, we believe that the genetic distinctness of the northeastern United States population is likely the result of a strong genetic bottleneck and/or rapid-local adaptation following its introduction from Central Europe.

### *Hybrid origins*

Hybridization has long been known to play an important role in the evolutionary trajectories of species and populations (Allendorf, Leary, Spruell, & Wenburg, 2001), and the promotion of the establishment of invasive species (e.g., Allendorf & Lundquist, 2003; Benvenuto, Cheyenne-Buchmann, Bermond, Ris, & Xavier, 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., 2019a). 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, Chandler, Andersen, Havill, & Elkinton, In Press). Therefore, we were surprised that only four individuals among our samples were classified as hybrids, suggesting that hybridization has not played a role in the establishment of winter moth populations, however, this result could be an artifact of our analyses given that only 12 of the sampled 24 loci have been shown to consistently co-amplify between the two species (Havill et al., 2017). Alternatively, given that the only hybrid individuals were detected in Oregon, a region from which winter moth has virtually disappeared after its initial establishment, it is also possible that some type of hybridization to extinction may be occurring in this system. This possibility needs to be examined further, perhaps with genome scans to look for introgression between the species, as it could present an example of hybridization between native and non-native species having a positive impact, counter to the “hybridization to extinction” paradigm that puts the overwhelming emphasis on risks to native species (Rhymer & Simberloff, 1996).

### *The 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 of instances where multiple introductions have been important for overcoming propagule pressures (Kolbe et al., 2004; Lavergne & Molofsky, 2007; Simberloff, 2009) and even an example of how bottlenecks may have aided the establishment of an invasive species (Tsutsui, Suarez, Holway, & Case, 2000). Interestingly, here we find evidence for multiple introductions of winter moth to North America, and that all four invasive populations have experienced dramatic bottlenecks in terms of the reductions of their effective population sizes (Supplemental Appendix S1 Table S3). Yet, we find no evidence for reductions in genetic diversity of these populations, as all invasive populations had as great or greater allelic diversity than populations found from across Europe (Supplemental Appendix S1 Table S2). 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.

### *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, Montgomery, Yu, Shiyake, & Caccone, 2006; Qin & Gullan, 1998). However, for sexual organisms where recombination and larger effective population sizes makes the results from phylogenetic inference ambiguous, a popular workflow for determining the origin of an introduced population includes the following steps: 1) to identify distinct genetic clusters either using Bayesian algorithms such as those implemented in Structure, FastStructure (Raj, Stephens, & Pritchard, 2014), and Admixture (Alexander & Lange, 2011), and/or to use measures of genetic distance (e.g., Latreille, Milesi, Magalon, Mavingui, & Atyama, 2019; Negawo et al., 2020; Rahi et al., 2020); 2) to create a random subset of equal-numbered individuals from each genetic or geographic cluster; and 3) 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 ‘popfiles’) presents a rapid and useful approach for the reconstruction of the regions of origins of nonnative 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 (after clustering runs have completed that is). 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.,  $Q$  [?] 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.

### *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. Most notable is the fact that populations in Nova Scotia and the Northeastern United States are genetically distinct from each other, despite having only ~ 300 km separating surveyed populations in Maine and Nova Scotia. 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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### Data Accessibility:

Genotype scores for all samples are provided in the Structure-formatted supplemental file titled "Winter-MothOriginsStructure.txt".

### Author Contributions:

All authors contributed equally to the writing of the manuscript. JCA and NPH performed genotyping, JCA conducted analyses, AC provided laboratory access, and JSE coordinated sample collection and secured funding.

### Tables and Figures:

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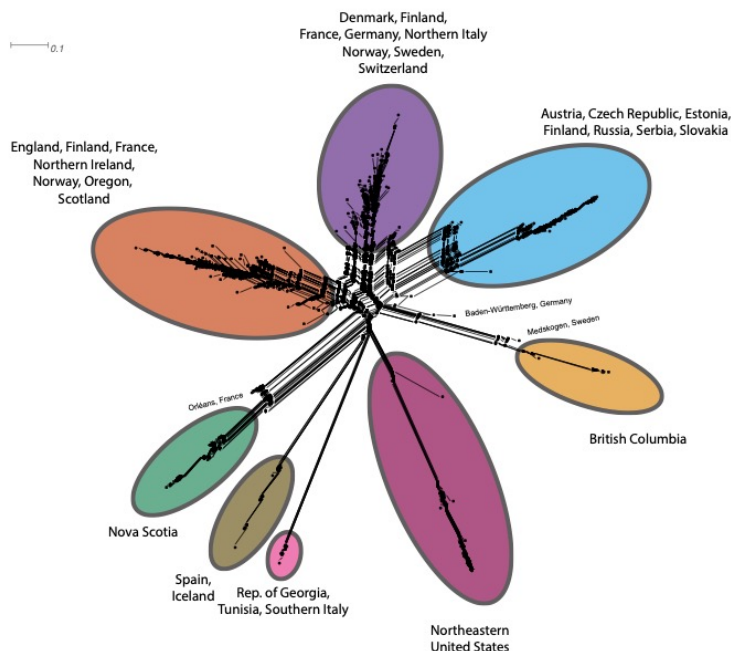
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**Figure 1.** Winter moth sample localities in the Pacific Northwest (top left); northeastern United States and Nova Scotia (top right); and in Europe, North Africa, and western Asia. Sample localities are colored following the results presented in Figure 3, except that the Icelandic, Spanish, Tunisian, southern Italian, and Georgian populations are shaded roughly following their assignments for  $K = 13$  in Figure 2.

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**Figure 2.** Major modes detected using CLUMPAK for supported partitions based on the results presented in Supplemental Appendix S1 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).



**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.

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**Figure 4.** DiyABC result from the final tournament that included all four invasive populations. Time along the y-axis is not drawn to scale. For each population, changes in colors represent different population size parameters (values presented in Supplemental Appendix S1 Table S3).

#### Supplemental Appendix S1:

**Table S1.** Locality information

**Table S2.** Population genetic summary statistics

**Table S3.** DiyABC Posterior estimates for Tournament 4

**Figure S1.** Graphical representation of scenarios from DiyABC Tournament 1

**Figure S2.** Graphical representation of scenarios from DiyABC Tournament 2

**Figure S3.** Graphical representation of scenarios from DiyABC Tournament 3

**Figure S4.** Graphical representation of scenarios from DiyABC Tournament 4

**Figure S5.** Negative log-likelihood results for Structure runs from  $K = 2$  through  $K = 14$

**Figure S6.** Results from the  $[?]K$  method implemented in StructureHarvester

**Figure S7.** Major Modes for all values of  $K$

**Figure S8.** NeighborNet for  $K = 6$

**Figure S9.** NeighborNet for  $K = 13$

**Figure S10.** DiyABC supported Scenarios from Tournaments 1, 2, and 3.

**Figure S11.** PCA of posterior values for Tournament 1

**Figure S12.** PCA of posterior values for Tournament 2

**Figure S13.** PCA of posterior values for Tournament 3

**Figure S14.** PCA of posterior values for Tournament 4