Phylogeographic Diversity of the Winter Moths *Operophtera brumata* and *O. bruceata* (Lepidoptera: Geometridae) in Europe and North America

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ABSTRACT The European winter moth, *Operophtera brumata* (L.), an invasive forest defoliator, is undergoing a rapid range expansion in northeastern North America. The source of this invasion, and phylogeographic diversity throughout its native range, has not been explored. To do this, we used samples from a pheromone-baited trap survey of *O. brumata* collected across its native range in Europe, and invasive range in North America. Traps in North America also attract a congeneric species, the Bruce spanworm *O. bruceata* (Hulst), and the western Bruce spanworm *O. b. occidentalis* (Hulst). From this sampling, we sequenced two regions of the cytochrome *c* oxidase subunit I mitochondrial gene; one region corresponds to the DNA 'barcode' region, the other is a nonoverlapping section. We used these sequences, in combination with sequence data from a recent survey of the Geometridae in western North America, for phylogenetic and phylogeographic analyses to characterize genetic divergence and variation for *O. brumata* in North America and Europe, and *O. bruceata* and *O. b. occidentalis* in North America. We found *O. brumata* mtDNA diversity to be dominated by a single widespread, and common haplotype. In contrast, *O. bruceata* shows high haplotype diversity that is evenly distributed throughout North America. Phylogeographic patterns indicate an introduction of *O. brumata* in British Columbia likely originated from Germany, and suggest the invasive population in northeastern North America may have its origins in the United Kingdom, and/or Germany. We found uncorrected pairwise sequence divergence between *Operophtera* species to be ≈7%. *O. b. occidentalis* is ≈ 5% divergent from *O. bruceata*, has a restricted range in the Pacific Northwest, and has unique morphological characters. Together these lines of evidence suggest *O. b. occidentalis* may be deserving of species status. Additionally, a single morphologically unique *Operophtera* specimen, similar to *O. bruceata*, was collected in southern Arizona, far outside the known range of *O. bruceata.* This suggests that North America may contain further, unsampled, *Operophtera* diversity.

KEY WORDS DNA barcoding, invasive species, winter moth, *Operophtera brumata*, *Operophtera bruceata occidentalis*

The winter moth, *Operophtera brumata* (L.) is a forest defoliator native to Europe and Asia that has become invasive in North America (Troubridge and Fitzpatrick 1993). Despite its detection in Nova Scotia in the 1930s (Embree 1965) it is only in the past decade that this species has rapidly expanded its range in the northeastern United States — causing persistent and widespread defoliation in eastern Massachusetts and nearby states (Elkinton et al. 2010). Outbreaks of winter moth have occurred in two other regions of North America, namely Nova Scotia during the 1950s (Cuming 1961), and the Pacific Northwest during the 1970s (Kimberling et al. 1986). In each case, a decade-long outbreak was successfully and permanently controlled by the introduction of two parasitoids from Europe: the tachinid fly, *Cyzenis albicans* (Fallén), and the ichneumonid wasp, *Agrypon flaveolatum* (Gravenhorst). Efforts are now underway to establish *C. albicans* in New England (J.S.E. et al., unpublished data).

In North America, winter moth co-occurs with its congener the Bruce spanworm, *Operophtera bruceata* (Hulst). Males of both species respond to the same pheromone compound (Roelofs et al. 1982), and are captured in the same pheromone-baited survey traps (Elkinton et al. 2010). Males of both species are difficult to tell apart based on wing patterns, but are readily distinguished by their genitalia (Eidt et al. 1966). However, Elkinton et al. (2010) found some individuals with 'intermediate' genitalia in central Massachusetts, where the two species overlap, and this observation is based on several thousand moths collected over 3 yr as part of an extensive pheromone trap

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survey conducted across North America. Additionally, Elkinton et al. (2010) used nuclear DNA analysis to demonstrate the existence of F1 hybrids in their pheromone trap survey. While the hybrids formed a very small percentage (1.9%) of the recovered moths, this result agrees with earlier lab-based hybridizations (Hale 1989, Troubridge and Fitzpatrick 1993). Although the Bruce spanworm and winter moth appear to share the same pheromone and hybridize, they differ in their North American distributions. The Elkinton et al. (2010) pheromone trap survey revealed that Bruce spanworm is widespread, occupying interior regions of Maine and New Brunswick, where winter moth is absent despite its long-term presence nearby in Nova Scotia. Elkinton et al. (2010) proposed that these distributions arise because Bruce spanworm is more cold tolerant than winter moth, and this pattern agrees with a recent study demonstrating that cold-temperature limits the distribution of winter moth in Norway (Jepsen et al. 2008). In addition, Bruce spanworm only rarely causes outbreaks (Rose and Lindquist 1982), whereas winter moth populations in New England are currently in perpetual outbreak phase (J.S.E. et al., unpublished data). These outbreaks are similar to those that occurred in Nova Scotia before the establishment of *C. albicans* (Embree 1965).

In western North America a subspecies of Bruce spanworm, *Operophtera bruceata occidentalis* (Hulst), occurs mainly west of the Cascade mountains in Oregon, WA, and British Columbia (Troubridge and Fitzpatrick 1993). *O. b. occidentalis* was originally described as the species *O. occidentalis* (Hulst 1896) but was subsumed as a subspecies of *O. bruceata* by Troubridge and Fitzpatrick (1993), despite possessing distinct morphological traits.

While the history of *O. brumata* outbreaks in North America is well documented (see Troubridge and Fitzpatrick [1993]), the sources and number of invasions are unknown. To explore this situation, we provided pheromone traps to colleagues throughout the range of *O. brumata* in Europe, Japan, and throughout the ranges of *O. brumata*, and *O. bruceata* in North America. Here we used specimens from this extensive sampling effort to describe phylogeographic diversity within both species and infer their phylogenetic relationships relative to other *Operophtera* species by sequencing two regions of cytochrome *c* oxidase subunit I (COI) mitochondrial DNA. We used our results to infer the potential number, and origin of invasions of *O. brumata* to North America, and explore the possibility for cryptic diversity in both *O. brumata* and *O. bruceata.*

Materials and Methods

Sampling. *Specimen Collection.* The majority of specimens are the species *O. brumata*, *O. bruceata*, and the subspecies *O. b. occidentalis*, that were collected as part of a recent, geographically extensive pheromone trap survey across North American and Europe reported in Elkinton et al. (2010). Initial identification of male moths was based on dissection of male genitalia as described by Elkinton et al. (2010) as well as hind wing and abdominal characteristics as described by Troubridge and Fitzpatrick (1993). We also included specimens of *O. rectipostmediana* (Inoue) collected from Japan. All specimens were collected in pheromone traps and were transferred to individual vials and stored at −80°C upon arrival at the University of Massachusetts, Amherst, with the exception of *O. brumata* from Norway, which were collected by J.S.E., and frozen as larvae.

DNA Sequence Collection. Mitochondrial (mtDNA) sequence variation has been shown to correspond to both morphological and nuclear DNA species-specific differences between *O. brumata* and *O. bruceata* (Elkinton et al. 2010). We used mtDNA sequences from several sources: novel sequences generated for this study (described below), those reported from Elkinton et al. (2010), those from a DNA barcode survey of geometrids in western Canada (deWaard et al. 2011), those from a DNA barcode survey of geometrids in Bavaria, Germany (Hausmann et al. 2011), as well as sequences from other species of *Operophtera* and other geometrids from GenBank. Several geometrid species were used as outgroups for phylogenetic analysis (described below), and these species were chosen based on relatively close, and more distant relationships as inferred in a recent phylogeny of the Geometridae (Yamamoto and Sota 2007). Sequences generated for this study were obtained as described below.

Genomic DNA was extracted from individual moth heads using the DNEasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The remainder of the body was retained at −80°C. We used polymerase chain reaction (PCR) to amplify two fragments of the mitochondrial gene cytochrome *c* oxidase subunit I (COI). One fragment corresponds to the mtDNA barcode region (Hebert et al. 2003), and the other fragment begins following the 3′ end of the barcode region; these fragments do not overlap. The barcode fragment used for analysis is a 615 bp fragment, and the additional COI fragment has 736 bp. Respectively, these fragments correspond to bases 11,886 through 12,501, and bases 12,616 through 13,352 in the *Bombix mori* (L.) mitochondrial genome (NCBI Reference Sequence NC_002355.1).

For the amplification of the barcode region, a 20 μl reaction mixture contained 10 μl of Qiagen HotStarTaq Master Mix (Cat. No 203446, Qiagen GmbH D-40724 Hilden), 2 μl of 10 μM forward primer LCO-1490 (Folmer et al. 1994) and 2 μl of 10 μM reverse primer HCO-2198 (Folmer et al. 1994), 0.8 μl of 25 mM $MgCl_2$, 3 μl of template DNA and 2.2 μl of sterile dH_2O. Amplification of the second gene fragment used the same 20 μl reaction mixture with the forward primer 'Jerry' (Simon et al. 1994) and the reverse primer 'Pat' (Simon et al. 1994). All amplifications were performed with a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) using the following conditions: 15 min at 95°C followed by 30 cycles of 30 s at 94°C, 30 s at 56°C for the

barcode region, and 52°C for the second fragment, 1 min at 72°C, and a final extension for 10 min at 72°C. PCR products were cleaned using Exo SAP-IT enzymatic digestion (USB Corporation, Cleveland, OH). Sequencing of PCR products in both directions was performed on a 3730 DNA Analyzer (Applied Biosystems) by Laragen, Inc. (Los Angeles, CA). Sequences were edited using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI). Alignment of the barcode and additional COI fragments was unambiguous.

Molecular Analyses. *mtDNA Phylogeny.* For all analyses, each unique haplotype is represented by a single sequence. Haplotypes are given a number designation relative to the individuals in that haplotype (e.g., haplotype 1 [H1] has the most individuals, haplotype 2 [H2] has the second most, etc.). The majority of specimens in our dataset are only represented by the barcode locus. To take advantage of any additional information provided by specimens for which we have the additional COI sequence, we include haplotypes based on the concatenated barcode plus additional COI sequence. We refer to these concatenated sequences as barcode+, which have the same base haplotype designation as the barcode haplotype (e.g., H1).

We conducted parsimony and Bayesian analyses to estimate phylogenetic relationships among several *Operophtera* species. Parsimony phylogenies were conducted with PAUP 4.0b10 (Swofford 2003) using a heuristic search with 10 random-addition-sequence starting trees per step and tree–bisection–reconnection branch swapping, and the maxtrees option equal to 500. Bayesian phylogenies were estimated using BEAST 1.6.1 (Drummond and Rambaut 2007). In this analysis, the loci were concatenated and partitioned by codon. All partitions were analyzed using a HKY substitution model (four gamma categories), with a constant size coalescent tree prior under the assumption of a strict clock, randomly generated starting tree, and a piecewise linear and constant root population size model. All other priors and operators were left at default values. All analyses were run on Mac Pro quad core computers of the Biology Computer Resource Center at the University of Massachusetts. Samples from the posterior were taken every 1×10^3 steps and chains were run for 5×10^7 steps. Posterior distributions for each analysis were summed from three independent chains after removing the first 25% of samples as burn-in from each chain. Convergence for individual runs was visually inspected using the program Tracer v1.5 (Rambaut and Drummond 2007). Posterior probabilities were also evaluated in Tracer, based on the effective sample size (ESS) where an ESS of >200 suggests sufficient sampling from the posterior (Ho and Lanfear 2010).

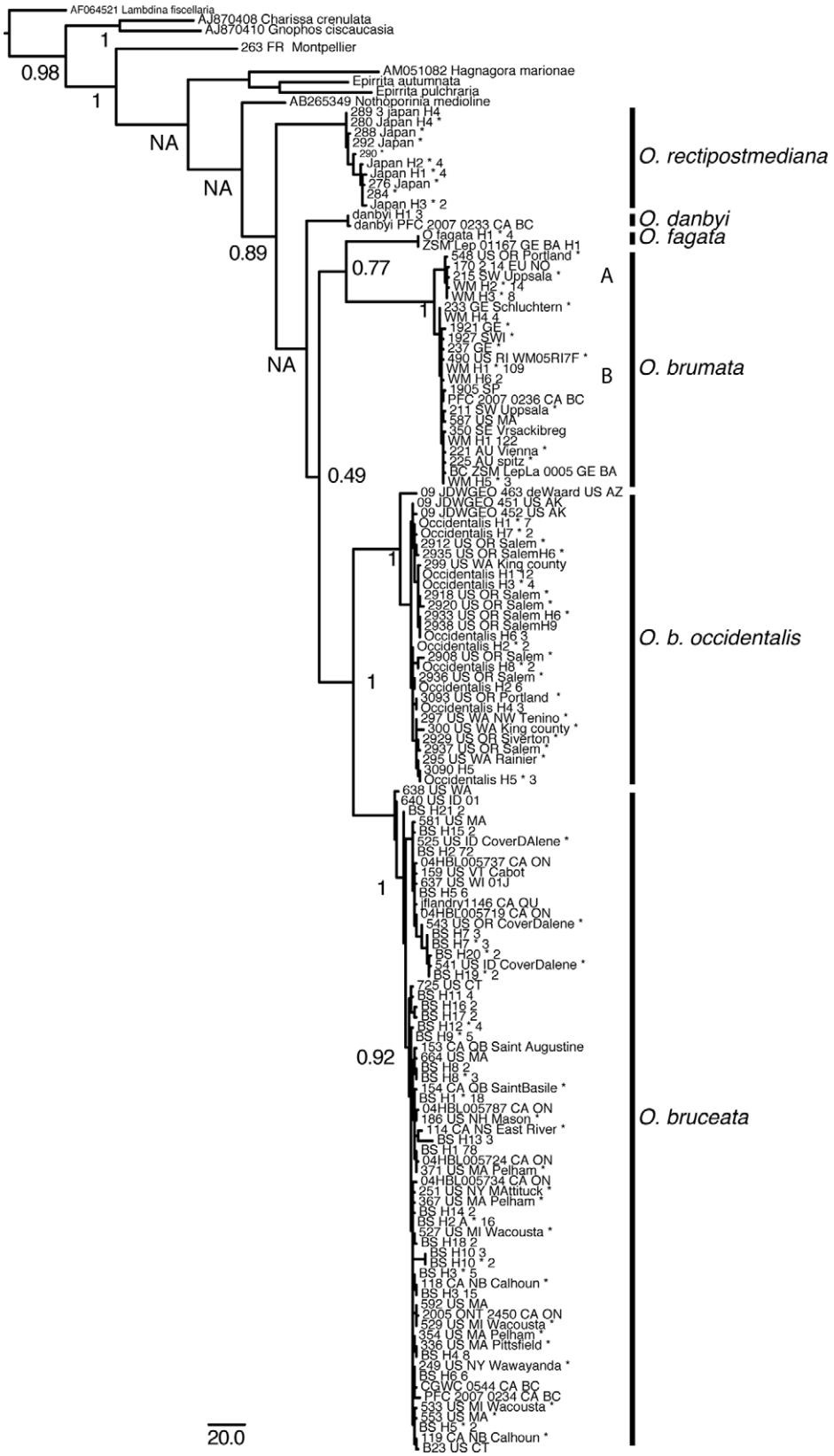
mtDNA Sequence Divergence. We characterized phylogenetic diversity in terms of potential species diversity based on sequence divergence within *Operophtera.* To do this, we used COI sequences to calculate pairwise uncorrected *P* values between the five nominate species of *Operophtera*, as well as several outgroup taxa and divergent *Operophtera* specimens. Pairwise comparisons were calculated using PAUP* 4.0b10 (Swofford 2003).

mtDNA Haplotype Networks. We diagramed haplotype networks for *O. brumata* collected from North America, and throughout the native range in Europe. We also diagramed networks for *O. bruceata* throughout North America, and *O. b. occidentalis* from the Pacific Northwest. Haplotype distances for both barcode and barcode+ sequences were calculated using Arlequin 3.5 (Excoffier and Lischer 2010) and initial networks were calculated using HapStar (Teacher and Griffiths 2011). Networks were edited so the area of a circle representing a haplotype is directly proportional to the number of individuals with that haplotype. The maps used in Fig. 1 are available from Wikimedia commons (http://en.wikipedia.org/wiki/File:North_america98.svg and http://en.wikipedia.org/wiki/File:Location_European_nation_states.svg). The specimens for which we have an additional fragment of COI (barcode+), offer a complimentary dataset that may provide additional resolution for a location of origin, and we calculated separate networks for the barcode and the concatenated barcode+ datasets.

Results

We used 632 barcode fragments of COI comprising 327 *O. brumata* specimens collected from 10 European countries, and 10 North American states and provinces, along with 305 *O. bruceata* specimens collected from 19 North American states and provinces. Specimens that we had both barcode and the additional COI fragment comprised 177 *O. brumata* from 8 European countries as well as 14 states and provinces in North America, and 80 *O. bruceata* from 16 states and provinces in North America. All novel sequences generated for this study have been deposited in GenBank and the Barcode of Life Data System (BOLD: dx.doi.org/10.5883/DS-PDOTWM, www.barcodinglife.org). The GenBank accession numbers for the barcode locus are JQ615968 - JQ616489, and the additional COI locus are JQ616490–JQ616772. Individual GenBank and BOLD accession numbers, along with all locality and ancillary information, are provided in supplementary Table 1 (available online only).

Phylogeographic Diversity. The overall topology of our mtDNA phylogeny (Fig. 1) is consistent with the recent geometrid phylogeny by Yamamoto and Sota (2007) where almost all specimens identified as *Operophtera* are placed within a monophyletic Larentiinae. A single moth (specimen 263) tentatively identified as "Operophtera sp." was placed among the outgroup taxa. Subsequent querying of specimen 263's COI barcode region on BOLD resulted in a perfect match to specimens of *Alsophila aceraria* Denis & Schiffermüller 1775, a distantly related geometrid moth that likewise flies late in the season. The parsimony analysis produced an overall topology that is consistent across all most parsimonious trees, but conflicts in several


AF064521 Lambdina fiscellaria
AJ870408 Charissa crenulata
AJ870410 Gnophos ciscaucasia
263 FR Montpellier
AM051082 Hagnagora marionae
Epirrita autumnata
Epirrita pulchraria
AB265349 Nothopoprinia medioline
289 3 japan H4
280 Japan H4 *
288 Japan *
292 Japan *
290 *
Japan H2 * 4
Japan H1 * 4
276 Japan *
284 *
Japan H3 * 2
danbyi H1 3
danbyi PFC 2007 0233 CA BC
O fagata H1 * 4
ZSM Lep 01167 GE BA H1
548 US OR Portland *
170 2 14 EU NO
215 SW Uppsala *
WM H2 * 14
WM H3 * 8
233 GE Schluchtern *
WM H4 4
1921 GE *
1927 SWI *
237 GE *
490 US RI WM05RI7F *
WM H1 * 109
WM H6 2
1905 SP
PFC 2007 0236 CA BC
211 SW Uppsala *
587 US MA
350 SE Vrsackibreg
WM H1 122
221 AU Vienna *
225 AU spitz *
BC ZSM LepLa 0005 GE BA
WM H5 * 3
09 JDWGEO 463 deWaard US AZ
09 JDWGEO 451 US AK
09 JDWGEO 452 US AK
Occidentalis H1 * 7
Occidentalis H7 * 2
2912 US OR Salem *
2935 US OR SalemH6 *
299 US WA King county
Occidentalis H1 12
Occidentalis H3 * 4
2918 US OR Salem *
2920 US OR Salem *
2933 US OR Salem H6 *
2938 US OR SalemH9
Occidentalis H6 3
Occidentalis H2 * 2
2908 US OR Salem *
Occidentalis H8 * 2
2936 US OR Salem *
Occidentalis H2 6
3093 US OR Portland *
Occidentalis H4 3
297 US WA NW Tenino *
300 US WA King county *
2929 US OR Siverton *
2937 US OR Salem *
295 US WA Rainier *
3090 H5
Occidentalis H5 * 3
638 US WA
640 US ID 01
BS H21 2
581 US MA
BS H15 2
525 US ID CoverDAlene *
BS H2 72
04HBL005737 CA ON
159 US VT Cabot
637 US WI 01J
BS H5 6
iflandry1146 CA QU
04HBL005719 CA ON
543 US OR CoverDalene *
BS H7 3
BS H7 * 3
BS H20 * 2
541 US ID CoverDalene *
BS H19 * 2
725 US CT
BS H11 4
BS H16 2
BS H17 2
BS H12 * 4
BS H9 * 5
153 CA QB Saint Augustine
664 US MA
BS H8 2
BS H8 * 3
154 CA QB SaintBasile *
BS H1 * 18
04HBL005787 CA ON
186 US NH Mason *
114 CA NS East River *
BS H13 3
BS H1 78
04HBL005724 CA ON
371 US MA Pelham *
04HBL005734 CA ON
251 US NY MAttituck *
367 US MA Pelham *
BS H14 2
BS H2 A * 16
527 US MI Wacousta *
BS H18 2
BS H10 3
BS H10 * 2
BS H3 * 5
118 CA NB Calhoun *
BS H3 15
592 US MA
2005 ONT 2450 CA ON
529 US MI Wacousta *
354 US MA Pelham *
336 US MA Pittsfield *
BS H4 8
249 US NY Wawayanda *
BS H6 6
CGWC 0544 CA BC
PFC 2007 0234 CA BC
533 US MI Wacousta *
553 US MA *
BS H5 * 2
119 CA NB Calhoun *
B23 US CT
1
0.98
1
NA
NA
0.89
0.77
1
NA
0.49
1
1
1
0.92
A
B
O. rectipostmediana
O. danbyi
O. fagata
O. brumata
O. b. occidentalis
O. bruceata
20.0

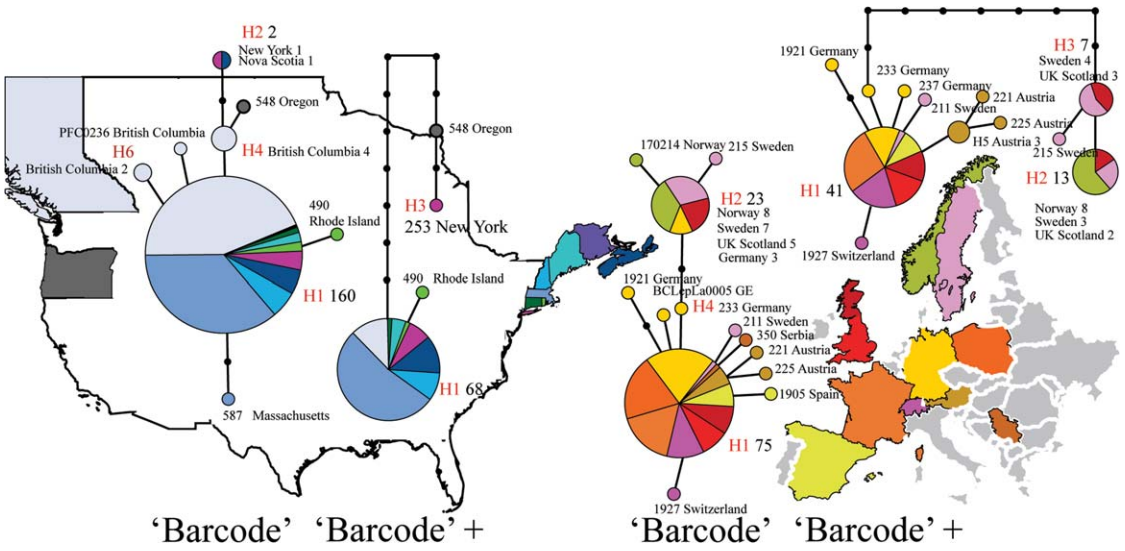


Fig. 2. Haplotype networks for *Operophtera brumata* collected in North America and Europe. The two sets of networks display the proportion of the same genotypes collected on the respective continent. Each circle is a unique haplotype, and the area of each circle is directly proportional to the number of specimens sharing that haplotype. Colors in each circle correspond to the proportion and geographic location of specimens sharing that haplotype. Each line segment connecting haplotypes is proportional to single character state changes (mutations) between haplotypes. Haplotype designations (e.g., H1) and the number of specimens in that haplotype are placed adjacent to the haplotype. Haplotype designations occurring in both the United States and Europe are indicated in red. Networks for each continent are divided into networks for the barcode region, as well as the combined barcode and barcode+ regions, to visualize any additional information added by the barcode+ region. (Online figure in color.)

places with a Bayesian consensus topology. The parsimony nodes supporting the *Hagnagora*/*Epirrita–Nothoporinia* split are reversed in the Bayesian consensus topology that also places *O. danbyi* (Hulst) as basal to the rest of the *Operophtera*, and shows poor resolution for the *O. fagata* (Scharfenberg)/*O. brumata–O. bruceata* split. Nodes that are well supported by both parsimony and Bayesian analysis distinguish two clades within *O. brumata*, a distinction between specimens recognized as *O. bruceata* and *O. bruceata occidentalis*, and a distinction between *O. b. occidentalis*, and a single specimen collected (in Arizona) from far outside the known range of *O. b. occidentalis* (Troubridge and Fitzpatrick 1993).

The population structure within our sampling of European *O. brumata* roughly corresponds to a geographic division where most haplotypes of clade A (Fig. 2) are predominantly from Norway, Scotland, and Sweden. However, three individuals of H2, from the study of Hausmann et al. (2011) are recovered from Bavaria, Germany. The individuals from clade A that occur in North America were only collected in Nova Scotia, and Long Island, NY. Clade B contains the remainder of the diversity recovered from Europe and the United States, including H1, which was present in all sampled localities except for Norway. The geographic distribution of all *O. brumata* haplotypes is presented in Fig. 1. The widespread H1 (clade B) was also present in Sweden, Scotland, and Germany along with haplotypes from clade A.

Within clade B, we found a haplotype (H4), which was only recovered from British Columbia and Germany. Several unique *O. brumata* haplotypes occur only in North America (H6, 490, PFC 2007, and 587). These samples are phylogenetically closely related to H1 (Fig. 2), suggesting they could also occur throughout most of southern Europe.

The species *O. bruceata* and subspecies *O. b. occidentalis* both show extensive haplotype diversity relative to *O. brumata* (Figs. 1–4), and are phylogenetically well supported as distinct clades by both Bayesian and parsimony analyses. Within *O. bruceata*, some haplotypes are unique to the Pacific Northwest and Alaska, but the overall lack of phylogeographic structure in our sampling (Fig. 2) is consistent with a large population occurring across most of North America (but see Bazin et al. 2006). Within *O. b.*

Fig. 1. Mitochondrial phylogeny of *Operophtera* and outgroup taxa. Shown is a maximum parsimony topology with node labels indicating presence and support of those nodes in a Bayesian analysis. NA indicates a node not present in the Bayesian analysis. Haplotypes are designated by haplotype number, and followed by the number of specimens in that haplotype (e.g., WM H1 109). Haplotypes and specimens marked with an asterisk comprise the barcode and barcode+ regions, those without an asterisk comprise just the barcode region. Branch lengths are directly proportional to the number of changes along that branch, and a scale bar is provided below.

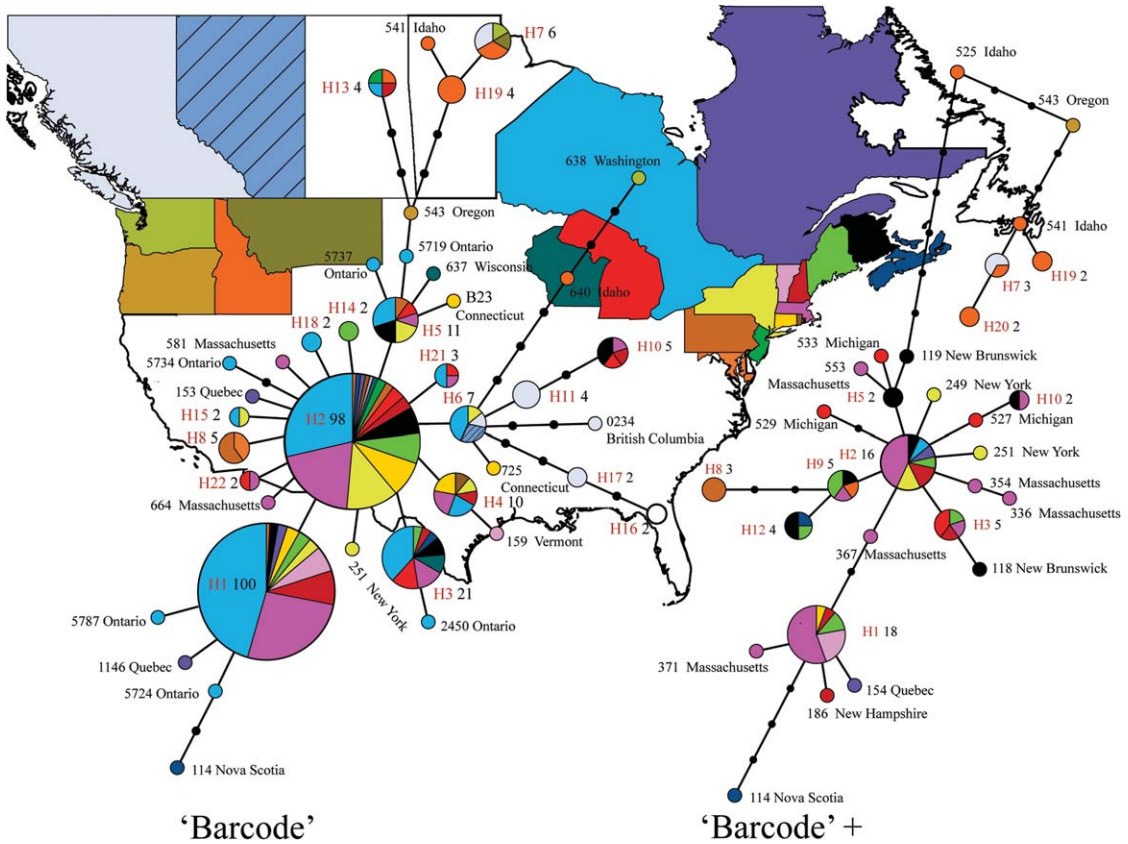


Fig. 3. Haplotype networks for *Operophtera brumata* collected throughout North America. Haplotype presentation is as described for the haplotypes in Fig. 2. (Online figure in color.)

occidentalis the phylogeographic pattern of haplotypes also suggests a large range-wide population (Fig. 4). Although our sampling for this subspecies is biased by a high density of specimens collected in Oregon, the pattern of haplotype diversity using the barcode region (Fig. 4) suggests this subspecies shows similar phylogeographic structure to *O. bruceata. O. b. occidentalis* does not appear to have a level of intraspecific mtDNA variation similar to *O. brumata* or *O. bruceata.* This pattern highlights the closely related, but phylogenetically distinct single specimen, 09–JDWGEO–463. This specimen, deposited in the National Museum of Natural History (USNM ENT 00718320), is geographically disjunct from the coastal range (Alaska to southern California) of *O. b. occidentalis.* It also has a much larger body and wing size than all other native North American *Operophtera* (J.R.W., unpublished data).

Interspecific Divergence. Mitochondrial uncorrected pairwise percent divergences between *Operophtera* species are ≈7–8% (Fig. 5). Notably the subspecies *O. b. occidentalis* is >7% divergent from other *Operophtera*, and ≈5% divergent from the nominate species *O. bruceata.* The unidentified specimen 09–JDWGEO–463 is ≈5% divergent from *O. bruceata*, and 2.4% divergent from *O. b occidentalis.*

Discussion

The vast majority (≈89%) of *O. brumata* in our samples from North America, and Europe belong to a single haplotype (H1). In North America, H1 comprises 93% of specimens, whereas in Europe, which has higher haplotype diversity, H1 comprises only ≈71% of specimens. The extreme prevalence of this haplotype on both continents reduces the available mtDNA diversity to infer the number and origin of introductions. Additionally, while H2 was found in the original introduction site in Nova Scotia and New York, and predominates in Norway, Scotland, and Sweden. It is also recovered in southern Germany that suggests this haplotype may have a distribution similar to H1, albeit at lower proportions in the population. Winter moths are known to have been established in Nova Scotia in the 1930s (Embree 1965), and given the large amount of commerce and trade between Nova Scotia and Britain, the latter is a likely source of North American winter moths. We found a unique haplotype match between three moths from the city of Vancouver, a major port in British Columbia, and a single moth collected in Germany (Fig. 1) that all belong to H4. This association probably represents a more recent, and separate introduction to Vancouver from Europe.

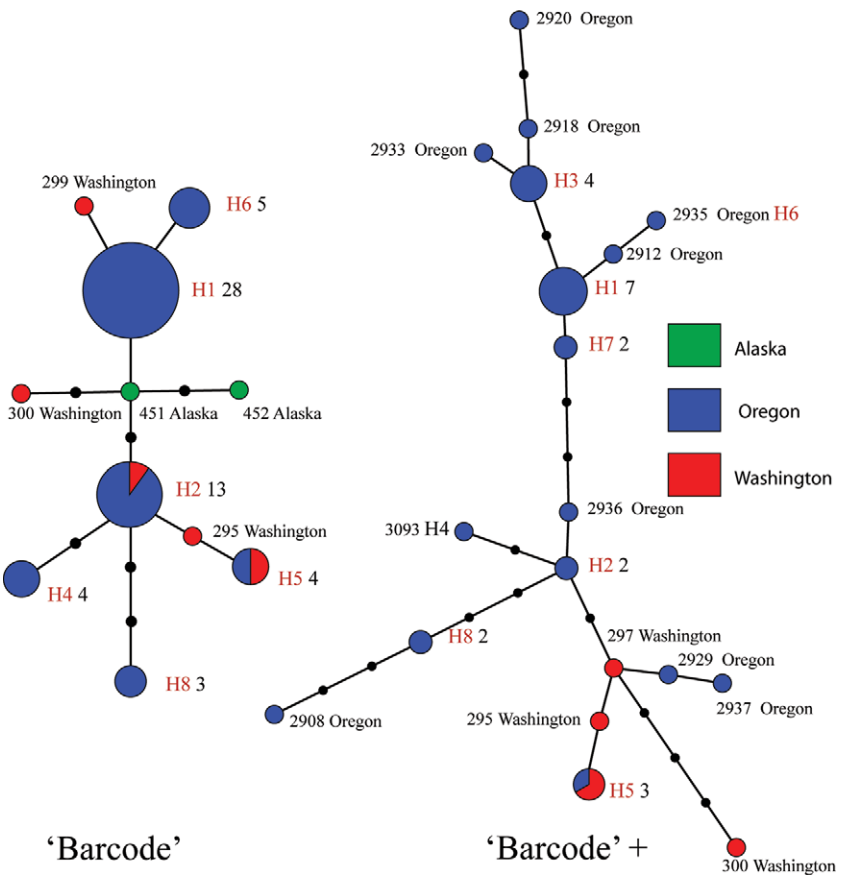


Fig. 4. Haplotype networks for *Operophtera brumata occidentalis* collected in North Western North America. Haplotype presentation is as described for the haplotypes in Fig. 2. (Online figure in color.)

And so, as H1 and H2 are both present in Germany, it is also possible this country may be a source of the North American invasion. An additional, albeit small, influence on *O. brumata* haplotype diversity may come from hybridization with *O. bruceata*, as these species hybridize in the lab (Troubridge and Fitzpatrick 1993), and in the field (Elkinton et al. 2010).

While the mtDNA loci used here may not have enough resolution to pinpoint the origins of introduction, the phylogeographic patterns suggest new inferences about the biology and diversity of winter moths in Europe and North America. Mitochondrial DNA variation in *O. brumata* is lower than in *O. bruceata* (Figs. 1 and 2), and this agrees with an earlier study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *Lambdina fiscellaria*	-													
2 *Epirrita pulchraria*	15	-												
3 *O. rectimpostmediana*	12	13	-											
4 *O. danbyi* H1	10	13	7.7	-										
5 *O. fagata* H1	13	15	7.2	7.7	-									
6 *O. brumata* H1	12	13	7.2	7.7	7	-								
7 *O. brumata* H2	13	13	6.7	8	6.9	0.5	-							
8 Undet. Arizona	13	16	8.1	8.8	7.4	7.7	8.1	-						
9 O. *occidentalis* H1	13	15	7.7	8.6	7.7	7.3	7.5	2.4	-					
10 O. bruceata 638 WA	13	14	7.5	8.2	8	7	7.5	5	4.8	-				
11 *O. bruceata* H1	12	13	7.5	8.3	8.6	7.7	8.1	5.9	5	1.2	-			
12 *O. bruceata* H20	13	13	6.7	7.9	8.3	7	7.4	4.8	4.3	1.8	1.6	-		
13 *O. bruceata* H5	13	13	7.5	8.1	8.4	7.5	8	5.5	5	0.9	0.3	1.2	-	
14 *O. bruceata* H3	12	13	7.5	8.1	8.7	7.7	8.1	5.9	5	1.2	0.3	1.6	0.3	-

> 10%
6–10%
3–6%
1–3%
0–1%

Fig. 5. Matrix of pairwise percent divergences between selected outgroup taxa (*Lambdina fiscellaria* (Guenee) and *Epirrita pulchraria* (Taylor)), and species of *Operophtera.* Haplotypes are labeled with their designation (e.g., H1); the Undet, AZ, haplotype is from specimen 09–TDWGEO–463. Cells are colored from light to dark according to a scale of increasing divergence in increments of 0–1, 1–3, 3–6, 6–10, and >10%.

finding low *O. brumata* allozyme variation in Belgium (Van Dongen et al. 1998). Despite this low variation, our sampling of *O. brumata* shows relatively higher phylogeographic structure than *O. bruceata.* In Europe, *O. brumata* H1 predominates in the southern part of the continent, and H2 is only found in northern Europe (Germany, Norway, Scotland, and Sweden), whereas our sampling of *O. bruceata* haplotypes shows very little structure across North America. It is possible these species-specific differences are because of disparities in population biology. *O. brumata* populations have extensive outbreaks of approximately decade-long cycles (Varley and Gradwell 1968), whereas *O. bruceata* populations outbreaks usually last 2–3 yr and tend to occur on local scales, particularly in Canada (Rose and Lindquist 1982). It is possible that selective sweeps occur more readily in *O. brumata* populations during these widespread outbreak phases, reducing overall mtDNA variation (Galtier et al. 2009). In addition, the complex glacial history of Europe has influenced the phylogeographic structure for many organisms (Hewitt 2000). The distribution of northern and southern *O. brumata* H1 and H2, could be because of isolation in glacial refugia.

Cryptic Diversity Within *O. bruceata.* *O. bruceata* has several groups that can be identified by congruent morphological, molecular, and geographic patterns, suggesting *O. bruceata* may be a complex of several species. The nominate *O. bruceata* (Fig. 2), has low phylogeographic structure, suggesting a large interbreeding population spread across most of northern North America (Fig. 3), possessing a relatively low range of within-group mtDNA divergence (0.3–1.8%; Fig. 5). *O. b. occidentalis* has unique morphological features of the wing and abdomen color (Troubridge and Fitzpatrick 1993), which are correlated with a restricted distribution along the Pacific coast. For these reasons this subspecies has sometimes been treated as a separate species (Miller and Cronhardt 1982). We found specimens collected in the range of *O. b. occidentalis* form a monophyletic group (Fig. 1), and mtDNA divergence between *O. b. occidentalis* and *O. bruceata* is ≈ 5% (Fig. 5). This divergence is similar to the ≈7% we observed in other species of *Operophtera* (Fig. 5), and is higher than ≈3% divergence corresponding to species-level divergence in other geometrids from the Pacific Northwest (deWaard et al. 2011). *O. b. occidentalis* has been considered a full species (*O. occidentalis* (Hulst)) until lowered to subspecific status by Troubridge and Fitzpatrick (1993) in a reasonable attempt to organize the genus. However, our data, combined with unique morphology noted by Troubridge and Fitzpatrick (1993), suggests that *O. b. occidentalis* could be elevated back to full species.

Additionally, we found a single morphologically distinct, and phylogenetically divergent (Fig. 1) specimen (09-JDWGEO-463 image available at http://www.barcodinglife.com/, public data:GNAU163–10) collected near Mount Lemmon, in the Sky Islands of southwestern Arizona, which was not collected at a pheromone trap. Despite our trap survey being the most extensive sampling conducted for *Operophtera* in North America, pheromone traps were not deployed in this region because it is considered to be outside the range of *O. bruceata* (Troubridge and Fitzpatrick 1993). This individual is most closely allied to *O. b. occidentalis* (Fig. 1), but its COI sequence is ≈2.5% divergent from *O. occidentalis*, whereas the largest intraspecific variation within *O. bruceata* is ≈1.6%, and much less in *O. b. occidentalis* (Fig. 5). In addition, this specimen has a considerably larger wing span, body size, and slightly lighter wing color than either *O. b. bruceata* or *O. brumata.* This specimen possibly represents the 'light form' of *O. occidentalis*, described as *Rachela* (now *Operophtera*) *latipennis* (Hulst 1896). However, Hulst only recorded this as from 'California', and as only a single type exists (as a dark morph) Troubridge and Fitzpatrick (1993) have synonymized *latipennis* with *O. b. occidentalis.* Despite the unclear species assignment of our specimen, its unique geographic, morphological, and genetic variation merits further taxonomic scrutiny for similar specimens in collections, and suggests further undiscovered *Operophtera* diversity may be present throughout western North America.

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