Phylogeographic structure in three North American tent caterpillar species (Lepidoptera: Lasiocampidae): *Malacosoma americana*, *M. californica*, and *M. disstria*

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While phylogeographic structure has been examined in many North American vertebrate species, insects have received much less attention despite their central ecological roles. The moth genus *Malacosoma* (Hübner, 1820), is an important group of forestry pests responsible for large-scale defoliation across much of the Nearctic and Palearctic. The present study uses sequence variation in the mitochondrial cytochrome c oxidase 1 (COI) gene to examine the population genetic structure of the three widespread *Malacosoma* species (*M. americana*, *M. californica*, and *M. disstria*). Populations of all three species showed highest diversity in the south, suggesting that modern populations derived from southern refugia with loss of variation as these lineages dispersed northwards. However, despite similar life histories and dispersal abilities, the extent of regional variation varied among the taxa. *M. americana*, a species restricted to eastern North America, showed much less genetic structure than the western *M. californica* or the widespread *M. disstria*. The regional differentiation in the latter reflects the likely derivation of modern lineages from several refugia, as well as taxonomic uncertainty in *M. californica*. In these respects, the three species of *Malacosoma* share phylogeographic patterns similar to those detected in vertebrates which are characterised by greater phylogeographic breaks in the western half of the continent and limited structure in the east.
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Running title: Phylogeography of three *Malacosoma*
ABSTRACT

While phylogeographic structure has been examined in many North American vertebrate species, insects have received much less attention despite their central ecological roles. The moth genus *Malacosoma* (Hübn, 1820), is an important group of forestry pests responsible for large-scale defoliation across much of the Nearctic and Palearctic. The present study uses sequence variation in the mitochondrial cytochrome *c* oxidase 1 (COI) gene to examine the population genetic structure of the three widespread *Malacosoma* species (*M. americana*, *M. californica*, and *M. disstria*). Populations of all three species showed highest diversity in the south, suggesting that modern populations derived from southern refugia with loss of variation as these lineages dispersed northwards. However, despite similar life histories and dispersal abilities, the extent of regional variation varied among the taxa. *M. americana*, a species restricted to eastern North America, showed much less genetic structure than the western *M. californica* or the widespread *M. disstria*. The regional differentiation in the latter reflects the likely derivation of modern lineages from several refugia, as well as taxonomic uncertainty in *M. californica*. In these respects, the three species of *Malacosoma* share phylogeographic patterns similar to those detected in vertebrates which are characterised by greater phylogeographic breaks in the western half of the continent and limited structure in the east.
INTRODUCTION

The patterns of genetic variation in species and the processes which underlie them are of particular interest to evolutionary biologists. Diverse factors, both historical and contemporary, influence how variation is distributed among populations; these include geological and climatic events, and the presence of physical and behavioural barriers (Avise, 2004). Past glaciations have had a major impact on the extent and patterning of genetic structure in Northern Hemisphere species (Hewitt, 2000). In North America, ice sheets covered much of present-day Canada and the northern United States, temperatures in ice-free areas were cooler than today, and sea levels dropped by up to 140 m (Pielou, 1991; Barendregt & Irving, 1998; Dyke et al., 2002). The distributions of many species were fragmented with their populations persisting in small ice-free refugia (Hewitt, 1996; Hewitt, 2000; Stewart & Lister, 2001). In addition, physical and ecological barriers influenced genetic structure by their impacts on dispersal and gene flow. Recent environmental changes, both anthropogenic and natural, are now causing range shifts and population changes with varied impacts on both inter- and intraspecific genetic variation (Walther et al., 2002; Chen et al., 2011).

Comparisons of genetic variation spanning multiple species can identify both general and species-specific patterns, revealing how particular life history characteristics impact population structure. Species-specific traits, such as dispersal ability or niche requirements, may affect how a species responds to environmental, climatic, and geological changes. For example, the majority of northern species experienced major population declines reflecting the loss of habitat during the Pleistocene glaciations, while the interglacials favoured range expansion and population growth (Nilsson, 1983; Pielou, 1991; Hewitt, 2000; Hewitt, 2004). In contrast, cold-adapted species experienced habitat loss, often retreating to high altitude and high latitude locations during interglacials (Stewart & Lister, 2001; Dalén et al., 2005; Galbreath et al., 2009; Stewart et al., 2009). Topographic features also have differing effects, with mountains and rivers restricting
gene flow in some species while acting as dispersal corridors for others. For example, the Rocky Mountains prevent gene flow between some populations (Crease et al., 1997; Burg et al., 2005), but provide habitat as “sky islands” with the intervening lowlands limiting gene flow in others (Knowles, 2000; DeChaine & Martin, 2005; Galbreath et al., 2009).

Past studies of phylogeographic structure in terrestrial organisms have largely examined vertebrates. Given their high diversity and abundance, phylogeographic patterns in insects have been understudied; past work has revealed diverse outcomes ranging from global panmixis (Alvial et al., 2007; Correa et al., 2017) to highly fragmented, structured populations (Dinca, Dapporto & Vila, 2011; Frantine-Silva et al., 2017; Karthika et al., 2017). Phylogeographic studies of Lepidoptera have employed both nuclear and mitochondrial markers, particularly the cytochrome c oxidase 1 (COI) locus (Vandewoestijne et al., 2004; Craft et al., 2010; Kirichenko et al., 2017). This study represents the first step in a broad investigation of phylogeographic patterns in North American Lepidoptera.

Malacosoma (Hübner, 1820) is a Holarctic genus found across much of North America, Europe, and Asia, with six species native to Canada and the United States (Franclemont, 1973). While males are strong fliers, females are usually sedentary until they deposit their egg mass (Stehr & Cook, 1968; Franclemont, 1973), suggesting that the dispersal of maternal markers will be low. As their name (tent caterpillar) suggests, they build large tents or moulting mats which can accommodate a large number of larvae (Franclemont, 1973). All six species feed on diverse deciduous trees and shrubs, with host preferences varying by taxon and region (Stehr & Cook, 1968; Franclemont, 1973; Parry & Goyer, 2004). The group contains a number of important forestry pests, species that experience cyclical outbreaks which often lead to extensive forest defoliation (Hildahl & Reeks, 1960; Stehr & Cook, 1968; Roland, 1993). Despite these impacts, there have been few studies of population genetic structure in these moths. One study, which assessed allozyme variation in Malacosoma americana (Fabricius, 1793) from eastern United
States, found limited variation and a lack of regional genetic differentiation (Costa & Ross, 1994). A second employed microsatellites and short DNA sequences to compare five populations of *Malacosoma californica pluviale* in coastal British Columbia (Franklin, Myers & Cory, 2014). Although high levels of variation were evident, there was little genetic differentiation between island and mainland populations. Both studies found limited genetic differentiation at a relatively small geographic scale.

This study examines the population genetic structure of the three widely distributed North American *Malacosoma* species, the eastern tent caterpillar *M. americana*, the western tent caterpillar *M. californica* (Packard, 1864), and the forest tent caterpillar *M. disstria* (Hübner, 1820) (Fig. 1). We employ the 659 bp cytochrome *c* oxidase I gene region at the continental scale to determine whether limited dispersal abilities and contemporary barriers are preventing movement in these species, what role the Pleistocene glaciations may have played in their current structure and distribution, and if these three *Malacosoma* species show concordant phylogeographic patterns or if there are differences in the patterns observed that may be explained by their life history characteristics. By understanding what influences have impacted the population genetic structure in these species we can add to our understanding of phylogeography in North American insects and we can begin to predict how the populations may expand with changing climate conditions.

**MATERIALS AND METHODS**

**Phylogenetic analyses**

Sequences of the 658 bp barcoding region of cytochrome *c* oxidase I (COI) for all *Malacosoma* specimens from the United States and Canada were downloaded from the Barcode of Life Data System (BOLD; see Supplemental Table S1; Ratnasingham & Hebert, 2007). Locations were recorded by state or province. Sequences were aligned in MEGA v6 (Tamura *et
In order to verify that the samples examined belonged to monophyletic species, a Bayesian network was constructed in BEAST v2.3 (Bouckaert et al., 2014) using the Hasegawa, Kishino, and Yano model with gamma-distributed rate variation and allowing for invariable sites (HKY + Γ + I). The analysis was run for 10,000,000 MCMC steps, and sampled every 2,000 steps with a 25% burn-in. The lasiocampid *Phyllosesma americana* (GenBank Accession Number JF842281) was used as the outgroup.

### Genetic analysis

Intraspecific analyses were performed on the three widespread *Malacosoma* species: *M. americana*, *M. californica*, and *M. disstria*. These species were selected based on distribution and available sample size (Table 1, Fig. 1). Haplotypes were assigned to each species with TCS v1.21 (Clement, Posada & Crandall, 2000) and confirmed manually. Genetic diversity was measured with haplotype (Hd) and nucleotide (π) diversity indices, calculated in DNAsp v5.10 (Rozas et al., 2003; Librado & Rozas, 2009). In order to test for population genetic structure an analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) and pairwise genetic differences (ΦST; 100,000 permutations) were calculated in Arlequin v3.5.1.2 (Excoffier & Lischer, 2010). Nearby sampling locations were grouped to increase sample sizes, and a modified false discovery rate was applied to correct for multiple tests (Benjamini & Yekutieli, 2001).

To test for the presence of genetic clusters two methods were used: a spatial analysis of molecular variance (SAMOVA) which identifies the maximum between group variance with the use of additional geographic information (K = 2 to 6; 1,000 iterations; Dupanloup, Schneider & Excoffier, 2002), and a clustering analysis as performed in Bayesian Analysis of Population Structure v5.2 (BAPS; Corander & Tang, 2007; Corander et al., 2008) which allows the assignment of individuals to genetic clusters with no *a priori* population information. Results
from the *Malacosoma* Bayesian analysis were also analysed at the species level. In order to
visualise the pattern of variation and relationship among haplotypes, a principal coordinates
analysis (PCoA) was run in GenAlEx v6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012),
and a statistical parsimony network was constructed in TCS v1.2.1 (Clement, Posada & Crandall,
2000).

RESULTS

Phylogenetic analyses

A total of 474 *Malacosoma* sequences from five species were downloaded from BOLD. The Bayesian network identified two main lineages within North America: the first group included specimens of *M. constricta* and *M. disstria* with each species forming a well-defined monophyletic group; the second included *M. americana, M. incurva*, and *M. californica* (Supplemental Figure S1). While *M. americana* was monophyletic, specimens assigned to *M. californica* were paraphyletic, suggesting a species complex. Of particular note, specimens of *M. californica* from Alberta (AB), Saskatchewan (SK), and New Brunswick (NB), as well as those identified as *M. californica pluviale*, did not group with the majority of *M. californica* samples. The taxonomic status of *M. californica* has been widely debated; it is currently viewed as including six largely allopatric subspecies, many of which have previously been viewed as distinct species, although Franclemont questioned the validity of *M. californica pluviale* as a subspecies rather than a species (Franclemont, 1973). By contrast, both *M. americana* and *M. disstria* have no described subspecies (Stehr & Cook, 1968; Franclemont, 1973). Further study with additional markers is required to clarify the taxonomic status of lineages within the *M. californica* complex, and to determine how many species should be recognized. As such, the intraspecific analyses here focus only on the large monophyletic portion of *M. californica* and do not include *M. californica pluviale* or the NB, AB, or SK samples (see Fig. 1).
Cytochrome c oxidase I sequences

Intraspecific analyses examined 79 *M. americana* samples, 207 *M. californica* samples, and 139 *M. disstria* samples (Supplemental Table S1). For each species the 658 bp gene region was highly polymorphic with 37 variable sites defining 33 haplotypes in *M. americana*, 61 variable sites defining 64 haplotypes in *M. californica*, and 43 variable sites defining 42 haplotypes in *M. disstria* (Table 1). There were 8, 13, and 13 anticipated amino acid substitutions, no frameshift mutations, and no stop codons. Fixed nucleotide differences were present in *M. californica* between sCA, AZ, and the other populations of this species, and in *M. disstria* between western (BC, AB, and SK) and eastern groups. There were no fixed differences in *M. americana*. Haplotype and nucleotide diversities were high in all species \( (H_d = 0.918 - 0.926; \pi = 0.0049 - 0.0097; \text{Table 1}) \), with diversity generally higher in southern and central populations \( (r = -0.284 \text{ to } -0.508; \text{Supplemental Table S2, Figure S2}) \). When populations with small sample sizes \( (n < 5) \) were removed from the correlation analysis, coefficients were much stronger for *M. americana* and *M. californica* \( (r = -0.937 \text{ and } r = -0.703, \text{respectively}) \), and slightly weaker for *M. disstria* \( (r = -0.360) \).

Genetic analyses

*Malacosoma americana*

Of the three species, *M. americana* showed the least population structure \( (\Phi_{ST} = 0.244, p < 0.0001) \), the fewest significant pairwise comparisons \( (7 \text{ out of } 15; \text{Table 2}) \), and the lowest diversity \( (\text{Table 1}) \). The greatest pairwise \( \Phi_{ST} \) values were seen between NB and the other populations. The SAMOVA analysis identified four groups \( (F_{CT} = 0.263, p = 0.02) \): MN, NB, and the separation of the remaining samples into northern (ON, MD/NC) and southern (TN,
AR/OK/TX) populations, while the Bayesian clustering analysis separated the samples into three clusters (Fig. 1, Supplemental Table S2): a "northern" group found primarily in ON and NB, and two "southern" groups, one found in all populations except NB, and a smaller group primarily in MN and OK. The distribution of haplotypes in the Bayesian groups was significantly non-random ($X^2 = 47.65, p < 0.0001$). The BEAST analysis generally supported the BAPS groups with BAPS1 and BAPS2 forming weakly supported clades while BAPS3 contained the remaining samples (posterior probability = 0.65 and 0.5, respectively; Supplemental Table S1, Figure S3).

The principal coordinates analysis showed a general separation of the samples into northern and southern groups along coordinate 1 (34.7%; Fig. 2a), while coordinates 2 and 3 explained 11.5% and 8.8% of the variation, respectively. The statistical parsimony network showed little pattern to the variation, with haplotypes generally being closely related, although more divergent haplotypes were present in ON, OK, and MN, and there was a general clustering of southern samples (OK, TX, AR, and TN) and NB samples despite a lack of fixed differences (Fig.3).

*M. californica*

*M. californica* had strong population structure (overall $\Phi_{ST} = 0.48, p < 0.0001$), and the highest nucleotide diversity (Table 1). All pairwise comparisons were significant except that between cBC and swBC, and those with WA (22 out of 28; Table 2). These are likely a result of the small WA sample size (n=3), and the small geographic distance between cBC and swBC. The greatest differences existed between sCA and all other populations ($\Phi_{ST} = 0.73 - 0.94$), and between AZ and all other populations ($\Phi_{ST} = 0.54 - 0.79$). The SAMOVA analysis identified genetic breaks between three groups: sCA, AZ, and all other populations ($F_{CT} = 0.57, p = 0.049$). Bayesian clustering analysis identified six clusters (Fig. 1, Supplemental Table S2): one in sCA, one in AZ, one in eBC, and three shared between multiple populations. The eBC population had
representatives in four clusters. The distribution of samples in the six clusters was highly significant ($X^2 = 486.6$, $p < 0.0001$). When sCA, CA, and AZ were removed from analysis, the distribution was still significantly different than random ($X^2 = 57.02$, $p < 0.0001$). The BEAST analysis identified four main clades with numerous subclades within them: two well-supported clades representing the AZ and sCA samples (posterior probability = 0.95 and 1, respectively), one clade found in eBC with one CA sample (posterior = 0.75), and one found across BC and WA (posterior = 0.77; Supplemental Figure S4). The CA samples were found outside of these clades.

The principal coordinates analysis separated the BC samples into two groups along coordinate 1, while the sCA and AZ samples were separated along coordinate 2 (Fig.2b). A total of 65.1% of the variation was allocated to the first three coordinates (39.0%, 16.4%, and 9.7%, respectively). The statistical parsimony network showed a similar pattern, separating the AZ, sCA, CA, and TX samples from the more northern populations, with two large groups containing all other samples (Fig.4). One group consisted of haplotypes from across the Pacific Northwest, and contained all samples from cBC, VI, swBC, and WA, as well as many eBC samples. There were several common haplotypes separated by one to three mutations. The second cluster was restricted to eBC; it was less diverse and contained two common haplotypes, one represented by 44 individuals. Most CA samples had unique haplotypes, while the sCA and AZ haplotypes were divergent with 13 - 15 (sCA) and 5 - 16 (AZ) mutations separating them from the nearest population (Fig.4).

**Malacosoma disstria**

*M. disstria* had the strongest population structure (overall $\Phi_{ST} = 0.52$, $p < 0.0001$), and 42 of 55 pairwise comparisons were significant following correction for multiple tests (Table 2). The 13 non-significant comparisons all involved ON (with NB/NS, with NC/FL/GA, or among the three ON locations). In contrast, the largest pairwise differences were between BC and all other
populations ($\Phi_{ST} = 0.49 - 0.98$). Diversity within populations was generally high with $H_d > 0.7$ in 10 of the 16 populations. The lowest values were in SK ($H_d = 0$) followed by eBC ($H_d = 0.20$; Supplemental Table S2). A similar pattern was seen with nucleotide diversity with the highest values in the three ON populations ($\pi = 0.0053 - 0.0065$) and TX ($\pi = 0.0137$; Supplemental Figure S2).

The SAMOVA analysis identified a genetic break between the BC populations and all other populations (including AB), possibly along the Rocky Mountains ($F_{CT} = 0.52$, $p = 0.015$). Bayesian clustering analysis showed a slightly different pattern with four identified clusters (Fig. 1, Supplemental Table S2): three were found in the north (two wholly) while one was primarily found in the south. The BC samples fell exclusively in one cluster, the AB, SK, and MB samples were in a cluster together, and the ON, QC, and NB/NS samples generally formed a third cluster. The allocation of samples to clusters was highly significant ($X^2 = 224.7$, $p < 0.0001$). The BEAST analysis identified four main clades, generally supporting the BAPS groups, although with slightly different membership: two well-supported clades found mostly in the west (posterior probability = 1 and 0.75), and two clades present mostly in the east and southeast (posterior = 0.77 and 0.39, respectively; Supplemental Figure S5).

The principal coordinates analysis separated the BC populations from most other populations along coordinate 1 (41.5%), and identified a general separation of northern and southern populations along coordinate 2 (14.8%). Coordinate 3 explained 12.6% of the variation (Fig.2c). The statistical parsimony network identified moderate variation, with five common haplotypes ($n \geq 10$): three found in a single region (BC, AB/SK, or ON), one shared by ON and NB/NS, and one found across several regions in the east and south (Fig.5). The BC samples formed a separate group (with a single AB sample), while AB, SK, and MB mostly grouped together. The southern samples generally grouped together.
DISCUSSION

Population genetic structure

The analysis of mitochondrial COI sequences from three North American *Malacosoma* species showed high levels of variation and diversity, with some highly divergent populations in *M. californica*. All three species show evidence of persistence in one or more southern refugia with subsequent recolonisation of northern regions. Diversity patterns exhibited the characteristic "southern richness, northern purity" (Hewitt, 2004) found across much of the previously glaciated Northern Hemisphere. This was particularly evident in *M. americana* where diversity in southern populations was twice that in northern regions (\(\pi = 0.0046 - 0.0061\) versus \(0.0013 - 0.0027\)), and in *M. californica* where diversity was four-fold higher in Arizona (\(\pi = 0.0093\)) than in Washington (\(\pi = 0.002;\) Supplemental Table S2). In *M. disstria* diversity levels were highest in Texas and in the three Ontario populations, likely representing admixture in the latter. All three species exhibited a negative correlation between latitude and nucleotide diversity (Supplemental Figure S2), with the strongest relationships seen in *M. americana* and *M. disstria*.

The two species found in the east, *M. americana* and the eastern portion of *M. disstria*, showed limited population structure consistent with a relatively young evolutionary history and/or high levels of gene flow. This pattern is common in species restricted to eastern North America, and in the eastern portion of the range for more widespread species. For example, many bird species found in the eastern half of North America exhibit limited genetic structure (Zink, Rootes & Dittmann, 1991; Vallianatos, Lougheed & Boag, 2001; Veit *et al.*, 2005), while widespread species show shallow divergence in the east (Klein & Brown, 1994; Graham & Burg, 2012; van Els, Cicero & Klicka, 2012), likely reflecting a single evolutionary origin and extensive contemporary gene flow. Other studies have identified limited structure in eastern trees...
Both *M. americana* and *M. disstria* exhibit this pattern: limited structure in eastern North America with some differences between northern and southern populations. This may be caused by limited ongoing gene flow between regions, likely a result of the limited dispersal capability of female *Malacosoma*. Interestingly, neither species have the genetic break between Atlantic and Gulf coast clades seen in many fish (Bermingham & Avise, 1986; Avise, 1992), insects (Vogler & Desalle, 1993; Ney & Schul, 2017), reptiles (Lamb & Avise, 1992), birds (Avise, 1992), and marine invertebrates (Herke & Foltz, 2002; Young *et al.*, 2002; see Soltis *et al.*, 2006 for additional references and an excellent description of this break), suggesting a single southeastern origin.

In addition to limited north-south diversification, *M. disstria* also exhibits an east/west separation (Table 2, Fig. 1), a pattern common in widespread North American species that often reflects multiple evolutionary origins (Sperling, Raske & Otvos, 1999; Gerardi *et al.*, 2010; Medina *et al.*, 2010; Lait & Burg, 2013). Lack of strong support for a western or southwestern refugium may be a result of the paucity of samples in this region; the presence of a western refugium may be suggested by the pattern found in *Populus tremuloides*, the favoured host of *M. disstria*, that shows evidence of two genetic clusters, one in the southwest and one in the north and east, with higher diversity in the southwest group (Callahan *et al.*, 2013). Many other continent-wide species also possess a large group with low diversity across northern and eastern areas, and multiple diverse groups west of the Rocky Mountains (Ball & Avise, 1992; Byun, Koop & Reimchen, 1997; Graham & Burg, 2012; van Els, Cicero & Klicka, 2012), a pattern linked to a single refugium for the east and multiple isolated refugia in the west. The high genetic diversity in the three Ontario populations (Supplemental Table S2, Figure S2), as well as the presence of all four Bayesian clusters meeting in these populations (Fig. 1), supports the
possibility of secondary admixture in this central region. Additional sampling between the
Ontario populations and the western populations, as well as from the southwestern portion of the
range, should help to clarify whether the mixing seen here is indicative of recolonisation from
multiple refugia, or if it suggests a diverse source population.

The western *M. californica* possessed a very different pattern of variation with strong
population genetic structure and very distinct populations (AZ and sCA) separated by multiple
fixed differences which may represent different subspecies or ecotypes. This pattern is common
in many southwestern and western species, and is explained by both the current and historical
topography of western North America: four major mountain ranges (Cascade, Coastal, Rocky,
and Sierra) run along a north-south axis, and large plains and deserts abound, all of which
contribute to a complex habitat mosaic. This heterogeneity, coupled with the resulting complex
or glacial histories of the region, has resulted in extensive structuring in many birds (Barrowclough
et al., 2004; Lait et al., 2012; van Els, Cicero & Klicka, 2012), mammals (Byun, Koop &
Reimchen, 1997; Riddle, Hafner & Alexander, 2000; Galbreath et al., 2009), insects (Brown et
al., 1997), and plants (Golden & Bain, 2000; Richardson, Brunsfeld & Klopfenstein, 2002;
Johansen & Latta, 2003). Given the divergence of the AZ and sCA populations (0.9 - 1.9%
divergence from the nearest population, CA), it is likely that they have been isolated for multiple
glacial cycles with limited recent gene flow. The AZ population shows relatively high diversity,
suggesting multiple refugia or impassable barriers within this small region. This has been seen in
a number of animal (Orange, Riddle & Nickle, 1999; Zink et al., 2001; Merrill, Ramberg &
Hagedorn, 2005; Graham et al., 2013) and plant (Frohlich et al., 1999) species, and is likely due
to the particularly heterogeneous nature of Arizona which contains seven ecoregions (Warshall,
1995; Poulos, Taylor & Beaty, 2007; Ober & Connolly, 2015; Powell & Steidl, 2015).

Physical barriers
Several physical barriers impede gene flow in North American species. In eastern North America, the Appalachian Mountains act as a barrier to movement in plants (Griffin & Barrett, 2004; Joly & Bruneau, 2004; Godbout et al., 2005), reptiles (Bushar et al., 2014; Krysko et al., 2017), and amphibians (Church et al., 2003; Jones et al., 2006), while the Mississippi, Tombigbee, and Appalichola Rivers prevent gene flow in many plant and animal species (see Bermingham & Avise, 1986; Avise, 1992; Soltis et al., 2006 and references therein). The two Malacosoma species in the east do not show genetic breaks along any of these traditional barriers. This may be due to recent colonisation from a single origin or ongoing gene flow in these regions. As the moths can fly, their dispersal capabilities should be greater than that of sedentary plant and reptile species, allowing them to cross rivers. The fact that the Appalachians have not prevented gene flow may indicate the importance of forested valleys as dispersal corridors. All three Malacosoma species are generalist herbivores, albeit with host preferences, thus they should encounter suitable habitat more often than strict specialists.

In western North America the main physical barriers are the Rocky, Coastal, Cascade, and Sierra Nevada Mountains (Crease et al., 1997; Nielson, Lohman & Sullivan, 2001; Johansen & Latta, 2003; Burg et al., 2005; Carstens et al., 2005). The Wyoming Basin and the Great Plains have also been shown to act as dispersal barriers, particularly for species associated with montane, forested, or wetland habitats (DeChaine & Martin, 2005; Wilson et al., 2005). Malacosoma disstria and M. californica both exhibit genetic breaks in western regions: M. disstria shows a break across the Rocky Mountains (between BC and AB), while M. californica has disjunct populations among many of the southwestern deserts (Table 2, Figs. 1 & 2). The Rocky Mountains may also act as a barrier in this species (or species complex); the samples identified as M. californica from AB and SK were very different than those in BC (4.9 - 6.7%), likely representing the described subspecies M. californica lutescens as a separate species (the Great Plains tent caterpillar; Franclemont, 1973).
Conclusions

Despite considerable overlap in their distributions and life history traits, the patterns of variation and levels of population structure in the three Malacosoma species varied considerably. The population genetic structure suggests a single origin in the east and a complex evolutionary history in the west. M. americana, restricted to the eastern half of the continent, shows limited structure with a north-south trend and greater diversity in the south. This is consistent with its expansion from a single southern refugium following the last glaciation with limited ongoing gene flow between distance regions. M. disstria shows a similar pattern in the east, supporting a single southern refugium, with one or more additional refugia possible in the west. Additional samples are required to elucidate whether the differentiation in its BC population reflects a founder event with subsequent divergence or additional structuring. M. californica shows the greatest degree of structure and differentiation, consistent with multiple evolutionary origins in the west and southwest. This study shows the utility of existing DNA barcodes in identifying patterns of genetic structure in insect species, which can uncover previously unknown evolutionary histories and suggest further avenues to explore.

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DATA ARCHIVING
All sequences are available on the Barcode of Life Database. Sampling locations and sequence data used in this study (BOLD IDs and available GenBank Accession Numbers) have been uploaded as online Supplemental Table S1.

**TITLES AND LEGENDS TO TABLES AND FIGURES**

**Table 1.** Sample size (n), number of sampling locations (loc), variable sites (VS), mean % pairwise distances (PD), number of haplotypes (h), overall haplotype (Hd) and nucleotide (π) diversities, and fixation index (ΦST) for three *Malacosoma* species. The three ΦST values were all highly significant (p < 0.0001).

**Table 2.** Population pairwise ΦST values for (a) *M. americana* (P<sub>crit</sub> = 0.015), (b) *M. californica* (P<sub>crit</sub> = 0.013), and (c) *M. disstria* (P<sub>crit</sub> = 0.011). ΦST values are given below the diagonal and p-values above the diagonal (* p< 0.05, ** p < 0.01, *** p < 0.001). Values significant following correction for multiple tests are shaded. Refer to Fig. 1 for locations.

**Fig. 1.** Approximate distributions (shaded) and sampling locations for (a) *Malacosoma americana*, (b) *M. californica*, and (c) *M. disstria*. The pie charts represent the distribution of BAPS groups, scaled for sample size. The green crosses represent the omitted AB, SK, and NB samples. Sampling locations are as follows: Alberta (AB), Arizona (AZ), British Columbia (BC; central [c], eastern [e], and southwest [sw]), California (CA; southern [s]), Kentucky (KY), Maryland (MD), Minnesota (MN), New Brunswick (NB), North Carolina (NC), Nova Scotia (NS), Ontario (ON; central [c], eastern [e], and southern [s]), Saskatchewan (SK), Tennessee (TN), Texas (TX), Vancouver Island BC (VI), and Washington (WA). The STH includes Arkansas.
(AR), Oklahoma (OK), and TX combined, and SE includes Florida (FL), Georgia (GA), and NC combined.

**Fig. 2.** Principal coordinates analysis for (a) *M. americana*, (b) *M. californica*, and (c) *M. disstria*. The northern populations are depicted by a circle or square, while southern populations are represented by a diamond. Samples are colour-coded by sampling location. Refer to Fig. 1 for locations.

**Fig. 3.** Statistical parsimony network showing the relationship among the 33 *M. americana* haplotypes. Each square represents one of the 79 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.

**Fig. 4.** Statistical parsimony network showing the relationship among the 64 *M. californica* haplotypes. Each square represents one of the 207 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.

**Fig. 5.** Statistical parsimony network showing the relationship among 42 *M. disstria* haplotypes. Each square represents one of the 139 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.
REFERENCES

Alvial I, Veliz D, Vargas H, Esquivel C, Vila I (2007). Lack of genetic structure in *Pantala flavescens* among Central and South American localities (Odonata: Libellulidae). *Odonatologica* **46**: 67-82. DOI: 10.5281/zenodo.572357.

Avise JC (1992). Molecular population structure and the biogeographic history of a regional fauna - a case history with lessons for conservation biology. *Oikos* **63**: 62-76. DOI: 10.2307/3545516.

Avise JC (2004). *Molecular markers, natural history, and evolution*, 2nd edn. Sinauer & Associates: Sunderland, MA.

Ball RM, Avise JC (1992). Mitochondrial-DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *The Auk* **109**: 626-636.

Barendregt RW, Irving E (1998). Changes in the extent of North American ice sheets during the late Cenozoic. *Canadian Journal of Earth Sciences* **35**: 504-509. DOI: 10.1139/e97-126.

Barrowclough GF, Groth JG, Mertz LA, Gutiérrez RJ (2004). Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). *Molecular Ecology* **13**: 1911-1922. DOI: 10.111/j.1365-294X.2004.02215.x.

Benjamini Y, Yekutieli D (2001). The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics* **29**: 1165-1188. DOI: 10.1214/aos/1013699998.

Bermingham E, Avise JC (1986). Molecular zoogeography of freshwater fishes in the southeastern United States. *Genetics* **113**: 939-965.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. *PLOS Computational Biology* **10**: e1003537. DOI: 10.1371/journal.pcbi.1003537.

Brown JM, LeebensMack JH, Thompson JN, Pellmyr O, Harrison RG (1997). Phylogeography and host association in a pollinating seed parasite *Greya politella* (Lepidoptera: Prodoxidae). *Molecular Ecology* **6**: 215-224. DOI: 10.1046/j.1365-294X.1997.t01-1-00171.x.
Burg TM, Gaston AJ, Winker K, Friesen VL (2005). Rapid divergence and postglacial colonization in western North American Steller's jays (Cyanocitta stelleri). *Molecular Ecology* 14: 3745-3755. DOI: 10.1111/j.1365-294X.2005.02710.x.

Bushar LM, Aborde CCB, Gao SS, Gonzalez MV, Hoffman JA, Massaro IK, Savitzky AH, Reinert HK. (2014). Genetic structure of timber rattlesnake (Crotalus horridus) populations: physiographic influences and conservation implications. *Copeia* 2014: 694-706. DOI: 10.1643/CE-14-047.

Byun SA, Koop BF, Reimchen TE (1997). North American black bear mtDNA phylogeography: Implications for morphology and the Haida Gwaii glacial refugium controversy. *Evolution* 51: 1647-1653. DOI: 10.2307/2411216.

Callahan CM, Rowe CA, Ryel RJ, Shaw JD, Madritch MD, Mock KE (2013). Continental-scale assessment of genetic diversity and population structure in quaking aspen (Populus tremuloides). *Journal of Biogeography* 40: 1780-1791. DOI: 10.1111/jbi.12115.

Carstens BC, Brunsfeld SJ, Demboski JR, Good JM, Sullivan J (2005). Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. *Evolution* 59: 1639-1652. DOI: 10.1554/04-661.1.

Chen I-C, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011). Rapid range shifts of species associated with high levels of climate warming. *Science* 333: 1024-1026. DOI: 10.1126/science.1206432.

Church SA, Kraus JM, Mitchell JC, Church DR, Taylor DR (2003). Evidence for multiple Pleistocene refugia in the postglacial expansion of the eastern tiger salamander, Ambystoma tigrinum tigrinum. *Evolution* 57: 372-383. DOI: 10.1111/j.0014-3820.2003.tb00271.x.

Clement M, Posada D, Crandall KA (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659. DOI: 10.1046/j.1365-294x.2000.01020.x.

Corander J, Marttinen P, Siren J, Tang J (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics* 9: 539. DOI: 10.1186/1471-2105-9-539.
Corander J, Tang J (2007). Bayesian analysis of population structure based on linked molecular information. Mathematical Biosciences 205: 19-31. DOI: 10.1016/j.mbs.2006.09.015.

Correa A, Vinson C, Braga L, Guedes R, de Oliveira L (2017). Ancient origin and recent range expansion of the maize weevil *Sitophilus zeamais*, and its genealogical relationship to the rice weevil *S. oryzae*. Bulletin of Entomological Research 107: 9-20. DOI: 10.1017/S0007485316000687.

Costa JT, Ross KG (1994). Hierarchical genetic structure and gene flow in macrogeographic populations of the eastern tent caterpillar (*Malacosoma americanum*). Evolution 48: 1158-1167. DOI: 10.1111/j.1558-5646.1994.tb05302.x.

Craft KJ, Pauls SU, Darrow K, Miller SE, Hebert PDN, Helgen LE, Novotny V, Weiblen GD. (2010). Population genetics of ecological communities with DNA barcodes: an example from New Guinea Lepidoptera. Proceedings of the National Academy of Sciences USA 107: 5041-5046. DOI: 10.1073/pnas.0913084107.

Crease TJ, Lee S-K, Yu S-L, Spitze K, Lehman N, Lynch M (1997). Allozyme and mtDNA variation in populations of the *Daphnia pulex* complex from both sides of the Rocky Mountains. Heredity 79: 242-251. DOI: 10.1038/hdy.1997.151.

Dalén L, Fuglei E, Hersteinsson P, Kapel C, Roth J, Samelius G, Tannerfeld M, Angerbjörn A. (2005). Population history and genetic structure of a circumpolar species: the arctic fox. Biological Journal of the Linnean Society 84: 79-89. DOI: 10.1111/j.1095-8312.2005.00415.x.

DeChaine EG, Martin AP (2005). Historical biogeography of two alpine butterflies in the Rocky Mountains: broad-scale concordance and local-scale discordance. Journal of Biogeography 32: 1943-1956. DOI: 10.1111/j.1365-2699.2005.01356.x.

Dinca V, Dapporto L, Vila R (2011). A combined genetic-morphometric analysis unravels the complex biogeographical history of *Polyommatus icarus* and *Polyommatus celina* common blue butterflies. Molecular Ecology 20: 3921-3935. DOI: 10.1111/j.1365-294X.2011.05223.x.
Dupanloup I, Schneider S, Excoffier L (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**: 2571-2581. DOI: 10.1046/j.1365-294X.2002.01650.x.

Dyke AS, Andrews JT, Clark PU, England JH, Miller GH, Shaw J, Veillette JJ. (2002). The Laurentide and Innuitian ice sheets during the Last Glacial Maximum. *Quaternary Science Reviews* **21**: 9-31. DOI: 10.1016/S0277-3791(01)00095-6.

Excoffier L, Lischer HEL (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564-567. DOI: 10.1111/j.1755-0998.2010.02847.x.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479-491.

Franclemont JG (1973). *Mimallonoidea: Mimallonidae and Bombycoidea: Apatelodidae, Bombycidae, Lasiocampidae. The Moths of North America North of Mexico, Fascicle 20.1*. The Wedge Entomological Research Foundation.

Franklin MT, Myers JH, Cory JS (2014). Genetic similarity of island populations of tent caterpillars during successive outbreaks. *PLOS One* **9**: e96679. DOI: 10.1371/journal.pone.0096679.

Frantine-Silva W, Giangarelli DC, Penha RES, Suzuki KM, Dec E, Gaglianone MC, Alves-dos-Santos I, Sofia SH. (2017). Phylogeography and historical demography of the orchid bee *Euglossa iopoecila*: signs of vicariant events associated to Quaternary climatic changes. *Conservation Genetics* **18**: 539-552. DOI: 10.1007/s10592-016-0905-7.

Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK (1999). A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular Ecology* **8**: 1683-1691.

Galbreath KE, Hafner DJ, Zamudio KR, Agnew K (2009). Isolation and introgression in the intermountain west: Contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography* **37**: 344-362. DOI: 10.1111/j.1365-2699.2009.02201.x.
Gerardi S, Jaramillo-Correa JP, Beaulieu J, Bousquet J (2010). From glacial refugia to modern populations: new assemblages of organelle genomes generated by differential cytoplasmic gene flow in transcontinental black spruce. *Molecular Ecology* **19**: 5265-5280. DOI: 10.1111/j.1365-294X.2010.04881.x.

Godbout J, Jaramillo-Correa JP, Beaulieu J, Bousquet J (2005). A mitochondrial DNA minisatellite reveals the postglacial history of jack pine (*Pinus banksiana*), a broad-range North American conifer. *Molecular Ecology* **14**: 3497-3512. DOI: 10.1111/j.1365-294X.2005.02674.x.

Golden JL, Bain JF (2000). Phylogeographic patterns and high levels of chloroplast DNA diversity in four *Packera* (Asteraceae) species in southwestern Alberta. *Evolution* **54**: 1566-1579. DOI: 10.1111/j.0014-3820.2000.tb00702.x.

Graham BA, Burg TM (2012). Molecular markers provide insight into contemporary and historic gene flow for a non-migratory species. *Journal of Avian Biology* **43**: 198-214. DOI: 10.1111/j.1600-048X.2012.05604.x.

Graham MR, Jaeger JR, Prendini L, Riddle BR (2013). Phylogeography of the Arizona hairy scorpion (*Hadrurus arizonensis*) supports a model of biotic assembly in the Mojave Desert and adds a new Pleistocene refugium. *Journal of Biogeography* **40**: 1298-1312. DOI: 10.1111/jbi.12079.

Griffin SR, Barrett SCH (2004). Post-glacial history of *Trillium grandiflorum* (Melanthiaceae) in eastern North America: inferences from phylogeography. *American Journal of Botany* **91**: 465-473. DOI: 10.3732/ajb.91.3.465.

Herke SW, Foltz DW (2002). Phylogeography of two squid (*Loligo pealei* and *L. plei*) in the Gulf of Mexico and northwest Atlantic Ocean. *Marine Biology* **140**: 103-115. DOI: 10.1007/s002270100680.

Hewitt GM (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58**: 247-276. DOI: 10.1111/j.1095-8312.1996.tb01434.x.

Hewitt GM (2000). The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907-913. DOI: 10.1038/35016000.
Hewitt GM (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**: 183-195. DOI: 10.1098/rstb.2003.1388.

Hildahl V, Reeks WA (1960). Outbreaks of the forest tent caterpillar, *Malacosoma disstria* Hbn., and their effects on stands of trembling aspen in Manitoba and Saskatchewan. *The Canadian Entomologist* **92**: 199-209. DOI: 10.4039/Ent92199-3.

Johansen AD, Latta RG (2003). Mitochondrial haplotype distribution, seed dispersal and patterns of postglacial expansion of ponderosa pine. *Molecular Ecology* **12**: 293-298. DOI: 10.1046/j.1365-294X.2003.01723.x.

Joly S, Bruneau A (2004). Evolution of triploidy in *Apios americana* (Leguminosae) revealed by genealogical analysis of the histone H3-D gene. *Evolution* **58**: 284-295. DOI: 10.1111/j.0014-3820.2004.tb01645.x.

Jones MT, Voss SR, Ptacek MB, Weisrock DW, Tonkyn DW (2006). River drainages and phylogeography: an evolutionary significant lineage of shovel-nosed salamander (*Desmognathus marmoratus*) in the southern Appalachians. *Molecular Phylogenetics and Evolution* **38**: 280-287. DOI: 10.1016/j.ympev.2005.05.007.

Karthika P, Vadivalagan C, Krishnaveni N, Murugan K, Nicoletti M, Canale A, Benelli G. (2017). Contrasting genetic diversity and intra-population polymorphism of the invasive pest *Henosepilachna viginti-octopunctata* (Coleoptera, Coccinellidae): a DNA barcoding approach. *Journal of Asia-Pacific Entomology* **20**: 23-29. DOI: 10.1016/j.aspen.2016.11.011.

Kirichenko N, Triberti P, Ohshima I, Haran J, Byun B-K, Li H, Augustin S, Roques A, Lopez-Vaamonde C. (2017) From east to west across the Palearctic: phylogeography of the invasive lime leaf miner *Phyllonorycter issikii* (Lepidoptera: Gracillariidae) and discovery of a putative new cryptic species in East Asia. *PLOS One* **12**:e0171104. DOI: 10.1371/journal.pone.0171104.
Klein NK, Brown WM (1994). Intraspecific molecular phylogeny in the yellow warbler (*Dendroica petechia*), and implications for avian biogeography in the West Indies. *Evolution* **48**: 1914-1932. DOI: 10.1111/j.1558-5646.1994.tb02223.x.

Knowles L (2000). Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. *Evolution* **54**: 1337-1348. DOI: 10.1111/j.0014-3820.2000.tb00566.x.

Krysko KL, Nunez LP, Newman CE, Bowen BW (2017). Phylogenetics of kingsnakes, *Lampropeltis getula* complex (Serpentes: Colubridae), in eastern North America. *Journal of Heredity* **108**: 226-238. DOI: 10.1093/jhered/esw086.

Lait LA, Burg TM (2013). When east meets west: population structure of a high-latitude resident species, the boreal chickadee (*Poecile hudsonicus*). *Heredity* **111**: 321-329. DOI: 10.1038/hdy.2013.54.

Lait LA, Friesen VL, Gaston AJ, Burg TM (2012). The post-Pleistocene population genetic structure of a western North American passerine: the chestnut-backed chickadee (*Poecile rufescens*). *Journal of Avian Biology* **43**: 541-552. DOI: 10.1111/j.1600-048X.2012.05761.x.

Lamb T, Avise JC (1992). Molecular and population genetic aspects of mitochondrial DNA variability in the diamondback terrapin, *Malaclemys terrapin*. *Journal of Heredity* **83**: 262-269. DOI: 10.1093/oxfordjournals.jhered.a111211.

Librado P, Rozas J (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452. DOI: 10.1093/bioinformatics/btp187.

McLachlan JS, Clark JS, Manos PS (2005). Molecular indicators of tree migration capacity under rapid climate change. *Ecology* **86**: 2088-2098. DOI: 10.1890/04-1036.

Medina RF, Rondon SI, Reyna SM, Dickey AM (2010). Population structure of *Phthorimaea operculella* (Lepidoptera: Gelechiidae) in the United States. *Environmental Entomology* **39**: 1037-1042. DOI: 10.1603/en09286.

Merrill SA, Ramberg FB, Hagedorn HH (2005). Phylogeography and population structure of *Aedes aegypti* in Arizona. *The American Journal of Tropical Medicine and Hygiene* **72**: 304-310.
Ney G, Schul J (2017). Population structure within the one-dimensional range of a coastal plain katydid. *PLOS One* **12**. DOI: 10.1371/journal.pone.0179361.

Nielson M, Lohman K, Sullivan J (2001). Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. *Evolution* **55**: 147-160. DOI: 10.1111/j.0014-3820.2001.tb01280.x.

Nilsson T (1983). *The Pleistocene: Geology and Life in the Quaternary Ice Age*. D. Reidel Publishing Company: Dordrecht, Holland.

Ober KA, Connolly CT (2015). Geometric morphometric and phylogenetic analyses of Arizona Sky Island populations of *Scaphinotus petersi* Roeschke (Coleoptera: Carabidae).

Peakall ROD, Smouse PE (2006). GenAlEx 6: Genetic Analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288-295. DOI: 10.1111/j.1471-8286.2005.01155.x.

Peakall ROD, Smouse PE (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* **28**: 2537-2539. DOI: 10.1093/bioinformatics/bts460.

Petersen SD, Stewart DT (2006). Phylogeography and conservation genetics of southern flying squirrels (*Glaucomys volans*) from Nova Scotia. *Journal of Mammalogy* **87**: 153-160. DOI: 10.1644/05-MAMM-A-062R1.1.

Pielou EC (1991). *After the Ice Age: The Return of Life to Glaciated North America*. University of Chicago Press: Chicago, IL.

Poulos HM, Taylor AH, Beaty RM (2007). Environmental controls on dominance and diversity of woody plant species in a Madrean, sky island ecosystem, Arizona, USA. *Plant Ecology* **193**: 15-30. DOI: 10.1007/s11258-006-9245-x.
Powell BF, Steidl RJ (2015). Influence of vegetation on montane riparian bird communities in the sky islands of Arizona, USA. *Southwestern Naturalist* 60: 65-71. DOI: 10.1894/mcg-09.1.

Ratnasingham S, Hebert PDN (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7: 355-364. DOI: 10.1111/j.1471-8286.2006.01678.x.

Richardson BA, Brunsfeld SJ, Klopfenstein NB (2002). DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (*Pinus albicaulis*). *Molecular Ecology* 11: 215-227.

Riddle BR, Hafner DJ, Alexander LF (2000). Comparative phylogeography of Baileys' pocket mouse (*Chaetodipus*) and the *Peromyscus eremicus* species group: historical vicariance of the Baja California peninsular desert. *Molecular Phylogenetics and Evolution* 17: 161-172. DOI: 10.1006/mpev.2000.0842.

Roland J (1993). Large-scale forest fragmentation increases the duration of tent caterpillar outbreak. *Oecologia* 93: 25-30. DOI: 10.1007/BF00321186.

Rozas J, Sanchez-DelBarrio JC, Messegue X, Rozas R (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497. DOI: 10.1093/bioinformatics/btg359.

Shaw J, Small RL (2005). Chloroplast DNA phylogeny and phylogeography of the North American plums (*Prunus subgenus Prunus section Prunocerasus*, Rosaceae). *American Journal of Botany* 92: 2011-2030. DOI: 10.3732/ajb.92.12.2011.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006). Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* 15: 4261-4293. DOI: 10.1111/j.1365-294X.2006.03061.x.

Sperling FAH, Raske AG, Otvos IS (1999). Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera : Geometridae).

Sperling FAH, Raske AG, Otvos IS (1999). Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera : Geometridae). *Insect Molecular Biology* 8: 97-106. DOI: 10.1046/j.1365-2583.1999.810097.x.

Stehr FW, Cook EF (1968). *A revision of the genus Malacosoma Hübner in North America (Lepidoptera: Lasiocampidae): systematics, biology, immatures, and parasites*, Vol 276.

Smithsonian Institution Press: Washington D.C.
Stewart JR, Lister AM (2001). Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology & Evolution* **16**: 608-613. DOI: 10.1016/S0169-5347(01)02338-2.

Stewart JR, Lister AM, Barnes I, Dálen L (2009). Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences* **277**: 661-671. DOI: 10.1098/rspb.2009.1272.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729. DOI: 10.1093/molbev/mst197.

Vallianatos M, Lougheed SC, Boag PT (2001). Phylogeography and genetic characteristics of a putative secondary-contact zone of the loggerhead shrike in central and eastern North America. *Canadian Journal of Zoology* **79**: 2221-2227. DOI: 10.1139/z01-157.

Vandewoestijne S, Baguette M, Brakefield PM, Saccheri IJ (2004). Phylogeography of *Uglais urticae* (Lepidoptera) based on DNA sequences of the mitochondrial COI gene and control region. *Molecular Phylogenetics and Evolution* **31**: 630-646. DOI: 10.1016/j.ympev.2003.09.007.

van Els P, Cicero C, Klicka J (2012). High latitudes and high genetic diversity: phylogeography of a widespread boreal bird, the gray jay (*Perisoreus canadensis*). *Molecular Phylogenetics and Evolution* **63**: 456-465. DOI: 10.1016/j.ympev.2012.01.019.

Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005). Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*). *Conservation Genetics* **6**: 159-174.

Vogler AP, Desalle R (1993). Phylogeographic patterns in coastal North American tiger beetles (*Cicindela dorsalis* Say) inferred from mitochondrial DNA sequences. *Evolution* **47**: 1192-1202. DOI: 10.1111/j.1558-5646.1993.tb02146.x.

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002). Ecological responses to recent climate change. *Nature* **416**: 389-395.

Warshall P (1995). *The Madrean sky island archipelago: a planetary overview*, Vol 264.

Wilson GM, Den Bussche RA, McBeek K, Johnson LA, Jones CA (2005). Intraspecific phylogeography of red squirrels (*Tamiasciurus hudsonicus*) in the central Rocky Mountains.
Mountain region of North America. *Genetica* **125**: 141-154. DOI: 10.1007/s10709-005-5154-5.

Young AM, Torres C, Mack JE, Cunningham CW (2002). Morphological and genetic evidence for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit crab *Pagurus longicarpus*. *Marine Biology* **140**: 1059-1066.

Zink RM, Kessen AE, Line TV, Blackwell-Rago RC (2001). Comparative phylogeography of some aridland bird species. *The Condor* **103**: 1-10. DOI: 10.1650/0010-5422(2001)103[0001:CPOSAB]2.0.CO;2.

Zink RM, Rootes WL, Dittmann DL (1991). Mitochondrial DNA variation, population structure, and evolution of the common grackle (*Quiscalus quiscula*). *The Condor* **93**: 318-329. DOI: 10.2307/1368947.
### Table 1 (on next page)

Sample details for three *Malacosoma* species.

Sample size (n), number of sampling locations (loc), variable sites (VS), mean % pairwise distances (PD), number of haplotypes (h), overall haplotype (Hd) and nucleotide (π) diversities, and fixation index (Φ<sub>ST</sub>) for three *Malacosoma* species. The three F<sub>ST</sub> values were all highly significant (p < 0.0001). 

| Sample size (n) | Number of sampling locations (loc) | Variable sites (VS) | Mean % pairwise distances (PD) | Number of haplotypes (h) | Overall haplotype (Hd) | Nucleotide (π) diversity | Fixation index (Φ<sub>ST</sub>) |
|-----------------|-------------------------------------|---------------------|-------------------------------|--------------------------|------------------------|--------------------------|---------------------------|
|                 |                                     |                     |                               |                          |                        |                          |                           |                           |
| Species       | n  | loc | VS  | PD ± SE    | h  | Hd   | π    | Φ<sub>ST</sub> |
|--------------|----|-----|-----|------------|----|------|------|--------------|
| *M. americana* | 79 | 12  | 37  | 0.490 ± 0.303 | 33 | 0.918 | 0.0049 | 0.244        |
| *M. californica* | 207 | 9   | 61  | 0.968 ± 0.668 | 64 | 0.925 | 0.0097 | 0.477        |
| *M. disstria* | 139 | 19  | 43  | 0.628 ± 0.383 | 42 | 0.926 | 0.0063 | 0.524        |
Population pairwise $\Phi_{ST}$ values for three *Malacosoma* species.

Population pairwise $\Phi_{ST}$ values for (a) *M. americana* ($P_{\text{crit}} = 0.015$), (b) *M. californica* ($P_{\text{crit}} = 0.013$), and (c) *M. disstria* ($P_{\text{crit}} = 0.011$). $F_{ST}$ values are given below the diagonal and p-values above the diagonal (* p< 0.05, ** p < 0.01, *** p < 0.001). Values significant following correction for multiple tests are shaded. Refer to Fig. 1 for locations.
### (a) NB ON/QC MN MD/NC TN STH¹

|       | NB  | ON/QC | MN  | MD/NC | TN  | STH¹ |
|-------|-----|-------|-----|-------|-----|------|
| NB    | -   |       |     |       |     |      |
| ON/QC | 0.195 | - |     |       |     |      |
| MN    | 0.637 | 0.395 | - | 0.298 | 0.257 | *    |
| MD/NC | 0.481 | 0.000 | 0.326 | - | 0.484 | 0.321 |
| TN    | 0.564 | 0.175 | 0.202 | 0.000 | - | 0.376 |
| STH¹  | 0.444 | 0.213 | 0.271 | 0.015 | 0.007 | -    |

### (b) CBC EBC SWBC VI WA CA sCA AZ

|       | CBC  | EBC  | SWBC | VI  | WA  | CA  | sCA | AZ  |
|-------|------|------|------|-----|-----|-----|-----|-----|
| CBC   | -    | ***  | 0.185| *** | *   | *** | **  | *** |
| EBC   | 0.249| -    | ***  | *** | *   | *** | **  | *** |
| SWBC  | 0.069| 0.233| -    | *** | 0.143| **  | **  | *** |
| VI    | 0.403| 0.313| 0.247| -   | 0.117| *** | *** | *** |
| WA    | 0.352| 0.258| 0.229| 0.138| -   | *   | *   | **  |
| CA    | 0.448| 0.241| 0.422| 0.641| 0.500| -   | **  | *** |
| sCA   | 0.892| 0.725| 0.907| 0.918| 0.935| 0.799| -   | **  |
| AZ    | 0.682| 0.618| 0.661| 0.789| 0.662| 0.544| 0.732| -   |

### (c) cBC eBC AB SK cON sON eON NB/NS STH¹ TN SE²

|       | cBC  | eBC  | AB   | SK   | cON  | sON  | eON  | NB/NS | STH¹ | TN  | SE² |
|-------|------|------|------|------|------|------|------|-------|------|-----|-----|
| cBC   | -    | 0.305| ***  | ***  | **   | ***  | ***  | ***   | ***  | *** | *** |
| eBC   | 0.044| -    | ***  | ***  | ***  | ***  | ***  | ***   | ***  | *** | *** |
| AB    | 0.677| 0.719| -    | 0.284| ***  | ***  | ***  | ***   | ***  | *** | *** |
| SK    | 0.948| 0.978| 0.026| -    | ***  | ***  | ***  | ***   | ***  | *** | *** |
| cON   | 0.541| 0.626| 0.357| 0.494| -    | 0.319| 0.897| *     | ***  | 0.057|     |
| sON   | 0.617| 0.660| 0.255| 0.362| 0.016| -    | 0.264| 0.109 | ***  | *   |     |
| eON   | 0.486| 0.530| 0.294| 0.367| 0.000| 0.012| -    | *     | **   | **  | *   |
| NB/NS | 0.769| 0.808| 0.284| 0.468| 0.201| 0.037| 0.134| -     | ***  | *** | **  |
| STH¹  | 0.612| 0.683| 0.354| 0.496| 0.259| 0.212| 0.201| 0.269 | -    | *** | 0.241|
| TN    | 0.873| 0.912| 0.473| 0.835| 0.440| 0.300| 0.313| 0.407 | 0.232| -   |     |
| SE²   | 0.824| 0.890| 0.349| 0.781| 0.272| 0.155| 0.188| 0.246 | 0.035| 0.317| -   |

³STH = AR, OK, TX ²SE = NC, GA, FL  * p < 0.05, ** p < 0.01, *** p < 0.001
Figure 1

Distributions, sampling locations, and Bayesian cluster membership for three *Malacosoma* species.

Approximate distributions (shaded) and sampling locations for (a) *Malacosoma americana*, (b) *M. californica*, and (c) *M. disstria*. The pie charts represent the distribution of BAPS groups, scaled for sample size. The green crosses represent the omitted AB, SK, and NB samples. Sampling locations are as follows: Alberta (AB), Arizona (AZ), British Columbia (BC; central [c], eastern [e], and southwest [sw]), California (CA; southern [s]), Kentucky (KY), Maryland (MD), Minnesota (MN), New Brunswick (NB), North Carolina (NC), Nova Scotia (NS), Ontario (ON; central [c], eastern [e], and southern [s]), Saskatchewan (SK), Tennessee (TN), Texas, (TX), Vancouver Island BC (VI), and Washington (WA). The STH includes Arkansas (AR), Oklahoma (OK), and TX combined, and SE includes Florida (FL), Georgia (GA), and NC combined.
Figure 2

Principal coordinates analysis for three *Malacosoma* species.

Principal coordinates analysis for (a) *M. americana*, (b) *M. californica*, and (c) *M. disstria*. The northern populations are depicted by a circle or square, while southern populations are represented by a diamond. Samples are colour-coded by sampling location. Refer to Fig. 1 for locations.
Figure 3

Statistical parsimony network for *M. americana*.

Statistical parsimony network showing the relationship among the 33 *M. americana* haplotypes. Each square represents one of the 79 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.
Figure 4

Statistical parsimony network for *M. californica*.

Statistical parsimony network showing the relationship among the 64 *M. californica* haplotypes. Each square represents one of the 207 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.
Figure 5

Statistical parsimony network for *M. disstria*.

Statistical parsimony network showing the relationship among 42 *M. disstria* haplotypes. Each square represents one of the 139 sequences colour-coded by location, inferred haplotypes are depicted by black circles, and each line represents a single nucleotide change. Refer to Fig. 1 for locations.