Phylogeographic structure in three North American tent caterpillar species (Lepidoptera: Lasiocampidae): *Malacosoma americana*, *M. californica*, and *M. disstria* (#22273)

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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. The present study begins to address this gap by examining population structure in members of the moth genus *Malacosoma*, an important group of forestry pests. The study begins by examining taxonomic boundaries among 474 specimens from five North American species based on sequence variation in the mitochondrial cytochrome *c* oxidase 1 (COI) gene. This work revealed that three of the species formed monophyletic lineages while *M. californica* and *M. incurva* were paraphyletic. The diversity of *M. californica* suggests a species complex, a conclusion supported by prior taxonomic work which assigned its members to six subspecies. Subsequent analysis examined the genetic structure of the three widespread 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 their 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. 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 than in the east.

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ABSTRACT

While phylogeographic structure has been examined in many North American vertebrate species, insects have received much less attention. The present study begins to address this gap by examining population structure in members of the moth genus *Malacosoma*, an important group of forestry pests. The study begins by examining taxonomic boundaries among 474 specimens from five North American species based on sequence variation in the mitochondrial cytochrome *c* oxidase 1 (COI) gene. This work revealed that three of the species formed monophyletic lineages while *M. californica* and *M. incurva* were paraphyletic. The diversity of *M. californica* suggests a species complex, a conclusion supported by prior taxonomic work which assigned its members to six subspecies. Subsequent analysis examined the genetic structure of the three widespread 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 their 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. 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 than 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 genetic variation.

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" for others with the intervening lowlands limiting gene flow (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. Here we examine three species in the genus *Malacosoma*, and describe the analytical framework that will be employed in later investigations on larger assemblages.

Malacosoma (Hübner, 1820) contains 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 structure in these moths. One study, which assessed allozyme variation in *Malacosoma americana* (Fabricius, 1793) from eastern United States, found limited variability 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 pluvial* coastal

British Columbia (Franklin, Myers & Cory, 2014). Although high levels of variation were evident, there was little genetic differentiation between island and mainland populations.

This study employs sequence variation in a 658 bp segment of the mitochondrial COI gene to examine genetic structure in 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~~). 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. By contrast, both *M. americana* and *M. disstria* have no described subspecies (Stehr & Cook, 1968; Franclemont, 1973). All three species feed on diverse deciduous trees and shrubs, with host preferences varying by taxon and region, and a narrower host range in *M. americana* (Stehr & Cook, 1968; Franclemont, 1973; Parry & Goyer, 2004). 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 mitochondrial markers will be low. While previous studies found ~~limited~~ genetic differentiation, they examined nuclear genes or very short mitochondrial sequences (<154 bp) at a relatively small geographic scale. By contrast, the present study assesses the genetic structure of a 658 bp mitochondrial gene region on a continental scale to ~~determine~~ if these *Malacosoma* species show concordant phylogeographic patterns.

MATERIALS AND METHODS

Species Relationships

A total of 474 COI sequences from specimens of five *Malacosoma* species collected in the United States and Canada were downloaded from the Barcode of Life Data System (BOLD;

see Supplemental Table S1; Ratnasingham & Hebert, 2007). These sequences were aligned in MEGA v6 (Tamura *et al.*, 2013) before a Bayesian network was constructed in BEAST v2.3 (Bouckaert *et al.*, 2014). The network was run with the HKY + Γ + I model for 10,000,000 MCMC steps, sampled every 2,000 steps, and had a 25% burn-in. Another lasiocampid, *Phyllodesma americana* (GenBank Accession Number JF842281), was used as the outgroup.

Genetic analysis

Haplotypes were assigned to each species manually and confirmed with TCS v1.21 (Clement, Posada & Crandall, 2000). Haplotype and nucleotide diversity indices were calculated in DNAsp v5.10 (Rozas *et al.*, 2003; Librado & Rozas, 2009), and an analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was performed in Arlequin v3.5.1.2 (Excoffier & Lischer, 2010). A spatial analysis of molecular variance (SAMOVA) was employed to detect genetic clusters (Dupanloup, Schneider & Excoffier, 2002). The SAMOVA was run for K = 2 to 6 with 1,000 iterations. Pairwise genetic differences (Φ_{ST}; 100,000 permutations) were calculated in Arlequin using K2P distances; a modified false discovery rate was applied to correct for multiple tests (Benjamini & Yekutieli, 2001). Nearby sampling locations were grouped to increase sample sizes. Isolation by distance was examined with a Mantel test as performed in Genepop v4.2 (10,000 permutations; Raymond & Rousset, 1995; Rousset, 2008); geographic distances were calculated with the Geographic Distance Matrix Calculator v1.2.3 (Ersts, 2015). Monmonier's algorithm, a 'splitting' analysis which uses Voronoi tessellation and Delauney triangulation, was employed to test for possible barriers to dispersal. The algorithm was implemented in Barrier v2.2 and used pairwise Φ_{ST} values as genetic distances (Manni, Guérard & Heyer, 2004).

A statistical parsimony network was constructed in TCS v1.2.1 to visualise the relationship among haplotypes. Cluster analyses were performed in Bayesian Analysis of Population Structure v5.2 (BAPS; Corander *et al.*, 2008) using the clustering with linked loci option (Corander & Tang, 2007). BAPS allows the assignment of individuals to genetic clusters with no *a priori* population information. A principal coordinates analysis (PCoA) was run in GenAlEx v6.5 (Peakall & Smouse, 2006; Peakall & Smouse, 2012) to identify genetic structure with no population constraints.

RESULTS

Species affinities and boundaries

The Bayesian network identified two main lineages within North American *Malacosoma*: the first group included specimens of *M. constricta* and *M. disstria* with each species forming a well-defined monophyletic ~~species;~~ the second included *M. americana*, *M. incurva*, and *M. californica* ~~(Fig. 2)~~. 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, Saskatchewan, and New Brunswick showed closer similarity to those identified as *M. californica pluviale* than to specimens in the west (likely *M. californica californica*). Based on their geographic origin, these populations likely represent *M. californica lutescens*, the Great Plains tent caterpillar, or *M. californica pluviale* with the remaining samples corresponding to *M. californica californica* and one or more of the three southern subspecies (*M. californica ambisimile*, *M. californica fragilis*, and *M. californica recenseo*). To further evaluate relationships, a Bayesian tree was run as above in BEAST v2.3 on the 474 North American samples plus 45 samples from four European *Malacosoma* species (*M. neustria*, *M. alpicola*, *M.*

franconica, *M. castrensis*), and four *M. incurva* samples from Mexico (see Supplemental Table S1). This analyses also identified two groups: one including the European taxa with *M. constricta* and *M. disstria*, all forming well-defined species, and a second group that generally mirrored the results from North American analysis. Interestingly, the four Mexican samples of *M. incurva* grouped with the *M. californica* AB and SK samples (Supplemental Fig. S1). As further study with additional markers is required to establish the taxonomic status of lineages within the *M. californica* complex, the current analyses does not include *M. californica pluviale* or the AB, SK, and NB samples (see Fig. 1).

Cytochrome *c* oxidase I sequences

Subsequent analyses examined 79 COI sequences for *M. americana*, 207 for *M. californica*, and 139 for *M. disstria* (Supplemental Table S1). 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; π = 0.0049 - 0.0097; Table 1), with diversity generally higher in southern populations (Supplemental Table S2).

Genetic analyses

175 ***Malacosoma americana***

176 Of the three species, *M. americana* showed the least population structure (overall Φ_{ST} =
177 0.24, $p < 0.0001$), the fewest significant pairwise comparisons (7 out of 15; Table 2), and the
178 lowest diversity (Table 1). The greatest pairwise Φ_{ST} values were seen between the MN and ON
179 populations, and between NB and the other populations. Monmonier's analysis supported the
180 separation of MN and NB from the other populations (Fig. 1), while the SAMOVA analysis
181 identified four groups ($F_{CT} = 0.263$, $p = 0.02$): MN, NB, and the separation of the remaining
182 samples into northern (ON, MD/NC) and southern (TN, AR/OK/TX) populations. There was
183 evidence of significant isolation by distance ($R^2 = 0.3$, $p = 0.006$)

184 The statistical parsimony network showed a general clustering of southern samples (OK,
185 TX, AR, and TN) and NB samples (Fig. 3). The haplotypes were generally closely related,
186 although ON, OK, and MN had more divergent haplotypes. The principal coordinates analysis
187 allocated 55% of the variation to the first three coordinates (34.7%, 11.5%, and 8.8%,
188 respectively). The samples separated into northern and southern groups along coordinate 1 (Fig.
189 4a). Bayesian clustering analysis separated the samples into three clusters (Fig. 1, Supplemental
190 Table S2): a "northern" group found primarily in ON and NB, and two "southern" groups, a
191 larger group found in all populations except NB, and a smaller group primarily in MN and OK.
192 The distribution of haplotypes was significantly non-random ($X^2 = 47.65$, $p < 0.0001$).

193

194 ***Malacosoma californica***

195 *M. californica* had the strongest population structure (overall $\Phi_{ST} = 0.48$, $p < 0.0001$),
196 and the highest nucleotide diversity (Table 1). All pairwise comparisons were significant except
197 those with WA (17 out of 21; Table 2), and only a single comparison lacked significance before

198 correction (WA with swBC, p = 0.15). The greatest differences existed between sCA and all
199 other populations (Φ_{ST} = 0.73 - 0.94), and between AZ and all other populations (Φ_{ST} = 0.54 -
200 0.79). Both Monmonier's analysis and SAMOVA analysis identified genetic breaks between
201 three groups: sCA, AZ, and all other populations (F_{CT} = 0.57, p = 0.049; Fig. 1). The Mantel test
202 showed moderate and significant isolation by distance (R^2 = 0.15, p = 0.016), with the three
203 highest genetic distances (sCA with cBC, swBC, and WA) at large, but not the maximum,
204 geographic distances.

205 The statistical parsimony network showed a dumbbell-like pattern, with two large groups
206 containing most of the samples except those from CA, sCA, and AZ (Fig. 5). One group
207 consisted of haplotypes from across the Pacific Northwest, and contained all samples from cBC,
208 VI, swBC, and WA, as well as many eBC samples. There were several common haplotypes
209 separated by one to three mutations. The second cluster was restricted to eBC; it was less diverse
210 and contained two common haplotypes, one represented by 44 individuals. Most CA samples
211 had unique, fairly divergent haplotypes, while the sCA and AZ haplotypes were divergent with
212 13 - 15 (sCA) and 5 - 16 (AZ) mutations separating them from the nearest population (Fig. 5).

213 The principal coordinates analysis allocated 65.1% of the variation to the first three
214 coordinates (39.0%, 16.4%, and 9.7%, respectively). The BC samples separated into two groups
215 along coordinate 1, while the sCA and AZ samples were separated along coordinate 2 (Fig. 4b).
216 Bayesian clustering analysis identified six clusters (Fig. 1, Supplemental Table S2): one in sCA,
217 one in AZ, one in eBC, and three shared between multiple populations. The eBC population had
218 representatives in four clusters. The distribution of samples in the six clusters was highly
219 significant (X^2 = 486.6, p <0.0001). When sCA, CA, and AZ were removed from analysis, the
220 distribution was still significantly different than random (X^2 = 57.02, p < 0.0001).

221

222 ***Malacosoma disstria***

223 *M. disstria* also had significant population structure (overall $\Phi_{ST} = 0.42$, $p < 0.0001$).
224 Forty-two of 55 pairwise comparisons were significant following correction for multiple tests
225 (Table 2). The 13 comparisons lacking significance involved ON with NB/NS, ON with
226 NC/FL/GA, or were among the three ON locations. In contrast, the largest pairwise differences
227 were between BC and all other populations ($\Phi_{ST} = 0.49$ - 0.98). Diversity within populations was
228 generally high with $H_d > 0.7$ in 10 of the 16 populations. The lowest values were in SK ($H_d = 0$)
229 followed by eBC ($H_d = 0.20$; Supplemental Table S2). A similar pattern was seen with
230 nucleotide diversity with the highest values in the three ON populations ($\pi = 0.0053$ - 0.0065)
231 and TX ($\pi = 0.0137$). Monmonier's analysis identified a genetic break between the BC
232 populations and all other populations (including AB), possibly along the Rocky Mountains. This
233 was supported by the SAMOVA analysis ($F_{CT} = 0.52$, $p = 0.015$; Fig. 1). A second barrier was
234 identified on the Great Plains (east of the SK and MB populations; $F_{CT} = 0.41$, $p = 0.002$), but
235 this was not supported by SAMOVA. The Mantel test showed significant isolation by distance (p
236 $= 0.0001$), although the pattern was very weak ($R^2 < 0.0001$) due to very high genetic distances
237 between two sites in BC (eBC and cBC) and one in SK, likely a result of the monomorphic
238 nature of the SK population. When the comparison was run without SK the pattern was much
239 stronger ($R^2 = 0.29$, $p = 0.0005$).

240 The statistical parsimony network identified moderate variation, with five common
241 haplotypes ($n \geq 10$): three restricted to a single region (BC, AB/SK, and ON), one shared by ON
242 and NB/NS, and a third found across several regions (Fig. 6). The BC samples formed a separate
243 group (with a single AB sample), while AB, SK, and MB generally grouped together. In general,

northern and southern samples were separated, although there were exceptions. The principal coordinates analysis revealed similar structure with BC separating along coordinate 1 (41.5%) and a general separation of northern and southern populations along coordinate 2 (14.8%). Coordinate 3 explained 12.6% of the variation (Fig. 4c). Bayesian clustering analysis identified four clusters (Supplemental Table S2): three were restricted to the north while one was primarily found in the south. The BC samples clustered with some ON samples, AB and SK were in a cluster together (with four NB samples), and the remaining ON and NB/NS samples formed a third cluster. The allocation of samples to clusters was highly significant ($X^2 = 224.7$, $p < 0.0001$).

DISCUSSION

Variation among species

The analysis of mitochondrial COI sequences from three North American *Malacosoma* species showed high levels of variation and diversity, with some highly divergent populations. Despite considerable overlap in their distributions, particularly between *M. disstria* and the other two, patterns of variation and levels of population structure varied considerably.

The eastern species, *M. americana,* 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 (McLachlan, Clark & Manos, 2005; Shaw & Small, 2005; Gerardi *et al.*, 2010) and mammals (Petersen & Stewart, 2006), with the exception of more distinct southern populations (e.g., Texas or Florida). Both *M. americana* and *M. disstria* exhibit this pattern: limited structure in eastern North America with some differences between northern and southern populations. This is likely caused by colonisation from a single source population with limited ongoing gene flow. Give the limited dispersal capability of female *Malacosoma*, one would expect less gene flow than in highly-mobile bird species.

The western *M. californica* possessed a very different pattern with strong population structure and very distinct populations (AZ and sCA) which may represent different subspecies or ecotypes. Both the current and historical topography of this region can help explain these patterns. Western North America contains four major mountain ranges (Cascade, Coastal, Rocky, and Sierra) which run along a north-south axis, large plains, and deserts, all of which contribute to a complex habitat mosaic. This heterogeneity, coupled with the resulting complex glacial histories of the region, has resulted in extensive structuring in 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 (Richardson, Brunsfeld & Klopfenstein, 2002; Johansen & Latta, 2003). Interestingly, the western populations of *M. disstria* also show increased structure.

In addition to regional diversification, *M. disstria* exhibits a strong east/west separation, 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). It is particularly frequent in species with low dispersal capability or strong natal

philopatry. The sustained separation of multiple genetic lineages, often with intervening zones of admixture, may reflect the presence of a conspecific population impeding the establishment of a new population, or by large areas of unsuitable habitat. Additional sampling from the central region could help to clarify if there is a distinct break, or whether secondary mixing is occurring.

Glacial refugia and recolonisation

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 (π = 0.0046 - 0.0061 versus 0.0013 - 0.0027; Supplemental Table S2), and in *M. californica* where diversity was four-fold higher in Arizona (π = 0.0093) than in Washington (π = 0.002). In *M. disstria* diversity levels decreased to the northwest, likely representing a founder event in the BC populations with limited ongoing gene flow across the Rocky Mountains. All three species showed strong isolation by distance, indicating that recolonisation likely occurred in a stepping-stone fashion.

The fact that *M. americana* lacked fixed differences among populations, while *M. disstria* had only a few between the western and eastern populations, suggests a single refugial origin for both species, likely in the south-eastern United States. This pattern has been reported in both plant (Gerardi *et al.*, 2010) and animal (Zink, Rootes & Dittmann, 1991; Veit *et al.*, 2005; Petersen & Stewart, 2006) species across eastern North America. Interestingly, neither of these *Malacosoma* 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).

As the BC populations are significantly different than those in the east, it remains possible that current populations of *M. disstria* derive from at least two refugia: one in the southeast and another in the west. There may have been multiple western refugia that were missed due to the lack of samples from the southwestern portion of the range; additional sampling is required to test this. This prospect is consistent with the pattern found in *Populus tremuloides*, its favoured host, 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.

M. californica showed multiple fixed differences among sCA, AZ, and the other populations. This pattern, which is common in many southwestern and western species (Golden & Bain, 2000; Riddle, Hafner & Alexander, 2000; Galbreath *et al.*, 2009; Hamilton, Formanowicz & Bond, 2011), suggests isolation in multiple glacial refugia. Given the divergence of these two 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 is 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 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 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, as they should encounter suitable habitat more often than specialists.

In western North America, the main physical barriers are the Rocky, Coastal, Cascade, and Sierra Nevada Mountains. These act as a barrier to gene flow in many species (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 clear break across the Rocky Mountains (between BC and AB), while *M. californica* has disjunct populations among many of the southwestern deserts. 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, likely representing a different species.

Conclusions

The population genetic structure of the three *Malacosoma* species suggest 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. *M. disstria* shows a similar pattern in the east, supporting a single southern refugium, with a second possible ~~origin in~~ the west. Additional samples are required to elucidate whether the differentiation in its BC population reflects a founder event or additional structuring. *M. californica* shows the greatest structure and differentiation, consistent with multiple evolutionary origins in the west and southwest. Further research into the taxonomic relationship within and among these species is required to determine where the *M. californica pluviale* and *M. californica* AB/SK samples fit within this species, or whether they actually represent cryptic species. 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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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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 locations (loc), variable sites (VS), mean % pairwise distances (PD), number of haplotypes (h), overall haplotype (Hd) and nucleotide (π) diversities, and fixation index (Φ_{ST}) for three *Malacosoma* species. The three Φ_{ST} values were all significant ($p < 0.0001$).

Table 2. Population pairwise Φ_{ST} values for (a) *M. americana* ($P_{crit} = 0.015$), (b) *M. californica* ($P_{crit} = 0.014$), and (c) *M. disstria* ($P_{crit} = 0.011$). Φ_{ST} values are given below the diagonal and p-values above the diagonal. Values significant following correction for multiple tests are shaded. Refer to Fig. 1 for locations.

405 **Fig. 1.** Approximate distributions and sampling locations for (a) *Malacosoma americana*, (b) *M.*
406 *californica*, and (c) *M. disstria*. The dashed lines indicate genetic breaks identified by
407 Monmonier's algorithm and SAMOVA. The pie charts represent the distribution of BAPS
408 groups, scaled for sample size. The green crosses represent the omitted AB, SK, and NB *M.*
409 *californica* samples. Sampling locations are as follows: Alberta (AB), Arizona (AZ), Arkansas
410 (AR), British Columbia (BC; central [c], eastern [e], and southwest [sw]), California (CA;
411 southern [s]), Kentucky (KY), Maryland (MD), Minnesota (MN), New Brunswick (NB), North
412 Carolina (NC), Nova Scotia (NS), Oklahoma (OK), Ontario (ON; central [c], eastern [e], and
413 southern [s]), Saskatchewan (SK), Tennessee (TN), Texas, (TX), Vancouver Island BC (VI), and
414 Washington (WA). In (a) STH includes AR, OK, and TX. In (c) STH includes AR, KY, and OK,
415 and SE includes FL, GA, and NC.
416
417 **Fig. 2.** Bayesian analysis based on 474 COI sequences from five North American species of
418 *Malacosoma*. The triangles represent multiple specimens from the same species, and the length
419 of the triangle is representative of the sequence variation. Posterior values > 0.8 are given. The
420 shaded boxes represent the samples used in this study.
421
422 **Fig. 3.** Statistical parsimony network showing the relationship among the 33 *M. americana*
423 haplotypes. The 79 samples are colour-coded by location, and inferred haplotypes are depicted
424 by black circles. Refer to Fig. 1 for locations.
425
426 **Fig. 4.** Principle coordinates analysis for (a) *M. americana*, (b) *M. californica*, and (c) *M.*
427 *disstria*. Samples are colour-coded by sampling location. Refer to Fig. 1 for locations.

428

429 **Fig. 5.** Statistical parsimony network showing the relationship among the 64 *M. californica*
430 haplotypes. The 207 samples are colour-coded by location, and inferred haplotypes are depicted
431 by black circles. Refer to Fig. 1 for locations.

432

433 **Fig. 6.** Statistical parsimony network showing the relationship among 42 *M. disstria* haplotypes.
434 The 139 samples are colour-coded by location, and inferred haplotypes are depicted by black
435 circles. Refer to Fig. 1 for locations.

436

437 **SUPPLEMENTARY MATERIAL**

438 **Supplemental Table S1.** Sample information for nine *Malacosoma* species. The BOLD sample
439 ID, GenBank Accession Number, and sampling location are given. GPS coordinates for *M.*
440 *americana*, *M. californica*, and *M. disstria* are given where available.

441

442 **Supplemental Table S2.** Sample size (n) and distribution of haplotypes (h), haplotype diversity
443 (Hd), and nucleotide diversity (π) among the (a) *M. americana*, (b) *M. californica*, and (c) *M.*
444 *disstria* sampling locations. Shared haplotypes have been allocated a letter. The allocation of
445 samples into Bayesian clusters (BAPS) is also given.

446

447 **Supplementary Fig. 1.** Bayesian analysis of nine *Malacosoma* species from North America and
448 Europe. *Phyllodesma americana* is used as outgroup. Posterior values are given.

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Table 1(on next page)

Sample details for three *Malacosoma* species.

Sample size (n), number of locations (loc), variable sites (VS), mean % pairwise distances (PD), number of haplotypes (h), overall haplotype (Hd) and nucleotide (π) diversities, and fixation index (Φ_{ST}) are given. The three Φ_{ST} values were all significant ($p < 0.0001$).

Species	n	loc	VS	PD ± SE	h	Hd	π	Φ_{ST}
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	0.478
M. disstria	139	19	43	0.628 ± 0.383	42	0.926	0.0063	0.419

1

2

Table 2 (on next page)

Population pairwise Φ_{ST} values for (a) *M. americana* (P_{crit} = 0.015), (b) *M. californica* (P_{crit} = 0.014), and (c) *M. disstria* (P_{crit} = 0.011).

Φ_{ST} values are given below the diagonal and p-values above the diagonal. Values significant following correction for multiple tests are shaded. Refer to Fig. 1 for locations.

(a)	NB	ON	STH	MN	MD/NC	TN
NB	*	**0.001**	**0.000**	**0.002**	**0.007**	**0.001**
ON	0.195	*	**0.000**	**0.001**	0.702	0.027
STH	0.444	0.213	*	0.019	0.321	0.376
MN	0.637	0.395	0.271	*	0.298	0.257
MD/NC	0.481	0.000	0.015	0.326	*	0.484
TN	0.564	0.175	0.007	0.202	0.000	*

STH = AR, OK, TX

(b)	CBC	swBC	eBC	AZ	sCA	CA	WA
CBC	*	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	0.014
swBC	0.329	*	**0.000**	**0.000**	**0.000**	**0.000**	0.154
eBC	0.249	0.303	*	**0.000**	**0.000**	**0.003**	0.018
AZ	0.682	0.785	0.618	*	**0.001**	**0.000**	**0.004**
sCA	0.892	0.905	0.725	0.732	*	**0.004**	0.029
CA	0.448	0.612	0.241	0.544	0.799	*	**0.012**
WA	0.352	0.099	0.258	0.662	0.935	0.500	*

(c)	AB	eBC	cBC	NB/NS	eON	sON	cON	SK	STH	SE	TN
AB	*	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.284	**0.000**	**0.001**	**0.000**
eBC	0.719	*	0.300	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
cBC	0.677	0.044	*	**0.000**	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.002**	**0.000**
NB/NS	0.284	0.808	0.769	*	0.021	0.110	0.026	**0.000**	**0.000**	**0.005**	**0.000**
eON	0.294	0.530	0.486	0.134	*	0.265	0.896	**0.002**	**0.000**	0.040	**0.001**
sON	0.255	0.660	0.617	0.037	0.012	*	0.317	**0.000**	**0.000**	0.049	**0.000**
cON	0.357	0.626	0.541	0.201	0.000	0.016	*	**0.000**	**0.000**	0.055	**0.000**
SK	0.026	0.978	0.948	0.468	0.367	0.362	0.494	*	**0.000**	**0.001**	**0.000**
STH	0.391	0.701	0.644	0.315	0.245	0.260	0.316	0.523	*	0.073	**0.000**
SE	0.349	0.890	0.824	0.246	0.188	0.155	0.272	0.781	0.111	*	0.015
TN	0.473	0.912	0.873	0.407	0.313	0.300	0.440	0.835	0.286	0.317	*

STH = AR, KY, OK; SE = NC, GA, FL

Figure 1

Approximate distributions and sampling locations for (a) *Malacosoma americana*, (b) *M. californica*, and (c) *M. disstria*.

The dashed lines indicate genetic breaks identified by Monmonier's algorithm and SAMOVA. The pie charts represent the distribution of BAPS groups, scaled for sample size. The green crosses represent the omitted AB, SK, and NB *M. californica* samples. Sampling locations are as follows: Alberta (AB), Arizona (AZ), Arkansas (AR), 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), Oklahoma (OK), Ontario (ON; central [c], eastern [e], and southern [s]), Saskatchewan (SK), Tennessee (TN), Texas, (TX), Vancouver Island BC (VI), and Washington (WA). In (a) STH includes AR, OK, and TX. In (c) STH includes AR, KY, and OK, and SE includes FL, GA, and NC.

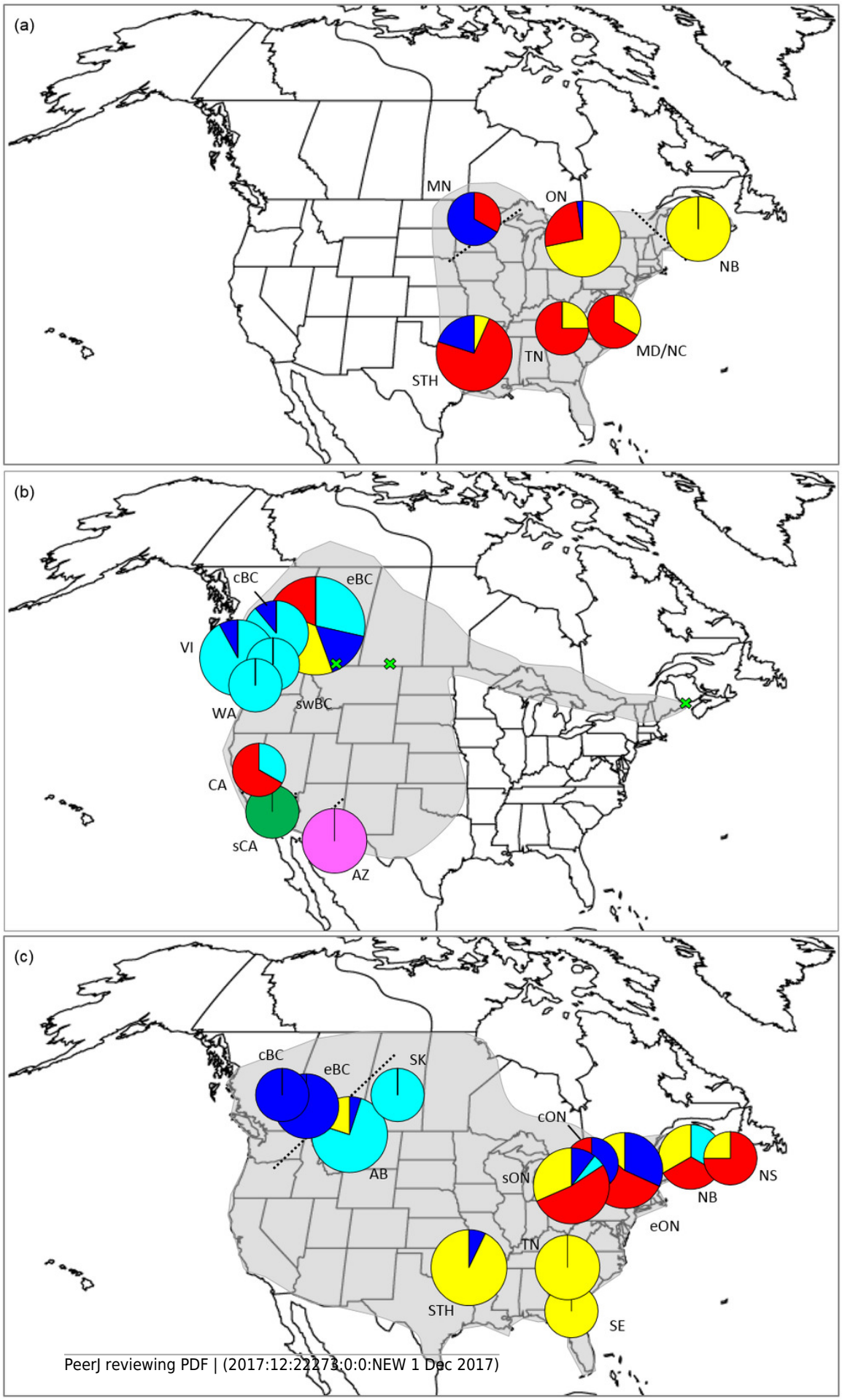

(a)
MN
ON
NB
TN
MD/NC
STH
(b)
cBC
eBC
VI
WA
swBC
CA
sCA
AZ
(c)
cBC
eBC
SK
AB
cON
sON
NB
NS
eON
TN
STH
SE

Figure 2

Bayesian analysis based on 474 COI sequences from five North American species of *Malacosoma*.

The triangles represent multiple specimens from the same species, and the length of the triangle is representative of the sequence variation. Posterior values > 0.8 are given. The shaded boxes represent the samples used in this study.

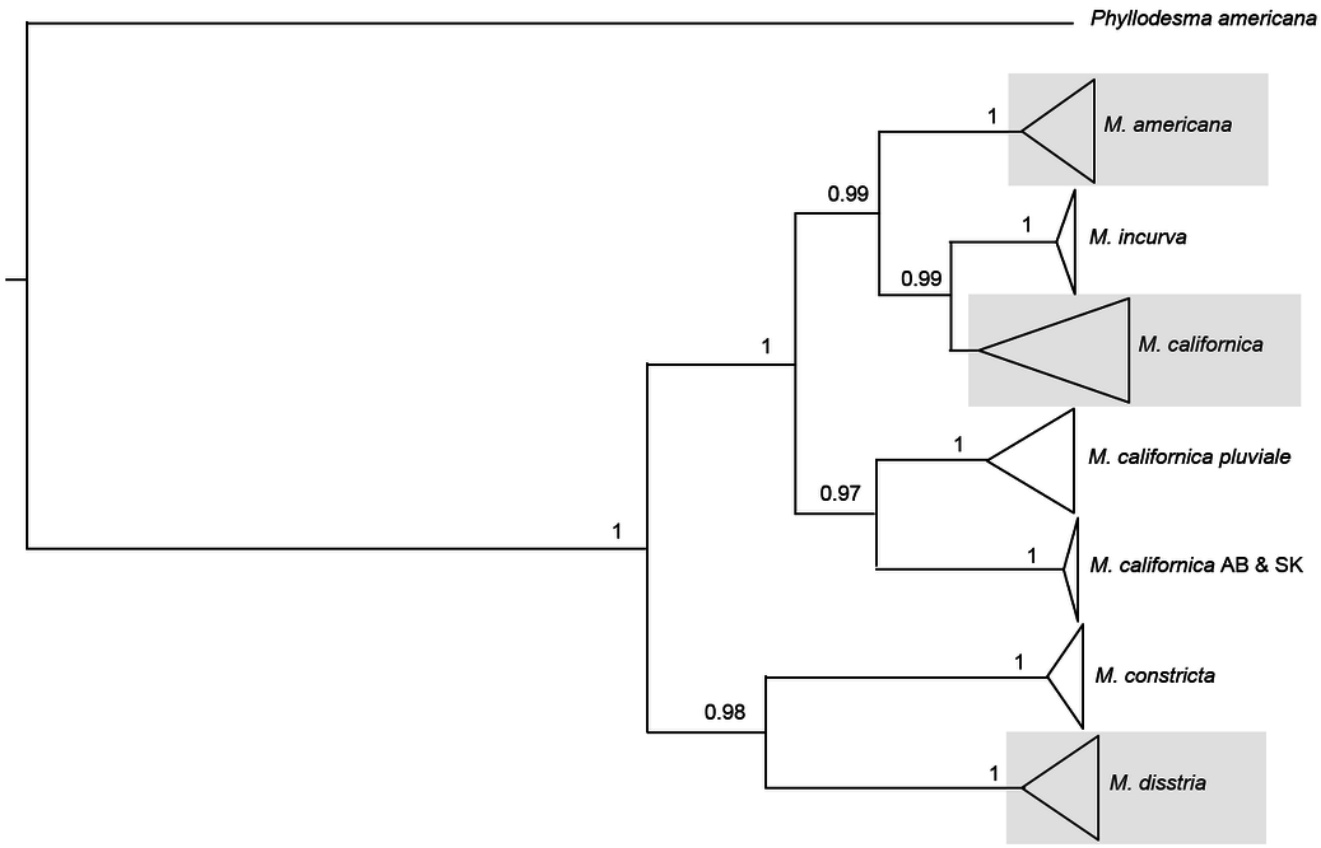


Figure 3

Statistical parsimony network showing the relationship among the 33 *M. americana* haplotypes.

The 79 samples are colour-coded by location, and inferred haplotypes are depicted by black circles. Refer to Fig. 1 for locations.

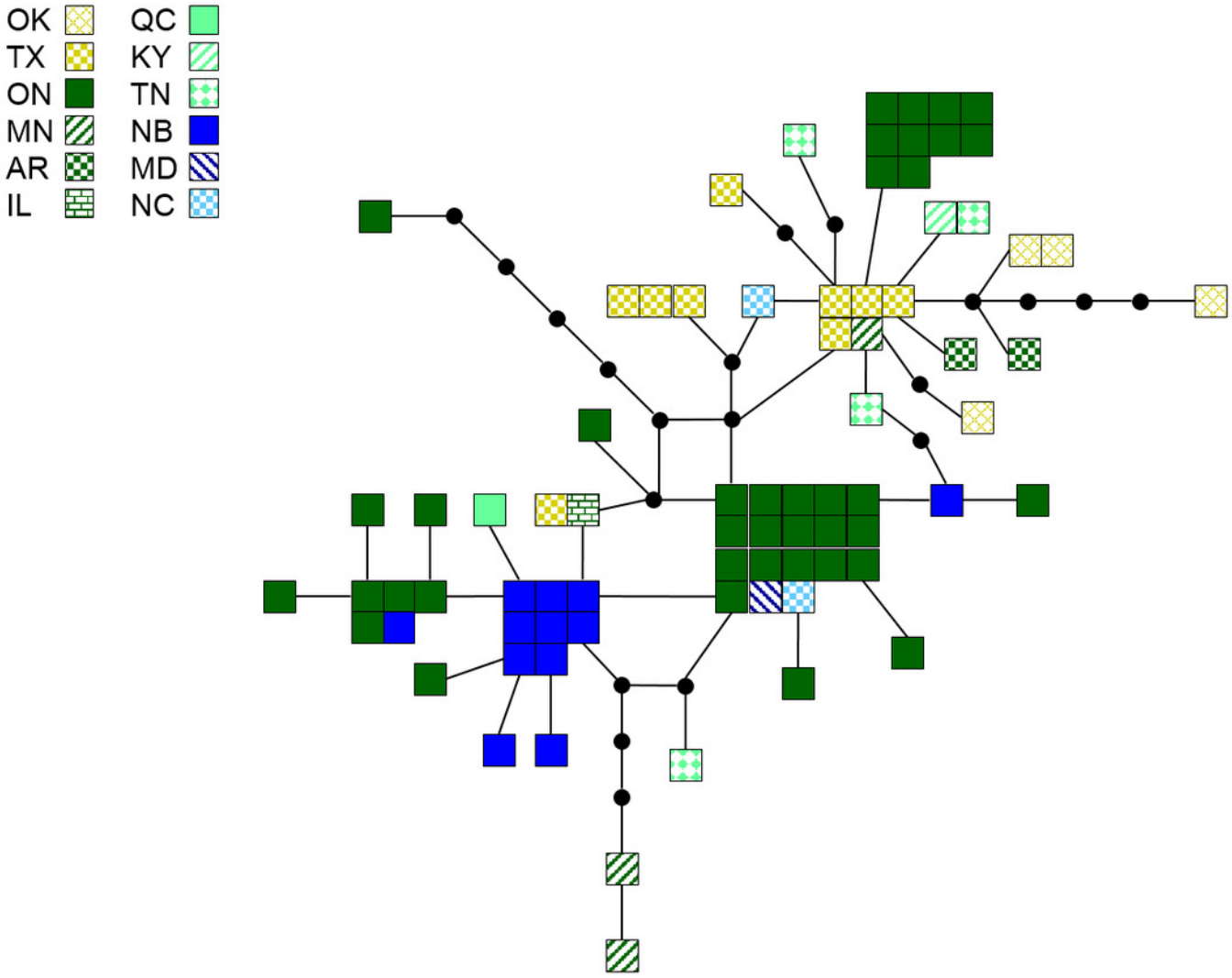
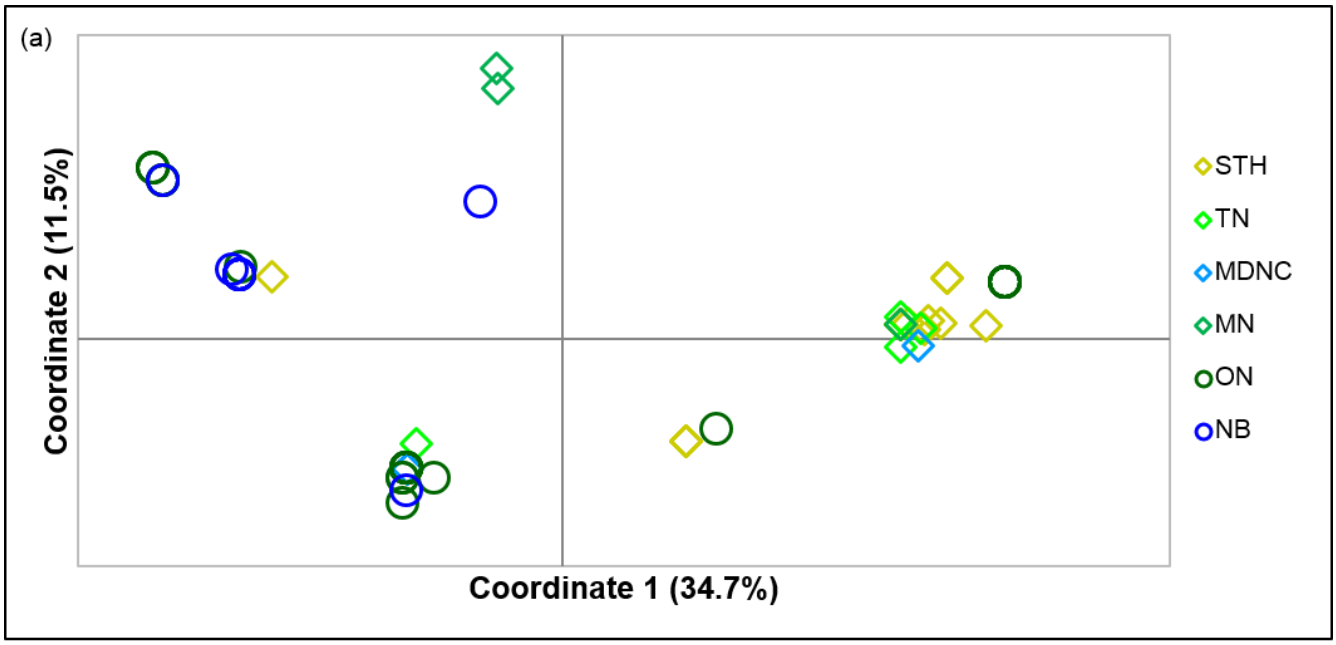
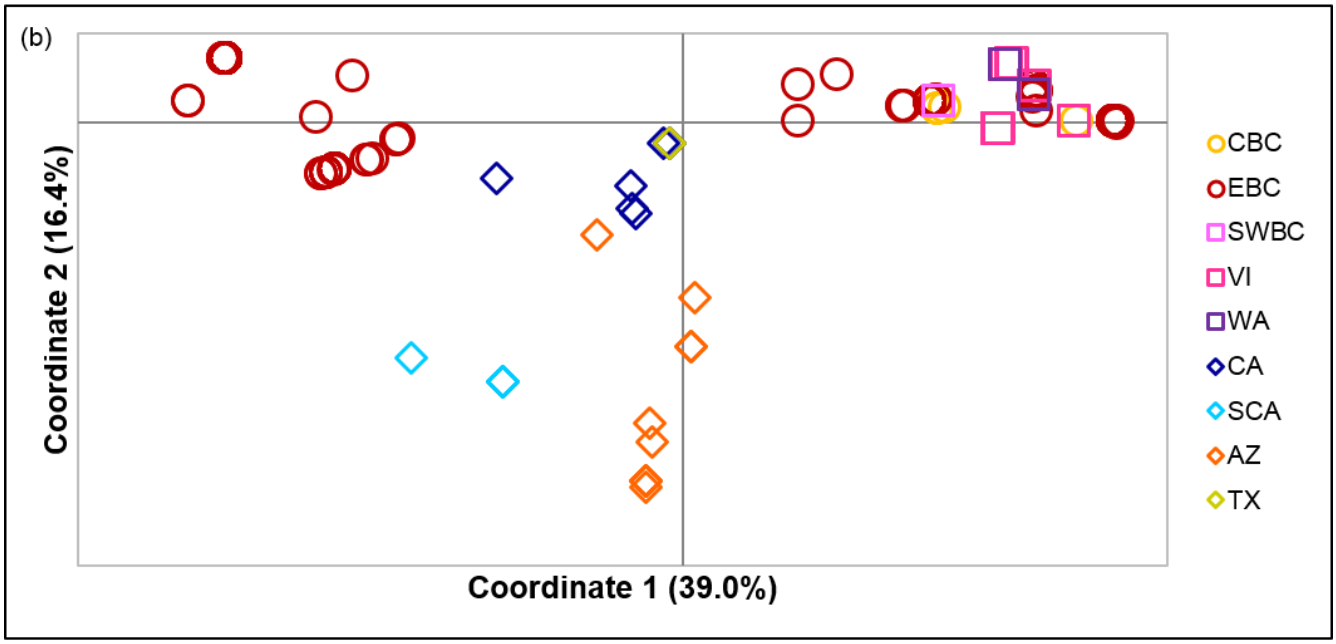


Figure 4

Principle coordinates analysis for (a) *M. americana*, (b) *M. californica*, and (c) *M. disstria*.

Samples are colour-coded by sampling location. Refer to Fig. 1 for locations.


(a)
Coordinate 2 (11.5%)
Coordinate 1 (34.7%)
STH
TN
MDNC
MN
ON
NB


(b)
Coordinate 2 (16.4%)
Coordinate 1 (39.0%)
CBC
EBC
SWBC
VI
WA
CA
SCA
AZ
TX

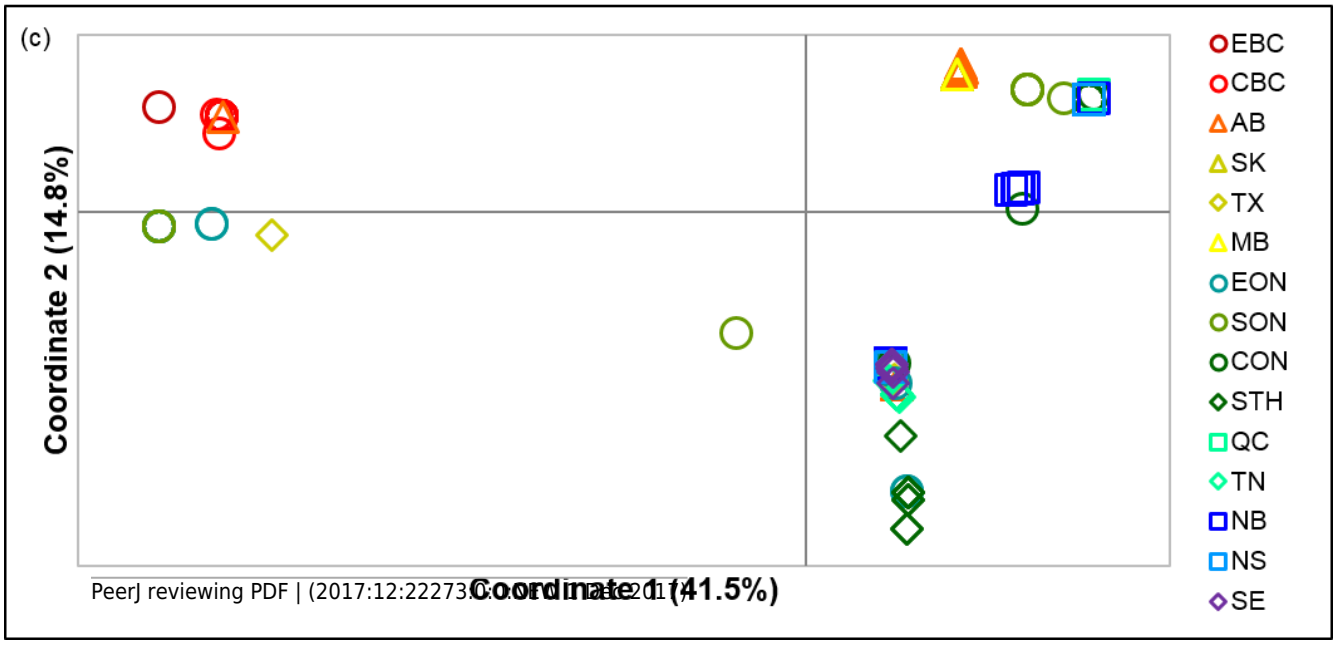

(c)
Coordinate 2 (14.8%)
Coordinate 1 (41.5%)
EBC
CBC
AB
SK
TX
MB
EON
SON
CON
STH
QC
TN
NB
NS
SE

Figure 5

Statistical parsimony network showing the relationship among the 64 *M. californica* haplotypes.

The 207 samples are colour-coded by location, and inferred haplotypes are depicted by black circles. Refer to Fig. 1 for locations.

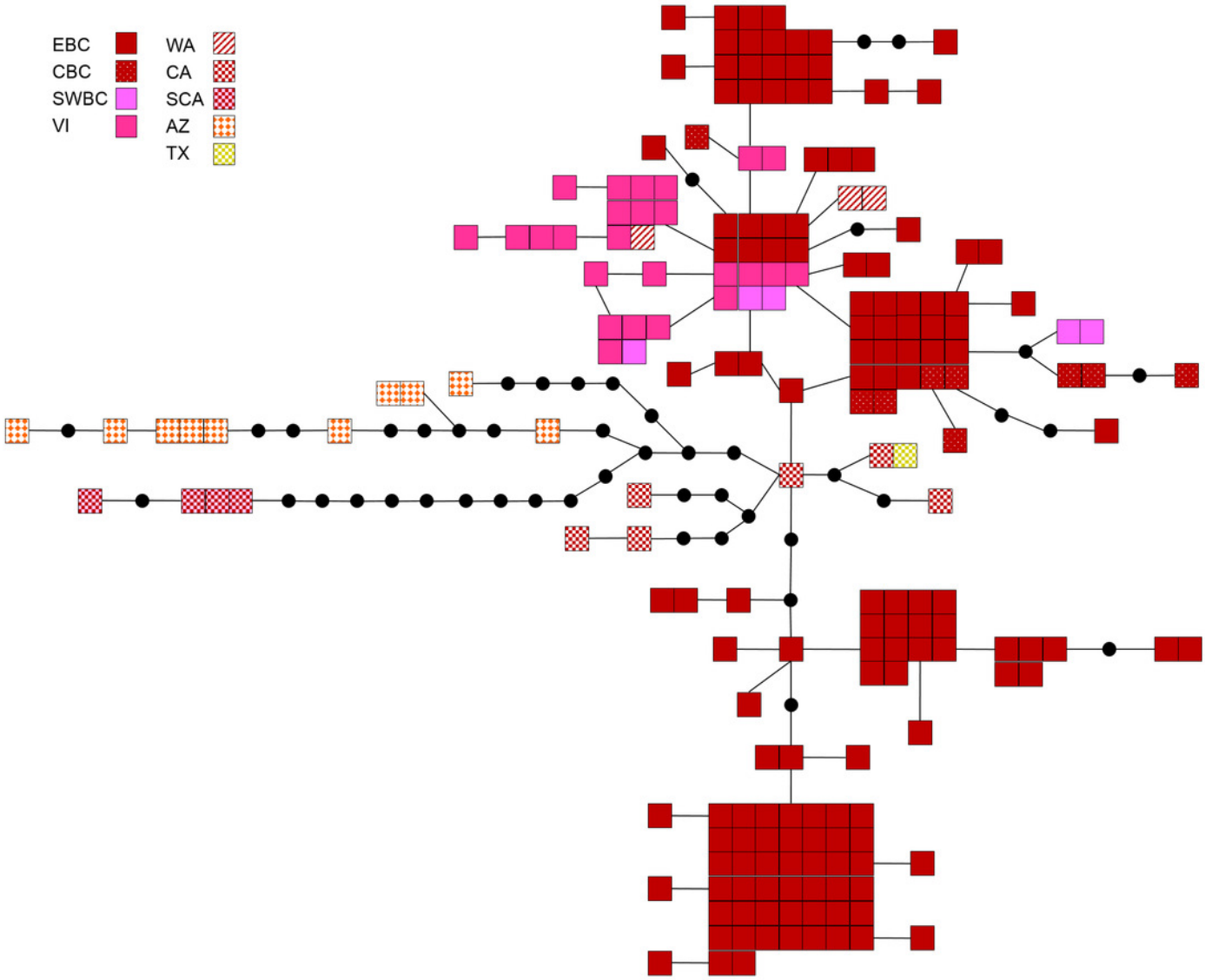


Figure 6

Statistical parsimony network showing the relationship among 42 *M. disstria* haplotypes.

The 139 samples are colour-coded by location, and inferred haplotypes are depicted by black circles. Refer to Fig. 1 for locations.