A survey of molecular diversity and population genetic structure in North American clearwing moths (Lepidoptera: Sesiidae) using cytochrome c oxidase I

Linda A. Lait*, Paul D. N. Hebert

Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada

* llait@uoguelph.ca

Abstract

The phylogeographic structure of insect species in North America is poorly understood. The moth family Sesiidae (Lepidoptera) contains many economically important pests of agriculture and forestry, as well as beneficial species used in biological control. Despite their significance, this study constitutes the first broad-ranging population genetic study on North American sesiids. It probes the population structure of eight species of sesiid moths based on sequence variation in cytochrome c oxidase I (N = 191). Haplotype diversity levels were high in seven of the eight species, while nucleotide diversity varied considerably. Patterns ranged from limited structure and a starburst pattern in the raspberry crown borer Pennesia marginata to highly geographically structured populations in the peachtree borer Synanthedon exitiosa and the maple callus borer Synanthedon acerni. These varied patterns suggest differing evolutionary histories and dispersal abilities. By elucidating population genetic structure and barriers to dispersal we can begin to devise conservation and management plans.

Introduction

Studies of population genetic structure can reveal where a species persisted through time, how it colonised new regions, and whether current populations experience ongoing gene flow. The evolutionary histories of North American species have been heavily influenced by the Pleistocene glaciations [1–3], with the most recent glaciation, the Wisconsin glaciation, resulting in alteration of habitat distribution [4, 5]. As a result, many species had to persist in ice-free refugia, primarily located south of the ice sheets and in a large ice-free region in Beringia, although recent studies have shown that periglacial regions on both coasts and in the Arctic may also have supported taxa [1, 6–8]. Contemporary dispersal capabilities are also reflected in the population genetic structure of a species. Highly vagile species such as birds, large mammals, and marine fishes often show limited geographic patterns [9–12], whereas species with restricted ranges, those affected by either physical or non-physical barriers, and those with poor dispersal
capabilities often have significant population genetic structure (e.g., [12]). Such insights into evolutionary history can aid management efforts, be it a recovery plan for a species of conservation concern, or control programs for invasive and pest species. For example, the identification of isolated populations can help to target conservation efforts. In addition, a deeper understanding of how a species moves between areas can help to devise strategies to limit its capacity to invade new regions.

Although insects comprise nearly two thirds of animal diversity [13, 14], they are underrepresented in the population genetics literature—particularly among studies of eastern North American taxa—relative to their richness and abundance. Studies have revealed varying phylogeographic patterns: limited genetic structure was found in the endangered burying beetle *Nicrophorus americanus* [15], the cabbage looper *Trichoplusia ni* [16], and the monarch butterfly *Danaus plexippus* [17]; whereas significant structure and distinct lineages have been identified in the wheat stem sawfly *Cephus cinctus* [18], the walnut twig beetle *Pityophthorus juglandis* [19], and the yellow fever mosquito *Aedes aegypti* [20]. Several studies have examined butterfly species: highly vagile and migratory species exhibit high levels of gene flow and little regional differentiation [17, 21, 22], while alpine species show significant differences between populations on different mountain ranges [23, 24]. The disparate findings among these taxa suggest that further study is necessary.

The Sesiidae (Lepidoptera), or clearwing moths, are a small, broadly distributed family of moths found globally [25]. Its most recent global checklist includes 1,452 species in 160 genera [26]. The North American fauna includes 133–135 species assigned to 20 genera [26, 27] with three recent introductions from the Palaearctic (*Sesia apiformis*, *Synanthedon myopaeformis*, and *Synanthedon tipuliformis*). In addition, two species have a natural Holarctic distribution and 41 species are shared with the Neotropics [26, 28]. The larvae of sesiids are primarily host specialists which bore into the roots and stems of a single genus or family of trees, shrubs, vines, or herbs [25]. As a result, many sesiids are pests of agricultural, forestry, or ornamental plants. For example, the peachtree borer *Synanthedon exitiosa* and the dogwood borer *Synanthedon scitula* cause substantial damage in peach and apple orchards, respectively [29–31], while the maple callus borer *Synanthedon acerni* and the ash borer *Podosesia syringae* cause significant damage to hardwood forests [30, 32–34].

Despite their importance as agricultural and forestry pests, and their corresponding use as biological control agents, there is little molecular data on sesiids. There is currently no comprehensive molecular phylogeny for this family, although two studies generated regional phylogenies using short fragments of the mitochondrial cytochrome oxidase I and II genes for 20 and 21 species from 10 and 12 genera, respectively [35, 36], while a single study has explored the population genetic structure of the sesiid *Synanthedon pictipes* revealing multiple genetic lineages within a small geographical area [37]. With over 6 million publicly available barcode records (www.boldsystems.org; [38]), and ~4.5 million insect records, there is a wealth of data available. The present study aims to increase our understanding of the population genetic structure of this group by making use of existing DNA barcode records to examine levels of genetic variation and both past and contemporary isolation in North American Sesiidae.

**Materials and methods**

**Sequences**

Sequences of the 658 bp barcode region of the mitochondrial cytochrome *c* oxidase I gene were downloaded from the Barcode of Life Database (BOLD) [38] in April 2018 for all 752 sesiids from Canada and the United States (see SI Table). Locations were recorded by state or province. Sequences were aligned in MEGA v6 [39]. In order to confirm the monophyly of
each species a phylogeny was constructed in Mr. Bayes v3.2 [40] using the generalised time reversible model with gamma-distributed rate variation and invariable sites (GTR+Γ+I). The analysis was run for 5 million runs with a 25% burn-in, standard deviation of split frequencies < 0.02, and final potential scale reduction factor (PSRF) > 0.9999. Xanthocastnia evalthe (Lepidoptera: Castniidae; GenBank Accession Number HM377853) was used as the outgroup.

Genetic analyses
Eight species were selected for further intraspecific analyses (Fig 1): Albuna pyramidalis, Carmenta mimuli, Pennisetia marginata, Synanthedon acerni, Synanthedon decipiens, Synanthedon exitiosa, Synanthedon sapygaformis, and Zenodoxus rubens. Haplotypes were assigned with TCS v1.21 [41] and confirmed by visual inspection. Haplotype (Hd) and nucleotide (π, per site) diversity measures and neutrality tests (Tajima D and Fu’s Fs) [42, 43] were run in DnaSP v5.10 [44, 45]. To test for population structure, an analysis of molecular variance (AMOVA; 100,000 permutations) [46] and pairwise genetic differences (FST; 100,000 permutations) for each species were calculated in Arlequin v3.5.2.2 [47]. The AMOVA allocated the genetic variation within and among sampling locations. For the pairwise comparisons, a modified false discovery rate correction (FDR) [48] was applied to correct for multiple tests. To test for evidence of population expansion a mismatch distribution analysis [49] was run in DnaSP v5.10 [44, 45].

In order to visualise the pattern of variation, a statistical parsimony network was constructed in TCS v1.21 [41] with a 95% connection limit. Bayesian clustering analysis was performed in BAPS v5.2 (Bayesian Analysis of Population Structure) [50] to assign specimens to clusters based on Bayes’ theorem with no a priori population information. Clustering analysis was run from K = 1 to 10 with the linked loci option [51].

Results
Samples
The 752 sesiid records downloaded included 662 specimens that could be assigned to one of 117 species either based on previous taxonomic identification or by membership in a Barcode Index Number (BIN) [52] assigned to a species. Of these 558 samples representing 100 species had full COI barcode sequences (≥ 540 bp; S1 Table).

Bayesian analyses
The Bayesian network supported the monophyly of most species, including the eight species selected for further analyses (Fig 2). Most genera formed monophyletic groups, with the exceptions that Sophona and Zenodoxus were paraphyletic while Palmia and Podesesia fell within a larger Synanthedon clade. Finally, Alcathoe, Hymenolea, Penstemonia, and Synanthedon rileyana all fell within a larger Carmenta clade. Many of these exceptions have been noted before [35, 36] and are likely due to 1) the recent separation of sister species, and/or 2) the generic misplacement of Synanthedon rileyana. Further work with additional specimens and markers is needed to increase the resolution of the phylogenetic relationships within this family.

Population structure
Eight species were selected for further analysis, with coverage ranging from eight to 47 specimens and from two to 13 sampling locations (Table 1). Levels of diversity varied considerably
Fig 1. Approximate distributions (shaded) and sampling locations for eight North American sesiid species. The points represent sampled sites; multiple sites in the same region (e.g., ON) were grouped for subsequent analyses.

https://doi.org/10.1371/journal.pone.0202281.g001
among taxa, ranging from two variable sites in *Pennisetia marginata* to 54 variable sites in *Synanthedon exitiosa*. Haplotype diversity ranged from 0.25 to 0.98, while nucleotide diversity ranged from 0.008 to 0.027 (Table 1). None of the neutrality tests showed significant results.

The overall AMOVAs produced $\Phi_{ST}$ values ranging from 0.16 to 0.88. P-values were significant for *Albuna pyramidalis*, *Pennisetia marginata*, *Synanthedon acerni*, *S. decipiens*, and *S. exitiosa*, but not for *Carmenta mimuli* or *Zenodoxus rubens* (Table 1). As *Synanthedon sapygaeformis* was primarily represented by a single population, neither overall nor pairwise $\Phi_{ST}$ values were calculated for this species. Pairwise mismatch distributions showed relatively low but non-significant Harpending’s raggedness index values, with that of *P. marginata* an order of magnitude higher than the others (Fig 3). There was a multimodal distribution in all but *Pennisetia marginata* (Fig 3), likely the result of population structure within the species rather than genuinely stable populations. This is supported by the lack of significance in all but two of the indices.

![Bayesian analysis based on 558 COI barcodes among 100 North American sesiid species](https://doi.org/10.1371/journal.pone.0202281.g002)

Table 1. Sample size (N), number of sampling locations (Loc), variable sites (VS), number of haplotypes (h), haplotype diversity ($H_d$), nucleotide diversity ($\pi$), and overall $\Phi_{ST}$ for eight North American sesiid species.

| Species                  | N   | Loc | VS  | h   | $H_d$ | $\pi$   | $\Phi_{ST}$ |
|--------------------------|-----|-----|-----|-----|-------|---------|-------------|
| *Albuna pyramidalis*     | 28  | 6   | 40  | 22  | 0.98  | 0.0132  | 0.234**     |
| *Carmenta mimuli*        | 17  | 3   | 27  | 10  | 0.84  | 0.0112  | 0.158       |
| *Pennisetia marginata*   | 36  | 8   | 2   | 3   | 0.25  | 0.0079  | 0.429**     |
| *Synanthedon acerni*     | 47  | 13  | 45  | 22  | 0.92  | 0.0134  | 0.881****   |
| *Synanthedon decipiens*  | 10  | 2   | 16  | 8   | 0.96  | 0.0078  | 0.480**     |
| *Synanthedon exitiosa*   | 27  | 8   | 54  | 18  | 0.96  | 0.0271  | 0.696****   |
| *Synanthedon sapygaeformis* | 17  | 2   | 24  | 13  | 0.96  | 0.0077  | n/a         |
| *Zenodoxus rubens*       | 8   | 2   | 34  | 7   | 0.96  | 0.0224  | 0.516       |

1 $p<0.05$, **$p<0.01$, ***$p<0.001$

https://doi.org/10.1371/journal.pone.0202281.t001
Fig 3. Mismatch distribution for eight North American sesiid species. Harpending’s raggedness index (r) and the sum of squared deviations (SSD) are given. Significant values are depicted with a ‘*’.

https://doi.org/10.1371/journal.pone.0202281.g003
The eight species had very different patterns of variation (Table 2, Fig 4). *A. pyramidalis*, *S. acerni*, and *S. exitiosa* all possessed extensive variation (40, 45, and 54 variable sites, respectively), but *A. pyramidalis* had no significant population pairwise comparisons. By contrast, *S. acerni* and *S. exitiosa* had significant values for most comparisons with both showing a distinct east/west pattern (Table 2, Fig 4). Both the statistical parsimony network and the Bayesian clustering analyses supported the separation of samples into multiple groups (Fig 4), with BAPS identifying three, four, and four groups with probabilities of 0.943, 1.0, and 1.0, respectively. In *A. pyramidalis*, one group consisted of most Manitoba samples along with the sole Saskatchewan sample, while a second contained a single Alberta sample showing 14 substitutions from any other lineage, and the third group contained all other samples from both eastern and western locations. In *S. acerni*, samples from Atlantic Canada, West Virginia, and Georgia formed one group, while samples from Ontario, Quebec, Connecticut, Illinois, Michigan, and Texas formed a second, and a third southern group included one sample each from Louisiana and Mississippi, and the fourth group with a single sample from Florida that possessed 21 substitutions from any other, although it still grouped within *S. acerni* (Fig 4). In *S. exitiosa*, the groups roughly represented California, Arizona, the south (Oklahoma, Louisiana, and Arkansas), and the east (Ontario and Georgia); Colorado samples were found in all but the California group (Fig 4). *Carmenta mimuli* and *Zenodoxus rubens* showed high levels of variation between Arizona and Texas (Fig 4), although none of the population comparisons were significant (Table 2). In both cases Bayesian clustering identified multiple groups (two in *C. mimuli* and four in *Z. rubens*; probability = 0.996 and 1, respectively). *Synanthedon decipiens* showed significant differences between the two sampled locations (Table 2, Fig 4), reflecting the fact that they shared no haplotypes, but Bayesian clustering could not detect any significant groups. *Synanthedon sapygaeformis* showed a diverse network of closely related haplotypes sampled almost entirely from a single location (Fig 4); BAPS identified three groups (probability = 0.946). In contrast, *P. marginata* showed limited variation (two variable sites) with a

---

Table 2. Population pairwise $\Phi_{ST}$ values for seven North American sesiid species. Significant comparisons after correction for multiple tests are shown in bold and shaded. Negative $\Phi_{ST}$ values are treated as 0.000.

|                | *A. pyramidalis*          | *S. acerni*         | *S. exitiosa*      | *C. mimuli*        | *S. decipiens* | *Z. rubens* |
|----------------|---------------------------|---------------------|--------------------|--------------------|----------------|-------------|
|                | **BC/AB** | **sAB** | **sBC** | **nMB** | **NL** | **BC** | **BC** | **NB/NS** | **ON** | **QC** | **NB** | **NL** | **ON** | **QC** | **CA** | **STH** | **LA** | **AZ** |
|                | 0.136      | 0.328   | 0.125   | 0.376   | 0.000 | 0.250 | 0.000 | 0.441 | 0.000 | 0.000 |
|                | 0.017      | 0.277   | 0.330   | 0.298   | 0.379 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
|                | 0.337      | 0.000   | 0.479   | 0.390   | 0.379 | 0.328 | 0.177 | 0.849 | 0.894 | 0.767 |
|                | 0.000      | 0.390   | 0.881   | 0.968   | 0.972 | 0.093 | 0.000 | 0.000 | 0.000 | 0.000 |
|                | 0.000      | 0.000   | 0.520   | 0.741   | 0.730 | 0.848 | | | | |
|                | 0.000      | 0.741   | 0.730   | 0.520   | 0.741 | 0.848 | | | | |

The eight species had very different patterns of variation (Table 2, Fig 4). *A. pyramidalis*, *S. acerni*, and *S. exitiosa* all possessed extensive variation (40, 45, and 54 variable sites, respectively), but *A. pyramidalis* had no significant population pairwise comparisons. By contrast, *S. acerni* and *S. exitiosa* had significant values for most comparisons with both showing a distinct east/west pattern (Table 2, Fig 4). Both the statistical parsimony network and the Bayesian clustering analyses supported the separation of samples into multiple groups (Fig 4), with BAPS identifying three, four, and four groups with probabilities of 0.943, 1.0, and 1.0, respectively. In *A. pyramidalis*, one group consisted of most Manitoba samples along with the sole Saskatchewan sample, while a second contained a single Alberta sample showing 14 substitutions from any other lineage, and the third group contained all other samples from both eastern and western locations. In *S. acerni*, samples from Atlantic Canada, West Virginia, and Georgia formed one group, while samples from Ontario, Quebec, Connecticut, Illinois, Michigan, and Texas formed a second, and a third southern group included one sample each from Louisiana and Mississippi, and the fourth group with a single sample from Florida that possessed 21 substitutions from any other, although it still grouped within *S. acerni* (Fig 4). In *S. exitiosa*, the groups roughly represented California, Arizona, the south (Oklahoma, Louisiana, and Arkansas), and the east (Ontario and Georgia); Colorado samples were found in all but the California group (Fig 4). *Carmenta mimuli* and *Zenodoxus rubens* showed high levels of variation between Arizona and Texas (Fig 4), although none of the population comparisons were significant (Table 2). In both cases Bayesian clustering identified multiple groups (two in *C. mimuli* and four in *Z. rubens*; probability = 0.996 and 1, respectively). *Synanthedon decipiens* showed significant differences between the two sampled locations (Table 2, Fig 4), reflecting the fact that they shared no haplotypes, but Bayesian clustering could not detect any significant groups. *Synanthedon sapygaeformis* showed a diverse network of closely related haplotypes sampled almost entirely from a single location (Fig 4); BAPS identified three groups (probability = 0.946). In contrast, *P. marginata* showed limited variation (two variable sites) with a
single widespread haplotype found in 86.1% (31/36) of the samples with representation from both eastern and western locations (see Figs 1 and 4). Bayesian clustering could not identify any groups in *P. marginata*, although there was a single significant pairwise comparison due to the private haplotype present in Utah (Table 2).

**Discussion**

The present study shows that North American sesiids exhibit a wide diversity of genetic variation and population genetic structure. The species are similar in size and dispersal ability, and are all specialists on a particular family or genus of host plant, although some target a single species while others feed on several [25]. While some of the variability in population genetic structure can be explained by differences in distribution, geographic coverage, and sample sizes, other patterns were unexpected.
Albuna pyramidalis

The extensive haplotype variation in *Albuna pyramidalis* without significant population structure between eastern and western locations suggests a postglacial expansion from a single glacial refugium, at least in the northern part of its range. Similar patterns have been reported in many northern species of trees, birds, and mammals. For example, Graham and Burg [53] found that *Picoïdes villosus* exhibits little or no structure in the boreal and eastern portion of its range; similarly, Milá *et al.* [54] identified a single genetic lineage in *Dendroica coronata* across Canada and Alaska. In both cases it was proposed that northern locations derived from a single refugium, likely in the south. Other species possess a single lineage across eastern North America east of the Rocky Mountains [55–58], or across all of Canada [59, 60]. Samples from the southern portion of the range are required to test whether the species as a whole persisted in a single refugium, or whether there is additional structure in the south, particularly in the southwest, as seen in some of the other sesiid species.

The high level of variation in the species may be reflective of the extensive morphological variation observed in *A. pyramidalis*; the species has been noted for its highly variable colouration with five colour forms described [25]. While three of the colour forms may be present in the region sampled (nominate, *coloradensis*, and *montana*), the groupings found with BAPS did not correspond to the colour variations suggesting that the different forms may breed extensively or have only recently diverged. The two most distinctive forms, *rubescens* and *beutenmulleri*, are found only in Utah and Colorado and were not sampled in this study.

Pennisetia marginata

The complete lack of population structure in *Pennisetia marginata* may reflect high levels of gene flow, a recent origin in North America, or a combination of these factors. High dispersal capability and gene flow among regions may occur due to a continuous distribution of wild and introduced populations of its host plant species (*Rubus*) [25]. While the suggested distribution of *P. marginata* is essentially disjunct, with only a few scattered records from the Great Plains, the species may traverse this central region without establishing large or long-term populations due to unfavourable conditions. Although extensive gene flow would explain haplotype sharing among geographically distant locations (i.e., British Columbia and Nova Scotia), it does not explain the limited variation despite a substantial sample size. A starburst pattern with a single common haplotype and a few others one or two mutations away has frequently been noted in fishes, birds, and mammals [61–65], and often indicates a recent rapid expansion following a bottleneck [66]. This is supported by the unimodal distribution in the mismatch analysis (Fig 3). The pattern could also be a result of an evolutionarily young species; Eichlin and Duckworth [25] noted that *P. marginata* is morphologically and behaviourally similar to the Palaearctic congeneric *P. hylaeiformis*, although consistent differences in male genitalia were able to distinguish the species. Examination of the cytochrome *c* oxidase I sequences supports the separation of these species with 3.2–4.0% divergences, suggesting that the divergence may have occurred relatively recently, possibly following a bottleneck.

Synanthedon acerni

*Synanthedon acerni* exhibited clear geographic differences between “eastern” (NB, NL, WV, GA) and “western” (ON, QC, IL, TX) locations, with southern locations forming two additional groups (LA and MS; and FL). This pattern suggests their derivation from multiple glacial refugia, a physical barrier preventing gene flow, separation due to host specificity, or a combination of factors. As discussed above, the presence of multiple glacial refugia in North America is certain [1], and genetic differentiation between populations from the Atlantic and Gulf
regions is common in species whose modern range spans these two regions [67, 68]. As well, several studies have shown that both the Mississippi River and Appalachian Mountains are often barriers to gene flow [69–71]. *S. acerni* is a specialist on *Acer* (Sapindaceae) species, particularly on red maple, *A. rubrum*, with which it shares its distribution, and silver maple, *A. saccharinum* [25]. Both species occur across eastern North America, with *A. rubrum* extending further east and *A. saccharinum* absent from Newfoundland [72]. Given this pattern it may suggest that the “eastern” lineage uses *A. rubrum* as its preferred host, while the “western” group is found on *A. saccharinum*. Studies of host species specificity could be used to test this further.

While three colour forms have been described for *S. acerni* [25], the eastern, western, and southern (LA and MS) groups all corresponded to the nominate form. The sample from Florida represented the colour form *buscki* which, given the high divergence between it and the other samples, may represent a separate species, or at the very least a form with a different host species. Additional work with nuclear markers and extensive sampling in the southeast region is required to confirm this.

**Synanthedon exitiosa**

*Synanthedon exitiosa* showed clear geographic structure with four distinct lineages: Arizona, California, the southern United States, and the eastern United States and Canada. This may reflect its isolation in multiple glacial refugia with differences retained due to low gene flow among regions, varying host preferences leading to adaptations, or incipient speciation. Distinct population structure as a result of multiple glacial refugia has been observed in many plant and animal species, particularly in the west [54, 56, 67, 73–75]. The current distribution of *S. exitiosa* includes a number of putative refugial areas in the southern United States and the coastal Pacific Northwest [1], supporting the suggestion that it persisted in multiple Pleistocene refugia. The importance of host specificity on genetic isolation and incipient speciation has also been demonstrated in several herbivorous insect species, with changes in host preference leading to reproductive isolation [76, 77]. *S. exitiosa* feeds extensively on peach trees *Prunus persica* (Rosaceae), an introduction from Asia, as well as several other *Prunus* species, both native and introduced [25]. Many of its introduced hosts have narrow distributions in North America (e.g., *P. amygdalus* only occurs in California and Utah), while there are numerous native Rosaceae species, many of which have small ranges [72].

*S. exitiosa* also exhibits extensive morphological variation, with numerous colour forms described [25]. The four groups identified by statistical parsimony and Bayesian clustering analyses (Fig 4) exhibit different colour morphs that partially correspond to named forms. Specimens from Arizona and California were black with little colouration corresponding to the form *graefi*, described as lacking the typical yellow bands, which has been described as the principal form found in the west [25]. Samples from the southern group have distinctive yellow bands along the body, possibly representing either the nominate *exitiosa* or the form *barnessii*, while those from the “eastern” group also exhibit yellow stripes, although to a lesser extent. The single female sample in the eastern group is *edwardsii*, evident by the distinct orange ring on the fourth and fifth segments of the abdomen [25].

**Carmenta mimuli, Synanthedon decipiens, Synanthedon sapygaformis, and Zenodoxus rubens**

*Carmenta mimuli* showed a surprising amount of variation in the three populations sampled, with two lineages identified although with no geographical correlation, possibly representing multiple invasions from a diverse Mexican population, multiple southern refugia with
subsequent admixture, or the presence of cryptic species. The two lineages identified are sympatric, and there are no described morphological variants in this species. Slight colour differences do not correspond to the lineages seen here. Extensive sampling of this species, as well as inclusion of nuclear markers, is required to identify whether these are in fact cryptic species. *Synanthedon decipiens* also showed clear divergence, with no shared haplotypes between the two southern locations sampled. As the divergence between the populations is minimal (and therefore likely more recent), perhaps these populations target different host plant species as the Sonoran scrub oak *Quercus turbinella* only extends as far east as Texas, while other *Quercus* (Fagaceae) species occur in Louisiana [72].

*Zenodoxus rubens* possessed multiple distinct lineages suggesting either multiple colonisation events into the southern United States, possibly as peripheral populations of a broader Meso-American species, or the presence of cryptic species. While little is known about Neotropical sesiids, two *Zenodoxus* species are known to occur in Baja California [78] and seven species have been described from the southwestern U.S. [25]. The samples studied here are consistent with the nominate form of *Z. rubens* as described by Eichlin and Duckworth [25], with the exception that the single divergent Texas sample has a hyaline region on the hindwings, identifying it as the *bexari* form. The divergences among groups range from 1.7% between the two Arizona groups and 3.3% between one Arizona group and the *bexari* Texas sample; distances such as the latter may suggest a separate species, or may be a result of missing intermediate samples. The lack of obvious morphological differences in the majority of samples suggests that the large divergences between the Arizona and Texas samples may represent a continuous cline of variation and limited dispersal capabilities. The presence of the *bexari* sample as the most distinct lineage raises additional questions; further sampling, particularly of *bexari* colour forms, is required to confirm its species status. The other species restricted to the southern states, *Synanthedon sapygaeformis*, may also represent a northern extension of a Meso-American species, or it may be endemic to the extreme south-east of the United States. *S. sapygaeformis* exhibited much higher variability than other southern species despite limited structure, possibly reflecting an older population. Eichlin and Duckworth [25] describe two colour forms for this species: the nominate form and the variant *floridensis*. All specimens examined here are of the more common *floridensis* form, suggesting that the differences in colour forms are not responsible for the variation.

**Conclusions**

Despite the lack of targeted taxonomic sampling, the COI sequences in BOLD provided an excellent overview of the population genetic structure in North American sesiid moths. A number of species showed distinct genetic lineages, likely representing the signature of isolation during glacial periods as well as limited ongoing gene flow. For example, *Synanthedon acerni* and *S. exitiosa* each showed four distinct lineages, suggesting historical separation followed by low levels of contemporary gene flow. In contrast, the widespread *Pennisetia marginata* exhibited a starburst pattern and almost complete lack of variation despite samples from both eastern and western locations, indicating a single glacial refuge and recent bottleneck and/or origin.

The results show that despite similar life history traits, this group of insects shows highly variable evolutionary histories. Future work should focus not only on species of agricultural importance, such as *Synanthedon exitiosa*, but also on those of evolutionary interest highlighted here, such as *Albuna pyramidalis*, *Synanthedon acerni*, and *Zenodoxus rubens*. In all of these species, additional samples targeting potential physical barriers and putative refugia will help to clarify their evolutionary history. In addition, by incorporating additional markers
it will be possible to clarify whether the variation seen here reflects diversity that arose in previously isolated populations or the presence of cryptic species.

**Supporting information**

S1 Table. Sample information for the 558 sesiid moths and outgroup castniid. The BOLD sample ID, GenBank Accession Number, Barcode Index Number (BIN), sampling location, and institute storing are given. GPS coordinates for the eight selected species are given where available.

(PDF)

**Acknowledgments**

We would like to acknowledge all of the contributors to the Barcode of Life Database including the collections, laboratory, and bioinformatics staff at the Centre for Biodiversity Genomics, and researchers from around the globe. A special thanks to all those who contributed specimens and sequences: the Biodiversity Institute of Ontario; the Canadian Forest Service, Northern Forestry Centre; the Canadian National Collection of Insects, Arachnids, and Nematodes; the College of Charleston; Colorado State University, C. P. Gillette Museum of Arthropod Diversity; the Florida Museum of Natural History; Michigan State University Museum; the Smithsonian Institution National Museum of Natural History; Texas A&M University; the University of Alberta, Strickland Museum of Entomology; the University of Alaska Museum; the University of Maryland; and the private collections of Alex Smith, Daniel Handfield, Franz Pühringer, Gregory Pohl, Hans Riefenstahl, and Jennifer Bundy. We would also like to thank the editor and reviewers for helpful comments that improved the manuscript.

**Author Contributions**

Formal analysis: Linda A. Lait.

Funding acquisition: Paul D. N. Hebert.

Investigation: Linda A. Lait.

Methodology: Linda A. Lait.

Supervision: Paul D. N. Hebert.

Visualization: Linda A. Lait.

Writing – original draft: Linda A. Lait.

Writing – review & editing: Linda A. Lait, Paul D. N. Hebert.

**References**

1. Pielou EC. After the Ice Age: The Return of Life to Glaciated North America. Chicago: University of Chicago Press; 1991.

2. Hewitt GM. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol J Linn Soc. 1996; 58: 247–276. https://doi.org/10.1111/j.1095-8312.1996.tb01434.x

3. Hewitt GM. The genetic legacy of the Quaternary ice ages. Nature. 2000; 405: 907–913. https://doi.org/10.1038/35016000 PMID: 10879524

4. Flint RF. Glacial Geology and the Pleistocene Epoch. New York: John Wiley and Sons, Inc; 1947.

5. Nilsson T. The Pleistocene: Geology and Life in the Quaternary Ice Age. Dordrecht, Holland: D. Reidel Publishing Company; 1983.
6. Stewart JR, Lister AM. Cryptic northern refugia and the origins of the modern biota. Trends Ecol Evol. 2001; 16: 608–613. https://doi.org/10.1016/S0169-5347(01)02338-2

7. Rowe KC, Heske EJ, Brown PW, Paige KN. Surviving the ice: northern refugia and postglacial colonization. Proc Natl Acad Sci USA. 2004; 101: 10355–10359. https://doi.org/10.1073/pnas.0401338101 PMID: 15249673

8. Swenson NG, Howard DJ. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. Am Nat. 2005; 166: 581–591. https://doi.org/10.1086/491688 PMID: 16224723

9. Lehman N, Wayne RK. Analysis of coyote mitochondrial DNA genotype frequencies: estimation of the effective number of alleles. Genetics. 1991; 128: 405–416. PMID: 2071019

10. Ball RM, Avise JC. Mitochondrial-DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. Auk. 1992; 109: 626–636.

11. Zink RM, Dittmann DL. Population structure and gene flow in the chipping sparrow and a hypothesis for evolution in the genus Spizella. Wilson Bull. 1993; 105: 399–413.

12. Ward RD, Woodwark M, Skibinski DOF. Phylogeography and population structure of the walnut twig bee-Psidium acerifolium (Hymenoptera: Apidae). Ann Entomol Soc Am. 2005; 98: 591–594. https://doi.org/10.1093/aesa/98.5.591 PMID: 15974849

13. Zhang Z-Q. Animal biodiversity: an update of classification and diversity in 2013. Zootaxa. 2013; 3703: 5–11. https://doi.org/10.11646/zootaxa.3703.1.1 PMID: 26146682

14. Zhang Z-Q. Phylum Arthropoda. Zootaxa. 2013; 3703: 17–26. https://doi.org/10.11646/zootaxa.3703.1.6

15. Szalanski AL, Sikes DS, Bischof R, Fritz M. Population genetics and phylogenetics of the endangered American burying beetle, _Nicrophorus americanus_ (Coleoptera: Silphidae). Ann Entomol Soc Am. 2000; 93: 589–594. https://doi.org/10.1603/0013-8746(2000)093[0589:PNAPCM]2.0.CO;2

16. Franklin MT, Rittand CE, Myers JH. Genetic analysis of cabbage loopers, _Trichoplusia ni_ (Lepidoptera: Noctuidae) , a seasonal migrant in western North America. Evol Appl. 2011; 4: 89–99. https://doi.org/10.1111/j.1752-4571.2010.00135.x PMID: 25567955

17. Brower AVZ, Jeansonne MM. Geographical populations and “subspecies” of New World monarch butterflies (Nymphalidae) share a recent origin and are not phylogenetically distinct. Ann Entomol Soc Am. 2004; 97: 519–523. https://doi.org/10.1603/0013-8746(2004)097[0519:GPPASM]2.0.CO;2

18. Lesieur V, Martin J-F, Weaver DK, Hoelmer KA, Morrill WL, et al. Phylogeography of the wheat stem sawfly, _Cephus cinctus_ Norton (Hymenoptera: Cephidae): implications for pest management. PLOS ONE. 2016; 11: e0168370. https://doi.org/10.1371/journal.pone.0168370 PMID: 27959958

19. Rugman-Jones PF, Seybold SJ, Graves AD, Stouthammer R. Phylogeography of the walnut twig beetle, _Pityophthorus juglandis_ (Coleoptera: Curculionidae), the vector of thousand canker disease in North American walnut trees. PLOS ONE. 2015; 10: e0118264. https://doi.org/10.1371/journal.pone.0118264 PMID: 26095760

20. Merrill SA, Ramberg FB, Hagedorn HH. Phylogeography and population structure of _Aedes aegypti_ in Arizona. Am J Trop Med Hyg. 2005; 72: 304–310. PMID: 15772327

21. Fordyce JA, Nice CC, Shaw K. Contemporary patterns in a historical context: phylogeographic history of the pipevine swallowtail, _Battus philenor_ (Papilionidae). Evolution. 2003; 57: 1089–1099. https://doi.org/10.1554/0014-3820(2003)057[1089:CPIAHC]2.0.CO;2 PMID: 12836825

22. Stump AD, Sperling FAH, Crim A, Scriber JM. Gene flow between Great Lakes region populations of the Canadian tiger swallowtail butterfly, _Papilio canadensis_, near the hybrid zone with _P. glaucus_ (Lepidoptera: Papilionidae). Great Lakes Entomol. 2003; 36: 41–53.

23. DeChaine EG, Martin AP. Historic cycles of fragmentation and expansion in _Parnassius smintheus_ (Papilionidae) inferred using mitochondrial DNA. Evolution. 2004; 58: 113–127. https://doi.org/10.1111/j.0014-3820.2004.tb01578.x PMID: 15058724

24. DeChaine EG, Martin AP. Historical biogeography of two Alpine butterflies in the Rocky Mountains: broad-scale concordance and local-scale discordance. J Biogeogr. 2005; 32: 1943–1956. https://doi.org/10.1111/j.1365-2699.2005.01356.x

25. Eichlin TD, Duckworth WD. Sesioidae: Sesioidea. The Moths of North America North of Mexico, Fascicle 5.1. The Wedge Entomological Research Foundation; 1988.

26. Pühringer F, Kallies A. Provisional checklist of the _Sesiidae_ of the world (Lepidoptera: Ditrysia). Mitteleuropäischen Arbeitsgemeinschaft Salzkammergut. 2004; 4: 1–85.

27. Pohl GR, Patterson B, Pelham JP. Annotated taxonomic checklist of the Lepidoptera of North America, North of Mexico. Working paper published online by the authors at ResearchGate.net. 2016. 766 pp.

28. Aurelian VM, Evenden ML, Judd GJR. Diversity and abundance of arthropod by-catch in semiochemical-baited traps targeting apple clearwing moth (Lepidoptera: Sesiidae) in organic and conventional...
apple orchards in British Columbia, Canada. Can Entomol. 2015; 147: 227–243. https://doi.org/10.4039/tce.2014.47

29. Russell WG, Stanley WW. Seasonal life history and control of peach tree borer, *Sanninoidea exitiosa* (Say), in Tennessee. J Tenn Acad Sci. 1969; 44: 85–91.

30. Solomon JD. Guide to insect borers of North American broadleaf trees and shrubs. Washington: U.S. Department of Agriculture Forest Service; 1995.

31. Bergh JC, Leskey TC. Biology, ecology, and management of dogwood borer in eastern apple orchards. Can Entomol. 2003; 135: 615–635. https://doi.org/10.4039/N02-089

32. Holland WJ. The Moth Book—A Popular Guide to a Knowledge of the Moths of North America. New York: Dover Publishers Inc.; 1968.

33. McKnight ME, Tunnock S. The borer problem in green ash in North Dakota shelterbelts. N Dak Agr Exp Sta Farm Res. 1973; 30: 8–14.

34. Solomon JD. Biology of an ash borer, *Podosia syringae*, in green ash in Mississippi. Ann Entomol Soc Am. 1975; 62: 1214–1215. https://doi.org/10.1093/aesa/68.2.325

35. McKern JA, Szalanski AL, Johnson DT, Dowling APG. Molecular phylogeny of Sesiidae (Lepidoptera) inferred from mitochondrial DNA sequences. J Agr Urban Entomol. 2008; 25: 165–177. https://doi.org/10.3954/1523-5475-25.3.165

36. Hansen JA, Klingeman WE, Moulton JK, Oliver JB, Windham MT, Zhang A, et al. Molecular identification of Synanthedonini members (Lepidoptera: Sesiidae) using cytochrome oxidase I. Ann Entomol Soc Am. 2012; 105: 520–528. https://doi.org/10.1603/AN11028

37. McKern JA, Szalanski AL. Genetic variation of the lesser peach tree borer, *Synanthedon pictipes* (Lepidoptera: Sesiidae) in Arkansas. J Agr Urban Entomol. 2008; 25: 25–35. https://doi.org/10.3954/1523-5475-25.1.25

38. Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol Ecol Notes. 2007; 7: 355–364.

39. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729. https://doi.org/10.1093/molbev/mst197 PMID: 24132122

40. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogeny. Bioinformatics. 2001; 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754 PMID: 11524383

41. Clement M, Posada D, Crandall KA. TCS: a computer program to estimate gene genealogies. Mol Ecol. 2000; 9: 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x PMID: 11050560

42. Tajima F. Statistical-method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123: 585–595. PMID: 25132525

43. Fu Y-X, Li W-H. Statistical tests of neutrality of mutations. Genetics. 1993; 133: 693–709. PMID: 8454210

44. Rozas J, Sanchez-DelBarrio JC, Messequer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics. 2003; 19: 2496–2497. https://doi.org/10.1093/bioinformatics/btg359 PMID: 14668244

45. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25: 1451–1452. https://doi.org/10.1093/bioinformatics/btp187 PMID: 19346325

46. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992; 131: 479–491. PMID: 1644282

47. Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010; 10: 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x PMID: 21565059

48. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Stat. 2001; 29: 1165–1188. https://doi.org/10.1214/aos/1013699998

49. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 1992; 9: 552–569. https://doi.org/10.1093/oxfordjournals.molbev.a040727 PMID: 1316531

50. Corander J, Marttinen P, Siren J, Tang J. Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinformatics. 2008; 9: 539. https://doi.org/10.1186/1471-2105-9-539 PMID: 19087322

51. Corander J, Tang J. Bayesian analysis of population structure based on linked molecular information. Math Biosci. 2007; 205: 19–31. https://doi.org/10.1016/j.mbs.2006.09.015 PMID: 17087977
Graham BA, Burg TM. Molecular markers provide insight into contemporary and historic gene flow for a non-migratory species. J Avian Biol. 2012; 43: 198–214. https://doi.org/10.1111/j.1600-048X.2012.05604.x

Miliá B, Smith TB, Wayne RK. Speciation and rapid phenotypic differentiation in the yellow-rumped warbler Dendroica coronata complex. Mol Ecol. 2006; 16: 159–173. https://doi.org/10.1111/j.1365-294X.2006.03119.x PMID: 17181728

Wooding S, Ward RD. Phylogeography and Pleistocene evolution in the North American black bear. Mol Biol Evol. 1997; 14: 1096–1105. https://doi.org/10.1093/oxfordjournals.molbev.a025719 PMID: 9364767

Zink RM. Microsatellite and mitochondrial DNA differentiation in the fox sparrow. Condor. 2008; 110: 482–492. https://doi.org/10.1525/cond.2008.8496

Knaus BJ, Cronn R, Liston A, Pilgrim K, Schwartz MK. Mitochondrial genome sequences illuminate cryptic diversity in a widespread North American songbird: phylogeography of the brown creeper (Certhia americana). Mol Phylogenet Evol. 2011; 58: 502–512. https://doi.org/10.1016/j.ympev.2010.12.003 PMID: 21168520

Jaramillo-Correa JP, Beaulieu J, Bousquet J. Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (Picea mariana), a transcontinental North American conifer. Mol Ecol. 2004; 13: 2735–2747. https://doi.org/10.1111/j.1365-294X.2004.02258.x PMID: 15315685

Avise JC, Helfman GS, Saunders NC, Hales LS. Mitochondrial DNA differentiation in North Atlantic eels: population genetic consequences of an unusual life history pattern. Proc Natl Acad Sci USA. 1986; 83: 4350–4354. https://doi.org/10.1073/pnas.83.12.4350 PMID: 16593712

Ball RM, Freeman S, James FC, Bermingham E, Avise JC. Phylogeographic population-structure of red-winged blackbirds assessed by mitochondrial-DNA. Proc Natl Acad Sci USA. 1988; 85: 1558–1562. https://doi.org/10.1073/pnas.85.5.1558 PMID: 16593914

Carr SM, Snellen AJ, Howse KA, Wroblewski JS. Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (Gadus morhua) from bay and offshore locations on the Newfoundland continental shelf. Mol Ecol. 1995; 4: 79–88. https://doi.org/10.1111/j.1365-294X.1995.tb00194.x PMID: 7711956

Cronin MA, Amstrup SC, Scribner KT. Microsatellite DNA and mitochondrial DNA variation in polar bears (Ursus maritimus) from the Beaufort and Chukchi seas, Alaska. Can J Zool. 2006; 84: 655–660. https://doi.org/10.1139/z06-039

Pulgarín-R PC, Burg TM. Genetic signals of demographic expansion in downy woodpecker (Picoides pubescens) after the last North American glacial maximum. PLOS ONE. 2012; 7: e40412. https://doi.org/10.1371/journal.pone.0040412 PMID: 22792306

Avise JC. Molecular markers, natural history, and evolution. Sunderland: Sinauer and Associates; 2004.

Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS. Comparative phylogeography of unglaciated eastern North America. Mol Ecol. 2006; 15: 4261–4293. https://doi.org/10.1111/j.1365-294X.2006.03061.x PMID: 17107465

Bermingham E, Avise JC. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics. 1986; 113: 939–965. PMID: 17246340

Austin JD, Lougheed SC, Boag PT. Discordant temporal and geographic patterns in maternal lineages of eastern North American frogs, Rana catesbeiana (Ranidae) and Pseudacris crucifer (Hylidae). Mol Phylogenet Evol. 2004; 32: 799–816. https://doi.org/10.1016/j.ympev.2004.03.006 PMID: 15288057

Burbrink FT, Lawson R, Slowinski JB. Mitochondrial DNA phylogeography of the polytypic North American rat snake (Elaphe obsoleta); a critique of the subspecies concept. Evolution. 2000; 54: 2107–2118. https://doi.org/10.1554/0014-3820(2000)054[2107:MDPOTP]2.0.CO;2 PMID: 11209786

Brant SV, Ortí G. Phylogeography of the northern short-tailed shrew, Blarina brevicauda (Insectivora: Soricidae); post fragmentation and postglacial recolonization. Mol Ecol. 2003; 12: 1435–1449. https://doi.org/10.1046/j.1365-294X.2003.01789.x PMID: 12755873

USDA. Plants Database; 2017. [online] https://plants.usda.gov/java/ [accessed June, 2017].
73. Lait LA, Burg TM. When east meets west: population structure of a high-latitude resident species, the boreal chickadee (*Poecile hudsonicus*). Heredity. 2013; 111: 321–329. https://doi.org/10.1038/hdy.2013.54 PMID: 23759728

74. Soltis DE, Gitzendanner MA, Strenge DD, Soltis PS. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Syst Evol. 1997; 206: 353–373. https://doi.org/10.1007/bf00987957

75. van Els P, Cicero C, Klicka J. High latitudes and high genetic diversity: phylogeography of a widespread boreal bird, the gray jay (*Perisoreus canadensis*). Mol Phylogenet Evol. 2012; 63: 456–465. http://dx.doi.org/10.1016/j.ympev.2012.01.019.

76. Feder JL, Opp SB, Wlazlo B, Reynolds K, Go W, Spisak S. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. Proc Natl Acad Sci USA. 1994; 91: 7990–7994. PMID: 11607491

77. Funk DJ. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neo-chlamisus bebbianae* leaf beetles. Evolution. 1998; 52: 1744–1759. https://doi.org/10.1111/j.1558-5646.1998.tb02254.x PMID: 28565322

78. Eichlin TD. Clearwing moths of Baja California, Mexico. Tropical Lepidoptera. 1992; 3: 135–150.