Mitochondrial DNA variation in two invasive birch leaf-mining sawflies in North America

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Abstract—Mitochondrial cytochrome oxidase I and II genes were sequenced for two invasive alien birch (*Betula* L. [Betulaceae]) leaf-mining sawflies, *Profenusa thomsoni* (Konow, 1886) (Hymenoptera: Tenthredinidae) and *Scolioneura betuleti* (Klug, 1816) (Hymenoptera: Tenthredinidae), accidentally introduced from Europe to North America. Ten North American and two European populations of *P. thomsoni* were sampled. As no genetic variation was observed for this parthenogenic species in Europe or North America, there is no evidence that this species was introduced more than once into North America. A single Canadian population of putative *S. betuleti* was genetically characterized and compared with populations of *S. betuleti* and *Scolioneura vicina* Konow, 1894 in Europe to resolve the species identity of the introduced Canadian population. Three haplotypes were present in European material but only one haplotype was represented in material collected in Canada. The haplotype in the Canadian population occurred in both *S. betuleti* and *S. vicina* in Europe. Thus, this preliminary genetic work cannot provide certain identity of the Canadian species. Moreover, there was no significant genetic difference between putative *S. betuleti* and *S. vicina* in Europe, leading us to suggest that *S. vicina* may not be reproductively isolated from *S. betuleti*, despite ecological differences.

Résumé—Les gènes d'ADN mitochondrial cytochrome oxydase I et II ont été séquencés chez deux espèces de tenthrèdes (Hymenoptera : Tenthredinidae) exotiques et invasives, mineuses des feuilles du bouleau (*Betula* L. [Betulaceae]), *Profenusa thomsoni* (Konow, 1886) et *Scolioneura betuleti* (Klug, 1816), introduites accidentellement en Amérique du Nord à partir d'Europe. Dix populations Nord Américaines et deux Européennes de *P. thomsoni* ont été échantillonnées. Puisqu'aucune variation génétique n'a été observée chez cette espèce parthénogénique en Europe et en Amérique du Nord, les données contredisent l'hypothèse selon laquelle cette espèce fut introduite plus d'une fois en Amérique du Nord. Une population canadienne de tenthrèdes présumément appartenant à l'espèce *S. betuleti* a été caractérisée génétiquement et comparée aux populations de *S. betuleti* et *S. vicina* Konow, 1894 d'Europe dans le but de clarifier l'identification à l'espèce de la population introduite au Canada. Trois haplotypes étaient présents dans le matériel européen mais seulement un était représenté dans le matériel collecté au Canada. L'haplotype de la population canadienne a été simultanément retrouvé chez des individus de *S. betuleti* et *S. vicina* provenant d'Europe. Conséquemment, ce travail génétique préliminaire ne peut pas confirmer l'identité de l'espèce canadienne. De plus, il n'y avait aucune différence génétique significative entre les spécimens européens présumés comme appartenant aux espèces *S. betuleti* et *S. vicina*, ce qui laisse croire que *S. vicina* n'est pas isolée reproductivement de *S. betuleti*, malgré les différences écologiques.

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Introduction

The origins and impacts of alien species are of increasing interest (Pimentel *et al.* 2000; Sakai *et al.* 2001; Simberloff *et al.* 2005). Knowing the identity and home range of an alien species is necessary to obtain access to the global cache of information about that species. Such information helps with assessment of potential risks to the economy and environment (Armstrong and Ball 2005), implementation of measures to slow or prevent further invasions (Baker *et al.* 2005), and elucidation of modes of entry (Walters *et al.* 2006). Genetic tools can be accurately and reliably applied to investigate species identity and origin. Many studies have used molecular tools to examine species identity, invasion routes, host range, and genetic diversity of alien arthropods (*e.g.*, Davies *et al.* 1999; Cognato *et al.* 2005; Navia *et al.* 2005), but few studies have applied genetic techniques to the study of alien Hymenoptera (Tsutsui *et al.* 2000; Baker *et al.* 2003; Hufbauer *et al.* 2004; Johnson and Starks 2004), and none have examined introduced sawflies (Symphyta).

Profenusa thomsoni (Konow, 1886) and *Scolioneura betuleti* (Klug, 1816) (Hymenoptera: Tenthredinidae) are two birch leaf-mining sawflies that are native to Europe but were accidentally introduced to North America. These species are rare or occasionally minor pests of birch (*Betula* L. [Betulaceae]) in Europe (Schönrogge and Altenhofer 1992; Kenis and Carl 1995; Pieronek 1995), but in North America they have become significant pests of birch (Benson 1959; Nystrom and Evans 1989; Digweed *et al.* 2003). The larvae of both species feed inside leaves, thus damaging the photosynthetic layer and causing aesthetic damage, a loss of tree vigor, and possibly death.

Profenusa thomsoni was first recorded in North America from the eastern United States in 1923 (Ross 1951) and from Canada in central Ontario in 1955 (Lindquist 1955). However, *P. thomsoni* was likely well established in Canada before 1955, as prior to that time it was commonly confused with another birch leaf-mining sawfly, *Fenusa pumila* Leach, 1817 (Hymenoptera: Tenthredinidae) (Martin 1960), and adults had been collected in Fort Gary, Manitoba, and Sault Ste. Marie, Ontario, in 1948 and 1950, respectively (Canadian National Collection of Insects, specimens Hymen No. 05-114 and Hymen No. 05-130). *Profenusa thomsoni* had become a significant pest in eastern Canada by the late 1950s (Martin 1960), in Alberta by the 1970s (Drouin and Wong 1984), and in Alaska by the 1990s (Snyder *et al.* 2007). The species has one generation per year in North America and is likely parthenogenic, as no males have been recorded (Benson 1959). Females emerge in late spring to early summer and lay eggs singly or in clusters on fully expanded birch leaves. Larvae complete development by early fall and final instars emerge and fall to the ground, where they construct earthen cells and overwinter as prepupae (Martin 1960).

Occurrence records of *P. thomsoni* (Lindquist 1955; Benson 1959) suggest the species was introduced somewhere in eastern North America, and likely much earlier than when it was first recorded (*i.e.*, 1923). Given that most sawflies are weak flyers (Benson 1950) and that *P. thomsoni* has only one generation per year (Martin 1960), it is unlikely that *P. thomsoni* was able to spread from Ontario to Alaska in less than 60–80 years solely under its own power. As birches are popular horticulture species, it is reasonable to infer that some introductions were associated with commercial transfer of infested plants in the horticultural trade. Thus, new populations were likely established by a small number of founder individuals, creating genetic bottlenecks from which it may be possible to identify the invasion history of the species.

Scolioneura betuleti is a more recent import to North America, first recorded from one site north of Toronto, Ontario, in 1983 (Evans *et al.* 1985). It has since expanded its range within Ontario, east to Prince Edward Island and Newfoundland, and west to British Columbia (S. Digweed, personal communication). Nystrom and Evans (1989) identified the species in Canada as *S. betuleti* using keys available at the time. Later, Altenhofer and Taeger (1998) split *S. betuleti* in Europe into *S. betuleti* and *Scolioneura vicina* Konow, 1894, basing their decision on adult phenology and host plant preference (the species are morphologically indistinct as both adults and larvae). *Scolioneura vicina* is described as a spring flyer, feeding only on *Betula pubescens* Ehrh. and *B. pendula* Roth, while *S. betuleti* flies in the fall and feeds on the same *Betula* species but also on *Alnus viridis* (Chaix) DC (Betulaceae). Thus, according to Altenhofer and Taeger (1998), the species found in Canada is more likely to be *S. vicina* than *S. betuleti*, based on flight phenology. While *S. betuleti* and *S. vicina* are not

currently considered pests of *Betula* spp. in Canada or Europe, there is a need to resolve this taxonomic issue as a prerequisite to future work on this species.

This study aims to reconstruct the invasion history of *P. thomsoni* in North America and resolve the taxonomy of *Scolioneura* Konow, 1890 species in Canada and Europe using mitochondrial DNA (mtDNA) sequence data. We used an 840-base-pair (bp) region of the mitochondrial genome of *P. thomsoni* and a 716-bp region from both putative *Scolioneura* species to assess (*i*) the source population in Europe for *P. thomsoni* and the number of separate introductions into North America and (*ii*) which of the two putative birch-inhabiting *Scolioneura* species recognized by Altenhofer and Taeger (1998) occurs in Canada. We selected mtDNA as our marker because sequence data are relatively easy to obtain and this genome has a high rate of evolution (Simon *et al.* 1994).

Methods

Specimen collection

Profenusa thomsoni larvae were extracted from leaves of *Betula* sp. from 10 North American sites and 2 central European sites (Table 1) and preserved in 80%–100% ethanol. Identification at the time of collection was based on host plant, larval morphology, and mine shape (Lindquist 1959) and was later confirmed by C.J.K. MacQuarrie. Attempts were made to collect throughout the range of *P. thomsoni*, but there were a few gaps in coverage because of unavailability of *P. thomsoni* populations or collectors.

Scolioneura larvae were collected in the same manner as *P. thomsoni* larvae at one site in eastern Austria and another in Ontario, Canada (Table 1). All European *Scolioneura* specimens were identified to species by E. Altenhofer at the time of collection (Table 1) and their phenology corresponds to that given for these species in Altenhofer and Taeger (1998) (Table 1). All Canadian material was identified by the collector and confirmed by C.J.K. MacQuarrie using descriptions and figures from Nystrom and Evans (1989). Collectors initially stored larvae in ethanol at room temperature or –20 °C until they were shipped. After arrival at the laboratory, specimens were stored at –20 °C.

DNA extraction, amplification, and sequencing

Mitochondrial DNA from *P. thomsoni* and *Scolioneura* species was extracted using a QIAamp DNA Mini Kit (QIAGEN, Mississauga, Ontario) according to the manufacturer's recommended protocol. Extractions made from the larval abdomen provided sufficient genetic material for amplification by polymerase chain reaction (PCR). The larval head and thorax for each sequenced specimen were stored in 100% ethanol at –70 °C and deposited as vouchers, along with a selection of intact larvae from the same collection locality and date, in the Strickland Entomological Museum at the University of Alberta, Edmonton, Alberta, Canada.

A fragment spanning the cytochrome oxidase I (COI), $tRNA^{Leu}$, and cytochrome oxidase II (COII) region of the mitochondrial genome was amplified from *P. thomsoni* using four heterologous primer pairs (Table 2). From this fragment we obtained 840 bp of sequence for a minimum of two specimens from all sites and an additional 1318 bp of sequence from one specimen from the most geographically separate sites, giving a total sequence of 2158 bp (Table 1). Only two to five specimens per site were sequenced. We observed no sequence variation between any of the 32 specimens, regardless of collection site, and therefore additional sequencing to assess haplotype frequencies was not necessary. A representative sequence of either 840 or 2158 bp from each site was deposited in GenBank (840 bp: accession Nos. EF445947–EF445951; 2158 bp: accession Nos. EF445956–EF445961).

A fragment of the COI gene was amplified from all *Scolioneura* specimens using primers Jerry and Pthom1 (set C, Table 2), from which 716 bp of sequence (GenBank accession Nos. EF445952–EF445955) was obtained. PCR amplification and sequencing reactions were carried out using standard laboratory methods, the exact details of which are presented elsewhere (Abe *et al.* 2005; Laffin *et al.* 2005; Roe *et al.* 2006). Additional sequence data obtained from GenBank were also used in our analysis, specifically 349 bp derived from *S. betuleti* from northern Finland (accession No. DQ302242) (Nyman *et al.* 2006). The differences in nucleotide sequence length between the GenBank sequence and ours were coded as missing data. A specimen from the same collection date and

Table 1. *Profenusa thomsoni* and *Scolioneura* sp. collection and sequencing information.

Collection site	Latitude	Longitude	Date	Collector	No. of specimens sequenced*
Profenusa thomsoni					
USA, AK, Anchorage	61.169	–149.918	27.vii–25.viii.2005	C. MacQuarrie	2[†,‡]
USA, AK, Eielson Air Force Base	64.689	–147.079	15.vii.2004	C. MacQuarrie	2[†]
USA, AK, Haines	59.150	–135.320	24.viii.2004	M. Schultz	2[†]
CAN, BC, Prince George	53.550	–122.450	28.viii.2005	C. MacQuarrie	3[†]
CAN, NWT, Yellowknife	62.272	–114.210	2.viii.2005	S. Digweed	3[†]
CAN, SK, La Ronge	55.070	–105.324	28.viii.2005	S. Digweed	3[†,‡]
CAN, MB, Swan River	52.062	–101.160	2.ix.2005	S. Digweed	3[†]
CAN, ON, Wawa	47.590	–84.470	4,8.viii.2005	K. Nystrom	3[†,‡]
CAN, NS, Dartmouth	44.476	–63.352	23.vii.2005	D. Williams	3[†,‡]
Austria, Lower Austria, Arbesbach	48.483	14.950	7.viii.2005	M. Kenis	5[†,‡]
Switzerland, Neuchâtel, Les Ponts-de-Martel	47.000	6.733	6.viii.2005	M. Kenis	3[†,‡]
***Scolioneura* sp.**					
Austria, Lower Austria, Etzen	48.566	15.033	17.vi.2005	E. Altenhofer	9A[§]
CAN, ON, Brantford	43.900	–80.140	23.vi.2005	L. Tucker	2A[§]
Austria, Lower Austria, Etzen	48.566	15.033	10–28.ix.2005	E. Altenhofer	2A[§], 4B[§]
Kilpisjärvi, Finland	69.046	20.795	10.viii.2001	T. Nyman/V. Vikberg	1C[‖]

Note: All specimens were collected as mid- to late-instar larvae from *Betula* sp. All *Profenusa thomsoni* specimens and *Scolioneura* sp. specimens from Austria and Canada are deposited at the University of Alberta, Strickland Entomological Museum, Edmonton, Alberta, Canada. *Scolioneura* specimens from Finland are deposited at the Zoological Museum of the University of Oulu, Oulu, Finland.

*Uppercase letters denote haplotypes of *Scolioneura* sp.; see text for details.

[†]840-bp sequence from all specimens, GenBank accession Nos. EF445947–EF445951.

[‡]2158-bp sequence from one specimen, GenBank accession Nos. EF445956–EF445961.

[§]716-bp sequence from all specimens, GenBank accession Nos. EF445952–EF445955.

[‖]349 bp from GenBank, accession No. DQ302242 (Nyman *et al.* 2006).

Table 2. Mitochondrial DNA primers used for *Profenusa thomsoni* and *Scolioneura* sp. COI–tRNA^{Leu}–COII amplification.

Primer set	Primer name	Direction and location	Sequence (5′–3′)	Reference
A	K698	TY-J-1460a	TACAATCTATCGCCTAAACTTCAGCC	Simon *et al*. 1994
A	K699	C1-N-1840	AGGAGGATAAACAGTTCA(C/T)CC	Sperling *et al*. 1995
B	Ron II	C1-J-1751d	GGAGCTCCAGATATAGCATTCCC	Simon *et al*. 1994
B	K525	C1-N-2329	ACTGTAAATATATGATGAGCTCA	Simon *et al*. 1994
C	Jerry	C1-J-2183a	CAACATTTATTTTGATTTTTTGG	Simon *et al*. 1994
C	Pthom1	C1-N-2934	GTTTTCATTCAATCGATG	This study
D	Pthom2	C1-J-2761	ATACCACGTCGATACTCGGA	This study
D	Barbara	C1-N-3661	CCACAAATTTCTGAACATTGACCA	Simon *et al*. 1994

Note: Direction and location of primers follows Simon *et al.* 1994.

location was acquired from T. Nyman and its identity confirmed by C.J.K. MacQuarrie.

Analysis

All *P. thomsoni* and *Scolioneura* sequences were aligned in Sequencher (Version 4.1, Gene Codes Corporation, Ann Arbor, Michigan, 2001) using default settings, with ambiguous base calls being corrected by eye; data derived from primer regions were removed prior to alignment. Contigs were assembled by aligning fragments generated from primer pairs and then aligning contigs from overlapping regions. Consensus strands were then generated from bidirectional contigs or from multiple unidirectional strands for the same region generated from independent sequencing reactions (with exceptions: 338 bp in one Anchorage, Alaska, specimen; no reverse complement between Ron II and K525 for one Eielson Air Force Base, Alaska, specimen and one Prince George, British Columbia, specimen). Base frequencies were calculated using PAUP* 4.4 (Swofford 2003). Alignment to the *Drosophila yakuba* Burla (Diptera: Drosophilidae) sequence (Clary and Wolstenhome 1985) was done by eye. Divergences between the *S. betuleti* sequence from GenBank and those obtained in this study were examined visually using MacClade 4.05 (Maddison and Maddison 2002) to evaluate the positions of amino acid base pair changes. Analysis of the *P. thomsoni* data set was done by eye.

Results

Profenusa thomsoni

We obtained sequences from 32 *P. thomsoni* specimens. The 2158-bp sequence (n = 12) corresponds to positions 1461–3564 of the *D. yakuba* sequence. Inserts in the *P. thomsoni* sequence were observed at the following positions relative to *D. yakuba*: 39 bp at position 1474, 15 bp at 3009, and 3 bp at 3062. The 840-bp sequence (n = 32) was aligned to positions 1736–2575 of *D. yakuba* with no indels. There was no variation among specimens in either the 840-bp sequence or the 2158-bp sequence.

Scolioneura

We obtained 716 bp of sequence from nine specimens of European *S. vicina*, six specimens of European *S. betuleti*, and two specimens of Canadian *Scolioneura* sp. This sequence corresponds to positions 2209–2925 of *D. yakuba*, with no indels. Additional Canadian material was collected and mtDNA extracted but this DNA could not be amplified despite repeated attempts. We identified three haplotypes: haplotype A was observed in all nine European *S. vicina* specimens, two of six European *S. betuleti* specimens, and both Canadian specimens; haplotype B was observed only in four European *S. betuleti* specimens; and haplotype C was observed only in the sequence obtained from GenBank. Genetic divergence between central European haplotypes A and B was 0.14%, or one nucleotide. Genetic divergence between the central European haplotypes (A + B) and the northern Finland haplotype (C) was 2.08%. The inferred amino acid sequence was the same for all haplotypes.

Discussion

To our knowledge this study is the first to use genetic methods to attempt to resolve the invasion history of an alien sawfly species and to

resolve a question of sawfly species identity. Furthermore, very few studies have used genetic characters to resolve the taxonomy of groups within the Symphyta (Schulmeister 2003; Heidemaa 2004; Nyman *et al.* 2006), and other attempts to reconstruct the invasion history of parthenogenic insects have been limited to the Hemiptera (*e.g.*, Downie 2002; Gwiazdowski *et al.* 2006; Havill *et al.* 2006).

Genetic variation in *P. thomsoni*

We observed no variation between *P. thomsoni* individuals from North America and those from Europe. This observation is consistent with a scenario in which there was one successful colonization event in North America, although that interpretation is preliminary in the context of the limited sampling in our study, especially in Europe. We do not know the full range of the single haplotype — only that it occurs at one locality in each of Switzerland and Austria — but the introduction to North America must have come from within the presumed European range of the haplotype. If a greater amount of genetic variation occurs in Europe, then it would be plausible that the original colonists to North America were relatively few and genetically uniform. In any case, these data are not consistent with the hypothesis of Schönrogge and Altenhofer (1992) that *P. thomsoni* is a native North American species (this hypothesis was based on the specialist parasitoid *Lathrolestes luteolator* Gravenhorst (Hymenoptera: Ichneumonidae) attacking the leaf miner only in North America). It is highly unlikely, if *P. thomsoni* were native to both Europe and North America, that there would be no genetic differentiation between populations from the two continents.

The lack of genetic divergence between the two sampled populations of *P. thomsoni* in its native Europe was unexpected, especially as we observed intrapopulation differences in *Scolioneura*. Further sampling over a broader geographic range may reveal the presence of other European haplotypes. This has been shown in other sawfly species over a similar geographic range using allozymes (Muller et al. 2004) and in the present study, where we observed two haplotypes over the same sampled range for *Scolioneura*. It is possible that we did not sample a sufficient number of individuals in Europe (n = 8) to detect the presence of rare haplotypes, although if these haplotypes did exist and comprised a significant portion of the population we should have detected some within our North American specimens (n = 24). Alternatively, there are multiple processes that could have caused a homogenization of mtDNA diversity in *P. thomsoni* prior to its introduction into North America (Hurst and Jiggins 2005). For example, selective sweeps by endosymbionts such as *Wolbachia* spp. have been suggested to cause low mtDNA diversity in other species (Hurst and Jiggins 2005) and parthenogenesis in haplodiploid groups (Werren 1997). If the mitochondrial genome we sequenced is representative of total genetic diversity, our data suggest that *P. thomsoni* may have a reduced adaptive capacity to deal with strong selective forces such as natural enemies, diseases, or abiotic factors that could be used for biological control.

***Scolioneura*: genetic variation and taxonomy**

The mtDNA sequences of putatively distinct *S. betuleti* and *S. vicina* are genetically very similar, differing only by 0.14% between haplotypes A and B. It is possible that more variation occurs throughout the range of *S. betuleti* / *S. vicina*, as suggested by the 2.08% variation between haplotypes from central Europe (haplotypes A and B) and northern Finland (haplotype C). However, this amount of variation is somewhat ambiguous in determining whether the *S. betuleti* specimen from northern Finland is part of a distinct species, as 2% variation has been suggested as a cutoff for species delimitation using mtDNA sequence data (Hebert *et al.* 2003; but see Cognato 2006).

The presence of only haplotype A in Canadian *Scolioneura* does not allow a clear genetic diagnosis of the Canadian material, as haplotype A is present in both putative European *Scolioneura* species (Table 1). In the absence of definitive genetic data the phenology of the Canadian population (*i.e.*, spring-flying adults) leads to the conclusion that this population is *S. vicina*, not *S. betuleti* as reported (Nystrom and Evans 1989). However, it is necessary to re-examine whether *S. vicina* is a valid species; Altenhofer and Taeger (1998) resurrected the name from synonymy on the strength of biological and host preference data. Our molecular data suggest that *S. betuleti* and *S. vicina* are possibly conspecific, and thus we suggest that *S. vicina* could be considered a junior synonym

of *S. betuleti* if these data can be supported by other molecular markers.

The observation that only fall-flying *S. betuleti* will utilize *A. viridis* (Altenhofer and Taeger 1998) in Europe should be examined further to understand why spring-feeding *S. vicina* do not utilize this host. It is possible that newly expanded *A. viridis* leaves are not accepted for oviposition by spring-flying *S. vicina*, but as leaves age, changes occur such that *A. viridis* becomes an acceptable host for fall-flying females. This could be tested by altering diapause cues in reared specimens so that spring- and fall-flying *Scolioneura* adults could be exposed to "fall" *A. viridis* foliage and *vice versa*.

Further avenues of investigation are provided by the finding that *Scolioneura* larval skins were recently recovered from sites in Newfoundland and British Columbia (S. Digweed, personal communication). It would be useful to sample these populations and any intermediates to evaluate the invasion history of *S. vicina* in Canada. Furthermore, surveys in Europe and Canada to delineate the ranges of mtDNA haplotypes may suggest where to search for new natural enemies to introduce against *S. vicina* in North America.

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