Lepidoptera wing scales: a new paleoecological indicator to reconstruct spruce budworm abundance

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Lepidoptera wing scales: a new paleoecological indicator for reconstructing spruce budworm abundance

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Running title: Wing scales, a new paleoecological proxy

Abstract

Natural disturbances have a major impact on boreal forest landscape dynamics and, although fire history is well documented at the Holocene scale, spruce budworm (SBW) dynamics are only known for the last three centuries. This is likely due to the difficulty in using and interpreting existing indicators (cephalic head capsules and feces). In this methodological study, we present an original approach using lepidopteran wing scales to reconstruct insect abundance. We analyzed two sediment cores from the boreal forest in central Quebec and extracted wing scales at every stratigraphic level. The required quantity of sediment for paleoecological analysis is relatively small given the large quantity of wing scales produced by Lepidoptera and their small size. Scales are well-
preserved due to their chitinous structure and their great variety of shapes offer a high potential for taxonomic identification. A statistical model based on the shape of scales of the three major epidemic lepidopterans in Quebec discriminated 68% of SBW scales. This indicator allows a more efficient and more precise reconstruction of SBW history with respect to the use of cephalic head capsules or feces.

Résumé
Les perturbations naturelles ont un impact majeur sur la dynamique des paysages en forêt boréale, et bien que l’historique des feux soit étudié à l’échelle holocène, la dynamique de la tordeuse des bourgeons de l’épinette (TBE) n’est connue que pour les trois derniers siècles. Ce manque peut s’expliquer par la difficulté d’utilisation et d’interprétation des indicateurs disponibles (capsules céphaliques et fèces). Cette étude méthodologique présente une méthode originale qui permet d’utiliser les écailles de lépidoptère pour reconstruire l’abondance de l’insecte. L’étude de deux carottes de surface échantillonnées dans la réserve faunique des Laurentides a permis de retrouver des microrestes à tous les niveaux stratigraphiques. La grande quantité d’écailles produite par papillons ainsi que la taille des microrestes a permis de réduire considérablement le volume de sédiments requis. Les écailles se conservent bien grâce à leur structure chitineuse et leur grande variété de formes présente un fort potentiel d’identification taxonomique. Un modèle statistique basé sur la mesure de la forme des écailles des trois principaux lépidoptères épidémiques au Québec a permis de discriminer 68% des écailles de TBE. Cet indicateur permet donc une reconstitution plus efficace et plus précise de l’historique des populations de TBE par rapport à l’utilisation des capsules céphaliques ou des fèces.

Keywords: boreal forest; insect outbreaks; moth; butterfly; paleoindicator
Introduction

Climate change is a major challenge in ecology, greatly affecting forest disturbance dynamics and ecosystem diversity (Gauthier et al. 2015). Boreal forests are the largest terrestrial ecosystems on the planet and produce more than a third of the world’s lumber (Gauthier et al. 2015, Montoro Girona et al. 2016). Due to the ecological and economic impacts of insect outbreaks in this ecosystem, it is important to develop a better understanding of how climate change affect these, often devastating, insect events. Forecasts project an increase in the frequency, duration, and timing of insect outbreaks (Nelson et al. 2013). However, there remains a shortage of indicators that permit a more thorough assessment of cycles and impacts of insect outbreaks at the Holocene scale (Simard et al. 2002).

Spruce budworm (Choristoneura fumiferana, Clemens) (SBW) is one of the most important agents of natural disturbance in the boreal forests of North America (Shorohova et al. 2011), causing major loss of productivity and death of host species (Hennigar et al. 2013). In the eastern Canadian boreal forest, SBW is, by far, the most damaging defoliator of conifers, reaching cyclically epidemic population densities that allow the insect to affect large forest areas (Pureswaran et al. 2015). During the last outbreak of the 20th century (1974–1988) SBW affected 55 million hectares causing the loss of 139 to 238 million m³ of fir and spruce (Boulet et al. 1996). This forest pests’ short life cycle, mobility, and high reproductive potential allow a rapid response to changes in environmental conditions (Menéndez 2007). Nevertheless, long-term studies of landscape natural variability focus on the use of charcoal fragments to determine fire activity and often conceal the effect of forest insect outbreaks (Bergeron et al. 1998).
However, in eastern Quebec SBW is considered a more important disturbance than fire and the lack of knowledge about its pluri-millennial dynamics is the cause of a serious bias in the understanding of landscape variability. It is therefore important to assess the relative importance of SBW outbreaks and the role played in the entire boreal forest ecosystem at the longest temporal scale possible.

Dendrochronological studies have revealed the epidemic history of SBW over the last three centuries (Boulanger et al. 2012). Tree-ring analysis provides important insights into impact from defoliation as well as the periodicity and synchronism of insect outbreaks across huge areas of forest. Nevertheless, dendrochronology is an indirect measure of SBW impact and its interpretation is limited as multiple factors may influence tree growth (Davis and Hoskins 1980). In addition, dendrochronology is restricted to the age of survivor trees and reconstruction of long-term chronologies using subfossil trees buried in lakes and ponds is promising but, for the moment, remains challenging.

Paleoecology allows a longer-term reconstruction of SBW activity. Macrofossils such as cephalic head capsules (Lavoie et al. 2009) or feces (Simard et al. 2006) have been used as direct indicators of the occurrence of insect outbreaks. However, the long-term dynamics remain poorly documented as analysis is time-consuming, and temporal resolution is low due to the amount of sediment to be examined. The number of cephalic head capsules extracted from a relatively large amount of sediment or peat material tends to be low and feces become increasingly difficult to identify with depth due to decomposition.

Butterfly and moth wings are covered with thousands of minute overlapping scales of variable shape, forming colour patterns on the wing surface (Dinwiddie et al. 2014).
These scales assume many different functions, from attracting and selecting mates (Burghardt et al. 2000) to camouflage (Brakefield and Liebert 2000), and thermal regulation (Srinivasarao 1999).

SBW scales are partly made of chitin fibrils (Richards 1947) that are well-preserved in sediments due to increasingly favorable conservation conditions with depth under anaerobic conditions (Sturz and Robinson 1986). As these scales are produced in a very high quantity, particularly during outbreaks, this should permit many of these microfossils to be retrieved from a very small quantity of sediments. There is also a great potential for taxonomic identification owing to the high diversity of scale shapes. These considerations led us to ask whether lepidopteran scales microfossils are a more suitable paleoindicator of SBW abundance. We hypothesize that this indicator can be extracted in a significant quantity and that its abundance peaks will match known periods of SBW outbreaks.

This methodological investigation proposes a practical technique to collect and extract fossil scales from sediment cores and discusses the potential benefits and difficulties of using scale shape as an indicator of Lepidoptera species. We also present examples from two short sediment cores recovered from a pair of lakes in the central boreal region of Quebec.

**Proposed methodology for scale extraction**

**Scale extraction and analysis**

To set the extraction parameters (sucrose solution density, sieve mesh size, centrifugation speed and duration…), we used a trial and error approach with test samples in which we
added a relatively constant quantity of scales (one entire forewing of a SBW per sample).

The parameters presented here are those that gave the best results.

We dried samples from a sectioned sediment core at 105 °C for 24 h. For each interval of interest, we collected a 0.5 g subsample of dry sediment (± 5 cm³) and heated it in a 100 mL 10 % potassium hydroxide (KOH) solution at 70 °C for 30 min or until complete deflocculation had occurred (Frey 1986). The slurry was then sieved through a 53 µm mesh to retain most of the scales. Small particles (mostly fragments of organic matter) were discarded to allow us to analyze a more concentrated sediment subsample. We centrifuged the samples at 500 RCF for 10 min in a 10 mL sucrose solution (relative density = 1.24) to remove higher density particles. This centrifuging was repeated three times. After each run, we recovered the supernatant, refilled the vial with the sucrose solution, and centrifuged again. We combined the three supernatants in a 50 mL plastic vial and, to precipitate scales and any remaining particles, we centrifuged the combined supernatant at 3900 RCF for 20 min. The final pellet was mounted onto microscope slides for microfossil counting. This operation permitted most of the scales to be easily extracted, at low cost, without using destructive chemicals.

**Taxonomic identification of scales and the potential of using scale shape**

There is very little existing literature about the taxonomic value of lepidopteran scales for paleoecological work. Species of Lepidoptera are usually distinguished by studying wing venation or genitalia, features that are not preserved in the sediment record. Among the other discriminant criteria, the most studied are the iridescent ultrastructure of scales and structural colour production (Dinwiddie et al. 2014). Ultrastructure analysis requires expensive and time-consuming scanning electron microscopy (SEM) that is not easily
applicable to paleoecological studies. In the case of moths, colour patterns are dull and extraction techniques, including the use of hot alkali solution, could alter the pigmentation of scales. In some rare cases, when these identification criteria are not sufficient to distinguish two sibling species, scale shape has been used (Anken and Bremen 1996, Yang and Zhang 2011).

To test the interspecific variability of shape parameters and their taxonomic potential, we selected three of the major epidemic defoliator lepidopterans in the North American boreal forest: spruce budworm (*Choristoneura fumiferana* Clemens), forest tent caterpillar (*Malacosoma disstria* Hübner) and hemlock looper (*Lambdina fiscellaria* Guénée) (Shorohova et al. 2011). We used eight specimens for each species, half of each sex to take sexual dimorphism into account, and studied scales from the upper and lower surface of each wing. Scales were separated from the wing using a fine brush and mounted on glycerol-coated microscope slides. To ensure random and systematic selection, we used a 10×10 grid beneath the slide and selected the first scale encountered in each cell. We analyzed 2400 scales per species. We used Elliptic Fourier Descriptors (EFDs) and some non-size correlated shape parameters as quantitative measures of scales shape. EFD coefficients were calculated from the chain-coded contours of each scale obtained with SHAPE package v.1.3 (Iwata and Ukai 2006) using 20 harmonics. These coefficients were normalized to be invariant with respect to the size, rotation, and position. This procedure generated multiple coefficients for each scale. As such, we used principal component analysis (PCA) to summarize the data. We used the six principal components scores as shape characteristics. Using image processing software ImageJ v.1.48v (Schneider et al. 2012), we also measured the aspect ratio (\(\frac{\text{MinFeret}}{\text{MaxFeret}}\)), circularity
(4\pi \times \frac{\text{Area}}{\text{Perimeter}^2})$, solidity ($\frac{\text{Area}}{\text{Convex hull area}}$) and the number of apical indentations. We used all these shape parameters to build a discriminating model that correctly classified 68% of the SBW scales, 79% of the hemlock looper scales and 62% of the forest tent caterpillar scales (Fig. 3). We used well-classified scales having a probability >0.8 to construct ten morphotypes for each species based on a K-mean cluster analysis (Fig. 4). Each cluster was characterized by the shape of the median scale and of the first two standard deviations. We also reconstructed the ten most common morphotypes (misclassified, probability <0.5). Specific shapes were observed such as the very deep indentations in *Malacosoma disstria* specimens as noted by Grodnitsky and Kozlov (1990) for *Lasiocampidae*. *Choristoneura fumiferana* morphotypes showed a higher circularity with a relatively high number of small indentations. In contrast, most of the *Lambdina fiscellaria* morphotypes have little to no indentation. Moreover, shape analysis revealed a high intra-specific variability for shape variation, confirming a hypothesis of mostly common scale shapes and some specialized ones (Ghiradella 1994). These morphotypes based on shape measurement still have limitations as a systematic taxonomic identification tool. Indeed, accurate identification of fossil scales extracted from sediment is not always possible due to the conservation state of scales (broken or folded) (Fig. 7e) or the soft focus effect. However, it can help differentiate peaks that may be caused by outbreaks of two or more lepidopteran species.

In Canada, 18 species, representing 1 to 2% of forest lepidopterans, are cyclically outbreaking (Faeth 1987, Mason 1987). From long-term abundance reconstruction studies of SBW in Quebec, we can exclude potentially introduced and western species. Moreover, since 1975, 95% of areas affected by defoliation were caused by SBW. In
addition, spatial extension of bioclimatic domains have been quite stable in eastern Canada since 5 ka BP (Dyke 2005). SBW feces in the sediment record from the northern part of its distribution area attests to its presence since 8 ka BP (Simard et al. 2006). As such, we can assume, with a low risk of misinterpretation, that high variations in fossil scales abundance in Quebec are mainly due to SBW dynamics.

**Case study: results and interpretation**

**Study sites**

The paleoecological protocol was tested on surface sediment cores from two lakes located at the Simoncouche Experimental Forest of the Université du Québec à Chicoutimi, Quebec. Lac Flévy (48°13’00.04”N; 71°12’57.21”W) covers an area of 2.33 ha at an altitude of 376 m. Lac Hautbois (48°12’30.65”N; 71°13’22.92”W) covers 3.9 ha and is at an altitude of 398 m (Fig. 2). We chose small lakes having low outflows, to ensure high sedimentation rates (Millspaugh and Whitlock 1995, Ali et al. 2009). Both studied sites lie on undifferentiated glacial till and fluvioglacial deposits constituted of loose or compact unsorted deposit (Ministère de l’Énergie des Mines et des Ressources 1976). Each lake is surrounded by even-aged trembling aspen (*Populus tremuloides*) stands and mixed even-aged stands of black spruce (*Picea mariana*) and poplar. The age structure indicated that these forest stands originated from an intense fire that occurred in 1922 (Gagnon 1989). SBW has been present in the area for a minimum of 8240 years (Simard et al. 2006) and recurrent outbreaks were reported during the last three centuries, becoming more frequent during the 20th century (Blais 1983). Aerial surveys indicate that defoliation has occurred in the study area since 2012 reaching severe levels after
Sediment core recovery and preparation

We collected two sediment cores from the deepest portion of each lake, one for isotopic dating and the second core for extracting scales. We used a Glew gravity corer for sediment sampling to ensure the sediment/water interface was not disturbed (Glew 1988). The core lengths for the microfossil cores were 32 cm and 24 cm for Lac Flévy and Lac Hautbois, respectively. The cores collected for the analysis of micro-remains were subsampled in the field at a 1-cm-thick resolution using a vertical extruder (Glew 1988).

Isotopic dating

Samples used for dating were dried (105 °C for 24 h) and then sent to the Radiochronology Laboratory at the Centre d’études nordiques, Université Laval for $^{210}$Pb and $^{137}$Cs analyses by gamma spectrometry (San Miguel et al. 2005). We selected a constant rate of supply (CRS) model for the $^{210}$Pb dating of both cores (Turner and Delorme 1996).

Results from scale extraction

Despite the small subsample size (0.5 g dry sediment) and the fact that surface samples are not the most suitable for this kind of analysis (high levels of bioturbation, high water content…), we obtained a substantial quantity of scales from each sampled interval of the sediment cores from both lakes (Fig. 5 and 6). This result is inconsistent with Kristensen and Simonsen’s (2003) assumption that Lepidoptera fossils are strikingly scarce in lacustrine sediments. All the collected microfossils were well-preserved and the
accumulation rate of scales presented a similar order of magnitude in both lakes. Lac Flévy presented two distinctive peaks, the first corresponding to the mid-20th century (± 15 years) and the second corresponding to the beginning of the 21st century (Fig. 5). Both peaks match with known periods of high SBW abundance in the study area. Although the stratigraphy of Lac Hautbois is less clear, it is comparable to that of Lac Flévy: we extracted the most scales from depths corresponding to the early 21st century and the scale accumulation rate decreases slightly prior to the 20th century (Fig. 6). Due to our conservative identification criteria, our results represent a quite low morphotype identification rate suggesting that this method should be used as a validation tool rather than as a systematic identification method. Nevertheless, in both lakes the species with the highest rate of morphotype match was *Choristoneura fumiferana* (Fig 5 and 6), confirming that SBW is the most common outbreaking Lepidoptera in the area. Finally, the identified SBW were similar to the unidentified scales suggesting that some SBW scales were unidentified due to a lack in identification criteria or a degraded physical condition. However, surface core scale abundance requires careful interpretation as $^{210}$Pb dating precision decreases with depth (Fig. 5). A lower resolution long-term $^{14}$C dated stratigraphy should be undertaken for SBW abundance analysis, which will be the subject of a later study.

**Advantages and shortcomings**

The use of fossil scales of Lepidoptera presents some advantages over other paleoecological proxies. Scales are less degradable than feces and much more abundant than cephalic head capsules. They have been found in nearly every sample analyzed to date, including the examples presented above and within longer sediment cores analyzed
for an upcoming publication. Moreover, the use of wing scales requires a relatively small amount of sediment to prepare, simplifying the sampling procedure and allowing wing scales to be analyzed along with additional proxies such as charcoal and pollen from the same core material. Insect infestations should thus be included in any future studies of long-term disturbance dynamics to allow forest management policies to be based on more complete and realistic information. Finally, scale count data can benefit from data processing methods such as the use of a LOESS smoothing to distinguish background variation from locally defined peaks (Higuera et al. 2010).

Nevertheless, as all paleoecological studies, this kind of research is time-consuming, restricting the replicability across multiple sites. In addition, the development of other discriminant criteria (texture, pigmentation, ultrastructure characteristic…) to improve the relevance of interpretation is pertinent due to the taxonomic potential of scales. This method could be adapted for use in different environments such as bogs, fens, ponds, or peat deposits to be directly comparable with existing proxies (feces and cephalic head capsules). Finally, future studies should explore the taphonomic processes affecting scale deposition, transport and sedimentation, and the magnitude of scale accumulation in endemic areas versus epidemic areas.

This study demonstrates that wing scales can be used as a paleoecological indicator for reconstructing insect outbreak history, being a more effective proxy than feces and cephalic head capsules. Fossil scales have a great potential to improve our understanding of the long-term dynamics of lepidopteran defoliator species that are still poorly known, especially in the boreal forest. This contribution will be essential for improving the accuracy of natural disturbance projections within the context of future climate change.
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Figure captions

Figure 1: Scale analysis protocol.

Figure 2: Location of the two studied lakes.

Figure 3: Discriminant analysis of the three species of outbreaking lepidopterans based on EFDs principal component scores and four scale shape parameters. Colour points represent highly specific shapes and faded points represent more common shapes.

Figure 4: Scales morphotypes extract from K-mean cluster analysis of (a) *Choritoneura fumiferana*, (b) *Lambdina fiscellaria*, and (c) *Malacosoma disstria* specific scales and (d) more common scales of the three species. The bold scale represents the median shape for each cluster and light grey scales represent the first standard deviation shape in each cluster.

Figure 5: Stratigraphy of Lac Flévy. (a) Lead-210 dating based on a constant rate of supply (CRS) model; (b) Total accumulation rate for the extracted scales; (c) Accumulation rate of unidentified scales (scales that did not match any morphotypes, common scale morphotypes, damaged scales, etc.); (d–f) Scales matching a specific morphotype of one of the three outbreaking species.

Figure 6: Stratigraphy of Lac Hautbois. (a) Lead-210 dating based on constant rate of supply (CRS) model; (b) Total accumulation rate of extracted scales; (c) Accumulation rate of unidentified scales (scales that did not match any morphotypes, common scale morphotypes, damaged scales, etc.); (d–f) Scales that matched a specific morphotype of one of the three outbreaking species.
Figure 7: Examples of (a) unidentified scales; (b) scales matching a *Choristoneura fumiferana* morphotype; (c) scales matching a *Lambdina fiscellaria* morphotype; (d) scales matching a *Malacosoma disstria* morphotype; (e) a damaged scale.
Scale analysis protocol.

134x142mm (300 x 300 DPI)
Location of the two studied lakes.

157x196mm (300 x 300 DPI)
Discriminant analysis of the three species of outbreaking lepidopterans based on EFDs principal component scores and four scale shape parameters. Colour points represent highly specific shapes and faded points represent more common shapes.

154x107mm (300 x 300 DPI)
Scales morphotypes extract from K-mean cluster analysis of (a) *Choritoneura fumiferana*, (b) *Lambdina fiscellaria*, and (c) *Malacosoma disstria* specific scales and (d) more common scales of the three species. The bold scale represents the median shape for each cluster and light grey scales represent the first standard deviation shape in each cluster.
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153x105mm (300 x 300 DPI)
Examples of (a) unidentified scales; (b) scales matching a Choristoneura fumiferana morphotype; (c) scales matching a Lambdina fiscellaria morphotype; (d) scales matching a Malacosoma disstria morphotype; (e) a damaged scale.

205x490mm (300 x 300 DPI)