Phylogeographic Analysis of the Douglas-fir Beetle *Dendroctonus pseudotsugae* Hopkins (Coleoptera: Curculionidae: Scolytinae)

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Introduction

Population genetic structure studies made in genus *Dendroctonus* have been conducted from the perspectives of allopatric and sympatric models. In the first case, host effect and historical contingency were not recognized as a source of variation, while the later considered the host itself as a source of reproductive isolation. Nevertheless, both models show that genus *Dendroctonus* has the highest values of genetic variation among Scolytidae (Anderson and others 1979; Anderson and others 1983; Higby and Stock 1982; Langor and Spence 1991; Namkoong and others 1979; Roberds and others 1987; Stock and Guenther 1979; Stock and others 1979; Sturgeon and Mitton 1986; Zuñiga and others, in press). In this sense, few studies have been performed dealing with genetic population differences in *D. pseudotsugae*. For instance, through the use of Isozymes produced by 13 gene loci, Stock and others (1979) found a pattern characteristic of populations differentiated to, or beyond, the race or subspecies level between Idaho and Oregon populations. This was a consistent result in later works (Bentz and Stock 1986). However, none of these works performed a population genetic structure analysis.

In a wide sense, classic theory of population genetics was used to describe how mutation, migration, genetic drift, and natural selection affect the distribution of genetic variation (Avise 2004). However, genetic population structure analyses provide us with limited information about historical processes involved in population differentiation. Consequently, a different approach that takes into account these population phenomena is needed to accurately estimate gene genealogies at the population level. The method of Templeton and others (1992) has been used with restriction site and nucleotide sequence data to infer population genealogies when divergence is low. In addition, this method has also been used, coupled with a nested clade procedure, to separate population structure from population history, and in this way, explore the phylogeography of many organisms (Templeton 1995). In this context, there are still few works using nested clade methods to understand phylogeography of bark beetles. Among these are those applied to *Ips typographus* and *Tomicus piniperda* (Ritzerow and others 2004; Stauffer and others 1999). Both species were demonstrated to be highly polymorphic when compared with other european scolytids, and last glaciations had a profound impact on

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their actual distribution ranges (as a result of more restricted host refugees). Another study with the bark beetle *Ips confusus* (Cognato and others 2003) revealed that past glaciation events better explain genetic population structure than isolation by neither the host type nor its past fragmentation. The purpose of this work is to perform a phylogeographic analysis to infer if mitochondrial haplotypes of *D. pseudotsugae* allow us to recognize which historical or demographical factors have been responsible for the actual genetic organization. Taxonomic status of *D. pseudotsugae* subspecies (*D. p. pseudotsugae* and *D. p. barragani*) *sensu* Furniss (2001) will be tested as well.

Methods

Until now, 38 different populations of *D. pseudotsugae* have been sampled from its distribution range in Mexico, United States, and Canada. mtDNA cytochrome oxidase I gene (COI) has been used to resolve relationships among populations due to its extensive intraspecific polymorphism and adequate nucleotide variation (Simon and others 1994). DNA extractions were carried out using DNeasy® Tissue Kit (QIAGEN Gmbh, Hilden, Germany), using whole, ground insect thorax (approximately 10 mg), following manufacturer's tissue protocol. To amplify a fragment of approximately 600bp of COI gene, primers C1-J-2441 and T12-N-3014 (Simon and others 1994) were used via polymerase chain reaction (PCR). Unincorporated dNTP's and oligonucleotides were removed using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Buckinghamshire, UK) and were directly sequenced using the Dye Terminator Cycle® Sequencing Reaction Kit (Perkin Elmer).

To test statements about two different subspecies in *D. pseudotsugae* (Furniss 2001), cladistic analyses of sequences were performed with program PAUP* (Swofford 1998). The trees were generated under a parsimony optimality criterion. A heuristic search of most parsimonious trees with the stepwise addition and tree bisection-reconnection algorithms were performed. All other settings were default. Bootstrap proportions were determined with 500 replicates and all other PAUP* settings were default. To test the null hypothesis of random association between haplotypes and geographical distribution, frequency and spatial distribution were used to create a statistical parsimony network (Templeton and others 1992) using the program TCS (v. 1.21, Clement and others 2000). Haplotypes in network were nested in a hierarchical series of 0-step, 1-step, 2-step, etc., clades until the entire network was nested into a single clade. Resulting nesting network was used to perform statistical test of geographic association of the nested clades with the program GeoDis (v. 2.5, Posada and others 2000). Then, inference keys outlined in Templeton and others (1995) were used.

Results

No nucleotide insertion or deletions were observed. Twenty-seven populations were analyzed (DNA extraction, PCR, sequencing), resulting in a total of 276 sequences, 550bp long. These sequences yielded 73 different haplotypes. It was found that nucleotide divergence (0.039 ± 0.019) and haplotype diversity ($\bar{h}$ 0.945) were higher when compared with those reported for other related species (Cognato and others 2003; Ritzerow and others 2004; Stauffer and others 1999), with the solely exception of *D. valens* ($\bar{h}$ 0.99, Cognato

and others 2005). Only 73 of 550 sites were parsimony informative. In one of the most parsimonious trees, it is clear separation was observed between northern (USA, CAN) and southern (MEX) populations, thus supporting statements about two different subspecies in *D. pseudotsugae* (Furniss 2001) (even though significant homoplasy was found, RC = 0.2146). Nested clade analysis is ongoing.

Future Work

Additional genetic work is planned because of the bias introduced in this study due to a lack of an appropriate sample size (inadequate collection effort). Although a statistical parsimony network was already constructed, it has not yet been used to make inferences about historical or demographic processes. In collaboration with Javier Victor, a total evidence approach (COI sequences + morphological traits) will be implemented in order to test subspecies level among *D. pseudotsugae* populations.

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