



Transcriptome and full-length cDNA resources for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major insect pest of pine forests

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ABSTRACT

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are major insect pests of many woody plants around the world. The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is a significant historical pest of western North American pine forests. It is currently devastating pine forests in western North America – particularly in British Columbia, Canada – and is beginning to expand its host range eastward into the Canadian boreal forest, which extends to the Atlantic coast of North America. Limited genomic resources are available for this and other bark beetle pests, restricting the use of genomics-based information to help monitor, predict, and manage the spread of these insects. To overcome these limitations, we generated comprehensive transcriptome resources from fourteen full-length enriched cDNA libraries through paired-end Sanger sequencing of 100,000 cDNA clones, and single-end Roche 454 pyrosequencing of three of these cDNA libraries. Hybrid *de novo* assembly of the 3.4 million sequences resulted in 20,571 isotigs in 14,410 isogroups and 246,848 singletons. In addition, over 2300 non-redundant full-length cDNA clones putatively containing complete open reading frames, including 47 cytochrome P450s, were sequenced fully to high quality. This first large-scale genomics resource for bark beetles provides the relevant sequence information for gene discovery; functional and population genomics; comparative analyses; and for future efforts to annotate the MPB genome. These resources permit the study of this beetle at the molecular level and will inform research in other *Dendroctonus* spp. and more generally in the Curculionidae and other Coleoptera.

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1. Introduction

The mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) is an endemic insect pest of western North American pine

forests, from Mexico to central British Columbia. It has had a long recorded history of major outbreaks both within Canada and the United States and attacks many species of pine (*Pinus* spp.) (Safranyik et al., 2010). The current epidemic in western Canada far exceeds the scope of any previously recorded bark beetle outbreak. Over 15 million hectares of pine forests, predominantly lodgepole pine (*Pinus contorta*), have been infested in British Columbia alone (Safranyik et al., 2010). In recent years, MPB has been observed attacking pine trees east of the northern Canadian Rockies, which was previously thought to be an effective geographical barrier. The new host range now includes pine species not previously encountered by the MPB, particularly jack pine (*Pinus banksiana*) and its hybrid with lodgepole pine (Cullingham et al., 2011). As Canada's boreal forest extends across the North American continent to the Atlantic coast, the potential for this beetle to spread further eastward is of major ecological, environmental and economic concern

Abbreviations: FLcDNA, full-length cDNA; GO, gene ontology; JH III, juvenile hormone III; KK-FSR, Kay Kay Forest Service Road; MPB, mountain pine beetle; P450, cytochrome P450 monooxygenase; ORF, open reading frame; UTR, untranslated region.

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(Kurz et al., 2008a; Safranyik et al., 2010). MPB is not endemic to Canada's boreal forest and thus the impact on this biome is not known. For instance, the potential for increased susceptibility of jack pine to MPB attack in the presence of pre-existing insect pests such as the defoliator jack pine budworm (*Choristoneura pinus pinus* Freeman), or vice versa, may pose a substantial additional risk for forest management (Colgan and Erbilgin, 2010). In addition, where population densities in British Columbia are high and suitable pine hosts are limited, MPB has also been observed successfully reproducing in spruce, which is not normally considered a viable host for this beetle (Huber et al., 2009).

Climate change is contributing to the present MPB epidemic and its current and expected host range expansion. Despite some uncertainty in underlying predictions, the area of climatically suitable habitat for MPB is expected to expand northward due to increasing temperatures and will overlap with at least the southern margins of the distribution of suitable pine hosts in the boreal forest (Safranyik et al., 2010). The severity of outbreaks of other bark beetles can also be associated with climate change (Sherriff et al., 2011). Conversely, large-scale insect infestations of forests can impact atmospheric carbon dynamics and ultimately feed back to climate change. Due to the impact of MPB infestations on the death and decay of forest trees, which normally serve as carbon sinks, these forests can become significant sources of carbon emissions exceeding some of the major anthropogenic sources (Safranyik et al., 2010). These scenarios of the MPB epidemic highlight the effects of climate change on expansion of an insect's host range, which may further magnify the change in climate through changes of the carbon sequestration balance in forest environments (Kurz et al., 2008a, 2008b).

The biology and life cycle of the MPB has been extensively studied and is well described (Safranyik and Carroll, 2006). Apart from a brief dispersal period of mature adults in late summer to find a new host tree and mate, all life stages remain under the bark of host trees. Upon emerging from their natal tree, mature adults disperse and use pheromones and host- and non-host volatiles to locate a suitable pine host and mate. The female beetle is the pioneering sex and upon feeding on a new host tree releases the sex-specific aggregation pheromone *trans*-verbenol, which synergizes with the host volatile myrcene to attract conspecifics. Once colonization has begun, additional beetles are recruited to the host tree by the male-produced aggregation pheromone *exo*-brevicomin. The pheromone-mediated mass-attack rapidly overcomes the constitutive chemical defences of the pine host, which are principally phenolic and terpenoid compounds (Franceschi et al., 2005; Keeling and Bohlmann, 2006a, 2006b). As the mass-attack increases, intraspecific competition is minimized by the anti-aggregation pheromone verbenone and the multi-functional male-produced pheromones frontalin and *exo*-brevicomin. Mated females oviposit on the sides of vertical galleries bored into the phloem, and upon hatching, the developing larvae feed on the phloem horizontally around the circumference of the tree away from the vertical parental gallery. While feeding, the larvae must contend with the substantial remaining host pine defence compounds (Clark, E.L., personal observation). MPB overwinter as larvae and considerable mortality occurs during the winter in this life stage, especially if an early cold spell catches the larvae before they have fully acclimatized to the decreasing temperatures. Larvae begin feeding again in the spring and then pupate in early summer. Adults eclose later in the summer and the teneral adults remain under the bark for a few weeks to mature prior to dispersal. In most cases MPB has one generation per year but warmer-than-average summer temperatures can result in parent adults re-emerging from one host and potentially producing a second brood in another host tree. Where summer temperatures are cooler, such as

at higher elevations, and potentially at higher latitudes, brood may take two years to mature (Amman, 1973).

MPB is successful in quickly killing pine host trees by a combination of pheromone-mediated mass attack of individual trees to overcome the host tree defences and by vectoring symbiotic fungi, some of which are pathogenic to the pine trees, such as the ascomycete *Grossmannia clavigera* (Lee et al., 2006). These fungi invade the sapwood of the tree, effectively blocking water transport within the tree, and may also detoxify host defences, making the tree more suitable for beetle colonization. Whether fungi play a critical role in initially overwhelming the tree defences and killing the tree has recently been reviewed (Six and Wingfield, 2011). In order to better understand the multi-organismal processes of this expanding epidemic, large-scale transcriptome resources (DiGuistini et al., 2007; Hesse-Orce et al., 2010) and a reference genome sequence (DiGuistini et al., 2009, 2011) have been developed for *G. clavigera* and have been applied in both functional and population genomics studies (DiGuistini et al., 2011; Tsui et al., 2009) as well as for the genomic delineation of fungal species (Alamouti et al., 2011). Likewise, the mechanisms by which trees defend themselves from insect and fungal attack are important for understanding this insect-pathogen-tree system (Keeling and Bohlmann, 2006b). Transcriptome resources have been developed for both the lodgepole pine (*P. contorta*) and jack pine (*P. banksiana*) host tree species for the characterization of defence mechanisms in both species (unpublished results and (Parchman et al., 2010)).

Despite being by far the most species-rich order of insects, Coleoptera (beetles) is underrepresented in the available arthropod sequence information. To date, the genome sequence of only one beetle species has been published, the red flour beetle (*Tribolium castaneum*) (Richards et al., 2008). The majority of available coleopteran sequences at NCBI dbEST are from this one species. A few coleopteran species have recently been targeted with next-generation sequencing technologies (e.g. Mittapalli et al., 2010; Pauchet et al., 2010). However, NCBI dbEST contains sequences for only three species of bark beetles (family Curculionidae, subfamily Scolytinae), the pine engraver beetle (*Ips pini*) (Eigenheer et al., 2003), the coffee berry borer (*Hypothenemus hampei*) and the MPB (Aw et al., 2010). Developing a comprehensive resource of sequence information in bark beetles is essential to better understand the biology of these ecologically and economically important pests and their impact on the sustainability of natural resources in forestry and agriculture around the world. The present research, together with a previous smaller-scale MPB EST analysis (Aw et al., 2010), contributed substantial new transcriptome sequences to the available sequence data for Coleoptera at NCBI.

With an emphasis on sequencing tissues believed to have direct involvement with the processes of cold tolerance and host colonization, including olfaction, pheromone biosynthesis, and the detoxification of host defence compounds, we have generated an extensive transcriptome resource for the MPB. We completed both traditional Sanger sequencing for long sequences of excellent quality, which also resulted in physical cDNA clones of many genes, often containing the full-length ORF, as well as 454 pyrosequencing for deep coverage of antenna and midgut/fat body tissues. In addition, we sequenced over 2300 cDNA clones fully to high quality that are putatively full-length. That is, these cDNA clones each contain a putatively full-length open reading frame, 3'-UTR sequence, and at least some 5'-UTR sequence. These sequences (publically available on NCBI) provide a critical dataset of genes for genome annotation, and population, comparative, and functional genomics studies of the MPB. As cytochrome P450 enzymes are expected to have important roles in the metabolism of host defence compounds as well as in pheromone biosynthesis, we targeted the MPB P450 gene family for deeper analysis.

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