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REVIEW

The contribution of genetics and genomics to understanding the ecology of the mountain pine beetle system[1]

Catherine I. Cullingham, Jasmine K. Janes, Richard C. Hamelin, Patrick M.A. James, Brent W. Murray, and Felix A.H. Sperling

Abstract: Environmental change is altering forest insect dynamics worldwide. As these systems change, they pose significant ecological, social, and economic risk through, for example, the loss of valuable habitat, green space, and timber. Our understanding of such systems is often limited by the complexity of multiple interacting taxa. As a consequence, studies assessing the ecology, physiology, and genomics of each key organism in such systems are increasingly important for developing appropriate management strategies. Here we summarize the genetic and genomic contributions made by the TRIA project — a long-term study of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) system encompassing beetle, fungi, and pine. Contributions include genetic and genomic resources for species identification, sex determination, detection of selection, functional genetic analysis, mating system confirmation, hybrid stability tests, and integrated genetic studies of multiple taxa. These resources and subsequent findings have accelerated our understanding of the mountain pine beetle system, facilitating improved management strategies (e.g., enhancements to stand susceptibility indices and predictive models) and highlighting mechanisms for promoting resilient forests. Further, work from the TRIA project serves as a model for the increasing number and severity of invasive and native forest insect outbreaks globally (e.g., Dutch elm disease and thousand cankers disease).

Key words: Dendroctonus, *Grosmannia*, *Leptographium*, *Ophiostoma*, *Pinus*, mountain pine beetle, forest management, genomics, population genetics.

Résumé : Les changements environnementaux modifient la dynamique des insectes forestiers à travers le monde. En changeant, ces systèmes posent des risques écologiques, sociaux et économiques significatifs par, à titre d'exemple, la perte d'habitats précieux, d'espaces verts et de bois. Notre compréhension de ces systèmes est souvent limitée par la complexité des interactions multiples entre taxons. Par conséquent, les études permettant d'évaluer l'écologie, la physiologie et la génomique de chaque organisme clé dans ces systèmes deviennent de plus en plus importantes pour développer des stratégies de gestion appropriées. Dans cet article, nous résumons les contributions d'ordre génétique et génomique du projet TRIA — une étude à long terme du système du dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopkins) comprenant un scolyte, des champignons et le pin. Les contributions incluent les ressources génétiques et génomiques pour l'identification des espèces, la détermination du sexe, la détection de la sélection, l'analyse génétique fonctionnelle, la confirmation des systèmes d'accouplement, les tests de stabilité des hybrides et les études génétiques intégrées de taxons multiples. Ces ressources et les découvertes subséquentes ont accéléré notre compréhension du système du dendroctone du pin ponderosa, ont facilité l'amélioration des stratégies de gestion (p. ex. des améliorations des indices de vulnérabilité des peuplements et des modèles prédictifs) et ont mis en lumière les mécanismes qui favorisent la résilience des forêts. De plus, les travaux du projet TRIA servent de modèle pour le nombre croissant et la sévérité accrue des épidémies d'insectes forestiers indigènes et envahissants globalement (p. ex. maladie hollandaise de l'orme et maladie des mille chancres du noyer). [Traduit par la Rédaction]

Mots-clés : Dendroctonus, *Grosmannia*, *Leptographium*, *Ophiostoma*, *Pinus*, dendroctone du pin ponderosa, gestion des forêts, génomique, génétique des populations.

Introduction

Management of forest insects is increasingly necessary as climate and forest management practices continue to change. Recent years have seen worldwide increases in the extent and intensity of outbreaks of forest insects (Logan et al. 2003; Lovett et al. 2006; Gauthier et al. 2014), and several species have increased their ranges (e.g., Cullingham et al. 2011). These outbreaks have typically resulted in widespread loss of valuable forest re-

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C.I. Cullingham* **and F.A.H. Sperling.** Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada.
J.K. Janes.* Biology Department, Vancouver Island University, Nanaimo, BC V9R 5W4, Canada; School of Environmental and Rural Sciences, University of New England, Armidale, NSW, Australia, 2351.
R.C. Hamelin. Department of Forest and Conservation Science, University of British Columbia, Vancouver, BC V6T 1Z4, Canada.
P.M.A. James. Département de sciences biologiques, Université du Montréal, Montréal, QC H3C 3J7, Canada.
B.W. Murray. Natural Resources and Environmental Studies Institute, University of Northern British Columbia, Prince George, BC V2N 4Z9, Canada.
Corresponding author: Catherine Cullingham (email: cathy.cullingham@ualberta.ca).
*These authors equally share first authorship.

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sources and altered ecosystem dynamics (Cooke and Carroll 2017), although a few positive impacts have been experienced in some areas (i.e., increases in real estate value and grazing area; Morris et al. 2018). A better understanding of the ecological and evolutionary dynamics of forest insect systems, prediction tools, and management strategies are essential to mitigating the negative impacts of insect outbreaks.

Genetic and genomic methods provide a means of answering questions that can be applied to forest management. For example, forest pathogens are often small cryptic organisms that are difficult to observe and identify. Using genetic and genomic tools, we can reliably identify species (Roe et al. 2010), characterize population structure to understand outbreak patterns (Samarasekera et al. 2012) and dispersal capacities (Janes et al. 2016), and identify adaptive variation, all of which can help us better understand pathogenicity and future outbreak potential (Janes et al. 2014; Ojeda et al. 2017). Genetic factors may contribute to resiliency in forest trees (Raffa 1989). Thus, hybrid genotypes may be an important bridge for pathogens to adapt to new species (Stukenbrock 2016) and provide new genetic combinations resulting in adaptive variation (Lewontin and Birch 1966). By genetically identifying hybrids and population-level genetic variation, we can better understand spatial variation in genetic resilience across the landscape. Information on host susceptibility is of paramount importance to safeguarding resilient populations for the future.

Understanding the mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins) system continues to benefit from long-term research (Negrón and Fettig 2014). This species recently increased its geographic (Samarasekera et al. 2012) and host (Cullingham et al. 2011) ranges and duration of outbreak (Aukema et al. 2008). Native to western North America, MPB's traditional range extends from Mexico to southwestern Canada and as far east as Colorado and South Dakota in the United States (US) (Wood 1982). Specifically, the historic range occupied 12 US states (Arizona, California, Colorado, Idaho, Montana, New Mexico, Nevada, Oregon, South Dakota, Utah, Washington, Wyoming) and three Canadian provinces (Alberta, British Columbia, Saskatchewan), with recent range expansion in the US (Nebraska; Costello and Schaupp 2011) and Canada (Alberta and northern British Columbia) (Cullingham et al. 2011). It colonizes several pine species, including lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. ex S. Watson), sugar pine (*Pinus lambertiana* Dougl.), western white pine (*Pinus monticola* Dougl. ex D. Don), ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson) (Safranyik and Carroll 2006), and, more recently, jack pine (*Pinus banksiana* Lamb.) (Cullingham et al. 2011).

Mountain pine beetle spends much of its life cycle under the bark of its host tree. The species exhibits sexual dimorphism; females are larger (Safranyik and Carroll 2006), typically having more lipids to fuel their pioneering dispersal flight in the summer (Evenden et al. 2014). Once females locate a suitable host tree, they emit a sex-specific aggregation pheromone to attract more MPB (Chiu et al. 2018). If successful in overcoming the tree's defences, beetles begin constructing breeding galleries (Safranyik and Carroll 2006). Larvae overwinter in three instar stages before emerging the following summer as adults (Safranyik and Wilson 2006). Typically, MPB are univoltine, surviving just one year, although some populations exhibit a semivoltine life cycle (Bentz et al. 2014). Bivoltinism, in the strict definition, is not considered likely in the current MPB range but may become possible under warmer climatic conditions (Bentz et al. 2014; Bentz and Powell 2014).

Mountain pine beetle is just one player in a complex system. Blue stain fungi (Ophiostomatales, Ascomycota) are symbiotic partners of several bark beetles, including MPB (Six and Wingfield 2011). These fungi benefit the beetle in two ways: (*i*) provision of supplementary nutrition and (*ii*) overcoming tree defences and modifying host tissues to favour brood development (Raffa and Berryman 1983; Paine et al. 1997). To date, three fungal species have been identified as common associates across most of the MPB range: *Grosmannia clavigera* ((Robinson-Jeffrey and Davidson) Zipfel, de Beer and Wingfield), *Leptographium longiclavatum* (Lee, Kim and Breuil), and *Ophiostoma montium* ((Rumbold) von Arx.) (Tsui et al. 2012). The ecology of these fungal partners suggests that they have different niches and pathogenicity.

Historically, MPB has averaged a population outbreak every 20 years, with each one lasting roughly 5 years (Safranyik and Wilson 2006). The most recent Canadian outbreak began in the late 1990s (Aukema et al. 2006), resulting in significant tree mortality (~50% of the merchantable pine volume in British Columbia (BC) was affected) (Walton 2012). The scale of this outbreak can be attributed to interacting factors of climate, forest management, and the distribution of suitable host trees (Taylor et al. 2006; Régnière and Bentz 2007), resulting in expansions in range (Carroll et al. 2003) and host (Cullingham et al. 2011). Such changes in the MPB system necessitate new research to better understand how MPB and its fungal symbionts will behave in a new environment.

The TRIA project (http://tria-net.srv.ualberta.ca/) was developed to establish a better understanding of the MPB system, focusing on informing forestry management and industry. It was named TRIA to represent the primary interacting organisms (tree, beetle, and fungi), but also three aspects — physiology, genomics, and ecology. Over 12 years, the project has examined several key species in the system and their interactions using physiology, field and lab experiments, modelling, and population genetics and genomics. In this synthesis, we summarize the contributions of the genetics and genomics components of the project, demonstrating how these studies have increased our knowledge of the system and contributed directly to forest management. We then suggest potential directions for future study into other relevant forest insect systems.

Developing genetic and genomic resources

To benefit from questions that can be addressed with genomic and genetic methods, we need access to appropriate resources and genetic markers. Prior to the TRIA project, there were few resources available for any of the species in the system. Here we briefly introduce the markers sets developed (summarized in Table 1), and in later sections, we highlight the questions that have been addressed using them.

Across the system, numerous genetic and genomic resources were developed under the TRIA project, including sequence data, microsatellites, single nucleotide polymorphisms (SNPs), reference genomes, and transcriptional data. Fungal genetic resources initially focused on sequence data (i.e., ITS) for reliable species identification (Roe et al. 2010; Alamouti et al. 2011). In contrast, work on pine and MPB relied on microsatellites. A set of 16 microsatellites was developed, including one sex-determining marker (Davis et al. 2009), which proved valuable in assessing population structure (Samarasekera et al. 2012) and in identifying sex ratio in MPB larval samples (James et al. 2016). Additional microsatellite markers, potentially linked to expressed genes, were developed later (Agata et al. 2011). For lodgepole and jack pine, we developed the first microsatellite markers to reliably amplify in both species (Cullingham et al. 2011), facilitating comparisons of genetic diversity. Greater genomic coverage, however, was necessary to address increasingly complex questions.

A draft MPB genome (Keeling et al. 2013) and a genomic reference for *G. clavigera* (DiGuistini et al. 2009, 2011) were provided through the TRIA project, and SNP panels were developed from these resources. These data facilitated studies of genetic (Batista et al. 2016) and adaptive population structure both in MPB (Janes et al. 2014) and in *G. clavigera*, *L. longiclavatum*, and *O. montium* fungi (Ojeda et al. 2014, 2017). As the genomes for pine are large and complex, transcript data were used to develop a panel of SNP

Table 1. Summary of genetic and genomic resources developed by the TRIA-Net project.

Species	Marker type	*N**	Application	Reference
Fungal associates†	DNA barcoding	4	Species identification	Roe et al. 2010
Grosmannia clavigera	Genomic	Draft genome	Resource development	DiGuistini et al. 2009, 2011
	Microsatellite	8	Population structure	Tsui et al. 2009
	SNPs	129	Identify adaptive variation	Ojeda et al. 2017
Leptographium longiclavatum	Microsatellite	6	Population structure	Tsui et al. 2009
	SNPs	147	Identify adaptive variation	Ojeda et al. 2014
Ophiostoma montium	SNPs	59	Identify adaptive variation	Ojeda et al. 2017
Lodgepole–jack pine	Microsatellite	11	Species discriminating	Cullingham et al. 2011
	SNPs	399	Population structure, identify adaptive variation	Cullingham et al. 2013*a*
	SNPs	25	Species discriminating	Cullingham et al. 2013*b*
Mountain pine beetle	Microsatellite	16	Population structure, sexing	Davis et al. 2009
	Genomic	Draft genome	Resource development	Keeling et al. 2013
	SNPs	764	Population structure, identify adaptive variation	Janes et al. 2014
	Microsatellite	48	Identify adaptive variation	Agata et al. 2011

*Number of loci, where applicable.
†Includes *G. clavigera*, *L. Longiclavatum*, and *O. montium*.

markers to assess potential adaptive differences (Cullingham et al. 2013*a*). These data also allowed development of a species-discriminating SNP panel to identify hybrids of lodgepole and jack pine (Cullingham et al. 2013*b*).

Species identification

One of the indirect, yet necessary, outcomes of the TRIA project has been the use of genetic and genomic resources to identify associated host and symbiont species. To better understand the biology of the MPB system, it has been essential to identify key species in the system, particularly for taxonomically challenging species such as fungi. For example, it is estimated that only 3%–8% of fungal species have been described in spite of estimates of global fungal diversity in the range of 1.5–3.8 million species (Hawksworth et al. 2017). This is primarily because morphological assessments of fungi are problematic; many diagnostic characters prove cryptic or absent at the time of collection, often leading to mis-identifications (Feau et al. 2009).

Recently, DNA barcoding using the internal transcribed spacer regions 1 and 2 (ITS) has become popular for fungal identification (Schoch et al. 2012; Batovska et al. 2017); however, Roe et al. (2010) showed that five independent loci varied in their efficacy for species identification across six closely related fungal species (Ophiostomataceae) in the MPB system. Further study demonstrated that ITS consistently failed to delimit closely related species (Roe et al. 2010), a pattern also found in 21 similar studies on different taxa. In contrast, multi-marker approaches provided greater resolution of species (Roe et al. 2010). In spite of the early warnings by Roe et al. (2010), the broader fungal community has been slow to change and this lack of ITS resolution can still create problems (Hawksworth et al. 2017). The consequences of such taxonomic confusion include artificial inflation or deflation of "species", followed by a lack of understanding about the ecology of functional groups or guilds, their geographic distribution, and the evolution and extent of pathogenicity and (or) symbiotic relationships in certain groups.

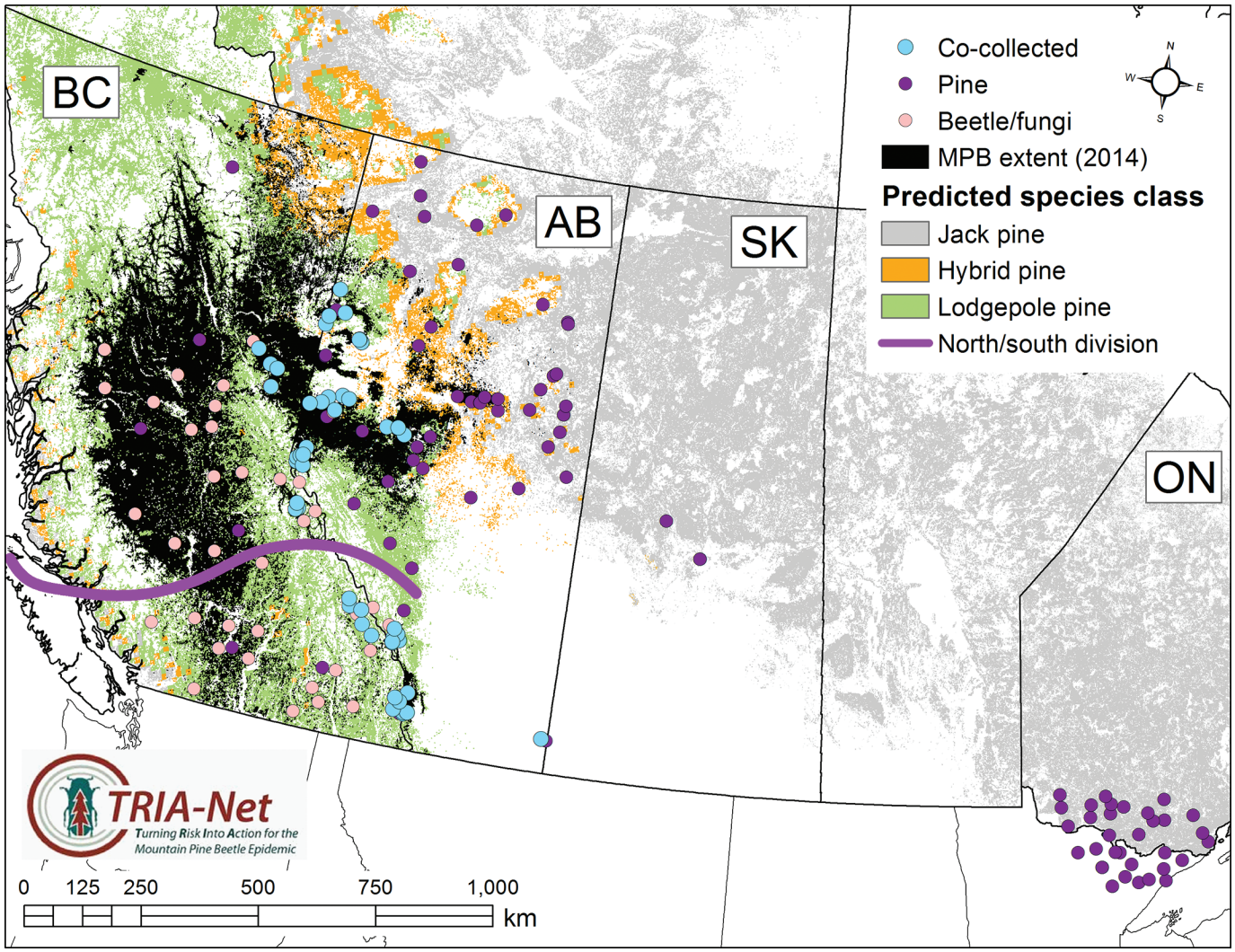
To further identify and delimit cryptic species, Alamouti et al. (2011) took advantage of the whole genome of *G. clavigera*. They sequenced 15 genomic regions from numerous *G. clavigera* using a phylogenetic concordance species concept approach (Taylor et al. 2000) to explore the presence of distinct phylogenetic subspecies. Two closely related fungal species were discovered and found to have different adaptations. The first species associates exclusively with MPB, lodgepole pine, and several other pine species. The second is associated with the MPB sister species *Dendroctonus jeffreyi* Hopkins, 1909 (Jeffrey pine beetle) and is found on Jeffery pine (*Pinus jeffreyi* Balf.) and ponderosa pine. Divergence of the two fungal taxa is likely the result of adaptation to the pine hosts, particularly to the defence compounds produced during beetle attacks. Genome sequencing and transcriptome analyses revealed that *G. clavigera* detoxifies tree defence compounds (e.g., terpenoids), using them as carbon sources (DiGuistini et al. 2011). Indeed, fungi carried by *D. jeffreyi* were more tolerant to oleoresin produced by Jeffrey pine than those from MPB. Cryptic host speciation in which fungal lineages adapt to different tree defence compounds could be important in range expansion. Monitoring the fungal complex during expansion to the east and north where naïve species such as jack pine are present will be important (Cullingham et al. 2011).

Expansion of MPB into lodgepole pine forests in Alberta (AB) in 2005 raised the question "Are nearby jack pine stands susceptible?" The range of lodgepole pine extends into central AB, where it overlaps with jack pine, creating a hybrid zone (Pollack and Dancik 1985). It was predicted that MPB would attack jack pine (Cerezke 1995), but the timely identification of either hybrids or jack pine attacked by MPB was critically missing for management. Identification of pure pine species versus hybrids was not reliable using methods including morphology, protein polymorphisms, and chemical profiles (Zavarin et al. 1969; Pollack and Dancik 1985; Rweyongeza et al. 2007; Yang et al. 2007). Using microsatellite markers, Cullingham et al. (2011) were able to confirm susceptibility of both hybrid and pure jack pine to MPB, facilitating a redefined potential host range for MPB that encompasses much of Canada and necessitating new research into the susceptibility of jack pine to MPB. For example, a number of researchers have investigated how jack pine will respond to beetle attack through the development of new genomic resources for lodgepole and jack pine (Hall et al. 2013), as well as studies investigating differences between pine species' chemical (e.g., Taft et al. 2015) and gene expression (Arango-Velez et al. 2014, 2016) profiles.

Phylogeography

Phylogeographic insights provide information on historical demographic and geographic patterns, allowing some evolutionary mechanisms to be inferred. Such studies have been used to identify source populations of invasive species (e.g., Kerdelhué et al. 2014), delineate conservation management areas (e.g., Haché et al. 2017), and predict range limits (e.g., Winkler et al. 2012); however, the application of phylogeographic studies in irruptive forest insect species has been questioned. This is primarily because fundamental niche spaces may be obscured by low within-species signatures of genetic diversity as a result of constant population growth and decline (Godefroid et al. 2016). Work from the TRIA project has shown that phylogeographic approaches can be used to better understand MPB range expansion dynamics but that they may have limited utility for identifying specific source

Fig. 1. Distribution of the most recent mountain pine beetle outbreak (as of 2014) and samples used during the TRIA-Net project. The north–south population structure observed in both beetle and fungi is indicated for reference. Sites where beetle, tree, and fungi were collected together are indicated by the "co-collected" sites. The pine species classes are predicted based on Burns et al. (2019). Mountain pine beetle data were obtained from Alberta Department of Agriculture and Forestry and British Columbia Ministry of Forests, Lands, and Natural Resources (http://www.for.gov.bc.ca/ftp/HFP/external/!publish/).



populations. For example, MPB patterns were consistent with isolation-by-distance at a continental scale, but more recent expansion signals and single-source populations could not be identified (Cullingham et al. 2012*a*). More recent studies using genome-wide SNPs (Dowle et al. 2017; Janes et al. 2018) have found similar patterns, demonstrating that both single- and multi-marker phylogeographic inferences have limitations in irruptive forest insect species.

In contrast, phylogeographic methods proved particularly useful in identifying variation in fungal symbionts. Multi-microsymbiont comparative phylogeography identified common historical factors that explain current distribution, relationships, and genetic structure for the three major fungal species. Roe et al. (2011*a*) identified landscape-scale population structuring, resulting in a north–south division for the fungal symbionts (Fig. 1). These patterns were attributed to historical outbreaks that saw MPB move into south-central BC and southern AB (Powell 1961), providing sufficient time for populations to differentiate. At a finer geographic scale, distinct differences were also observed. *Grosmannia clavigera* and *L. longiclavatum* had similar population structure and genetic diversity, whereas *O. montium* exhibited higher levels of genetic diversity (Roe et al. 2011*a*). These differences were attributed to modes of transmission: *G. clavigera* and *L. longiclavatum* are transported preferentially in MPB mycangia, whereas *O. montium* can be transported in mycangia or on the exoskeleton (Six et al. 2003). Thus, a greater diversity of *O. montium* is transmitted relative to other fungal species. These genetic data illustrated previously unknown variation in fungal symbionts that could affect MPB fitness (Roe et al. 2011*a*). For example, different fungi may have varying nutritional value and pathogenicity. Therefore, differential transport of fungal symbionts may impact fungal growth, tree mortality, and ultimately MPB survivorship. This information helps us disentangle some ecological complexity in the MPB system and may have future management implications for tree genetic resistance or antifungal applications by targeting the fungus that is most important to the beetles.

Population genetic structure

Using microsatellite markers developed for MPB (Davis et al. 2009), Samarasekera et al. (2012) analyzed MPB population genetic structure throughout BC and AB at the peak of the BC outbreak.

They found evidence for two population clusters in BC, a widespread northern cluster centered in the Fraser Plateau in the Central Interior and a southern cluster ranging from the South Coast to the Kootenays (Fig. 1). Populations with mixed ancestry were noted along the interface of the geological divide (e.g., Lillooet, Lac la Hache, and Valemount). Overall, the northern cluster exhibited lower genetic diversity, consistent with predicted northern postglacial expansion from southern glacial refugium. Conversely, southern populations showed patterns consistent with previous spatiotemporal modeling of outbreaks in southern BC (Aukema et al. 2006), including isolation-by-distance indicative of long-term habitation in the area and multiple contemporary outbreaks in southern BC at the time. Further, these patterns have been confirmed by studies using different genetic markers and (or) an expanded range of samples (e.g., Janes et al. 2014; Batista et al. 2016). These results highlight two major implications: (*i*) warming climates can affect MPB outbreak dynamics, facilitating synchronized independent outbreaks, and (*ii*) contrasting patterns of population structure can be used to infer dispersal capacity and source populations for outbreaks.

Batista et al. (2016) identified additional population structure using a panel of SNPs subdivided into neutral and potentially adaptive sets. Evidence for four genetics clusters was shown from a collection of 62 sampling locations throughout the species range in western North America. This included a northern and southern Canadian cluster (similar to Samarasekera et al. 2012), as well as a US west coast cluster and an eastern cluster (South Dakota, Colorado, and Arizona). Similar genetic structure has been noted in an independent analysis of SNP variation (Dowle et al. 2017).

Understanding population structure in fungal pathogens is important because it can reveal epidemiological patterns not seen in incidence data (Milgroom and Fry 1997). Rates of migration and gene flow, sexual reproduction, population size, and demographic events such as population bottlenecks can be estimated using population genetics approaches. As fungi associated with MPB appear to be dependent on beetles for large-scale dissemination and host colonization, the genetic structure of fungal associates were expected to mirror that of the beetle with clear north–south clusters (Samarasekera et al. 2012). Correlation among genetic distance matrices of the fungal associates and the MPB and the presence of similar genetic clusters in fungal associates supported this hypothesis and the theory of multiple infection foci (Aukema et al. 2006; Tsui et al. 2012, 2014). Overall, fungus population structure was consistent with that of MPB, supporting evidence of MPB migration via the northern Rocky Mountains (Samarasekera et al. 2012). A high level of gene flow was also observed across fungal populations separated by hundreds of kilometres, a reflection that fungi benefit from long-distance transport by the beetle.

Another intriguing application of genetic structure and relatedness estimates is genetic tracking of beetles (Trevoy et al. 2018). The ability to "track" certain population signatures on the landscape could help refine spread risk models by better informing dispersal kernel estimates. An advantage may come from keeping abreast of the level of genetic diversity present in the species. Genetic diversity provides the basis for adaptive potential, thus identifying regions of high diversity may help to target management activities to these areas when resources are limited.

Mating systems and sex skew

Mating system and fine-scale spatial genetic structure assessments can be useful in understanding genealogical relationships and demographic history. The mating system of MPB was confirmed using molecular methods (Janes et al. 2016) as observations of natural mating behaviour were limited by a life cycle mostly completed under the bark of trees. Parentage data proved that individuals are polygamous and supported previous observations (e.g., Safranyik and Wilson 2006; Bleiker et al. 2013) that approximately 5% of females will be mated by male siblings prior to dispersal. Further, Janes et al. (2016) highlighted the extent to which MPB can disperse, as individuals at the target population were found to be most closely related to a population ~700 km away (Janes et al. 2016). The level of gene flow, as a result of continued long-distance migration and polygamy, prevented fine-scale genetic structure from developing. These findings indicate that populations separated up to a few thousand kilometres can be similar and therefore respond similarly to management, provided that other factors are equivalent. However, for predictive modelling, the highly dispersive nature of MPB over long distances complicates estimates of spread risk and founding population size (Goodsman and Lewis 2016; Powell et al. 2018).

Understanding sex ratio in insect populations is essential for forecasting population dynamics. This is especially true for MPB, which exhibits significant departure from parity during outbreaks. Previous work suggested that spatial differences in sex ratio respond to tree diameter heterogeneity and "outbreak age" (Amman and Cole 1983). James et al. (2016) tested the relationships between spatial heterogeneity and MPB sex ratio using molecular sexing data from Samarasekera et al. (2012). They also tested the role of weather and climate on sex-ratio skew, finding that tree diameter, year of outbreak, and weather all influenced sex-ratio skew. However, factors determining skew differed between adult and larval beetles. On the basis of these differences, James et al. (2016) were able to infer that sex-ratio skew arises early during development through differential mortality of males. Under- or over-estimating brood production and population growth may bias forecasting models and subsequent expectations of outbreak dynamics; the findings of James et al. (2016) help refine the commonly used 2:1 sex ratio for calculating stand susceptibility (Shore and Safranyik 1992).

The ability to reproduce sexually is an important aspect of fungal pathogen epidemics; fungi with mixed mating systems (reproducing asexually and sexually) have a higher adaptive potential (McDonald and Linde 2002). Heterothallism (two distinct mating types for sexual reproduction) was discovered by sequencing the genome of one fungal associate, *G. clavigera* (DiGuistini et al. 2011). To understand how frequent sexual reproduction is among fungal associates, the level of clonality was analyzed among the fungal associates of MPB and was found to be remarkably low in species in which sexual stage is rarely observed (Ojeda et al. 2017). In fact, most sampled populations of fungal associates were at linkage equilibrium and the mating type genes were in a 1:1 ratio, indicating that sexual reproduction, and the generation of new genotypic variants, is frequent (Ojeda et al. 2017; Tsui et al. 2013). The emerging picture is that fungal associates have the ability to migrate, recombine, and expand, aided by their beetle vector, providing greater potential for adaptation and colonization of new habitats.

Spatial community structure

Studies assessing spatial variation and fundamental niches of MPB-associated fungi have revealed interesting patterns. For example, both *G. clavigera* and *O. montium* are considered relatively common in southern areas, although *G. clavigera* tolerates lower temperatures than *O. montium* (Six and Bentz 2007) in southern populations. In contrast, *L. longiclavatum* was considered rare in these southern areas (Lee et al. 2006). However, the majority of this work was conducted in the traditional range of MPB. As MPB expanded into more northern and eastern parts of Canada, opportunities to better assess ectosymbiont spatiotemporal patterns in response to different climates, host tree physiology, and potentially even insect host tree preference may have arisen. While Roe et al. (2011*b*) found that fungal communities maintained the broad north–south clustering found in MPB (Fig. 1), new patterns emerged with the continued expansion. *Grosmannia clavigera* is

increasingly replaced with *L. longiclavatum* in northern latitudes, supporting previous findings of differential climatic tolerances (Rice and Langor 2009) but also suggesting competitive exclusion as these two species rarely co-occur, despite some overlap in optimal conditions (Roe et al. 2011*b*). Interestingly, co-occurrence of all three species is possible. Roe et al. (2011*b*) suggested that the inclusion of the physiologically dissimilar *O. montium* forces each species to narrow its realized niche as a result of high local competition. Thus, this hypothetical re-shuffling of niche space, in conjunction with dispersal and repeated re-colonization, may facilitate the co-existence of each species (Roe et al. 2011*b*). This research serves to highlight the complexity in the MPB system. As MPB continues to disperse over long distances in consecutive outbreak waves, it likely introduces different strains of fungi that could change community composition over time as a result of gene flow and local adaptation. Thus, to better predict and understand MPB dynamics, it is essential to remain abreast of initial variation in ectosymbiont associations and their changes over time.

Lodgepole and jack pine hybridize in AB and, potentially, in the Northwest Territories, but the distribution of this hybrid zone had not been well characterized despite numerous attempts (e.g., Wheeler and Guries 1987; Rweyongeza et al. 2007; Yang et al. 2007). Having an accurate species distribution map of each species, particularly hybrids, is important both for general forest management and for assessing MPB spread risk. Recent research demonstrated that these species and their hybrids have different responses to MPB and its fungal associates (Lusebrink et al. 2013; Clark et al. 2014) and therefore will likely have different outbreak potentials. Developing a high-resolution distribution map is challenging, however, due to the spatial extent of species' ranges, morphology-based identification issues, and the cost of genotyping. However, these species show different ecological adaptations (Kenkel et al. 1997; Carlson et al. 1999; Yang et al. 1999), thus Cullingham et al. (2012*b*) used this information together with microsatellite resources (Cullingham et al. 2011) to create a species distribution map based on niche modelling. Using logistic regression, spatial variation in genetic ancestry was modelled using climate and spatial variables, including elevation, drought, precipitation, summer heat–moisture ratio, extreme cold, and location (latitude and longitude). The predicted distribution of species demonstrated that the hybrid zone is larger and more spatially complex than previous range maps indicated. More accurate maps of these species' distributions can be used in spread-risk models. For example, Cooke and Carroll (2017) used this predicted distribution map to set the initial conditions for simulations to predict MPB spread in eastern pine forests. The relationship between species and climate could also be used to adjust forest management guidelines under different climate change scenarios (e.g., Gray et al. 2016). As a resource, this map has proven valuable as a planning tool for researchers and government to identify regions for research studies and for assessing relative forest stand risk to MPB. An updated range map has been completed using similar methods (Burns et al. 2019), which encompasses the entire hybrid zone for lodgepole and jack pine (Fig. 1).

Adaptive variation

Understanding how MPB have rapidly expanded their range and how that might impact genetic diversity and selection processes was essential given the prospect of continued forest loss. Using the draft MPB genome, the TRIA project developed a SNP chip with over 1500 loci to address such questions. A combination of population genetic structure, landscape genetics, and outlier detection methods provided the first insights into how MPB might have breached the Canadian Rocky Mountains and established in areas traditionally viewed as less suitable habitat (Janes et al. 2014). Similar to Samarasekera et al. (2012), Janes et al. (2014) also found genomic variation to be distributed in north–south clusters (Fig. 1). Additional patterns were surprising as typical founder signatures (e.g., heterozygosity and allelic diversity) were not found at all sites. Rather, these findings served to emphasize the high level of functional connectivity among populations over large geographic distances (>100 km). Meanwhile, outlier detection tests revealed 32 SNPs with annotated genes associated with cholesterol synthesis, actin filament contraction, and membrane transport. Janes et al. (2014) suggested that these genetic signatures were involved in dispersal capacity, via muscle contraction, and endothermic regulations, essentially selecting for beetles with greater flight and cold tolerance. However, the paucity of SNPs that could be linked to annotated genes reflected a significant gap in our ability to describe genomic architecture and how MPB is expanding its range and adapting to novel environments. Several of the genes identified had only speculative functions in other insects, highlighting the need for functional genetic experiments to fully understand the role that these genes have and what that means in terms of ecology. While Janes et al. (2014) provided much needed information about MPB genetics and dispersal, its greatest impact has been in (*i*) highlighting how functional and population genetics can be used to understand evolutionary potential (e.g., Janes and Batista 2016; Batista et al. 2016) and (*ii*) prompting experimental work to verify the function of specific gene families identified and their physiological implications (e.g., Keeling et al. 2016; Robert et al. 2016; Fraser et al. 2017).

In an effort to identify specific genes that might be involved in physiological adaptations of the MPB, Horianopoulos et al. (2018) examined a suite of gene-linked microsatellites (Agata et al. 2011). They detected a strong signature of selection for a sex-linked, neo-X, "inhibitor of apoptosis" gene. Spatial genetic analysis of this gene showed an allele found predominantly in northern Canadian populations. Further, a temporal analysis of allele-specific gene expression of MPB larva preparing to overwinter found that this allele was upregulated earlier than the alternative alleles. This functional difference between alleles suggests a possible mechanism of early upregulation of the stress response "inhibitor of apoptosis" gene in northern populations that may provide a selective advantage in response to early season cold events in the harsh northern range of the MPB.

Genetic variation plays an important role in the susceptibility of populations to pathogens (King and Lively 2012). Examples from agriculture demonstrate that genetically depauperate populations (monoclones) are highly susceptible to disease (Pilet et al. 2006), similar to natural populations of endangered species with limited genetic diversity (Thorne and Williams 1988). These observations demonstrate that host genotype influences susceptibility of individuals. Cudmore et al. (2010) demonstrated that naïve lodgepole pine in BC supported higher reproductive success of MPB than previously exposed lodgepole pine, suggesting a genetic component to MPB susceptibility. In an attempt to identify genetic variation underlying susceptibility in lodgepole and jack pine, Cullingham et al. (2014) analyzed over 400 genetic loci across populations of lodgepole pine in BC and AB and jack pine populations in AB, Saskatchewan, and Ontario. They identified a list of 15 candidate genes in lodgepole pine and four in jack pine, but these loci did not overlap. Interestingly, the loci identified in each species were related to contrasting environmental variables. While they were not able to determine whether these candidates were directly related to MPB–fungal resistance, these loci are a first step in identifying putative candidates. This study demonstrates the power of a genomic approach for genetic conservation and monitoring of these important forest species (Aravanopoulos 2016). The genetic data provide an important baseline to compare future generations against to ensure that adaptive potential is maintained for continued stand resiliency.

Species interactions

A particularly interesting feature of the TRIA project was the co-collection of tree, beetle, and fungi (Fig. 1). This unique dataset was the foundation for an analysis by James et al. (2011) representing the first genetically informed demonstration of how landscape structure affects movement of MPB. This work also presented a new methodological approach to investigating shared spatial genetic structure among interacting species (MPB and *G. clavigera*). Using spatial genetic analysis, they identified north–south genetic clusters in both MPB and *G. clavigera*. Then, using a landscape genetics approach, they also determined that different environmental features drive spatial genetics structure within these northern and southern subregions. This suggests that there may be missing links in our understanding of basic spatial life-history dynamics. The main contribution here is insight into environmental features that determine movement of MPB. Specifically, different factors were implicated in the recent northern (pine volume and climate) vs. historical southern (fungal genetic structure) ranges. Further, they verified the role of pine volume in determining spatial connectivity (e.g., Safranyik et al. 2010). Finally, on the methodological front, their integrated approach to landscape genetics using one species as a "resistance" surface to model connectivity of an associated species was unique. This approach has been taken up for the analysis of other systems, including Lyme disease in eastern Canada (Leo et al. 2016).

In spite of our increased understanding of the role of bark beetle–fungal symbionts (Six and Wingfield 2011; Paine et al. 1997), one question that remains unresolved is why multiple assemblages of fungal symbionts are maintained. The three fungal associates are consistently found in association with the MPB across its range and were reported (under different names) almost 50 years ago (Whitney 1971). Although fungal symbionts do not appear to directly interfere with each other (Bleiker and Six 2009), temperature and the ability to capture resources likely play a role in interspecific dynamics (Moore and Six 2015). One explanation for the maintenance of this complex is that niche partitioning and adaptive radiation generate slightly different adaptive profiles for each fungus.

Ojeda et al. (2017) tested this hypothesis by collecting and genotyping 900 fungal samples from 35 locations in Canada and the US. A genotype–environment association analysis found that both common (temperature seasonality and the host species) and distinct (drought, cold stress, precipitation) environmental and spatial factors shaped the genomes of these fungi, with contrasting outcomes. For example, fungal associates possessed distinct temperature optimum profiles, with *G. clavigera* growing faster than *L. longiclavatum* and *O. montium* at all but the highest temperatures (Ojeda et al. 2017). Importantly, variation among the genetic clusters within each species (Tsui et al. 2012, 2014; Ojeda et al. 2017) was highly significant and heritable, suggesting differential temperature adaptation even within species. Symmetrical replacement between some of the fungi along a latitudinal gradient was previously shown, supporting the hypothesis of complementarity among fungal associates (Roe et al. 2011*b*). This study provides evidence that MPB could reduce the risk of becoming aposymbiotic by transporting fungal associates with different and complementary characteristics (Six and Bentz 2007). Thus, maintaining a multi-partite fungal symbiosis could be instrumental in allowing MPB to colonize new habitats, survive in highly variable climatic regimes, and withstand adverse environmental conditions. This is a particularly important consideration in light of recent climate projections.

Beyond mountain pine beetle

We are seeing increasing pressure on our forest systems by both native fungal and insect species in response to changes in climate (Gauthier et al. 2014) and invasive pests as a result of international trade (Tobin et al. 2013; Ramsfield 2016). From our experiences, the strength of this project has stemmed from co-collection of the interacting species, allowing us to develop a multi-taxa approach to understanding both individual species parameters and interactions among the species. This approach has increased our understanding of the MPB system and provided information for use in risk-analysis frameworks. For example, genetic-based findings from TRIA provide the means to refine stand susceptibility indices (e.g., James et al. 2016), predictive spread models (e.g., Tsui et al. 2014; Cullingham et al. 2013*a*), and species distributions (e.g., Cullingham et al. 2013*b*; Roe et al. 2011*b*) and to develop better means of monitoring (e.g., Trevoy et al. 2018) and managing (e.g., Janes et al. 2014) MPB and associated forest resources (e.g., Cullingham et al. 2014). Other forest systems may benefit from the analyses that we have presented here. Indeed, population genetic analysis of spruce budworm has already identified source populations of outbreaks (James et al. 2015). Moving forward, a better understanding of Dutch elm disease (*Ophiostoma ulmi* (Buisman) Nannfeldt (Ophiostomataceae), *Ophiostoma novo-ulmi* Brasier) in Canada could be gained from a genetic–genomic approach. Genetics could address questions regarding the role that the two insects (native elm bark beetle (*Hylurgopinus rufipes* Eichhoff) and an invasive bark beetle (*Scolytus schevyrewi* Semenov)) (Ramsfield 2016) play in vectoring the fungi, as well as what contributes to the spatial distribution of the fungi.

Conclusion

In response to the recent and rapid expansion of MPB, particularly within Canada, TRIA researchers coordinated to address pressing questions regarding the ecology, physiology, and genomics of key taxa within the MPB system with a goal of developing new knowledge that would be pertinent to forest management. This review highlights the main findings and advances made in relation to genetic and genomic studies conducted under TRIA in the past 12 years. TRIA-related research has clearly made significant contributions to improving our understanding of the MPB system in the form of species identification, genetic and genomic resources, phylogeographic and population genetic structure insights, and the identification of putatively adaptive genes. These findings have clear implications for both "basic" and "applied" research contexts. From an applied perspective, we have improved managers' capacity to include sex-ratio estimates in calculating stand susceptibility indices through the use of genetic markers that discriminate sex in morphologically sexless larvae (James et al. 2016). Similarly, timely confirmation that MPB populations had established in lodgepole pine × jack pine hybrids would not have been possible without genetic tools to accurately distinguish among pure and hybrid pines (Cullingham et al. 2011). We believe that our basic findings will serve as a strong foundation upon which further fundamental and applied work will be built.

Another key contribution of TRIA has been the significant advances in our understanding of the interactions and cumulative impacts among key species in the MPB system that were made possible through a coordinated and collaborative multi-taxa approach. By collecting samples from MPB, fungal symbionts, and pine species over space and time, TRIA has identified correlations among genetic patterns and markers across multiple species (e.g., James et al. 2011). While these findings are not causal per se and may not be incorporated into management frameworks immediately, they are essential to developing a framework in which abiotic and biotic factors influencing genetic variation can be quantified. Further, such a framework would facilitate greater understanding of the interactive influence of both genotypes among individuals within a species, as well as individual genotypes with the environment (e.g., similar to a community genetics approach). As genetic variation forms the basis for adaptation,

identifying and quantifying these influences and, perhaps, generalizing individual responses to species responses will be particularly relevant in developing new research directions and management strategies in the future. For example, studies highlighting the potential for rapid adaptation in MPB (e.g., Janes et al. 2014) have already prompted directed research into MPB cold tolerance (Robert et al. 2016; Fraser et al. 2017).

We advocate that similar coordinated multi-taxon approaches be applied to emerging and existing forest insect systems to better understand forest–insect–fungus pathogenicity at the landscape level. Further, we urge resource managers and researchers alike to increase the use of genetic methods in future studies and to make better use of existing genetic resources and findings. We see the benefits of incorporating genetic and genomic studies by (*i*) complementarity to ecological and physiological studies, often enhancing them by providing data and answers that could not be gained through direct means (e.g., observation alone), and (*ii*) a greater breadth of studies using genetic and genomic methods that strengthen our ability to generate and mine genomic resources, which we foresee facilitating novel advancements in forest management, forestry, and conservation.

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