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ORIGINAL ARTICLE

Polygamy and an absence of fine-scale structure in *Dendroctonus ponderosae* (Hopk.) (Coleoptera: Curculionidae) confirmed using molecular markers

This article has been corrected since Advance Online Publication and a corrigendum is also printed in this issue

JK Janes[1,2], AD Roe[2], AV Rice[2], JC Gorrell[2], DW Coltman[2], DW Langor[3] and FAH Sperling[2]

An understanding of mating systems and fine-scale spatial genetic structure is required to effectively manage forest pest species such as *Dendroctonus ponderosae* (mountain pine beetle). Here we used genome-wide single-nucleotide polymorphisms to assess the fine-scale genetic structure and mating system of *D. ponderosae* collected from a single stand in Alberta, Canada. Fine-scale spatial genetic structure was absent within the stand and the majority of genetic variation was best explained at the individual level. Relatedness estimates support previous reports of pre-emergence mating. Parentage assignment tests indicate that a polygamous mating system better explains the relationships among individuals within a gallery than the previously reported female monogamous/male polygynous system. Furthermore, there is some evidence to suggest that females may exploit the galleries of other females, at least under epidemic conditions. Our results suggest that current management models are likely to be effective across large geographic areas based on the absence of fine-scale genetic structure.
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INTRODUCTION

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), is a native forest pest throughout western North America (Maness *et al.,* 2012). Its pre-2005 range extended from northern Mexico to central British Columbia and southwestern Alberta, and an isolated population was located in the Cypress Hills in southeastern Alberta and Saskatchewan, Canada (Mock *et al.,* 2007; Safranyik *et al.,* 2010). *Dendroctonus ponderosae* is one of the few North American bark beetles that can kill live, healthy trees (primarily *Pinus* species) in order to feed and complete its life cycle (Lee *et al.,* 2006; Reeve *et al.,* 2012). Populations naturally cycle between phases of endemic (small, low-density populations attacking low-quality hosts) and epidemic (wide-spread, high-density populations attacking mature healthy trees) (Safranyik and Wilson, 2006) approximately every 20 years (Aukema *et al.,* 2006; Safranyik *et al.,* 2010). Typically, these epidemic phases have been regulated by natural predators (for example, woodpeckers; Fayt *et al.,* 2005), interspecific competition (for example, *Ips pini*; Rankin, 1988), intraspecific competition (Trzcinski and Reid, 2009) and temperature (Régnière and Bentz, 2007). However, in recent years warmer temperatures and altered forest management practices have resulted in what is possibly the biggest epidemic outbreak recorded in history (Taylor and Carroll, 2004).

Around 2005, *D. ponderosae* began to invade boreal Canada and spread throughout much of north-central Alberta and into the southern Northwest Territories (Lee *et al.,* 2006; Safranyik *et al.,* 2010). This outbreak has devastated over 18 million hectares of forest (Aukema *et al.,* 2008; Natural Resources Canada, 2015a), causing significant economic, social and environmental damage (Aukema *et al.,* 2006). The range expansion raised concerns that *D. ponderosae* would continue to spread through the boreal. A hybrid zone of lodgepole pine × jack pine in parts of north central Alberta (Cullingham *et al.,* 2011) provides a potential bridge, allowing *D. ponderosae* to become better suited to jack pine as a host, thereby providing access to the boreal forests across North America. Resultantly, management of *D. ponderosae* in boreal forests is of national importance (Natural Resources Canada, 2015b); however, initial research has shown that the biology (that is, brood development and survival) of *D. ponderosae* populations in the boreal forest is different to populations in western and southern forest types (Rice *et al.,* 2008; Safranyik *et al.,* 2010; Myrholm and Langor, 2015). These differences highlighted the potential risk of simply extending management based on historical knowledge of *D. ponderosae* directly to the boreal. It was clear that effective management of *D. ponderosae* in the boreal required new research about mountain pine beetle biology and its ecological interactions within boreal systems (Safranyik *et al.,* 2010), including information about life cycle, mortality, dispersal and mating systems.

Throughout much of its historical range in Canada, female beetles typically initiate host attack and egg gallery construction between July and August (Safranyik and Wilson, 2006). Males join females later, attracted by female-emitted pheromones, and mating occurs in the galleries (Safranyik and Wilson, 2006). Larvae develop and pass

[1]Environment and Parks, Government of Alberta, Edmonton, Alberta, Canada; [2]Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada and [3]Northern Forestry Centre, Canadian Natural Resources, Edmonton, Alberta, Canada
Correspondence: Dr JK Janes, Environment Sustainable Resource Development, Government of Alberta, Edmonton, Alberta T5J1G4, Canada.
E-mail: jasmine.janes@gov.ab.ca
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through four instars, overwintering largely in the third and fourth instar, before pupating in June (Aukema *et al.,* 2006). Teneral adults undergo hardening before emerging from the host tree gallery and embarking on their own dispersal flights in June and July of the following year (Aukema *et al.,* 2006). However, observations suggest that these events occur earlier in boreal systems as less thermal units are required to achieve a generation (Bentz *et al.,* 2014; DW Langor, unpublished data). *Dendroctonus ponderosae* populations typically exhibit univoltine reproduction (Sturgeon and Mitton, 1986).

The mating system of *D. ponderosae* is complex. Males join females in freshly constructed egg galleries where mating takes place (Reid, 1962). However, recent reports suggest that only 1–2% (Safranyik and Wilson, 2006) or up to 3–12% (Bleiker *et al.,* 2013) of females have mated before they emerge from maternal galleries (that is, pre-emergence mating). It has been widely accepted that male beetles may be polygynous but there is no clear published indication that females engage in polyandry. Safranyik and Wilson (2006) state that sperm remains viable in the spermatheca for at least a year, suggesting that females would not have a biological need to mate again even if they survived winter and continued reproducing in the following year. In order to contribute to *D. ponderosae* management at the front of the boreal invasion in Alberta, and build on models predicting patterns and rates of invasion and success of *D. ponderosae* populations, a detailed understanding of beetle mating systems and resultant fine-scale population genetic structure would be extremely useful.

Studies of parentage and sibship relationships within *D. ponderosae* populations appear to be absent in the literature, presumably because of difficulties in conducting field-based studies (Berger-Wolf *et al.,* 2007). However, information on genealogical relationships can be particularly valuable in studies of mating systems and spatial genetic structure in natural populations (Wang and Santure, 2009). The level of kinship among individuals within a population can be used to infer the predominant mating system (Berger-Wolf *et al.,* 2007) and show the local pedigree structure that, in turn, provides information about the level of spatial genetic structure present (Jacquemyn *et al.,* 2006). With the rapid development and growing application of molecular markers, studies into these areas are far more tractable. Single-nucleotide polymorphisms (SNPs) are particularly informative. Here we investigate the fine-scale genetic structure and mating system of *D. ponderosae* using SNPs. More specifically, we aim to determine whether *D. ponderosae* shows genetic evidence of: (1) fine-scale population genetic structure, (2) mating systems other than polygyny (that is, are females also polyandrous?) that may influence dispersal patterns and fine-scale population genetic structure and (3) pre-emergence mating.

MATERIALS AND METHODS

Specimen collection and DNA extraction

Adult beetles and larvae were collected from a boreal stand of lodgepole pine (*Pinus contorta* Douglas ex Loudon) trees near Fairview, Alberta (56.490 N, −118.518 W). Sampled trees were attacked by outbreak populations of *D. ponderosae* in July and August 2008 and were felled on 3 September 2008. Five trees ranging in height from 13.5 to 21.4 m were felled. Each tree was delimbed and cut to provide five sections with the following designations and heights above ground level: ST = 0.5–1.0 m; B = 2.0–3.0 m; D = 4.0–5.0 m; F = 6.0–7.0 m; and H = 8.0–9.0 m. Sections were transported back to the laboratory where the bark was removed to expose individual egg gallery systems. These samples were given unique identifiers that would allow them to be traced back to individual trees, sections and galleries (that is, tree no.–section no.–gallery no.). Separately, to test for relatedness among proximate sites (within 750 km), additional adult beetles were genotyped: 11 beetles from Fort St James, British Columbia (56.704 N, −121.712 W; collected 2005); 20 from Grande Prairie, Alberta (54.992 N, −118.614 W; collected 2007); and 20 from Fox Creek, Alberta (54.481 N, −116.635 W; collected 2007) (Figure 1). Adults and larvae were rinsed in sterile distilled water and stored at −20 °C.

Excavated galleries often contained adults, larvae or both. Where possible, gallery systems containing one or more adult beetles and late-instar larval progeny were selected for DNA extraction to best include potential parents. Thus, we used a number of galleries in which both adults and larvae were present to assess the mating system through parentage assignment; a number of galleries that contained only adults or larvae were then added to the analysis to further test the assignment and relatedness. The sex of adults was determined using characters of posterior margin of the seventh tergite (Safranyik and Wilson, 2006). Larvae could not be sexed because of an absence of diagnostic characteristics. Genomic DNA was extracted from adult and larval *D. ponderosae* specimens using QIAGEN (Toronto, ON, Canada) DNeasy 96 Blood & Tissue kits as per the manufacturer's instructions, with modification to the lysis step. Lysis was completed by incubating the samples at 56 °C overnight. All DNA samples were checked for quality and normalized using Qubit (Invitrogen, Life Technologies, Carlsbad, CA, USA) flourometry and milliQ to a concentration of 20 ng μl^{-1} before genotyping.

Genotyping and filtering

A panel of 1536 SNPs was previously developed using the Illumina (San Diego, CA, USA) GoldenGate assay (Janes *et al.,* 2014). From this GoldenGate panel, we selected 114 SNPs to develop a Sequenom panel. In total, 404 samples from the five Fairview trees (see Supplementary Table 1) were genotyped at McGill University and Génome Québec Innovation Centre using the Sequenom iPLEX Gold genotyping assay (Sequenom, 2008).

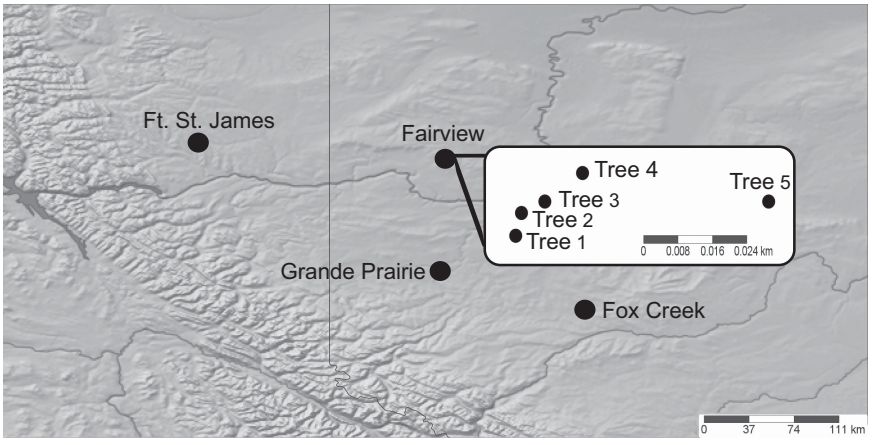

Figure 1 Map of beetle sites used in this study. The inset box shows the spatial distribution of the five trees sampled at Fairview.

Genotyped samples and SNPs were filtered to ensure high quality and reproducibility. First, the quality of SNP and sample calls was assessed using the call rate. SNPs and samples with a call rate of $\geqslant 80\%$ were considered 'good', whereas SNPs and samples with call rates $<80\%$ were removed. Second, samples were checked for reproducibility. Third, samples and SNPs with $>10\%$ missing information (that is, NN) were removed.

Data analysis

Observed (H_O) and expected (H_E) heterozygosities were assessed using GenePop Version 4.1.3 (Rousset, 2008) to detect deviations from Hardy–Weinberg equilibrium. A Bonferroni adjustment (Holm, 1979), using an initial α of 0.05, was used to assess the statistical significance of multiple *P*-values. Outlier detection was used to identify SNPs under directional selection. BayeScan was run using the methodology described in Janes *et al.* (2014). To investigate fine-scale genetic structure and variation, the total genetic variance was assessed using a hierarchical analysis of molecular variance in GenAlEx 6.4 (Peakall and Smouse, 2006). Data were organized according to the following hierarchical partitions: (1) within individuals, among individuals within galleries (*F*is) and among galleries (*F*st); (2) within individuals, among individuals within sections from individual trees (*F*is) and among sections within individual trees (*F*st); (3) within individuals, among individuals within trees (*F*is) and among trees (*F*st); (4) within individuals (*F*is), among individuals within galleries (*F*st), among galleries within sections (*F*sr) and among sections (*F*rt); and (5) within individuals (*F*is), among individuals within sections (*F*st), among sections within trees (*F*sr) and among trees (*F*rt). Tests for significant departure from the null hypothesis that subpopulations are part of a single large, random mating, genetic population were performed using 999 random permutations.

Pairwise relatedness estimates were calculated using SPAGeDi 1.4 (Hardy and Vekemans, 2002). These relatedness estimates assess the biological relationships among individuals based on genotypic similarity through the proportion of shared alleles by calculating allele frequencies across the entire population, and standardizing average relatedness to a mean of zero. Therefore, a positive value between two individuals implies they are more related than expected by chance, whereas a negative value implies that they share fewer alleles than expected relative to the total population. The Fairview population was compared with the following spatially proximal populations: Grande Prairie, Fort St James and Fox Creek. To prevent biases from uneven sampling, allele frequencies from GenAlEx were averaged and used as an input for SPAGeDi.

Parentage and sibship were assigned using CERVUS 3.0.7 (Kalinowski *et al.*, 2007) with the following parameters: (1) proportion of candidate mothers/fathers sampled 0.5; (2) proportion of loci mistyped 0.01; (3) strict confidence level 95%; and (4) accepted number of mismatches $\leqslant 2$. These assignments were performed for galleries containing putative parents with larvae, and for all individuals separately. Probability of identity was calculated using CERVUS.

RESULTS

Fine-scale spatial genetic structure

In total, 2155 individuals were harvested, providing an average density of 431 (s.e. $\pm$ 0.133) and 86 (s.e. $\pm$ 0.062) individuals per tree and section, respectively. Because of limited availability of gallery systems containing one or more presumed parent adults with larvae, several galleries were selected that contained only larvae or adults. In total, 404 individuals (203 larvae and 201 adults) were selected for genotyping. Of the 201 adults, 140 were female, 56 were male and 5 were of undetermined sex. Adult females and males were found in galleries 80% and 32% of the time, respectively. These 404 individuals represented 174 galleries from 24 cross-sections across the 5 trees (one section did not yield suitable material). From these 174 galleries, 15 (8.6%) contained both adults and larvae, 15 (8.6%) did not have an adult present and 144 (82.7%) did not have larvae present. The 15 galleries that contained both adults and larvae yielded 119 larvae and 22 adults (11 female and 11 male), and were used to perform initial parentage assessments. Later, all 404 individuals were used to test for relatedness and further assess the mating system through parentage analyses. Using a call rate threshold of $>80\%$, and removing SNPs and samples with $>10\%$ missing information, 21 (15.7%) SNPs were removed. The sample reproducibility error rate was $<5\%$. Of the remaining 93 SNPs, 2 were monomorphic and removed, leaving a total of 91 SNPs for analyses. These 91 SNPs comprised 44 exonic, 25 intronic and 22 noncoding SNPs. Outlier detection tests revealed one directionally selected locus. The inclusion of this locus did not change the results obtained. We retained all 404 samples in our analyses.

Table 1 Analysis of molecular variance (AMOVA) results for hierarchical partitions of individuals, galleries, sections and trees

Hierarchical partition	*Tree*	F*is*	F*st*	F*sr*	F*rt*
(1) Individuals–galleries		0.129	0.118		
(2) Individuals–sections per tree	1	0.203	0.053		
	2	0.182	0.016		
	3	0.173	0.022		
	4	0.159	0.048		
	5	0.194	0.040		
	All	0.185	0.037		
(3) Individuals–trees		0.209	0.008		
(4) Individuals–galleries–sections		0.129	0.119	0.105	0.015
(5) Individuals–sections–trees		0.185	0.037	0.037	0.000[a]

All results yielded a significant *P*-value <0.05 unless indicated.
[a]Indicates this value had a nonsignificant *P*-value >0.05.

Tests for deviation from Hardy–Weinberg proportions indicated that there were no significant deviations after sequential Bonferroni correction. All hierarchical analysis of molecular variance results (Table 1) suggest that the majority of molecular variance is best explained at the individual level, typically 76–78%. Very little genetic variance was explained at the individual tree (1%) or section levels (2–5%), with a modest degree explained at the level of galleries (10–12%). Mean pairwise Queller and Goodnight relatedness estimates within each population ranged from -0.04 to 0.01 and -0.021 to 0.001 among populations. Individuals within Fort St James were significantly less related to each other than expected when compared with individuals from within Fairview. Among populations, Fort St James and Grande Prairie were significantly less related to Fairview than the Fox Creek population. Figure 2 provides the estimates for each grouping with 95% confidence intervals.

Mating system

We identified six galleries from two trees that contained both males and females in the presence of larvae; an additional five galleries across four trees were found with females and larvae, and four galleries from two trees were found with males and larvae. One gallery (gallery 5-5-170; that is, tree no.—section no.—gallery no.) contained two males in the same gallery with larvae, but no female. These galleries contained a total of 119 larvae. In all, 6 of the 11 (54.55%) females and 8 of the 11 males (72.72%) were excluded from being the mother/father of at least one of the larvae found in their respective galleries. Three males (FV0993, FV1025 and FV1031) were found to be unrelated to all of the larvae found within their respective galleries. The gallery 5-5-166 contained both a male and female; however, the male (FV0993) was unrelated to the five larvae present in gallery, whereas the female (FV0994) was considered a parent (see Tables 2 and 3).

Across all Fairview samples, CERVUS identified a number of parental cross-assignments among the adult beetles present in the 15 galleries containing adults and larvae. For example, females FV0694

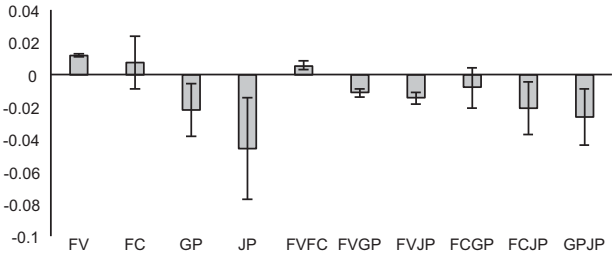

Figure 2 Mean relatedness estimates (Queller and Goodnight) within and among populations showing 95% confidence limits. FC, Fox Creek; FSJ, Fort St James; FV, Fairview; GP, Grande Prairie.

Table 2 CERVUS maternity assignment results for the 11 galleries containing females with larvae

Female ID	*Gallery*	*Total larvae present*	*Total larvae unrelated to female*	*Total larvae cross-assigned (gallery)*
FV0694	3-1-58	7		1 (5-2-135)
FV0753	3-5-89	7	3	
FV0772	4-1-95	6		
FV0547	1-1-2	8		
FV0613	1-5-21	3	1	
FV0890	5-1-134	22	2	
FV0929	5-2-141	14	2	
FV0950	5-3-146	15	4	
FV0994	5-5-166	5		1 (5-1-132)
FV1005	5-5-169	5		
FV1024	5-5-173	5	1	

All results met the strict 95% confidence and ≤2 loci mismatches.

Table 3 CERVUS paternity assignment results for the 11 galleries containing males with larvae

Male ID	*Gallery*	*Total larvae present*	*Total larvae unrelated to male*	*Total larvae cross-assigned (gallery)*
FV0640	2-2-34	4		
FV0695	3-1-58	7	2	
FV0870	5-1-133	13	3	
FV0928	5-2-141	14		1 (4-5-131)
FV0949	5-3-146	15		
FV0993	5-5-166	5	5	
FV1006	5-5-169	5	2	2 (5-1-134)
FV1012	5-5-170	2	1	
FV1013	5-5-170	2	1	
FV1025	5-5-173	5	5	
FV1031	5-5-174	3	3	

All results met the strict 95% confidence and ≤2 loci mismatches.

(gallery 3-1-58) and FV0994 (gallery 5-5-166) were assigned as the mothers of larvae FV0916 (gallery 5-2-135) and FV0864 (gallery 5-1-132), respectively. Males FV0928 (gallery 5-2-141) and FV1006 (gallery 5-5-169) were assigned as the fathers of larvae FV0851 (4-5-131), and FV0901 and FV0906 (both from 5-1-134), respectively (see Tables 2 and 3). The probability of identity from CERVUS indicated that all individuals had unique genotypes (probability of identity = 0).

Using all available Fairview samples, we assessed the occurrence of full and half-sibling relatedness within galleries, and among galleries within sections, sections within trees and among trees. CERVUS showed that in a single gallery, larvae can be full-siblings, half-siblings or unrelated (see Table 4). A number of larvae present in different trees were identified as being full-siblings, although a large proportion of individuals were unrelated (89.54%). The occurrence of half-siblings was greatest among different trees, followed by larvae within the same gallery. SPAGeDi indicated that 5.69% of larvae were the product of pre-emergence mating between full-siblings ($r \geqslant 0.625$). Four adult–larva combinations were highly inbred according to the pairwise relatedness estimates obtained (>0.70). Overall, the average female reproductive success was 2.89 (s.e. ± 0.39) larva, whereas the male success was 4.73 (s.e. ± 1.15).

Table 4 Proportion of larvae assigned as related or unrelated from pairwise comparisons

Relation	*Tree location*	*%*
Half-sib	Same gallery	2.23
	Different gallery, same section	0.37
	Different section, same tree	1.31
	Different tree	4.41
Full-sib	Same gallery	0.40
	Different gallery, same section	0.01
	Different section, same tree	0.00
	Different tree	0.05
Unrelated	Same gallery	1.64
	Different section and tree	89.54

Larvae were compared within the same gallery, among galleries within the same section, among sections within the same tree and among trees.

DISCUSSION

Fine-scale spatial genetic structure

Previous studies investigating the genetic structure of *D. ponderosae* have focused on the landscape scale and typically reported weak spatial genetic structure resulting in partitioning of northern and southern sampling sites, irrespective of the spatial extent of sampling (Mock *et al.*, 2007; James *et al.*, 2011, 2014; Samarasekera *et al.*, 2012). However, these results did not indicate whether *D. ponderosae* populations were exhibiting fine-scale spatial genetic structure. The extent and location of habitat patches for *D. ponderosae*, typically stands of 60–160 years in lodgepole pine (Shore and Safranyik, 1992; Aukema *et al.*, 2006), are spatially and temporally dynamic in context because of stand aging, harvesting patterns and natural disturbances (especially wildfire). Changes in availability of suitable habitat or large changes in *D. ponderosae* population densities may cause the species to change its dispersal behavior or mating system (Row *et al.*, 2010), ultimately affecting genetic structure. Although changes in genetic structure may not be detected at the landscape scale because of a homogenizing effect from long-distance dispersal (Aukema *et al.*, 2006; Safranyik *et al.*, 2010), they may be observed at the stand scale (Pierson *et al.*, 2013).

Our study revealed no evidence of fine-scale spatial genetic structure at the stand level. Sampling of Fairview was conducted in 2008 at the height of the epidemic phase in an area where *D. ponderosae* had not been recorded before 2006. We had originally believed that 2008 attacks in this area would have come from beetles dispersing short distances as the majority of insect invasions are characterized by *occasional* long-distance dispersal (Liebhold and Tobin, 2008). For example, Turchin and Thoeny (1993) observed ~50% of mark-recapture southern pine beetle (*Dendroctonus frontalis* Zimmerman) dispersing <600 m. Once an area is colonized, fine-scale genetic structure can develop during the time the area is occupied as a result

of family groups and continued limited dispersal, and subsequently gene flow (Jacquemyn *et al.,* 2006; Pierson *et al.,* 2013). Thus, we believed the Fairview stand would be colonized via localized migration, providing a signal of fine-scale spatial genetic structure. However, our results suggest that homogenization, as a result of long-distance dispersal, may also influence fine-scale spatial genetic structure through the contribution and combination of different genotypes from multiple source locations, similar to the pattern observed by Janes *et al.* (2014). Similar conclusions have been made in locusts (*Locusta migratoria*), although in this instance it was suggested that the propensity to outbreak also played a major role in homogenizing genetic variation (Chapuis *et al.,* 2009).

Individuals from Fairview appeared to be more closely related to individuals from Fox Creek, ~700 km away. However, it is not clear whether this pattern is an artifact of temporal differences in sampling and population establishment times. Fort St James, which was sampled earlier (2005) and had been established longer than either Fox Creek or Fairview, was more unrelated to Fairview than expected. These results suggest that Fox Creek, not Fort St James or Grande Prairie, was potentially the source population for Fairview—or vice versa. Our results are similar to those found in *D. frontalis*. Schrey *et al.* (2008) compared epidemic populations of *D. frontalis* across national forests in Mississippi, incorporating a study area of $<500\ km^2$. They did not observe significant spatial genetic structure at this scale and proposed that the beetles in the outbreak area comprised a single interbreeding population in which genetic differentiation had not yet occurred. These results are also consistent with findings from Blanchett *et al.* (2012), in which rates of genetic differentiation were found to be lower in outbreak locust species compared with other *Calliptamus* species. We were unable to find previous studies of fine-scale spatial genetic structure in *D. ponderosae*.

Mating system

Mating behavior in which males or females have more than one mate at a time (that is, polygamy) is believed to evolve when the environmental potential for multiple mates is energetically defendable and individuals have the ability to utilize such potential (Emlen and Oring, 1977; Ptak and Lachmann, 2003). Polygyny is hypothesized to evolve when environment or behavior brings about female clumping, an abundance of female mates during a limited period of time, providing an opportunity to increase overall fitness (Emlen and Oring, 1977; Baena and Macias-Ordonez, 2015). Similarly, polyandry is believed to provide genetic benefits such as improving the likelihood that a female will acquire 'good' genes, increase genetic diversity among offspring and ensure fertilization if some males have low-quality sperm (Yasui, 1998; Bird *et al.,* 2012). Using molecular methods we directly assessed the likelihood of polygyny vs polyandry within the Fairview *D. ponderosae* population.

Previous empirical evidence had proposed a mating system where males are polygynous and females are monogamous (Reid, 1958, 1962). Males were assumed polygynous because, under natural conditions, several authors indicate that a male beetle will occasionally leave a mated female and her egg gallery to mate with another (Reid, 1958, 1962; Safranyik and Wilson, 2006; Bleiker *et al.,* 2013). Females were thought to be monogamous from laboratory observations that: (1) females can create a second egg gallery and brood in the absence of a second mating (Reid, 1958); and (2) because the male beetle arriving in the egg gallery blocks the entrance after mating, first with his body and eventually with boring dust and frass (Safranyik and Wilson, 2006). Thus, it was assumed that there was no biological need for further mating, with respect to females. However, our data clearly show, through the identification of cross-gallery and cross-tree parental assignments, that polyandry occurs under natural conditions. Biologically, this result is supported by the fact that a number of males leave the egg gallery before female oviposition (Safranyik and Wilson, 2006), and that a number of unmated males were apparently still searching for mates. Thus, 'new' males may take advantage of previously mated females after the original male has vacated the gallery. It is also possible that some females leave the gallery in search of more male mates to ensure fertilization and increasing fitness through multiple breedings, similar to the behavior observed in *Leptinotarsa undecimlineata* (Baena and Macias-Ordonez, 2015). Genetic confirmation of polyandry supports observations of polygamy in beetles from southwestern Alberta and adjacent British Columbia in the 1980s (DW Langor, unpublished data). Thus, the data presented herein suggest that *D. ponderosae* is best treated as polygamous, at least in epidemic phases.

When numerous adults are breeding in close proximity, these parents may interact with other parents and nonrelated offspring, expanding the potential network of social interactions. These between-family interactions may be beneficial, in the case of cooperative behaviors, or costly, in the case of competition or brood parasitism (Wong *et al.,* 2013). Our study revealed a number of galleries containing unrelated individuals. Reasons for this may include: (1) misidentified samples, (2) larval coalescence or (3) brood parasitism. Sampling errors are unlikely as every precaution was taken to ensure errors did not occur. At high brood densities, related pupae may coalesce in a common feeding chamber before emerging (Bleiker *et al.,* 2013). However, larval behavior may be different because of the risk of competition and subsequent density-dependent mortality. Amman and Cole (1983) state that larvae within a brood gallery will occasionally cross sibling feeding galleries, whereas Safranyik and Wilson (2006) state that larvae will rarely cross nonrelated galleries, preferring to backtrack and feed in another direction. Larvae crossing into nonrelated galleries may occur but we did not detect larval coalescence during this study.

Another explanation for the high rate of nonrelated individuals within galleries may be brood parasitism. Several insects are known to engage in brood parasitism, ranging from bees and wasps (Hymenoptera; Field, 1992) to lace bugs (Hemiptera; Zink, 2000) and beetles (Coleoptera; Müller and Eggert, 1990). Brood parasitic strategies are thought to evolve when breeding sites are in close proximity and there is an opportunity for parental care to be misdirected (Wong *et al.,* 2013). Although brood parasitism has not been reported in *Dendroctonus* it is possible that, under epidemic conditions, female *D. ponderosae* may engage in such behavior. Brood parasitism is known to alleviate some of the energetic costs involved with parental care by making use of unrelated conspecifics (Field, 1992). It can also be initiated when breeding sites are limited as it may increase fecundity of the parasitic female in relation to the primary (Zink, 2000). Under epidemic conditions, competition among females for access to gallery space may be high, even if density-dependent competition is somewhat regulated by the cessation of aggregation pheromone production (Safranyik and Wilson, 2006). For example, endemic populations of *D. ponderosae* typically display a density of 7–13 individual attacks per tree (Carroll *et al.,* 2006) with females constructing galleries up to 1.5 m in length (Reid, 1962). Under epidemic conditions the attack rate increases to ~60 individuals per m^2 (Raffa and Berryman, 1983). In such a competitive environment, it may make biological sense for some females to become 'parasitic' and enter another's gallery, thereby trading valuable energetic resources normally spent on gallery construction and

parental care (for example, removing frass from the galleries and remaining within the gallery after egg laying) for greater egg-laying and reproductive fitness. Brood parasitism, or sharing, cannot be confirmed by our data alone, though it is consistent with earlier anecdotal observations of brood parasitism by one of us (DW Langor, unpublished data). For the current study, the probability of multiple individuals possessing the same genotype (probability of identity) was effectively zero, making brood parasitism a plausible explanation for the presence of nonrelated larvae within galleries.

Pre-emergence mating among siblings has been reported in the ranges of 1–2% (Safranyik and Wilson, 2006) and 3–12% (Bleiker *et al.*, 2013) for *D. ponderosae*. Field-caught females with full spermathecal sacs (Reid, 1958; Bleiker *et al.*, 2013) are presumably the result of sibling crosses as only related males should be present with newly emerging females at that time. Using molecular methods in an epidemic population, our results suggest that the number of individuals resulting from matings among siblings is comparable (5%) to those previously reported. Levels of pre-emergence mating may be higher in endemic populations when density of beetles may be lower. At higher densities, there will be greater mate choice and opportunity compared with periods of low population density. Therefore, it may be more common for pre-emergence mating to occur during endemic phases. The confirmed individuals with relatedness estimates well within the range of offspring resulting from sibling matings further corroborates pre-emergence mating behavior.

The identification of highly related individuals, and the identification of half-siblings in the same gallery, adds support to a polyandrous mating system as it suggests that the pre-emergence mated females were later joined by an unrelated male in the egg gallery, resulting in two sources of sperm. We have made several anecdotal observations of two males being present in a single gallery in both field and laboratory studies (DW Langor, unpublished data). Until now, there appears to have been no published record of this behavior in *D. ponderosae*, although a genetic study of *Dendroctonus micans* Kugelann has revealed polyandry through multiple paternities in single-female broods (Fraser *et al.*, 2014). We identified two male beetles in the same gallery (5-5-170) and genetically confirmed that both males had sired offspring present within the gallery. This finding changes our understanding of mountain pine beetle mating behavior and confirms previous unpublished observations.

CONCLUSION

In summary, we have confirmed that fine-scale spatial genetic structure is absent from *D. ponderosae* populations and that the mating system is most likely polygamous. Furthermore, we have demonstrated the effectiveness of high-throughput molecular markers in assessing population structure and their applicability in parentage studies of forest insect pests. These results have important implications in the management of current and future *D. ponderosae* outbreaks. A polygamous mating system, coupled with long-distance dispersal, effectively dilutes any signal of fine-scale spatial genetic structure through considerable genetic heterogeneity. The absence of fine-scale genetic structure at a localized scale implies a level of genetic uniformity (that is, each stand will exhibit a high degree of genetic variation), suggesting that any attempts to tailor forest management practices to a specific genetic component will be fruitless. This notion corroborates the idea put forth by Schrey *et al.* (2008) that management practices designed for one forest stand will be suitable across a broader geographic area. Our results suggest that current proactive forest management practices in which stand age cohorts are thinned by the removal of trees meeting a specific diameter at breast height, and reactive management in which 'red' trees are identified and removed, are suitable for managing mountain pine beetle on the landscape. It seems appropriate, given this information, that more investment should be placed on managing beetle outbreaks on a landscape scale and that the ongoing research of the population dynamics and epidemiology of *D. ponderosae* is key to developing effective long-term management of this pest.

DATA ARCHIVING

Genotype data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.kb418.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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