Isolation and characterization of 16 microsatellite loci in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae)

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Abstract

We isolated 16 polymorphic microsatellite loci in the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and developed conditions for amplifying these markers in four multiplex reactions. Three to 14 alleles were detected per locus across two sampled populations. Observed and expected heterozygosities ranged from 0.000 to 0.902 and from 0.100 to 0.830, respectively. Three loci deviated from Hardy–Weinberg equilibrium in one sampled population. One of these loci may be sex linked. These markers will be useful in the study of population structure in this important pest species.

Keywords: *Dendroctonus ponderosae*, microsatellite, mountain pine beetle

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Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is native to western North America, reproducing in the phloem of many western pine species (*Pinus* spp.). *Dendroctonus ponderosae* is a classic outbreak species; populations remain at endemic levels for many decades, but changes in biotic and abiotic factors can lead to a rapid population expansion or outbreak, resulting in substantial host plant mortality (Raffa *et al*. 2008). Despite the large economic and environmental impacts of *D. ponderosae*, little information is available on the genetic structure of this species. Population genetic studies of *D. ponderosae* will provide important insights into the dynamics and origins of eruptive populations.

Genomic libraries were constructed at the Savannah River Ecology Laboratory (SREL; Glenn & Schable 2005) and the University of Alberta (UA; Hamilton *et al*. 1999). At SREL, genomic DNA was extracted from thorax tissue of adult beetles (Mullenbach *et al*. 1989), digested with *Rsa*I, ligated to SuperSNX linkers, independently hybridized to three mixes of biotinylated oligonucleotides (Mix 2, 3 and 4 from Glenn & Schable 2005) and captured on streptavidin beads. DNA was recovered by polymerase chain reaction (PCR) using SuperSNX24-F primer and cloned (TOPO TA Cloning Kit, Invitrogen); 260 clones were sequenced on an Applied Biosystems (AB) 3100 DNA sequencer. Sequences were assembled using Sequencher 4.2.2 (Gene Codes). Primers for 36 candidate loci were developed using Primer 3 (Rozen & Skaletsky 2000). At UA, genomic DNA was extracted from flight muscle tissue from surface sterilized beetles using a standard cetyltrimethyl ammonium bromide method (Sambrook & Russell 2001), digested with *Rsa*I, *Alu*I and *Nhe*I, ligated to SNX linkers, hybridized to a pool of biotinylated probes [$(GT)_{12}$, $(CT)_{12}$, $(GACA)_6$, $(GATC)_6$] and captured on streptavidin beads. Enriched DNA was recovered using SNX-F, digested with *Nhe*I, ligated into pBSIISK$^+$ (Stratagene) and transformed into XL1-Blue competent cells. Inserts from 1536 clones were sequenced on an AB 3730 DNA Analyser. Sequences were aligned in SeqMan (Lasergene, DNA*) and primers designed for 134 loci using Primer 3.

Amplification of 36 loci isolated at SREL was tested at Utah State University (USU) using genomic DNA of 25 individuals collected in the Sawtooth National Recreation Area, Idaho. Amplification tests for the 134 loci isolated at UA used a three primer labelling system (Schuelke 2000) with DNA extracted from seven *D. ponderosae* larvae collected in Alberta (DNeasy, QIAGEN). Amplifications for all loci were also attempted on genomic DNA extracted from single spore cultures of the symbiotic fungal species

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Grosmannia clavigera, *Grosmannia aurea*, *Ophiostoma montium*, *Leptographium longiclavatum* and *Leptographium terebrantis*. Markers were considered to be *D. ponderosae* specific when amplification was successful using mountain pine beetle template but failed using fungal genomic DNA. Loci that amplified successfully using both fungal and beetle template were also excluded as potential fungal specific markers.

Five of 36 (USU) and 11 of 134 (UA) primer sets yielded reliable polymorphic banding patterns and were specific to *D. ponderosae*. Conditions for multiplex PCR and co-loading were developed for efficient genotyping using these 16 markers (Table 1). Multilocus genotypes were determined for 80 individuals from two locations in Alberta, Canada (Canmore, $N = 41$ and Fairview, $N = 39$). Four multiplexed 10-μL reactions (A–D; Table 1) were performed containing 25 ng of genomic DNA, 1× PCR buffer, 0.16 mM dNTPs, optimized $MgCl_2$ and primer amounts (Table 1) and 0.5 U *Taq* DNA polymerase (AB), using thermocycling consisting of 94 °C for 2 min, 35 cycles of 92 °C for 30 s, 53 °C for 30 s, and 72 °C for 15 s, and a final extension step at 72 °C for 30 min. Fragments were co-loaded into two injections (I & II; Table 1) on an AB 3730 DNA Analyser. Band sizes were determined relative to GeneScan-500 LIZ (AB) and scored using GeneMapper software.

Genetic diversity statistics (Table 2) were calculated using the Excel Microsatellite Toolkit (Park 2001) and tests of disequilibrium performed using GenePop 1.2 (Raymond & Rousset 1995). Three loci displayed significant heterozygote deficiency following a strict Bonferroni correction (Table 2). Micro-Checker (van Oosterhout *et al*. 2004) estimated a null allele frequency of 0.0993 at Dpo520 in Canmore and 0.0898 at MPB054 in Fairview. Locus Dpo486 may be sex linked, amplifying regions on both the X (odd length alleles) and Y (even length alleles) chromosomes. Even length alleles ($P = 0.122$ and 0.167 in Canmore and Fairview, respectively) were never homozygous within an individual and were present in three male beetles. Even alleles were not detected in three female beetles. Considering only individuals with odd length alleles ($N = 31$ and 26 in Canmore and Fairview, respectively), locus Dpo486 displayed significant heterozygote deficiency in Canmore with an estimated null allele frequency of 0.1213. No locus pairs were in genotypic disequilibrium following Bonferroni correction.

These loci will be valuable tools for use in population genetic studies of *D. ponderosae* examining the dynamics, and origins of eruptive populations.

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Table 1 Primer sequences and PCR conditions for 16 microsatellite markers isolated from *Dendroctonus ponderosae*. Dpo markers were cloned at UA and MPB markers were cloned at SREL and developed at USU. X_F, X_V, X_N and X_P refer to 6-FAM, VIC, NED and PET fluorescent dyes (AB) respectively. *Reverse primer tailed with GTT to promote adenylation. †Loci were amplified in four reactions (A–D) and detected in two co-loaded sets (I and II)

Locus	GenBank Accession no.	Forward primer	Reverse primer	Repeat motif	Expected amplicon size (bp)	†Multiplex PCR and co-loading	Primer concentration (nmol)	$MgCl_2$ (mM)
Dpo028	FJ487588	X_VGTGACGATTTTTCCTGATGA	TTCATTTTATTGCATTTTGGA	$(TG)_{11}$	234	IIC	80	3.5
Dpo103	FJ487589	X_PGAACGACGTCTCGTACACAT	ATTCATTCCTTAACCGCATT	$(TG)_{16}T$	192	IIC	320	3.5
Dpo160	FJ487590	X_NACACTTTTCCATCGGAATTT	TTTTCGAAGCAATAACTCCA	$(GT)_{12}$	190	IB	160	3.0
Dpo453	FJ487591	ATTCGGGCACCTAGAATAAG	X_FAAATTGGTAGCAAAATGACG	$(CA)_5G(AC)_{10}$	173	IIC	160	3.5
Dpo479	FJ487592	TTATTGGGGTTCTGGAGTCT	X_FTCGATTTCCATTCAACAATC	$(GT)_{10}$	114	IA	160	3.0
Dpo486	FJ487593	CAGCCAAAGCACTTAACAAC	X_NTCGACTAAAGTCCAATCACG	$(TG)_4G(TG)_4N_6(TG)_7$	116	IA	160	3.0
Dpo530	FJ487594	*GTTTCACCGCTTCAGTATGAAAT	X_FCTGGCTTTATTTGACTCGTG	$(AC)_8$	280	IID	640	3.5
Dpo566	FJ487595	GCTCCAATGCAGATACAAAG	X_FTTTGGTCTGGTTCGTCTAAA	$(GT)_9$	131	IB	160	3.0
Dpo760	FJ487596	X_FCCAAATGAGGATGTTCGTAA	AGCATGTTTGCGCTATTTT	$(TG)_{12}$	220	IID	160	3.5
Dpo780	FJ487597	X_FATCCAGACTCCCATCCATTTC	TGGGCTGATTTCTTCCAT	$(GT)_{10}$	197	IB	160	3.0
Dpo793	FJ487598	X_VCGAAGAATTCGCTGACTAC	CGGTCATAACTTTGGTTCAC	$(CA)_{11}$	159	IA	160	3.0
MPB011	FJ487599	X_PCGTTTTCCAGCATCTCAAGG	TTGTTTCCTCAGTGGGAATTG	$(ATC)_8$	214	IB	160	3.0
MPB012	FJ487600	X_PCCGCCGTTTTGTACATACT	TCACGACAGATCTCCAACCA	$(CA)_{10}$	238	IID	320	3.5
MPB017	FJ487601	GCTTTGGTTCAACCCAGAAA	X_FTCACGTCTCGCTTATTGGTG	$(TAG)_6$	239	IB	160	3.0
MPB038	FJ487602	AAACCGCCCTTGTTTCTTCT	X_NCACGGTGACCCAGATTTCTT	$(AAC)_8$	242	IID	80	3.5
MPB054	FJ487603	X_NAATCGGTATCGCACAAAAGC	AGCAAAGCACTCCACGAAAT	$(ATC)_6$	203	IIC	80	3.5

Table 2 Genetic diversity statistics and results of tests of disequilibrium for 16 microsatellite markers genotyped in two populations (Canmore $N = 41$ and Fairview $N = 39$) of *Dendroctonus ponderosae*

Locus	Size range (Total N_A)	Population	N_A	H_O	H_E	P (HWE)
Dpo028	216–231 (8)	Canmore	6	0.488	0.520	0.4670
		Fairview	5	0.231	0.258	0.5635
Dpo103	180–231 (12)	Canmore	10	0.902	0.830	0.6499
		Fairview	8	0.692	0.782	0.7455
Dpo160	177–221 (14)	Canmore	14	0.756	0.819	0.9623
		Fairview	7	0.692	0.619	0.2511
Dpo453	160–180 (9)	Canmore	8	0.756	0.702	0.8251
		Fairview	6	0.590	0.695	0.5656
Dpo479	102–118 (7)	Canmore	4	0.707	0.636	0.0502
		Fairview	6	0.513	0.630	0.0905
§Dpo486	109–115 (3)	Canmore	3	0.317	0.404	*0.0103
		Fairview	3	0.333	0.303	0.1468
Dpo530	275–283 (5)	Canmore	4	0.488	0.649	*< 0.0000
		Fairview	4	0.718	0.647	0.4219
Dpo566	118–136 (8)	Canmore	7	0.512	0.479	0.5750
		Fairview	3	0.154	0.189	0.3168
Dpo760	211–231 (9)	Canmore	8	0.585	0.555	0.8460
		Fairview	5	0.539	0.565	0.8305
Dpo780	195–203 (6)	Canmore	5	0.732	0.678	0.1753
		Fairview	5	0.487	0.525	0.7407
Dpo793	145–167 (9)	Canmore	9	0.805	0.791	0.4024
		Fairview	4	0.410	0.408	0.9360
MPB011	203–218 (4)	Canmore	4	0.512	0.565	0.2408
		Fairview	4	0.590	0.494	0.6965
MPB012	234–265 (4)	Canmore	2	0.171	0.158	1.0000
		Fairview	4	0.667	0.572	0.6353
MPB017	241–259 (5)	Canmore	5	0.561	0.529	0.7893
		Fairview	3	0.333	0.303	0.1360
MPB038	231–243 (6)	Canmore	6	0.488	0.544	0.1260
		Fairview	3	0.103	0.124	0.1313
MPB054	194–226 (6)	Canmore	5	0.390	0.428	0.2456
		Fairview	3	0.000	0.100	*0.0001

N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; *significant deviation from Hardy–Weinberg equilibrium (HWE) following strict Bonferroni correction; †may be sex linked.

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