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Landscape-scale genetic variation in a forest outbreak species, the mountain pine beetle (*Dendroctonus ponderosae*)

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Abstract

The mountain pine beetle *Dendroctonus ponderosae* is a native species currently experiencing large-scale outbreaks in western North American pine forests. We sought to describe the pattern of genetic variation across the range of this species, to determine whether there were detectable genetic differences between *D. ponderosae* occupying different host trees in common localities, and to determine whether there was molecular evidence for a past demographic expansion. Using a combination of amplified fragment length polymorphism (AFLP) and mitochondrial sequencing analyses, we found evidence of genetic structuring among populations that followed a broad isolation-by-distance pattern. Our results suggest that the geographical pattern of gene flow follows the core distribution of the principal *D. ponderosae* host species, around rather than across the Great Basin and Mojave Deserts. Patterns of haplotype diversity and divergence were consistent with a range-wide population expansion. This signal was particularly pronounced in the northern part of the species' range, where outbreak activity is currently increasing. Using AFLP markers, we were unable to detect significant differences among groups of insects sampled from different host trees in common locations. Incidentally, we found that a large proportion of the polymorphic AFLP markers were gender-specific, occurring only in males. While we did not include these markers in our analyses, this finding warrants further investigation.

Keywords: AFLP, *Dendroctonus ponderosae*, gender-associated, mountain pine beetle, phylogeography, range expansion

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Introduction

In an era of rapid environmental and ecological change, populations of endemic organisms are expected to experience dramatic shifts in distribution and prevalence (Huntly *et al.* 1997; Thomas *et al.* 2004). Although the focus in conservation is often on endemic organisms that are at risk of decline or extinction, it is also important to consider organisms that may be favoured by current environmental changes. Of particular concern are native, eruptive insect species that have the potential to undergo rapid population expansion and can be important drivers of ecological change. Because insect life cycles are highly sensitive to temperature, climate change is having a particularly large impact on the development, survival, and distribution of insect herbivores

worldwide (Williams & Liebhold 1997; Coley 1998; Hill *et al.* 1999; Hansen *et al.* 2001; Bale *et al.* 2002; Watt & McFarlane 2002). Describing and tracking gene flow in such organisms can provide important information about their movements and trajectories over time. This information can be a critical component of effective management strategies and a foundation for understanding and predicting the evolution of these species.

The mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is a bark beetle native to western North American forests, spanning wide latitudinal and elevational gradients (Fig. 1). *D. ponderosae* infest and reproduce within the phloem of most *Pinus* species from northern Baja California in Mexico to central British Columbia in Canada, and their geographical range is dictated by the distribution of both suitable host species and favourable climatic regimes (Safranyik 1978; Wood 1982; Logan & Bentz 1999). *D. ponderosae* populations,
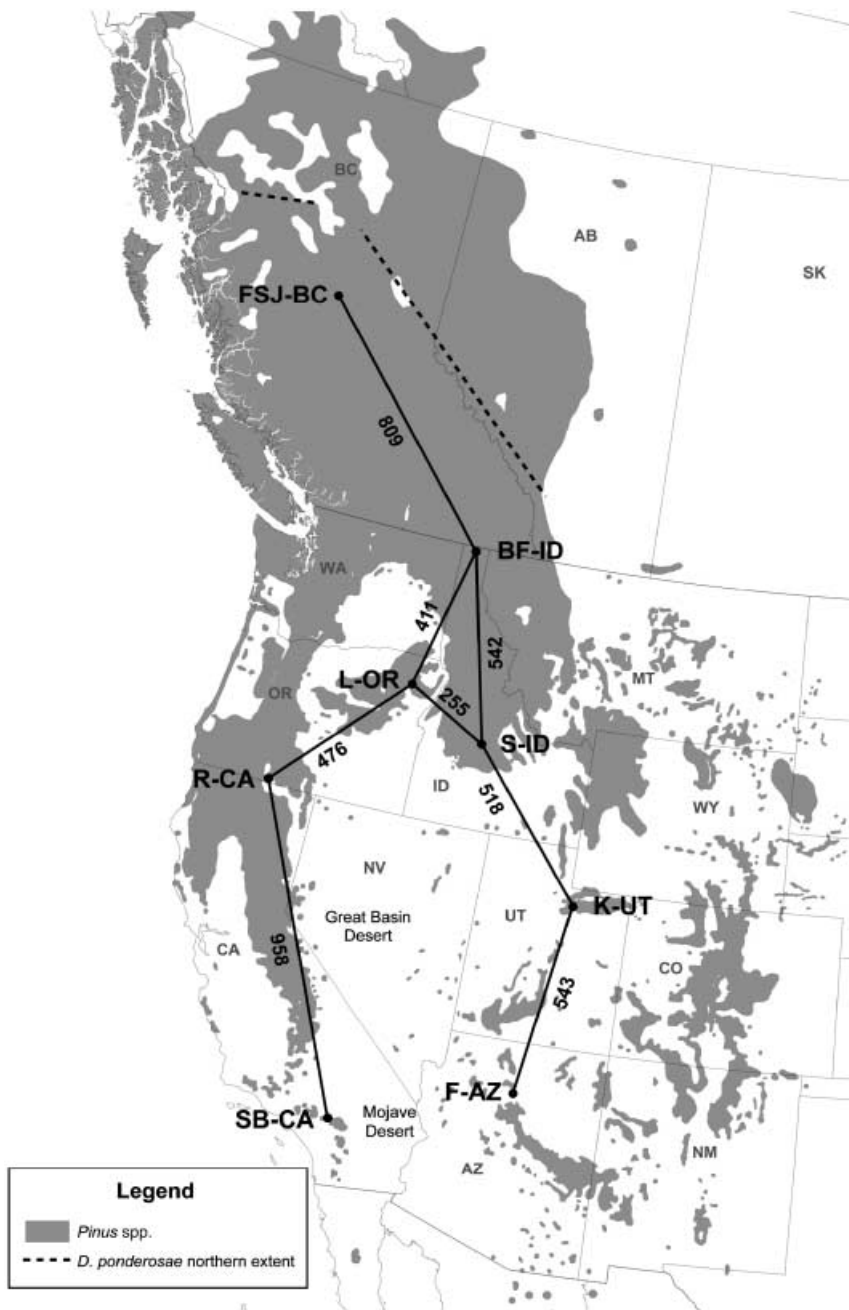
Fig. 1 Geographic distribution of the principle host tree species of *Dendroctonus ponderosa* including *Pinus contorta*, *P. ponderosae*, *P. albicaulis*, *P. lambertiana*, and *P. monticola*. The geographical distribution of *D. ponderosae* generally follows that of its principle host species except at the northern and southern extents (Wood 1982; Tovar *et al.* 1995). The northeastern extent of *D. ponderosae* distribution in British Columbia and Alberta, based on recorded observations since 1975 (S. Taylor unpublished data), is represented as a dashed line. Sample locations for *D. ponderosae* populations included in this study (see Table 1) and linear distances (km) between sample locations (used for corrected geographical distance calculation) are also shown.

which typically remain at low levels within *Pinus* forests for many decades, can rapidly erupt to outbreak levels, killing vast areas of susceptible host trees (Raffa 1988). As an eruptive species, outbreaks of *D. ponderosae* can be impressive events, with annual losses often greater than fire or other natural disturbances (Dale *et al.* 2001). In 2004 alone, *D. ponderosae* affected more than 1.2 million ha in the western United States (Anonymous 2005) and 8 million ha in British Columbia (www.for.gov.bc.ca). Native forest outbreak species, such as *D. ponderosae*, are historically important drivers of vegetation dynamics in western pine

forests (Amman 1977). Although the adverse impacts of *D. ponderosae* on forest values have long been recognized, it has also become apparent that bark beetle disturbances, in concert with other natural disturbance agents, play a major role in maintaining the structure and function of healthy forest ecosystems (Romme *et al.* 1986; Bebi *et al.* 2003).

Lodgepole pine (*Pinus contorta*), a primary host for *D. ponderosae*, extends north into the Yukon and Northwest Territories and east across much of Alberta, areas where *D. ponderosae* outbreaks have not previously been recorded

(Fig. 1). With recent warming trends, the number of infestations in these areas has risen at an increasing rate, in large part due to a shift in the thermal suitability for sustaining *D. ponderosae* populations (Carroll *et al.* 2004). Similar shifts in thermal suitability have resulted in increased levels of *D. ponderosae* activity in other ecologically important, high elevation ecosystems in the western United States such as those dominated by whitebark pine (*Pinus albicaulis*) (Keane & Arno 1993; Meyer 2005). This increased activity is similar to the major outbreaks that occurred in these ecosystems during the warm periods of the 1930s and 1940s (Perkins & Swetnam 1996; Furniss & Renkin 2003). Although *Dendroctonus* were present in high elevation systems during the Holocene (A. Brunelle, personal communication), lifehistory strategies of high elevation pines (e.g. longer lifespan, smaller annual reproductive effort) suggest that they may not be as adapted to *D. ponderosae* infestations as shorter-lived species such as *P. contorta* that are maintained by periodic disturbances (Logan & Powell 2001). Additionally, continued increases in temperature could result in expansion of *D. ponderosae* outbreaks into jack pine, *Pinus banksiana*, a species not currently considered a host of *D. ponderosae* but which is contiguous with lodgepole pine in Alberta (Logan & Powell 2001). Expansion of *D. ponderosae* into jack pine would dramatically increase the range of this species into eastern Canada and central United States. Climate change-induced shifts of *D. ponderosae* into new habitats and hosts will undoubtedly result in significant ecological and socio-economic consequences (Ayres & Lombardero 2000).

In order to understand and effectively study the evolutionary and demographic trajectory of *D. ponderosae* under a changing climate regime, it is essential that we have a clear understanding of the evolutionary and demographic history of the species in western landscapes. Although previous investigations have found pronounced differences in allozyme allele frequencies among geographically distinct populations (Stock *et al.* 1984; Sturgeon & Mitton 1986a; Langor & Spence 1991; Kelley *et al.* 2000), the range-wide geographical distribution of molecular variation in *D. ponderosae* has never been investigated. Several researchers have also assessed genetic divergence among *Dendroctonus* populations occupying different sympatric host trees (Stock & Guenther 1979; Higby & Stock 1982; Stock *et al.* 1984; Stock & Amman 1985; Sturgeon & Mitton 1986a, b; Kelley *et al.* 1999; Kelley *et al.* 2000), although results in *D. ponderosae*, based on allozyme differentiation, have been inconclusive and/or inconsistent.

The specific goals of the present study were to (i) describe the pattern and degree of genetic variation in *D. ponderosae* across its range, (ii) determine whether there are detectable genetic differences among *D. ponderosae* populations occupying different host trees in common localities, and (iii) determine whether there is a molecular signature of demographic expansion range-wide and at the northern edge of the current range.

Materials and methods

Sample collection and DNA extraction

Live *Dendroctonus ponderosae* specimens were collected from eight locations across the species' range (Fig. 1, Table 1) by directly sampling under the bark of infested trees or using Lindgren pheromone traps (Lindgren 1983) baited with the mountain pine beetle lure (*trans*-verbenol, myrcen, *exo*-brevicomin, PheroTech, Inc., Delta, BC). In two of the locations, insects were collected separately for different

Table 1 Sample populations of mountain pine beetle (*Dendroctonus ponderosae*) included in this study, sampling method used to obtain these individuals, number of individuals used in AFLP analysis (N), and diversity indices for populations of *D. ponderosae* based on AFLP profiles. H, average heterozygosity (standard error); %P, percentage polymorphic loci (99% criterion)

Population identifier	Location	Host tree sampled	Sampling Method	N^{AFLP}	H (SE)	%P
BF-ID	Bonner's Ferry, Idaho	*Pinus albicaulis*	Infested trees	17	0.2057 (0.0168)	64.15
F-AZ	Flagstaff, Arizona	*Pinus flexilis*	Infested trees	17	0.1526 (0.0152)	46.54
FSJ-BC	Ft St James, British Columbia	*Pinus contorta*	Infested trees	21	0.1651 (0.0170)	45.91
K-UT	Kamas, Utah	*Pinus contorta*	Pheromone trap	21	0.2307 (0.0151)	74.21
L-OR	La Grande, Oregon	*Pinus contorta*	Infested trees	20	0.1933 (0.0159)	65.41
S-ID	Stanley, Idaho	*Pinus contorta*	Pheromone trap	20	0.2181 (0.0147)	74.84
R-CAW	Klamath, Oregon	*Pinus albicaulis*	Pheromone trap	20(R-CA)	0.1916 (0.0149) (R-CAW & R-CAL combined)	71.70
R-CAL	Klamath, Oregon	*Pinus contorta*	Pheromone trap	20(R-CA)		
SB-CAL	San Bernardino, California	*Pinus contorta*	Infested trees	18(SB-CA)	0.1626 (0.0151) (SB-CAL & SB-CAS combined)	64.78
SB-CAS	San Bernardino, California	*Pinus lambertiana*	Infested tree bolts	16(SB-CA)		

host tree species (*Pinus lambertiana* (SB-CAS, *n* = 16) and *Pinus contorta* (SB-CAL, *n* = 18) in San Bernardino California and *Pinus albicaulis* (R-CAW, *n* = 20) and *P. contorta* (R-CAL, *n* = 20) in Klamath, Oregon) for a total of 10 sample populations (Table 1). Insects were preserved in 95% ethanol and frozen upon arrival in the laboratory.

Dendroctonus ponderosae is known to carry symbiotic fungi, *Grosmannia clavigera* (formerly *Ophiostoma clavigerum*) (Zipfel *et al.* 2006) and *Ophiostoma montium*, in specialized maxillary compartments (mycangia), as well as a number of other fungi and yeast on external exoskeleton surfaces (Six 2003; Lim *et al.* 2005). Internal bacteria and yeasts are also known to play an important role in *Dendroctonus* biology (Conn *et al.* 1983; Delalibera *et al.* 2005) although few have been identified. We took three measures to minimize the probability of DNA from these associated organisms interfering with downstream nuclear analyses: First, we decapitated all beetles to reduce the chance of contamination by mycangial fungi. Second, we separated the thorax from the abdomen in 22 samples, including 2–3 individuals from each of the 10 study populations, and treated these samples as replicates. We replicated five additional samples prior to DNA extraction without separating the abdomen and thorax, for a total of approximately 14% of the total sample set. Third, we included in our nuclear analyses DNA extracted from pure cultures of two fungal associates (*G. clavigera* and *O. montium*, three isolates each) and two yeast species (*Pichia capsulatum* and *Pichia holstii*) isolated from *D. ponderosae*. DNA was extracted from our samples and replicates using a salt-chloroform protocol with isopropanol precipitation (Mullenbach *et al.* 1989).

Amplified fragment length polymorphism analyses

Amplified fragment length polymorphism (AFLP) analysis was used to assess nuclear variation among sampled populations of *D. ponderosae*. Although AFLP is an anonymous marker system that may include markers on the mitochondrial genome, the relative size of the nuclear genome dictates that the majority of AFLP bands will be nuclear. AFLP markers, while dominant, are widely used in phylogeographical studies in a broad range of taxa and can provide a valuable contrast to mitochondrial data sets in studies of population structure (e.g. Sullivan *et al.* 2004; Gompert *et al.* 2006). While mitochondrial sequence data has a long history of being useful in phylogenetic reconstruction, population-level gene genealogies based on any single linkage group or locus may not be representative of the broader genomic dynamics over time (Maddison 1996; Bazin *et al.* 2006), necessitating the use of multiple markers to infer organismal history. AFLP analysis can generate large numbers of markers distributed broadly across the nuclear genome (Vos *et al.* 1995; Mueller & Wolfenbarger 1999).

In this study, AFLP marker profiles were generated for a total of 190 individuals representing 10 populations (Table 1) using the protocol originally described by Vos *et al.* (1995) with modifications described in Mock *et al.* (2004). We used the following seven selective primer combinations (*5′ 6-FAM labelled): *Eco-AGG/Mse-AC; *Eco-AGG/Mse-AG; *Eco-ACG/Mse-AC; *Eco-ACG/Mse-AG; *Eco-ATG/Mse-AC; *Eco-ACC/Mse-AG; *Eco-ATC/Mse-AG. The amplicons were separated on a sequencing gel with a ROX 400 (Applied Biosystems) size standard using an ABI 3100 automated sequencer. Individual profiles were visualized and scored for each selective primer combination using GENOGRAPHER 1.6 software (Benham 2001). Markers were scored if they were polymorphic (95% criterion) and could be scored unambiguously across the data set. Scoring was performed without reference to sample or population identity. Initially, a total of 185 loci were scored across all samples. Ten of these loci were excluded because they were similar in size to bands observed in the fungal isolates. An additional 16 loci were excluded because they were sex-associated (see Results). Thus, the final AFLP data set included data from 159 polymorphic loci.

Population nuclear diversity was assessed by estimating percentage polymorphic loci (%P) and heterozygosity (H). Because AFLP markers are dominant, heterozygosity estimates required the assumption of Hardy–Weinberg equilibrium within populations. Allele frequency estimates were made using the Taylor expansion method (Lynch & Milligan 1994) implemented in the software TFPGA (Tools For Population Genetic Analysis) (Miller 1997). Heterozygosity and its standard error were estimated for each population using DISPAN software (Ohta 1993).

Genetic structuring among populations was characterized using Weir & Cockerham (1984) estimate (θ_{ST}) of Wright's F_{ST}. The 95% confidence intervals about θ_{ST} were calculated by bootstrapping 1000 times over loci. Genetic distances (D_a; Nei *et al.* 1983) among all pairwise populations were estimated and used to construct an unrooted neighbour-joining dendrogram using DISPAN software (Ohta 1993). The percentage of 1000 bootstrap pseudoreplicates (over loci) reproducing a particular node in this dendrogram was calculated as a measure of nodal support. Genetic distances among individuals, without pre-assigning population membership, were calculated based on AFLP profiles using a simple matching metric with NTSYSPC software (Rohlf 2000). A principle coordinates analysis (PCoA) plot was constructed to visualize these distances among pairs of individuals. The PCoA plot incorporated eigenvalues from the three eigenvectors capturing the most variation in the data set.

Pairwise population differentiation was assessed using Fisher's exact test (Raymond & Rousset 1995). This test was implemented using TFPGA software (Miller 1997), which uses a Markov chain Monte Carlo (MCMC) approach

(Manly 1991; Raymond & Rousset 1995) to approximate exact probabilities of the observed AFLP marker phenotype frequency differences. A combined probability test (Fisher 1954; Sokal & Rohlf 1995) was used to assess significance over all loci. Landscape-scale patterns of pairwise population genetic differentiation were described by plotting genetic distance (average pairwise simple matching distance between profiles of individuals from different populations, corrected by subtracting average distances within populations) against geographical distance. Geographical distance between population pairs was estimated in two ways: first by using linear map distances between adjacent populations (uncorrected geographical distance), and second by using cumulative stepwise distances among populations, excluding large regions lacking host tree species (corrected geographical distance, Irwin *et al.* 2005) (Fig. 1). The correlation between genetic and geographical distance matrices was assessed using a Mantel test (Mantel 1967; Sokal & Rohlf 1995), in which the probability of the observed Z statistic was estimated using 1000 random permutations of the genetic distance matrix to generate a distribution of Z-statistic values.

Mitochondrial DNA sequencing

The polymerase chain reaction (PCR) was used to amplify a region of mtDNA which included portions of the cytochrome oxidase I (COI) and cytochrome oxidase II (COII) coding regions, and the complete tRNA-LEU. This region of the mtDNA was selected based on the availability of published PCR primer sequences and the demonstration that there is an appropriate level of sequence variation to examine closely related species and divergent populations within bark beetles (Kelley & Farrell 1998; Kelley *et al.* 1999, 2000). These three regions were initially amplified as a single contiguous 1971-bp fragment with the primers C1-J-1718 and TK-N-3785 (Table 2). Internal primers listed in Table 2 were used in subsequent amplifications with the amplified 1971-bp fragment as template to generate overlapping segments for DNA sequencing. Amplifications were performed in 50 µL reactions using 2 µL of genomic DNA extraction, 5 µL 10× PCR buffer, 5 µL 8 mм dNTP mix, 5 µL of each 2 µм

primer, 0.2 µL *Taq* DNA polymerase (Perkin Elmer Cetus) and water to final volume. Reactions consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 92 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s, and a final extension step at 72 °C for 5 min. Negative controls were performed with all reactions. PCR products were purified using QIAGEN spin columns (QIAGEN) The DNA sequences of purified PCR products were obtained using an Applied Biosystems 3100 automated DNA sequencer using Dye Terminator Cycle Sequencing. PCR products were sequenced from both the forward and reverse directions. The sequence of single stranded DNA was obtained using these primers to generate overlapping fragments, which were assembled into contiguous sequences and aligned using sequencher 4.2.2 (Gene Codes). The final alignment of 71 *D. ponderosae* sequences comprised 1909 bp, including 1257 bp of COI, the complete tRNA-LEU (59 bp), and 591 bp of COII. The sequences of all unique *D. ponderosae* haplotypes were deposited in GenBank (Accession nos DQ865977–DQ866021), and referenced by haplotype designations provided below. *D. brevicomis* was used as an outgroup for phylogenetic analysis based on the hypothesis of specific relationships in Kelley & Farrell (1998).

Mitochondrial DNA analyses

Patterns of molecular diversity for mtDNA sequences within populations were assessed by estimating haplotype diversity (Nei 1987); nucleotide diversity (π) (Nei 1987), number of segregating sites (S); and the average number of nucleotide differences (k) (Tajima 1983) using the software dnasp version 4.10.3 (Rozas *et al.* 2003). The net number of nucleotide substitutions per site (D_a; Nei 1987) between populations was calculated, and a matrix of pairwise values constructed. Mantel testing (Mantel 1967; Sokal & Rohlf 1995) was used to assess (i) the correlation between this matrix and the corresponding matrix of pairwise population genetic distances from AFLP data (average corrected simple matching distances, see above), and (ii) the correlation of D_a between populations and both corrected and uncorrected geographical distances (as described for AFLP analyses, above). The Mantel tests were conducted

Primer	Sequence (5′–3′)	Source
C1-J- 1718	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al.* (1994)
C1-J-2193	CCAGGGTTTGGTATAATTTCTTA	This study
C1-N-2611	GCAAAAACTGCACCTATTGA	Cognato & Sperling 2000
C1-N-2650	CAATGATTCCCTCTTTTTACAGG	Kelley & Farrell (1998)
TL-J-3037	TAATATGGCAGATTAGTGCATTGGA	Contreras-Diaz *et al.* 2003
C2-N-3147	TTAGAGGGGAAGACCTATCTTGT	This study
TK-N-3785	GAGACCATTACTTGCTTTCAGTCATCT	Contreras-Diaz *et al.* 2003

Table 2 Primers used to amplify portions of mtDNA cytochrome oxidase I (COI), cytochrome oxidase II (COII) gene, and the tRNA^LEU in *D. ponderosae*. Primers were used for both PCR amplification and sequencing

using TFPGA software (Miller 1997), and the probabilities of the observed correlations were estimated using 10 000 random permutations of matrix elements.

Genetic structuring among populations was characterized using a hierarchal analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) with ARLEQUIN 2.0 software (Schneider *et al.* 2000) to estimate the distribution of mtDNA sequence variation within and among populations. Significance of the partitioning of molecular variance and the associated estimate of F_{ST} was assessed by conducting 1000 random permutations of the data. We estimated evolutionary relationships among haplotypes by constructing phylogenetic trees with the neighbour-joining (NJ) algorithm using PAUP* 4.0b10 (Swofford 2002). A dendrogram was constructed using the NJ algorithm with Jukes–Cantor distance correction (Jukes & Cantor 1969), chosen based on the recommendations of Nei & Kumar (2000) for data sets with an average number of mutations per site of 0.05 or less. Support for the internal nodes in this dendrogram was assessed by 1000 bootstrap psuedoreplicates of the data set.

We examined the sequence data for evidence of demographic expansion using three approaches: (i) distribution of pairwise differences (mismatch distribution), (ii) Tajima's *D* statistic (Tajima 1989), and (iii) a Markov chain Monte Carlo approach (Kuhner *et al.* 1998). The distribution of pairwise sequence differences at equilibrium is expected to yield a multimodal distribution, while sudden demographic expansions are expected to produce a star-like phylogeny and yield a unimodal distribution of pairwise differences (Slatkin & Hudson 1991; Rogers & Harpending 1992). Mismatch distributions of pairwise differences among haplotypes were generated with 1000 randomizations of the data using ARLEQUIN 2.0 software (Schneider *et al.* 2000). We calculated the probability of observing by chance a higher value of the sum of squared deviations between the observed and expected mismatch distribution under a model of demographic expansion (Excoffier 2004). An alternative approach, Tajima's *D* statistic (Tajima 1989) compares the population genetic parameter theta ($\theta = 2N_e\mu$), based on pairwise nucleotide differences (θ_π), with an estimate based on the number of segregating sites (θ_S). Assuming neutrality, the *D* statistic can be used to infer demographic history. For stable populations at equilibrium the expected value of these two estimators is equal. Since θ_π takes into account the frequency of polymorphisms, it is more sensitive to recent changes in effective population size. Negative values of Tajima's *D* suggest recent demographic expansion. Significance of Tajima's *D* was evaluated by comparison to a distribution generated from 10 000 random simulations of the data (using a coalescent simulation algorithm adapted from Hudson (1990)) in ARLEQUIN 2.0 (Schneider *et al.* 2000). Finally, the likelihood growth parameter *g* was estimated with a Metropolis–Hastings algorithm (Kuhner *et al.* 1995) as implemented in FLUCTUATE 1.3 (Kuhner *et al.* 1998). We obtained estimates of *g* using MCMC runs of 10 short chains (sampling increments of 100:2000 steps per chain) and two long chains (sampling increment 100:100 000 steps per chain). Because MCMC-based methods can become fixed on local optima, we conducted multiple independent runs, increasing the number of steps in the long chains until the SE of three consecutive estimates was less than 1% of the mean parameter estimate. Significant evidence of population growth based on the MCMC analyses was assessed by two criteria: first, a lower 99% CI of the likelihood estimate greater than zero, and second, a value of *g* greater than 3SD(*g*) (Lessa *et al.* 2003).

Results

Amplified fragment length polymorphism analyses

Initially, 175 loci were scored across all samples following exclusion of 10 bands comigrating with fungal AFLP bands. Using this data set, a PCoA plot was constructed based on simple matching distances among all individuals. The resulting plot (Fig. 2a) indicated the presence of two clusters of individuals, each with representatives from all populations. Prior to DNA extraction, gender had been determined microscopically (Lyon 1958) for 16 individuals (10 females, 6 males) from the San Bernardino, California population from *Pinus lambertiana* (SB-CAS). To test the possibility that sex-associated AFLP marker linkage was driving this clustering pattern, we examined gender-specific allele frequency differences across all loci in this population (Fig. 3). Sixteen loci showed striking gender-specific frequency differences. Twelve of these loci were fixed for the presence of the band in males and absence in females, and the remaining four loci showed male-to-female frequency ratios of 1.00:0.10, 0.83:0.10, 1.00:0.30, and 1.00:0.10, respectively. Although we lacked gender identification on the remaining samples, these markers tended to behave in a concerted fashion in other populations, and to occur at frequencies of near 0.5 (data not presented). When these 16 loci were removed from the data set and a new PCoA plot constructed, individual clustering by population was evident, and the population clusters were juxtaposed in a manner concordant with geographical locations (Fig. 2b). Taken together, this evidence suggests that there are a large proportion of male-associated AFLP markers in the mountain pine beetle. This pattern has been confirmed with subsequent independent AFLP analysis of 20 sex-identified insects (10 males, 10 females) from central Idaho (data not shown). The 16 gender-associated markers were excluded from our data set for further analyses.

The overall scoring error rate in the final AFLP data set (159 loci) was 1.99%. Mismatches were concentrated in
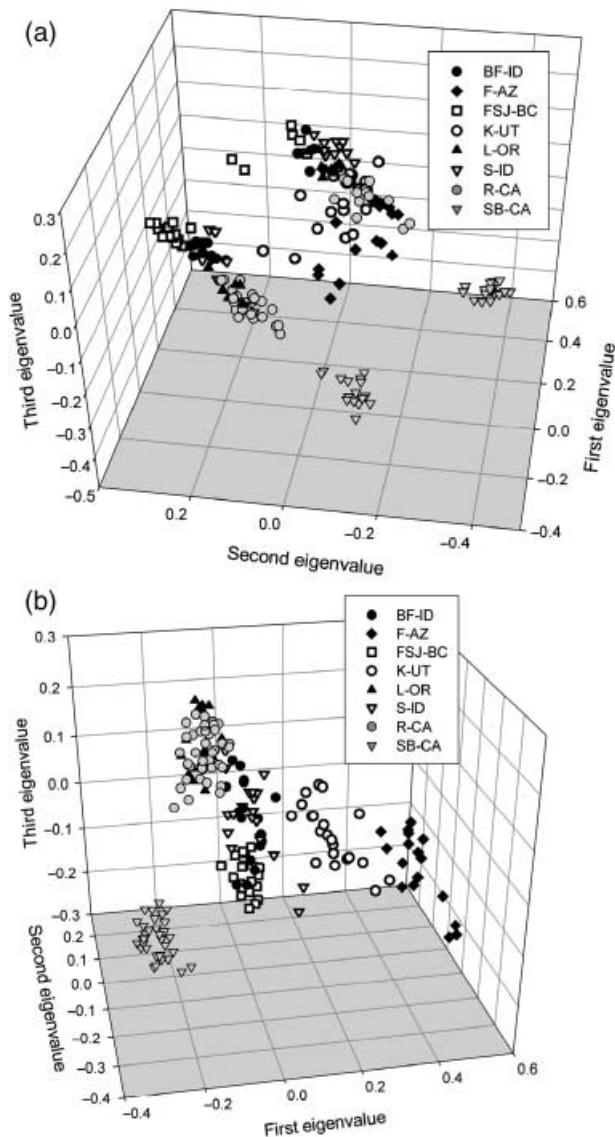
Where insects were sampled from two different host tree species in a common location (Redding and San Bernardino, California), no significant host-specific differences were found. The combined probability over loci of the observed AFLP marker phenotype frequency differences was greater than 0.999 in both the R-CAW and R-CAL populations and the SB-CAL and SB-CAS populations. Based on this finding, the sample populations from different host trees were combined for further analysis (R-CAW and R-CAL combined to R-CA; SB-CAS and SB-CAL combined to SB-CA).

AFLP-based estimates of genetic diversity were generally similar among populations (Table 1). The 95% confidence intervals (CI) for average estimated H across loci overlapped in all populations except K-UT (higher and nonoverlapping 95% CI with respect to F-AZ, SB-CA, and FSJ-BC) and S-ID (higher and nonoverlapping 95% CI with respect to F-AZ). Percentage polymorphic loci (%P) was strikingly lower in both FSJ-BC and F-AZ compared to the remaining populations, and these populations were among the three least diverse populations by both measures (H and %P) (Table 1). The rank order of population diversity was different depending on the metric (%P or H) (Table 1), but by both measures S-ID and K-UT were the most diverse. There was a tendency for the populations sampled using pheromone traps (S-ID, K-UT, and R-CA) to be more diverse than those sampled from infested trees, but it is unclear whether this tendency was a sampling artefact.

Population-level structuring across the sampling range based on AFLP data was pronounced: θ_{ST} over all populations was 0.304 (95% CI 0.267–0.341). This structuring was also evident in the PCoA plot of individual AFLP data (Fig. 2b). Patterns of genetic distance among populations were generally consistent with the geographical juxtaposition of the sample populations, as evidenced in the neighbour-joining dendrogram (Fig. 4) with the exceptions of the FSJ-BC population and the SB-CA population. The FSJ-BC population was similar to the S-ID and BF-ID populations (Figs 2b and 4) despite its large geographical distance from them (Fig. 1). The SB-CA population was geographically closest to the F-AZ population, but these two were genetically the most distant from one another. Genetic relationships among populations depicted with a neighbour-joining dendrogram (Fig. 4) were consistent with the PCoA plot (Fig. 2b) in inferring that the BF-ID and FSJ-BC populations are similar, that the SB-CA and F-AZ populations are the most distinct, and that the F-AZ population is most similar to the K-UT population. Pairwise population exact testing indicates, consistent with the PCoA plot, that neither the BF-ID and S-ID nor the L-OR and R-CA populations are significantly distinct (Table 3). Exact testing results also suggest that the BF-ID and L-OR populations and the L-OR and S-ID populations were not significantly distinct at the Bonferroni-corrected significance level of $P = 0.0018$, but
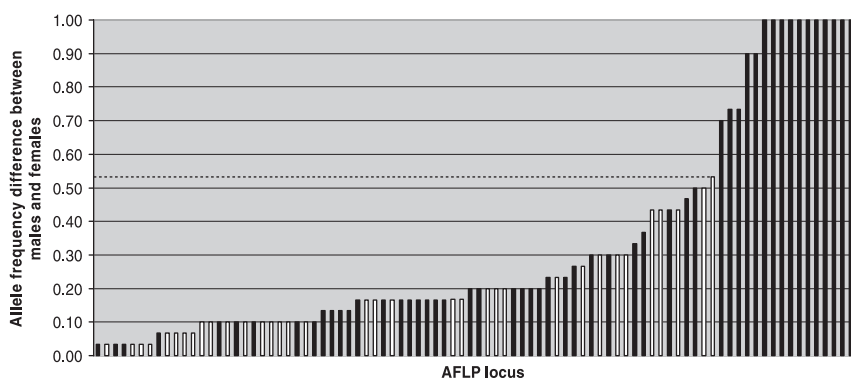

Fig. 2 Principle coordinates diagram of *Dendroctonus ponderosae* individuals; (a) based on a simple matching distance matrix of AFLP profiles at 175 loci, including 16 sex-associated loci that (b) were subsequently excluded from the data set for a total of 159 loci. Data from the first three eigenvectors (axes) are presented, which capture (a) 31.84% and (b) 29.2% of the total variation in the data. Sampling locations of individuals (see Table 1) are indicated by symbols.

particular replicates, with three individuals accounting for 62% of the mismatches. Among the abdomen/thorax replicates, there was no strong tendency among mismatches for the AFLP band to be present in the abdomen samples, as might be expected if gut endosymbionts were contributing additional bands: in 51.85% of the mismatches, bands were present in the abdomen samples but absent in the thorax samples.

Fig. 3 Absolute values of allele frequency differences between male and female *Dendroctonus ponderosae* from the San Bernardino CA population (6 males and 10 females). Black bars represent loci with bands more commonly present in males, and white bars are loci with bands more common in females. In all loci with frequency differentials > 53%, AFLP bands were generally present in males and absent in females. Loci with sex-associated frequency differentials > 53% (denoted by dashed line) were excluded from population analyses.

Table 3 Results of Fisher's exact tests for pairwise population differentiation in *Dendroctonus ponderosae*: values represent the probability of the observed pattern of allele frequencies under the null hypothesis that each pair of populations is actually a single, randomly mating group. Probabilities greater than the Bonferroni-corrected level of 0.0018 are in bold italics. Populations include Bonner's Ferry, Idaho (BF-ID); Flagstaff, Arizona (F-AZ); Fort St James, British Columbia (FSJ-BC); Kamas, Utah (K-UT); La Grande, Oregon (L-OR); Stanley, Idaho (S-ID); Redding, California (R-CA); and San Bernardino, California (SB-CA)

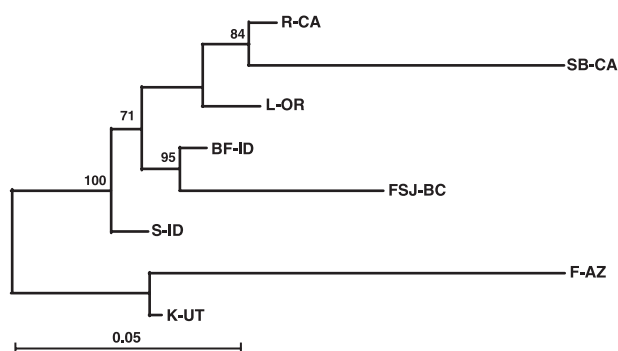|	BF-ID	F-AZ	FSJ-BC	K-UT	L-OR	S-ID	R-CA	SB-CA
BF-ID	**							
F-AZ	< 0.0001	**						
FSJ-BC	< 0.0001	< 0.0001	**					
K-UT	< 0.0001	< 0.0001	< 0.0001	**				
L-OR	*0.0073*	< 0.0001	< 0.0001	< 0.0001	**			
S-ID	*0.9807*	< 0.0001	< 0.0001	< 0.0001	*0.0670*	**		
R-CA	< 0.0001	< 0.0001	< 0.0001	< 0.0001	*0.1900*	< 0.0001	**	
SB-CA	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	**

Fig. 4 Unrooted neighbour-joining dendrogram of *Dendroctonus ponderosae* populations based on AFLP marker data (159 loci) using D_a genetic distance (Nei *et al.* 1983). The percentage of 1000 bootstrap pseudoreplicates (over loci) reproducing a particular node are provided. Population abbreviations correspond to Table 1.

both of these population contrasts were associated with probabilities less than 0.07 (Table 3).

The correlation between geographical and AFLP-based genetic distances among population pairs, assessed using both corrected and uncorrected geographical distances

(Fig. 5a, b), suggested that (i) a general range-wide pattern of isolation by distance exists in *Dendroctonus ponderosae* ($r = 0.82$, $P = 0.001$ with corrected geographical distances); (ii) the use of corrected geographical distances significantly improved the correlation between the genetic and geographical distance matrices, particularly for the comparison between F-AZ and SB-CA; (iii) FSJ-BC tended to be genetically more similar to other populations in comparison to the range-wide pattern; (iv) that F-AZ tended to be genetically more distant from other populations in comparison to the range-wide pattern; and (v) that long distance dispersal is unlikely to occur via routes that are not populated by host trees (particularly across the Mojave and Great Basin Deserts).

Mitochondrial sequencing analyses

Forty-five unique mitochondrial haplotypes were recovered from the 71 individuals sequenced. The proportion of unique haplotypes in our sample yielded a haplotype diversity of 0.975 (SD = 0.008). In contrast to this high haplotypic diversity, there was an overall low level of nucleotide polymorphism. Of the total 1909 bp, there were 111 variable sites with an average uncorrected pairwise
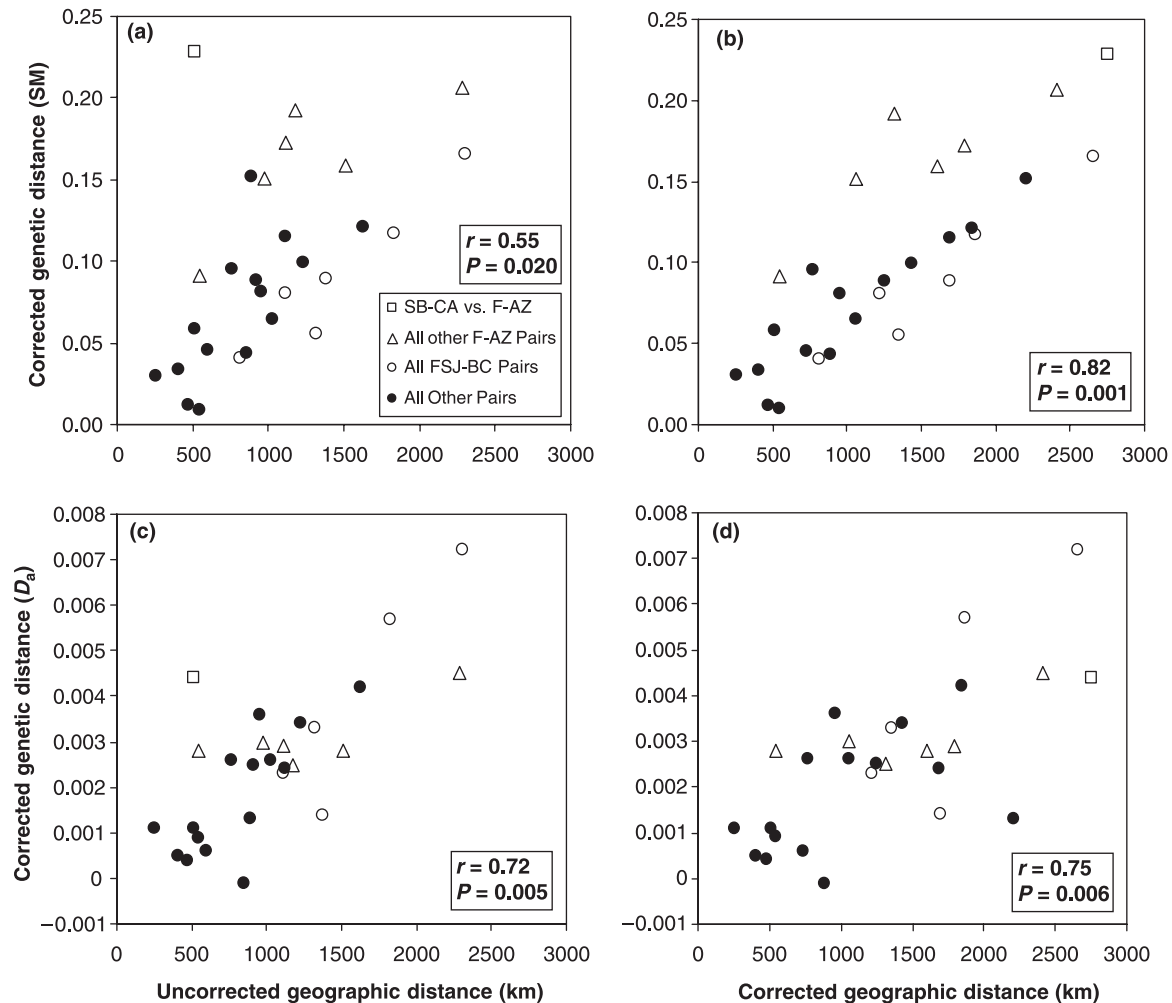
Fig. 5 Genetic distance between populations vs. geographical distance among pairs of mountain pine beetle populations in western North America using uncorrected (a, c) and corrected (b, d) geographical distances. Genetic distances estimated using AFLP profile phenotypes (SM) (a, b) and mitochondrial sequence divergence (D_a) (c, d) are assessed. In both analyses, pairwise genetic distances were corrected by subtracting average within-population distances from between population distances. Geographic distances were corrected by excluding pairwise connections across landscapes lacking appropriate host trees (See Fig. 1). Correlations (r) between matrices and associated probabilities, obtained through permutation testing, are provided.

sequence distance between unique haplotypes (P distance) of 0.74% with a range of 0.05–1.57%. The average uncorrected sequence distance between *Dendroctonus brevicomis* and *D. ponderosae* haplotypes was 11.06%.

Mitochondrial sequence data was obtained for 7–10 individuals per population. The within-population diversity measures are summarized in Table 4. The haplotype diversity (H_d) within populations ranged from 0.417 to 1.000 with an average of 0.808. The lowest H_d was found in the northernmost population (FSJ-BC), with three unique haplotypes out of 10 individuals sampled. In two populations, BF-ID and L-OR, all sampled individuals had unique haplotypes (H_d = 1.000). The number of segregating sites (S) and average number of nucleotide substitutions (k) among haplotypes followed a similar pattern: haplotypes

in the northernmost population (FSJ-BC) had the fewest segregating sites (2) and the lowest value of k (0.444). The low levels of polymorphism and diversity in the northern population was consistent with a recent range expansion and loss of genetic diversity due to founder effects.

Population-level structuring of mtDNA sequence variants across the sampling range was pronounced. The AMOVA partitioned 35.04% of the total molecular variance into the among-populations component, resulting in a highly significant F_{ST} over all populations (0.35, P < 0.00001). Regional structuring of mitochondrial haplotypes based on the AMOVA was equivalent to the structuring based on AFLP markers. The neighbour-joining tree showed little concordance with geographical structure and low support for most of the branching patterns (Fig. 6). While there was

Table 4 Summary statistics based on 1909 bp of COI and COII mtDNA sequence in *Dendroctonus ponderosae*. Number of individuals (N), number of haplotypes (N_H), haplotype diversity (H_d), nucleotide diversity (π), number of segregating sites (S), and average number of nucleotide differences (k) are presented. Populations include Bonner's Ferry, Idaho (BF-ID); Flagstaff, Arizona (F-AZ); Ft St James, British Columbia (FSJ-BC); Kamas, Utah (K-UT); La Grande, Oregon (L-OR); Stanley, Idaho (S-ID); Redding, California (R-CA); and San Bernardino, California (SB-CA)

Population	N	N_H	H_d	$SD(H_d)$	π	S	k
BF-ID	9	9	1.000	0.052	0.0058	42	11.056
F-AZ	10	6	0.778	0.137	0.0018	9	3.400
FSJ-BC	9	3	0.417	0.191	0.0002	2	0.444
K-UT	10	6	0.778	0.137	0.0075	40	14.356
L-OR	8	8	1.000	0.063	0.0076	37	14.571
S-ID	7	3	0.762	0.115	0.0063	22	12.000
R-CA	10	9	0.978	0.054	0.0072	51	13.667
SB-CA	8	4	0.750	0.139	0.0067	31	12.857
Total	71	45	0.975	0.008	0.0077	111	14.604

a high level of haplotypic endemism within populations, the well-supported nodes were generally restricted to small groups of haplotypes shared across multiple populations. The low levels of divergence among haplotypes and the lack of resolution at interior nodes in the tree were consistent with a recent origin for the populations across the range of *D. ponderosae* sampled for this study. Geographical clustering among haplotypes was apparent only in the FSJ-BC population. There was a significant correlation ($r = 0.654$; $P = 0.004$) between the pairwise population genetic distance matrices resulting from mitochondrial sequence data (D_a) and AFLP data (average corrected simple matching distance) (Table 5).

There was also a significant correlation between average pairwise D_a and both uncorrected ($r = 0.715$, $P = 0.006$) and corrected ($r = 0.747$, $P = 0.005$) pairwise geographical distances (Fig. 5c, d), consistent with the AFLP-based pattern of range-wide isolation by distance. However, the use of corrected geographical distances did not improve the correlation between the genetic and geographical distance matrices with mitochondrial sequence data as it did with AFLP data. Further, there was no tendency for mitochondrial sequence-based distances (D_a) to be greater between F-AZ and other populations, or for the genetic distances between FSJ-BC pairs to be less distant than other pairs. The differences between AFLP and mitochondrial sequence data may reflect differences in sample sizes, levels of variation, demographic processes, or coalescence times between these markers.

The mismatch distribution of observed mitochondrial haplotypes (mean 14.61, variance 51.76) failed to reject the model of sudden demographic expansion (sum of squared deviations 0.0063; $P = 0.27$). This result was consistent with
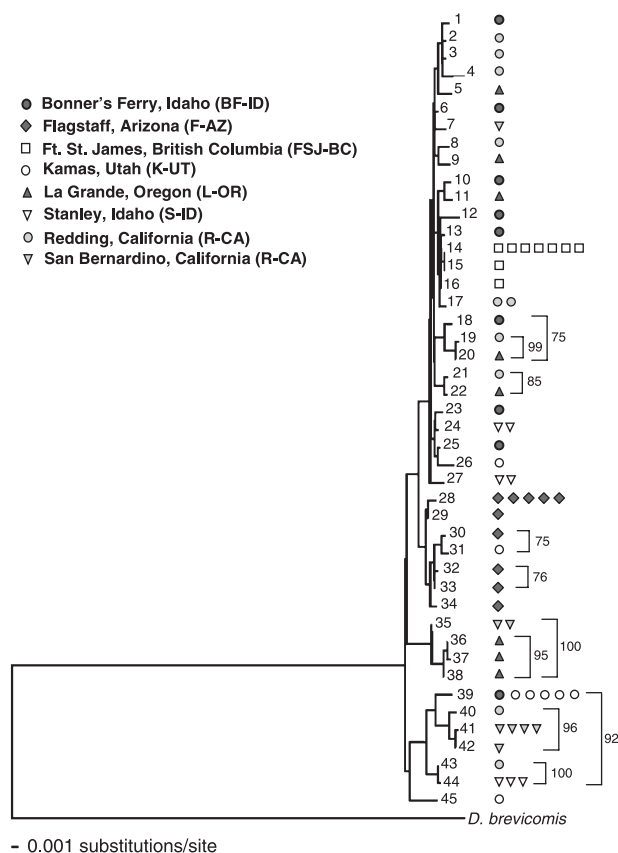

Fig. 6 Neighbour-joining dendrogram of 45 unique mitochondrial haplotypes (1909 bp) in *Dendroctonus ponderosae* (GenBank accessions DQ865977 – DQ866021), constructed using Jukes-Cantor (Jukes & Cantor 1969) corrected distance. Bootstrap support values greater than 75% based on 1000 pseudoreplications of the data are shown to the right of supported groups.

the positive exponential growth parameter from the likelihood analysis. The mean value of the likelihood growth parameter (g) from three independent runs (long chain length 100 000 steps) was 574.97 (SD = 71.34). The standard error of the mean value of g from these independent runs was 4.97 (0.9% of the mean). All MCMC runs indicated a significant positive estimate of g. In all cases the lower 99% CI was greater than zero and the estimated value of g was greater than 3SD(g). The value of Tajima's D for the entire range was −1.246. While not statistically different from zero, the negativity of this value is consistent with a demographic expansion event.

Discussion

Gender-associated AFLP loci

Our finding of a strong male–biased association in high proportion (9.1%) of our AFLP loci (Figs 2 and 3) is

Table 5 Matrix of genetic distances between *Dendroctonus ponderosae* population pairs. Genetic distances based on mitochondrial sequence data (net number of nucleotide substitutions per site (D_a; Nei 1983) are below the diagonal. Genetic distances based on AFLP data (average simple matching distances between AFLP profiles corrected by subtraction of average within-population distances) are above the diagonal

	BF-ID	F-AZ	FSJ-BC	K-UT	L-OR	S-ID	R-CA	SB-CA
BF-ID	**	0.1591	0.0404	0.0649	0.0339	0.0094	0.0437	0.1210
F-AZ	0.0028	**	0.2062	0.0916	0. 1921	0.1512	0. 1725	0.2279
FSJ-BC	0.0009	0.0045	**	0.1169	0.0805	0.0554	0.0890	0.1653
K-UT	0.0026	0.0028	0.0057	**	0.0956	0.0584	0.0884	0.1516
L-OR	0.0005	0.0025	0.0023	0.0026	**	0.0301	0.0121	0.0996
S-ID	0.0009	0.003	0.0033	0.0011	0.0011	**	0.0456	0.1153
R-CA	−0.0001	0.0029	0.0014	0.0025	0.0004	0.0006	**	0.0811
SB-CA	0.0042	0.0044	0.0072	0.0013	0.0034	0.0024	0.0036	**

consistent with observations by Lanier & Wood (1968) of a 'relatively large heteromorphic pair of sex chromosomes' in *Dendroctonus ponderosae* and *Dendroctonus jeffreyi*, and suggests that some portion of the Y chromosome may be nonrecombining. This marker-based phenomenon has not been described in other *Dendroctonus* species, but could be an important consideration in population genetic studies of this genus, potentially leading to erroneous conclusions about genetic structuring within populations or among host trees. Alternatively, gender-associated markers could be used to identify the sex of adults, eggs and larvae in future studies. Further investigation of these markers could also prove useful in understanding chromosome structure and evolution in these insects.

Landscape-scale genetic variation in D. ponderosae

Both AFLP and mitochondrial sequencing analyses revealed significant structuring (F_{ST} estimates 0.304 and 0.35, respectively) among sample populations, with gene flow in an approximately horseshoe shape around the Great Basin and Mojave Deserts (Figs 1, 4, and 5), a large area where the principal hosts of *D. ponderosae* are sparse. Within the *D. ponderosae* range sampled, populations generally followed a pattern of isolation by distance (Fig. 5). In this analysis, the use of geographical distances corrected for host tree distribution (Fig. 1) significantly improved the correlation between genetic and geographical distance between pairwise populations (Fig. 5). The effect was particularly pronounced for the comparison of SB-CA and F-AZ, which are separated by the Mojave Desert (Fig. 1). These findings suggest that although sparse islands of potential host trees exist in the Mojave Desert, gene flow between SB-CA and F-AZ is more likely to occur via routes populated by host trees along the eastern, northern and western edges of the Great Basin.

Mitochondrial sequencing revealed a very high level of haplotype diversity in all populations except FSJ-BC (discussed below) (Table 4). Only three of the 45 observed

haplotypes were shared by more than one population. Sequence diversity, by contrast, was low, with an overall average pairwise divergence of only 0.7% (Table 4). Relationships among haplotypes were generally unresolved (Fig. 6), and there was no tendency for similar haplotypes to cluster geographically except in F-AZ and FSJ-BC. It is likely that the high haplotypic diversity and low divergence in this system obscured geographical clustering that might have been apparent with a larger sample of individuals at each site, particularly if additional sampling allowed detection of more shared haplotypes. This observation, and the expectation that these eruptive beetles may have an extremely large population size, is consistent with incomplete lineage sorting. In contrast to the phylogenetic analysis, a high level of population structuring was detected by partitioning the molecular variance with an AMOVA analysis. The level of population structure for mtDNA sequence data ($F_{ST} = 0.35$) was slightly higher than that for AFLP data. This result is consistent with genetic drift acting more strongly on mitochondrial genes due to the lower effective population size of mitochondrial genes vs. nuclear genes. Overall, the two data sets are highly concordant, yielding comparable estimates of population subdivision and similar patterns of association between genetic distances and geographical distances. A direct comparison of the two data sets using a Mantel test showed a significant correlation between the matrices of pairwise population genetic distances using mitochondrial (D_a) and AFLP (average corrected simple matching distance) data (Table 5).

Dendroctonus ponderosae was originally described by Hopkins (1909) as two species, based on geographical distribution and morphological differences in both adults and immature forms. The boundary between the two original species, *D. ponderosae* and *Dendroctonus monticolae*, was in the narrow band of host tree distribution along the Utah and Idaho border near our K-UT sample population (Fig. 1). The range of *D. ponderosae* extended south and east into Wyoming, South Dakota, Utah, Colorado, Arizona

and New Mexico, and *D. monticolae* was found in Idaho, Montana and British Columbia as well as south into California along the west coast. These species were synonymized to *D. ponderosae* by Wood (1963) based on subsequent morphological (Blackman 1938) and hybridization (Hay 1956) studies. Lanier & Wood (1968) confirmed the synonymy using phenological and karyological observations. In our study, there was a tendency for the F-AZ population to be more divergent (based on AFLP analysis) from the other populations than expected given its geography (Figs 4 and 5), suggesting that the previous taxonomic boundary between *D. ponderosae* and *D. monticolae* (Hopkins 1909) may indeed reflect a genetic discontinuity. Interestingly, we also found that the K-UT and F-AZ populations shared two mitochondrial haplotypes (Fig. 6), possibly as a result of intermixing along this boundary (although incomplete lineage sorting cannot be ruled out). K-UT also shared a haplotype with BF-ID, a population well to the north, and had the highest levels of both haplotype sequence diversity (k) and AFLP diversity (H and percentage *P*) among all the sample populations (Tables 1 and 4). These patterns are consistent with the expectations under the scenario of recent contact between two distinct genetic stocks of *D. ponderosae*. It seems unlikely, however, that this genetic subdivision was a species-level phenomenon if the shared haplotypes are due to recent gene flow. By contrast, Kelley *et al*. (1999) found striking differentiation in populations of *Dendroctonus brevicomis* between western and eastern regions across its range (separated by the Great Basin), corresponding to two varieties of its only host tree *Pinus ponderosa*. As with *D. ponderosae*, *D. brevicomis* was originally described as two species (Hopkins 1909), based partially on geographical distribution, that were later synonymized (Wood 1982). *Dendroctonus pseudotsugae* also infests two different varieties of its host, *Pseudotsuga menziesii*, and fixed allozyme differences were found in beetles collected in the two geographically separated areas (Stock *et al*. 1979). Similarly, mitochondrial lineages in a related scolytine in this landscape, *Ips confusus*, have pronounced geographical structure that follows the Pleistocene distribution of pinyon pines (Cognato *et al*. 2003). Collectively, these results suggest that the phylogeography of many scolytines found in western North America, including *D. ponderosae*, is dictated by historical distributions of their host tree species. To fully asses comparative genetic structure between the two synonomized species of *D. ponderosae*, however, additional samples from the easternmost portion of the current range will be necessary.

Population-level variation among host trees

Although allozyme differentiation of *D. ponderosae* populations along host lines was previously reported (Stock & Amman 1985; Sturgeon & Mitton 1986a; Langor & Spence 1991;

Kelley *et al*. 2000), using AFLP profiles we were unable to detect statistically significant differences between *D. ponderosae* samples collected from different host trees in the same vicinity (*Pinus albicaulis* and *Pinus contorta* at R-CA; *P. contorta* and *Pinus lambertiana* at SB-CA). These results suggest that *D. ponderosae* is not mating assortatively by host tree species in these two populations, and are consistent with the current classification of *D. ponderosae* as a host generalist (Kelley & Farrell 1998). As suggested by Langor & Spence (1991), the sympatric host-specific differentiation observed using allozymes may reflect selective pressures on developing brood imposed by the different tree species. Such selective pressure is not likely to be detected using a panel of multilocus AFLP markers. Using mtDNA, Cognato *et al*. (2003) also found that host specificity did not greatly influence lineage diversification of *I. confusus*, and no differences were found between populations of *D. brevicomis* or *D. ponderosae* collected from two sympatric host species (Kelley *et al*. 1999, 2000). Cognato *et al*. (2003) reasoned that herbivore species spending only one or a few generations in a particular host are less likely to become genetically isolated because in subsequent generations alternative hosts within the same forest can be used.

Molecular evidence for demographic expansion in D. ponderosae

When a population undergoes an expansion, new mutations and lineages are preserved at a higher rate than expected in a stable population at mutation-drift equilibrium, leading to a shallow, starlike phylogeny. Older lineages may be lost or minimized as the expansion continues, and the population may come to be dominated by an array of closely related haplotypes. On the whole, our mitochondrial sequence data clearly showed this pattern, with high haplotypic diversity, little resolvable phylogenetic structuring among haplotypes, low levels of sequence divergence, and an absence of older (more divergent) lineages (Table 4 and Fig. 6). We specifically tested the hypothesis of demographic expansion using three different statistical treatments of the mitochondrial data: a mismatch distribution, calculation of Tajima's *D*, and calculation of a likelihood growth parameter *g*. The results of all three analyses were consistent with demographic expansion, although the negative Tajima's *D* was not significant. These results suggest a history of post-Pleistocene demographic expansion at a large geographical scale, perhaps in response to range-wide factors directly or indirectly favouring population growth such as fluctuations in host species abundance. The genus *Pinus* experienced a north to south dispersion from the northernmost part of North America into Mexico during the Early and Middle Tertiary (Van Dyke 1940). Subsequent climate and geological shifts

favoured a northward retreat during the Pleistocene (Mirov 1967) which is thought to have contributed to geographical distribution of the genus *Dendroctonus* (Zuniga *et al.* 2002).

The molecular signal of range expansion would be expected to have some of the same features as the signal of population expansion, since population expansions likely accompany range expansion. Additionally, at the leading geographical edges of a range expansion, recently established populations would be expected to have lower genetic diversity (manifest via both nuclear and mitochondrial markers) due to founder effects (Ibrahim *et al.* 1996; Rowe *et al.* 2004). Peripheral populations at these range edges would also be expected to bear a close resemblance to their source populations, perhaps sharing haplotypes. In *D. ponderosae*, this pattern is evident at the northern edge of the range, where new infestations have been documented. The FSJ-BC population, sampled from the northern edge of the *D. ponderosae* range, clearly shows reduced nuclear (Table 1) and haplotype (Table 4) diversity, and is most closely associated with BF-ID, the next most northerly population sampled (Figs 1, 4, and 6). In addition, the FSJ-BC population was somewhat less divergent from other populations than expected given its geographical distance from them (Fig. 5), also consistent with expectations for recent establishment via range expansion. Historically, the distribution of *D. ponderosae* has undoubtedly followed that of its primary host, lodgepole pine, which experienced a northward expansion into British Columbia and the Northwest Territories following the last glaciation period (Marshall *et al.* 2002). Contemporary increases in *D. ponderosae* activity are likely a result of a recent warming trend (Carroll *et al.* 2004). Because *D. ponderosae* is not limited by host availability in the northern portion of its range, our results suggest that given current climate change trajectories, *D. ponderosae* will continue to expand northward.

In the two southernmost populations sampled, the signals of range and population expansion were less clear. The F-AZ population had relatively low nuclear (H, %P; Table 1) and mitochondrial sequence (S, k; Table 4) diversity, but was also remarkably divergent from other populations in terms of nuclear allele frequencies (Figs 4 and 5). F-AZ was most similar to K-UT, and shared two haplotypes with that population (see Discussion of this result above). This pattern could be the result of range expansion (presuming that the source population was not sampled) or historical isolation and less population expansion than is seen in other localities. Flagstaff, Arizona is the southernmost locale where *D. ponderosae* is currently found on the eastern side of the Mojave Desert, although collections were recorded in 1907 from the Chiricahua National Forest in southern Arizona (Hopkins 1909). *D. ponderosae* voltinism and population success is significantly influenced by the seasonality of temperature (Logan & Bentz 1999), which undoubtedly plays a large role in determining the southern distribution of *D. ponderosae.* Bentz *et al.* (2001) hypothesized that current temperatures in southern Arizona were too warm for a successful univoltine population, but not warm enough for a bivoltine strategy. Discernment between the scenarios of range expansion, population isolation, and demographic stability or contraction in the F-AZ population would require sampling of additional populations and increased phylogenetic resolution. The SB-CA population was also divergent from other populations in terms of nuclear allele frequencies (Figs 4 and 5), although most similar to R-CA and consistent with expectations for isolation by distance (Fig. 5). The SB-CA population was not unusually lacking in nuclear diversity (H, %P; Table 1) or mitochondrial haplotype or sequence diversity (Table 4), and therefore lacked the hallmarks of recent range expansion. Similar to the F-AZ population, SB-CA is considered the southernmost distribution of *D. ponderosae* on the western side of the Mojave Desert, although collections have been recorded farther south in Baja California, Mexico (Wood 1982).

Conclusion and recommendation for further study

Overall, our results suggest that (i) *D. ponderosae* is not panmictic across its range, (ii) that populations generally bear the molecular signal of range-wide population expansion and range expansion to the north, and (iii) that there may be some discontinuity in an otherwise range-wide pattern of isolation by distance that is geographically congruent with the previous subdivision between *D. ponderosae* and *D. monticolae*. This study adds to a growing body of literature describing phylogeographical patterns driven by pre- and post-Pleistocene processes across a broad range of taxonomic groups in western North America. The emerging consensus based on numerous studies of plants (e.g. Soltis *et al.* 1997; Brunsfeld *et al.* 2001), vertebrates (e.g. Nielson *et al.* 2001; Carstens *et al.* 2005), and arthropods (e.g. Kelley *et al.* 1999; Cognato *et al.* 2003), is that taxa vary widely in phylogeographical structure across this region. The evolutionary and demographic processes contributing to this variation may be best described as a mosaic of relatively old vicariance events, Pleistocene refugia, and more recent patterns of range expansion and dispersal (Carstens *et al.* 2005). The differences in pattern observed among taxa are likely the result of differences in life histories and dispersal capabilities and in some cases may reflect co-evolutionary association between taxa. While studies of the phylogeographical structure in other members of the genus *Dendroctonus* have shown patterns of divergence consistent with older vicariance events (Kelley *et al.* 1999), the pattern in *D. ponderosae* suggests recent range expansion and divergence. These differences may be, in part, due to the outbreak dynamics and lack

of strict host specificity of *D. ponderosae*. In the northern portion of the range, the lack of pronounced genetic structure and shallow divergence among haplotypes may also be associated with the phylogenetic patterns of host trees. For example, lodgepole pine (*P. contorta*) has a similar phylogenetic pattern of shallow divergence suggesting recent range expansion (Marshall *et al.* 2002). We recommend that future studies in this system include sampling of additional populations in the eastern portion of the species' range, incorporation of codominant markers such as microsatellites (not currently available for this species), and the exploration of more variable regions of the mitochondrial genome. Additionally, the discovery of gender-biased AFLP markers in this study presents the possibility of interesting chromosomal and gender-specific evolutionary research in this genus. In an era of rapid climate change, *D. ponderosae*, as a major ecological driver that is extremely sensitive to thermal shifts, will continue to be an important model system for evolutionary ecology and natural resource management (Logan & Powell 2001; Hicke *et al.* 2006; Taylor *et al.* 2006). Our results contribute to a growing understanding of the status and evolutionary history of this important species.

Acknowledgements

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