

Geographic Variation in Bacterial Communities Associated With the Red Turpentine Beetle (Coleoptera: Curculionidae)

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ABSTRACT Bacterial communities are known to play important roles in insect life histories, yet their consistency or variation across populations is poorly understood. Bacteria associated with the bark beetle *Dendroctonus valens* LeConte from eight populations, ranging from Wisconsin to Oregon, were evaluated and compared. We used the culture-independent technique of denaturing gradient gel electrophoresis to visualize bacterial diversity, or individual operational taxonomic units (OTUs), from individual beetles. One-way analysis of similarities was used to test for differences of bacterial communities between sites. Analysis of community profiles showed that individual beetles on average contained 10 OTUs, with frequency of association from 2 to 100% of beetles. OTU sequences most closely matched β - and γ -proteobacteria, and one each matched Bacilli and Actinobacteria. Several OTUs were particularly abundant, most notably an Actinobacterium from 100% and two Proteobacteria from 60% of beetles sampled. Some OTUs were similar to previously described bacteria with known biochemical capabilities and ecological functions, suggesting that some bacterial associates of *D. valens* may contribute to its ability to exploit a resource low in nutrients and high in defensive compounds. There were significant differences of bacterial communities between sites. The strength of these differences was positively correlated with distance between sites, although additional unexplained factors also contribute to the variation.

KEY WORDS community analysis, microbial diversity, symbiosis, bark beetle

Associations with bacteria can have important implications to insect reproductive success, community interactions, and niche diversification (Mueller et al. 2005, Zilber-Rosenberg and Rosenberg 2008, Douglas 2009), yet little is known about how the distributions and frequencies of association of bacteria vary across insect populations. Some endosymbionts are known to show spatial variation (Funk et al. 2001, Tsuchida et al. 2002, Hansen et al. 2007), but geographic variation among bacteria with other relationships is largely unknown.

Microorganisms contribute to insect development and survival in harsh environments through acquisition of scarce nutrients, metabolism of toxins, and protection against natural enemies. Such relationships seem to be especially prevalent among insects that exploit woody substrates, which are relatively nutrient poor, heavily chemically defended, and available to competitors once defenses are depleted (Martin et al. 1991, Ayres et al. 2000, Cardoza et al. 2006, Geib et al. 2008, Scott et al. 2008, Morales-Jiménez et al. 2009).

Thus, many subcortical-feeding insects such as ambrosia and bark beetles, woodwasps, and termites possess fungal symbionts that confer a variety of benefits (Breznak 1982, Breznak and Brune 1994, Six and Klepzig 2004, Douglas 2009). However, the role of bacterial symbionts in the reproductive success of wood-boring insects has been comparatively understudied (Vasanthakumar et al. 2006, Warnecke et al. 2007, Klepzig et al. 2009). Such relationships can be complex, as for example, the ability of some bark beetle-associated bacteria to stimulate or inhibit growth and asexual reproduction of fungal associates can be affected by host tree chemistry (Adams et al. 2009).

The red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae), is a solitary bark beetle that colonizes the lower stems of many species of pine. In its native range from northern Central America to southern Canada, it usually prefers trees weakened by disease or stress and typically completes development without killing the host (Klepzig et al. 1991, Owen et al. 2005). In the late 1990s, *D. valens* invaded parts of China, where it became a primary mortality agent of *Pinus tabulaeformis* Carr. (Yan et al. 2005). *D. valens* is associated with several symbiotic ascomycete fungi within the family Ophiostomataceae: *Leptographium procerum* (Kendrick) Wingfield, *Leptographium terebrantis* Barras and Perry, and

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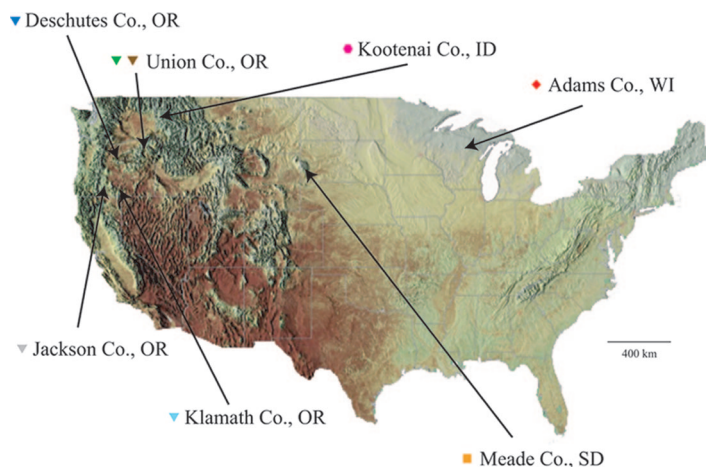


Fig. 1. Collection sites of *D. valens*. Colored symbols match site locations in Figs. 3 and 4. (Online figure in color.)

Ophiostoma ips (Rumbold) Nannfeldt (Klepzig et al. 1991). The exact roles of these fungi in the ecology of *D. valens* are unknown, although *L. procerum* and *L. terebrantis* are weakly virulent phytopathogens (Wingfield 1986) and thus may facilitate tree colonization.

Bacteria associated with guts of *D. valens* have been identified from three divisions, γ -proteobacteria, Firmicutes, and Actinobacteria, with a total of 13 species detected (Morales-Jiménez et al. 2009). We quantified the bacterial community variability and determined the frequency of association of specific bacterial phylotypes with individual *D. valens* across beetle populations, at sites ranging from Wisconsin to Oregon. Components of the bacterial community profiles were excised and sequenced to determine taxonomic identification using the culture-independent method of denaturing gradient gel electrophoresis (DGGE) (Nocker et al. 2007). We hypothesized that beetles from all sites would share a small number of bacterial taxa. Because geographic isolation and habitat variability can affect symbiotic community structure, we further hypothesized that between-site variability in bacterial communities would exceed within-site variability.

Materials and Methods

Beetle Sampling and Site Location. Five adult beetles were sampled from each of eight sites in the northwestern Rocky Mountain and Great Lakes regions of the United States in 2007 and 2008, with the exception of four beetles from one site (Fig. 1). Beetles were collected from host tissues (Adams Co., WI; Kootenai Co., ID; Deschutes Co., OR) or flight traps baited with Δ -3-carene (Meade Co., SD; two sites in Union Co., OR; Jackson Co., OR; Klamath Co., OR). The forest types included a red pine plantation (WI), lodgepole pine (SD), ponderosa pine (ID; Union Co., OR), mixed ponderosa pine/Douglas fir (Union Co., OR), mixed ponderosa pine, sugar pine, Douglas-fir, white fir, cedar (Jackson Co., OR), and mixed pon-

derosa pine, sugar pine, lodgepole pine, white fir (Klamath Co., OR). All beetles were placed in 100% ethanol in separate vials in the field and transported to the laboratory.

DNA Extraction, Amplification, and DGGE. DNA extraction was performed on whole beetles. Beetles were manually crushed, and DNA was extracted following the manufacturer's directions of the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA).

Polymerase chain reaction (PCR) amplification conditions and DGGE conditions are as described previously (Feris et al. 2003, Newberry et al. 2004). Briefly, nested PCR was performed under sterile conditions using the conserved 16S rRNA gene primers. Initial PCR was performed using 27f (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492r (5'-GGT TAC CTT GTT ACG ACT T-3') primers (Newberry et al. 2004). Reaction conditions were as follows: 1 μ l of template DNA was amplified with Promega *Taq* (Promega, Madison, WI) with thermal cycler conditions: 20 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Targeted PCR was performed with the clamped primer 536fC (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCCC CWT AAT GGC GCC GMC GAC-3') and 907r (5'-CCC CGT CAA TTC CTT TGA GTT T-3'). PCR amplicons were separated via DGGE using a D-Code system (Bio-Rad Laboratories, Hercules, CA). Each DGGE gel contained two sets of internal standards to allow cross-gel comparisons: 100-bp ladder (Sigma-Aldrich, St. Louis, MO) and a separate lane with $\approx$ 700 ng of PCR product amplified from two *D. valens* samples. A linear gradient of denaturant ranging from 40 to 60% (7 M urea : 40% [wt:vol] formamide) in a 6% acrylamide gel matrix was used. Gels were run at 130 V for 5 h. DGGE bands were visualized by staining with SYBR Safe Stain (Invitrogen, Carlsbad, CA) and photographically captured (Fotodyne, Hartland, WI).

Bands that appeared to be prevalent across samples were excised from gels (Gene Catcher; The Gel Company, San Francisco, CA) and reamplified with 535f and 907r, nonclamped primers. Clone libraries were

created using the pT7Blue-2 Perfectly Blunt Cloning Kit (Novagen, Darmstadt, Germany), purified with the Qiagen Plasmid Mini Kit, and sequenced (Polymorphic DNA Technologies, Alameda, CA). Clones obtained were reamplified with the clamped primers and run on DGGE to verify that the sequences obtained were from targeted bands. Sequences of bands were compared with those deposited in GenBank, and the closest matches to named bacteria are reported.

Community Analysis. Determination of presence and location of bands in the gel was conducted using Bionumerics (Applied Maths, Austin, TX) band match function. Each unique band was considered an operational taxonomic unit (OTU). Sørensen's similarity index (Sørensen 1948) was used to compute the similarity of bacterial communities of the beetles (Primer E software v. 6; Clarke and Gorley 2006). This index incorporates the presence of community members (OTUs) common to paired samples and does not include OTU abundance in the calculation. The similarity matrix was used to construct a dendrogram from group average cluster analysis (Primer E software v. 6, Clark and Gorley 2006). Additionally, the matrix was analyzed with a one-way analysis of similarities (ANOSIM, Primer E software v. 6) to test the null hypothesis that association of OTUs with beetles was independent of site. In this test, permutations of random mixing of OTUs with beetles were used to compare with actual associations. A similar dendrogram was constructed from group average cluster analysis, with presence of OTUs tallied across beetles from each site.

These values were used to construct a multidimensional scaling (MDS) plot (Primer E software v. 6, Clark and Gorley 2006). The MDS plot was used to arrange samples in two-dimensional space according to their relative similarities. In this procedure, a stress value was calculated to indicate the efficiency of sample placement. A stress value of <0.05 represents an ordination with nearly perfect congruency between placement in the MDS and the similarity matrix. A stress value of 0.3 or greater represents an ordination in which sample placement in the plot is random (Clarke and Warwick 1994). To determine whether bacterial community differences between sites were related to distances between them, a correlation Z test was used. Percentage of dissimilarity was determined by Sørensen's similarity index.

Once the MDS plot was constructed the BvSTEP procedure was used to select the OTUs that were the best predictors of the pattern (Clarke and Warwick 1994). After the BvSTEP procedure, the OTUs most responsible for the overall pattern were separated from those considered to be outliers, and separate MDS plots were made for each group.

The similarity percentages–species contributions one-way analysis (SIMPER) was used to quantify the contribution of each OTU to within-site similarity and between-site dissimilarity (Primer E software v. 6, Clarke and Gorley 2006).

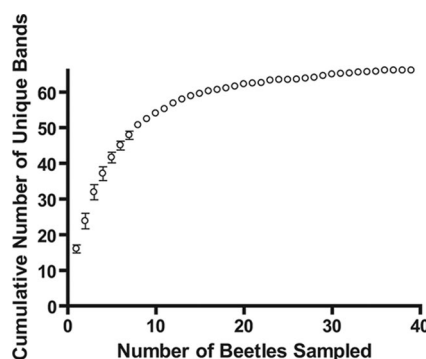


Fig. 2. Cumulative number of unique bands accumulated with sampling intensity. Values were calculated by averaging the number of unique bands given 10 permutation of random ordering.

Results

Identification of Bacteria Associated With *D. valens*. Bacteria detected by DGGE seem to be effectively sampled, as seen by the leveling of the rarefaction curve after a sampling intensity of ≈ 14 beetles (Fig. 2). Four classes of bacteria were detected from sequencing of bands: Actinobacteria, Bacilli, β -proteobacteria, and γ -proteobacteria (Table 1). β - and γ -proteobacteria were the most OTU-rich, with six β -proteobacteria and 11 γ -proteobacteria detected (Table 1). Sequences of β -proteobacteria most closely matched *Burkholderia phenazinium*, *Massilia timonae*, *Delftia tsuruhatensis*, and *Tetrathibacter kashmirensis* (Table 1). Sequences from γ -proteobacteria most closely matched *Enterobacter amnigenus*, *Pantoea endophytica*, *Pseudoxanthomonas spadix*, *Rahnella aquatilis*, *Yersinia ruckeri*, and *Xanthomonas* sp. (Table 1). Several sequenced OTUs most closely matched *Rahnella* sp. or *Rahnella aquatilis* and *Yersinia ruckeri*. The *Rahnella* sequences also closely matched sequences of bacteria in the genera *Yersinia*, *Serratia*, *Hafnia*, and *Aranicola*, and the *Yersinia* sequences also closely matched sequences of *Serratia* sp. The sequence of one OTU matched that of an uncultured Actinobacterium in Rubrobacteridae and another matched a Bacilli, *Bacillus pumilus* (Table 1).

Bacterial Community Analysis. The MDS procedure resulted in two-dimensional arrangement of bacterial profiles grouped by site (Fig. 3). In this MDS, the stress value of 0.26 indicates that the spatial orientation of the points in two-dimensional space was loosely representative of their relative similarity. By removing bacteria that were not the best indicators of within-site communities (Fig. 4A), the two-dimensional arrangement of the MDS improved slightly (Fig. 3, stress = 0.24). Additionally, the spatial patterns of similarities between these two datasets were very similar. In contrast, the arrangement of the outlying OTUs was nearly equal to random (stress = 0.29) and bore little resemblance to the MDS of either the full dataset or the dataset using only the best indicators of community similarity (Fig. 3).

Table 1. Bacteria associated with *D. valens*: designations indicate closest match of bands sequenced from DGGE profiles represented in Fig. 4A

Phylogenetic group (class)	OTU no. ^a	Number of clones	Accession no.	Closest named match ^b	S _{ab} score ^c	Frequency of detection (%)
Actinobacteria	66	5	GQ423617	Uncultured Rubrobacteridae	95	100
Bacilli	38	3	GQ423616	<i>Bacillus pumilus</i>	100	38.5
β-Proteobacteria	41	3	GQ423602	<i>Tetrathlobacter kashmirensis</i>	96	60.0
	46	2	GQ423612	<i>Delftia</i> sp.	100	48.7
	26	3	GQ423601	<i>Massilia timonae</i>	100	25.6
	49	3	GQ423613	<i>Delftia tsuruhatensis</i>	99	25.6
	24	4	GQ423599	<i>Burkholderia phenazinium</i>	97	20.5
	23	4	GQ423600	<i>Massilia</i> sp.	100	7.7
γ-Proteobacteria	56	2	GQ423615	<i>Pseudoxanthomonas spadix</i>	99	60.0
	40	3	GQ423606	<i>Yersinia ruckeri</i>	100	48.7
	44	5	GQ423607	<i>Yersinia ruckeri</i>	99	46.2
	63	2	GQ423614	<i>Yersinia ruckeri</i>	100	38.5
	35	3	GQ423610	<i>Pantoea endophytica</i>	98	35.9
	37	3	GQ423611	<i>Xanthomonas</i> sp.	100	33.3
	43	6	GQ423608	<i>Enterobacter amnigenus</i>	99	30.8
	36	2	GQ423603	<i>Yersinia ruckeri</i>	99	20.5
	42	3	GQ423604	<i>Yersinia ruckeri</i>	99	15.4
	7	3	GQ423609	<i>Rahnella aquatilis</i>	100	12.8
	28	2	GQ423605	<i>Yersinia ruckeri</i>	100	7.7

^a DGGE band placement as determined by BioNumerics to OTU no., represented in Fig. 4A.
^b Top match as determined by the BLAST method in GenBank. 16S rRNA gene sequences from cloned DGGE bands.
^c S_{ab} score [percentage of similarity of clone sequences] reported from GenBank.

A majority of the bacterial community profiles from each site clustered closely in the MDS with both all detected OTUs and only those OTUs selected by the BvSTEP procedure (Fig. 3). Spatial groupings were especially striking for four beetles from Adams Co., WI, and the combined grouping of four beetles from Meade Co., SD, and five from Kootenai Co., ID. The two-dimensional spacing of bacterial community similarity was supported by cluster analysis (Fig. 4A) and ANOSIM, which detected significant dif-

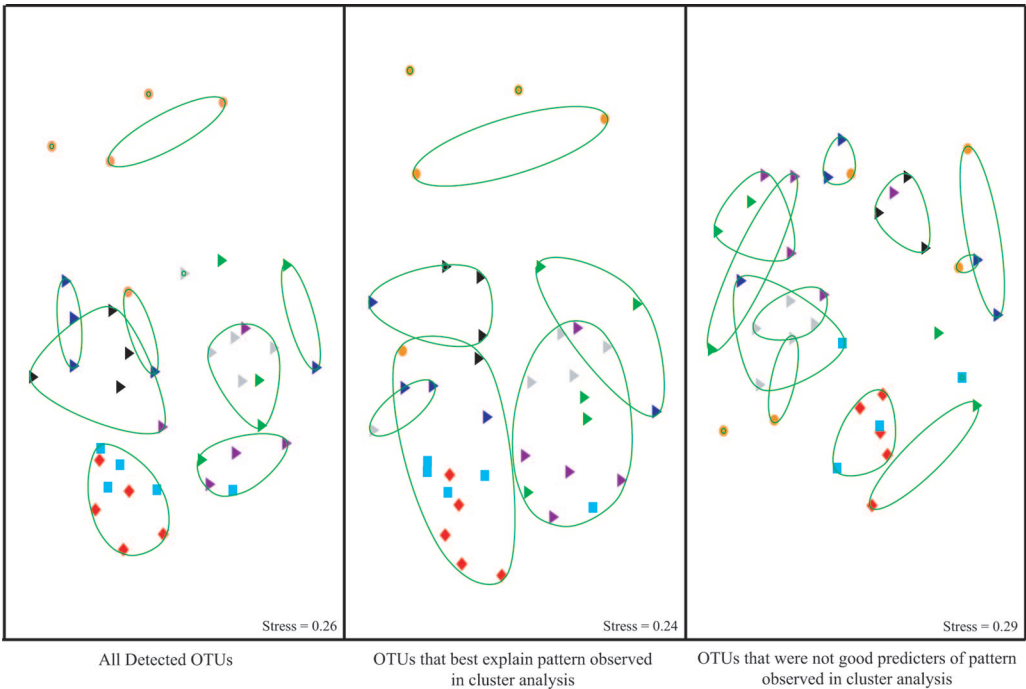


Fig. 3. MDS plots of similarities of bacterial communities associated with *D. valens*. MDS plots compare communities from all OTUs detected (left), those selected by BvSTEP procedure (center), and those removed by BvSTEP procedure (right). Two-dimensional arrangement was determined by cluster analysis and bacterial communities more similar than 41% are enclosed by circles. Symbols correspond to collection site locations as labeled in Figs. 1 and 3. (Online figure in color.)

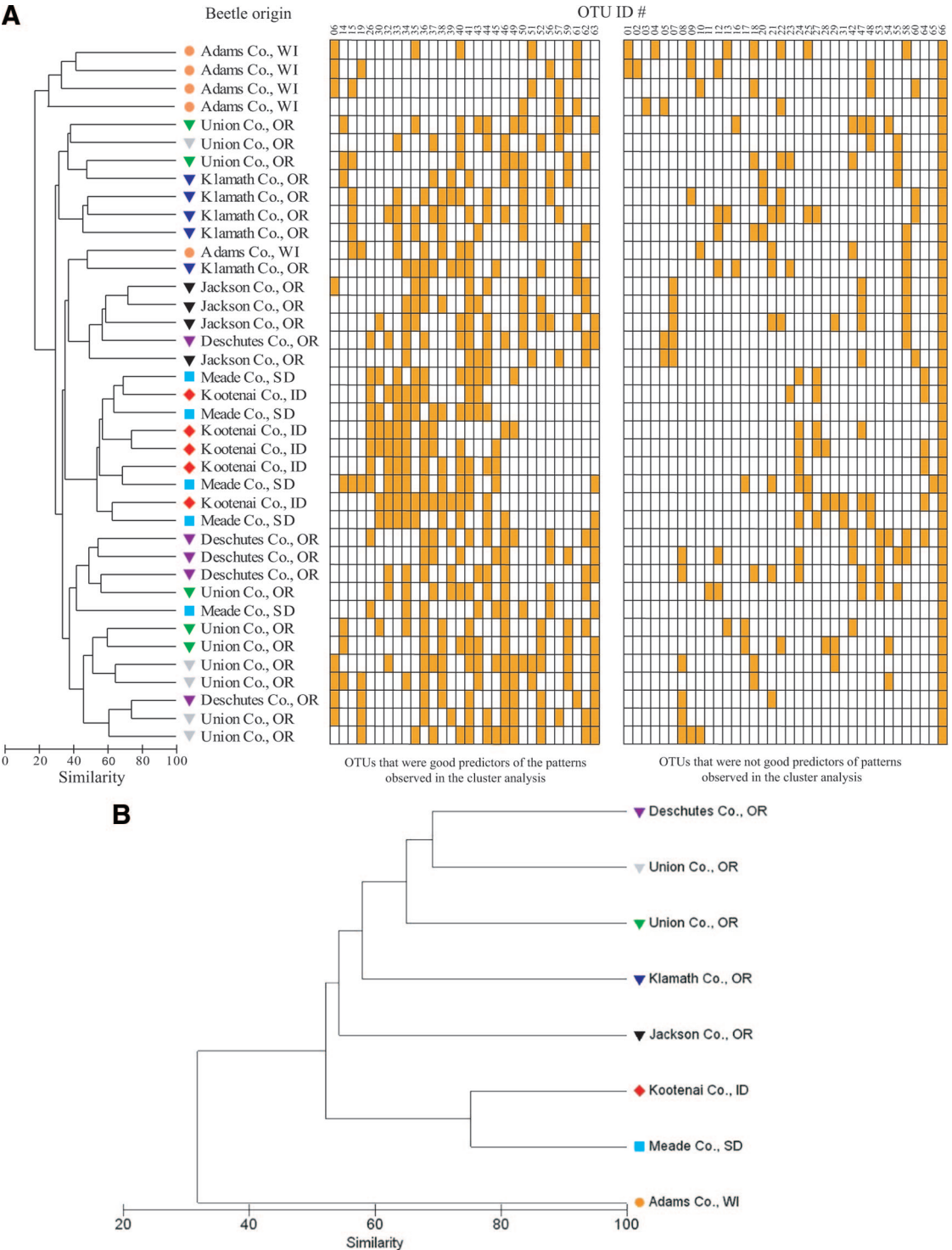


Fig. 4. (A) Graphic depiction of bacterial profiles from *D. valens* beetles. Representations contain OTUs selected to best explain community patterns of association (left) and OTUs that were not good predictors of community patterns of association (right) as determined by BvSTEP. The dendrogram clustered bacterial profiles according to Sorensen's similarity index performed on all OTUs detected from DGGE. Sequenced OTUs are listed in Table 1. (B) Dendrogram from group average cluster analysis of bacterial community similarity across beetles from each site. (Online figure in color.)

Table 2. Average dissimilarity (*R* statistic, *P* value) of bacterial communities of *D. valens* between sites

	Adams Co., WI	Deschutes Co., OR	Klamath Co., OR	Jackson Co., OR	Kootenai Co., ID	Meade Co., SD	Union Co., OR A	Union Co., OR B
Adams Co., WI								
Deschutes Co., OR	80.5 (0.74, 0.008)							
Klamath Co., OR	75.1 (0.42, 0.016)	69.6 (0.57, 0.008)						
Jackson Co., OR	74.9 (0.47, 0.024)	63.7 (0.53, 0.016)	66.7 (0.46, 0.016)					
Kootenai Co., ID	84.5 (0.86, 0.008)	64.9 (0.75, 0.008)	69.6 (0.68, 0.008)	69.1 (0.98, 0.008)				
Meade Co., SD	81.7 (0.79, 0.008)	62.9 (0.42, 0.032)	67.5 (0.40, 0.016)	66.8 (0.72, 0.008)	46.9 (−0.02, 0.532)			
Union Co., OR A	81.3 (0.72, 0.008)	63.1 (0.26, 0.079)	67.7 (0.25, 0.079)	67.2 (0.49, 0.032)	72.0 (0.72, 0.008)	67.8 (0.49, 0.016)		
Union Co., OR B	77.2 (0.61, 0.008)	56.6 (0.11, 0.214)	70.6 (0.47, 0.024)	68.8 (0.69, 0.008)	70.3 (0.79, 0.008)	65.6 (0.51, 0.024)	61.8 (0.13, 0.190)	

Values indicate average percentage of dissimilarity according to SIMPER and pairwise *R* statistics and significance levels according to ANOSIM test.

ferences from 22 of 28 pairwise comparisons between sites (Table 2).

Differences between the bacterial communities associated with beetles were positively correlated with distance between sites (correlation coefficient = 0.528, *Z* value = 2.936, *P* < 0.005; Fig. 5). Most notably, dissimilarity tended to be greatest between the most geographically isolated site, Adams Co., WI, and all other sites. The single greatest dissimilarity was 84.5% between Kootenai Co., ID, and Adams Co., WI (Table 2). The sites with the most similar bacterial communities were usually near each other, such as those between beetles from Union Co., OR sites A and B, and between Union Co. and Deschutes Co., OR. An exception was in the most similar communities, which were between the distantly separated Kootenai Co., ID and Meade Co., SD sites. These patterns are especially apparent when comparing pooled communities within each site (Fig. 4B).

All beetles from all sites were associated with one common OTU, an uncultured Actinobacterium (Table 1; Fig. 3, OTU 66). At least one additional OTU was detected from all beetles from each of four sites: Deschutes Co., OR (OTU 36, 46, closest match *Delftia* sp., and 63, closest match *Yersinia ruckeri*); Jackson Co., OR (OTU 41, closest match *Tetrathiobacter kashmirensis*); Kootenai Co., ID (OTU 32, 33, 34, and 36); and Union Co., OR B (OTU 46, closest match *Delftia* sp.)

(Table 3; Fig. 4A). These OTUs were relatively large contributors to overall community similarities within these sites. The bacterial communities of beetles from these four sites had relatively higher within-site similarity than those of the other four (Table 3). Communities from Adams Co., WI, and Klamath Co., OR, were least similar, with all but one OTU detected in less than four beetles (Table 3). Communities from Meade Co. and Union Co., OR, A were moderately similar, having no OTUs common to all beetles, other than the Acinobacterium, but several common associates from four of the five beetles (Table 3).

Discussion

Four classes of bacteria were found to be associated with *D. valens*, with frequencies of association ranging from 2 to 100% of beetles. The composition of these communities varied among sites, and geographic distance partially explains these differences. For example, bacteria communities of beetles from the most isolated site, Adams Co., WI, were most different from those at all other sites. Likewise, bacterial communities from beetles collected in comparatively close sites, e.g., Jackson Co. and Klamath Co., OR, were often more similar. Community differences resulting from geographic isolation and environmental variation have been detected in other bacterial associations, such as

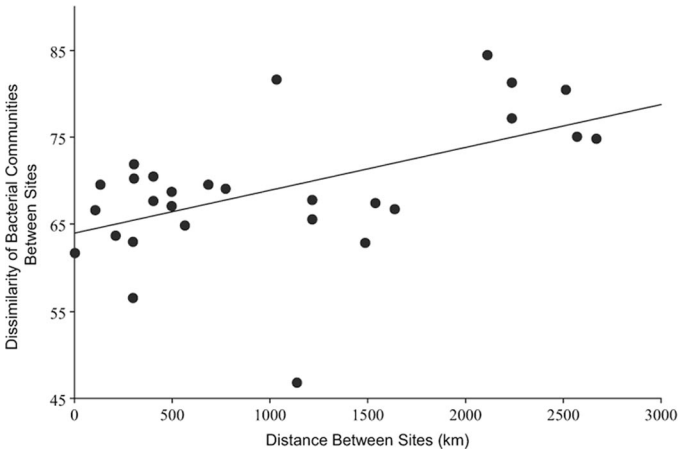


Fig. 5. Dissimilarity of bacterial communities associated with *D. valens* between sites plotted against distance between counties of beetle sampling.

Table 3. Contributions to total within-community similarity of the four most frequently detected OTUs (in rank order) at each site of only OTUs selected by BvSTEP to be the best predictors of community similarity

County	Community similarity (%)	OTU in rank order of contribution			
		Freq (%): Con (%): OTU	Freq (%): Con (%): OTU	Freq (%): Con (%): OTU	Freq (%): Con (%): OTU
Adams Co., WI	29.5	80: 42.7: 61	60: 23.5: 6	40: 9.7: 57	40: <8.0: 4 others
Deschutes Co., OR	47.4	100: 21.6: 36	100: 21.6: 46	100: 21.6: 63	60: 6.13: 41, 44, 62
Klamath Co., OR	35.2	80: 19.7: 50	60: 9.6: 15	60: 9.6: 33	60: 9.6: 5 others
Jackson Co., OR	47.5	100: 11.5: 41	75: 13.2: 62	75: 12.3: 44	75: <12.0: 34, 35, 50
Kootenai Co., ID	64.5	100: 16.3: 32	100: 16.3: 33	100: 16.3: 34	100: 16.3: 36
Meade Co., SD	47.7	80: 11.8: 26	80: 11.8: 33	80: 11.8: 34	80: 11.8: 40
Union Co., OR A	40.8	80: 15.5: 14	80: 15.5: 40	80: 15.5: 46	80: 15.5: 59
Union Co., OR B	45.29	100: 19.4: 46	80: 11.8: 52	80: 10.4: 49	80: 10.4: 63

OUT, no. corresponds to those listed in Fig. 4A.

with marine sponges (Taylor et al. 2005) and mussels (DeChaine et al. 2006). Some other factors that might contribute to bacterial community differences include host tree species, presence of sympatric bark beetles, climate, elevation, slope, and forest structure (Nishiguchi 2000, Dillon et al. 2002, Izumi et al. 2006, Adams et al. 2008, Webster et al. 2008).

Several bacteria were detected consistently from beetles regardless of location. The most frequent associate had a sequence most closely matching an uncultured actinobacterium, and five other bands (phylotypes) were detected in >45% of the beetles. Many of these bacteria were not detected from the guts of *D. valens* collected in Mexico (Morales-Jiménez et al. 2009), most notably the uncultured actinobacterium that we detected from all beetles, the β -proteobacteria that ranged in frequency of association between 7 and 60%, and the γ -proteobacterium *Pseudoxanthomonas spadix* detected in 60% of beetles. Bacteria detected in guts of *D. valens* from Mexico common with those detected in this study are *Rahnella aquatilis* and bacteria in the genera *Pantoea* and *Enterobacter* (Morales-Jiménez et al. 2009).

Although bacteria function cannot be ascertained from these patterns, this community includes some species whose functions have been characterized in other systems. For example, *Bacillus pumilus* in oral secretions of the spruce beetle *Dendroctonus rufipennis* Kirby inhibit *Aspergillus* and *Trichoderma* fungi that invade their galleries (Cardoza et al. 2006). Likewise, some Actinobacteria produce antibiotics that protect the mutualistic fungi of *Dendroctonus frontalis* Zimmermann and leaf-cutting ants (Currie et al. 1999, Scott et al. 2008) or have cellulolytic activity in termites (Pasti and Belli 1985). *Burkholderia* (Coenye and Vandamme 2003), *Delftia* (Liu et al. 2002), *Mycobacterium* (Hennessee et al. 2009), *Massilia* (Bodour et al. 2003), and *Pseudoxanthomonas* (Kim et al. 2008) can metabolize a wide range of hydrocarbons and therefore might contribute to detoxification of tree defensive compounds. Finally, the presence of *Serratia* and *Yersinia* suggests potentially pathogenic relationships (Grimont and Grimont 1978).

Although bacteria in the classes β - and γ -proteobacteria (Table 1) are common associates of insects, the richness of bacteria detected from *D. valens* is rela-

tively low at the class level, at least compared with the bacterial community of another tree-colonizing bark beetle, *D. frontalis* (Vasanthakumar et al. 2006). This suggests either that our technique underestimated diversity or that diversity associated with *D. valens* is lower than that of some other subcortically feeding beetle species. Underestimation of diversity could arise from biases in PCR for abundant species or hidden diversity in bands (Crosby and Criddle 2003). However, in agreement with our findings, Morales-Jiménez et al. (2009) also found low diversity of bacteria associated with *D. valens* using culture-dependent and -independent techniques. Although larger sample sizes would likely yield higher diversity, leveling of the rarefaction curve indicates that our sampling intensity was adequate (Fig. 2) and that diversity of bacteria associated with *D. valens* is relatively low compared with many other reported systems.

In summary, the community of bacteria associated with *D. valens* showed both consistency of association with several OTUs and variation of the bacterial communities across the northern United States, with distance being a major factor of this variation. The high frequency of association of several bacteria across a large geographic range, and their close relation to bacteria with known symbiotic importance in other systems, suggests that some likely affect the development or survival of *D. valens*. Further study is necessary to determine what factors influence the association of various bacteria with this beetle and how they affect their host. Additionally, this baseline characterization of diverse populations provides an opportunity to evaluate how bacterial symbionts may affect an insect's ecological role between its native and invaded ranges.

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