# Population Genetic Structure of an Edaphic Beetle (Ptiliidae) Among Late Successional Reserves within the Klamath-Siskiyou Ecoregion, California

RYAN M. CAESAR,[1] NANCY GILLETTE,[2] AND ANTHONY I. COGNATO[3]

Department of Entomology, Texas A&M University, College Station, TX 77843-2475

Ann. Entomol. Soc. Am. 98(6): 931–940 (2005)

**ABSTRACT** The Klamath-Siskiyou ecoregion of northern California is one of the most diverse temperate coniferous forests. A network of "late successional reserves" (LSRs) has been established to maintain characteristics of late successional forest and to promote late successional characteristics in younger stands. Also, an important goal of conservation management is the maintenance of genetic diversity of ecologically important species. However, this management strategy has not yet been implemented among the LSRs. This study examined the level of genetic diversity among populations of a soil-inhabiting beetle, *Acrotrichis xanthocera* (Matthews) (Ptiliidae), within the LSR network. Using a partial DNA sequence of the mitochondrial cytochrome oxidase I gene, a total of 31 haplotypes were identified for 117 individuals. Genetic fixation indices and phylogenetic and nested clade analyses all suggest moderate gene flow among five LSR and five non-LSR populations. In addition, haplotype diversity was high and the occurrence of unique haplotypes was common for most populations, which suggests current or past isolation of some populations. These results suggest the LSR network maintains considerable genetic variation for this beetle. However, the genetic variation was not equally distributed among the LSRs. Thus, to facilitate gene flow throughout the ecoregion, it is suggested that future LSRs should reduce gaps among the current LSRs.

**KEY WORDS** biodiversity, Coleoptera, conservation genetics, nested clade analysis, phylogeography

[123]THE KLAMATH-SISKIYOU ECOREGION (Fig. 1) is one of the most diverse temperate coniferous forests in the world with high levels of endemism and rare habitat types (Sawyer 1996, DellaSala et al. 1999, Noss et al. 1999). For example, there are >3,500 documented plant species, of which 281 are endemic to the region (Sawyer 1996). The topography and geology of the Klamath is considerably heterogeneous, with steep gradients of altitude, temperature and precipitation (Sawyer 1996). Long- and short-term environmental factors (e.g., volcanic eruptions and fire) likely are crucial to the creation and maintenance of high biodiversity (Sawyer 1996, Taylor and Skinner 1998). The terrestrial diversity of the region is considered endangered (DellaSala et al. 1999) due to fragmentation of critical habitat and native species and competition with invasive species (DellaSala et al. 1999).

Most of the Klamath-Siskiyou ecoregion falls within National Forests, although only 10.5% has legal protection (DellaSala et al. 1999). The U.S. Forest Service has established a management policy for this region based primarily on habitat preservation for the northern spotted owl, *Strix occidentalis caurina* (Noss et al. 1999). To protect spotted owl habitat, the Northwest Forest Plan developed by the U.S. government (U.S. Department of Agriculture 1994) designated a network of "late successional reserves" (LSRs) throughout the Klamath-Siskiyou ecoregion. The purpose of the LSR system was to maintain characteristics of late successional forest and old-growth ecosystems and to promote the development of late-successional characteristics in younger stands (Taylor and Skinner 1998).

Although this approach may be appropriate for protection of the spotted owl, several aspects of the management plan and broader conservation goals have not been sufficiently addressed. This type of species management, in which protection of one charismatic species is presumed to afford protection for all sympatric species, has proven ineffective when vertebrates are used as surrogates for invertebrates (Rubinoff 2001, Moritz 2002). This umbrella species management plan does not meet the broader goals for conservation in the region particularly the preservation of species and genetic diversity (Noss et al. 1999). Furthermore, abiotic factors have been identified that will maximize the promotion of late successional conditions (Taylor and

[1] Current address: Department of Entomology, The Ohio State University, Columbus, OH, 43212.

[2] USDA Forest Service, Pacific Southwest Research Station, Berkeley, CA 94701.

[3] Corresponding author, e-mail: a-cognato@tamu.edu.

Skinner 1998), but these have not been considered in the establishment of LSRs.

Comparatively little research has been conducted to characterize the organisms and evolutionary processes that have contributed to the distinctiveness of this region. This is especially true for the soil and litter inhabiting macroarthropods that make up a significant portion of the metazoic diversity of temperate conifer forests. These arthropods are the most diverse organisms in the forests of the Klamath-Siskiyou ecoregion (Moldenke 1999) and play a crucial role in decomposition and nutrient cycling that is essential for the long-term sustainability of forests. Thus, leaf litter arthropods can be useful indicators of forest health (Van Straalen 1997). In addition, patterns of genetic variation among these organisms will likely provide insight into the evolutionary processes that have shaped the history of species within the Klamath ecoregion (Roderick 1996). Phylogenetic analysis provides a means to assess the historical and contemporary processes that shape both species and genetic diversity (Slatkin and Maddison 1989, Avise 2000), and which have immediate implications for species conservation (Avise 2000, Moritz 2002). Information regarding the distribution of genetic variation and population histories can be used to enhance conservation efforts and develop more effective land management policies (Hibbet and Donoghue 1996, Moritz 2002).

Feather-wing beetles (Ptiliidae) are tiny, ubiquitous edaphic insects that are found in most forests throughout North America (Hatch 1953, Sörensson 2003). Ptiliids, along with other edaphic beetles, have been implicated as potentially useful bioindicators (Sawada and Hirowatari 2002). They are often abundant in suitable microhabitats within their range (Dybas 1990), but their size and obscure life histories tend to limit their detection. It has been presumed that ptiliids, due to their size and preferred habitats, tend to avoid flight (Sörensson 1997). For this reason, they might be expected to exhibit significant population structure on the geographic scale of the Klamath. Alternatively, it has been proposed that the well-developed feather-wings of certain ptiliids allow for passive, but relatively infrequent, dispersal over long distances (Dybas and Dybas 1981, Dybas 1990).

The purpose of this study is to assess the geographic distribution of genetic diversity among populations of the ptiliid *Acrotrichis xanthocera* (Matthews 1884) within the Klamath-Siskiyou ecoregion. Phylogenetic analysis and measures of population structure were used to measure contemporary gene flow among populations. A partial DNA sequence of the mitochondrial cytochrome oxidase I gene was sequenced from specimens collected from the Klamanth-Siskiyou LSR and neighboring regions. The evolutionary relationship among haplotypes was used to examine the relationship between geography locality and genetic variation and the processes responsible for observed patterns. The results of these analyses were used to estimate historical biological factors contributing to the current population structure of *A. xanthocera.* This information was then discussed in relation to the LSR network and to conservation of genetic diversity.

## Materials and Methods

Five LSRs throughout the Klamath-Siskiyou ecoregion and five sites outside of the region (Fig. 1; Table 1) were sampled in May and June 2002 and 2003. LSRs were chosen based on elevation, successional stage, vegetation, and terrain (DellaSala et al. 1999). Non-LSR sites outside of the Klamath-Siskiyou ecoregion shared some of the LSR characteristics, but they were dominated by redwood forests; old and secondary growth forests were sampled. These sites were lower in elevation, with gentle terrain and were considerably less mesic compared with the LSRs.

Arthropods were extracted from samples of soil, moist litter, mammal dung, or detritus with Berlese funnels that were placed in a refrigerated room. Over the course of 2 to 3 d, arthropods were extracted under 60-Watt light bulbs into 80% ethyl alcohol. Ptiliid individuals were then sorted to morphospecies into 100% ethyl alcohol and stored at −20°C.

M. Sörensson (Lund University, Lund, Sweden) identified representative specimens of each morphospecies and from each sample locality. Only *A. xanthocera* specimens were used in subsequent molecular and population analyses. All voucher specimens were deposited in the Texas A&M University insect collection (voucher number 642).

**Laboratory Methods.** Specimens (117) were analyzed for this study. Genomic DNA was extracted from dismembered beetles by using DNeasy tissue kits (QIAGEN, Valencia, CA). The manufacturer's protocol was followed with the exception of the addition of 40 μl of proteinase K and an extended incubation period of 8–20 h. The sclerotized portions of the exoskeleton were retained after digestion for confirmation of identification.

A total of 750 bp of the mitochondrial DNA (mtDNA) protein coding gene cytochrome oxidase I (COI) was sequenced from each individual (position 2235 of *Drosophila yakuba* Burla 1954; Clary and Wolstenholme 1985). Universal insect primers CI-J-2183 (5′-CAA CAT TTA TTT TGA TTT TTT GG-3′), CI-J-2441 (5′-CCT ACA GGA ATT AAA ATT TTT AGT TGA TTA GC-3′), and TL2-N-3014 (5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′) (Simon et al. 1994) were used for amplification of COI via the polymerase chain reaction (PCR). A new primer CI-N-2641 (5′-AAT GAA TAT CAA TGA ACG AAC CC-3′), specific to *A. xanthocera*, was designed for use with CI-J-2183. PCR reactions were carried out in 25-μl volumes using puRe*Taq* Ready-To-Go PCR beads (Amersham Biosciences Inc., Piscataway, NJ) following manufacturer's protocol. Either 10 or 25 μl of purified DNA was used in each PCR reaction depending on the relative DNA concentration. Ten microliters was used for concentrations >25 ng, and 25 μl was used for concentrations <25 ng. The following steps were performed on a programmable thermal cycler: cycle 1, 95°C for 2.0 min.; cycles 2–36, 94°C for 0.5 min.,

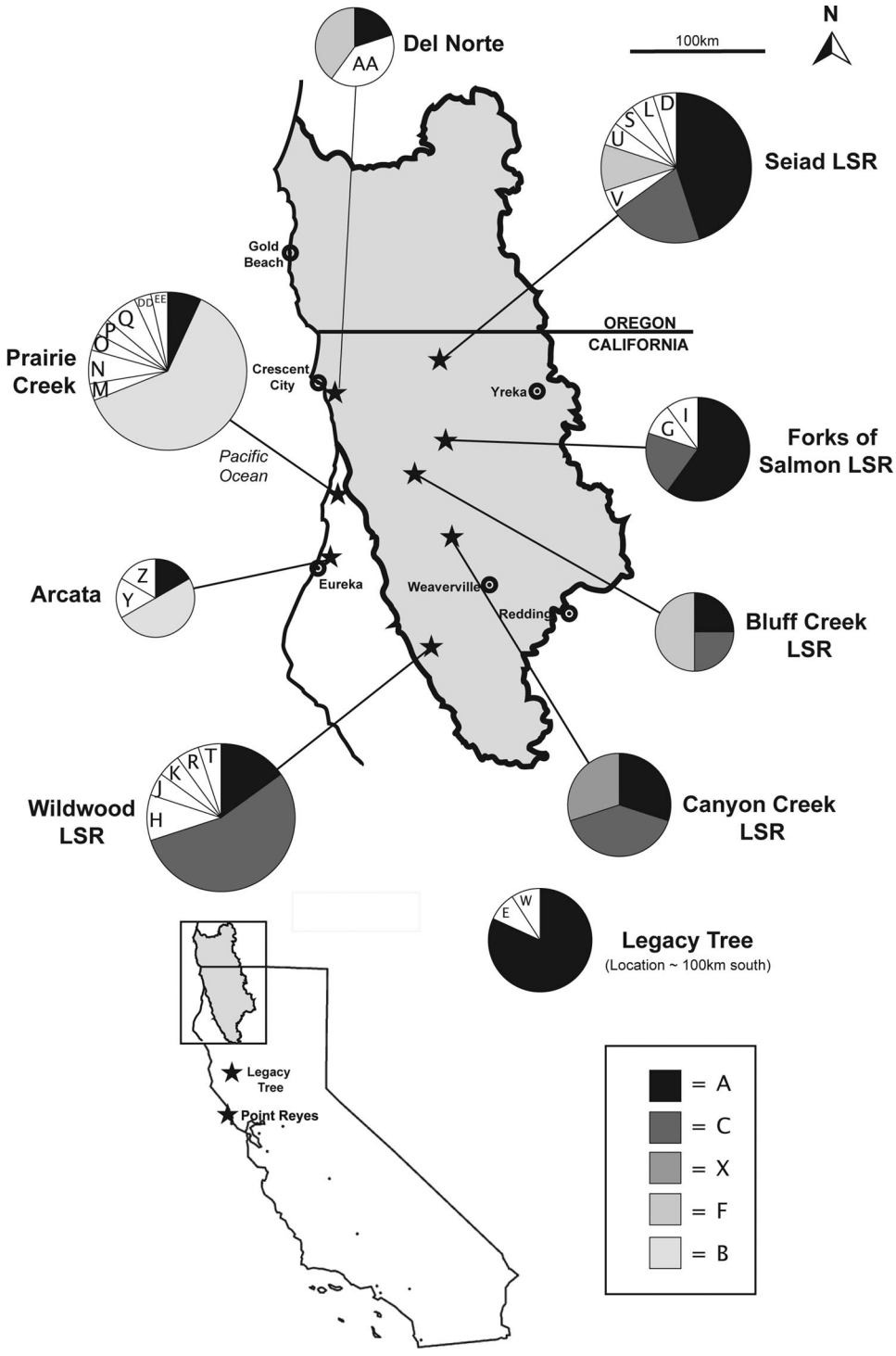


**Fig. 1.** Distribution of *A. xanthocera* haplotypes in the Klamath-Siskiyou Ecoregion (shaded region) in California. Stars indicate collection sites of *A. xanthocera* specimens. Legend refers to most common haplotypes, with unique or singleton haplotypes designated by letters on pie charts. The size of each pie chart reflects relative sample size of that sample. Point Reyes, located ≈200 km to the south, is not shown due to its small sample size.

**Table 1. Sample locality and haplotype information**

| Pop | County | Coordinates (dd) | Haplotype (N) | *h* |
|---|---|---|---|---|
| Arcata (Ar) | Humboldt Co. | 40.87474 N 124.07297 W | A(1), B(3), Y(1), Z(1) | 0.80 |
| Bluff Creek (BC) | Humboldt Co. | 41.25942 N 123.68818 W | A(1), C(1), G(2) | |
| Canyon Creek (CC) | Trinity Co. | 40.25942 N 123.02259 W | A(3), C(4), X(3) | 0.73 |
| Del Norte (DN) | Del Norte Co. | 41.70495 N 124.12583 W | A(1), B(2), AA(2) | |
| Forks of Salmon (FS) | Siskiyou Co. | 41.33226 N 123.20353 W | A(6), C(2), G(1), I(1) | 0.64 |
| Legacy Tree (LT) | Mendocino Co. | 39.47120 N 123.54788 W | A(9), E(1), W(1) | 0.35 |
| Point Reyes (PR) | Marin Co. | 38.08662 N 122.86308 W | BB(1), CC(1) | |
| Prairie Creek (PC) | Humboldt Co. | 41.36080 N 124.02308 W | A(2), B(18), M(1), N(2), O(1), P(1), Q(2), DD(1), EE(1) | 0.62 |
| Seiad (Se) | Siskiyou Co. | 41.90323 N 123.08062 W | A(9), C(4), D(1), F(2), L(1), S(1), U(1), V(1) | 0.77 |
| Wildwood (Ww) | Humboldt Co. | 40.39632 N 123.03267 W | A(3), C(11), H(2), J(1), K(1), R(1), T(1) | 0.69 |

Haplotype diversity, *h*, is not reported for populations with $n < 6$.

45°C for 0.75 min., 72°C for 1.0 min.; cycle 37, 72°C for 5.0 min.

PCR products were visualized under UV light after electrophoresis through a 1.5% agarose gel. Relative concentration of amplified DNA was estimated with a DNA standard included in all agarose gels.

The amplified PCR products were purified using the QIAquick PCR purification kit (QIAGEN) and sequenced using the BigDye Terminator version 1.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) according to manufacturer's recommendations. Both 5′ and 3′ strands were sequenced using the same primers that were used for PCR. Cycle sequencing reaction products were applied to polyacrylamide gels on either an ABI PRISM 377 automated sequencer or an ABI PRISM 3100 Genetic analyzer (Applied Biosystems, Foster City, CA) for visualization of the cycle sequencing reaction products.

Sequences were manually edited using Sequence Navigator version 1.0.1 (Applied Biosystems) or Sequencher version 4.1 (Gene Codes Corporation, Ann Arbor, MI) and aligned by eye with the aid of Se-Al version 1.0a1 (Rambaut 1996). Alignment of the COI sequences did not require the insertions of gaps to represent insertion or deletion events. Sequences for each haplotype were deposited in GenBank nucleotide sequence database (AY550852–AY550882).

**Population Genetic, Phylogenetic, and Nested Clade Analyses.** Analysis of gene flow and genetic differentiation among populations was based on $N_{ST}$ and $G_{ST}$ fixation indices (Lynch and Crease 1990). A Mantel test was implemented to test for correlations between genetic and geographic distances (Le Progiciel R version 4.0d6; Casgrain 2001). DnaSP version 3.99.6 (Rozas et al. 2003) was used to analyze DNA polymorphism, gene flow, and population differentiation. Nucleotide diversity ($\pi$; Nei 1987) was calculated using Matrix 2.0 (Posada 2001). Haplotype diversity was calculated as $h = n(1 - \Sigma p_i^2)/(n - 1)$, where n is the number of haplotypes, and $p_i$ is the frequency of the *i*th haplotype (Nei 1987).

Phylogenetic relationships of 117 *A. xanthocera* COI sequences were estimated using PAUP* version 4.0b2a (Swofford 1998) under the maximum parsimony (MP) optimality criterion. A heuristic search was performed for the MP analyses using the tree bisection reconnection (TBR) algorithm and 1000 random addition sequence replicates, with all characters equally weighted and unordered. For each replicate, no more than 1000 trees with a score greater than 1 were saved to reduce the computational time of analyses. Support for individual nodes was assessed with 1000 pseudo-replicates with the bootstrap procedure with the same search parameters as the MP search.

A nested clade analysis was performed to test the null hypothesis of random association of maternal lineages and geographic location. A network of haplotypes was created by TCS version 1.12 (Clement et al. 2000) using the statistical parsimony (Templeton et al. 1992), and clades therein were nested according to the rules delineated in Templeton et al. (1995). Tests for statistically significant associations between haplotype frequency and spatial distribution were carried out with the program GEODIS version 2.0 (Posada et al. 2000). With this procedure, 10,000 permutations were performed to create a null distribution of clade ($D_c$) and nested clade ($D_n$) distances for comparison to observed distance values. The results of the GEODIS analysis were interpreted via the inference key of Templeton (1998), which allows the investigator to choose among the likely historical biological factors that explain the current geographic distribution of haplotype variation.

## Results

Of the 750 bp of mtDNA COI, 56 sites were variable, and 6, 3, and 47 of these differences occurred at first, second, and third codon positions, respectively (11% first, 5% second, and 84% third). Mean base frequencies were A, 0.311; C, 0.150; G, 0.147; and T, 0.392 (Fig. 2).

The Mantel test indicated a weak but statistically significant positive correlation between genetic and geographic distances (Table 2) ($r = 0.167$). Tests for population structure indicated moderate genetic differentiation with limited gene flow among these populations ($G_{ST} = 0.291$, $N_{ST} = 0.727$). Pairwise comparisons of $G_{ST}$ and $N_{ST}$ values among populations indicated a geographic component to genetic variation with haplotype frequencies partitioned among

| Haplotype | Pos | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z | AA | BB | CC | DD | EE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2239 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | C | C |
| 2245 | 3rd | C | * | * | * | * | * | * | * | * | * | * | T | T | T | T | T | T | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2248 | 3rd | T | * | * | * | * | * | * | * | * | * | * | C | C | C | C | C | C | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2257 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | G | G | G | G |
| 2269 | 3rd | A | * | * | * | * | * | * | * | T | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2275 | 3rd | G | * | * | * | * | * | * | * | * | * | * | A | A | A | A | A | * | * | ? | * | * | * | * | * | * | * | * | A | A | A | A |
| 2284 | 3rd | G | * | * | * | * | * | * | * | * | * | * | A | A | A | A | * | A | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2296 | 3rd | A | * | * | * | * | C | * | * | * | * | C | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2308 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | C | C |
| 2311 | 3rd | C | * | * | * | * | * | * | * | * | T | * | T | T | T | T | T | T | * | ? | * | * | * | * | * | * | * | * | T | T | T | T |
| 2332 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | G | G | G | G |
| 2335 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | T | * | * | * |
| 2341 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | C | C | C | C |
| 2356 | 3rd | A | * | * | * | * | * | * | * | * | * | * | G | G | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | * | * | * |
| 2362 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | C | C | C | C |
| 2365 | 3rd | C | * | * | * | * | * | * | * | * | * | T | * | * | T | T | T | T | T | ? | * | * | * | * | * | * | * | * | T | T | T | T |
| 2408 | 1st | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * | * | A | * | * | * | * | * | * | * | * |
| 2434 | 3rd | T | * | * | * | * | * | * | * | A | A | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2443 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | * | G | G | * | * | * | * | * | * | * | * | * | * | * | * | G | G | * | * |
| 2473 | 3rd | T | * | * | * | * | * | * | * | * | * | * | C | C | C | C | C | C | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2509 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2512 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | T | T | T |
| 2518 | 3rd | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2530 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2551 | 3rd | G | A | * | * | * | * | * | * | * | * | * | A | A | A | A | A | A | * | * | * | * | * | * | A | A | A | A | A | A | A | A |
| 2567 | 1st | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2575 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2581 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2584 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2590 | 3rd | A | * | * | * | * | * | * | * | * | * | * | G | G | G | G | G | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2605 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2614 | 3rd | A | T | * | * | * | * | * | * | * | * | * | T | T | T | T | T | T | * | T | * | * | * | * | T | T | T | C | C | T | T |
| 2617 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | G | G | G | G |
| 2644 | 3rd | G | * | * | * | * | * | * | * | * | * | * | * | * | A | A | * | * | * | * | * | * | * | * | * | * | * | * | A | A | A | * |
| 2645 | 1st | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | C | * | * | * | * | * |
| 2647 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | * | * | * | * |
| 2648 | 1st | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | A | A | A | A |
| 2659 | 3rd | T | * | * | * | * | * | * | * | * | * | * | C | C | C | C | * | C | * | C | * | * | * | * | * | * | * | * | * | * | * | * |
| 2665 | 3rd | A | * | G | G | * | * | * | G | G | G | G | G | * | * | * | * | * | * | G | G | * | * | * | * | * | * | * | * | * | * | * |
| 2723 | 1st | T | * | * | * | * | * | * | * | * | * | * | C | C | C | C | C | C | * | * | * | * | * | * | * | * | * | * | C | C | C | C |
| 2737 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | T | T | T |
| 2746 | 3rd | C | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | T |
| 2749 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | * | C | C |
| 2776 | 3rd | A | * | * | * | * | G | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2815 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | A | * | * | * | * | * | * | * |
| 2851 | 3rd | C | * | * | * | * | * | * | * | * | * | * | T | T | * | * | T | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2893 | 3rd | A | * | * | * | * | * | * | * | * | * | * | * | * | * | * | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 2900 | 1st | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | G | * | * | * | * | * | * | * | * | * | * | * |
| 2922 | 2nd | C | * | * | T | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * |
| 2926 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | A | * | * | * | * | * | * | ? | * | * |
| 2950 | 3rd | A | * | * | * | * | * | * | * | * | * | * | G | G | G | G | * | * | * | * | * | * | * | * | * | * | * | * | * | ? | * | * |
| 2962 | 3rd | T | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | C | * | * | * | ? | * | * |
| 2964 | 2nd | C | * | * | * | * | * | ? | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | A | * | * | * | ? | * | * |
| 2965 | 3rd | G | A | * | * | * | * | ? | A | * | * | * | A | A | A | A | A | A | * | * | * | * | * | * | C | A | A | * | ? | A | A |
| 2967 | 2nd | C | * | * | * | * | * | ? | * | * | * | * | * | * | * | T | * | * | * | * | * | * | * | * | * | * | * | * | ? | T | * |
| 2983 | 3rd | T | * | * | ? | ? | * | ? | * | * | * | * | * | C | * | ? | ? | * | * | C | C | ? | * | * | ? | * | * | * | ? | C | ? |

**Fig. 2.** Nucleotide variation. Numbers in the first column correspond to base positions of *D. yakuba* COI sequences (Clary and Wolstenholme 1985). Positions that were invariant among all individuals were excluded. Asterisks refer to invariant sites, and question marks indicate sites with missing data.

**Table 2. Pairwise geographic distances (kilometers) between populations**

| | BC | CC | FS | Se | Ww | Ar | DN | PC | LT | PR |
|---|---|---|---|---|---|---|---|---|---|---|
| BC | 0 | | | | | | | | | |
| CC | 72 | 0 | | | | | | | | |
| FS | 41.3 | 55 | 0 | | | | | | | |
| Se | 88 | 116.3 | 64.1 | 0 | | | | | | |
| Ww | 110.7 | 50.9 | 104.7 | 167.2 | 0 | | | | | |
| Ar | 53.4 | 88.6 | 89.2 | 141.5 | 102.9 | 0 | | | | |
| DN | 61.4 | 132.1 | 87.7 | 89.6 | 171.8 | 92.4 | 0 | | | |
| PC | 30.3 | 100.7 | 68.6 | 98.8 | 135.3 | 54.3 | 39.4 | 0 | | |
| LT | 198.6 | 166.5 | 208.8 | 272.9 | 112 | 162 | 253 | 213.5 | 0 | |
| PR | 359.4 | 307.7 | 361.5 | 424.1 | 257 | 326.5 | 416 | 376.6 | 164.7 | 0 |

Geographic distances were calculated using the Distance/Bearing tool of MapSource version 3.02 (Garmin Corporation, Olathe, KS). Average distance between sites is 128.9 km. See Table 1 for key to population abbreviations.

the Klamath-Siskiyou LSRs, the coastal populations, and the southern populations (Table 3).

A total of 31 mtDNA haplotypes indicated a relatively high haplotype diversity ($h = 0.839$) and low nucleotide diversity ($\pi = 0.0494$). There were 2–9 haplotypes in each population (Fig. 1), with haplotype A the most abundant in all populations except Point Reyes, with highest frequencies at Seiad and Legacy Tree. Haplotypes B and C were the next most abundant, but B was present only in the coastal populations (PC, Ar, and DN) and C only in the Klamath region (Se, Ww, FS, CC, and BC; Fig. 1). All populations except Bluff Creek ($n = 4$) contain unique haplotypes. The spatial variation in both haplotype frequency and composition (Fig. 1) suggested that coastal and inland populations are genetically different.

The phylogenetic analysis yielded 25,000 equally most parsimonious trees of length 79. Although resolution among haplotypes was low, tree indices indicated that homoplasy was not likely the cause of the lack of phylogenetic resolution (CI = 0.747, RI = 0.935, RC = 0.699) but rather attributed to a lack of parsimony informative characters in these data. The individuals sampled from the Klamath populations formed an unresolved monophyletic clade in the strict consensus with the exception of haplotype R from Wildwood (Fig. 3). There are no obvious phylogenetic patterns for *A. xanthocera* populations within the Klamath, i.e., regions within the Klamath were not monophyletic. The statistical parsimony network similarly failed to recover monophyletic relationships within the Klamath region, but it did provide greater resolution at the regional scale that increased with nesting level (Fig. 4). For example, haplotypes clustered within clades 3-3 and 2-3 defined two geographic lineages associated with the Klamath and coastal sampling areas, respectively (Fig. 1). An exception to this pattern was clade 2-7 that consisted of several intermediate haplotypes and only one sampled haplotype, R. Although reticulations in this clade could not be unambiguously resolved, they did not affect the inference of demographic events. Haplotypes BB and CC, from Point Reyes, and haplotypes DD and EE, from Prairie Creek, were not connected to the parsimony network because the number of steps between these haplotypes and others in the network was greater than the 95% parsimony limit (Templeton et al. 1992). This suggests that Point Reyes and Prairie Creek populations are genetically isolated from the other sampled localities. Omission of these haplotypes from the analysis did not influence the interpretation of demographic events in the Klamath and Coastal regions inferred from the nested clade analysis.

Within the Klamath, the nested clade analysis revealed a complex history of *A. xanthocera.* The most common haplotype, A, is shared among all geographically contiguous populations and was absent only from the geographically distant Point Reyes region. Coalesence theory predicts that the frequency and widespread distribution of haplotype A makes it the most likely root of the statistical network (Templeton 1998). Its ubiquitous presence suggests that ancestral lineage sorting is incomplete or alternatively gene flow among the populations. Conversely, the restricted locations of the majority of haplotypes (Fig. 1) suggest past isolation of some populations. However, the null hypothesis of a random association between geographic locality and haplotype frequencies cannot be rejected. There is statistically significant evidence for a contiguous northwest range expansion. Recently this expansion gave way to increased long distance isolation between southern and northern populations. Gene flow continues between the Klamath and coastal populations, but it is restricted. Within the Klamath, gene flow also is limited, albeit to a lesser degree (Table 3; Fig. 4). Inference of the total cladogram is not possible, because it involves the nesting of two tip clades (4-1 and 4-2). Both tip and interior clades are required to infer demographic events. This is not prob-

**Table 3. Measures of gene flow and inferred population structure based on fixation indices for *A. xanthocera* populations in northern California**

| Geographic comparison | No. populations | No. haplotypes | $N_{ST}$ | $G_{ST}$ | Gene flow and structure inference |
|---|---|---|---|---|---|
| All | 10 | 31 | 0.727 | 0.291 | Low, structure |
| Klamath | 5 | 16 | 0.107 | 0.110 | High, no structure |
| Coast | 3 | 11 | 0.126 | 0.029 | High, no structure |
| South | 2 | 5 | 0.954 | 0.294 | Low, structure[a] |
| Klamath-coast | 8 | 27 | 0.453 | 0.200 | Moderate-high, structure |
| Klamath-south | 7 | 20 | 0.134 | 0.080 | Moderate, structure[a] |
| Coast-south | 5 | 15 | 0.245 | 0.194 | Moderate, structure[a] |

[a] Indices including Point Reyes are inflated due to small sample size. However, they do not affect the interpretation.

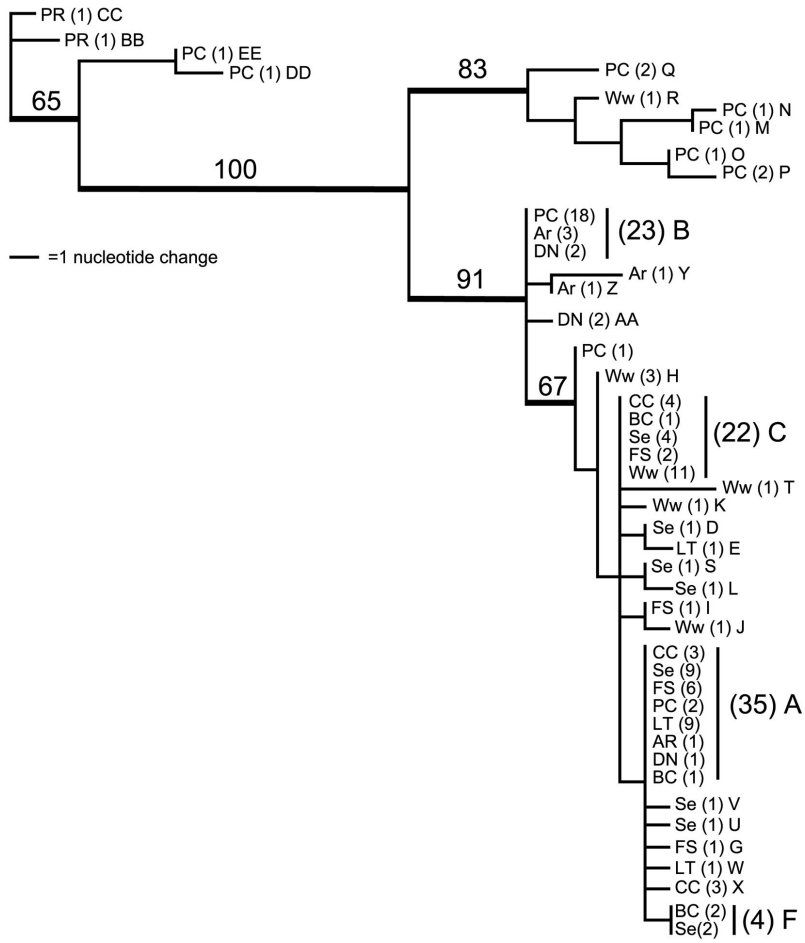


**Fig. 3.** One of 25,000 equally most parsimonious trees of 117 *A. xanthocera* individual mtDNA COI sequences, sampled from 10 populations in northern California. Nodes recovered in the strict consensus of all trees, are indicated by bootstrap values labeled above branches. Terminal labels reference the population (Table 1). In parentheses is the number of individuals at the given node, followed by the letter of the haplotype. The largest haplotype groups are designated in larger font type with number of individuals in parentheses followed by letter of haplotype.

lematic, as the inference from lower level clades corroborates the inferences obtained from the genetic and phylogenetic analyses

## Discussion

**Population Structure of *A. xanthocera*.** Populations of *A. xanthocera* display little structure at the scale of the Klamath-Siskiyou ecoregion but demonstrate significant evidence of genetic structure on a broader geographic scale. Haplotype diversity is relatively high, and most populations have one or more haplotypes associated only with specific regions (Fig. 1). Measures of population structure and nested clade analysis support partitioning of genetic variation among regions. Cladistic analysis reveals little phylogenetic resolution (Fig. 3), and the distribution of haplotypes indicates some moderate-to-high gene flow among adjacent populations (Table 4). Nested clade analysis (Fig. 4; Table 4) suggests a stepping stone-like (Crow and Kimura 1970) movement of *A. xanthocera* individuals with short-range dispersal among adjacent populations, despite past isolation.

Assumption of low vagility of other soil arthropods has been questioned. Population structure of a collembolan is influenced by long-distance dispersal, despite its edaphic and wingless condition (Van der Wurff et al. 2003). Among ground beetle species, genetic heterogeneity is more closely correlated with environmental factors than with a priori dispersal capability based on wing development (Liebherr 1988). Ptiliid feather-wings likely provide means for passive wind dispersal over long distances (Dybas 1990). Winds in this area are predominately eastwardly; therefore, the results provide evidence that these winds may act as a mechanism for occasional long-distance dispersal of ptiliids. However, more samples collected from localities between the east and west regions are needed to further test this hypothesis (Fig. 1).

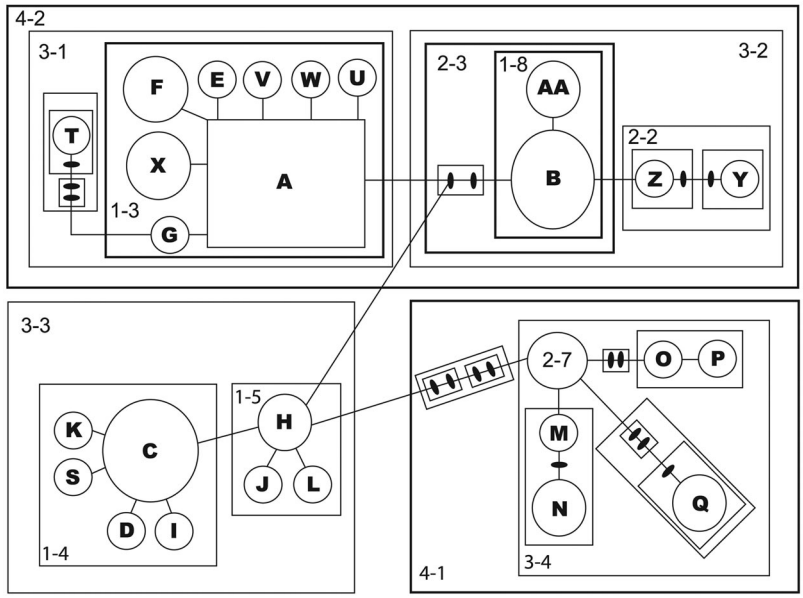


**Fig. 4.** Nested network of *A. xanthocera* haplotypes. Hollow circles and squares indicate individual haplotypes with frequencies proportional to size. Haplotype A has the greatest root probability. Filled ovals represent inferred intermediate haplotypes based on the statistical parsimony analysis. Lines between haplotypes and intermediates represent one nucleotide change. Dashed numbers refer to nested clades. Clade 2-7 is left unresolved during the analysis and does not alter the inferences derived from network patterns. Clades with heavy-lined boxes are significant (Table 4); unnumbered clades are not significant.

**Table 4. Summary of inferences regarding demographic events deduced from clades with significant nested clade values**

| Clade | $\chi^2$ | Nested clades | $D_C$ (km) | $D_N$ (km) | Chain of inference | Demographic event |
|---|---|---|---|---|---|---|
| 1-3 | n.s. | A (INT) | 95.31,>,$P = 0.075$ | 94.04,>,$P = 0.077$ | 1 yes, 2 yes, 3 no, 4 no | Restricted gene flow, isolation by distance |
| | | V (TIP) | n.s. | n.s. | | |
| | | W (TIP) | n.s. | n.s. | | |
| | | X (TIP) | 0.00,<,$P = 0.033$ | 32.88,<,$P = 0.0012$ | | |
| | | E (TIP) | n.s. | n.s. | | |
| | | F (TIP) | 24.27,<,$P = 0.018$ | n.s. | | |
| | | U (TIP) | n.s. | n.s. | | |
| | | I-T | 83.83,>,$P = 0.0012$ | n.s. | | |
| 1-8 | n.s. | AA (TIP) | n.s. | n.s. | 1 yes, 2 yes, 3 no, 4 no | Restricted gene flow, isolation by distance |
| | | B (INT) | 38.61,>,$P = 0.02$ | 36.26,>,$P = 0.02$ | | |
| | | I-T | 38.61,>,$P = 0.02$ | n.s. | | |
| 2-3 | $P = 0.032$ | 1–8 (TIP) | n.s. | n.s. | 1 yes, 2 yes, 3 no, 4 no | Restricted gene flow, isolation by distance |
| | | 1–9 (INT) | 38.61,>,$P = 0.022$ | 36.26,>,$P = 0.022$ | | |
| | | I-T | 38.61,>,$P = 0.022$ | n.s. | | |
| 4-1 | $P = 0.00$ | 3–4 (TIP) | 44.89,<,$P = 0.075$ | 94.45,>,$P = 0.00$ | 1 yes, 2 no, 11 yes, 12 yes, 13 no, 14 no | Range expansion[a] |
| | | 3–3 (INT) | 57.17,<,$P = 0.003$ | 58.97,<,$P = 0.012$ | | |
| | | I-T | n.s. | −35.48,<,$P = 0.00$ | | |
| 4-2 | $P = 0.00$ | 3–1 (TIP) | 87.05,>,$P = 0.095$ | 86.85,>,$P = 0.077$ | 1 yes, 2 no, 11 yes, 12 no | Contiguous range expansion |
| | | 3–2 (INT) | 41.49.<,$P = 0.00$ | 65.84,<,$P = 0.0045$ | | |
| | | I-T | −45.56,<,$P = 0.002$ | −21.01,<,$P = 0.056$ | | |
| Total cladogram | $P = 0.00$ | 4–1 (TIP) | 63.62,<,$P = 0.047$ | 69.05,<,$P = 0.099$ | N/A | N/A |
| | | 4–2 (TIP) | n.s. | n.s. | | |

TIP, tip clades; INT, interior clade; I-T, interior-tip average clade distances; n.s., nonsignificant. Greater-than or less-than symbols indicate a $D_C$ or $D_N$ value that is significantly larger or smaller than expected if haplotypes were distributed randomly. *P* values indicate probability that the $D_C$ or $D_N$ estimated from the data is by chance. Inferences were drawn from the nested clade analysis interpretation key provided by Templeton (1998). The steps in the chain of inference can be examined by comparison with this key.

[a] Sample design inadequate to discriminate between isolation by distance versus long-distance dispersal.

**Implications for Conservation in the Klamath-Siskiyou Ecoregion.** Little old growth or late successional forest remains intact, which is mostly a result of logging practices (Sawyer 1996, DellaSala et al. 1999, Noss et al. 1999). To preserve significant molecular biodiversity, genetic variation in local populations must be considered. Populations frequently exist in small patches of suitable litter, which increases the potential for isolation among populations as habitat becomes increasingly fragmented. Because each site studied harbors a unique subset of the genetic diversity, a potential for reduced genetic variation exists. Reserves could be viewed as poor if they contain less genetic diversity relative to other populations (Moritz 2002). Extinction of an organism can be considered the ultimate failure of a reserve. At present, there does not seem to be a threat to reduced genetic variation or the survival of *A. xanthocera* because there is a wide distribution of haplotypes.

The population genetic patterns shown by this common beetle suggest that it disperses locally with occasional geographic expansion (Tables 3 and 4). Given that many soil arthropods are likely less vagile, fragmentation of the ecoregion could have a severe effect on the edaphic community. Our phylogeographic study is a step toward a revision of management plans for the edaphic community of the Klamath-Siskiyou ecoregion. Moritz (1994) suggests a "community genetics" approach for the establishment of conservation priorities. This procedure involves examination of multiple species within a geographic region for unique population structure. Congruent demographic processes have been shown across multiple lineages within a geographic region (Moritz 1994, 2002; Calsbeek et al. 2003). Calsbeek et al. (2003) reported a strong concordance among the structure of populations of higher level taxa in the major Californian biogeographic regions. We would predict phylogenetically and ecologically related soil arthropods would exhibit similar areas of genetic endemism as *A. xanthocera.* However, considerable research of soil arthropod population genetics remains before a community genetics approach can be implemented for the establishment of conservation priorities in the Klamath-Siskiyou ecoregion.

Variation of genetic diversity among geographically isolated populations of *A. xanthocera* could be used to guide the establishment of future reserves or amend the current network of LSRs. The LSRs protect a variable amount of haplotype diversity and uniqueness (Fig. 1). For example, Seiad and Wildwood LSRs harbor substantial haplotype diversity and contain a greater number of unique haplotypes. However, mostly common haplotypes occur in the remaining LSRs (Table 1). A non-LSR site, Prairie Creek, also exhibits high haplotype diversity and uniqueness. For nearly a century, logging was absent from this 5,666-hectares (14,000-acre) sanctuary of old-growth forest that may explain the high haplotype diversity. Guidelines that established the LSRs and laws that protect them are ambiguous or nonexistent; however, it is possible to improve the LSR system in light of these results. Local dispersal of *A. xanthocera*, and likely other edaphic arthropods, suggests that future LSRs should be established as a connective network. Given that rare haplotypes are spatially segregated (Fig. 1), new sites should reduce gaps among the current LSRs. Thus, the increased proximity of LSRs would help to facilitate gene flow throughout the ecoregion. In addition, it is acknowledged that resources are limited and preservation of all late successional forest patches is not possible. In these cases, decision makers should use multiple measures of biodiversity to assess conservation priority. Consideration of long-term protection of biodiversity within the ecoregion must include revaluation of the current uses of the LSRs. Based on the Prairie Creek results and although speculative, logging prohibition may best maintain genetic diversity. However, more detailed studies of species ecology, biology, and genetic diversity are needed to maximize the preservation of the evolutionary processes that shape the biodiversity within the Klamath-Siskiyou ecoregion.

## Acknowledgments

We thank Mike Camann and Karen Lamoncha for logistical support during fieldwork, Mikael Sörensson for specimen identifications and collecting advice, Joe Gillespie for laboratory assistance, April D. Harlin for assistance with nested clade analysis and review, and Jenny Caesar for help with figure production. This study was funded by grants from the Texas A&M University Entomology Graduate Student Organization to R.M.C., startup funds provided to A.I.C. and by cooperative agreement number 01-CA-11272162-160 from the USDA Forest Service, Pacific Southwest Research Station to N.G.

## References Cited

**Avise, J. C. 2000.** Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA.

**Casgrain, P. 2001.** Le Progiciel R package. http://www.fas.umontreal.ca/biol/casgrain/en/labo/R/.

**Calsbeek, R., J. N. Thompson, and J. E. Richardson. 2003.** Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. Mol. Ecol. 12: 1021–1029.

**Clary, D. O., and D. R. Wolstenholme. 1985.** The mitochondrial DNA molecule of *Drosophila yakuba:* nucleotide sequence, gene organization, and genetic code. J. Mol. Evol. 22: 252–271.

**Clement, M., D. Posada, and K. Crandall. 2000.** TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657–1660.

**Crow, J. F., and M. Kimura. 1970.** An introduction to population genetics theory. Harper & Row, New York.

**DellaSala, D. A., S. B. Ried, T. J. Frest, J. R. Strittholt, and D. M. Olson. 1999.** A global perspective on the biodiversity of the Klamath-Siskiyou Ecoregion. Nat. Areas J. 19: 300–319.

**Dybas, H. S. 1990.** Insecta: Coleoptera: Ptiliidae, pp. 1093–1112. *In* D. L. Dindal [ed.], Soil biology guide. Wiley, New York.

**Dybas, L. K., and H. S. Dybas. 1981.** Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. Evolution 35: 168–174.

**Hatch, M. H. 1953.** The beetles of the Pacific Northwest, University of Washington Press, Seattle, WA.

**Hibbet, D., and M. Donoghue. 1996.** Implications of phylogenetic studies for conservation of genetic diversity in Shiitake mushrooms. Conserv. Biol. 10: 1321–1327.

**Liebherr, J. K. 1988.** Gene flow in ground beetles (Coleoptera: Carabidae) of differing habitat preference and flight-wing development. Evolution 42: 129–137.

**Lynch, M., and T. J. Crease. 1990.** The analysis of population survey data on DNA sequence variation. Mol. Biol. Evol. 7: 377–394.

**Matthews, A. 1884.** Synopsis of North American Trichopterygidae. Trans. Am. Entomol. Soc. 11: 113–156.

**Moldenke, A. R. 1999.** Soil-dwelling arthropods: their diversity and functional roles, pp. 33–44. *In* R. T. Meurisse, W. G. Ypsilantis, and C. Seybold [eds.], Proceedings: Pacific Northwest Forest and Rangeland Soil Organism Symposium. USDA-FS Pacific Northwest Research Station, Portland, OR.

**Moritz, C. 1994.** Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3: 401–411.

**Moritz, C. 2002.** Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst. Biol. 51: 238–254.

**Nei, M. 1987.** Molecular evolutionary genetics. Columbia University Press, New York.

**Noss, R. F., J. R. Strittholt, K. Vance-Borland, C. Carroll, and P. Frost. 1999.** A conservation plan for the Klamath-Siskiyou ecoregion. Nat. Areas J. 19: 392–411.

**Posada, D., K. A. Crandall, and A. R. Templeton. 2000.** GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9: 487–488.

**Posada, D. 2001.** MATRIX 2.0. Available at http://inbio.byu.edu/Faculty/kac/crandall_lab/programs.htm/.

**Rambaut, A. 1996.** Se-Al: sequence alignment. http://evolve.zoo.ox.ac.uk/.

**Roderick, G. K. 1996.** Geographic structure of insect populations: gene flow, phylogeography, and their uses. Annu. Rev. Entomol. 41: 325–352.

**Rozas, J., J. C. Sanchez-Delbarrio, X. Messeguer, and R. Rozas. 2003.** DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496–2497.

**Rubinoff, D. 2001.** Evaluating the California gnatcatcher as an umbrella species for conservation of southern California coastal sage scrub. Conserv. Biol. 15: 1374–1383.

**Sawada, Y., and T. Hirowatari. 2001.** A revision of the genus *Acrotrichis* Motschulsky (Coleoptera: Ptiliidae) in Japan. Entomol. Sci. 5: 77–101.

**Sawyer, J. O. 1996.** Northern California, pp. 20–41. *In* R. Kirk [ed.], The enduring forests: northern California, Oregon, Washington, British Columbia, and Southeast Alaska. The Mountaineers, Seattle, WA.

**Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Lui, and P. Flook. 1994.** Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

**Slatkin, M., and W. P. Maddison. 1989.** A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123: 603–613.

**Sörensson, M. 1997.** Morphological and taxonomical novelties in the world's smallest beetles, and the first Old World record of Nanosellini (Coleoptera: Ptiliidae). Syst. Entomol. 22: 257–283.

**Sörensson, M. 2003.** New records of featherwing beetles (Coleoptera: Ptiliidae) in North America. Coleopt. Bull. 57: 369–381.

**Swofford, D. L. 1998.** PAUP*: phylogenetic analysis using parsimony, version 4.0b. 1. Smithsonian Institute, Washington DC.

**Taylor, A. H., and C. N. Skinner. 1998.** Fire history and landscape dynamics in a late-successional reserve, Klamath Mountains, California, USA. For. Ecol. Manag. 111: 285–301.

**Templeton, A. R. 1998.** Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7: 381–397.

**Templeton, A. R., E. Routman, and C. Phillips. 1995.** Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum.* Genetics 140: 767–782.

**Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992.** A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data III. Cladogram estimation. Genetics 132: 619–633.

**U.S. Department of Agriculture. 1994.** Record of decision for amendments to Forest Service and Bureau of Land Management planning documents within the range of the northern spotted owl: standards and guidelines for management of habitat for late successional and old growth forest related species within the range of the northern spotted owl. U.S. Forest Service and U.S. Bureau of Land Management, Washington, DC.

**Van der Wurff, A.W.G., A. Isaaks, G. Ernsting, and N. M. Van Straalen. 2003.** Population substructures in the soil invertebrate *Orchesella cincta*, as revealed by microsatellite and TE-AFLP markers. Mol. Ecol. 12: 1349–1359.

**Van Straalen, N. M. 1997.** Community structure of soil arthropods as a bioindicator of soil health, pp. 235–264. *In* C. E. Pankhurst, B. M. Doube, and W.S.R. Gupta [eds.], Biological indicators of soil health. CAB International, New York.

*Received 10 June 2004; accepted 29 May 2005.*