# POPULATION STRUCTURE OF *Acrotrichis xanthocera* (Matthews) (COLEOPTERA: PTILIIDAE) IN THE KLAMATH ECOREGION OF NORTHWESTERN CALIFORNIA, INFERRED FROM MITOCHONDRIAL DNA SEQUENCE VARIATION

A Thesis

by

RYAN MATTHEW CAESAR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2004

Major Subject: Entomology

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Approved as to style and content by:

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May 2004

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# ABSTRACT

Population Structure of *Acrotrichis xanthocera* (Matthews) (Coleoptera: Ptiliidae) in the Klamath Ecoregion of Northwestern California, Inferred from Mitochondrial DNA Sequence Variation.

(May 2004)

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The Klamath-Siskiyou Ecoregion of northern California and southern Oregon has extremely high biodiversity, but conservation centers on the protection of habitat for the northern spotted owl. A network of late successional reserves has been established without consideration of potential for protecting overall biodiversity, including genetic diversity. Mitochondrial DNA sequences are used to examine the population structure of *Acrotrichis xanthocera* (Coleoptera: Ptiliidae) sampled from five late successional reserves within the Klamath-Siskiyou Ecoregion and five comparison sites from northern California. Measures of gene flow, phylogenetic analysis, and nested clade analysis are employed to infer historical demographic and phylogeographic processes. Results show that *A. xanthocera* populations have undergone past range expansion, but gene flow is currently limited. Individual late successional reserves do not adequately protect the genetic variation in this species. Although further research is needed, these results are likely to be congruent for other edaphic arthropod species. Improvement of the late successional reserve system is warranted for maximum protection of the genetic diversity of soil arthropod populations.

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# INTRODUCTION

Based on high species richness, high endemism, and rarity of habitat types the Klamath-Siskiyou Ecoregion (Fig. 1) is one of the most diverse temperate coniferous forests in the world (DellaSala *et al*. 1999, Noss *et al*. 1999, Sawyer 1996). For example, there are over 3,500-documented plant species, including 281 endemics (Sawyer 1996). Extraordinary timber production and exceptional fisheries exemplify the economic importance of the area. The terrestrial biodiversity of the region is considered endangered, and the most significant threats are fragmentation and loss of critical habitat due to logging, grazing, and mining, and competition between native and invasive species (DellaSala *et al*. 1999). Despite an increased awareness of its uniqueness by concerned citizens and scientists, federal resource and land managers have failed to alter conservation strategies in the Klamath-Siskiyou Ecoregion.

Most of the Klamath-Siskiyou Ecoregion consists of national forests and is under the supervision of the United States Forest Service, although only roughly 25% of the habitat is undisturbed and only 10.5% of it has legal protection (DellaSalla *et al*. 1999). The U.S. Forest Service has established a management policy for the Klamath-Siskiyou Ecoregion based largely on habitat preservation for the northern spotted owl, *Strix occidentalis caurina* (Noss *et al*. 1999). This type of "species management" in which protection of one charismatic species is presumed to afford protection for all sympatric species, has been shown to be ineffective, especially when vertebrates are used as surrogates for

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This thesis follows the style and format of *Molecular Ecology*.

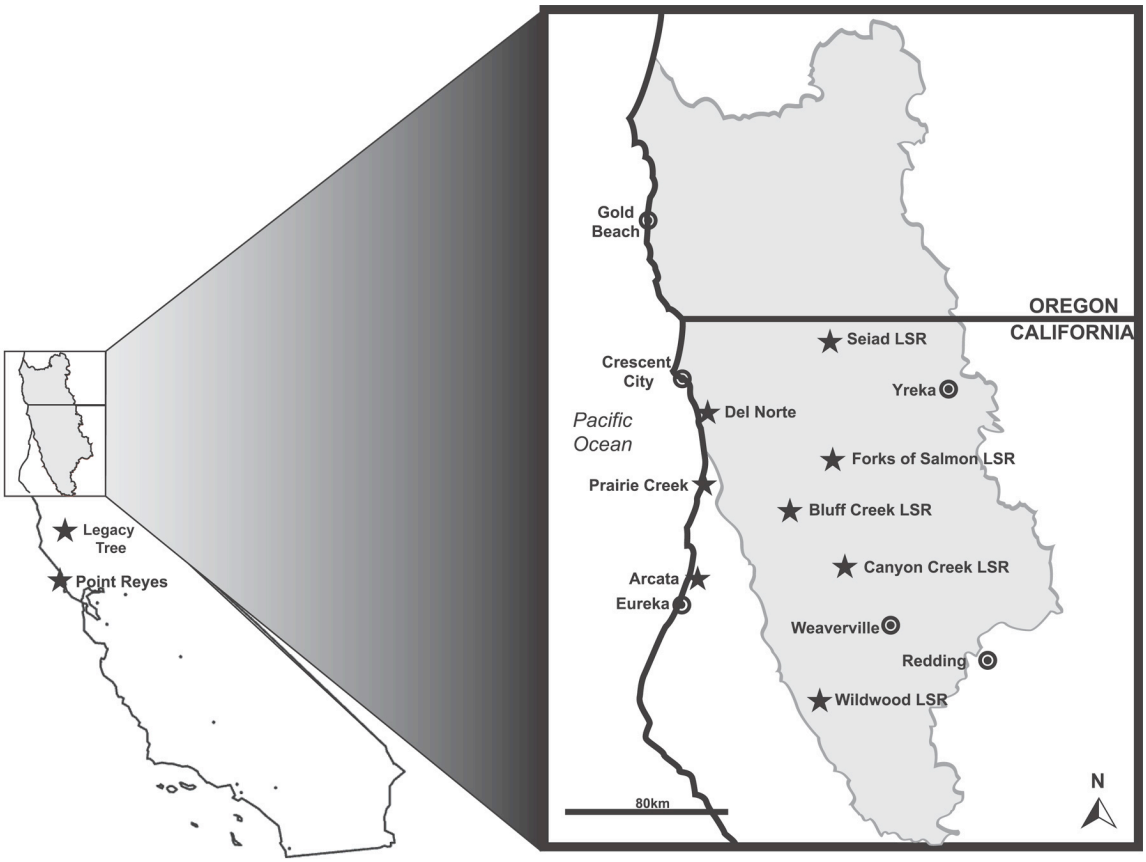


**Fig. 1**. The Klamath-Siskiyou Ecoregion (shaded area; after Noss *et al*. 1999). Stars indicate sites where *A. xanthocera* specimens were collected for this study.

invertebrates (Rubinoff 2001; Moritz 2002). This "umbrella" species management plan does not meet the broader goals for conservation in the region (Noss *et al*. 1999).

The Northwest Forest Plan (U.S. Department of Agriculture & U.S. Department of Interior 1994) designated a network of "late successional reserves" (LSRs) throughout this region. The purpose of the LSR system is to maintain characteristics of late successional forest and old growth ecosystems, and to promote the development of late-successional characteristics in younger stands (Taylor & Skinner 1998). LSRs compose about 15% of the region, although they are not truly protected areas due to sporadic timber extraction (Noss *et al*. 1999) and the potential for future development (DellaSala *et al*. 1999). A lack of unifying principles to define LSRs may facilitate this encroachment into conserved areas. Abiotic factors have been identified that will maximize the promotion of late successional conditions (Taylor & Skinner 1998), but these have not been considered in the establishment of LSRs. Measures of species and genetic diversity have not been incorporated into the establishment of LSRs. As Noss *et al*. (1999, p. 400) point out, "…the Northwest Forest Plan does a mediocre job of protecting biodiversity in the Klamath-Siskiyou Ecoregion."

Broad goals have been outlined for a comprehensive conservation plan for the Ecoregion, based on: (1) "protection of special elements, such as rare hotspots, old-growth forests, and key watersheds;" (2) "representation of physical and vegetative habitat types;" and (3) "maintenance of viable populations of focal species…" (Noss *et al*. 1999, p. 392). While this approach is appropriate, the third goal has not been sufficiently addressed. The terms "viable populations" and "focal species" are exceedingly vague and subjective. In practice, such terms typically refer to charismatic megafauna and/or threatened or

endangered species. In addition to species diversity and habitats, a primary goal of conservation is to promote the maintenance of genetic variation (which is essential to the third goal). Insufficient baseline knowledge exists for the application of such goals to broad-scale conservation practices. Broad goals and comprehensive strategies are often the claim of conservation plans; however, few of these plans are either broad or comprehensive. Often they are based on existing knowledge that is inherently limited. In order to approach a broad and comprehensive conservation strategy, basic biological research must be incorporated and plans must be dynamic enough to incorporate this new knowledge. Studies of soil arthropod community structure (MA Camann in prep.) and fire ecology (Taylor & Skinner 1998) have been recently undertaken in an attempt to improve Pacific southwestern forest management policies that focus on LSRs. In addition to such ecological studies, incorporation of historical information concerning species relationships, population structure, and movement can aid in conservation (Avise 2000; Moritz 2002).

Phylogenetic analysis of populations reveals processes involved in population structure and movement of individuals (Slatkin & Maddison 1989; Avise 1994). Gene flow over large spatial and temporal scales is difficult to observe directly, particularly for small cryptic animals. A phylogenetic approach provides an indirect means for assessment of historical and contemporary processes that shape both species and genetic diversity (Avise 2000). Patterns of gene flow provide much more insight into the evolutionary processes (both historical and current) of interest for conservation. This information can be used to improve conservation efforts and target more effective land management policies (Hibbet & Donoghue 1996; Moritz 2002).

Comparatively little biological research has been conducted to catalog, describe, and 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 macro-arthropods (including insects, mites, myriapods, copepods, etc.) that make up a significant portion of the metazoic diversity inhabiting temperate conifer forests. These arthropods are the most diverse organisms in the temperate northwest forests, and make up a significant portion of the biomass (Moldenke 1999). They play a crucial role in the ecological processes of decomposition and nutrient cycling, and are consequently essential for the long-term sustainability of forests. As such, they are useful bioindicators of forest health (Van Straalen 1997). Numerous features of insect biology, such as their relatively short life cycles and large population sizes, make them ideal study organisms for assessing the functions of geographic structure in evolutionary and ecological processes (Roderick 1996). These processes are consequently vital for management and conservation decisions. Given their importance to the health of forest ecosystems, increased ecological and systematic studies are necessary for a more complete understanding of the role these organisms play within this ecosystem.

The purpose of this thesis is to assess the geographic distribution of genetic diversity among populations of a common edaphic arthropod, *Acrotrichis xanthocera* (Matthews 1884) (Coleoptera: Ptiliidae), within a complex landscape in Northern California. Fixation indices are employed to measure contemporary gene flow among populations, a genealogical tree is reconstructed with a portion of the mitochondrial DNA cytochrome oxidase I gene, and nested clade analysis is used to test hypotheses concerning the

relationship between geography and genetic variation. The results of these analyses are used to estimate historical biological factors contributing to the current population structure of *A. xanthocera* in the Klamath-Siskiyou Ecoregion. This information is then discussed in relation to the LSR network, to provide a better understanding of how to effectively protect the genetic diversity of soil macroarthropods in the Pacific Southwest and to contribute to the overall knowledge of biodiversity in the region.

# MATERIALS AND METHODS

*Study organism*

The choice of organisms for population genetic and conservation studies requires consideration of ecological factors, availability of specimens, and availability of taxonomic expertise. An edaphic ptiliid beetle was chosen for this study because of its abundance in preliminary samples from LSRs and the availability of PCR primers for mitochondrial DNA amplification. Little is known about the biology of ptiliid beetles. They are among the smallest of known insects, rarely exceeding 1 mm in length (Dybas 1990; Hall 2001). Ptiliids feed mainly on fungi and detritus, and are found in soil, litter, under the bark of rotting logs and other moist habitats (Hall 2001; Sörensson 2003). Hence, they likely play a crucial role as decomposers in forest ecosystems, making them important contributors to the overall health of soils and forests. Ptiliids, along with other edaphic beetles, have been implicated as potentially useful bio-indicators (Sawada & 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.

Currently, there are approximately 27 genera and 120 species known in North America. This is almost certainly a severe underestimate based on the number of undescribed taxa found in museum collections (Dybas 1990; Hall 2001; Sörensson 2003). Parenthetically, collections made for the present study have potentially yielded three new species, including one new genus. Populations can be highly polymorphic in terms of gross external morphology (overall shape, presence of wings, etc.; Hall 2001), making species recognition difficult. The spermathecae of females appears to be of great value in species diagnoses (Hatch 1953; Hall 2001). Currently, M Sörensson (personal communication) is

revising the higher-level relationships of the entire family.

The genus *Acrotrichis* Motschulsky contains the largest number of described species within the Ptiliidae, although the taxonomy and phylogenetics of this genus remain equivocal (Dybas 1990; Sawada & Hirowatari 2002). Many species of *Acrotrichis* are widely distributed, and there are over 35 described species in North and Central America (Hall 2001). The species of *Acrotrichis* are normally dark brown to black, and occur in decaying and moist forest litter, dung, vertebrate nests, etc. and like other soil arthropods play a direct and crucial role in the maintenance of healthy forest ecosystems (Dybas 1990; Moldenke 1999). No accurate taxonomic keys are available for the North American species, and the genus is in need of revision (Dybas 1990). Knowledge of the Palaearctic fauna is much more complete (Johnson 2001).

This thesis centers on *Acrotrichis xanthocera* (Matthews), a common hemiedaphic Nearctic species that is dark brown with yellow legs and antennae, approximately 1 mm in overall length (Hatch 1953; Matthews 1884). It is found throughout much of the North American continent, though not occurring in the southeast (Sörensson 2003). It occurs in litter of conifer forests in the Rockies and the coastal mountain chains of the western USA and Canada. *Acrotrichis xanthocera* can be extremely abundant in moist litter, although this abundance is patchy (personal observation).

*Acrotrichis xanthocera* often occurs with apparently unisexual populations, believed to be parthenogenetic, although populations of the usual 50/50 mixture of males/females are also well known (M Sörensson personal observation, unpublished data). These parthenogenetic populations may contribute to geographic structure by increasing

reproductive isolation of populations (Roderick 1996).

An organism's potential for dispersal is of great importance to patterns of population structure. Vagile insects may have significantly less genetic population structure when compared to more sedentary organisms, as they tend to disperse over large geographic distances and there are less potential barriers to gene flow. Nonetheless, apparent population structure is not necessarily tied to an organism's vagility (Avise 1994; Roderick 1996). Small edaphic insects, despite being winged, could be expected to exhibit genetic population structure similar to that of a sedentary organism. If so, the genetic diversity would vary greatly among populations (gene flow limited by short distance dispersal). Other ecological factors such as habitat fragmentation and elevation can add to the complexity of genetic variation exhibited by insects (Liebherr 1988).

The extremely small size of ptiliids makes direct observation of dispersal and flight behavior difficult in a natural setting, and none have been reported in the literature. It has been assumed that ptiliids, due to their size and preferred habitats, may 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 featherwings of certain ptiliids allow for passive dispersal over long distances (Dybas & Dybas 1981; Dybas 1990). If the latter case applies to *A. xanthocera*, then dispersal by adults may be limited only by wind, allowing at least occasional long distance colonization. Such a situation might result in a lack of significant population structure.

*Study area and sampling*

The Klamath-Siskiyou Ecoregion is a mountainous forest of mixed conifers and hardwoods, approximately 44,000 $km^2$ in area. Near the coast the climate is wet and mild, but inland it is Mediterranean (Noss *et al*. 1999). A majority of precipitation occurs in fall and winter (Taylor & Skinner 1998), making the summers rather mesic. Douglas fir, sugar pine, pacific madrone, tanoak, and the endemic Port-Orford-Cedar are common tree species found in the forest. Adjacent Ecoregions include coastal redwood forests to the southwest, the Pacific Ocean to the west, the central California valley to the east and south, and the Cascade Range to the north and east.

The topography and geology of the Klamath are considerably heterogeneous, with steep gradients of altitude, temperature and precipitation (Sawyer 1996). Frequent natural disturbances such as fire, wind, volcanic eruptions, insect outbreaks, etc. (Taylor & Skinner 1998), as well as logging, development and other anthropogenic disturbances, further contribute to a dynamic and complex interaction between environment and biota. These long and short-term environmental factors likely are crucial to the creation and maintenance of high biodiversity (Sawyer 1996).

Samples were collected from five LSRs throughout the Klamath, as well as from five sites outside of the Klamath (Fig. 1, Table 1) throughout May and June of 2002 and 2003. LSRs were chosen for this study based on similarity of elevation, successional stage, vegetation, terrain, as well as accessibility. Non-LSR sites from outside of the Klamath-Siskiyou Ecoregion were chosen to maximize the likelihood of adequately sampling *A. xanthocera*. Prairie Creek and Del Norte sites are in a national park that contains much of

the remaining old growth redwood forest. Arcata samples are from a "community forest" that is secondary growth forest. These sites were lower in elevation, with less rugged terrain, and are considerably less mesic.

All samples were selected based on the presence of moisture in litter to maximize the chance of collecting ptiliids. Soil, moist litter, mammal dung, detritus, etc. was placed into cotton bags and taken to the laboratory, where samples were placed in Berlese funnels in a refrigerated room. Over the course of 2-3 days, arthropods were extracted under 60 watt light bulbs into 80% ethyl alcohol. Ptiliids were removed from coarse arthropod samples and sorted to morphospecies. Individuals were isolated from one another and placed in individually labeled vials into 100% USP ethyl alcohol. These vials were stored at –20°C for molecular study.

Individuals were initially determined to genus and morphospecies, and representatives from each morphospecies and sample locality were sent to M. Sörensson (Lund University, Lund, Sweden) for species identification. To allow visualization of spermathecae, representative specimens were slide-mounted following the method of Hall (2001). Specimens not slide mounted were stored in 100% ethyl alcohol. All specimens used in this study, as well as additional individuals from all populations sampled, have been designated as voucher specimens. All voucher specimens used in this study are permanently deposited in the Texas A&M University insect collection (voucher number 642).

**Table 1**. Sample locality and haplotype information. Haplotype diversity, *h*, is not reported for populations with $n < 10$.

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

*Rationale for molecular marker*

A total of 750 bp of the mitochondrial (mt) protein coding gene cytochrome *c* oxidase I (COI) was sequenced from each individual ptiliid. MtDNA is a useful neutral marker for assessment of genetic variation (Avise 1994). Maternal inheritance of mtDNA results in short coalescence time (4 times the rate of nuclear DNA; Moritz 1994), accordingly genetic variation caused by recent barriers to gene flow may be revealed. MtDNA sequences are particularly well suited for this purpose, provided that enough variation exists to resolve a phylogeny. Intraspecific phylogenetics require that species be made up of populations corresponding to independent lineages that can be examined in a bifurcating, hierarchical context. Some authors suggest that this is not the case, and this brings up arguments about how gene trees relate to species trees (Avise 1989; Brower *et al*. 1996; Maddison 1997). Because it is a well-known gene that codes for an evolutionarily conservative protein, COI has been informative for systematic study at the population level in numerous insect species (Cognato & Sperling 2000; Roderick 1996; Simon *et al*.1994).

*Laboratory methods*

Prior to genomic DNA extraction, individuals were assigned a number code based on the order in which they were extracted; several individuals from different populations were extracted concurrently. Individual specimens were placed on a Petri dish and, with the aid of a stereomicroscope, forceps, and insect pins, the abdomen was separated from the head (all tools were sterilized by 100% ethyl alcohol and flame). Extraction generally preceded specimen identification, so the bodies of individuals were not ground in the usual fashion; rather, the sclerotized portions of the body were retrieved following digestion for subsequent

identification. Genomic DNA was extracted from each individual using DNeasy tissue kits (Qiagen Incorporated, Valencia, CA). The manufacturer's animal tissue protocol was followed, except the amount of proteinase K added during lysis was double the recommended volume and the incubation period was overnight (~20 hours) to maximize the yield of DNA from these very small specimens.

Universal insect primer pairs (stock oligonucleotides supplied by Integrated DNA Technologies, Incorporated, Coralville, Iowa) were used for amplification of this 750 bp region: 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'). A new primer (affectionately referred to as BOBO, formal naming in progress; 5'- AAT GAA TAT CAA TGA ACG AAC CC -3'), specific to *A. xanthocera*, was designed for use with CI-J-2183 (5'- CAA CAT TTA TTT TGA TTT TTT GG –3') (Simon *et al*. 1994). These overlapping primer sets were used together to ensure accurate nucleotide sequence designation. The extraction procedure often yielded inconsistent amounts of genomic DNA; to compensate for this, variable quantities of DNA and $H_2O$ were added for the PCR, depending on the relative DNA concentration. PCR reactions were carried out in 25 µl volumes using puRe*Taq* Ready-To-Go™ PCR beads (Amersham Biosciences, Piscataway, NJ). The following steps were performed on a programmable thermal cycler: cycle 1, 95° for 2.0 min.; cycles 2-36, 94° for 0.5 min., 45° for 0.75 min., 72° for 1.0 min.; cycle 37, 72° for 5.0 min.

5.0 µl of PCR product from each individual was mixed with 2.0 µl 5X loading buffer (Applied Biosystems, Foster City, CA) and applied to an ethidium bromide stained 1.5%

agarose gel at 100v for 30 min. To verify amplification the gel was illuminated under an ultraviolet light source and photographed. Relative concentration of amplified DNA was estimated against a 100 kb ladder.

The amplified PCR products were purified using the QIAquick PCR purification kit (Quiagen Incorporated, Valencia, CA) and sequenced using the BigDye® Terminator v.1.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) according to manufacturer recommendations. The same primers used for PCR were used in the cycle sequence reactions. Sequences were applied to polyacrylamide gels (Long Ranger® Singel® Packs, Cambrex Corporation, East Rutherford, NJ) on either an ABI PRISM® 377 automated sequencer or an ABI PRISM® 3100 Genetic analyzer (Applied Biosystems, Foster City, CA) for visualization of the products of cycle sequencing reactions.

Sequences were manually edited using Sequence Navigator version 1.0.1 (Applied Biosystems, Foster City, CA) 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/deletion (indel) events. All individuals were sequenced in both the 5' and 3' directions to verify nucleotide designation at each position. Unanimous nucleotide designations at each position among individual sequences supplied additional verification of sequence identity. Resulting consensus sequences were compared with published sequences using BLAST sequence similarity searching (NCBI, Bethesda, MD) to confirm the identity of the COI fragment. The closest matching sequences were those of other beetles in the staphylinoidea (e.g. GenBank accession number AJ293079). Reference sequences from

each population have been deposited in the GenBank nucleotide sequence database under accession numbers AY550852-AY550882 (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html).

Sites with missing data were considered in the identification of haplotypes. Often these sites are excluded from haplotype designation, but this is an extremely conservative approach that ignores variation present in those taxa for which data exists at these sites.

*Population genetic, phylogenetic and nested clade analysis*

Several software packages were used to explore sequence polymorphism, divergence between populations, and gene flow. A Mantel test, which tests for correlations between genetic and geographic distances, was implemented in 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 using the fixation indices $N_{ST}$ and $G_{ST}$. $N_{ST}$ and $G_{ST}$ values are analogues of Wright's $F_{ST}$ that are better suited for nucleotide and haplotype data, respectively (Lynch & Crease 1990). $G_{ST}$ is similar to $N_{ST}$ except that it deals with haplotype frequency rather than nucleotide divergence, and estimates of $N_{ST}$ and $G_{ST}$ tend to be highly correlated (Lynch & Crease 1990). Nucleotide diversity ($\pi$, Nei 1987) was calculated using Matrix 2.0 (Posada 2001). Haplotype diversity was calculated as $h = n(1-\sum p_i^2)/(n-1)$ where $n$ is the number of haplotypes and $p_i$ is the frequency of the $i$-th haplotype (Nei 1987).

Phylogenetic relationships of 117 *Acrotrichis xanthocera* mtDNA COI sequences were estimated using PAUP* version 4.0b2a for Macintosh (Swofford 1998). A heuristic search was performed for the maximum parsimony 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 one were saved, so as to reduce the computational time of the analysis. In order to assess the confidence of individual nodes on the trees, bootstrapping (1000 pseudo-replicates) was performed.

A nested clade analysis of the 117 mtDNA COI sequences 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 statistical parsimony, 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 using GEODIS version 2.0 (10 000 permutations; Posada *et al*. 2000). The results were interpreted via Templeton's (1998) inference key, 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 % 1st, 5 % 2nd, and 84 % 3rd). The first nucleotide of this sequence corresponds to nucleotide 2235 of the *Drosophila yakuba* mtDNA COI sequence (Fig. 2; Clary & Wolstenholme 1985). Mean base frequencies are A: 0.311, C: 0.150, G: 0.147, and T: 0.392.

An initial test for an association between genetic (uncorrected "p") and geographic distances (Table 2) showed a weak, yet statistically significant (at the $\alpha = 0.05$ level) positive correlation (r = 0.167). Analysis of gene flow and genetic differentiation among populations were based on fixation indices. The estimates for all populations of $G_{ST} = 0.291$and $N_{ST} = 0.727$ indicates moderate genetic differentiation and limited gene flow for these haplotypes. Pairwise comparisons of $G_{ST}$ and $N_{ST}$ values among populations indicate that genetic variation follows geographic pattern by grouping together the Klamath-Siskiyou LSRs (referred to as Klamath in Table 3), the coastal populations, and the southern populations (Table 3). Fixation indices are useful measures of genetic differences among populations. However, they do not allow the assignment of cause for the observed patterns, nor do they necessarily reflect historical patterns.

A total of 31 mtDNA haplotypes were identified for 117 *A. xanthocera* individuals. Overall haplotype diversity is high ($h = 0.839$) and nucleotide diversity is low ($\pi = 0.0494$). The number of haplotypes per population ranges from two to nine (Fig. 3). Haplotype A is the most abundant and is present in all populations except Point Reyes, with high

| 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 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| **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 |
| **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 *Drosophila yakuba* COI sequences (Clary & Wolstenholme 1985). Asterisks refer to invariant sites, question marks indicate sites with missing data.

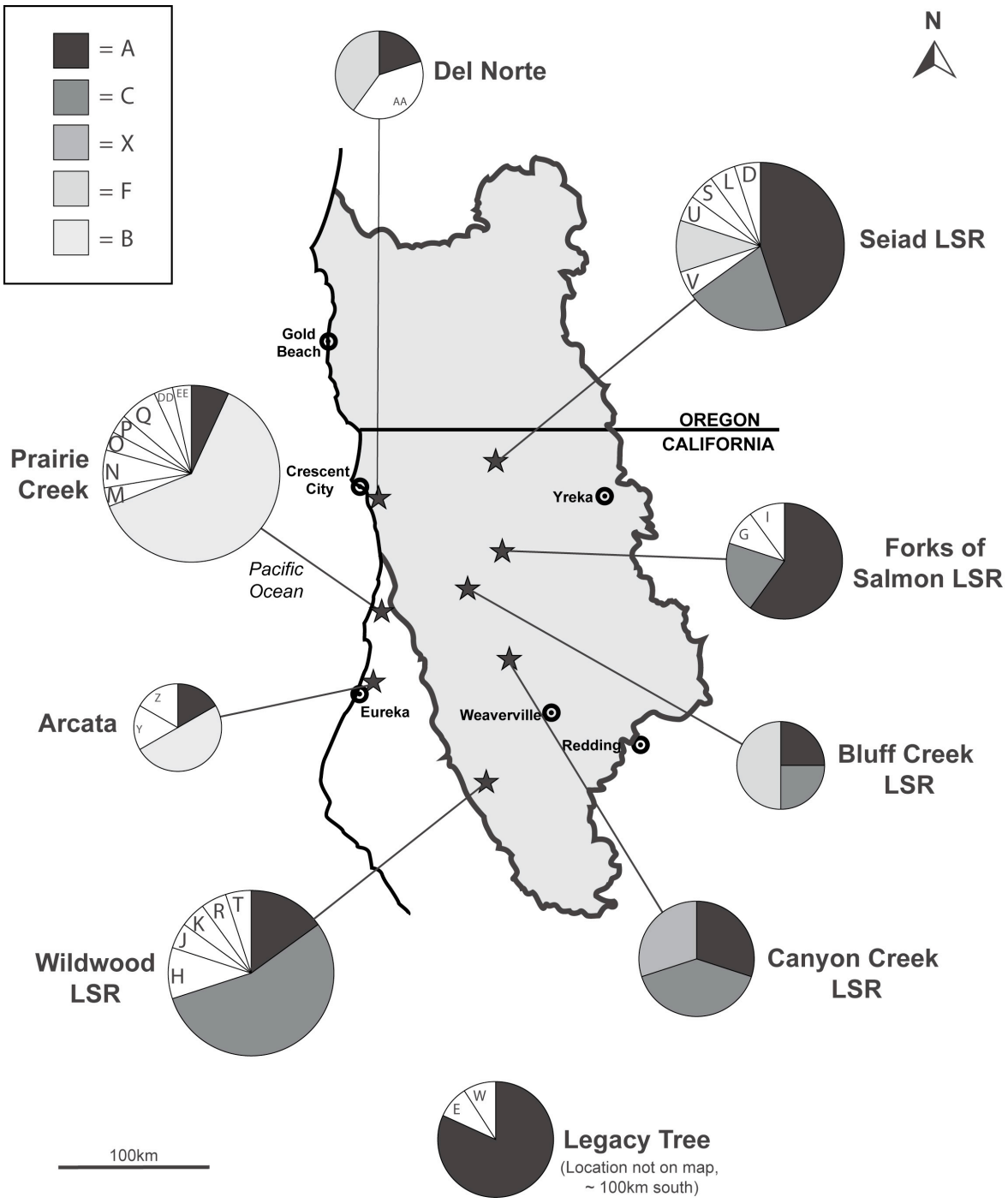


**Fig. 3**. Distribution of *A. xanthocera* haplotypes in the Klamath-Siskiyou Ecoregion (shaded region). Legend refers to most common haplotypes, with unique or singleton haplotypes designated by letters on pie charts. The size of each pie chart reflects relative sample size of that sample. Point Reyes, located ~200 km to the south, is not shown due to its small sample size.

**Table 2**. Pairwise geographic distances (lower diagonal; km) and genetic distances (upper diagonal; uncorrected "p") between populations. 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.

| | *BC* | *CC* | *FS* | *Se* | *Ww* | *Ar* | *DN* | *PC* | *LT* | *PR* |
|---|---|---|---|---|---|---|---|---|---|---|
| BC | 0 | 0.0015 | 0.0013 | 0.0017 | 0.0035 | 0.0052 | 0.0034 | 0.0012 | 0.0009 | 0.0333 |
| CC | 72 | 0 | 0.0013 | 0.0016 | 0.0029 | 0.0035 | 0.0029 | 0.0106 | 0.0010 | 0.0332 |
| FS | 41.3 | 55 | 0 | 0.0015 | 0.0029 | 0.0032 | 0.0027 | 0.0105 | 0.0008 | 0.0329 |
| Se | 88 | 116.3 | 64.1 | 0 | 0.0028 | 0.0031 | 0.0027 | 0.0095 | 0.0013 | 0.0326 |
| Ww | 110.7 | 50.9 | 104.7 | 167.2 | 0 | 0.0047 | 0.0044 | 0.0102 | 0.0030 | 0.0327 |
| Ar | 53.4 | 88.6 | 89.2 | 141.5 | 102.9 | 0 | 0.0028 | 0.0103 | 0.0027 | 0.0324 |
| DN | 61.4 | 132.1 | 87.7 | 89.6 | 171.8 | 92.4 | 0 | 0.0103 | 0.0021 | 0.0322 |
| PC | 30.3 | 100.7 | 68.6 | 98.8 | 135.3 | 54.3 | 39.4 | 0 | 0.0102 | 0.0310 |
| LT | 198.6 | 166.5 | 208.8 | 272.9 | 112 | 162 | 253 | 213.5 | 0 | 0.0324 |
| PR | 359.4 | 307.7 | 361.5 | 424.1 | 257 | 326.5 | 416 | 376.6 | 164.7 | 0 |

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

| Geographic comparison | Number of populations | Number of haplotypes | $N_{ST}$ | $G_{ST}$ | Gene flow & 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* |
| Klamath-Coast | 8 | 27 | 0.453 | 0.200 | Moderate-high, structure |
| Klamath-South | 7 | 20 | 0.134 | 0.080 | Moderate, structure* |
| Coast-South | 5 | 15 | 0.245 | 0.194 | Moderate, structure* |

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

frequencies at Seiad and Legacy Tree. Haplotypes B and C are the next most abundant; B is present in the coast populations only (PC, Ar, and DN) while C is present in the Klamath populations only (Se, Ww, FS, CC, and BC). Most haplotypes are singletons, represented by one individual. All populations except Bluff Creek ($n$ = 4) contain unique haplotypes. The spatial variation in both haplotype frequency and composition (Fig. 3) suggests that there has been restricted gene flow with either occasional long distance colonization or past range expansion.

The phylogenetic analysis yielded 25, 000 equally most parsimonious trees of length 79. Although resolution among individuals is low, homoplasy is not likely to be the cause (CI = 0.747, RI = 0.935, RC = 0.699). The low resolution can thus be attributed to a lack of parsimony informative characters in these data. Alternatively, this high number of trees is an artefact of missing data in the matrix. Some individuals had up to 25% missing data. Missing data are known to increase the number of equally most parsimonious trees while decreasing resolution (Kitching *et al*. 1998). In an attempt to increase resolution, only individual haplotypes were included and reanalyzed under the same conditions. Fewer equally most parsimonious trees were found, but the resulting tree topologies do not differ from the analysis of individuals.

Clearly, the intraspecific genealogy of *A. xanthocera* mtDNA haplotypes lacks resolution. Individuals sampled from the Klamath populations (with one exception, haplotype R, from Wildwood) formed an unresolved monophyletic clade in the strict consensus (Fig. 4). There are no obvious phylogenetic patterns for *A. xanthocera* populations within the Klamath, with no cases of reciprocal monophyly.

The statistical parsimony analysis confirmed the results of the cladistic analysis while providing greater resolution among haplotypes (Fig. 5). Haplotypes group into a network comprised of three geographic lineages, corresponding to the Klamath, coastal, and southern sampling areas (Fig. 3). Clade 2-7 is left unresolved in Fig. 5, because it is comprised of inferred intermediate haplotypes and only one observed haplotype (R). The reticulations in this clade could not be unambiguously resolved, and do 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 remaining haplotypes, which is an artefact of the statistical parsimony analysis. These haplotypes are isolated both phylogenetically and geographically and have no influence on the interpretation of demographic events in the nested clade analysis.

Within the Klamath, the null hypothesis of a random association between geographic locality and haplotype frequencies cannot be rejected. The nested clade analysis provides 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 (Table 4). Within the Klamath, gene flow is also limited, albeit to a lesser degree (Table 4). Inference of the total cladogram is not possible, as it involves the nesting of two tip clades (4-1 & 4-2). Both tip and interior clades are required to infer demographic events. This is not problematic, as the inference from lower level clades corroborates the inferences obtained from the genetic and phylogenetic analyses.

The fixation indices based on haplotype frequency, gene trees obtained through cladistic analysis, and the haplotype network and nested clade analysis all corroborate a pattern of historical range expansion at the scale sampled, and more recent limitation of gene flow between southern, coastal, and Klamath lineages.

# DISCUSSION

## *Population structure of* Acrotrichis xanthocera

Populations of *Acrotrichis xanthocera* display little structure at the scale of the Klamath-Siskiyou Ecoregion, but on a broader geographic scale genetic subdivision is apparent. Overall haplotype diversity is high, and most populations have one or more unique haplotypes (Fig. 3). Three different lines of inquiry support these results: conventional population genetic measures, phylogenetic analysis, and nested clade analysis. Gene flow among populations is moderate to high; cladistic analysis reveals little phylogenetic resolution (Fig. 4), and the spatiotemporal distribution of haplotypes indicates some contemporary gene flow among adjacent populations (Table 4). These results provide a means for assessing evolutionary and ecological processes and for making judgments concerning establishment of management plans based on genetic variation (Moritz 1994).

Measurements of gene flow, especially when coupled with a cladistic analysis of population structure, can provide indirect estimates of the dispersal capabilities of species' for which direct observation is difficult. Fixation indices suggest that gene flow is moderate to high between the Klamath and coastal populations (Table 3), but this approach does not take into consideration historical processes. It could be that the presence of the shared haplotype A and small sample sizes inflate the $N_{ST}$ values. The most common haplotype, A, is shared among all geographically contiguous populations, absent only in the far removed Point Reyes sample. The presence of haplotype A in these populations can be explained by a lack of ancestral lineage sorting and little contemporary gene flow between these regions,

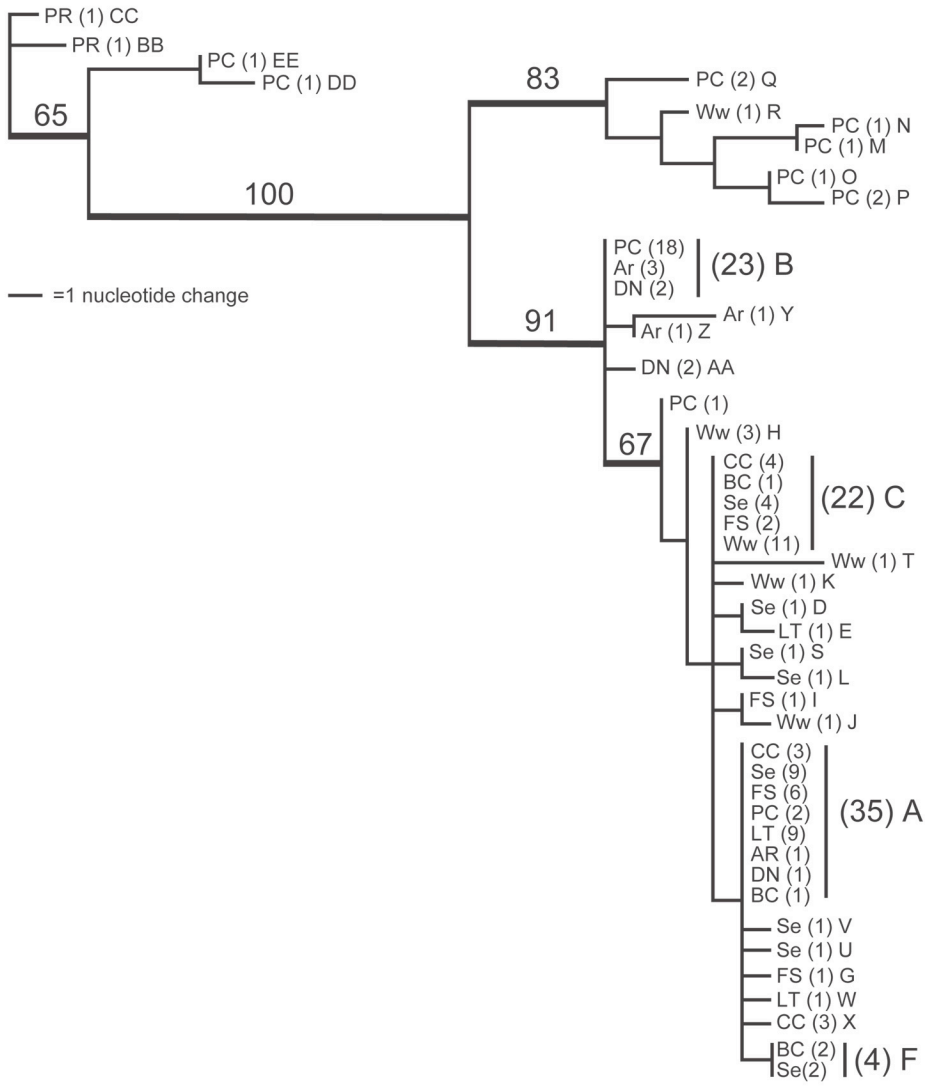


**Fig. 4.** 1of 25 000 equally most parsimonious trees of 117 *A. xanthocera* individual mtDNA COI sequences, sampled from 10 populations in northern California, USA. Darker lines indicate branches and nodes recovered in the strict consensus of all trees, and numbers are bootstrap values for those nodes. 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 haplotpye.

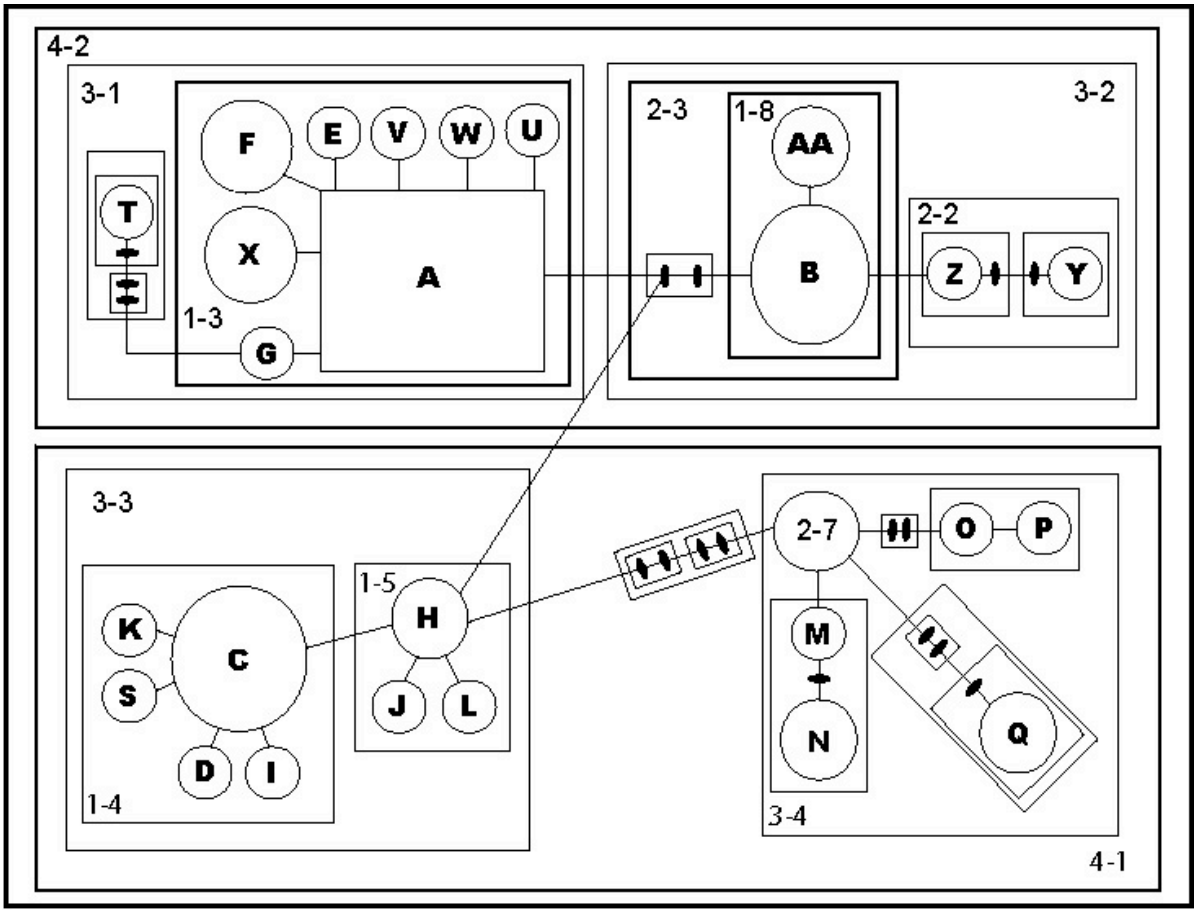


**Fig. 5.** Nested network of *A. xanthocera* haplotypes. Hollow circles and squares indicate individual haplotypes with frequencies proportional to size. Haplotype A has the greatest root probability. Filled ovals represent inferred intermediate haplotypes based on the statistical parsimony analysis. 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 darker 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* |
| | | 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. = non-significant. 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.

*Sample design inadequate to discriminate between isolation by distance versus long distance dispersal

which is supported by the nested clade analysis. Haplotype A represents the root of the statistical parsimony network (Fig. 5). It follows that A is the ancestral haplotype, and populations have not been isolated long enough for complete lineage sorting to occur. The partitioning of haplotypes B and C, together with the persistence of haplotype A in most populations, suggests that populations are becoming increasingly isolated, but ancestral lineage sorting is incomplete. Phylogeographic analysis further indicates that historically these populations experienced a contiguous range expansion (Table 4). More recently, gene flow has been restricted resulting in isolation by distance, as indicated by the higher incidence of private haplotypes in most populations (Fig. 3). The nested clade analysis (Fig. 5; Table 4) supports a stepping stone model (Crow & Kimura 1970) of contemporary gene flow for *A. xanthocera*, with short-range dispersal among adjacent populations. The apparent lack of population structure for *A. xanthocera* in the Klamath is probably due to a lack of long-term barriers to gene flow. *Acrotrichis xanthocera* females are apparently more vagile than their edaphic lifestyles might imply.

Soil macroarthropods may be generally considered sedentary, or with limited dispersal capabilities on the order of meters (Van der Wurff *et al*. 2003). A recent study of collembolans showed that one species' population structure is influenced by long distance dispersal, despite its edaphic and wingless condition (Van der Wurff *et al*. 2003). A study of closely related carabid beetle species demonstrated genetic variation of an order of magnitude among species and that genetic heterogeneity is more closely correlated with environmental factors than with *a priori* dispersal capability based on wing development (Liebherr 1988). Dybas (1990) noted that ptiliid wings might function primarily for passive

flotation; if so, then prevailing winds and local air currents are the only limiting factors for the dispersal patterns of ptiliids. The cladogram and fixation indices support a pattern of long distance gene flow for *A. xanthocera* females. The nested clade analysis, however, suggests that female dispersal in *A. xanthocera* is frequent, but limited by geographic proximity. Together, these studies indicate that perceived dispersal capabilities are potentially unreliable predictors of population structure and that environmental factors such as habitat preference, habitat fragmentation, and climate influence the geographic distribution of genetic diversity to a greater extent.

*Implications for conservation in the Klamath-Siskiyou Ecoregion*

Soil arthropods in the Klamath-Siskiyou Ecoregion have experienced habitat fragmentation largely as a result of logging practices. Little old growth or late successional forest remains intact (Sawyer 1996; DellaSala *et al*. 1999; Noss *et al*. 1999). In order to preserve significant molecular biodiversity, genetic variation in local populations must be considered. Populations frequently exist in small patches of suitable litter, setting the stage for increased isolation among populations as habitat becomes increasingly fragmented. Because each site studied harbors a unique subset of the genetic diversity of the species, a potential for a reduction of genetic variation exists, which can lead to inbreeding depression, loss of population size, and ultimately extinction. At present there does not appear to be a threat to the survival of *A. xanthocera*, but the patterns shown by this common beetle can be used to assess the situation for other edaphic arthropods. The use of common organisms to look for patterns that might apply to rare and unique sympatric organisms is clearly desirable as such research is often disruptive or harmful to the study organisms. There is support for

the use of population structure in widespread species to direct conservation plans (Fréville *et al*. 1998), as this approach may predict areas of endemism for phylogenetically and ecologically related taxa. Moreover, the present status of *A. xanthocera* in the Klamath-Siskiyou Ecoregion can only be expected to persist as long as further habitat reduction and fragmentation is proscribed.

Examining the community structure of soil arthropods provides an effective means of assessing the relative health of soil and forest ecosystems (Van Straalen 1997). Studies of this nature are ongoing in the Klamath-Siskiyou Ecoregion (M Camman *in prep*.) The community structure approach requires detailed knowledge of single species fluctuations due to environmental factors that can occur from year to year or even seasonally (Van Straalen 1997), and these studies do not necessarily incorporate a historical perspective on the value of particular localities in terms of their contribution to sustaining genetic diversity among sympatric organisms. Measurements of species richness and community structure are impeded by both a lack of taxonomic knowledge (Crozier 1997) and a taxonomic bias in conservation research (Clark & May 2002). The use of indicator species for conservation involves additional uncertainties that can be avoided by using alternate approaches such as those used here (Crozier 1997). Phylogenetic measures of biodiversity are better than species richness indices for assessing conservation worth (Crozier 1997), but knowledge of historical biogeography based on molecular evidence is often lacking at the regional scale (Moritz 2002). Sampling organisms for genetic diversity to identify patterns of biodiversity and examining evolutionary processes circumvents the need for long-term studies of

populations required in a community structure approach and accommodates the lack of taxonomic knowledge for soil insects.

Moritz (1994) suggested a "community genetics" approach for establishing conservation priorities, which involves examining multiple species within a geographic region for unique population structure. It has been shown that demographic processes can be congruent across multiple lineages within a geographic region (Mortitz 1994, 2002; Calsbeek *et al*. 2003), provided that these processes are inferred from common molecular markers (Crozier 1997). This congruence will vary with geographic area, vagility of organisms examined, and vicariance among biogeographic entities (Mortiz 2002). Calsbeek *et al*. (2003) reported a strong concordance among the structure of populations of higher-level taxa in the major Californian biogeographic regions. However, within the Klamath-Siskiyou Ecoregion there have been no investigations across multiple taxa to test for geographic patterns that might test the efficacy of the LSR system or guide the future establishment of biological reserves. Given the taxonomic bias against insects in conservation research and literature (Clark & May 2002), considerable work remains before such an approach based on soil arthropods can be implemented for the Klamath-Siskiyou Ecoregion.

Although the present study does not incorporate this community genetics approach, the results can be used to provide a preliminary assessment of the LSR system in meeting the concerns of conservation geneticists. Variation in genetic diversity among geographically isolated populations can be used to guide the establishment of future reserves or to adjust the current network of LSRs. The LSRs protect variable levels of haplotypic

diversity and uniqueness (Fig. 3). For example, the Seid and Wildwood LSRs harbor substantial haplotype diversity, and both contain five unique haplotypes. The Forks of Salmon LSR, however, contains only two unique haplotypes and has poorer haplotype diversity. Likewise, mostly common haplotypes occur in the remaining LSRs sampled. A non-LSR, the Prairie Creek site, harbors substantial haplotype diversity and contains a greater number of unique haplotypes than any of the LSRs. Prairie Creek is a 14, 000 acre sanctuary of old-growth forest has been absent of logging for at least a century, which may explain the high genetic diversity therein. Considering the quality and quantity of genetic variation present at Prairie Creek, it is apparent that many of the LSRs do not protect comparable genetic diversity. Local dispersal of *A. xanthocera* (and likely sympatric edaphic arthropods) suggests that future LSRs should be established as a connective network. An increased proximity of LSRs would help to facilitate gene flow throughout the ecoregion.

The LSRs sampled for this study do appear to protect a substantial amount of the genetic diversity of *Acrotrichis xanthocera*. However, considering the disparity among individual LSRs in haplotype diversity and uniqueness, the LSR system may need improvement. Furthermore, the guidelines to establish LSRs and the laws that protect them are ambiguous or non-existent. It is acknowledged that resources are limited and therefore preservation of all late successional patches is not possible. In these cases, decision makers should use multiple measures of biodiversity in order to assess conservation priority. If the U.S. Forest Service is concerned with long-term protection of biodiversity within its forests, LSRs should be further evaluated for their potential to protect genetic diversity.

# CONCLUSIONS

Despite recognition of the Klamath-Siskiyou Ecoregion as having extremely high biodiversity (DellaSalla *et al*. 1999), little research has addressed questions of genetic diversity in all but the most charismatic and economically important taxa. The structure of genetic variation among populations and subpopulations is the best basis for inference of historical gene flow (Avise 2000) and other biogeographic events that shape patterns of biodiversity within and among species. There are no studies on the genetic structure of ecologically important edaphic arthropods in this region. Little is known about the range and dispersal of ptiliid beetles.

I have presented the first study of population structure in an edaphic arthropod, *Acrotrichis xanthocera* (Coleoptera: Ptiliidae), for the Klamath-Siskiyou Ecoregion. The primary outcomes of this study are: 1) there is little population structure within the Klamath-Siskiyou Ecoregion, although at broader geographic scales population structure is apparent and 2) genetic diversity is not uniformly distributed across the sampled areas, with almost all populations containing unique haplotypes. These patterns are consistently explained by a historical period of range expansion, followed by restriction of gene flow and isolation by distance in more recent times.

This little known, yet common and ecologically important beetle exhibits geographic patterns that may be consistent with those of sympatric organisms that are ecologically similar. The results support the findings of others (DellaSalla *et al.* 1999; Noss *et al*. 1999) that the Klamath-Siskiyou is a unique and diverse ecoregion. Additionally, it contributes to progress in meeting one of the primary goals of a broad conservation plan for the region

(Noss *et al*. 1999). By reporting the geographic distribution of genetic variation in the Klamath-Siskiyou Ecoregion, I have provided information that may be useful to making decisions that will enable the maintenance of viable populations.

Studies of this nature are most valuable to the concerns of conservation biologists and land managers when they are done in comparison with diverse taxa within the same region (Mortiz 1994, 2002; Crozier 1997). Hence, similar studies of numerous and diverse sympatric taxa are warranted. If the goal of conservation biology is to protect and enhance biodiversity, then the evolutionary processes that create it cannot be ignored.

Further research using more populations and increased geographic sampling is needed to assess population structure on a finer scale as well as to examine the situation in the entire range of the species. Similar studies on other soil arthropods should be conducted, such that a community genetics approach can be taken to assess conservation value of particular locations.

The fundamental goal of conservation biology is to preserve biodiversity and the processes that shape it, in spite of antagonistic anthropogenic disturbances (Heywood 1994; Crozier 1997; Moritz 2002). However, in practice such a broadly defined goal is open to severe misinterpretation and oversimplification. There is not likely to be any single solution to the problems associated with the identification and protection of biodiversity, even on regional levels. The definition of biodiversity itself is multi-faceted (Heywood 1994), and does not acknowledge the potential mutual exclusivity of the concepts of diversity and uniqueness. The development of comprehensive conservation plans that can accommodate

multiple levels of biodiversity and the evolutionary and ecological processes that shape it are increasingly advocated (Heywood 1994; Noss *et al*. 1999; Mortiz 2002).

Biological conservation is ultimately more of an economic and political problem (Crozier 1997) than a scientific one. Numerous factors go into making management decisions concerning public lands, and scientific criteria are rarely considered. Crozier (1997) pointed out that one of the remaining voids that plagues conservation biology is the lack of basic biological research for species that are not "model organisms." This thesis has ventured to fill part of this void for one beetle species in the Klamath-Siskiyou Ecoregion.

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