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Patterns of Morphological, Biochemical, and Molecular Evolution in the *Oeneis chryxus* Complex (Lepidoptera: Satyridae): A Test of Historical Biogeographical Hypotheses

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Surveys of allozyme allele frequency and mitochondrial DNA (mtDNA) sequence variation were employed to test historical biogeographical hypotheses on the origin and unique distribution of the synchronized biennial, high-altitude butterflies of the *Oeneis chryxus* complex in western North America. Populations of *O. c. stanislaus* and *O. ivallda* from the central and northern Sierra Nevada are indistinguishable by use of allozyme allele frequency data, possessed nearly identical mtDNA cytochrome oxidase subunit 1 (COI) haplotypes, and were found to be relatively distantly related to *O. c. chryxus* from the Snake Range in eastern Nevada. However, individuals of *O. ivallda* from Piute Pass in the southern Sierra Nevada are more variable, with some individuals sharing mtDNA characteristics with *O. c. chryxus*. We find little support for the hypothesis proposed by W. Hovanitz in 1940 that *O. c. stanislaus* invaded the central Sierra Nevada from across the Great Basin and displaced *O. ivallda*, but cannot reject the hypothesis that ancestral *Oeneis* dispersed across the Great Basin to California. This result is congruent with hypotheses of dispersal across the Great Basin for the origin of some Sierran alpine organisms.

***Key Words:* *Oeneis*; phylogeography; mitochondrial DNA; Great Basin; Sierra Nevada; Lepidoptera.**

INTRODUCTION

Hovanitz (1940) proposed that the alpine butterfly *Oeneis chryxus stanislaus* in the Sierra Nevada of California originated via dispersal from the Rocky Mountains. He suggested that these butterflies dispersed by "island-hopping" across Great Basin mountain ranges. Paleovegetation reconstructions based on data from packrat (*Neotoma* sp.) middens and palynological data accumulated since 1940 indicate that Pleistocene climate changes resulted in significant elevational shifts in vegetation zones and that the lowest elevation areas in the Great Basin were probably occupied by subalpine parkland/tundra/steppe vegetation dominated by Bristlecone (*Pinus aristata*) and Limber Pines (*P. flexilis*) at the end of the Pleistocene (Wells, 1983). This late glacial Great Basin environment could have facilitated the dispersal of alpine organisms, including *Oeneis*, from the Rocky Mountains or eastern Great Basin to the Sierra Nevada (Billings, 1978; Wells, 1983). Paleobotanical and geological evidence suggests that the mean elevation of the Great Basin was as much as 1.5 km higher in the Miocene and that the current Basin and Range topography is the result of subsidence rather than uplift (Wernicke *et al.*, 1988; Wolfe *et al.*, 1997). Depending on the rate of subsidence, higher elevations in the Great Basin during the Pleistocene could have augmented the dispersal of alpine organisms from the east. We used a phylogeographic approach (Avise, 1994) to (1) examine the systematic relationships of *O. c. stanislaus* and its close relatives *O. c. chryxus* and *O. ivallda* and (2) test the historical biogeographical hypothesis of migration across the Great Basin. This approach employed an analysis of allozyme allele frequency data and variation in mitochondrial DNA (mtDNA) sequences from across a large portion of the range of the *O. chryxus* complex to determine the evolutionary relationships among butterfly populations. These relationships should reflect the history of dispersal or vicariance in these organisms (Avise, 1994; Morrone and Crisci, 1995).

MATERIALS AND METHODS

The Oeneis chryxus Complex

The *O. chryxus* complex is distributed across much of northern North America (Fig. 1) and these butterflies are biennial (=semivoltine), requiring 2 years to complete their life cycle and overwinter twice, presumably

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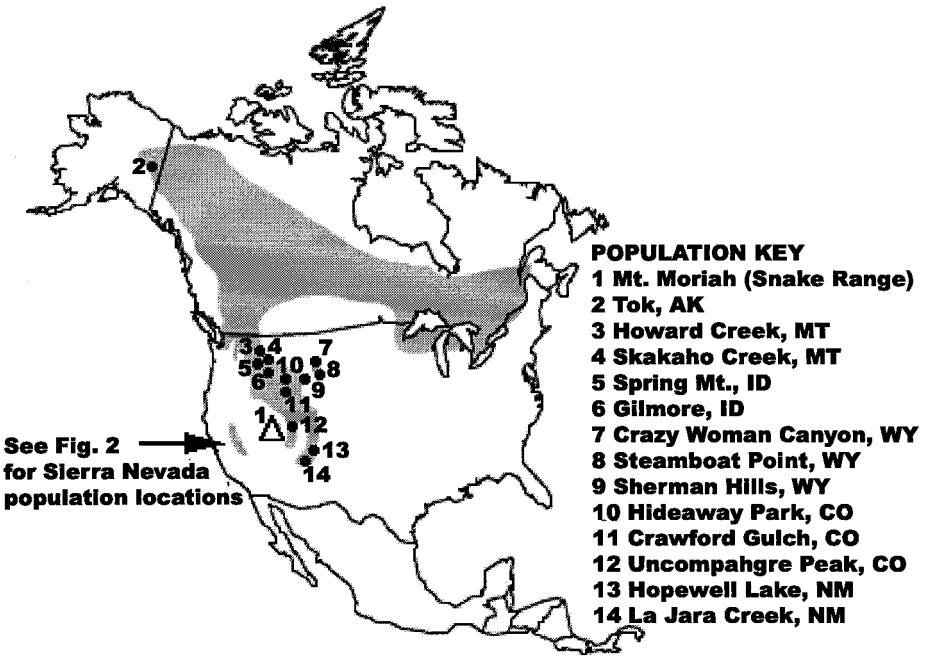

FIG. 1. The approximate range of the *Oeneis chryxus* complex (following Scott, 1986; Opler, 1992; Stanford and Opler, 1996). Localities of *O. chryxus chryxus* mtDNA samples are indicated. The Mt. Moriah (Snake Range), Nevada population was also included in the survey of allozyme variation. The locations of *O. ivallda* and *O. c. stanislaus* populations in California and western Nevada are provided in Fig. 2. See Appendix B for locality and collection data for mtDNA samples.

as larvae both times. In the eastern portion of their range these butterflies occupy the Canadian and Hudsonian life zones. They occur in alpine areas in western North America: in the Rocky Mountains from Alaska to New Mexico, in the northern Cascade Range in northern Washington, in the Bighorn Mountains of Wyoming, in some eastern Great Basin ranges including the Snake and Schell Creek ranges, and in the Sierra Nevada and Sweetwater Mountains of California and western Nevada (Garth and Tilden, 1986; Opler, 1992; Scott, 1986; Stanford and Opler, 1996; G. Austin, personal communication). There are no populations known in the central or southern Cascade Range in Oregon or in the Klamath–Siskiyou Mountains of southern Oregon and northern California. The California–western Nevada populations are geographically isolated from the rest of the complex. The island-like alpine habitats occupied by these butterflies in western North America are also isolated by uninhabitable, low-elevation habitats.

There are three taxa in western North America that are distinguished by the color of the dorsal wing surface. All three are similarly cryptic on the underside. In the Rocky Mountains, the dark, orange-brown-colored *O. c. chryxus* occurs. In the Sierra Nevada of California, where *O. c. chryxus* does not occur, two other forms exist in a parapatric distribution. The lightly colored, whitish-yellow *O. ivallda* occurs in the northern and southern Sierra Nevada and is replaced by the darker, orange-brown *O. c. stanislaus* in the central part of the range (Fig. 2). No other Sierran organisms exhibit this distribution pattern.

Although they differ only in color and pigment chemistry (Tilden and Smith, 1986), California lepidopterists historically have treated these two entities as separate species: *O. ivallda* and *O. c. stanislaus*. Where the two forms meet (in the north between Carson Pass and Reynolds Peak and in the south in the vicinity of Tioga Pass, Yosemite National Park), populations contain individuals that are phenotypically pure *O. ivallda* or *O. c. stanislaus* and individuals that are intermediate in phenotype. The differences in wing color form a continuous series and the entire range of phenotypes from darkest to lightest is present among individuals from populations in these areas of contact (Hovanitz, 1940; Remington, 1958; C. C. Nice, personal observation). Unfortunately, the genetic control of wing color has not been studied directly because the animals are difficult to culture.

Hovanitz (1940) first described the unique distribution of *O. ivallda* and *O. c. stanislaus* (Fig. 2) and proposed a biogeographical hypothesis for its origin. The close phenotypic resemblance of *O. c. stanislaus* to *O. c. chryxus* suggested that ancestral *O. c. stanislaus* had dispersed across the Great Basin and displaced *O. ivallda* from the central Sierra Nevada. (This hypothesis does not explicitly explain the origin of *O. ivallda*, which presumably was the result of an earlier dis-

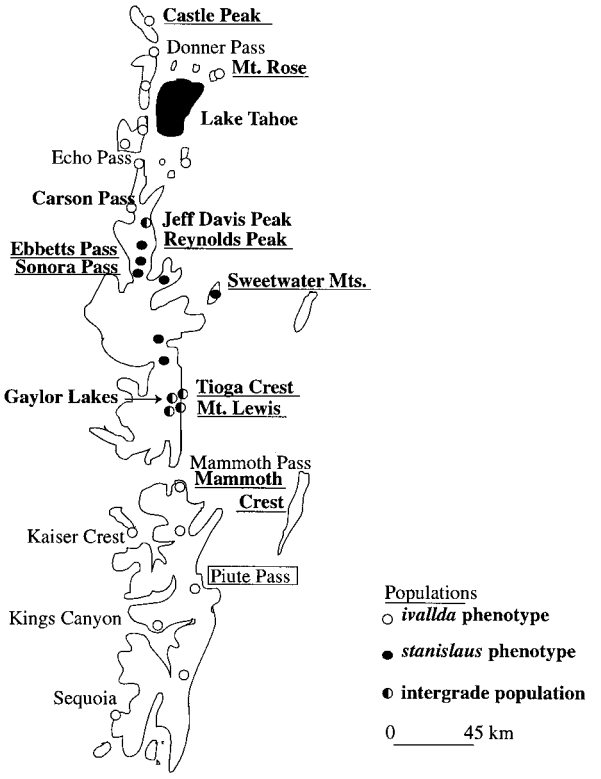

FIG. 2. The distribution of alpine habitats is shown in outline for the Sierra Nevada and adjacent ranges of California and western Nevada. Localities of *Oeneis ivallda* and *O. c. stanislaus* are indicated with circles. Populations sampled in this study by allozyme electrophoresis are indicated in boldface; underlined populations were sampled for both allozyme and mtDNA sequence variation. The Piute Pass population was sampled for mtDNA variation only. This figure was redrawn from Hovanitz (1940) with additional localities added.

persal event. There are no other *chryxus*–complex populations that match *ivallda* in color, though similarly colored entities occur in several complexes of Old World *Oeneis*.)

Porter and Shapiro (1989) investigated the relationship between *O. ivallda* and *O. c. stanislaus* in an allozyme electrophoretic analysis of three California populations (one *O. ivallda* population, one intergrade population, and one *O. c. stanislaus* population). They found that the genetic distance between the two taxa was very low and suggested that they are conspecific (Porter and Shapiro, 1989). However, because of the limited geographic sampling in that study, Porter and Shapiro (1989) were not able to fully evaluate the evolutionary relationships among the three taxa or test the biogeographical scenario suggested by Hovanitz (1940).

Reconstruction of the evolutionary relationships among populations of the *O. chryxus* complex in western North America by use of allozyme allele frequency and mtDNA sequence data provides a framework for the testing of biogeographical hypotheses on the origins of *Oeneis* in the Sierra Nevada of California. Sierran alpine organisms may have dispersed from the east, across the Great Basin, or from the Cascade Range located north of the Sierra Nevada. Alternatively, Sierran alpine organisms may have evolved *in situ* from low-elevation forms (Billings, 1978; Chabot and Billings, 1971; Major and Bamberg, 1967; Major and Taylor, 1990; Stebbins, 1982; Taylor, 1977). This last hypothesis appears to be unlikely for Sierran *Oeneis* because only the somewhat distantly related *O. nevadensis* occurs in California. If Hovanitz's hypothesis of dispersal across the Great Basin is correct, *O. c. stanislaus* (among California populations) should be most closely related to *O. c. chryxus* from the eastern Great Basin and Rocky Mountains. This study amounts to a test of the general hypothesis of dispersal across the Great Basin for Sierran alpine organisms.

Allozyme Data

We surveyed 527 individuals from 13 populations with allozyme electrophoresis: 3 populations of the northern *O. ivallda*, 4 populations of *O. c. stanislaus*, 1 population of the southern *O. ivallda*, 4 populations from within the contact zones that are intergrade populations, and 1 population of *O. c. chryxus* from the Snake Range of eastern Nevada. Appendix A provides the locality and collection data for these samples and Figs. 1 and 2 illustrate their locations. Specimens were captured and maintained alive until frozen in either a −80°C freezer or in a liquid nitrogen thermos in the field. Those frozen in liquid nitrogen were later transferred to a −80°C freezer. Wings and the posterior portions of the abdomens (for males) or the whole abdomens (for females) were removed and stored in numbered and dated glassine envelopes inscribed with locality and date of capture data and are stored as vouchers. The entire female abdomen was removed to avoid complications arising from possible genotyping of spermatophore proteins or DNA. The remaining bodies of each individual were placed in a 1.5-ml microcentrifuge tube and prepared for allozyme analysis as described in Porter and Mattoon (1989). Voucher specimens were deposited in the Bohart Museum of Entomology, University of California at Davis.

Twenty-four presumptive loci were surveyed, of which 11 were reliably scorable and 9 were polymorphic. These 9 loci, listed with their abbreviations and their IUBNC Enzyme Commission numbers (Shaklee *et al.*, 1990), are aspartate aminotransferase (AAT) (2.6.1.1), glucose-6-phosphate dehydrogenase (G6PDH) (1.1.1.49), glucose-6-phosphate isomerase (GPI) (5.3.1.9), glycerol-3-phosphate dehydrogenase (G3PDH) (1.1.1.8), fumarate hydratase (FH) (4.2.1.2), isocitrate dehydrogenase (IDH-1) (1.1.1.42), malate dehydrogenase (MDH-1) (1.1.1.37), malic enzyme (NAD+) (ME) (1.1.1.38), and phosphoglucomutase (PGM) (5.4.2.2). Electromorphs

were given letter designations arbitrarily. Potentially ambiguous electromorphs were rerun in adjacent lanes to confirm scoring.

Genotype data for each individual for the nine polymorphic loci were analyzed with the BIOSYS computer program (Swofford and Selander, 1981) and the Genetic Data Analysis (GDA) computer program version 1.0 (Lewis and Zaykin, 1996). The GDA software was also used to calculate hierarchical *F* statistics (Crow and Aoki, 1984; Wright, 1951) with the estimators of Weir and Cockerham (1984) and Weir (1996). Confidence intervals for these estimators were calculated from 1000 bootstrap replicates over all loci. Pairwise genetic distances among populations and measures of genetic variability were calculated. A maximum-likelihood phylogeny was constructed from allele frequency data with the CONTML program of the PHYLIP software package (Felsenstein, 1993). Nonmetric multidimensional scaling (Lessa, 1990) of pairwise coancestry coefficients with the NCSS 97 computer statistical package was also performed to illustrate the relationships among populations. Pairwise coancestry coefficients are the appropriate distance metric for recently diverged populations where divergence is due to drift only (Weir, 1996).

MtDNA Data

The sampling of mtDNA sequence variation was designed to cover populations sampled with allozyme electrophoresis and to extend the geographic coverage to another southern *O. ivallda* population at Piute Pass in the Sierra Nevada (Fig. 2) and to the remaining alpine portion of the *O. chryxus* complex from Alaska to the central Rockies to New Mexico (Fig. 1). Unfortunately, most of these additional individuals were dried specimens and could not be included in the allozyme analysis. A 480-bp region of the mtDNA cytochrome oxidase subunit 2 (COII) region was sequenced for 60 specimens of the *O. chryxus* complex plus 3 specimens of *Oeneis nevadensis,* a low-elevation congener used as an outgroup taxon. Appendix B provides locality data for these specimens. Samples used in the mtDNA sequence survey included specimens from several sources. Twenty-six specimens were previously processed for allozyme electrophoresis (see above) and the material from these homogenates was extracted. Thirteen fresh-caught specimens were either kept alive until placed in −80°C freezers or frozen in liquid nitrogen in the field and transported to −80°C freezers. The rest were dried specimens.

Wings and abdomens were removed in the same fashion as described in the allozyme electrophoresis methods (above). For those samples that were originally prepared for electrophoresis, an unmeasured portion of the electrophoresis homogenate was transferred to a new autoclaved 1.5-ml microcentrifuge tube. Phenol–chloroform extractions were performed following standard methods (Hillis *et al.,* 1996) and extracted DNA was rehydrated in 50 μl of H_2O. From these solutions, diluted solutions (1/200 dilutions of the original 50 μl) were made yielding a concentration of DNA of approximately 1–10 ng/μl. These solutions were used in PCRs (as described in Kocher *et al.,* 1989; and Palumbi, 1996) employing the mtDNA COII primers STROM (5′ TAA TTT GAA CTA TYT TAC CNG CA 3′) and EVA (5′ GAG ACC ATT ACT TGC TTT CAG TCA TCT 3′) (Caterino and Sperling, 1999). These reactions yielded an approximately 480-bp PCR product which was then sequenced. Fluorescently labeled dideoxy terminators were used for single-stranded sequencing reactions according to Applied Biosystems Inc. specifications. Labeled extension products were separated on a gel and analyzed with an automated DNA sequencer (Applied Biosystems Model 377). Forward sequences were obtained for all 60 *O. chryxus* complex specimens plus outgroup specimens, and eight arbitrarily chosen samples were sequenced in the reverse direction as a check of sequencing errors. From these sequences, 440 continuous nucleotides could be reliably read for all specimens. This portion of the COII region of the mtDNA genome is approximately homologous to positions 3294 through 3734 of the *Drosophila yakuba* reference sequence (Clary and Wolstenholme, 1985).

Neighbor-joining and maximum-parsimony analyses and phylogeny reconstructions based on these sequences were performed with the PAUP 4.0b3a program (Swofford, 2000). We also employed an analysis of molecular variance (AMOVA) (Excoffier *et al.,* 1992) to determine whether mtDNA variation was distributed in accordance with the current taxonomic boundaries within the *O. chryxus* complex. AMOVA provides a nonhistorical assessment of the distribution of genetic variance. A nested AMOVA was performed by the partitioning of the total sum of squares into components representing variation among individuals within populations, among populations within taxa (*O. ivallda, O. c. stanislaus,* and *O. c. chryxus;* intergrade populations were omitted), and among taxa with the ARLEQUIN software (Schneider *et al.,* 1997).

RESULTS

Allozyme Data

Allele frequencies of the polymorphic loci (Nice, 1998; also available from the corresponding author) and measures of population genetic variability (percentage polymorphic loci and heterozygosity) are similar in all Sierran populations surveyed (Table 1). Genetic distances (Nei's unbiased genetic distance) among all populations ranged from 0.000 to 0.676, with the largest distances being those between the *O. c. chryxus* population in the Snake Range in eastern Nevada and the *Oeneis* populations in California and

TABLE 1

Genetic Variability Statistics for the 23 *Oeneis* Populations for 9 Polymorphic Loci Surveyed Electrophoretically

Population	$\bar{x}_{alleles}$	P	H_{obs}	H_{exp}
O. ivallda (northern)				
Mt. Rose 93	1.8 (0.4)	44.4	0.191 (0.087)	0.186 (0.085)
Mt. Rose 95	1.7 (0.3)	44.4	0.132 (0.067)	0.146 (0.074)
Castle Peak 91	1.9 (0.5)	44.4	0.176 (0.078)	0.193 (0.088)
Castle Peak 92	1.7 (0.3)	44.4	0.188 (0.077)	0.201 (0.082)
Castle Peak 93	1.9 (0.5)	33.3	0.170 (0.085)	0.193 (0.094)
Castle Peak 94	1.9 (0.4)	44.4	0.139 (0.062)	0.166 (0.074)
Castle Peak 95	1.7 (0.3)	44.4	0.178 (0.079)	0.185 (0.087)
Carson Pass 91	2.2 (0.3)	55.6	0.228 (0.087)	0.236 (0.082)
Carson Pass 93	2.3 (0.3)	44.4	0.158 (0.062)	0.199 (0.070)
Carson Pass 95	2.3 (0.4)	44.4	0.205 (0.084)	0.200 (0.079)
Intergrade populations northern				
Jeff Davis Peak 93	2.0 (0.2)	55.6	0.144 (0.057)	0.188 (0.062)
Jeff Davis Peak 95	1.9 (0.4)	55.6	0.169 (0.072)	0.175 (0.068)
O. c. stanislaus				
Reynolds Peak	2.1 (0.3)	66.7	0.231 (0.080)	0.260 (0.069)
Ebbetts pass	2.4 (0.4)	66.7	0.218 (0.081)	0.249 (0.081)
Sweetwater Mts. 93	2.1 (0.4)	44.4	0.172 (0.077)	0.214 (0.086)
Sweetwater Mts. 95	2.6 (0.4)	55.6	0.213 (0.069)	0.235 (0.080)
Sonora Pass 89	1.7 (0.3)	44.4	0.183 (0.085)	0.180 (0.084)
Sonora Pass 95	2.6 (0.4)	55.6	0.232 (0.084)	0.250 (0.084)
Intergrade populations southern				
Tioga Crest	2.1 (0.5)	44.4	0.196 (0.084)	0.212 (0.090)
Gaylor Lakes	2.2 (0.4)	55.6	0.238 (0.095)	0.221 (0.088)
Mt. Lewis	2.4 (0.4)	44.4	0.259 (0.094)	0.242 (0.086)
O. ivallda (southern)				
Mammoth Crest	2.3 (0.4)	44.4	0.244 (0.097)	0.248 (0.095)
O. c. chryxus				
Mt. Moriah	2.4 (0.3)	77.8	0.289 (0.070)	0.296 (0.070)
Means	2.1 (0.29)	50.2	0.193 (0.054)	0.212 (0.035)

Note. Mean number of alleles per locus, $\bar{x}_{alleles}$; percentage of loci polymorphic, P; observed heterozygosity, H_{obs}; H_{exp}, Hardy–Weinberg expected heterozygosity; standard errors in parentheses.

western Nevada. Distances among populations of *O. ivallda* and *O. c. stanislaus* in the Sierra Nevada are low (Nei's D ranging from 0.000 to 0.168) despite being slightly exaggerated by the inclusion of only polymorphic loci in their calculation. These distances are within the range of distances observed for conspecific populations of invertebrates (Thorpe, 1983) and other butterflies (Nice and Shapiro, 1999; Porter and Geiger, 1988; Porter and Mattoon, 1989; Shapiro and Geiger, 1986). The largest of these distances is between the Castle Peak 1994 and the Mt. Rose 1995 samples, both *O. ivallda* populations. Indeed, the average distance among *O. ivallda* populations is greater than the average distance between *O. ivallda* and *O. c. stanislaus*

TABLE 2

Nei's (1978) Unbiased Genetic Distances Averaged between the Three Taxa: *O. ivallda, O. c. staislaus,* and *O. c. chryxus*

Taxon	1	2	3
1 *O. ivallda*	0.037 (0.000–0.168)		
2 *O. c. stanislaus*	0.036 (0.000–0.147)	0.012 (0.000–0.032)	
3 *O. c. chryxus*	0.549 (0.477–0.676)	0.508 (0.487–0.521)	No comparison

Note. Ranges are given in parentheses. There is no possible comparison for *O. c. chryxus* because there was only one population of this taxon in the allozyme analysis.

TABLE 3

Summary of Hierarchical *F* Statistic Means at Different Levels

	Hierarchical level	Value	95% confidence interval
A	F_{IS}	0.070	0.190–0.001
	F_{IT}	0.258	0.403–0.189
	F_{ST}	0.202	0.294–0.160
	Comparison		*F*
B	X Y		XY
	Population–taxon		0.103
	Population–total		0.191
	Taxon–total		0.098

Note. (A) Fixation indices calculated with the estimators of Weir and Cockerham (1984) and Weir (1996) from allozyme allele frequencies of the nine polymorphic loci surveyed pooled over 23 population samples of *O. ivallda*, *O. c. stanislaus*, and *O. c. chryxus*. Confidence intervals were calculated from 1000 bootstrap replicates over all loci. (B) A hierachical analysis of genetic variance at the population, taxon, and total range levels with the intergrade populations omitted from the analysis. (Appendix A provides locality data.)

populations (Table 2), though the difference is not statistically significant. Hierarchical analysis of the genetic variance revealed that the largest component of the variance was among populations, and the among-taxa component was the smallest (Table 3).

Phylogenetic analyses using maximum-likelihood revealed that *O. ivallda* and *O. c. stanislaus* are indistinguishable (Fig. 3). The California taxa are distantly related to *O. c. chryxus* from the Snake Range, Nevada. In other words, *O. ivallda* and *O. c. stanislaus* are more closely related to each other than either is to *O. c. chryxus* from eastern Nevada. Multidimensional scaling may outperform hierarchical algorithms in situations of recent divergence and may be particularly illuminating in cases of reticulation or clinal variation (Lessa, 1990). The *O. c. chryxus* population from Mt. Moriah, Nevada was excluded from the multidimensional scaling analysis to allow for inspection of the patterns within the Sierra Nevada (Fig. 4). This analysis confirms the general patterns discussed above and emphasizes the position of the northernmost populations (Mt. Rose and Castle Peak) showing the most differentiation. There is no evidence of any obvious clines or other geographic patterns in allozyme alleles that mirror the patterns in wing coloration.

mtDNA Data

Sixteen haplotypes were observed in this analysis of the 440 bp mtDNA sequences of 60 individuals of the *O. chryxus* complex (GenBank Accession Nos. AF155172–AF155190 and AF166529–AF166547). Mean A-T content for the 16 haplotypes was 75.4%. This value is commonly observed in insect mtDNA sequences (Simon *et al.*, 1994). Seventeen (3.9%) of the 440 bp sequenced were polymorphic. The most common haplotype, haplotype A, was present in 24 specimens (Appendix B provides haplotypes for all specimens). Uncorrected sequence divergence between the 16 haplotypes ranged from 0.2 to 2.3%. Haplotype diversity (h) was 0.796 and nucleotide diversity (π) for the entire data set was 0.0047 (equation 10.5 in Nei, 1987). Protein sequences were inferred with a mtDNA code from *Drosophila* with eight codons for serine (de Bruijn, 1983). Three of the 146 amino acid residues varied across all specimens; one of these amino acid changes results from a 3-bp deletion present in some sequences.

Twenty-one of 22 specimens of *O. ivallda* and *O. c. stanislaus* from California and western Nevada that represent most of the populations examined using allozyme electrophoresis (i.e., *O. ivallda* and *O. c. stanislaus* populations sampled except the Piute Pass population) have identical sequences (haplotype A). Haplotype P from one individual from the Mammoth Crest population possesses a single nucleotide transition difference from haplotype A. Two individuals from Crazy Woman Canyon and one individual from Steamboat Point (Fig. 1), both in the Bighorn Mountains of Wyoming, also possess haplotype A. The sample from the southern Sierra Nevada *O. ivallda* population at Piute Pass contains four haplotypes (C, G, H, and K), two of which (G and H) contain a 3-bp deletion that corresponds to amino acid 130 in the *D. yakuba* reference sequence (Clary and Wolstenholme, 1985). This is a deletion relative to other *Oeneis* sequences (including the outgroup, *O. nevadensis*) and the *D. yakuba* sequence. This deletion is also present in all six sequences from the Snake Range *O. c. chryxus* population

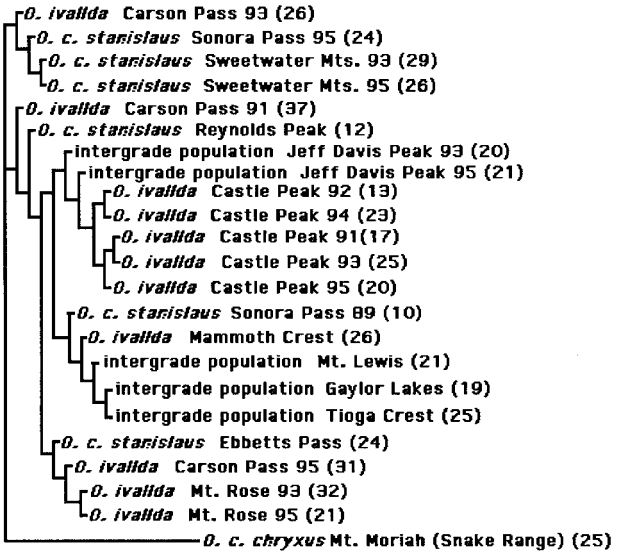

FIG. 3. Maximum-likelihood cladogram calculated from allozyme allele frequency data. Samples sizes are given in parentheses. Appendix A provides locality and collection data for allozyme samples.

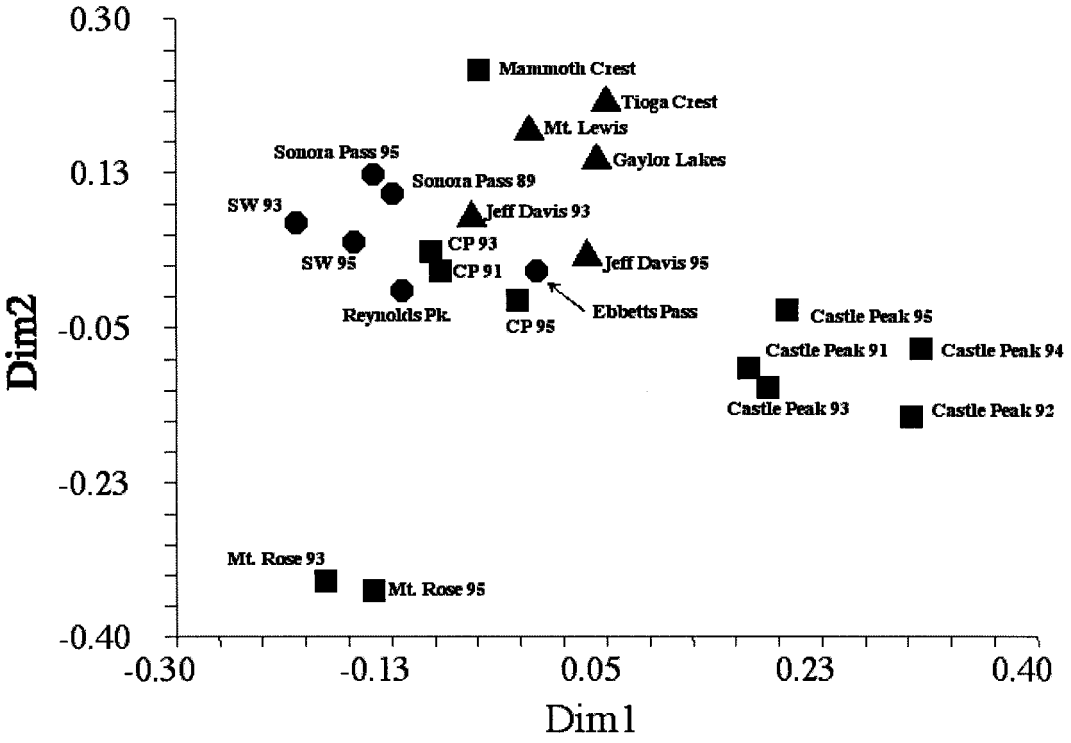

FIG. 4. Multidimensional scaling of 22 population samples of the *Oeneis* complex from the Sierra Nevada. Dimension 1 is plotted against dimension 2. Circles indicate populations of *O. c. stanislaus*, squares indicate *O. ivallda*, and triangles indicate intergrade populations. SW, Sweetwater Mountains populations.

(haplotype D) and in all sequences from individuals of *O. c. chryxus* in Alaska, Idaho, Montana, Colorado (except the Uncompahgre Peak population), and southeastern Wyoming (haplotypes B, J, and E). Sequences from Uncompahgre Peak in southwestern Colorado (haplotypes L, M, and N) and Hopewell Lake, New Mexico (haplotype F) do not contain this deletion. One of two individuals sequenced from La Jara Creek, New Mexico (haplotype I) also contains this deletion.

Analysis of molecular variance reveals that the largest component of total variance was found among populations (66.5%) (Table 4). The among-taxa component of variance was much smaller (22.3%) (Table 4), indicating relatively little taxonomic differentiation.

Phylogenies were inferred from cladograms constructed by neighbor-joining and maximum-parsimony methods with the PAUP* 4.0b3a program (Swofford, 2000). The existence of an apparently informative indel in the COII sequences of the *O. chryxus* group justified treatment of the indel as an extra character and we weighted it as equal to a transition. Maximum-parsimony produced 138 equally parsimonious trees and a majority-rules consensus tree was computed. Neighbor-joining and maximum-parsimony phylogenies differed significantly only in the position of haplotype E, and only the neighbor-joining tree is presented (Fig. 5). In the maximum-parsimony tree, haplotype E is the sister

TABLE 4

Results of a Hierarchical Analysis of Molecular Variance of the COII mtDNA Sequence Data Grouping Populations by Taxonomic Designation (i.e., *O. ivallda*, *O. c. stanislaus*, and *O. c. chryxus*)

Source of variation	*df*	SSD	Variance component	% of total	*P* value
Among taxa	2	22.5	0.36	22.3	0.058
Among populations/within taxa	19	48.3	1.07	66.5	<0.001
Within populations	34	6.1	0.18	11.2	<0.001

Note. This analysis excluded the intergrade populations because of their uncertain taxonomic status.

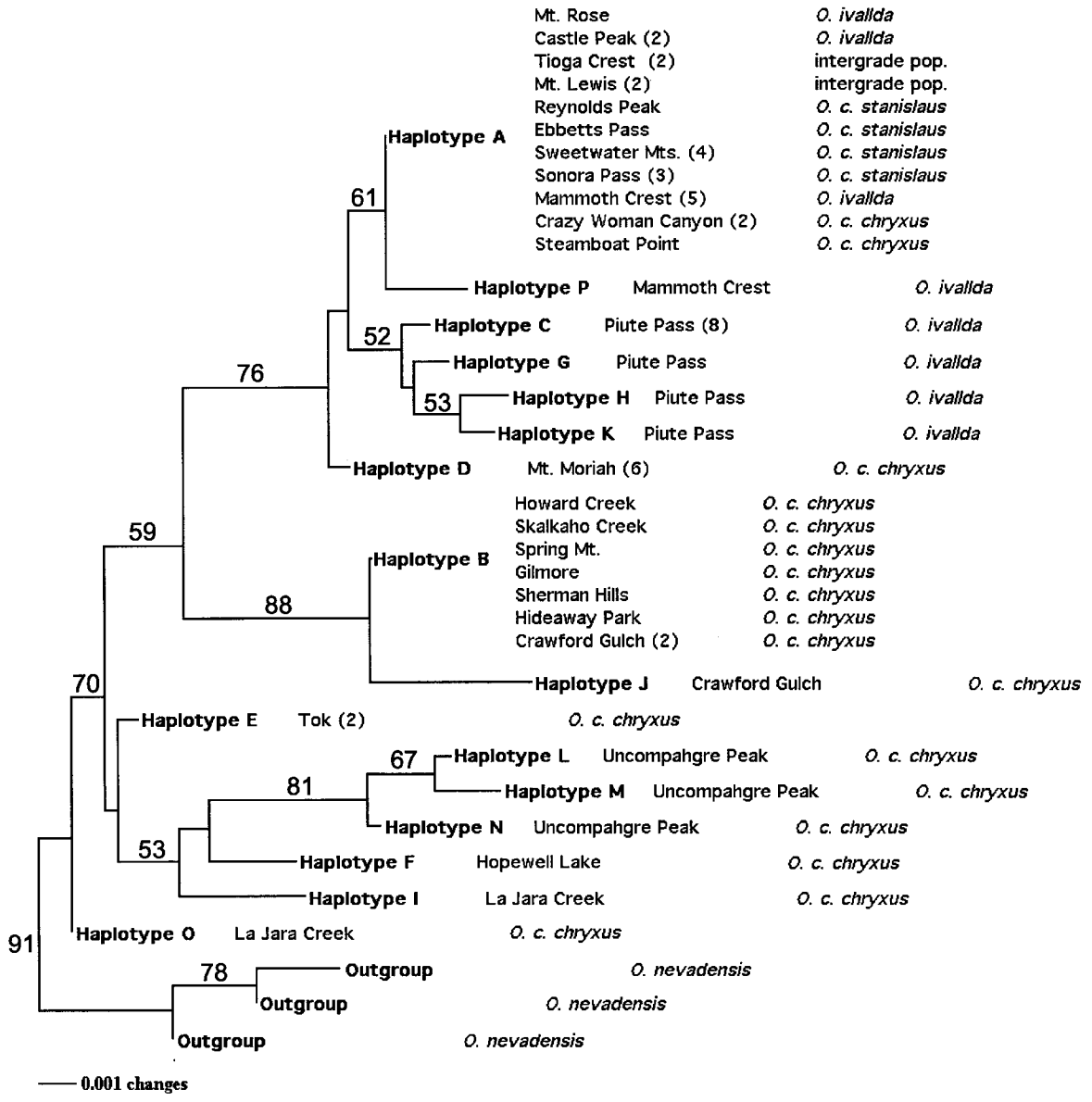

FIG. 5. Neighbor-joining tree of *Oeneis* mtDNA COII haplotypes. Bootstrap values from 1000 replicates are given above branches. Population haplotypes are provided in Appendix B.

haplotype to the group including haplotypes B, J, K, H, G, C, P, and A.

The California and western Nevada COII sequences are the most derived with the sequences from the *O. c. chryxus* populations in the Snake Range forming their sister group. The large group of sequences from the central Rocky Mountains form a sister group to the Great Basin–Sierra Nevada clade. Under the assumption that the variation detected in our samples reflects population-level variation, this arrangement is consistent with the prediction of dispersal from the Rockies to the Great Basin to the Sierra Nevada (Morrone and Crisci, 1995). However, bootstrap support for this topology is low (Fig. 5).

DISCUSSION

The Taxonomic Status of O. ivallda and O. c. stanislaus

O. ivallda and *O. c. stanislaus* do not appear as monophyletic groups in our analyses of allozyme allele frequencies and mtDNA sequence variation. Furthermore, *O. c. stanislaus* is clearly more closely related to *O. ivallda* than to *O. c. chryxus*. We agree with the conclusion of Porter and Shapiro (1989) that *O. ivallda* and *O. c. stanislaus* are conspecific. There is no genetic differentiation or boundary between these taxa. The averaged genetic distances calculated from allozyme

data among *O. ivallda* and *O. c. stanislaus* (Table 2) are actually smaller than the average distances among populations within *O. ivallda*. A hierarchical analysis of detected genetic variance at allozyme loci reveals that the largest component of the variance is due to genetic differences among populations, not among nominal taxa (Table 3). Similarly, an analysis of molecular variance (AMOVA, Table 4) within the mtDNA sequence data reveals that the largest component of variance is that among populations, not among taxa. The wing color and its distribution appear to be the only characters distinguishing *O. ivallda* from the rest of the complex. We suggest that *O. ivallda* be considered a subspecies of *O. chryxus*.

Biogeographical Hypotheses

Floristically based investigations into the origins of the alpine plants of the Sierra Nevada have suggested that alpine organisms may have dispersed to the range from northern (alpine or arctic) areas or from the east (i.e., from the Rocky Mountains across the Great Basin). Alternatively, endemic organisms may have evolved *in situ* from low-elevation taxa (Billings, 1978; Chabot and Billings, 1971; Major and Bamberg, 1967; Major and Taylor, 1990; Stebbins, 1982; Taylor, 1977). The phylogeographic history of the *O. chryxus* complex reconstructed from allozyme data and mtDNA COII sequences supports the hypothesis of dispersal across the Great Basin. *O. ivallda* and *O. c. stanislaus* in the Sierra Nevada are each other's closest relatives and their nearest relatives include individuals from the Mt. Moriah population in the Snake Range of eastern Nevada (Figs. 3 and 5). The populations in the Rocky Mountains form the sister group to the Sierra Nevada and Snake Range clade (Fig. 5). The source of dispersants to the Sierra Nevada appears to lie to the east of that range. However, bootstrap values on many of the internal branches are low. Consequently, our phylogenetic results must be considered as provisional hypotheses. More markers and longer sequences will be required to definitively test these biogeographical hypotheses.

These results constitute the first demonstration of dispersal across the Great Basin with the use of genetic evidence as an independent test. Our data are consistent with the hypothesis that altitudinal shifts in vegetation during the Pleistocene (Heusser and King, 1988) and the development of Bristlecone- and Limber Pine-dominated steppe vegetation at low elevations in the Great Basin permitted dispersal of alpine organisms across western North America (Billings, 1978; Thompson, 1990; Wells, 1983). The only inconsistency in our data is the presence of the northern California haplotype (A) in three phenotypically *O. c. chryxus* individuals from the Bighorn Mountains of Wyoming (Crazy Woman Canyon and Steamboat Point; Fig. 1). These three sequences are identical to those from northern California and distinctly different from sequences from nearby populations in southern Wyoming, Montana, and Colorado. It is intriguing that these populations in the Bighorns are biennial, with adults flying on the odd years like most California populations, but unlike the even-year *O. c. chryxus* populations nearby in Sherman Hills, Wyoming and throughout the Rockies. A possible explanation is that haplotype A is widespread but rare and was not detected with our small sample sizes. Dispersal could have occurred from the Sierra Nevada to the Bighorn Mountains, though it is difficult to envision since this hypothesis would require a reversion to the *O. c. chryxus* wing phenotype or the introduction of California mtDNA into a Rocky Mountain population.

Oeneis appears to have colonized the southern Sierra Nevada first, somewhere near Piute Pass, and then dispersed northward (Fig. 5). This hypothesis is supported by the occurrence of a 3-bp deletion in the COII sequences from some Piute Pass and all sampled Snake Range individuals and by the distribution of haplotype diversity within the Sierra Nevada. Haplotype diversity should be greater within populations that have been in place longer and diversity should decrease along the path of colonization if colonization proceeds by repeated founder events (Cann *et al.*, 1987; Templeton, 1998). In accordance with this prediction, the Piute Pass population contains at least four distinct haplotypes (haplotypes C, G, H, and K), whereas there are only two haplotypes in all of the 22 individuals sampled from north of Piute Pass. Twenty-one individuals from nine populations have haplotype A and 1 individual from Mammoth Crest (the population closest to Piute Pass in our sampling) has haplotype P, which is different from haplotype A at a single nucleotide site. The alpine zone becomes significantly narrowed in the vicinity of Mammoth Pass, Madera County, and this may act as a geographic restriction on gene flow. However, we failed to detect the expected heterozygosity excesses in our allozyme data that would be associated with population bottlenecks (unpublished data).

An alternative to this colonization hypothesis is a hypothesis of range fragmentation. Up-slope movement of alpine habitats at the end of the Pleistocene quite probably lead to a restriction in areal extent of these habitats. This effect would be exacerbated in the northern Sierra Nevada which has significantly less area above treeline than does the southern Sierra Nevada (Billings, 1978) (Fig. 3). Formerly contiguous alpine habitat would have been fragmented into isolated alpine "islands," especially in the northern Sierra Nevada. Consequently, the southern Sierra Nevada may support larger and/or demographically more stable populations and thus more genetic diversity. The nonmetric multidimensional scaling analysis of allozyme data (Fig. 4) emphasizes that the northernmost popu-

lations in the Sierra Nevada (Mt. Rose and Castle Peak) are also the most differentiated. These populations lie near the northern limit of alpine habitat and may be the most isolated as a result of fragmentation of alpine habitat as vegetation zones moved up slope at the end of the Pleistocene. Falsification of these hypotheses is not currently possible and requires broader geographic sampling within the Sierra Nevada and the employment of more markers.

Much more extensive sampling is required to explore the history of the Rocky Mountains *O. chryxus* populations. However, some intriguing hypotheses are generated by our data. The clade formed of haplotypes found in populations from the south, in Colorado and New Mexico (haplotypes F, I, L, M, N, and O), is the least derived (Fig. 5). Since this boreal species group has circumpolar (rather than temperate or tropical) affinities, it seems likely that the southwestern populations are relicts of the Pleistocene and that *O. c. chryxus* was distributed in unglaciated parts of the Rockies and the mountains of New Mexico during the Pleistocene. This is congruent with scenarios explaining the distributions of other relictual butterfly populations such as those of *Boloria acrocnema* (Family: Nymphalidae) (Britten and Brussard, 1992) in the Rocky Mountains.

Taken together, phylogenetic analyses of allozyme and mtDNA data provide some support for the hypothesis that *Oeneis* colonized the Sierra Nevada via dispersal across the Great Basin. Hovanitz's (1940) hypothesis that *O. c. stanislaus* dispersed across the Great Basin and displaced *O. ivallda* from the central Sierra Nevada is not supported by our data because *O. c. stanislaus* is most closely related to *O. ivallda*. However, we cannot falsify the general hypothesis of dispersal across the Great Basin because the best hypothesis of evolutionary relationships within this group generated from our mtDNA data (Fig. 5) supports this scenario. If this is true, the morphological evolution and divergence of *O. ivallda* and *O. c. stanislaus* must have occurred in the Sierra Nevada after the arrival of *Oeneis*. How this divergence resulted in the parapatric distribution observed in the Sierra Nevada (Fig. 2) remains an open question. Hovanitz (1940) suggested that the distribution of the two taxa in the Sierra Nevada is maintained by selection for protective coloration, driven by predators. Hovanitz (1940) argued that the darkly colored *O. c. stanislaus* occurs in the central Sierra Nevada corresponding to a large region containing darkly colored volcanic (andesitic) or metamorphic rocks and that the northern and southern Sierra Nevada have predominantly lighter-colored granitic rocks at the surface above tree line. Thus, *O. ivallda* and *O. c. stanislaus* match the substrates of their habitats and are protected from predation in this protective coloration hypothesis. We are currently attempting to test this hypothesis.

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APPENDIX A: Locality and Collection Data for Allozyme Electrophoresis Samples

Population Locality	N	Collection Date	Collector	County, State
***O. ivallda* (northern)**	**245**			
Mt. Rose 93	32	18.vii.93 (17)	AMS	Washoe Co., NV
		24.vii.93 (11)	CCN	
		25.vii.93 (4)	CCN	
Mt. Rose 95	21	31.vii.95	CCN	Washoe Co., NV
Castle Peak 91	17	13.vii.91	AMS	Nevada Co., CA
Castle Peak 92	13	9.vii.92 (5)	AMS	Nevada Co., CA
		24.vii.92 (8)	AMS	
Castle Peak 93	25	6.vii.93 (5)	AMS	Nevada Co., CA
		7.vii.93 (20)	AMS	
Castle Peak 94	23	18.vi.94 (1)	AMS	Nevada Co., CA
		27.vi.94 (11)	AMS	
		2.vii.94 (10)	AMS	
Castle Peak 95	20	31.vii.95	AMS	Nevada Co., CA
Carson Pass 91	37	3.vii.91	AMS, CCN	Alpine Co., CA
Carson Pass 93	26	29.vi.91	AMS, CCN	Alpine Co., CA
Carson Pass 95	31	15.vii.95	CCN, S. Graves	Alpine Co., CA

Population Locality	N	Collection Date	Collector	County, State
Intergrade Populations	**106**			
Jeff Davis Peak 93	20	1.vii.93	CCN	Alpine Co., CA
Jeff Davis Peak 95	21	13.vii.95 (4)	CCN	Alpine Co., CA
		13.viii.95 (17)	CCN	
Tioga Crest	25	4.viii.95 (8)	CCN	Mono Co., CA
		14.viii.95 (17)	CCN, K. Roberg	
Gaylor Lakes	19	5.viii.95	CCN	Tuolumne Co., CA
Mt. Lewis	21	15.viii.95 (4)	CCN	Mono Co., CA
		24.viii.95 (17)	CCN	
O. stanislaus	**125**			
Reynolds Peak	12	19.vii.93	CCN	Alpine Co., CA
Ebbetts Pass	24	30.viii.95 (9)	CCN	Alpine Co., CA
		6.ix.95 (15)	CCN	
Sweetwater Mt.s 93	29	7.vii.93 (25)	CCN	Mono Co., CA
		8.viii.93 (4)	CCN	
Sweetwater Mt.s 95	26	23.vii.95 (19)	CCN	Mono Co., CA
		30.viii.95 (1)	CCN	
		8.ix.95 (6)	CCN	
Sonora Pass 89	10	13.vii.89	G. Kareofelas	Alpine Co., CA
Sonora Pass 95	24	26.vii.95	CCN	Alpine Co., CA
***O. ivallda* (southern)**	**26**			
Mammoth Crest	26	7.viii.93	CCN	Madera Co., CA
O. c. chryxus	**25**			
Mt. Moriah (Snake Range)	25	7.vii.94	CCN	White Pine Co., NV

APPENDIX B: Locality and Collection Data for mtDNA Samples

Population Locality	N	Collection Date	Collector	County, State	Haplotypes
***O. ivallda* (northern)**	**3**				
Mt. Rose	1	19.vii.95	CCN	Washoe Co., NV	A
Castle Peak 94	1	2.vii.94	AMS	Nevada Co., CA	A
Castle Peak 95	1	31.vii.95	AMS	Nevada Co., CA	A
Intergrade Populations	**4**				
Tioga Crest	2	4.viii.95	CCN	Mono Co., CA	A
Mt. Lewis	2	24.viii.95	CCN	Mono Co., CA	A
O. c. stanislaus	**9**				
Reynolds Peak	1	19.vii.93	CCN	Alpine Co., CA	A
Ebbetts Pass	1	30.viii.95 (9)	CCN	Alpine Co., CA	A
Sweetwater Mt.s 92	1	22.vii.92	CCN	Mono Co., CA	A
Sweetwater Mt.s 95	3	23.vii.95 (19)	CCN	Mono Co., CA	A
Sonora Pass	3	26.vii.95	CCN	Alpine Co., CA	A
***O. ivallda* (southern)**	**17**				
Mammoth Crest 93	2	17.viii.93	CCN	Madera Co., CA	A
Mammoth Crest 95	4	29.viii.95	CCN	Madera Co., CA	A (3), P
Piute Pass	11	15.viii.97	T. Armstrong	Inyo Co., CA	C (8), G, H, K
O. c. chryxus	**27**				
Mt. Moriah (Snake Range)	6	7.vii.94	CCN	White Pine Co., NV	D
Tok	2	1.vii.97	C. Ferris	Alaska	E
Howard Creek	1	11.vi.92	S. Kohler	Missoula Co., MT	B
Skalkaho Creek	1	10.vi.92	S. Kohler	Ravalli Co., MT	B
Spring Mt.	1	6.vi.92	C. Ferris	Lemhi Co., ID	B
Gilmore	1	7.vi.92	C. Ferris	Lemhi Co., ID	B
Crazy Woman Canyon	2	4.viii.95	J. Scott	Johnson Co., WY	A
Steamboat Point	1	3.viii.95	J. Scott	Sheridan Co., WY	A
Sherman Hills	1	2.vi.94	C. Ferris	Albany Co., WY	B
Hideaway Park	1	5.vii.84	J. Scott	Grand Co., CO	B
Crawford Gulch	3	20.vi.90	J. Scott	Jefferson Co., CO	B (2), J
Uncompahgre Peak	3	19.vii.80	J. Scott	Hinsdale Co., CO	L, M, N

Population Locality	N	Collection Date	Collector	County, State	Haplotypes
Hopewell Lake	2	20.vi.78	J. Scott	Rio Arriba Co., NM	F
La Jara Creek	2	28.v.78	J. Scott	Sandoval Co., NM	I, O
Outgroup	**3**				
Oeneis nevadensis					
Twain Valley	3	21.vi.96	CCN	Plumas Co., CA	Outgroup

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