Introduction:

Phylogenetic and phylogeographic methods that associate a specie's genetic ancestry with current geographic location have been utilized in studying the history of the North American desert adapted biota. Most studies have focused upon the evolution of warm desert biota with an emphasis upon reptiles and mammals (e.g., Riddle et al., 2000; Lowe et al. 1967). In contrast, little work has been done to explore the evolution of cold desert adapted invertebrate species. Organisms native to the cold Great Basin desert, which includes northern Nevada, western Utah, parts of southern Oregon, and eastern California (Fig. 1), have a phylogenetic history that has been shaped by the geological history of the continent, including Pleistocene glacial fluctuations that occurred from 2 million to 12,000 years ago (Fiero, 1986; Epps et al., 1998). Specifically, periods of isolation within glacial refugia in tandem with flooding and evaporating lakes such as Lake Lahontan caused species' ranges to constrict and expand. After the last glacial maximum, Holocene warming events created dune environments that are found throughout Nevada and are seen as "islands" of diversity because they provide unique and isolated habitats for dune specialized species (Epps et al., 1998). This highly variable climactic history may have influenced the current ranges and species compositions of Great Basin ecosystems.

Nevada houses as many as 316 endemic species and subspecies, which include the iconic Lahontan cutthroat trout (*Oncorhynchus clarki*), the Amargosa toad (*Bufo nelsoni),* countless buckwheat species (*Eriogonum* spp.), and 57 insects (Nevada Natural Heritage Program, 2004). Nevada's unique biota is threatened by anthropogenic disturbances such as intensive agriculture, livestock grazing, infrastructural development, and off-road vehicle use. Due to these

perturbations, many endemic species and subspecies appear on the Nevada Natural Heritage Program's (NNHPO) at risk or endangered species list (NNHPO, 2004).

One of the most threatened ecosystems in Nevada is the region's expansive dune systems. Dune-adapted organisms are particularly vulnerable for they exhibit both restricted ranges and typically rely upon specialized host plant relationships (Porter & Rust, 1996). Pollinators such as bees and butterflies serve as essential components to dune ecosystem stability by maintaining populations of dune specialized flora while adding to the overall dune habitat diversity (Wilson et al., 2007). One specialized herbivore is the Sand Mountain blue butterfly (*Euphilotes pallescens arenamontana*), a subspecies of the Pallid Dotted-blue complex and a member of the gossamer-winged butterfly Family Lycaenidae. This at risk and endemic butterfly is a specialist upon a single host plant, the Kearney buckwheat (*Eriogonom nummulare)* (Opler & Tilden, 1999). Although locally abundant throughout the Great Basin Desert, this buckwheat only persists in sandy soil like those found around Sand Mountain Recreational Area, located on highway 50, 25 miles east of Fallon, Nevada (NNHPO 2004; Nevada BLM, 2010).

Over the past ten years, as the use of off-road vehicles has become popularized, this unique dune habitat has become increasingly degraded. In 2004, members of the Center for Biological Diversity, Xerces Society, Public Employees for Environmental Responsibility, and Nevada Outdoor Recreation Society filed a petition with the U.S. Nevada Fish and Wildlife Service (USFWS) to add the Sand Mountain blue butterfly to the endangered species list so that it could be federally protected. After several more petitions, in 2007, the USFWS concluded that the Sand Mountain blue butterfly had a stable population and would not be protected under the Endangered Species Act (Nevada Fish and Wildlife Service, 2010). It is believed that the butterfly may be able to persist despite the presence of off-road vehicles if butterfly and host

plant population numbers do not continue to decline. In order to protect the remaining Sand Mountain blue butterfly population and its host plant, the Nevada Bureau of Land Management (BLM) created voluntary designated off-road vehicle routes in 2007 (Nevada Bureau of Land Management, 2010). Since then, little else has been done to investigate the current status of this endemic butterfly despite being considered a special status species by the Nevada BLM due to its role as a major pollinator of the Kearney buckwheat.

The Sand Mountain blue butterfly is one of seven morphologically defined subspecies of *Euphilotes pallescens* that are found in Nevada, some of which are dune specialists and also considered "at risk" by the NNHPO (2004). Their range is distributed as scattered populations throughout the Great Basin, southeast California, southern Utah, and portions of the Colorado Plateau (Fig. 1). Much like the Sand Mountain blue butterfly, other populations of *E. pallescens* specialize upon perennial buckwheat (*Eirogonum*) during both adult and larval stages, adults only flying about 200 meters from a host plant throughout their lifetime (Opler & Tilden, 1999)

While the Sand Mountain blue and its related subspecies are morphologically distinct (though highly variable) and geographically isolated to various alkaline and dune habitats, no previous attempt has been made to explore patterns of genetic diversity or gene flow among them. Here we offer an initial exploration within *E. pallescens* in particular and also within the genus. An understanding of phylogenetic and population-genetic patterns in this group of butterflies could inform future management and conservation as well as clear up past taxonomic confusion associated with this complex. This study may also serve as a foundation for future investigations into the evolution of cold desert adapted butterflies, which may help our understanding of factors contributing to their current distribution and morphological diversity.

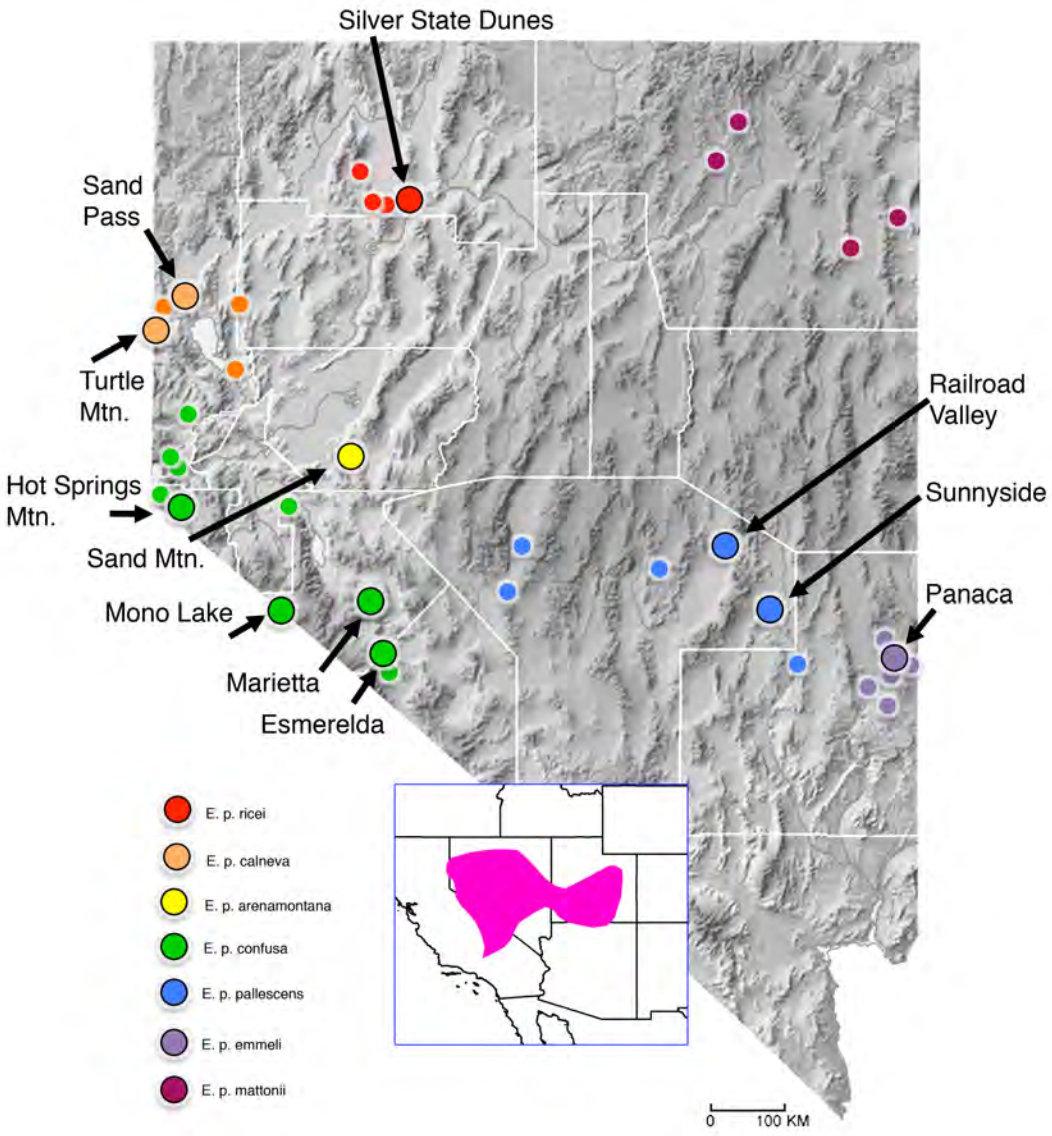

Figure 1. Sampling and population localities for focal Nevada *Euphilotes pallescens* subspecies (complete *E.* pallescens range shown in the inset) (Opler & Tilden, 1999). Larger dots are populations sampled in this study.

Materials and Methods:

Sampling:

Butterfly specimens were collected at known historical locations representing six of the seven subspecies of *E. pallescens* as well as from other *Euphilotes* species and outgroups (Fig. 1). Other *Euphilotes* taxa included *E. enoptes, E. ancilla,* and *E. glaucon. E. pallescens* specimens were collected by D. Murphy, other *Euphilotes* by C. Nice and J. Fordyce. After collecting, individuals were assigned a voucher number and placed in a freezer at the University of Nevada, Reno to preserve DNA integrity (appendix 1).

Molecular Methods:

DNA was extracted from the proximal end of the abdomen of each specimen to preserve genitalia using QIAGEN DNAeasy tissue kit (appendix 1). A portion of the mitochondria gene, cytochrome oxidase subunit-1 (CO1) was amplified using primer pair LepF1 (ATTCAACCAATCATAAAGATATTGG) and LepR1 (TAAACTTCTGGATGTC CAAAAAATCA) (Foottit, Maw, Von Dohlen, & Herbert, 2008). Primers amplified an approximately 586 bp long DNA fragment of the CO1 gene. PCR occurred in a 25 µL volume with the following conditions: 3 mM $MgCl_2$, 200 pM dNTPs, 2 units of Taq polymerase, 1mM of each primer, and standard PCR buffer concentration. For each PCR, approximately 40 ng of template DNA was added to the reaction. The PCR program included an initial step of 94^{o}C for 180 sec, followed by 35 cycles of 94^{o}C for 30 sec, 50^{o}C for 90 sec, and 94^{o}C for 12 min, with a final step of 86^{o}C for 60 sec.

Amplified fragments were then visualized using electrophoresis techniques, which include agarose gels stained with ethidium bromide. Successful amplifications were sent to the

Nevada Genomics Center and were sequenced in both directions (nuclear sequences were always obtained from both directions; mitochondrial sequences, which were always very clear, were often obtained in a single direction). Sequences were assembled using Clustal W (Thompson et al., 1994) as implemented in Sequencher 4.0 (Gene Code Corp., Ann Arbor, MI), and alignments were visually reviewed using MacClade 4.07 (Maddison & Maddison, 2005).

Two additional nuclear DNA regions were amplified, including elongation factor 1-alpha (EF1-α) and internal transcribed spacer region 1 (ITS1). Both were examined from a subset of the specimens to further illuminate genetic relationships among subspecies and various outgroups. Primers used to amplify EF1-α were EF44 5'- GCYGARCGYG ARCGTGGTATYAC- 3' and EF51r 5'-CATGTTGTCGCCGTGCCAAC- 3'. Primers used to amplify the ITS1 nuclear region were 5′ -GATTACGTCCCTGCCCTT- TG-3′ (forward-18S) and 5′- CGATGATCAAGTGTCCTG- CA-3′ (reverse-5.8S) (Pilgrim, 2002) and resulted in approximately 729 bp . The PCR program entailed an initial temperature of 94^{o}C for 150 sec, followed by 35 cycles of 94^{o}C for 30 sec, 52^{o}C for 60 sec, and 72^{o}C for 60 sec, and a final step of 72^{o}C for 10 min.

Phylogentic & Haplotype Network Analysis:

All nuclear and mitochondrial loci were evaluated separately with Bayesian phylogenetic methods in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). Each sequence was analyzed according to the constraints of the general time-reversible model of sequence evolution (Lanave et al., 1984) with invariant sites and gamma-distributed rate variation across sites (GTR + G) and with all parameters unlinked across loci. Bayesian analyses required four independent runs with one heated and three cold chains in each run. MCMC (Markov Chain Monte Carlo) for the CO1

region was set for 6,000,000 generations, which were sampled every 1,000 generations. Chains were run until the average standard deviation of the split frequencies dropped below 0.01.

For both EF1-α and ITS1, MCMC were set for 3,000,000 generations with sampling every 1,000. Again, chains were run until the average standard deviation of the split frequencies dropped below 0.01. A burn-in period of 10 % of each sample was removed from every analysis after graphical determination of stationarity. Other *Euphilotes* sister species and additional outgroups were included (*E. enoptes, E. ancilla,* and *E. glaucon)* in these analyses (Mattoni, 1988).

We also constructed a parsimony-based haplotype network using aligned CO1 sequences for all *E. pallescens* and outgroups specimens using TCS version 1.21 (Clement et al., 2000). The program estimated parsimonious relationships among haplotypes within 95% confidence limits with gaps treated as missing data.

Gene Flow, & Morphological Analysis:

Analysis of molecular variance (AMOVA) to estimate gene flow between populations was performed on mitochondrial haplotypes using Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) and a neighbor-joining tree of populations was generated based on a table of pairwise Fst values (calculated with haplotype frequencies and corrected average pairwise differences among haplotypes).

Figure 2. Example of morphometric analysis displaying percent black and percent orange threshold measurements.

Underside wing morphology of both the hind and forewing of *E. pallescens* subspecies from each population was analyzed in a total of 210 specimens (Fig. 1) Diagnostic characters were informed by historical classification methods (Mattoni, 1988). Size and percent black of the forewing were calculated. Size and percent black and percent orange of aurorae on the

hind wing were also quantified (Fig. 2). All morphometrics were calculated with ImageJ (Abramoff et al., 2004) and quantitatively analyzed by resemblance matrices and nonmetric multidimensional scaling with Primer-E (Clark & Gorley, 2006)

Results:

Phylogentic & Haplotype Networks:

Phylogenetic analyses revealed little genetic structure between subspecies of *E. pallescens* (i.e. subspecies do not form distinct clades). The nuclear marker, EF1-α resulted in a largely unresolved tree topology with a large polytomy placing other *Euphilotes* intermixed with *E. pallescens* specimens (Fig. 3). Conversely, the nuclear marker ITS1 places *E. pallescens* subspecies clustered together in a relatively well-supported clade with other *Euphilotes* species clustering together (Fig. 4). The mitochondrial genaeology however, revealed 23 unique haplotypes and indicates a relatively deep historical divergence, with *E. pallescens* haplotypes and other *Euphlotes* taxa on either side of that divergence (Fig. 5a). This divergent genealogy is corroborated by two haplotype networks, which were separated by 95% parsimony limits (Fig. 5b). Each corresponds to the divergent clades on the mitochondrial gene tree. Network one displays the most common haplotype A, which is found in every sampled population of *E. pallescens,* with haplotypes that exhibit a single base pair mutation radiating outward. Network two indicates a more complex genealogy. Specifically, haplotype H is found in populations of *E. p. calneva* and is more genetically similar to *Euphilotes* outgroup haplotypes than to other *E. p.* haplotypes. Also, more haplotype diversity was uncovered in southern Nevada (Fig. 6).

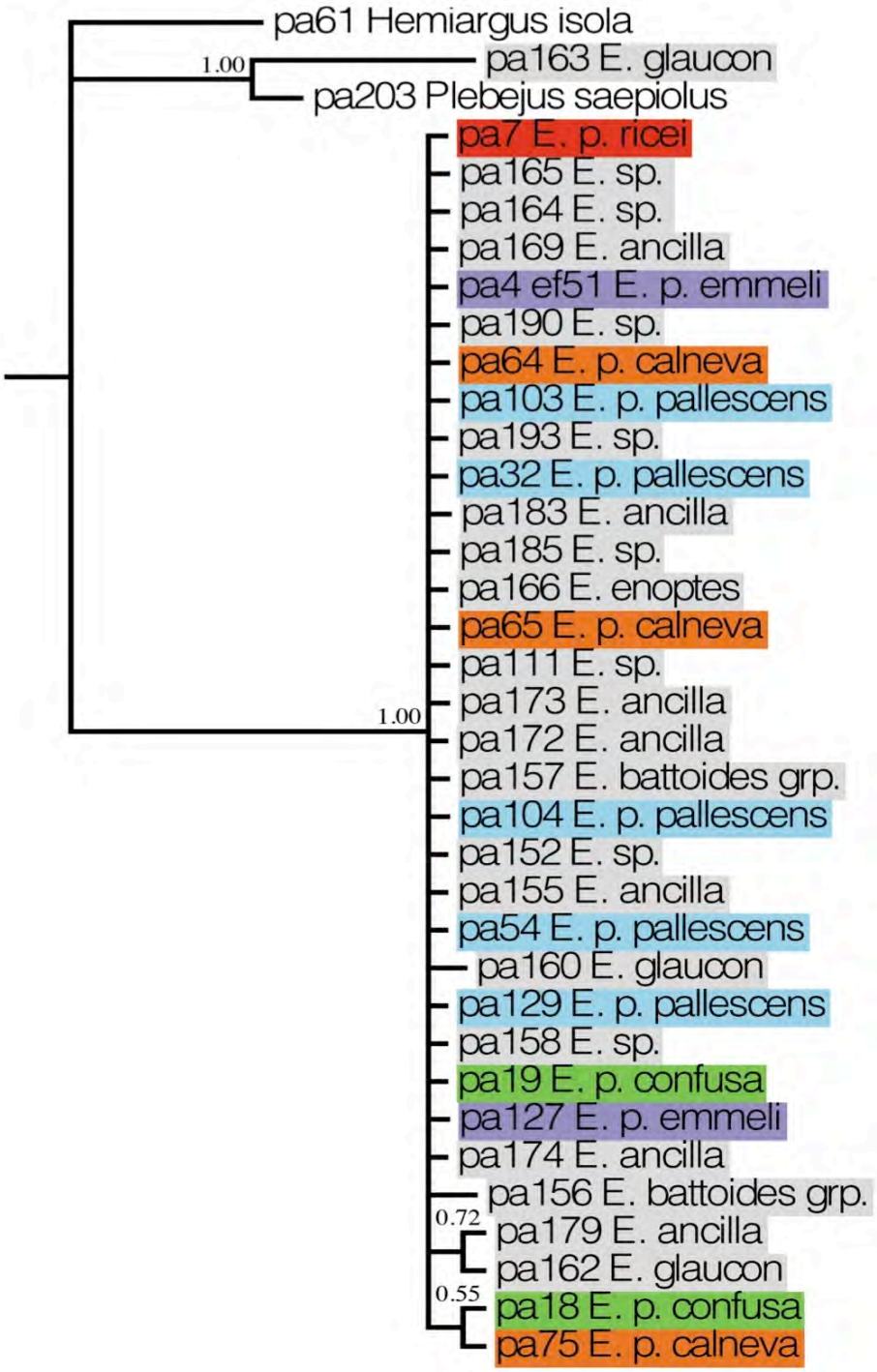

Figure 3. Phylogenetic tree of the nuclear region EF1-α. Colors correspond to *E. pallescens* subspecies (gray = other *Euphilotes* taxa).

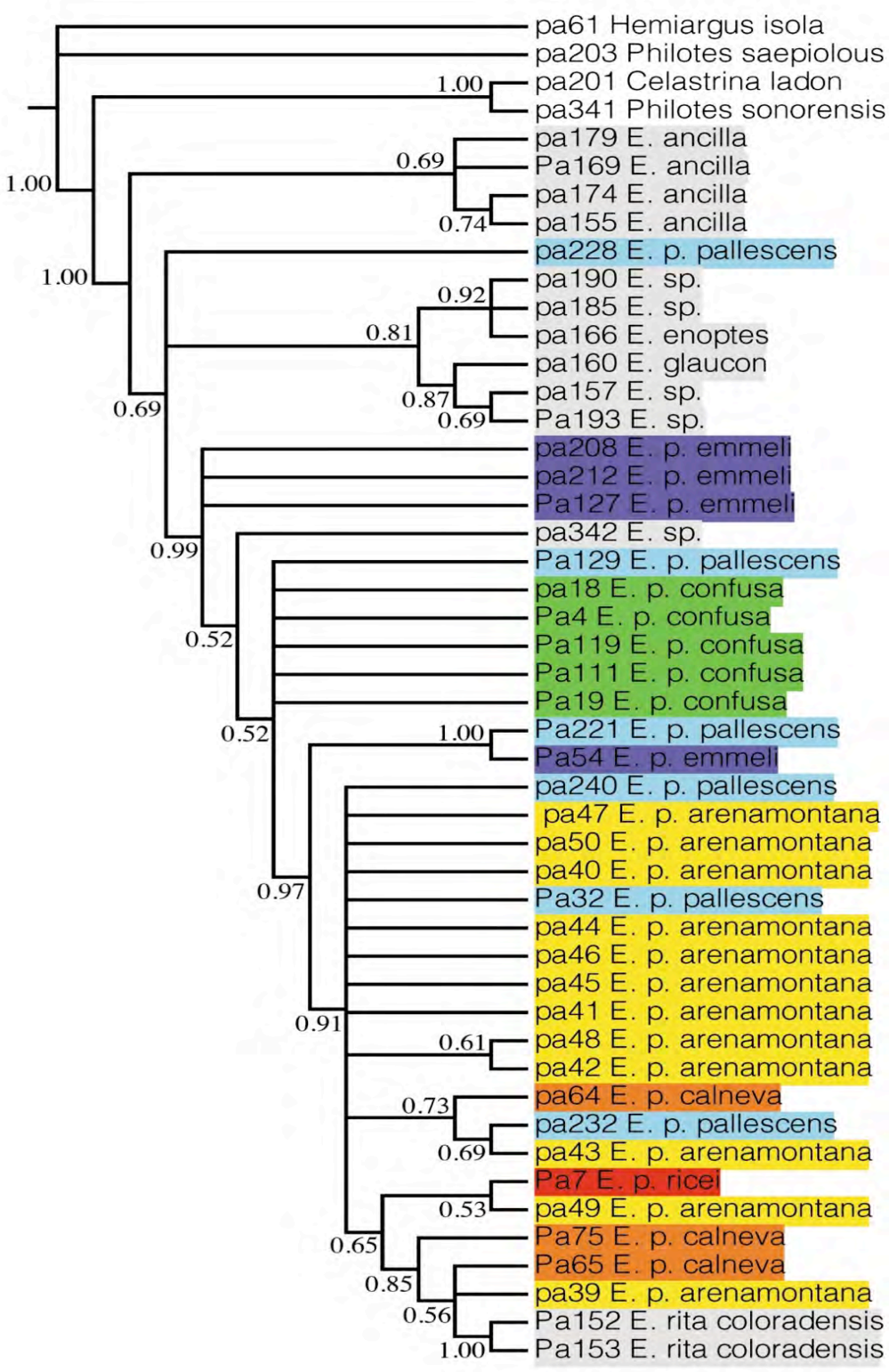

Figure 4. Phylogenetic tree of the nuclear region ITS1 region. Colors correspond to *E. pallescens* haplotypes; gray = other *Euphilotes taxa.*

Figure 5a.

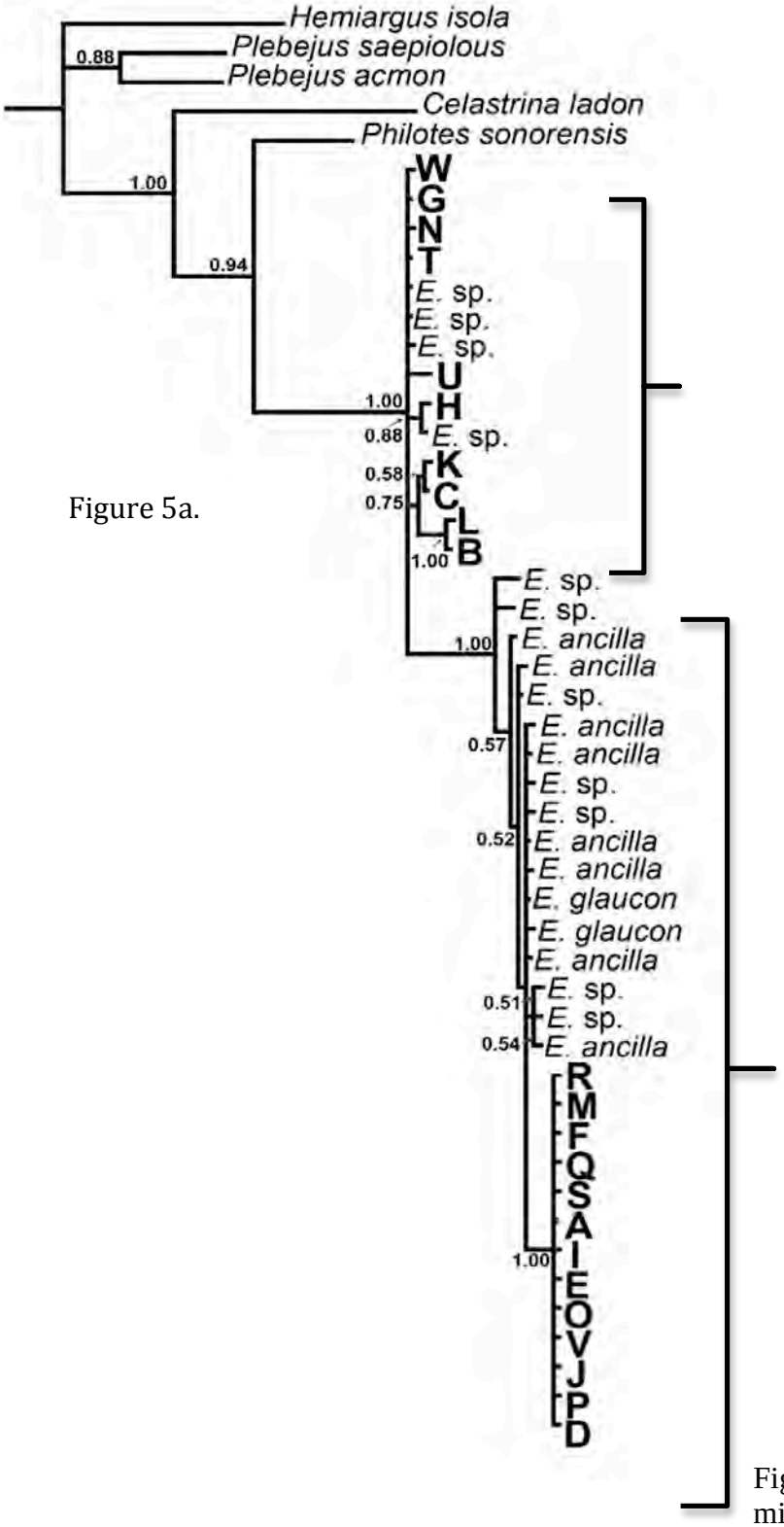

Figure 5a. Phylogenetic tree of the mitochondrial region C01. *E. pallescens* haplotypes are indicated with letters (see Fig. 1 for distributions of haplotypes in Nevada).

Figure 5b.

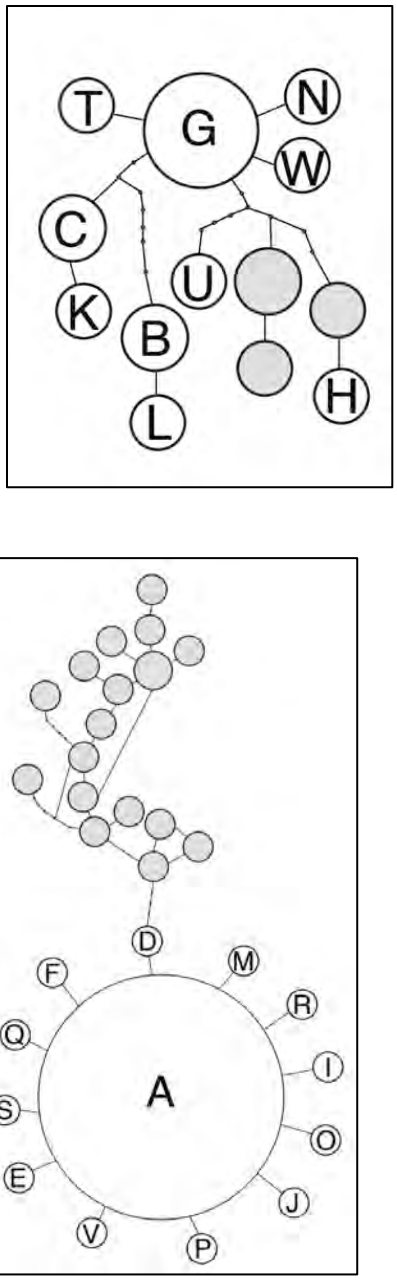

Figure 5b. Haplotype networks for mitochondrial DNA. Both networks correspond to the adjacent section of the phylogenetic tree. Steps in the network indicate base pair changes that distinguish each unique haplotype. The two networks shown could not be joined within 95% parsimony limits. Grey circles indicate haplotypes found in other *Euphilotes* taxa (not *E. pallescens*).

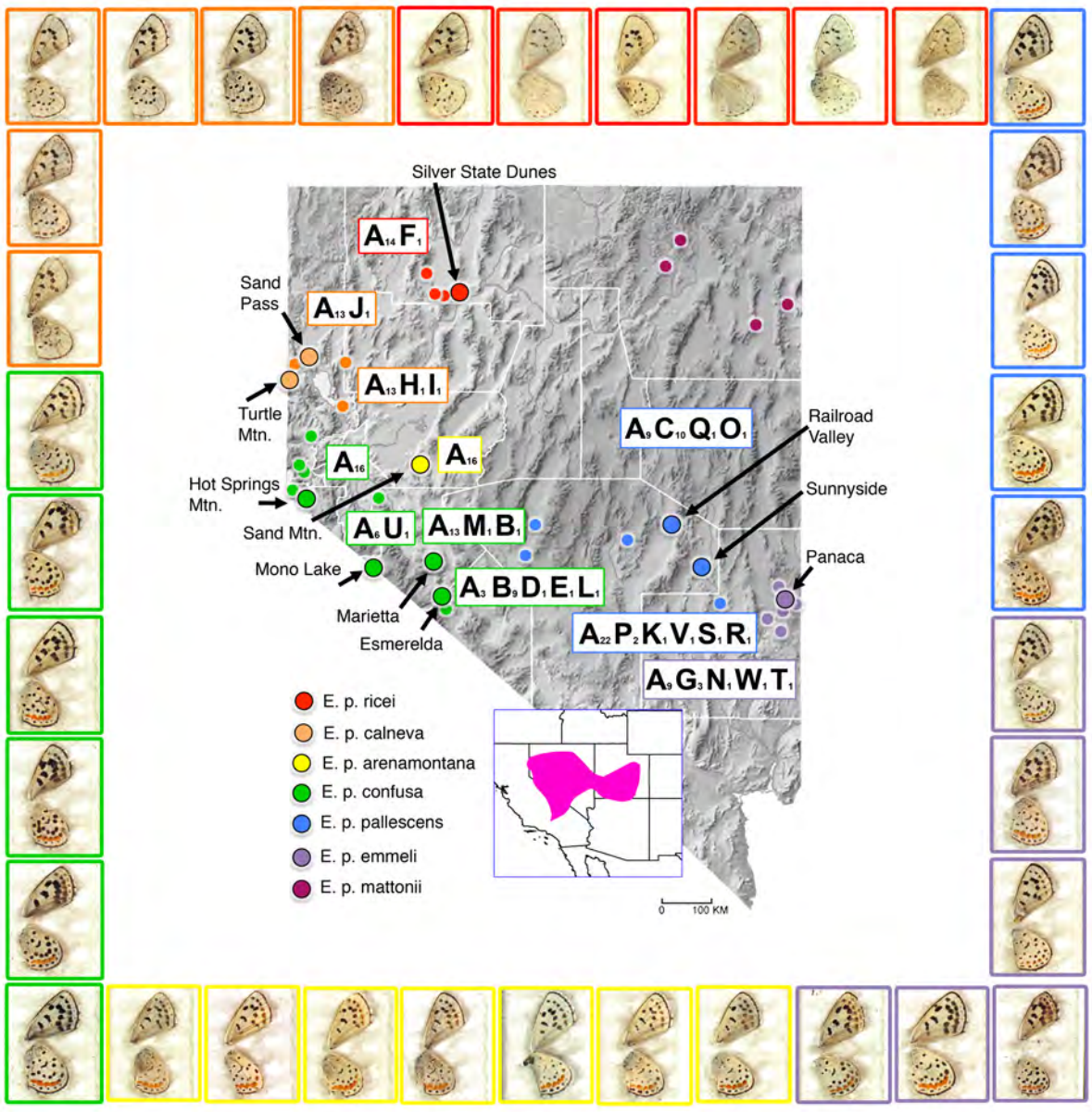

Figure 6. Sampling and population localities for focal Nevada *Euphilotes pallescens* subspecies (complete *E.* pallescens range shown in the inset). Haplotypes and their relative abundances are indicated in the boxes for each location. Around margin, the wing morphology of six of the subspecies is displayed, color surrounding wing picture correlates to collection location and subspecies.

Gene Flow, & Morphological Results:

The AMOVA indicated significant genetic structure among *E. pallescens* populations, though haplotype A was found in every sampled population (Fst = 0.26, $P < 0.0001$) (Table 1). This is consistent with some level of restricted gene flow. A neighbor-joining dendrogram was created with Fst values (based on haplotype frequencies and corrected average pairwise differences). This dendrogram suggests a certain amount of geographic structure associated with different haplotypes (Fig. 7). Haplotypes from Sand Pass are most distinct. This is a population that is found in the north west region of Nevada and is followed by the branches from Silver State Dunes, Hot Springs, Sand Mountain, and Turtle Mountain populations, which are also located in the north west. Haplotypes from Mono Lake, Marietta, and Sunnyside, Panaca, Esmeralda, and Railroad Valley are then most similar to each other. These are the furthest south and east populations and exhibit the most haplotype diversity.

Table 1. Results from analysis of molecular variance among populations of *Euphilotes pallescens.*

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	10	10.648	0.05678 Va	26.25
Within populations	166	26.476	0.15950 Vb	73.75
Total	176	37.124	0.21627	
Fixation Index	0.26253			

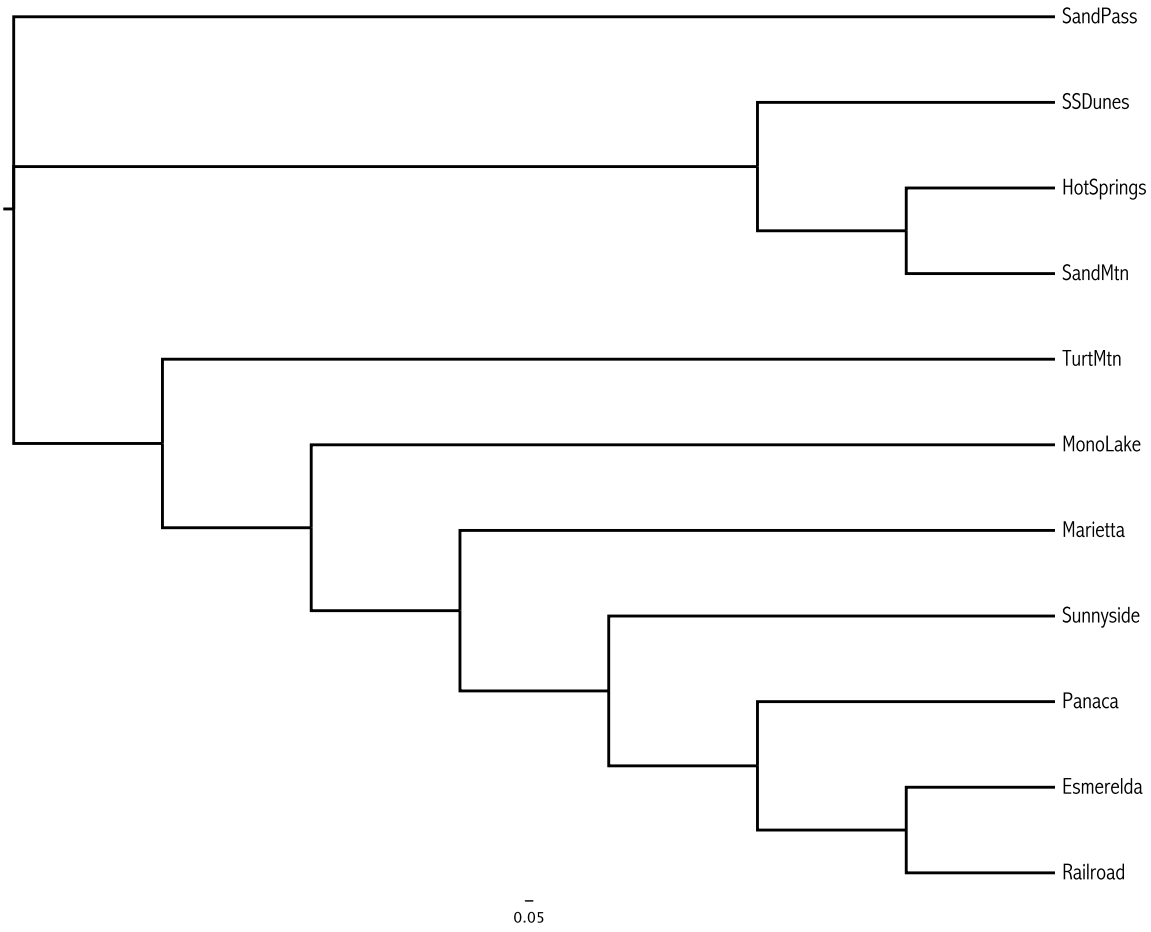

Figure 8. Neighbor joining dendrogram based on Fst values among populations.

Morphometric analyses revealed a fair amount of phenotypic divergence among the populations analyzed. A non-metric multidimensional scaling plot displays the relative similarity and differences among the various morphologies of the subspecies (Euclidean distances in Fig. 8 correspond to differences among individuals)**.** *E. p. ricei* and *E. p. calneva* have the most divergent morphologies with very diminished spots and mostly absent orange aurorae. Our focal

subspecies, *E. p. arenamontana* is placed near other northern populations in the middle of the scatter plot. This suggests a certain degree of morphological differentiation and that the Sand Mountain blue butterfly more closely resembles northern morphotypes than southern morphotypes. Subspecies *E. p. confusa* and *E. p. emmeli* appear at the other end of the scatter plot due to their bolder spot patterns, and well defined orange aurorae. Overall, underside wing pattern varies among populations that are geographically isolated and is marked between the specimens collected from the extreme northern and southern populations.

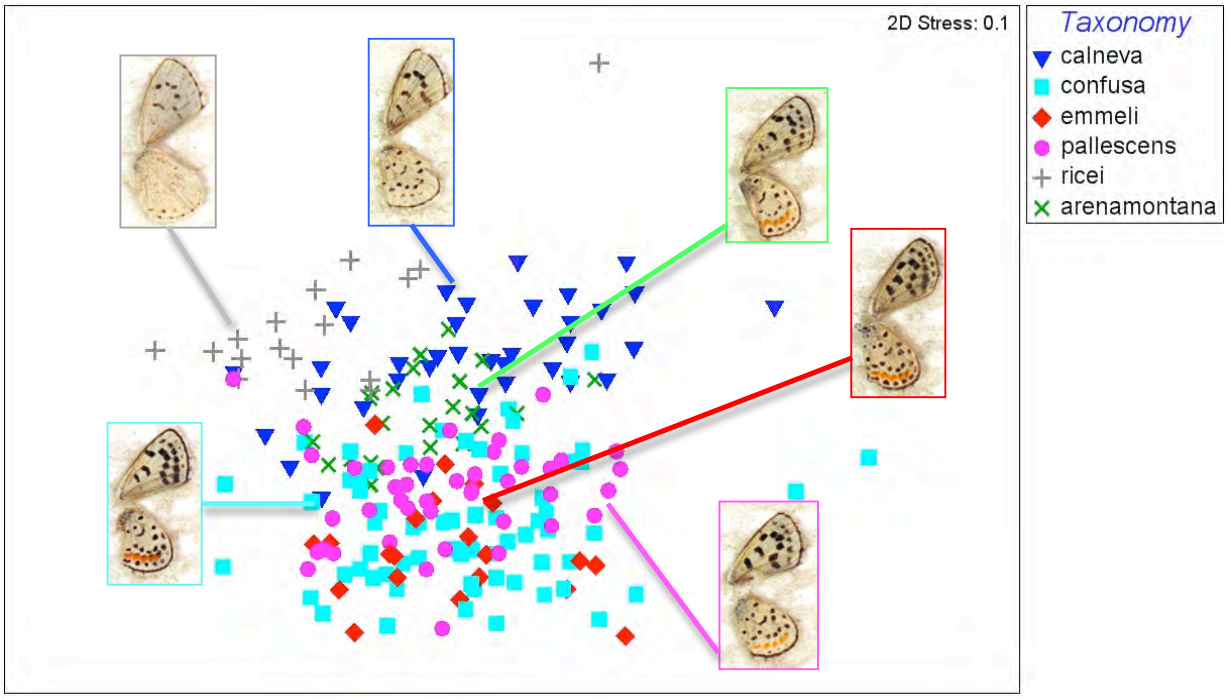

Figure 9. Non-metric multidimensional scaling plot depicting relative differences among *Euphilotes pallescens* subspecies morphotypes.

Discussion:

Phylogenetic Patterns of Mitochondrial and Nuclear Sequences

Bayesian analyses of the genetic markers amplified in this study resulted in phylogenetic hypotheses that display little genetic distinction among *Euphilotes pallescens* subspecies (Figs. 3-5a). The nuclear marker EF1-α showed little genetic structure between *Euphilotes pallescens*

and other *Euphilotes* species (Fig. 3). In other studies, this marker has been shown to be less variable than CO1 and ITS1 (Roe & Sperling, 2007), but has been successfully utilized to illuminate relationships at the species level within the Order of Lepidoptera (Casner & Tomasz, 2010). This lack of tree structure may be because the marker does not work well for this particular group at the species level or divergences in genetic sequences may not have accumulated over the Pleistocene time scale, which supports a relatively recent diversification of this group. These phylogenetic relationships might be expected from historical and nominal designations of this complex in that relations among *E. pallescens* and other *Euphilotes* have been problematic (Mattoni, 1988).

Unlike EF1-α, ITS1 separated *E. pallescens* from other *Euphilotes* species (Fig. 4). However, this marker also showed little structure between subspecies. Our focal subspecies, *E. p. arenamontana* was placed on the tree among other subspecies suggesting that this population is not completely genetically isolated. Two specimens of *Euphilotes rita* fall within the larger *Euphilotes pallescens* clade suggesting that *E. rita* may not be a distinct species.

Conversely, a deep divergence is displayed by the CO1 phylogenetic tree, which places *E. pallescens* subspecies at either side of that division (Fig. 5a). The most striking feature of this tree is that haplotypes found within the *E. pallescens* complex are in some cases more closely related to haplotypes of other *Euphilotes* species than to other *E. pallescens* haplotypes. The mitochondrial haplotype networks (Fig. 5b) also illustrate this relationship. Haplotype A was the most abundant and was found in each sampled population (Fig. 6). Our focal subspecies, *E. p. arenamontana*, (yellow population in Fig. 1) is fixed for the most common haplotype, suggesting that this population is not genetically distinct from other subspecies populations, at least at the level of mitochondrial variation. However, some level of restricted gene flow among

geographically isolated populations in Nevada is suggested by the distribution of mitochondrial variation which is also illustrated in the neighbor-joining tree (Fig. 7).

Phylogeographic History

Patterns derived from the nuclear markers differed from those based on mtDNA sequences, which may be the result of a complex evolutionary history. The presence of two distinct mtDNA networks, both composed of a mixture of *E. pallescens* and other *Euphilotes* species, suggests there may have been one or more ancient hybridization events among nominal species. The nuclear markers may not show evidence of hybridization because mtDNA retains this evidence longer due to its tendency to introgress beyond species boundaries, while any nuclear markers transferred during hybrization may be lost due to selection or drift after the hybridization event.

The lack of structure displayed by both nuclear genes is consistent with recent morphological evolution within the *Euphilotes pallescens* complex that may have been influenced in part by Pleistocene climate oscillations. Nevada along with much of the American west experienced extreme weather modulations between glacial maxima and minima (Fiero, 1986; Epps et al., 1998). These weather patterns often shaped the phylogeographic history of species as repeated glaciations forced populations into restricted refugia (Epps et al., 1998; Hewitt, 2004).

Gene flow and Morphology

Euphilotes pallescens subspecies are known for their geographic isolation throughout their range and for being morphologically distinct (Mattoni, 1988; Opler & Tilden, 1999). In

concordance with these assumptions, the AMOVA reflected some degree of gene flow restriction between *E. pallescens* subspecies (Table 1.) A dendrogram based on Fst values also implies a geographic pattern (Fig. 7) This dendrogram indicates that pairwise differences among haplotypes may be distributed non-randomly among populations implying a geographic pattern in mitochondrial diversity. Our focal population, Sand Mountain, is placed among northern haplotype associations along with haplotypes from Sand Pass, Silver State Dunes, Hot Springs, and Turtle Mountain populations. Turtle Mountain, Mono Lake, Marietta, Sunnyside, Panaca, Esmeralda, and Railroad Valley appear in continuous stepwise association with one another, and make up a largely southern cluster.

Morphological differences among subspecies are marked and also appear to have a geographic component (Fig. 8). *E. p. pallescens, confusa, and emmeli* subspecies make up the southern most locations of Nevada and have similar morphological characteristics, which include more bold black spots on the underside of the forewing and vibrant orange aurorae on the underside of the hind wing (see also Fig. 6). Located at the top of the scatterplot, with reduced spots on the underside of the forewing and almost absent orange aurorae on the underside of the hind wing, *E. p. calneva* and *ricei* are morphologically distinct from more southern subspecies. *E. p. arenamontana* is placed between both extreme morphotypes but more closely resembles individuals collected from northern populations. Morphological distinctness between northern and southern populations could be the result of relatively recent evolution, too recent to be reflected in the distribution of neutral genetic variation, phenotypic plasticity, or characteristics that are maintained in the face of gene flow among populations. The two latter hypotheses are less likely given the extent of gene flow restriction among populations (Table 1). This implies

that the Sand Mountain blue and its related subspecies have recently evolved both unique ecological and morphological characteristics.

Conservation

Euphilotes pallescens subspecies, members of the pallid-dotted blue butterfly complex, are found throughout remote areas of Nevada, southern California, western Utah, and northern Arizona (Opler & Tilden, 1999). These gossamer winged lycaenids have been described as geographically isolated as well as ecologically and morphologically distinct (Austin & Murphy, 1987). With life history traits such as specializing upon various species of dune and playa adapted buckwheat (*Eriogonum* spp.) and limited migration abilities associated with single annual generations (Opler & Tilden, 1999), populations of this butterfly complex are vulnerable to environmental degradation and habitat fragmentation. Four out of the seven described subspecies from Nevada are deemed as "at risk," including our focal subspecies, the Sand Mountain blue butterfly (*E. p. arenamontana*), which has been the focus of almost a decade of conservation efforts (NNHPO, 2004; Nevada BLM, 2010). With this study we have suggested that the Sand Mountain blue butterfly is not genetically distinct at the markers we studied given that it exhibits the most common mtDNA haplotype, which is found in every population of *E. pallescens* subspecies in Nevada (Fig. 7). This would be consistent with the possibility that the Sand Mountain blue butterfly is a relatively recently evolved addition to the *E. pallescens* subspecies complex. Additionally, this study's finding of a high degree of gene flow restriction between subspecies populations (see AMOVA Table 1) and the Sand Mountain blue's geographic isolation (Fig. 9) serve to support the assumption that the Sand Mountain blue butterfly is vulnerable to changes in effective population size.

Current conservation efforts have focused upon the persistence of the butterfly's host plant, the Kearny Buckwheat (*Eriogonum nummulare),* and the prevention of further degradation of the Sand Mountain dune habitat (Nevada BLM, 2010). This study may inform future conservation attempts for the Sand Mountain blue and its related subspecies that focus upon the population genetics of this group. Various population genetic conservation methods have been suggested for butterflies and lycaenids in general, which include captive breeding of individuals from an at risk population for re-release into the population of origin, or breeding individuals form a robust population and releasing them into an at risk population (Matthew et al., 2011; Crone at al., 2007). Although these methods may seem straightforward and relatively easy to implement, the tendency for isolated butterflies to become locally specialized and adapted create unforeseen complications that can have dramatic consequences. Issues such as gene flow, temperature preferences, phenology, host plant associations, symbiotic relationships with other insects, and bacterial infections such as Wolbachia all present hurdles to captive rearing programs (Matthew at al., 2011; Nice at al., 2009). For instance, genomic incompatibility and outbreeding depression may have deleterious consequences if individuals of one subspecies were to be released into a small population composed of a different subspecies. These complications may arise in this complex because of the private haplotypes found in each population of *E. pallescens* (Fig. 7) along with the AMOVA results that indicate gene flow is not common among populations throughout Nevada (Table 1.) Dune adaptation may also present a problem with the Sand Mountain blue butterfly specifically, for dunes present uniquely harsh temperatures that playa adapted *E. pallescens* subspecies such as individuals from Railroad Valley may not be capable of surviving (Chapman et al., 1926). Selecting subspecies from ecologically similar habitats should be stressed when developing future captive rearing programs. Lastly, Wolbachia

is a bacterial endosymbiont that can impact the effective population size of previously uninfected insect populations by dangerously skewing sex ratios and has presented a large problem for arthropod conservation (Nice at al., 2009). Future studies of the *E. pallescens* complex should focus upon determining if Wolbachia infections are present among subspecies and populations. This information could dramatically impact the course of future captive rearing programs.

Conclusions:

The Sand Mountain blue and its related *E. pallescens* subspecies are not genetically distinct according to both nuclear markers (Figs. 3-4), which supports recent evolution in this group. Conversely, a more ancient history of isolation and hybridization among *Euphilotes* species is suggested by the mtDNA phylogenetic tree (Fig. 5a), which displays both a deep split among *E. pallescens* and other *Euphilotes* spp., and complex haplotype relationships among *E. pallescens* subspecies and haplotypes of other *Euphilotes* spp. (Fig. 5b). Additionally, these findings suggest that DNA barcoding, which uses percent mtDNA sequence divergence to distinguish between species (Hebert et al., 2003), may not be useful for the differentiation and conservation of this group (Rubinoff, 2006).

The unique morphology (Fig. 8) and restricted gene flow among subspecies and populations (Table 1) suggest a recent evolution of the unique characteristics of this group, which were possibly catalyzed by isolation during Pleistocene climactic fluctuations (Fiero, 1986; Epps et al., 1998) and may present hurdles to future conservation efforts (Matthew et al., 2011; Crone at al., 2007). Further investigation into this complex group is required to develop an adequate conservation program.

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APPENDIX 1.0

Species	**Voucher ID**	**Haplotype**	**Collection Location**
Euphilotes pallescens ricei	Pa1	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa2	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa5	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa6	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa7	F	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa8	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa9	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa10	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa11	A	Silver State Dunes, NV

Euphilotes pallescens ricei	Pa12	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa13	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa14	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa15	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa16	A	Silver State Dunes, NV
Euphilotes pallescens ricei	Pa140	A	Silver State Dunes, NV
Euphilotes pallescens calneva	Pa75	J	Sand Pass, NV
Euphilotes pallescens calneva	Pa76	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa77	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa78	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa79	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa80	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa81	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa82	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa83	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa84	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa85	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa86	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa139	A	Sand Pass, NV
Euphilotes pallescens calneva	Pa63	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa64	H	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa65	I	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa66	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa67	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa68	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa69	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa70	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa71	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa72	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa73	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa74	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa149	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa150	A	Turtle Mountain, NV
Euphilotes pallescens calneva	Pa151	A	Turtle Mountain, NV
Euphilotes pallescens confusa	Pa95	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa96	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa97	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa98	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa99	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa100	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa101	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa102	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa112	A	Hot Springs Mountain, NV

Euphilotes pallescens confusa	Pa113	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa114	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa115	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa116	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa117	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa118	A	Hot Springs Mountain, NV
Euphilotes pallescens confusa	Pa342	U	Mono Lake, CA
Euphilotes pallescens confusa	Pa343	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa344	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa345	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa346	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa347	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa348	A	Mono Lake, CA
Euphilotes pallescens confusa	Pa87	A	Marietta, NV
Euphilotes pallescens confusa	Pa88	A	Marietta, NV
Euphilotes pallescens confusa	Pa89	A	Marietta, NV
Euphilotes pallescens confusa	Pa90	A	Marietta, NV
Euphilotes pallescens confusa	Pa91	A	Marietta, NV
Euphilotes pallescens confusa	Pa92	A	Marietta, NV
Euphilotes pallescens confusa	Pa93	A	Marietta, NV
Euphilotes pallescens confusa	Pa94	A	Marietta, NV
Euphilotes pallescens confusa	Pa119	M	Marietta, NV
Euphilotes pallescens confusa	Pa120	A	Marietta, NV
Euphilotes pallescens confusa	Pa121	B	Marietta, NV
Euphilotes pallescens confusa	Pa122	A	Marietta, NV
Euphilotes pallescens confusa	Pa123	A	Marietta, NV
Euphilotes pallescens confusa	Pa124	A	Marietta, NV
Euphilotes pallescens confusa	Pa125	A	Marietta, NV
Euphilotes pallescens confusa	Pa3	A	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa4	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa17	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa18	D	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa19	E	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa20	A	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa21	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa22	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa23	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa24	B	Esmerelda, Mineral Co. line,

			NV
Euphilotes pallescens confusa	Pa25	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa26	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa27	A	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa28	B	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens confusa	Pa111	L	Esmerelda, Mineral Co. line, NV
Euphilotes pallescens arenamontana	Pa39	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa40	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa41	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa42	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa43	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa44	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa45	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa46	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa47	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa48	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa49	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa50	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa134	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa135	A	Sand Mountain, NV
Euphilotes pallescens arenamontana	Pa136	A	Sand Mountain, NV
Euphilotes pallescens pallescens	Pa29	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa30	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa31	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa32	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa33	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa34	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa35	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa36	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa37	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa38	C	Railroad Valley, NV

Euphilotes pallescens pallescens	Pa129	O	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa130	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa131	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa234	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa235	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa236	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa237	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa238	C	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa239	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa240	Q	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa241	A	Railroad Valley, NV
Euphilotes pallescens pallescens	Pa103	K	Sunnyside, NV
Euphilotes pallescens pallescens	Pa104	P	Sunnyside, NV
Euphilotes pallescens pallescens	Pa105	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa106	P	Sunnyside, NV
Euphilotes pallescens pallescens	Pa107	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa108	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa109	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa110	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa141	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa142	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa143	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa144	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa145	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa146	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa147	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa148	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa219	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa221	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa222	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa224	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa225	V	Sunnyside, NV
Euphilotes pallescens pallescens	Pa227	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa228	S	Sunnyside, NV
Euphilotes pallescens pallescens	Pa229	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa230	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa231	A	Sunnyside, NV
Euphilotes pallescens pallescens	Pa232	R	Sunnyside, NV
Euphilotes pallescens pallescens	Pa233	A	Sunnyside, NV
Euphilotes pallescens emmeli	Pa51	G	Panaca, NV
Euphilotes pallescens emmeli	Pa52	G	Panaca, NV
Euphilotes pallescens emmeli	Pa53	A	Panaca, NV
Euphilotes pallescens emmeli	Pa54	G	Panaca, NV

Euphilotes pallescens emmeli	Pa55	A	Panaca, NV
Euphilotes pallescens emmeli	Pa56	G	Panaca, NV
Euphilotes pallescens emmeli	Pa57	G	Panaca, NV
Euphilotes pallescens emmeli	Pa58	G	Panaca, NV
Euphilotes pallescens emmeli	Pa59	A	Panaca, NV
Euphilotes pallescens emmeli	Pa60	G	Panaca, NV
Euphilotes pallescens emmeli	Pa62	G	Panaca, NV
Euphilotes pallescens emmeli	Pa126	G	Panaca, NV
Euphilotes pallescens emmeli	Pa127	N	Panaca, NV
Euphilotes pallescens emmeli	Pa128	G	Panaca, NV
Euphilotes pallescens emmeli	Pa204	G	Panaca, NV
Euphilotes pallescens emmeli	Pa205	G	Panaca, NV
Euphilotes pallescens emmeli	Pa207	G	Panaca, NV
Euphilotes pallescens emmeli	Pa208	W	Panaca, NV
Euphilotes pallescens emmeli	Pa209	G	Panaca, NV
Euphilotes pallescens emmeli	Pa210	W	Panaca, NV
Euphilotes pallescens emmeli	Pa211	G	Panaca, NV
Euphilotes pallescens emmeli	Pa212	T	Panaca, NV
Euphilotes pallescens emmeli	Pa213	G	Panaca, NV
Euphilotes pallescens emmeli	Pa214	G	Panaca, NV
Euphilotes pallescens emmeli	Pa215	A	Panaca, NV
Euphilotes pallescens emmeli	Pa216	A	Panaca, NV
Euphilotes pallescens emmeli	Pa217	G	Panaca, NV
Euphilotes pallescens emmeli	Pa218	G	Panaca, NV