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ORIGINAL ARTICLE

Complex evolutionary history of the pallid dotted-blue butterfly (Lycaenidae: *Euphilotes pallescens*) in the Great Basin of western North America

Joseph S. Wilson[1]*, Michelle Sneck[2], Dennis D. Murphy[3], Chris C. Nice[4], James A. Fordyce[5] and Matthew L. Forister[3]

[1]*Department of Biology, Utah State University, Tooele, UT, 84074, USA,* [2]*Department of Ecology and Evolutionary Biology, Rice University, Houston, TX, 77005, USA,* [3]*Department of Biology, University of Nevada, Reno, NV, 89557, USA,* [4]*Department of Biology, Population and Conservation Biology Program, Texas State University, San Marcos, TX, 78666, USA,* [5]*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, 37996, USA*

*Correspondence: Joseph S. Wilson, Department of Biology, Utah State University Tooele, 1021 W. Vine Street, Tooele, UT 84074, USA.
E-mail: joeswilson@gmail.com

ABSTRACT

Aim The aim of this study was to investigate patterns of genetic isolation associated with populations in discrete habitat patches in a North American cold desert through analysis of the pallid dotted-blue butterfly, *Euphilotes pallescens*. This small butterfly is largely restricted to low-elevation habitats across the Great Basin. The apparent geographical isolation and reported morphological variation among *E. pallescens* populations makes this species an ideal candidate with which to investigate patterns of genetic isolation and diversification in this arid region.

Location Great Basin, western North America.

Methods We used sequence data from nuclear and mitochondrial genes to investigate genetic diversity among *E. pallescens* populations, and among *E. pallescens* and a number of closely related *Euphilotes* species, using Bayesian phylogenetic analyses, as well as population genetic analyses. In conjunction with genetic variation, morphological variation was examined in the context of geographical and subspecific differentiation.

Results Our genetic and morphological analyses suggest a moderate amount of isolation among populations, consistent with the hypothesis of restricted gene flow among isolated dune habitats, and possibly associated with isolation in distinct Pleistocene refugia. The patterns of diversification within *E. pallescens* and among closely related species are complicated by discordance among phylogenetic reconstructions based on nuclear and mitochondrial genes. Discordance among gene genealogies suggests a complex evolutionary history, perhaps involving alternating periods of reticulation and divergence in isolation.

Main conclusions Although *E. pallescens* may be a vagile species, we find that persistence on isolated dunes in the Great Basin is associated with appreciable genetic and morphological differentiation among populations. However, genetic, morphological and taxonomic axes of variation are only partially in agreement. More generally, the discordance we find among genetic regions is consistent with the ascendant paradigm in phylogenetic reconstruction: gene genealogies often do not perfectly match species trees. Thus we present *Euphilotes* as a model for future biogeographical and phylogenetic reconstructions employing larger data sets of independent sequence markers.

Keywords

Gene tree, genetic isolation, Great Basin Desert, Lycaenidae, phylogeography, Pleistocene refugia, reticulation, species tree, USA, vicariance.

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INTRODUCTION

Genetic studies investigating the history of biotic diversification in the Nearctic deserts have focused primarily on warm deserts (Mojave, Sonoran, Peninsular and Chihuahuan deserts; e.g. Riddle *et al.*, 2000a,b; Zink *et al.*, 2001; Jaeger *et al.*, 2005; Smith & Farrell, 2005; Riddle & Hafner, 2006). In those deserts, multiple late Neogene barriers appear to have isolated populations vicariantly, which led to diversification in a variety of taxonomic groups (summarized by Riddle & Hafner, 2006). The uplift of the Sierra Madre/Colorado Plateau axis and the extension of the Gulf of California (the Bouse Embayment), for example, have been cited as the main drivers of diversification in rodents, toads, snakes, plants and velvet ants (Riddle *et al.*, 2000a; Jaeger *et al.*, 2005; Devitt, 2006; Douglas *et al.*, 2006; Moore & Jansen, 2006; Wilson & Pitts, 2010a; Wilson *et al.*, 2012). The shared historical biogeography of the warm desert taxa indicates that late Neogene vicariance played an essential role in the development of this biota.

While much of the recent work investigating isolation and diversification in desert landscapes has focused on the warm deserts of North America, the regional cold desert (the Great Basin Desert) has received relatively little attention. Some patterns are, however, beginning to emerge. Studies investigating phylogeographical patterns in taxa inhabiting the Great Basin have found that many populations are genetically distinct from those in neighbouring cold desert regions (such as the Colorado Plateau), and that populations within the Great Basin tend to exhibit east–west patterns of genetic divergence (Epps *et al.*, 1998; Orange *et al.*, 1999; Hafner *et al.*, 2008; Wilson & Pitts, 2010a; Hafner & Upham, 2011; Schultheis *et al.*, 2012). This pattern of east–west divergence could have resulted from isolation in distinct Pleistocene refugia, as has been suggested from studies of velvet ants (Wilson & Pitts, 2010a), or from Neogene mountain-building events (Hafner *et al.*, 2008) that may have created barriers isolating eastern and western populations. While most of the studies investigating isolation and diversification in the Great Basin are based on organisms that exhibit limited dispersal (e.g. flightless beetles, rodents, velvet ants), additional studies on more vagile taxa can lead to a better understanding of the factors associated with population isolation and diversification in organisms adapted to cold deserts.

An apt target organism with which to investigate isolation and diversification in highly mobile taxa inhabiting the Great Basin is the pallid dotted-blue butterfly, *Euphilotes pallescens* (Tilden & Downey, 1955), which is found in apparently isolated populations often associated with dune habitats (Fig. 1). Beyond our general interest in historical population dynamics in the cold deserts, *E. pallescens* is of evolutionary interest because complex relationships among populations and lineages within the genus *Euphilotes* have spawned confusion and controversy. The species *Euphilotes pallescens* has itself been separated into eight subspecies (Pratt & Emmel, 1998) – namely *Euphilotes pallescens ricei* Austin, 1998, *E. p. calneva* Emmel Emmel, 1998; *E. p. arenamontana* Austin, 1998, *E. p. confusa* Pratt & Emmel, 1998; *E. p. pallescens* Tilden & Downey, 1955, *E. p. emmeli* Shields, 1975, *E. p. mattonii* Shields, 1975 and *E. p. elvirae* (Mattoni, 1966) – seven of which are found only in the Great Basin (Austin, 1998a). Each subspecies is known from a relatively narrow geographical range and is distinguished either by morphological characteristics or by host-plant associations (e.g. Austin, 1998b,c; Emmel & Emmel, 1998; Pratt & Emmel, 1998). The putative geographical isolation of subspecies, as well as the reported ecological diversity, makes *E. pallescens* a worthy candidate for investigations into the historical processes associated with isolation and diversification in the Great Basin biota.

Because of its evident isolation and close association with a distinct habitat and a single host plant at each occupied site, several *E. pallescens* subspecies have been targeted for conservation actions. For example, the Nevada Natural Heritage Program (NNHP) considers four *E. pallescens* subspecies (*E. p. ricei*, *E. p. calneva*, *E. p. arenamontana* and *E. p. mattonii*) at risk of extinction and in need of protection (Nevada Natural Heritage Program, 2010). In 2004, several conservation groups including the Center for Biological Diversity, Xerces Society, Public Employees for Environmental Responsibility, and Nevada Outdoor Recreation Society filed a petition with the US Fish and Wildlife Service (USFWS) requesting federal protection for the Sand Mountain blue butterfly (*E. p. arenamontana*), which is known from only a single location in central Nevada. In this case, the USFWS determined that the subspecies maintained stable populations and that federal protection was not necessary (Nevada Fish & Wildlife Service, 2010).

While a number of the widely dispersed populations that have been assigned to *E. pallescens* have received attention from taxonomists and conservationists, little is known regarding genetic diversity among the numerous subspecies distributed across the Great Basin Desert. Here we examine those patterns of diversity through phylogenetic and population genetic analyses of three genes (two nuclear DNA regions and one mitochondrial DNA region), combined with morphometric analyses of wing pattern variation. We utilize genetic data gathered from *E. pallescens* as well as from other *Euphilotes* species in western North America, allowing patterns of diversification and isolation in *E. pallescens* to be placed in a broader context. Using the molecular and morphological data we address the following questions. (1) How isolated are *E. pallescens* populations in the Great Basin? (2) Do *E. pallescens* subspecies designations reflect molecular and morphological variation? (3) Do the patterns of biogeographical diversification in *E. pallescens* coincide with those found in other Great Basin taxa?

MATERIALS AND METHODS

Taxon sampling

Collections were made from multiple sites that were located from historical records (e.g. Austin, 1998a) that spanned the

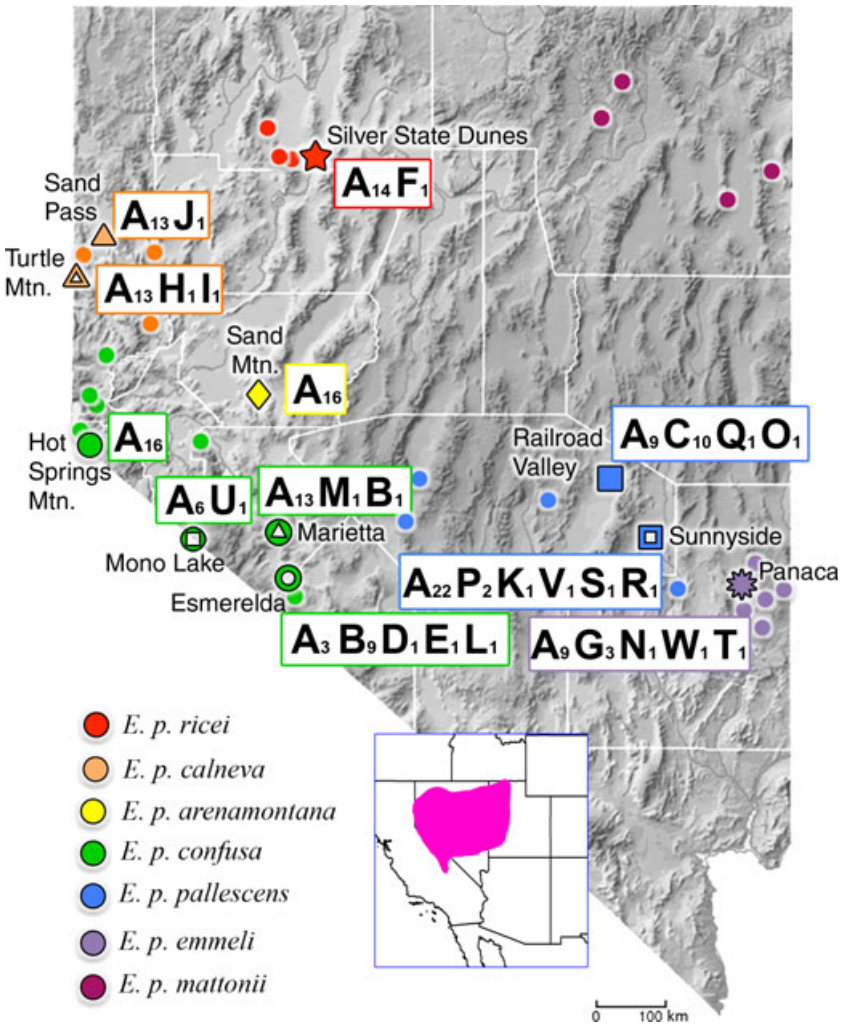

Figure 1 Map of *Euphilotes pallescens* locations in western North America showing historical records for subspecies populations (small circles) and populations from which we made collections (larger symbols with labels). Symbols correspond to those in Figs 3–5. Haplotypes are given for each sampled population as capital letters with subscripts that denote the number of each individual haplotype collected from that population. The inset map illustrates the boundaries of the Great Basin.

known range of *E. pallescens*. Samples were hand-collected from populations assigned to six of the seven *E. pallescens* subspecies inhabiting the Great Basin during the summers of 2008 and 2009 (Fig. 1, Appendix S1 in Supporting Information). At least 14 individuals were collected from all populations except Mono Lake, which yielded only 7 individuals. Specimens were net-collected, placed into glassine envelopes and stored in a freezer. Multiple attempts were made to collect specimens representing *E. p. mattonii*, which is known only from north-eastern Nevada, but no specimens were found at any of the sites from which this subspecies is historically known, nor from any other locality, potentially indicating subspecific extirpation. In addition, only a few *E. pallescens* populations are historically known from the eastern Great Basin (i.e. western Utah). No samples were obtained from these areas, as the majority of historical localities are now on military property and are currently inaccessible. Some authors have suggested that additional *E. pallescens* populations can be found in the Colorado Plateau in eastern Utah, but because the status of these populations and of other *Euphiolotes* species remain poorly resolved, with as few as three to as many as eleven distinct species being recognized (Austin *et al.*, 2008), and because the focus of the present study is on isolation and diversification in the Great Basin, samples were not taken from the Colorado Plateau. Specimens used for molecular examination have been labelled as voucher specimens and are stored at the University of Nevada, Reno (UNR). In addition to samples from *E. pallescens* populations, a limited number of specimens representing congeneric taxa were collected from sites across western North America (Appendix S1). Additional taxa included *Euphilotes enoptes*, *E. ancilla*, *E. glaucon*, *E. battoides*, *E. rita* and some unidentified *Euphilotes* species. Other taxa were also collected to be used as more distant outgroups. These included *Philotes sonora*, *Celastrina ladon*, *Plebejus acmon*, *Plebejus saepiolus* and *Hemiargus isola*, the latter being designated as the outgroup in all phylogenetic analyses. Current nomenclature and a list of authorities can be found at http://www.butterfliesandmoths.org/.

Molecular methods

DNA was isolated from thoracic tissue using the DNeasy Tissue Kit (Qiagen Inc., Alameda, CA, USA). Three loci were

amplified: one mitochrondrial DNA (mtDNA) region [a portion of the cytochrome *c* oxidase subunit I (*COI*: 586 bp)], and two nuclear DNA regions [the internal transcribed spacer region 1 (ITS1: 729 bp) and elongation factor 1-alpha (*EF1*-α: 329 bp)]. *COI* was amplified using the primer pair LepF1 and LepR1 (Hajibabaei *et al.*, 2006) with an annealing temperature of 52 °C. Most of our analyses focus on *COI*, which has often been used for phylogeographical and population genetic analyses. We focused on variation in mtDNA when investigating population-genetic patterns. Nuclear loci were used only in phylogenetic analyses and were amplified from a subset of specimens, targeting those that had unique mtDNA haplotypes based on preliminary analyses, as well as from various outgroups. Primers used to amplify *EF1*-α were EF44 and EF51r (Monteiro & Pierce, 2001), with an annealing temperature of 53–56 °C. Primers used to amplify the ITS1 nuclear region were forward-18S and reverse-5.8S (Pilgrim, 2002), with an annealing temperature of 50 °C. All PCR programmes included 35 cycles. Following amplification, fragments were sequenced in both directions using an AB1 3730xl DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA) and assembled in Sequencher 4.10.1 (Gene Codes Corp., Ann Arbor, MI, USA). All sequences have been deposited in GenBank (Appendix S2).

Phylogenetic and population genetic analyses

Each of the three genetic loci was separately subjected to Bayesian phylogenetic analysis using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Appropriate models of nucleotide substitution were determined in MrModeltest 2.3 (Nylander, 2004). The *COI* data set was partitioned by codon position, with the following models applied to each partition: first position, GTR + I; second position, F81; third position, GTR + Γ. The nuclear locus *EF1*-α was also partitioned by codon position, with the following models applied to each partition: first position, HKY; second position, F81; third position, F81. Because ITS1 is a non-coding locus, the GTR + Γ model was applied to the entire data set. Bayesian analyses of each locus included four independent runs, with three heated chains and one cold chain in each run. The Markov chain Monte Carlo (MCMC) chains were set for 6,000,000 generations and sampled every 100 generations (with the first 600,000 generations discarded as burn-in). Convergence and burn-in were assessed using Tracer 1.4.1 (Rambaut & Drummond, 2007).

We constructed a haplotype network for the *COI* sequences for all *E. pallescens* specimens using parsimony in tcs 1.21 (Clement *et al.*, 2000). The program estimated the 95% reconnection limit between haplotypes, with gaps treated as missing data. (The *COI* data set, however, contained no internal gaps.) Analysis of molecular variance (AMOVA) was performed on mitochondrial haplotypes using Arlequin 3.5.1.2 (Excoffier & Lischer, 2010) to investigate the genetic differentiation between the 11 populations (Fig. 1). Finally, a spatial analysis of molecular variance (SAMOVA; Dupanloup *et al.*, 2002) was used to explore the organization of mitochondrial variation within *E. pallescens* across the Great Basin, and to determine if molecular variation is concordant with contemporary subspecific taxonomic designations.

Morphological analysis

To investigate morphological divergence among *E. pallescens* populations, and to compare morphological characteristics with patterns of molecular divergence, the ventral surfaces of the hindwings and forewings were photographed using a Canon Powershot digital camera and examined for all *E. pallescens* specimens (male and female) that yielded *COI* sequences. Approximately equal numbers of male and female specimens were sampled from each population. Characteristics previously used to distinguish among *E. pallescens* subspecies (Mattoni, 1988), specifically the area of black markings on the ventral forewings and hindwings (i.e. the sum of all black markings) and the area of the orange of aurorae on the hindwings, were measured using ImageJ (Abramoff *et al.*, 2004). In addition, the size of the black band on the apex of the forewing was measured by standardizing the width of the band to the length of the wing for each specimen. All photographs included a ruler in order to standardize the measurements. Measurements were analysed using non-metric multidimensional scaling (NMDS) based on a matrix of Bray–Curtis dissimilarities generated using the R package vegan 2.0-3 (Oksanen *et al.*, 2011). Differences among populations were examined using permutational analysis of variance (PERMANOVA; Anderson, 2005) implemented in vegan 2.0-3, with significance based on 99,999 permutations of the matrix.

RESULTS

Molecular and phylogenetic patterns

Sequences were obtained from 190 *E. pallescens* individuals, as well as from 41 specimens representing other *Euphilotes* species and five outgroup taxa. Of the 190 *E. pallescens* mtDNA sequences that were obtained, 23 represented unique haplotypes. Nuclear genes were amplified from individuals, focusing on each of the 23 unique mitochondrial haplotypes. Additional specimens were included from the Sand Mountain population because of the conservation concerns that have been raised for this population. For ITS1, a total of 25 *E. pallescens* sequences were obtained, along with sequences from 12 individuals representing other *Euphilotes* species and four outgroups. For *EF1*-α, a total of 13 *E. pallescens* sequences were obtained, along with sequences from 21 individuals representing other *Euphilotes* species and three outgroups.

Bayesian analysis of the *COI* data produced a phylogeny with several well-supported clades, indicating a monophyletic *Euphilotes* at the genus level, but a polyphyletic *E. pallescens* composed of two divergent clades (Fig. 2). Each of

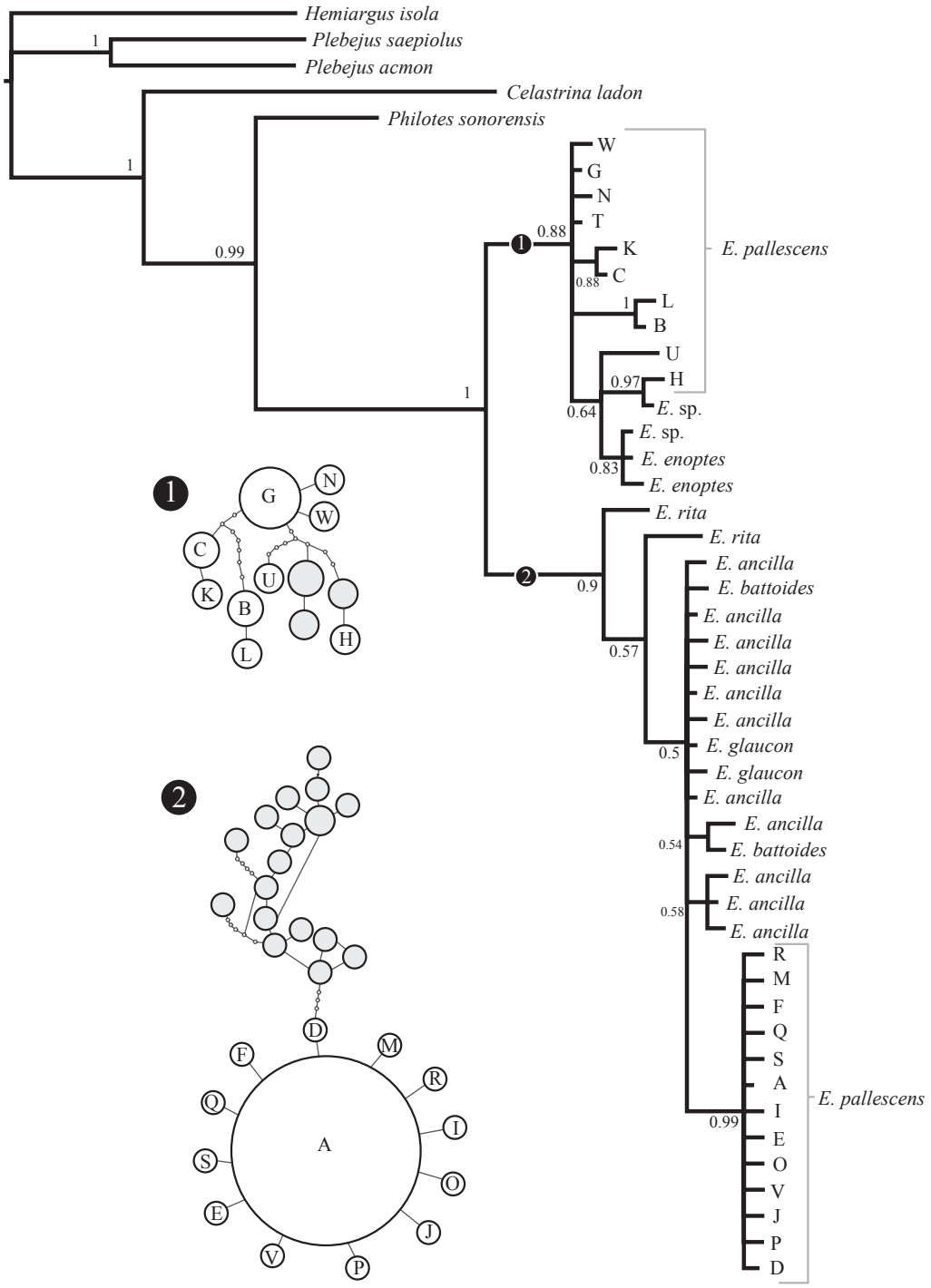

Figure 2 Consensus tree of the Bayesian analysis of *Euphilotes pallescens* individuals from western North America for cytochrome *c* oxidase subunit I (*COI*) with posterior probabilities given for each node. *Euphilotes pallescens* populations are named based on their haplotype. Haplotype networks are also given and correspond to clades marked '1' and '2', with *E. pallescens* populations marked with their corresponding haplotype and other *Euphilotes* species marked in grey. The circle size reflects the number of individuals exhibiting a haplotype.

the two *Euphilotes* clades contains both *E. pallescens* individuals and other *Euphilotes* species. Bayesian analysis of the ITS1 data produced a phylogeny with several well-supported clades showing a monophyletic *Euphilotes*, but, in contrast to the mitochondrial data, a moderately supported *E. pallescens* clade (Fig. 3). While *EF1-α* has successfully been used to recover patterns of species-level diversity in other Lepidoptera (e.g. Monteiro & Pierce, 2001), our analyses found

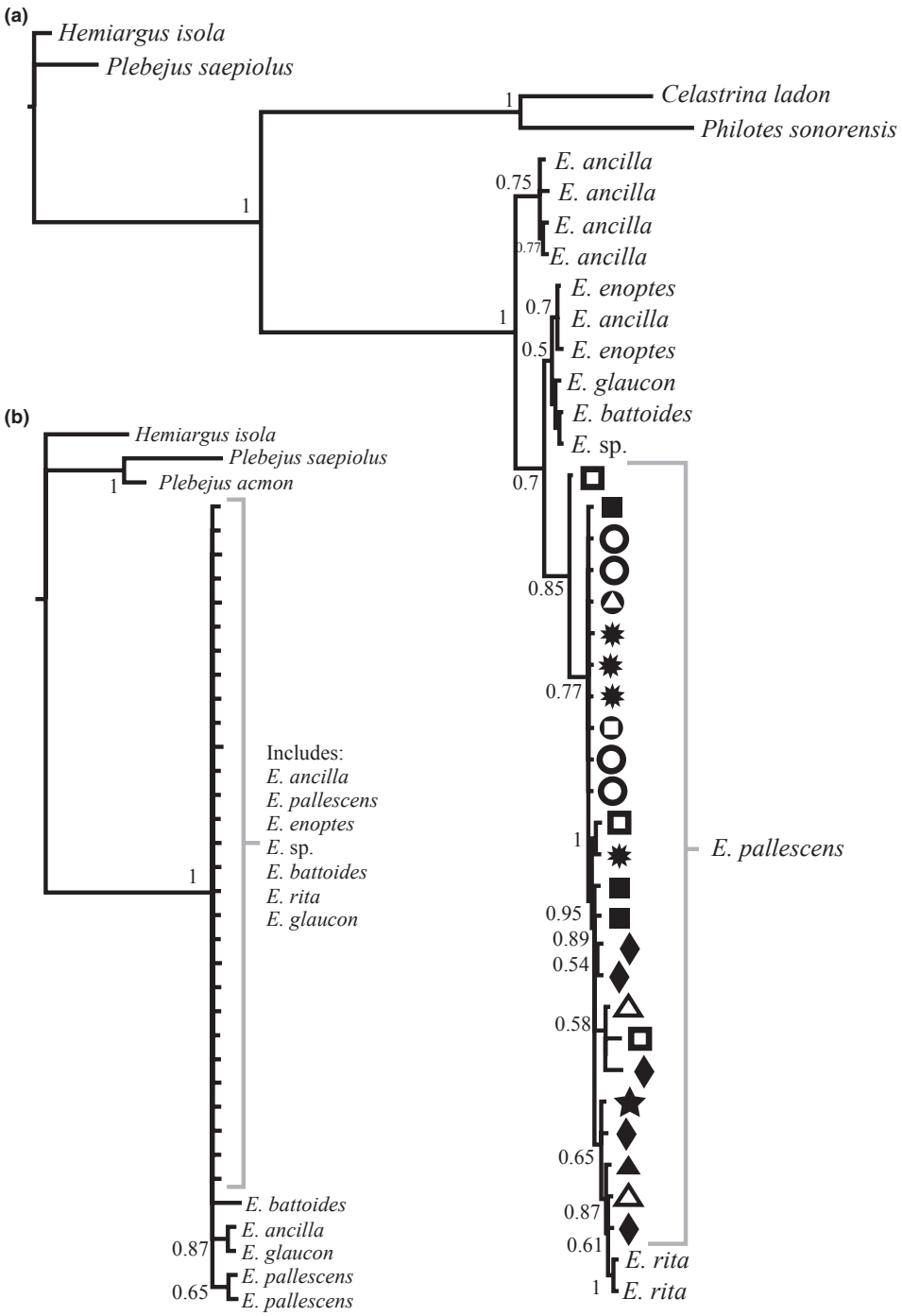

Figure 3 Consensus trees of the Bayesian analyses of *Euphilotes pallescens* individuals from western North America for (a) the internal transcribed spacer region 1 (ITS1) and (b) elongation factor 1-alpha (*EF1-α*), with posterior probabilities given for each node. *Euphilotes pallescens* populations are marked with symbols corresponding to those in Figs 1, 4 & 5.

little species-level variation in this marker. Although the genus *Euphilotes* was supported as a clade in *EF1-α* analyses, none of the species within the genus was monophyletic (Fig. 3). Within *E. pallescens*, none of the nominal subspecific entities can be associated with exclusive or well-supported phylogenetic clades, although evidence of restricted gene flow is indicated by population genetic analyses, as described below.

Gene flow and population structure

Network analysis of the *Euphilotes COI* data set resulted in two separate networks (Fig. 2), which could not be joined within 95% confidence limits of parsimony. As with the two major clades of the mitochondrial phylogeny (Fig. 2), each of the networks contains *E. pallescens* as well as other *Euphilotes* species. These two networks correspond to the two

clades recovered in the *COI* phylogenetic analysis (Fig. 2). The most common haplotype in *E. pallescens*, haplotype A, was found in each of the sampled *E. pallescens* populations and was the only haplotype recovered from *E. p. arenamontana* (the Sand Mountain population). Haplotype diversity was highest in *E. p. pallescens*, *E. p. confusa* and *E. p. emmeli* populations, which are found primarily in the southern Great Basin (Fig. 1).

Although a single haplotype (A) is found throughout all of the *E. pallescens* populations, there are many haplotypes that are found in one or a limited number of populations (see for example the southernmost populations in Fig. 1), which suggests some level of genetic isolation or restricted gene flow among these geographically isolated desert populations. The possibility of restricted gene flow is supported by the AMOVA results, which revealed an overall significant level of among-population genetic differentiation (Φ_{ST} = 0.26, $P < 0.0001$; see Table 1 for pairwise Φ_{ST} values). When spatial information was included, the analysis using SAMOVA indicated that a grouping of populations into four groups ($K = 4$) explained a high proportion of among-group variation ($\Phi_{CT} = 0.485$, $P < 0.01$; Fig. 4a). Population groupings of $K = 2$ and $K = 5$ also indicated high proportions of among-group variation; the populations grouped in a manner similar to the $K = 4$ grouping (Fig. 4b), but were less biologically informative (when $K = 2$, the population from Esmeralda formed one group, while the remaining 10 populations formed the other group). When $K = 5$, populations were grouped as in the analysis when $K = 4$, except that Railroad Valley and Panaca formed two distinct groups. The four groups associated with $K = 4$ included a large group containing the more north-western populations, with the southernmost and eastern populations included in the remaining groups (Fig. 4b).

Morphological variation

In addition to genetic variation, we quantified morphological variation to investigate the putative distinctness of subspecific taxa, and as another line of evidence for understanding biogeographical relationships among populations. Morphometric analyses based on ventral wing traits indicated appreciable phenotypic divergence among populations and among at least some of the nominal subspecific taxa (Fig. 5). PERMANOVA revealed statistically significant morphological differences among populations ($F = 3.0934$, $R^2 = 0.120$, $P < 0.0001$; Fig. 5). Sex was also a significant factor in the morphological differences among populations ($F = 5.2177$, $R^2 = 0.023$, $P = 0.001$). As with mitochondrial analyses (Table 1, Fig. 4), the observed morphological variation suggests differences in morphological divergence in the northwest and in the south, although the relative extent of differentiation among populations that is associated with the two types of data (mitochondrial and morphological) is reversed in these two regions, as discussed below.

DISCUSSION

Our objectives in the analysis of *E. pallescens* genetic and morphological data were to investigate isolation among populations, to examine subspecific taxonomic designations, and to compare our results with other biogeographical findings from the region. To address the first objective: our population genetic analyses suggested some degree of isolation in the majority of comparisons between populations (Table 1). Isolation can also be readily seen in the large number of private mitochondrial haplotypes (haplotypes found in a single population; Fig. 1). Similarly, a large number of locally distributed haplotypes were observed in Great Basin populations of rodents (Hafner *et al.*, 2008; Hafner & Upham, 2011; Jezkova *et al.*, 2011) and in a species of velvet ant (Wilson & Pitts, 2010a). In contrast, in another velvet ant species, a single haplotype was found in populations across the Great Basin (Wilson & Pitts, 2012). While our findings of genetic isolation among Great Basin populations of *E. pallescens* are not unique, it is interesting to note the higher dispersal potential of *E. pallescens* compared with the other Great Basin taxa that have been analysed. This common pattern of isolation among dispersal-limited and more vagile taxa suggests the possibility that the Great Basin could be an

Table 1 Pairwise Φ_{ST} values for populations of *Euphilotes pallescens* from across the Great Basin that were included in the analysis. Significant values at $P < 0.05$ are marked with an asterisk.

	Sand Mtn	Sand Pass	Turtle Mtn	Silver State Dunes	Esmeralda	Hot Springs Mtn	Marietta	Panaca	Railroad Valley	Sunnyside
Sand Mtn	–									
Sand Pass	0.00986	–								
Turtle Mtn	0.00444	−0.00437	–							
Silver State Dunes	0.00444	0.00017	0	–						
Esmeralda	0.57876*	0.55654*	0.4631*	0.56669*	–					
Hot Springs Mtn	0	0.00986	0.00444	0.00444	0.57876*	–				
Marietta	0.00444	−0.00439	−0.05381	0	0.44524*	0.00444	–			
Panaca	0.3608*	0.33769*	0.21551*	0.34791*	0.18764*	0.3608*	0.21379*	–		
Railroad Valley	0.40868*	0.38978*	0.29402*	0.39837*	0.15048*	0.40868*	0.28839*	0.01414	–	
Sunnyside	−0.01948	−0.02259	−0.02897	−0.01983	0.57983*	−0.01948	−0.02741	0.33271*	0.39572*	–

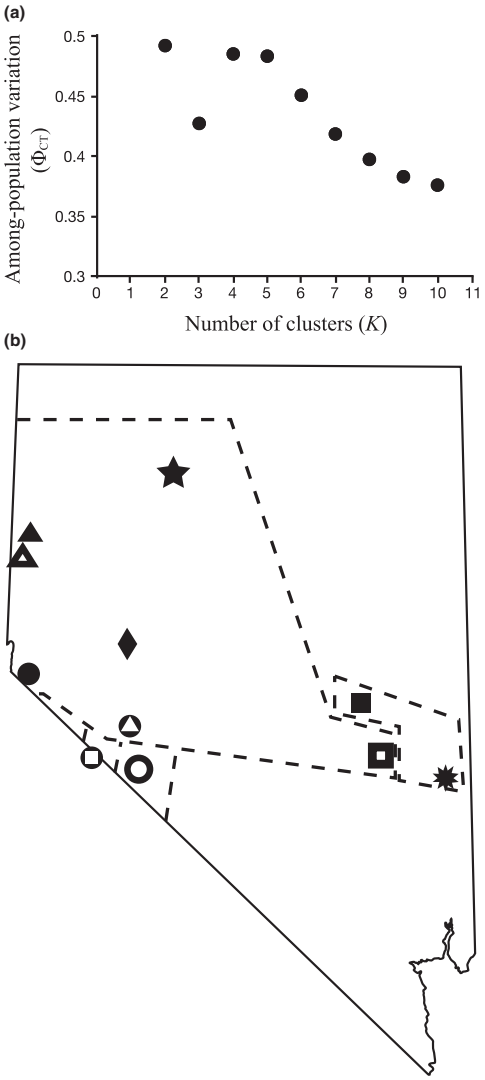

Figure 4 Spatial analysis of molecular variance (SAMOVA) of *Euphilotes pallescens* populations (n = 11) from western North America. (a) Graph of the proportion of among-population variation (Φ_{CT}) on the y-axis for each number of clusters (K) on the x-axis, and (b) map of *E. pallescens* populations showing groupings based on molecular and geographical data, with the dashed lines indicating SAMOVA results when K = 4.

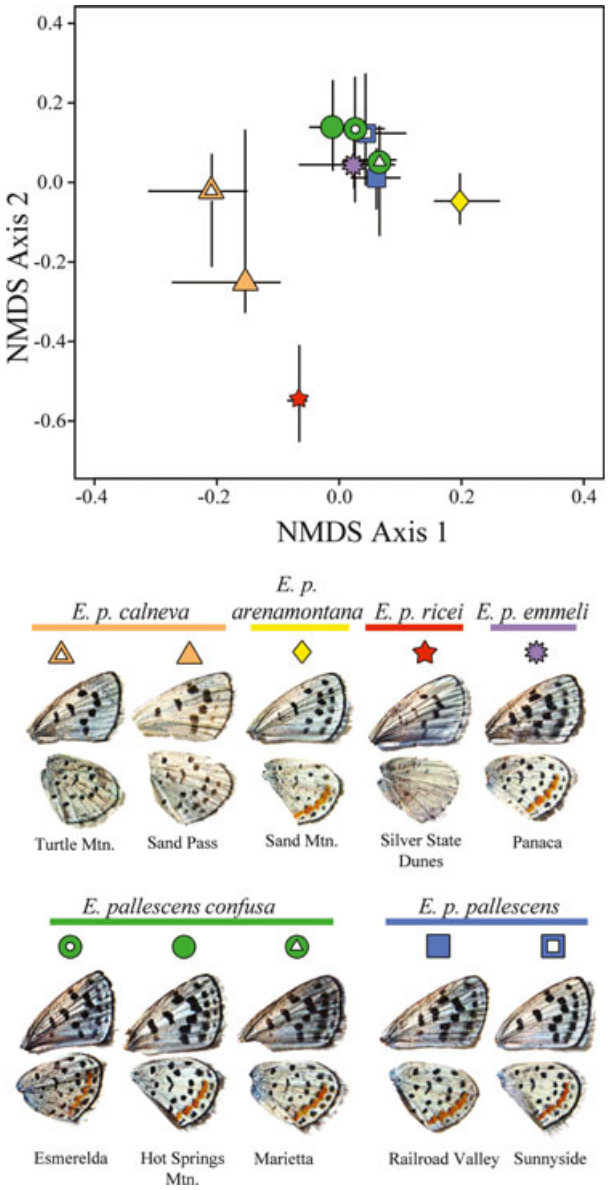

Figure 5 Non-metric multidimensional scaling (NMDS) on wing morphology of *Euphilotes pallescens* populations (n = 10) from western North America. Symbols indicate the median for each *E. pallescens* population. Bars indicate the interquartile distances. Symbols for each population correspond to those in Figs 1, 3 & 4. Representative wings are presented to show the variation present among different populations.

important and useful system for investigating central themes in conservation biology and biogeography.

Before discussing taxonomic designations, we turn to biogeographical and regional patterns. Among the small number of case studies investigating patterns of isolation and diversification in the cold deserts of North America, a generalized pattern is beginning to emerge. This pattern, which is informed by several organisms (primarily taxa that exhibit limited dispersal), is characterized by genetic divergence across the Great Basin, with genetic diversity partitioned into eastern and western regions (Epps *et al.*, 1998; Orange *et al.*, 1999; Hafner *et al.*, 2008; Wilson & Pitts, 2010a; Hafner & Upham, 2011). This pattern of an east–west genetic split has been attributed both to Pleistocene (Wilson & Pitts, 2010a) and to Neogene (Hafner *et al.*, 2008) events. For example, a recent analysis of Great Basin kangaroo mice found a clear set of temporally hierarchical patterns with a deep east–west split that was attributed to Neogene events, and also a more recent north–south pattern of genetic differentiation (Hafner & Upham, 2011).

Our results indicate isolation and differentiation among populations along both latitudinal (north–south) and longitudinal (east–west) geographical axes. In particular, results from SAMOVA suggest a weak east–west pattern among *E. pallescens* populations, with those populations in the

north-western Great Basin largely grouping together and those from the eastern Great Basin also forming a group (Fig. 4b). Although *E. pallescens* populations from southern Nevada appear to be differentiated with respect to mitochondrial variation (Fig. 4), they are relatively homogenous with respect to morphological variation (Fig. 5). In contrast, populations from northern Nevada are similar with respect to mitochondrial variation, but appear to be morphologically quite distinct. Regardless of the lack of a clear pattern of divergence in morphological and molecular data, the Φ_{ST} values among *E. pallescens* populations (Table 1) suggest some degree of restricted gene flow between populations.

Populations from north-western areas of the Great Basin, including Silver State Dunes, Sand Pass, Turtle Mountain, Sand Mountain and Hot Springs Mountain, are all situated in or around the Lahontan Basin, which was filled with several large pluvial lakes during the Pleistocene (Grapes *et al.*, 2008; Wilson & Pitts, 2010b). *Euphilotes pallescens* populations could have been restricted to shoreline areas that provided cold-desert refugia for some Great Basin taxa throughout the Pleistocene (Wilson & Pitts, 2012), or they may have experienced temporary niche evolution during glacial cycles, as has been suggested for other Great Basin taxa (Jezkova *et al.*, 2011), enabling populations to persist in isolated locations across the Great Basin. This isolation and associated genetic bottlenecks and drift in small populations might account for both the morphological distinctness and the lack of genetic diversity in those north-western populations. Other populations, including Marietta, Esmeralda, Railroad Valley, Sunnyside and Panaca, that are found in the southern Great Basin, which probably would have experienced dramatically reduced gene flow from the Lahontan Basin populations during the Pleistocene owing to barriers such as the mountain ranges in central Nevada. Given the greater amount of genetic diversity that we observed in southern populations, it is possible that the history of *E. pallescens* populations in the south was different from that experienced by populations in the north-west. The southern history might have involved a larger refugial population or populations, because of the predicted climatic stability of the region during glacial cycles (Jezkova *et al.*, 2011; Wilson *et al.*, 2012), although such hypotheses cannot be directly tested at this time.

Subspecies designations

The results of the morphological and population genetic analyses suggest some degree of isolation among *E. pallescens* subspecies (Table 1). With mitochondrial haplotypes shared among all *E. pallescens* populations, none of the nominal subspecific entities shows evidence of complete isolation. This pattern could be a result of contemporary gene flow or of shared ancestral polymorphism (Forister *et al.*, 2008). Distinguishing between these options is not possible from the data available, although the latter possibility (shared ancestral polymorphism rather than contemporary gene flow) seems more likely given the highly localized populations and the severe nature of the desert environment that separates these butterfly populations. As summarized above, populations and subspecies are differentiated to greater or lesser degrees with respect to both morphological and molecular data, although patterns of differentiation in the southern region are different from those in the north-western region.

Despite the amount of genetic isolation observed among populations of *E. pallescens* (Table 1), our data fail to entirely reject or support the current subspecies designations. For example, our morphological data suggest that *E. p. arenamontana*, *E. p. calneva* and *E. p. ricei* can be distinguished from each other and from other taxa, while *E. p. confusa*, *E. p. pallescens* and *E. p. emmeli* are morphologically indistinguishable (Fig. 5). Our molecular data, however, show the opposite pattern: *E. p. arenamontana*, *E. p. calneva*, *E. p. ricei* and two populations of *E. p. confusa* are genetically indistinct, while populations of *E. p. confusa*, *E. p. pallescens* and *E. p. emmeli* are characterized by greater differentiation among populations (Fig. 4). The historical complexities suggested by discrepancies between different genetic markers derived from mitochondrial and nuclear DNA (Figs 2 & 3) and the differences in morphological and molecular patterns indicate that a further dissection of relationships among populations and subspecies will have to wait for larger, more comprehensive data sets, such as multilocus data sets, or data sets that incorporate ecological, behavioural, morphological and molecular data.

Complex evolutionary history

Beyond population genetic data, the phylogenetic patterns revealed in nuclear and mitochondrial genes point to a complex evolutionary history for this species. Of particular note is the grouping of mitochondrial haplotypes into two distinct clades (labelled 1 and 2 in Fig. 2), with each clade containing sequences from individuals of both *E. pallescens* and other *Euphilotes* taxa. This contrasts with the pattern in the nuclear ITS1 region, which recovered a clade (albeit only moderately supported) of *E. pallescens* individuals. This kind of discordance perhaps poses more questions than it answers, and certainly highlights the fact that gene trees should not be mistaken for species trees (see Maddison, 1997).

Nevertheless, we find it opportune to raise at least one hypothesis based on the observed discordance between mitochondrial and nuclear data. Mitochondrial genetic variation has been known in many cases to cross species boundaries (Galtier *et al.*, 2009). The mechanism is presumably one of genetic neutrality or superiority (in the variant that spreads) that does not negatively interact with differing or divergent genetic backgrounds. While the mitochondrial variant spreads, nuclear genetic material can be selected against and does not spread. This dynamic has been documented in other lycaenid butterflies: a mitochondrial variant was shown to have spread into the endangered Karner blue butterfly (*Lycaeides melissa samuelis*) from a closely related, geographi-

cally proximate, and more abundant species (Gompert *et al.*, 2006). Infection with endosymbiotic bacteria in the genus *Wolbachia* was implicated in the spread of the foreign mitochondrial variant into the Karner blue (Nice *et al.*, 2009). *Wolbachia* infection can have a number of effects in insects, including male-killing and feminization of males, which can cause the infection, as well as associated mitochondrial DNA, to spread rapidly within and among populations (Charlat *et al.*, 2003). Preliminary studies have documented *Wolbachia* presence in *E. pallescens*, although infections have not yet been identified to strain (J.S.W., unpublished data).

Thus one possible explanation for the mitochondrial–nuclear discordance in *Euphilotes* is a history of hybridization in which mitochondria crossed species boundaries, creating an interdigitated mitochondrial genealogy (Fig. 2) while leaving a more straightforward history of diversification in nuclear genes (Fig. 3). This interpretation is posed as a hypothesis for further investigation, as we know little about the phylogenetic history of this apparently complex genus. Host-specific lycaenid butterflies have been characterized in other groups as having the capacity for rapid diversification as well as for hybridization, which has in fact been suggested previously for *E. battoides* and *E. enoptes* (Austin & Murphy, 1987). Historical complexity is perhaps, therefore, to be expected, and a simple contrast of one introgressed gene versus a central 'species history' might ultimately be overly simplistic.

In addition to the history of reticulation that is suggested by the mitochondrial phylogeny presented in Fig. 2, it is interesting to note that none of the mitochondrial haplotypes are shared among *E. pallescens* and any of the other *Euphilotes* taxa. This is significant because inference can be drawn that any hybridization is likely to have been relatively far in the past, as very recent hybridization among nominal species could have left a signature of identical in different taxa.

Despite the complex histories suggested by conflicting lines of genetic evidence, these data from *E. pallescens* have added to our still growing understanding of diversification in the cold-desert biota in western North America. In the case of *E. pallescens*, the range may have been more fragmented during glacial cycles, resulting in areas where populations experienced distinct histories. This suggests that there is still much to be learned from the Great Basin, a vast geographical area encompassing a relatively unappreciated diversity of habitats and biogeographical history.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Map showing collection localities for each of the *Euphilotes pallescens* populations and species sampled for this study.

Appendix S2 Information for each of the *Euphilotes* and outgroup specimens used in our phylogenetic and population genetic analyses.

BIOSKETCH

Joseph S. Wilson is an assistant professor at Utah State University Tooele in the Department of Biology. His research interests focus on the historical biogeography of the North American deserts and arid regions, concentrating on the history of diversification in arid-adapted insects. He is especially interested in the ecological factors that drive evolution in insects.

Author contributions: J.S.W. collected molecular data, analysed the data and led the writing; M.S. collected molecular and morphological data and assisted in data analysis; D.D.M. conceived the ideas and collected insect specimens; C.C.N. and J.A.F. collected specimens and assisted in data analysis and writing; and M.L.F. collected specimens, analysed data and assisted in writing.

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