Arnold Schwarzenegger
Governor

MEASUREMENT OF LARGE-SCALE GENE FLOW: A PATHWAY TOWARD UNDERSTANDING ADAPTATION AND THE GENETICS OF CLIMATIC TOLEARNCE

PIER FINAL PROJECT REPORT

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Preface

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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- Renewable Energy Technologies
- Transportation

Measurement of large-scale gene flow is the final report for the [project name] project contract (contract number XXX-XX-XXX, work authorization number [insert #] or grant number [insert #]) conducted by the University of Notre Dame. The information from this project contributes to PIER's [insert RD&D program area from bulleted list above] Program.

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Abstract

This report outlines the findings of research on two contrasting butterflies, *Erynnis propertius* and *Papilio zelicaon*. The species co-occur in California, primarily in oak-savanna ecosystems that are threatened by various forms of global change. Collections were made throughout California and combined with collections from elsewhere in the Pacific Northwest to generate a latitudinal gradient capturing the northern and southern extensions of the species' ranges. Results reveal genetic differentiation of northern (British Columbia) and southern (San Diego County) populations from the rest of the study populations. Northern populations are expected to increase under climate warming, but their divergence suggests the potential for local adaptation, a factor that could population reductions under climate change. The southern populations are likely to decline under climate change as conditions become unsuitable, and genetic diversity that occurs there may be at risk. The study also revealed differences in the extent of genetic differentiation between the study species such that the smaller and more specialized butterfly was more differentiated across its range. This establishes the possibility that local adaptation may differ among taxa. Further research is needed to reveal if genetic differences uncovered with neutral markers correspond to functional differences related to climatic factors.

Keywords

Butterfly, climate change, *Erynnis propertius*, gene flow, genetic marker, genetic structure, *Papilio zelicaon*, microsatellite, mtDNA, population divergence

Executive Summary

Introduction

Earth's plants and animals are vitally important to humans, including Californians, and climate change from fossil fuel combustion has been identified as a major threat to those natural systems on which humanity depends (*Thomas et al.*, 2004). We must predict how and which species will be affected by climate change to manage the goods and services that biodiversity provides. Changes in species' distributions will be one of the most dramatic biotic responses to climate change.

Purpose

This project uses molecular tools to reveal genetic differences among populations within a species' range. Such differences may underlie distinctive responses of populations under climate change. If populations respond in unique ways to climate change, then the simple expectation of poleward range shifts may not be realized. Further, this mechanism may be more pronounced in some species than in others, suggesting a need to compare the geographic structure of multiple species. In this study, a comparison of two contrasting butterfly species is performed.

Project objectives

This project had three objectives: 1) gather collections throughout southern and central California of two butterfly species, *Erynnis propertius* and *Papilio zelicaon*; 2) build genetic markers for these species for use in population genetic analysis; and 3) screen samples collected in California and elsewhere along the West Coast for these markers, revealing locations with distinctive genetic composition and differentiation.

Project outcomes

By the conclusion of the project, field and museum specimens were gathered, microsatellite markers were built for both study species, and these markers, together with the sequence of a mitochondrial gene, were used to assess the genetic composition of populations in California, Oregon, Washington, and British Columbia. Analysis revealed that populations at the northern (British Columbia) and southern (San Diego County) range edge of the species were genetically differentiated from the core (southern Oregon and northern and central California). Species differences in the extent of differentiation also were revealed particularly at the northern range edge in British Columbia, with greater genetic differences among populations appearing the smaller and more specialized, *E. propertius*.

Conclusions

Conservation attention should be paid to populations of both species in San Diego County as they harbor unique genetic composition in comparison to other sites in the study region. These populations occur on the lagging edge of climate change, and they are the most likely to experience deteriorating conditions as the climate warms. If these populations go extinct, the diversity that they harbor could be lost. Genetic differentiation also was observed in two species with strongly differing life history traits. Therefore, population differentiation within a

species' range, possibly leading to distinctive responses under climate change, could be more common than previously assumed.

Recommendations

Further research is needed to identify if the populations that are genetically differentiated in this study also are functionally differentiated with respect to climate. Experiments or functional genomic studies are necessary to take this next step. In the meantime, conservation biologists in California should consider the ways in which populations in distinct locations will respond differently to climate change as greenhouse gas emissions continue to rise.

Benefits to California

This project frames the impacts of climate change in terms of an organism that many Californians care deeply about. Butterflies capture the public's imagination, helping to build broad understanding about the consequences of human modification of the environment. As well, butterflies are an essential component of California's natural landscape, particularly those inhabiting the oak habitats that are so uniquely Californian. Rate payers and California residents can use the findings of this study to determine if the biotic risks faced by potential geographic range shifts and genetic loss is sufficient to warrant restrictions on greenhouse gas emissions. To reveal the precise extent of this risk, however, additional studies are needed.

Introduction

Greenhouse gas emissions are altering climatic conditions in California (*Field et al*., 1999), and evidence is steadily accumulating that human activities in California and elsewhere are altering Earth's atmosphere (*Houghton et al*., 2001). Concentrations of atmospheric CO_2 and other greenhouse gases have increased, in part, due to electric utilities fed by fossil fuel combustion. Due to the heat-trapping capacity of these gases, the mean climate in California is expected to warm 3-5 degrees C by 2100 with associated changes in precipitation and possible increases in the frequency of extreme weather events (*National Assessment Synthesis Team,* 2001).

Californians, given the state's large human population and its unique natural diversity, need information to properly manage natural resources in the face of climatic change. Voters and public utility users must decide if the ecological risks from greenhouse gas emissions (i.e., the degree to which California ecosystems will be affected negatively) warrant restrictions in the consumption of fossil fuels. Ecologists, meanwhile, are racing to fill this information gap.

This project provides an important step toward understanding the drivers of ecological responses to climate change. In particular, the project tests critical assumptions about genetic differences in populations that may cause differential responses of populations to changing climate. Specifically, the project uses two flagship butterflies that inhabit one of California's most prized ecosystems to study if species with differing traits have differing potential for adaptation to local conditions.

Some evidence suggests that many, but not all, species are shifting their geographic ranges (or geographic distribution) in response to regional warming (*Parmesan et al*., 1999). In general, scientists predict that species will shift toward the poles as they track a warming climate (*Parmesan et al.,* 1999) assuming that reductions in habitat availability or fragmentation do not restrict movement (*Hill et al*., 1999, 2001; *Thomas et al*., 2001; *Warren et al*., 2001). This project examines the inherent capacity of species to shift their ranges due to genetic differences and local adaptation within a species' range. Differences in the capacity for range shifts are largely unknown for species (*Gerber and Eckhart*, 2005), but McLaughlin et al. (2002a, 2002b) have shown that at least one species of butterfly in California is vulnerable to extinction under climate change in the center of its range. This vulnerability suggest that simple range shifts may not apply, at least not to all Californian butterflies.

The objective of this project was to advance the science of climate change biology using model organisms with importance to California. To begin exploring differential responses of species' distributions under climate change, it is essential to know if populations are genetically differentiated. Such work lays the foundation for future research investigating the functional differences among populations related to climate.

1.1. Local adaptation under climate change

If populations are adapted to local conditions across their range, climate change could result in widespread declines or extinction rather than a steady geographic range shift. This project tests the potential for local adaptation using genetic techniques that identifying neutral genetic differences among populations. To further determine if species with differing life history traits may differ in this population differentiation, analyses are pursued with two contrasting species.

Most ecologists assume that climate change, as caused by the emission of greenhouse gases, will cause species to shift their geographic distributions poleward (*Parmesan et al*., 1999; *Hill et al.*, 1999; *Davis et al*., 1998; *Kaustuv et al*., 2001; *Crozier*, 2003, 2004). This will happen because conditions at one edge of the range (northern range edge in the northern hemisphere) will improve relative to the tolerances of the individuals that occur there, while the other edge (southern range edge in the northern hemisphere) will decline as favored conditions deteriorate. This outcome should occur if populations of a species are relatively similar, with similar tolerances to the same climatic conditions. Local adaptation, however, can change this picture of geographic shifting under regional warming (*Davis and Shaw*, 2001). If populations throughout a range are adapted to the local conditions that occur in a habitat, populations may respond in distinct ways that could be independent of position within a species' range and create complex geographic patterns of population increase and decrease.

Therefore, species in California may move northward across the state, tracking change as it occurs, or populations over a wide area in the state could be perturbed locally by climate change and possibly decrease. A review of range dynamics and the ecology of species borders points to the "paucity" of studies comparing populations over wide areas (*Hoffmann and Blows*, 1994), and such studies are needed to address which response – systematic range shifts or local perturbation – is more likely under climate change. This project fills this research void.

1.2. The causes of population differentiation

Rates of inter-population gene flow are a primary determinant of differentiation among populations within a species' range (*Pease et al.*, 1989; *García-Ramos and Kirkpatrick*, 1997; *Kirkpatrick and Barton*, 1997; *Case and Taper*, 2000), and differentiation is necessary for local adaptation and localized responses to climate change. High rates of gene flow tie populations together, limiting the potential for adaptation to local conditions as genes from other locales swamp opportunities for genes of local benefit to be selected (*Avise*, 1994; *Bossart and Prowell*, 1998). Divergence can take place under high gene flow only if selection is very strong (e.g., an extremely unique or distinct climate). Low rates of gene flow, in contrast, allow for greater differentiation among populations (*García-Ramos and Kirkpatrick*, 1997; *Wright*, 1931). Less gene-swamping occurs and populations can adapt in response to smaller forces of natural selection. Genetic drift due to isolation or small population size at the edge of a range also may promote divergence (*Hoffmann and Blows*, 1994). Life history factors of a species, including resource use and dispersal capability, are likely to be important determinants of interpopulation movement and thus genetic differentiation (*Wright*, 1943; *Brouat et al*., 2003).

A number of phylogeographic studies have examined the genetic differentiation of populations across space using molecular genetic tools. Studies have been pursued with a tremendous diversity of organisms from insects (*Roderick*, 1996), trees (*Oubrog et al.*, 1999), fish (*DeWoody and Avise*, 2001), and even bacteria (*Horner-Devine et al.*, 2004). Few of these studies, however, quantify gene flow with the goal of detecting possible climatic adaptation and thus predicting geographic responses to climate change (*Gerber and Eckhart*, 2005).

2.0 Methods

The project quantifies genetic differences among populations of two species with differing life history traits. Specifically, it identifies if populations in some portions of the species' ranges are more or less genetically similar than other locations.

2.1. Study species

Butterflies are known to be responsive to climatic factors and climatic change (*Dennis*, 1993; *Ayres and Scriber* ,1994; *Hill et al*., 1999, 2001; *Parmesan et al*., 1999; *Hellmann*, 2001, 2002; *Roy et al*., 2001), and two butterfly species were examined because they capture a large difference in body size and resource specialization. *Erynnis propertius* is a specialist butterfly that feeds on trees in the genus *Quercus* (oaks). It is a fairly small and sedentary butterfly restricted to habitats where oaks co-occur with flowering plants (i.e., nectar resources) (*Scott*, 1986; *Opler et al.*, 1995; *Guppy and Sheppard*, 2001). *Papilio zelicaon* is a generalist that feeds on plants in the parsley family Apiaceae (umbellifers) (*Scriber et al*., 1995; *Wehling*, 1994). It is a large butterfly that occurs in native habitats where flowers and its native hosts occur as well as in the agricultural and suburban landscape where non-native host plants (e.g., sweet fennel) are abundant (*Wehling*, 1994; *Wehling and Thompson*, 1997).

The geographic distribution of these species is shown in Figure 1. *Erynnis propertius* is confined to coastal states; *Papilio zelicaon* has a broader distribution across the west. Both species also reach their southern range boundary in the southern reaches of the state (and in Baja California, Mexico). The butterflies co-occur in oak grasslands and woodlands, an ecosystem type widely threatened in California. Oak savannas have long played an important role in the state's history, culture, and ecology (*Pavlik et al*., 1991).

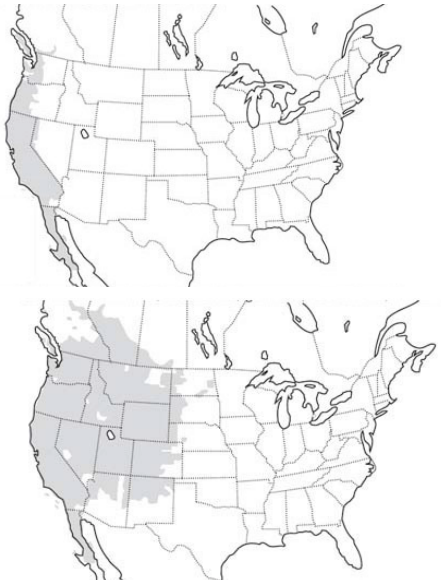

Figure 1: Geographic distribution (range) of *Erynnis propertius* (top) and *Papilio zelicaon* (bottom). Illustrations from Scott (1986).

2.2. Genetic analysis

To measure the history and degree of population isolation, the amount of shared genetic material occurring among California populations was assessed with molecular genetic techniques. This determines if habitats throughout the state are occupied by parts of a panmictic population or by subdivided demes (i.e., genetically distinct populations). Panmixia arises from high gene flow or a high degree of genetic sharing among populations. Differentiation arises from reduced gene flow or intense local selection that reduces the genetic similarity of populations in distinct locales. Molecular genetic tools also indicate the amount of genetic variation occurring within a given population. Such genetic variation will be the basis for any future adaptation to changing conditions as may occur under climate change (*Gunter et al*., 2000).

Specimens of both species were collected throughout the state of California. Collections under this grant were focused from San Francisco southward, but these data were combined with previous and recently augmented collections from the northern California, Oregon, Washington, and British Columbia in all analyses. Sampling in this northern region was supported by a grant to J. Hellmann by the Program for Ecosystem Research, US Department of Energy (DE-FG02-05ER64023).

Adults were collected by hand during the flight season. A maximum of 20 individuals were collected from each population, where "population" was defined as individuals inhabiting non-contiguous habitats separated by at least 20 km or contiguous habitats separated by 30 km. These values are arbitrary but are generally longer than the flight distance of most butterflies (*Scott*, 1986). All collection points were recorded with a handheld GPS recorder. To supplement the collection of adults, stands of host plant for *P. zelicaon* (e.g., sweet fennel) were searched for caterpillars in various locations. To augment field collections, the San Diego Natural History Museum and the Essig Museum of Entomology at UC Berkeley also were contacted for historical specimens. These specimens were analyzed in the same fashion as field-collected stock.

Specimens were examined for genetic diversity using microsatellite and mitochondrial (mtDNA) markers at the University of Notre Dame. These markers are widely used in phylogenetic and gene flow studies of Lepidoptera (e.g., *Caterino and Sperling*, 1999; *Caterino et al*., 2000; *Nice and Shapiro*, 2001; *Sperling*, 2003).

2.2.1. Microsatellite markers

Microsatellite markers are one of the most useful tools for population studies because they are co-dominant, highly polymorphic, and allow examination of allelic variation at discrete loci (*Jarne and Lagoda*, 1996; *Parker et al*., 1998). Microsatellites have been developed in a number of Lepidoptera, e.g., *Parnassius*, *Melitaea*, *Heliconius*, *Lycaeides*, *Speyeria* and *Polyommatus* (*Meglécz et al*., 1997; *Meglécz and Solignac*, 1998; *Saccheri et al*., 1998; *Keyghobadi et al*., 1999, 2003; *Harper et al*., 2000, 2003; *Anthony et al*., 2001; *Flanagan et al*., 2002; *Williams et al*., 2002). Previous studies with microsatellites indicate that 15 markers is a reasonable target for assessing population structure (*Mitton*, 1994; *Boecklen and Howard*, 1997; *Sannucks*, 2000; *Kalinowski*, 2005).

Toward the development of microsatellite markers in our study species, a pooled DNA sample from twelve whole-bodies of *E. propertius* was previously sent to Genetic Identification Services (GIS) for development of a library enriched for microsatellites. From the resulting library, GIS sequenced 60 clones and all but one contained a GA or CA repeat with an average length of 19-20 repeats. Based on this library, GIS designed 46 primers (*Zakharov et al*., 2007). For *P. zelicaon*, a sample of six individuals was sent to GIS, and they sequenced 112 clones , obtained microsatellites containing CA, GA, CAG, and ACC motifs, and designed 68 primers (*Zakharov and Hellmann*, 2007). These microsatellite libraries and suggested primers were purchased using funds from the University of Notre Dame and the Center for Insect Genomics. The suggested primers then was screened at Notre Dame for markers that reliably amplified and were sufficiently polymorphic. Fifteen markers for *E. propertius* and 17 markers for *P. zelicaon* were identified (*Zakharov and Hellmann*, 2007; *Zakharov et al.* 2007).

Microsatellite fragments were amplified and screened for fragment size using a Beckman CEQ automated sequencer (see method details in Zakharov (2007) and Hellmann and Zakharov et al.

(2007)). Samples were scored for the number of microsatellite repeats per individual for each marker in each species.

2.2.2. Mitochondrial DNA markers

The mitochondrial gene, *ND5*, also was used to explore the genetic composition of populations (*Avise et al*., 1987; *Martin and McKay*, 2004). Known primers from the literature (*Yagi et al*., 1999) were used to sequence 851bp of the gene with the use of an ABI-3730 automated sequencer at Notre Dame. Sequences defined "haplotypes" or forms of the *ND5* gene represented in each individual of each species. See detailed methods in Zakharov and Hellmann (in review).

2.3. Analysis

The data for both marker types were analyzed using statistical tools from the field of population genetics. First, a variety of summary statistics including nucleotide diversity and haplotype diversity (mtDNA) and allelic richness, heterozygosity, inbreeding coefficient, and linkage disequilibrium (microsatellites) were calculated for each marker type and species. Second, a neighbor-joining phenogram was built to capture the relatedness of populations based on microsatellite alleles (*Nei*, 1978; *Felsenstein*, 1993), and the software package STRUCTURE (*Pritchard et al*., 2001) was used to determine if geographical regions could be differentiated based on microsatellite differences. Third, the distribution of mtDNA haplotypes was plotted on a map to examine geographic pattern of ND5 sequences. Finally, F_{ST} (*Wright*, 1951) was calculated for both marker types using AMOVA to distinguish higher versus lower rates of gene exchange among sampling locations *(Dieringer and Schlotterer*, 2003; E*xcoffier et al*., 2005). Details of these methods are given in Zakharov and Hellmann (in review).

3.0 Results

During the spring of 2006, Zakharov visited sites throughout coastal California from the Mexican border to the San Francisco Bay. Zakharov and Hellmann selected dates for the collecting trip based on the known flight periods of *E. propertius* and *P. zelicaon* in southern California, adjusted for weather conditions occurring in the later part of February and early part of March, 2006. Early-spring weather reports indicated a warm and dry spring; therefore, March 12 through April 23 was selected for Zakharov's travel. The weather in later March, however, turned cool and wet forcing an extension of the trip to May 2, 2006.

A total number of 41 *E. propertius* and 11 *P. zelicaon* were collected during the field trip. This is much smaller than the 20 individuals per site that were targeted. In general, Zakharov saw fewer individuals than anticipated. This population depression seems to have affected both *E. propertius* and *P. zelicaon*, as well as other species, and the anomaly was widely noted by entomologists in California. Arthur Shapiro (UC Davis), for example, reported that butterfly numbers along the I-80 corridor were lower than he has observed anytime in his 30-year record of butterfly sampling (*San Francisco Chronicle*, 2006). To supplement field sampling, therefore, we obtained 22 *P. zelicaon* samples from the Museum of Natural History in San Diego and 6 *E. propertius* from the UC Berkeley Mueseum. Most of these samples were collected within the last ten years.

For the purposes of analysis, all data collected in southern and central California (above) were combined with samples gathered from northern California, Oregon, Washington, and British

Columbia. (These samples were gather using funds from other sources; see above.) In combining the data together, a picture of the entire latitudinal gradient of both species' ranges emerges (Fig. 1). Results below are given for the entire latitudinal gradient, but recommendations and conclusions are drawn for California only.

3.1. Microsatellite markers

Detailed results are given in Zakharov and Hellmann (in review) for both microsatellite and mtDNA markers. The following synopsis highlights their findings.

Fourteen of the 15 markers designed for *E. propertius* were used in population analysis; all 17 were used for *P. zelicaon*. The amount of polymorphism revealed by microsatellite markers was moderate in *E. propertius* and higher in *P. zelicaon*. The average number of alleles per locus, for example, was 3.5 for *E. propertius* and 4.5 for *P. zelicaon*. Neither species showed a geographic pattern in the allelic richness per population summed over all markers except that richness was lowest in *E. propertius* for sites at the northern-most portion of the species' range in British Columbia. Observed heterozygosity differed from expected heterozygosity in both species, and 14/14 markers and 15/17 markers deviated from Hardy-Weinberg equilibrium in *E. propertius* and *P. zelicaon* respectively.

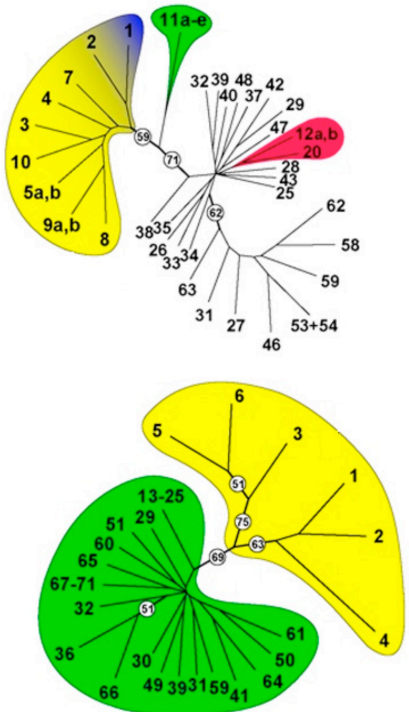

Figure 2: Neighbor-joining phenograms of *Erynnis propertius* (top) and *Papilio zelicaon* (bottom). Numbers correspond to population sampling locations in Fig. 4. Colors correspond to the colors in STURCTURE analysis shown in Fig. 3. In (top), populations are divided into distinct clades for Vancouver Island (yellow and blue), the northern portion of the species' range (red = mainland British Columbia and Green = northern Washington), and all other, undifferentiated sites (uncolored). In (bottom), two clades emerge, one (yellow) corresponding to Vancouver Island and the other (green)

Figure 2 shows a neighbor-joining phenogram linking populations based on Nei's standard genetic distance (*Nei*, 1978). Figure 3 indicates the result of STRUCTURE analysis dividing populations into groupings that are distinguishable from one another. The colors in both Figures 2 and 3 correspond such that population groups in Figure 2 are identified by the colored results of STRUCTURE analysis in Figure 3. Figures 2 and 3 indicate two major lineages in both species corresponding to northern locations (British Columbia) and the rest of the range. In *E. propertius*, greater differentiation at the northern range margin was found such that populations on Vancouver Island and on mainland British Columbia and Washington could be differentiated from the rest of the study region.

Estimates of gene flow among population groupings (F_{ST}) were in the range of 0.06 for *E. propertius* and 0.05 for *P. zelicaon*. Both values are quite low, indicating high gene flow and high genetic similarity among populations. These values were robust to varying the number of population groupings. Calculations were first made using two groups of populations (all of the mainland versus Vancouver Island); then the number of groups was steadily increased to more than ten. In each case, the populations per group were clustered based on geographic proximity of sampling.

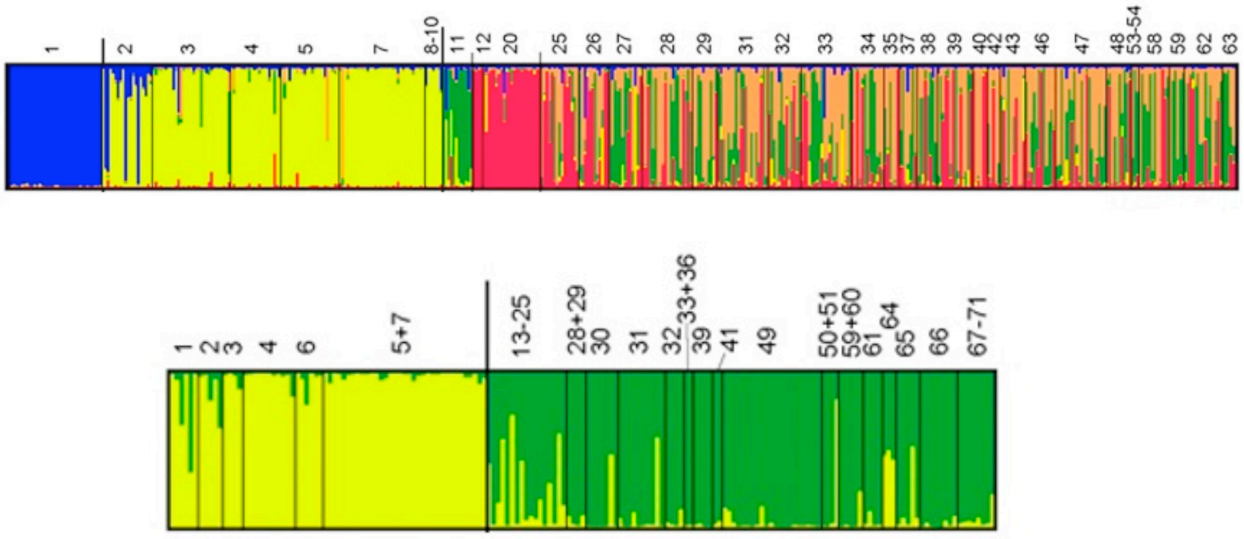

Figure 4: Results of STRUCTURE analysis for microsatellites of *E. propertius* (top) and *P. zelicaon* (bottom). Numbers at the top of each subfigure refer to sampling locations given in Fig. 4. Each verticlal bar in the plots refers to a sampled individual where the color of each bar refers to the probability that it can be assigned to a genetically differentiated group. Colors also correspond to the phenogram in Fig. 2. The microsatellites of *E. propertius* (top) show differentiation into five groupings corresponding to Hornby Island, Vancouver Island, mainland British Columbia, northern Washington, and the remainder of the species range (referring to colors listed left to right from blue through yellow, green, red and an undifferentiated mixture of colors). The microsatellites of *P.*

3.2. Mitochondrial DNA markers

Analysis of sequence variation in the mitochondrial gene, *ND5*, revealed 28 haplotypes in *E. propertius* and 18 haplotypes in *P. zelicaon*. (Again, for additional details, see Zakharov and Hellmann (in review).) One of the haplotypes in *E. propertius* was highly divergent with 43 nucleotide substitutions relative to the other, closely-related haplotypes. Dissection of genetialia and microsatellite data suggest that these individuals are correctly assigned to *E. propertius*. Instead, they seem to capture a historical introgression event in the mitochondrial genome only between *E. propertius* and *E. horatius* (Zakharov and Hellmann, in prep). This haplotype reaches its highest frequency in the northern portion of the species' range but occurs throughout the latitudinal gradient.

Figure 4 shows a map of haplotype diversity and frequency per sample population. Unlike the microsatellite data, mtDNA indicates differentiation at both the northern (British Columbia)

and southern (San Diego County) range edges. For *E. propertius*, the most common haplotype was found in all locations throughout the range except for sites in San Diego County. For *P. zelicaon*, sampling locales in San Deigo were characterized by the presence of a haplotype not found elsewhere in the entire study region.

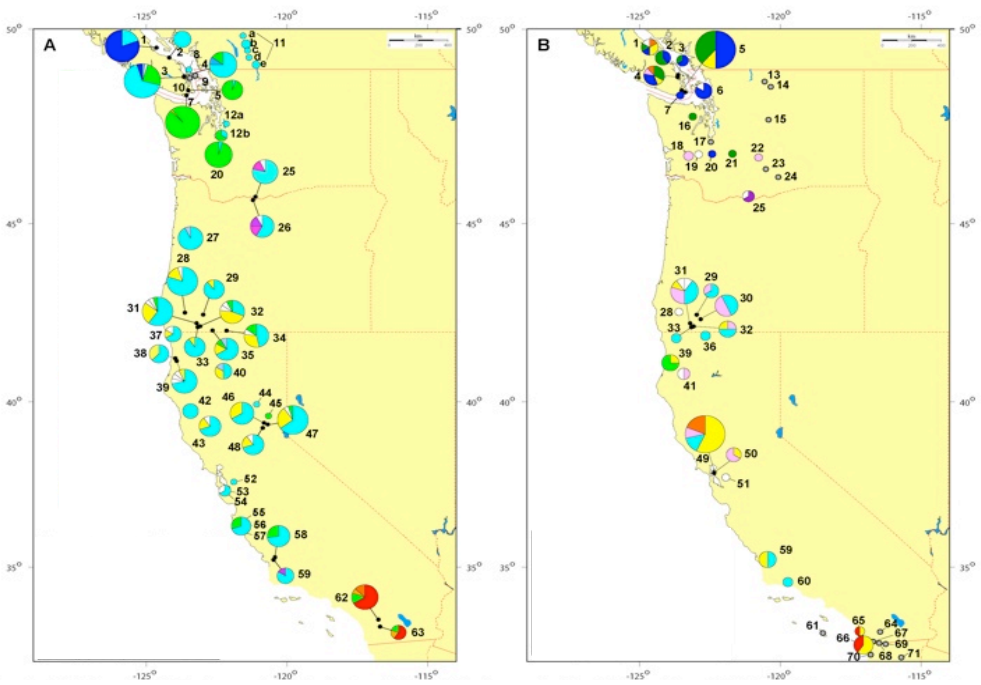

Figure 4: Distribution of mtDNA haplotypes across the study region for *Erynnis propertius* (A) and *Papilio zelicaon* (B). Each color in the pie charts indicates a unique haplotype; white slices indicate a haplotype sampled in only one individual. The size of the pie indicates the number of sampled individuals. Numbers refer to site localities also shown in Figs. 2 and 3. Populations at the northern edge of the species' range, in proximity of the Columbia River, and

Estimates of gene flow were calculated for the same population groupings as for microsatellite markers. Values were markedly higher than the values calculated for microsatellites (0.7 for *E. propertius* and 0.3 for *P. zelicaon*) and indicate a low rate of gene flow (high degree of population differentiation) in both species. The amount of gene flow also was higher for *P. zelicaon* than for *E. propertius*, a difference that was much smaller for the microsatellite estimate above. When the highly divergent haplotype for *E. propertius* was included in the analyses, the value of F_{ST} slightly increased to 0.75.

4.0 Conclusions and Recommendations

This project is a first step in the exploration of genetic differences and their implications under climate change. The results suggest three important features about two contrasting butterflies that occur in California with implications for conservation and possible ecological responses under climate change.

First, there are several lines of evidence that point to significant geographic differentiation at the northern limit of the species' ranges. This may be due, in part, to an island effect associated with Vancouver Island, but in *E. propertius*, populations in the northern portion of the mainland (British Columbia and Washington) also are differentiated from the rest of the range. This supports the hypothesis of population divergence within a species' range and allows the

possibility that populations could have distinctly different responses to climate change based on underlying genetic differences. In particular, populations in British Columbia could have different climatic tolerances (i.e., local adaptation) than populations in California.

Second, mtDNA data suggest that the southern portion of both species' ranges (San Diego County) are distinctive from the species' range core (central and northern California and Oregon). These populations harbor some unique genetic diversity and occur on the lagging edge of climate change -- the edge of the range most likely to become unsuitable under climate warming. Recent research suggests that southern boundaries deserve greater attention from climate change researchers as they may maintain a large amount of genetic diversity and can be genetically distinct from central locations (*Hampe and Petit*, 2005). Habitat conservation and genetic preservation in *E. propertius* and *P. zelicaon* populations in San Diego County, therefore, should be a priority in an era of climate change. As southern divergence is found in both of our study species despite strong differences in their life history, this phenomenon may apply widely to butterfly species in California.

Third, there are differences between the study species in the degree of population differentiation, including greater geographic differentiation and more restricted gene flow in *E. propertius* relative to *P. zelicaon*. This difference corresponds to the expectation that a smaller-bodied species with greater resource specialization (conferring reduced dispersal capability) should be more differentiated than a large-bodied, generalist species. If population differences lead to differential responses and a complex mosaic of population-specific responses under climate change, this complexity may be more pronounced in small, specialized species. Conservation efforts under climate change could prioritize such species.

Subsequent research in this study system should examine functional differences among the populations identified as distinctive with microsatellite and mtDNA markers. For example, individuals from San Diego County should be compared in experimental studies to individuals from central and northern California and from Vancouver Island, British Columbia to determine if differences in mtDNA reflect differences in climatic tolerance. Where such functional differences are found, techniques such as microarray analysis could be used to explore the genetic expression of differing genotypes under differing climatic conditions. This research could reveal genes controlling differential responses to climatic conditions.

5.0 References

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6.0 Glossary

CO_2 –

F_{ST} – fixation index, a measure of the genetic similarity of subpopulations compared to the total population

AMOVA – molecular analysis of variance, a statistical analysis of population genetic data

mtDNA – sequence data from the mitochondrial gene, ND5

ND5 – dehydroenase subunit 5, an enzyme coded in the mitochondrial genome

STRUCTURE – software program for the anslysis of genotype data to investigate population structure (http://pritch.bsd.uchicago.edu/structure.html)