

Genetic Study of the Sacramento Mountain Checkerspot Butterfly
(*Euphydryas anicia cloudcrofti*):
A Species of Greatest Conservation Need

2009 Project Report

Prepared for the Share with Wildlife Program
New Mexico Department of Game and Fish

Prepared by
Rachael Ryan and Brook Milligan
Department of Biology
New Mexico State University
Las Cruces, New Mexico 88003

15 June 2009

1 Overview

In July 2008 our proposal entitled "Genetic Study of the Sacramento Mountain Checkerspot Butterfly (*Euphydryas anicia cloudcrofti*): A Species of Greatest Conservation Need" was submitted to the Share with Wildlife Program of the New Mexico Department of Game and Fish. The goal of the proposal was to undertake an analysis of genetic relationships among the 10 populations of Sacramento Mountain Checkerspot Butterfly (*Euphydryas anicia cloudcrofti*) in order to ascertain the degree to which individual populations are interconnected via gene flow. Greater numbers of individuals moving from one population to another increase the genetic homogeneity of the entire species, whereas fewer numbers moving increase the genetic heterogeneity. Because those genetic patterns may be quantified using molecular techniques, it is possible to infer the degree of interconnectedness of the populations.

From a management perspective, knowledge of the population structure of *E. anicia cloudcrofti* is important for at least two distinct reasons. First, it provides valuable information on the species itself that is relevant to its current evaluation by the U.S. Fish and Wildlife Service as a potential endangered species. For example, the relative risk of extinction may depend on the population structure. At one extreme is a situation in which these butterfly populations exist as highly isolated subpopulations with very little interchange of individuals. At another extreme is a situation in which they are connected populations with greater interchange and greater opportunity for recolonization of locally extinct populations. Thus, this study will inform the policy decisions being undertaken currently to establish the formal status of *E. anicia cloudcrofti*.

Second and more generally, species such as this checkerspot that at least have the potential to move from one site of suitable habitat to another may help inform forest managers about how species respond to landscape structure and about the nature of corridors between habitats. One species may indeed be informative about the use of forest and non-forest habitats by other species. Thus, this study will help identify connectivity among canyon habitats that could be applicable to other species.

In line with these general objectives, the main scientific goals of this project were as follows.

1. Conduct a genetic analysis of the Sacramento Mountain checkerspot butterfly metapopulation in New Mexico.
2. Amplify microsatellite DNA at a minimum of 3 loci from 50 Sacramento Mountain checkerspot butterflies (5 individuals each from the 10 canyon subpopulations) using polymerase chain reaction (PCR).
3. Genotype the DNA from the PCR amplification in 2 above.
4. Analyze DNA for comparisons with Hardy-Weinberg equilibrium, indicators of genetic diversity, and estimates of genetic distance.
5. Assess the metapopulation structure of the Sacramento Mountain checkerspot butterfly to determine if cryptic population structure exists.
6. Use the results of 1 through 5 above to provide information regarding identification of habitat connectivity among patches and potential population linkages in the Sacramento Mountains, and effects of fragmentation from habitat loss or degradation, wildfires, recreation, impacts to host plants, or other factors.

Population	Individuals
Bailey Canyon	4
Cox Canyon	5
Deerhead Canyon	5
Horse Pasture	5
Pines Campground	5
Pumphouse Canyon	5
Silver Springs	4
Sleepy Grass	5
Spudpatch Canyon	4
Yard Plot	5
Total	47

Table 1: Distribution of individuals prepared for sequencing at the CINX-35 locus.

2 Current status

Despite proposing this study almost 12 months ago, we only received funding to undertake it in May 2009. Consequently, this report is predicated upon the fact that funds have been available for barely one month.

Aim 1 Conduct a genetic analysis of the Sacramento Mountain checkerspot butterfly metapopulation in New Mexico.

This aim essentially subsumes all other aims and represents the sum total activities associated with them. As a result, the discussion of each aim below describes a specific component of this one, and those accomplishments will not be repeated here.

Aim 2 Amplify microsatellite DNA at a minimum of 3 loci from 50 Sacramento Mountain checkerspot butterflies (5 individuals each from the 10 canyon subpopulations) using polymerase chain reaction (PCR).

We have identified 12 nuclear microsatellite gene regions that should reveal genetic variation suitable for quantifying the genetic population structure of *E. anicia cloudcrofti*. Of these 12 loci, we have successfully verified that at least four can be amplified and genotypes assessed. The remainder have yet to be completely tested, so additional loci may be available as the study progresses. Due to the major delay in funding this project, at this time only one (CINX-35) of these gene regions has been amplified in individuals from all ten canyon subpopulations. Our gel electrophoresis for quality control reveals successful amplification of the intended gene region. Nevertheless, we currently have a set of 47 individuals fully prepared for sequencing (Table 1), which will commence shortly. We expect to have data on these 47 individuals within weeks, and will then be able to identify the exact genotype of each individual at this locus.

Aim 3 Genotype the DNA from the PCR amplification in 2 above.

As mentioned above, sequencing reactions required for genotyping the first set of individuals are currently underway. Consequently, we anticipate having these individual genotyped before

the first project year is complete.

Aim 4 Analyze DNA for comparisons with Hardy-Weinberg equilibrium, indicators of genetic diversity, and estimates of genetic distance.

This aim focuses on one step of the analysis of our genotypic data. Consequently, no progress can be made until at least some genotypic data is available. As soon as our first sequencing results are available, however, we will be able to perform a preliminary analysis as intended. Thereafter, we will update our analysis as each new set of genotypic data is obtained.

Aim 5 Assess the metapopulation structure of the Sacramento Mountain checkerspot butterfly to determine if cryptic population structure exists.

This aim focuses on another step of the analysis of our genotypic data. Again, no progress can be made until at least some genotypic data is available. As soon as our first sequencing results are available, however, we will be able to perform a preliminary analysis as intended. Thereafter, we will update our analysis as each new set of genotypic data is obtained.

Aim 6 Use the results of 1 through 5 above to provide information regarding identification of habitat connectivity among patches and potential population linkages in the Sacramento Mountains, and effects of fragmentation from habitat loss or degradation, wildfires, recreation, impacts to host plants, or other factors.

This aim focuses on the most synthetic component of the analysis of our genotypic data. As a result, it is expected that little progress will be made on this aim until a greater amount of genotypic data is available. As we add data, however, we intend to undertake preliminary versions of this analysis so that we can track the growth of knowledge and provide preliminary indications of the results.

3 Future plans

It is unfortunate that the process of funding this project was so greatly delayed during the past year. As a result, we have achieved less with respect to data collection than we would have liked. However, we have laid a foundation for much greater progress during the upcoming year. For example, our amplifications appear robust. This is a critical step, as everything else depends on these. We will have sequence-based genotype data in the very near future, thereby completing the validation of our initial analysis. From this foundation and with more timely funding, we will be able to make much more rapid progress during the upcoming year.

4 Related work

In addition to preparing for data collection on this project, we have continued work on related studies Ryan (2007) that will help inform our broader understanding of *E. anicia cloudcrofti*. These projects are relevant to this particular study, because they either provide a broader context for the results obtained here or they could dramatically increase the quantity of data, and hence quality of inferences, we can obtain.

During this year we have brought our initial study of the phylogenetic relationship between *E. anicia cloudcrofti* and other Euphydryas species almost to conclusion. Based upon sequences

from the cytochrome c oxidase I (mitochondrial) and 16S rRNA (nuclear) genes from 100 *E. anicia cloudcrofti* individuals, it is clear that this taxon may be as distinct from its 'parent' species *E. anicia* as several other *Euphydryas* species. This indicates that its status as an endemic subspecies may be incorrect. Instead, it may in fact be an endemic species. We are currently embarking on a much more extensive survey to better ascertain whether or not this hypothesis is supported by more data.

Another study may enable us to expand the dataset proposed for this study. If successful, the quality of our inferences regarding population structure will be greatly increased. As a result of a funded NSF grant, we have recently placed into service a Roche Genome Sequencer FLX instrument. This has the capacity to generate sequences simultaneously from millions of molecules in a single run. We are currently adapting it to the task of surveying microsatellite loci. If successful, we will be able to genotype all the individuals and all the loci proposed for this study in a single run. Clearly, several runs will greatly expand the scope and lead to a much clearer understanding of population structure.

5 Conclusion

Although delayed in initiation due to substantial administrative delays in receiving funding, this project has made substantial progress during its first month of laboratory activity. Samples have been amplified, verified, and prepared for sequencing. In short order we will have initial genotype data that we can use to for preliminary analysis. Although those results will not yet yield particularly significant inferences, they will be useful for verifying that the analyses can be completed once more data is available. Thus, quite soon all the elements of our scientific aims will have been accomplished at a very preliminary level. Subsequently, continual addition of data, which will occur steadily through the next year, will expand the quality of our inferences.

In addition, however, we have made major progress on related projects with direct bearing on this one. Thus, the delay associated with initiating this project has been used fruitfully in ways that should ultimately better inform the conclusions we are able to make here.

References

Rachael Ryan. Update summary for the Sacramento Mountain Checkerspot (*Euphydryas anicia cloudcrofti*). Technical report, U.S. Forest Service, Sacramento Ranger District, Lincoln National Forest, 2007.