

Final Report on Research Funded by Research Enhancement Program Grant to Chris Nice, Department of Biology (January to August, 2004)

I. Abstract

Project Title: Population Genetics of an Adaptive Radiation

Principle Investigator: Chris Nice

Department: Biology

REP funds were used in an investigation of an adaptive radiation in the North American butterfly genus *Lycaeides*. Specifically, patterns of population genetic variation were surveyed by amplification and sequencing of single copy nuclear genes, mitochondrial genes, and by the development of a new genetic marker system: Amplified Fragment Length Polymorphism markers (AFLPs). This work has taken the investigation of the radiation of these butterflies in new directions. We have uncovered unexpected patterns of variation and developed a novel approach to testing hypotheses on the contributions of multiple reproductive isolation mechanisms. REP money was used to support one undergraduate honors thesis project, another undergraduate project, and four graduate student projects in addition to the PI's own research. These funds also supported one undergraduate research assistant during the summer, 2004. These projects formed the foundation for a NSF Graduate Research Fellowship awarded to Zachariah Gompert and an NSF Proposal to the Population Biology Panel.

What follows is a brief summary of the results, extensions and accomplishments of this project:

II. List of Publications

1. Nice, C. C. and J. A. Fordyce. The adaptive significance of microhabitat shifts and a color polyphenism in pipevine swallowtail (*Battus philenor*) larvae. In review.

III. Presentations on the research funded by REP 2004 to Chris Nice

1. Gompert, Z. and C. C. Nice. 2004. Paper. Discord between Molecules and Morphological Characters in *Lycaeides*: Parallel Evolution or Hybridization?. Southwestern Association of Biologists Annual Meeting. October, 2004. Southwest Research Station, Portal, AZ.
2. Nice, C. C. and J. A. Fordyce. 2004. Paper. The adaptive significance of microhabitat shifts and a color polyphenism in pipevine swallowtail (*Battus philenor*) larvae. Southwestern Association of Biologists Annual Meeting. October, 2004. Southwest Research Station, Portal, AZ.
3. Gompert, Z., Forister, M. Fordyce, J. and C. Nice. 2005. Paper. DNA barcoding: Boon or boondoggle? Society for the Study of Evolution Annual

Meeting. June, 2005. Fairbanks, Alaska.

4. Nice, C. C. and J. A. Fordyce. 2005. Paper. How caterpillars avoid overheating: behavioral and phenotypic plasticity of pipevine swallowtail larvae. Society for the Study of Evolution Annual Meeting. June, 2005. Fairbanks, Alaska.

IV. Grants submitted based on work funded by REP 2004

1. NSF Graduate Research Fellowship to Zachariah Gompert 2005 (Funded).
2. NSF Proposal No. 0444699 (Submitted July 2004 -Not Funded) Title: The evolution of genetic, morphological and ecological discontinuities during speciation. \$368,358.
3. Texas Parks and Wildlife, Section 6 Grant (Submitted-Pending). Title: Genetic Isolation of Comal Springs Riffle Beetle Populations. \$80,165

VI. Student Projects funded by REP 2004 to Chris Nice. The following is a short summary of student projects. Each of these will result in one or more publications with the students as lead authors.

- A. Zachariah Gompert, Undergraduate Honor Thesis Project, Biology. Project Title: The utility of Amplified Fragment Length Polymorphism (AFLP) markers for investigation of speciation in *Lycaenid* butterflies.
- B. Eric VanGasbeek, Undergraduate Project, Biology. Project Title: Hybridization and estimation of post-zygotic reproductive isolation between *Lycaeides idas* and *L. melissa*.
- C. Chris Farmer, Graduate Student, Biology. Project Title: A preliminary investigation of putative hybridization and ecological divergence in the Hackberry butterflies of the genus *Asterocampa* in central Texas.
- D. Maurine Spencer, Graduate Student, Biology. Project Title: Population genetic structure in the Acmon complex of *Plebejus* (Lepidoptera: Lycaenidae) in western North America.
- E. Zachariah Gompert, Graduate Student, Biology. Project Title: Population Divergence in North American *Lycaeides*: An Examination of Possible Character Differences Driving Speciation.
- F. Lauren Lucas. Graduate Student, Biology. Project Title: Nabokov's Blues: A Reexamination of the Morphological Systematics of the Genus *Lycaeides* in North America.

VII. Current progress.

Work with the gene *wingless* has provided sufficient levels of sequence variation to test hypotheses and to measure gene flow. In fact, the patterns of variation in *wingless* found within the focal populations were so intriguing that the scope of the project was expanded to examine populations from across North America. To date, amplification and sequencing of *wingless* has been accomplished for 120 individuals from 30 populations.

Amplified Fragment Length Polymorphism markers (AFLPs) are dominant, nuclear markers that are located throughout the genome. We have successfully optimized the protocols for these markers for use in the *Lycaeides* study system. These genetic markers have proven their utility in cases of recent or ongoing divergence. For the *Lycaeides* system, AFLP data has been generated for 12 populations (approximately 25 individuals/population) for three primer pairs providing more than 100 polymorphic nuclear markers. Data from these markers have allowed us to examine the fine-scale phylogeographic structure and to test specific hypotheses about evolution in these butterflies.

Two significant findings are:

1. Populations of *Lycaeides melissa* from above treeline in the Sierra Nevada of California appear to be a unique entity within the species complex. This falsifies the hypothesis that these populations are more closely related to *L. idas* and that *L. melissa* is polyphyletic. These data also apparently falsify the hypothesis that these populations are of hybrid origin between the two nominal species.
2. Patterns of relationship from all three kinds of genetic markers (mitochondrial sequence, nuclear sequence and AFLPs) have allowed us to accurately estimate evolutionary relationships among populations. These estimates can now be used to test hypotheses on the efficacy of reproductive isolation mechanisms. This represents one of the first attempts to examine reproductive isolation from a multidimensional perspective.
3. Populations of the federally endangered Karner Blue butterfly, *L. m. samuelis*, are monophyletic with respect to *wingless* sequence variation and AFLP data. This stands in sharp contrast to patterns observed for mitochondrial genes where populations on either side of a phylogeographic barrier at Lake Michigan contain significantly different haplotypes. The implication from the contrasting patterns in cytoplasmic and nuclear markers is that some gene flow is occurring or has occurred recently in this region. The Karner Blue butterfly populations appear to represent a significant third distinct lineage within *Lycaeides* that has been unrecognized until now. Introgression of mitochondrial DNA into Karner populations from the west has obscured the phylogenetic relationships. The possibility of introgressive hybridization poses some serious questions regarding conservation management of this endangered taxon. This kind of geographic variation represents a challenge to the recently proposed “DNA Barcoding” methods of assessing biodiversity.

VIII. Extensions of the original plan of work

A. Comparison of patterns in *Lycaeides* to other taxa.

1. **Investigation of divergence in the genus *Plebejus*.** The discovery of useful levels of genetic variation in *wingless* sequences forms the basis for comparative studies of phylogenetic and phylogeographic patterns in other butterfly taxa that share similar life histories and may share a similar pattern of divergence and speciation. A master's student, Maurine Spencer, has just finished a survey of mitochondrial DNA and *wingless* sequence variation in North American members of the genus *Plebejus* for comparison with the patterns observed in *Lycaeides*. Interestingly, the nuclear gene *wingless* proved to be relatively invariant, indicating that this species complex is younger perhaps than the *Lycaeides* complex. This work provides an excellent basis of comparison to the findings from *Lycaeides* and other taxa. The data from this project also forms the foundation for careful examination of the ecological and morphological factors that may be playing a role during diversification in these butterflies.
2. **Investigation of the Hackberry Butterflies.** The development of single copy genes for the *Lycaeides* project has been extended to an investigation of gene flow and speciation in a group of butterflies from the San Marcos area. The three nominal species of Hackberry butterflies from North America occur sympatrically in central Texas. The three species are distinguished on the basis of wing color pattern differences and exhibit additional ecological and behavioral differences. However, putative hybrids have been collected in San Marcos, suggesting that reproductive isolation is incomplete in these butterflies. A master's student, Chris Farmer, is conducting a preliminary investigation of levels of gene flow and divergence using mitochondrial genes and the single copy nuclear gene *wingless*. The development of primers for *Lycaeides* has allowed use to extend the funded work to another group of butterflies belonging to a different family, in this case, the Family Nymphalidae. The central question with this research is: are the mechanisms and patterns of evolutionary divergence observed in *Lycaeides* similar to those found in this distantly related group of butterflies?