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Gene flow of the variegated fritillary butterfly as an indicator of ecological connectivity in Boulder County, Colorado

Introduction
Landscape connectivity is recognized as a key component of biodiversity conservation in the face of global habitat loss and fragmentation; however, there is still much to learn about the influence of the matrix – the area of unsuitable habitat between habitat patches – on metapopulation persistence (Ferreras 2001, Damschen et al. 2006). Described as spatially isolated, local populations connected by some degree of gene flow, metapopulations are dependent on the movement of individuals across the matrix for maintaining genetic diversity, as well as for patch recolonization after local extinction events (Hanski and Gaggiotti 2004). Once treated as a homogenous zone of unsuitable habitat, the matrix is in actuality often diverse in pattern and structure, and may impose varying levels of resistance or permeability to animal movement (Ricketts 2001, McRae et al. 2005). My dissertation research examines butterfly movement between habitat patches around Boulder, Colorado to determine key areas of landscape connectivity, and areas in which restoration efforts may enhance connectivity for local biota. Differential levels of gene flow across the landscape are used to infer the permeability of anthropogenic and natural land cover types. This research will enhance our understanding of butterfly ecology at a local scale, and will have broader implications for land-use management and ecological conservation planning at the interface between urban and rural areas.
Like many urban areas, Colorado’s Front Range has experienced decades of land-use conversion and urban expansion, resulting in the fragmentation of what were once large swaths of contiguous prairie into smaller patches, bounded by a matrix of agricultural fields, urban developments, and transportation corridors. This system provides an excellent opportunity to examine the varying impacts of anthropogenic land cover types as well as open space areas and natural corridors on functional connectivity between isolated habitat patches. Butterflies are especially pertinent for this study as they are often considered indicator species (Fleishman et al. 2005), and can act as umbrella species whose conservation confers protection to much of the associated ecological community (Fleishman et al. 2000). Accordingly, the results of my research will have conservation implications beyond the butterfly species examined. This segment of my graduate research focuses on the distribution and landscape genetics of the variegated fritillary (*Euptoieta claudia*), which utilizes a variety of nectar sources and hostplants found in open areas on the Front Range (Scott 1986).

**Methods**

Preliminary sampling was conducted on Boulder Open Space and Mountain Park lands between 10 May to 30 September, 2011. OSMP trails were utilized to locate *E. claudia* butterflies, which were then caught with a mesh butterfly net. Two individuals of were collected and frozen as voucher specimens. All other individuals were released after obtaining a 3x3mm nonlethal wing clip. Wing clips were immediately stored in 95-100% ethanol, for subsequent DNA extraction.

DNA extractions were performed using QIAGEN DNeasy Blood and Tissue kits. Extracted DNA will be used to develop panels of variable microsatellites and single nucleotide polymorphisms (SNPs) specific to *E. claudia*, as genetic markers for this species do not yet exist in the published literature. Although the isolation of variable microsatellite loci is notoriously difficult for butterflies (e.g., Keyghobadi et al. 2002), recent advances in sequencing technology will facilitate the completion of this step in the genetic analysis process. Next Generation Sequencing (NGS) methods will be used during spring 2012 to identify genetic markers, and generate preliminary fine-scale genetic data. The advent of NGS technology in recent years has allowed for landscape
genetics research to occur in a much more timely and cost effective manner. Rather than creating a panel of 10-20 genetic markers – which can take years and thousands of dollars – NGS technology “shotgun” sequences the entire genome of each individual, allowing for the immediate identification of tens of thousands of variable loci (for example, see Yu et al. 2011). This methodology is ideal for this research given the historic difficulty of marker isolation in butterflies, and is funded for this project by the Dewind Award for butterfly conservation (Xerces Society, $3,750).

**Preliminary mapping**

The results of the genetic analyses will be used to evaluate alternative models of landscape connectivity, generated using Circuitscape (McRae and Shah 2009). These models are parameterized by hypothesized permeability of matrix land cover types – based on the biology of the study species – using raster land cover files (USGS National GAP data) in ArcGIS 10 (ESRI, Redlands, CA). Circuitscape uses a combination of isolation-by-resistance and least-cost path modeling within the framework of circuit theory, to generate models of landscape connectivity (McRae et al. 2008). Figure 1 shows one possible connectivity map for *E. claudia* in the Boulder area. Multiple alternative models will be generated with varying relative permeabilities. For example, residential developments may pose lower resistance to movement than agriculture fields, due to the presence of nectar resources found in landscaped gardens. Alternatively, agricultural fields may be more permeable to movement than residential areas due to the absence of buildings, streets, and traffic. The models will be evaluated using genetic coalescence data: a higher degree of gene flow between isolated populations indicates greater landscape permeability.
Figure 1. Aerial image of the study area (top), and hypothesized connectivity map for *E. claudia* (bottom). Dark areas show low landscape permeability, and bright areas show high connectivity between habitat patches.
Conclusion
The results of my research will describe the specific impacts of different land cover types on the genetic connectivity of variegated fritillaries in the Boulder area. This research will contribute to our understanding of butterfly ecology in the Boulder area – specifically, how movement and gene flow are influenced by the landscape. The GIS models of landscape connectivity, based on the genetic analyses, will highlight important corridors for butterfly movement that should be targeted for conservation planning. The models will also delimit areas that could enhance ecological connectivity if restoration efforts were implemented. For example, Figure 1 shows high connectivity throughout open space areas south of Boulder, but relatively lower connectivity between north and south Boulder. This provides support for a restored prairie corridor to the east of the city, or a “stepping stone” system of restored patches. I predict that matrix types providing heterogeneous vegetative structure, such as native prairie and gardens in residential developments, will impose relatively lower levels of resistance to butterfly movement, shown by higher levels of gene flow across these matrix types. In contrast, matrix types composed entirely of built infrastructure are likely to show high resistance to movement, and may act as complete barriers to gene flow. This research will provide insight into the qualitative gradients of habitat and non-habitat areas, which have primarily been treated in a binary manner until recent years.

Understanding the effects of anthropogenic habitat fragmentation on butterfly metapopulation connectivity will have local and global implications for land-use management and conservation planning. This project has the potential to inform management practices at the urban-rural interface for local agencies such as Boulder Open Space, as well as federal agencies like the U.S. Fish and Wildlife Service. Funds from the Boulder County Nature Association facilitated essential preliminary sampling, which will enable the identification of variable genetic markers in spring 2012. In addition to their necessity in determining gene flow for this research project, these markers will also be greatly beneficial to other ecologists working on butterfly genetics. Accordingly, these markers will be made available to the scientific community through immediate publication. Future
research plans include further sampling of additional Boulder County *E. claudia* populations during the 2012 and 2013 flight seasons, as well as other butterfly species with varying levels of vagility to assess the relationship between dispersal ability, host plant specificity, and landscape connectivity in an anthropogenically fragmented system.

References


