



## Investigating movement and population genetic structure of *Parnassius clodius* butterflies in Grand Teton National Park

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**Abstract** Numerous species are responding to warming climate by shifting distributions northward and poleward. Butterflies have been instrumental in documenting such climate-induced range shifts. *Parnassius clodius* is a montane butterfly found throughout the Greater Yellowstone Ecosystem in metapopulations of disconnected dry, gravelly sagebrush meadows. Dispersal among metapopulations will likely strongly determine whether the species can move in response to changing climate. We collected *P. clodius* in 41 study sites spread throughout the GYE for which we also have occupancy data. Future analysis of genotyping-by-sequencing data for these 209 samples will help describe population structure across the landscape and identify potential landscape features that are barriers to movement for this species.

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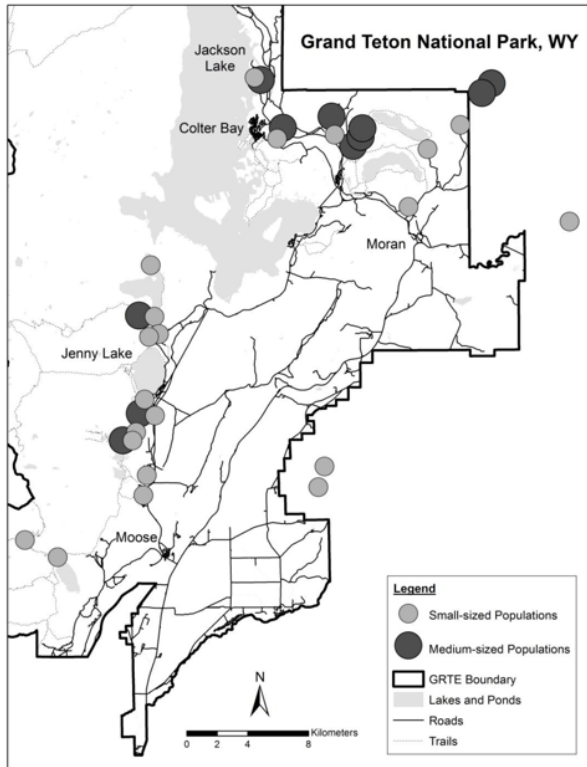
### Introduction

Climate models predict that climate change will cause general shifts in species ranges toward the poles and high elevations, and empirical studies confirm that these range shifts are occurring (Parmesan et al., 1999; Kerr et al., 2015). A species' or population's ability to disperse is a key factor determining whether a range shift is possible, and a detailed understanding of how and under what circumstances a particular species or population is able to disperse is therefore crucial.

Our research group has been studying the butterfly *Parnassius clodius* in the Greater Yellowstone Ecosystem since 1992, and recently completed an occupancy modeling analysis of 41 meadows in Grand Teton National Park (GRTE) (Szcodronski, 2014). We found a high percentage of suitable meadows occupied by *P. clodius* (78%), but the total number of butterflies recorded at each study site was very small. In 21 out of the 32 meadows, we saw

fewer than 8 individuals total over all 4 butterfly surveys (see the small-sized populations depicted in Figure 1). The remaining 11 medium-sized populations ranged from 14 to 35 total individuals recorded throughout the study.

It is unclear whether the current population configuration is a stable one. Forest encroachment and shrinking meadow habitat related to climate change could threaten the viability of the *P. clodius* population in GRTE. Recent studies of sister-species *P. smintheus* have been conducted in alpine meadows along the Rocky Mountain range of Alberta, Canada. Roland et al. (2000) found that treeline changes reduced the average meadow size by 78% from 1952 to 1993, impairing habitat connectivity and reducing the number of *P. smintheus* moving between meadows by 41%. Butterflies in the Alberta system do not disperse among meadows if there is more than 1 km of forest separating the populations (Roland and Matter, 2007). Montane meadow butterflies such as



**Figure 1.** Map of *Parnassius clodius* populations in Grand Teton National Park (Szcodronski, 2014).

*Parnassius* that are constrained to isolated, shrinking habitats and that avoid forest edges are highly vulnerable to genetic isolation and could be under an increased extinction threat (Keyghobadi et al., 1999; Roland et al., 2000; Keyghobadi et al., 2005; Roland and Matter, 2007; Dirnböck et al., 2011).

Keyghobadi et al. (2003) found that *P. smintheus* movement was significantly different between meadow areas and forested areas, i.e., forests are more “viscous” than meadows, resulting in fewer butterflies moving through forests than through meadows. Data collected during our 2016 field work will allow us to use similar genetic techniques to answer important questions about *P. clodius* movement across the landscape in GTNP. In this study, we investigate how a more diverse set of landscape features—including forests, large and small meadows, lakes, willow shrubland, sagebrush flats, and developed areas—affect the potential dispersal ability of *P. clodius*.

The genetic data collected during the 2016 field season will allow us to detect population subdivision at a very fine spatial scale (<25km). We will examine whether genetic distance increases with geographic distance to determine if isolation by distance limits gene exchange among subpopulations (Wright, 1943), and we will identify any landscape barriers to gene flow. Our genetic analyses will provide critical estimates of the frequency of movement between populations (effective gene flow) and the overall genetic variation within the metapopulation, as well identify any isolated subpopulations that may be at an increased risk of extinction. Our population genetics analysis combined with our landscape analysis will ultimately help us better understand the long-term viability of *P. clodius* in the Greater Yellowstone Ecosystem.

## Methods and preliminary results

### Study organism

*Parnassius clodius* are moderately large (5 to 7 cm wingspan), predominantly white butterflies found in western Canada and the western United States. The highest densities of *P. clodius* are typically found in dry, gravelly sagebrush meadows (Auckland et al., 2004). *P. clodius* males emerge several days before the females in June–July and adults fly for 3–4 weeks. Adult females oviposit on vegetation near the host plant species, *Dicentra uniflora*, a spring ephemeral that grows near snowmelt edges. *P. clodius* larvae feed on the host plant throughout the spring until pupation.

### Study sites

This study was conducted in 41 sagebrush meadows in Grand Teton National Park and Bridger-Teton National Forest (see Figure 1). The Teton region contains a unique heterogeneous distribution of habitat types along elevation and hydrological gradients that include conifer and aspen forests, willow shrubland, sagebrush flats, and montane meadows. Landsat satellite multispectral imagery data were used to classify six montane meadow types within the GYE, ranging from hydric willow and sedge mead-

ows to mesic meadows with high forb cover to xeric sagebrush meadows (Jakubauskas et al., 1996; Debinski et al., 1999). Based on *P. clodius* known habitat preferences, meadow sites were restricted to meadows with montane mesic herbaceous vegetation and montane xeric herbaceous vegetation with sagebrush cover. GIS vegetation data layers provided by the 2002-2005 Grand Teton National Park Vegetation Mapping Project (Cogan et al., 2005) were used in ArcGIS 10.1 to locate potentially suitable *Parnassius* habitat in the Teton region. Using these habitat criteria, Szcodronski (2014) identified 41 study sites as potential habitat; our study surveyed these same sites.

### Data collection and analysis

Between June 6<sup>th</sup> and July 9<sup>th</sup> 2016 our team surveyed each meadow twice (i.e., two different days) for a total of 82 surveys. During each survey, two researchers wandered the meadow randomly and independently for 20 minutes. Using an aerial net, *P. clodius* butterflies were captured to obtain up to 7 butterflies per meadow total over both surveys. A single hind leg was removed from each captured butterfly using clean surgical scissors and the samples were stored at -18 °C in 100% ethanol until DNA extractions could be performed in the lab. Males were preferentially sampled and butterflies were released after sampling. A total of 220 samples were collected over the 2016 field season, of which 200 were males and 20 were females. No *P. clodius* butterflies were sighted or sampled in 3 of the 41 meadows.

DNA extractions occurred from March 13th through March 17th 2017 in Madison, WI, using DNeasy<sup>®</sup> Blood and Tissue Kit (QIAGEN<sup>®</sup>) with the addition of RNase A treatment (Thermo Fisher Scientific Inc.) A total of 209 samples were sent for genotyping-by-sequencing (GBS) on March 17th 2017. GBS is a next-generation sequencing approach that provides genome-scale measures of genetic variation. Genetic data from GBS will be analyzed to determine the genetic structure of the metapopulation, i.e., which subpopulations are more closely related to each other. This genetic information will be combined with a spatially-explicit habitat map to deter-

mine which landscape features serve as barriers or corridors for *P. clodius* movement through the park.

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