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Spatio-temporal ecological and evolutionary dynamics in natural butterfly populations

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Abstract Spatial and temporal variation in the strength and nature of natural selection could help explain genetic diversity in natural populations and data on short term evolutionary responses to fluctuations in temperature and rainfall could facilitate predictions of climate change impacts. In 2012, we began a long term study of genome-wide molecular evolution in populations of *Lycaeides idas* in the Greater Yellowstone Ecosystem (GYE). In 2016, we used distance sampling to estimate population densities of 10 butterfly populations spread across the GYE in Wyoming and Montana. In parallel, we estimated host plant cover and conducted insect community surveys at each site. We also completed a genotyping-by-sequencing survey for eight populations sampled in 2013 and 2015 to estimate contemporary variance in effective population sizes. Based on 480 samples across sites, we found significant variation in population sizes (as estimated by distance sampling) among sites and years. Host plant abundance, climate, and insect communities varied among sites but were not consistently predictive of population size. Estimates of effective population sizes among sites showed pronounced variation that was uncorrelated with genetic diversity, possibly due to widespread fluctuating selection.

Introduction

The study of evolution in natural populations has advanced our understanding of the origin and maintenance of biological diversity. For example, long term studies of wild populations indicate that natural selection can cause rapid and dramatic changes in traits, but that in some cases these evolutionary changes are quickly reversed when periodic variation in weather patterns or the biotic environment cause the optimal trait value to change (e.g., Reznick et al., 1997; Grant and Grant, 2002). In fact, spatial and temporal variation in the strength and nature of natural selection could explain the high levels of genetic variation found in many natural populations (Gillespie, 1994; Siepielski et al., 2009). Long term studies of evolution in the wild could also be informative for biodiversity conservation and resource management, because, for example, data on short term evolutionary responses to annual fluctuations in temperature or rainfall could be used to predict longer term evolution in response to directional climate change. Most previous research on evolution in the wild has considered one or a few observable traits or genes (e.g., Kapan, 2001; Grant and Grant, 2002; Barrett et al., 2008). We believe that more general conclusions regarding the rate and causes of evolutionary change in the wild and selection's contribution to the maintenance of genetic variation could be obtained by studying genome-wide molecular evolution in a suite of natural populations. Thus, in 2012 we began a long term study of genome-wide molecular evolution in a series of natural butterfly populations in the Greater Yellowstone Area (GYA). This study will allow us to quantify the contribution of environment-dependent natural selection to evolution in these butterfly popu-

lations and determine whether selection consistently favors the same alleles across space and through time.

The focal species, *Lycaeides idas*, is one of five nominal species of *Lycaeides* butterflies that occur in North America (Figure 1; Nabokov, 1949; Guppy and Shepard, 2001; Gompert et al., 2006). These species are descended from one or more Eurasian ancestors that colonized North America about 2.4 million year ago (Vila et al., 2011). *L. idas* hybridizes with a second species, *L. melissa*, in the GYA (Gompert et al., 2010, 2012). *L. idas* is a holarctic species that is found in Alaska, Canada, and the central and northern Rocky Mountains of the contiguous USA (Scott, 1986). *L. idas* is univoltine and adults generally fly from mid-July to early August. In the GYA *L. idas* populations often occupy mesic forest and montane habitat at elevations ranging from 2000-3500 m above sea level. Most populations of *L. idas* in the GYA feed on *Astragalus miser* as larvae, but some populations feed on other native legumes (most notably, other species of *Astragalus* and *Lupinus*; Gompert and Messina, 2016). We selected *L. idas* as the focal species for this study because of our experience with this species, extensive data on the location and natural history of *L. idas* populations, the availability of genomic resources for this species, and several key aspects of this species's natural history (e.g., *L. idas* have non-overlapping generations with one generation per year, well-defined populations, and modest genome sizes, and *L. idas* are found in various different habitats that might experience different environment-dependent selection pressures).

The specific goals of this study are to: (i) quantify genetic variation and molecular evolution in *L. idas* and their relationship with population size, insect community, and environmental variation across space (i.e., different populations) and through time (i.e., from generation to generation) and (ii) test the hypothesis that the nature and strength of environment-dependent selection varies among populations and over generations and that this variation is sufficiently large to contribute to the maintenance of genetic variation in *L. idas*. This report documents the results from the fifth year of this long term study. The first year (2012) was a pilot study in which we collected *L. idas* for DNA sequencing and tested the distance sampling technique to estimate population sizes. In our second year (2013) we collected *L. idas* and started distance sampling at four populations. In 2014 we collected *L. idas*, and used distance sampling at ten populations. In 2015 we collected *L. idas* from our ten focal populations. In 2016, we collected *L. idas*, attempted distance sampling at all ten populations, and tested our insect community survey method.

Figure 1. Female *L. idas* butterfly perched above its host plant (*Astragalus miser*) on Blacktail Butte (BTB).

Methods

We collected 480 specimens from the ten populations included in this study between July 12 and August 4, 2016 (Figure 2, Table 1). Four of the populations are within park boundaries (BTB and RNV in GRTE and BNP and HNV in YNP). These whole adult butterflies are stored at -80 °C for later DNA extraction and sequencing. In addition, we used a distance sampling protocol to estimate adult population sizes in *L. idas*. Distance sampling involves counting individuals and recording their distance from a transect line or point (Buckland et al., 2001). This distance information is used to estimate a detection function

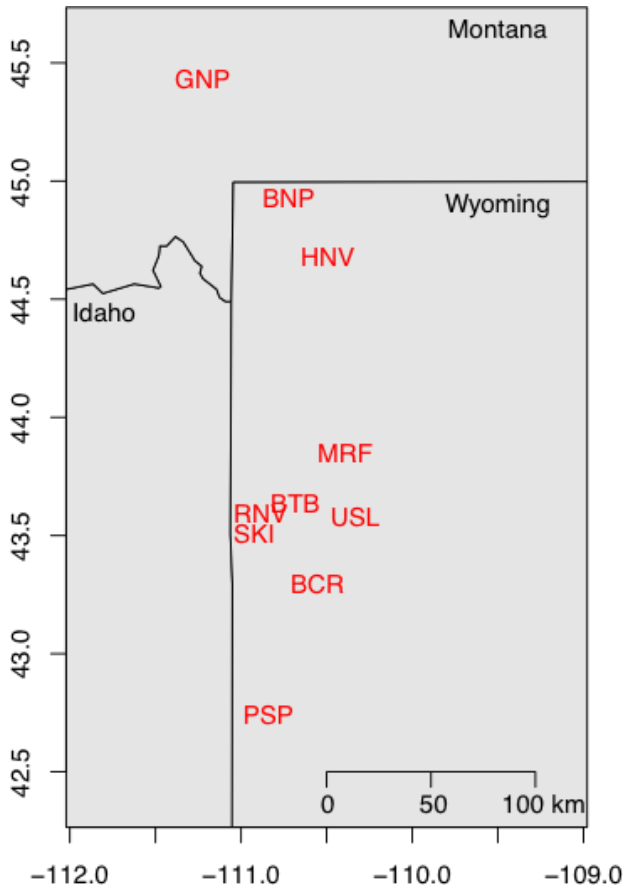

Figure 2. Map of the ten *L. idas* populations in the GYA involved in this long term study.

that accounts for imperfect detection away from the transect line. We performed the distance sampling method one or two times per *L. idas* population over the course of the four weeks. For each population we randomly chose ten or fewer points within a defined area of suitable habitat (we identified suitable habitat from ground surveys and satellite images). At each of these points, four trained observers (ZG, LKL, two USU Biology graduates, Amy Springer and Samridhi Chaturvedi, and two USU undergraduates, Chase Gabbitas and Britney Allen) walked an approximately 100-meter transect and: 1) counted the *L. idas* we saw along the way, recorded the sex and measured their distance from the transect line, and 2) quantified the abundance of butterfly host plant (Figure 1). We recorded a 0, 1 or 2 to denote whether there were no butterfly host-plants, less than 50% of the ground cover was host-plants, or more than 50% of the ground cover was host-plants within a meter of each transect line, respectively. The host-plant species recorded depended on the population: *Astragalus miser* (BCR, BTB, MRF, HNV, BNP, GNP, SKI, USL), *Astragalus bisulcatus* (USL), *Lupinus sp.* (PSP) or *Hedysarum sp.* (RNV). We only performed distance sampling between 10:00 am and 2:00 pm under sunny or partly sunny skies.

We estimated population densities (adult butterflies per square kilometer) using the *distsamp* function in the *unmarked* R package. We binned the detection distances of butterflies into 1 meter bins prior to analysis (e.g., 0 to 1 m, 1 to 2 m, etc.). We used a half-normal detection function and estimated the detection function and density model parameters using maximum likelihood (Royle et al., 2004). This model assumes the latent transect-level abundance distribution is Poisson and that the detection process is multinomial with a different detection probability for each distance class or bin. We then estimated population size by first multiplying density by the area of habitat (km^2) and then by three because adult *L. idas* live for about a week but the population flies for about three weeks.

To preliminarily explore whether differences in population size across space (populations) can be explained by climate, we used 19 weather variables averaged over 1950-2000 (source: http://www.worldclim.org/bioclim), summarized as one variable via a Principal Component Analysis (PCA) using the *prcomp* function in R.

We conducted an insect community survey at the end of at least one transect per site (6 at BTB, 2 at PSP, 1 at BCR, 10 at USL, 6 at GNP, 7 at BNP, 3 at HNV, 2 at MRF, 7 at SKI, 2 at RNV). The survey consisted of 1-3 sweeps of *L. idas* host plant with a sweep net. Insects in the sweep net were identified to family, and the number of individuals of each family were recorded. These data were used in a principal component analysis (PCA) to characterize differences in insect community across sites. We used the *prcomp* function in *R* to perform this PCA.

We completed a genotyping-by-sequencing survey of genetic variation in *Lycaeides* (as in Gompert et al.,

Pop	2013 size	2014 size	2016 size	host-plant	Climate PC score
BTB	1838.7	1978.5	2763.3	0.8	-1.5
BCR	2382	1241.7	NA	0.5	-3.8
BNP	633.9	1273.2	NA	0.8	1.2
GNP	1119.9	1024.5	NA	0.2	3.6
HNV	NA	5291.4	NA	0.1	1.1
MRF	NA	977.7	NA	0.2	-1.5
PSP	NA	366.6	NA	0.4	-3.9
RNV	NA	NA	NA	0.3	5.7
SKI	NA	1348.8	1242.2	0.7	1.6
USL	NA	1708.2	2927.1	0.5	-2.4

Table 1. Population abbreviations ("pop"), population size estimates via distance sampling in 2013, 2014, and 2016, average host-plant abundance (estimated in 2016; "host plant"), and a representation of long term climate at each population (PC1 represented 52.6% of the variance in the original dataset).

2014) from samples collected from eight populations sampled in 2013 and 2015. Genetic change in these sites across years was used to estimate contemporary variance effective populations sizes (as in Gompert and Messina, 2016).

Results

We collected 12 males and 17 females from Bull Creek (BCR), 29 males and 21 females from Bunsen Peak (BNP), 33 males and 17 females from Blacktail Butte (BTB), 21 males and 29 females from Garnet Peak (GNP), 35 males and 15 females from Hayden Valley (HNV), 32 males and 13 females from Mt. Randolf (MRF), 28 males and 19 females from Periodic Springs (PSP), 30 males and 18 females from Rendezvous Mountain (RNV), 37 males and 17 females from Ski Lake (SKI), and 42 males and 15 females from Upper Slide Lake (USL). BNP and HNV are within Yellowstone National Park, and BTB and RNV are in Grand Teton National Park. We were unable to collect our target 50 individuals from a few of our populations: BCR, MRF, PSP and SKI. Populations BCR, MRF and PSP are some of our driest sites, and we may have visited them too late in the season. Conversely, we may have visited SKI too early in the season. Similarly, *L. idas* abundances were high enough at only three sites to analyze distance sampling data.

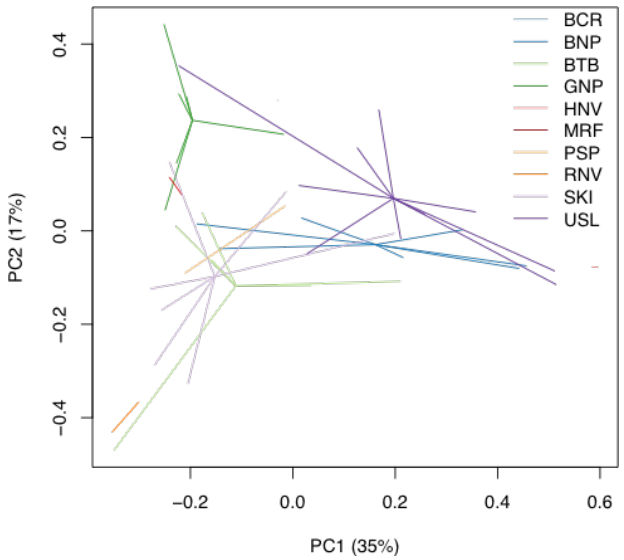

Figure 3. Differences in relative abundances of insect families as summarized by a PCA across all 10 *L. idas* sites, as summarized by a PCA.

In Table 1 we include our population size estimates from distance sampling in summers 2013-2014 and 2016 (Buckland et al., 2001; Royle et al., 2004; *distsamp* function in the *unmarked* R package), as well as average host-plant abundance collected during 2016. When comparing estimates between 2016 and 2013-2014, we observed that SKI stayed about the same, and BTB and USL increased. The range of host-plant abundance across sites was 0.1 to 0.8, with the highest abundance at BTB and BNP and the lowest at HNV. The climate variable ranged from -0.91 to 5.69 across sites. Negative numbers represent hotter and drier climates, whereas positive values represent colder and wetter climates. We found that PSP and BCR were the hottest/driest. PSP was -3.91 and BCR was -3.75. The coldest and wettest were RNV at 5.69 and GNP at 3.55 (Table 1).

The PCA performed on the insect community data revealed differences among sites (Figure 3). Principal component 1 (PC1) explained 35% of the variance in the dataset, and PC2 explained 17% of the variance. PC1 was a bit different for USL and BNP than for all other sites, and GNP was different from the other sites for PC2.

Contemporary estimates of effective population sizes

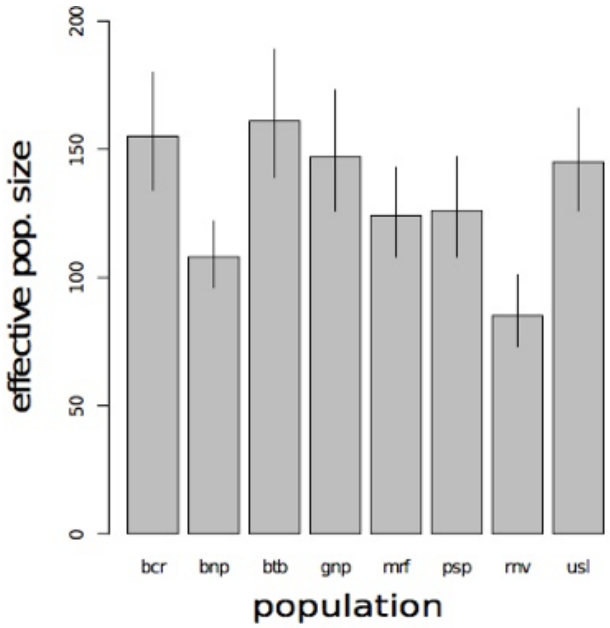

Figure 4. Variance in effective population size estimates from 8 focal populations based on genome-wide measures of allele frequency changes.

(Figure 4), based on genetic data, varied by an order of magnitude more than and were uncorrelated with genetic diversity levels (coefficient of variation = 0.196 vs 0.014 for expected heterozygosity). These results suggest that diversity might not reflect drift-mutation equilibrium as posited by standard neutral theory, and that alternative hypotheses, such as widespread fluctuating selection, warrant consideration.

Discussion

Based on our moderate population size estimates, we predict that both genetic drift and selection are important drivers of evolution in this system (Lynch and Walsh, 2007). The comparison of population size estimates among years are potentially interesting and could reflect demographic variability across time. The difference in habitat (i.e., host-plant and insect community) and climate across populations highlight the spatial variation in this study system.

We will continue this study during the 2017 summer field season. During this and subsequent field seasons, we will collect samples and estimate population sizes at all ten sites listed in Table 1. We will also continue collecting habitat data that will be useful for fitting causal models of molecular evolution. We also will collaborate with both undergraduate and graduate researchers during the 2017 field season.

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