

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 third 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 (population size is an important parameter for our evolutionary models). In our second year (2013) we collected *L. idas* and started distance sampling at four populations. This year we collected *L. idas* and used distance sampling at ten populations.



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

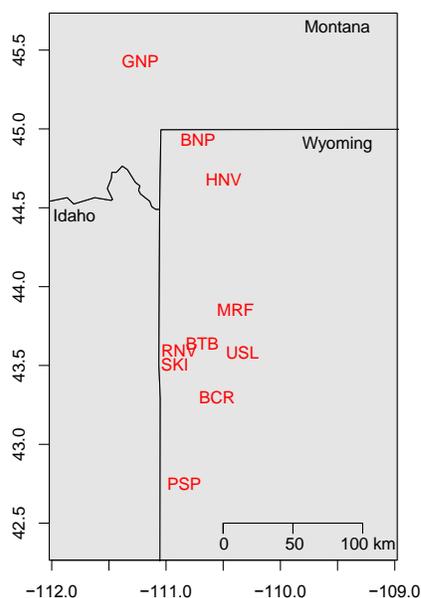
## ◆ METHODS

We collected 596 specimens from twelve locations: the ten populations involved in this study (Figure 2, Table 1), as well as two other locations near Dubois, WY with which we are investigating putative current hybridization between two *Lycaeides* species. Four of the populations are within national park boundaries (BTB and RNV in GTNP and BNP and HNV in YNP). We are storing these whole adult butterflies at  $-80^{\circ}\text{C}$  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 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 four weeks (July 7 – August 3). For each population we randomly chose ten or fewer random 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 and two USU Biology undergraduates, Robert Olsen and Peter Nelson) 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 plants (Figure 3). 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 2004). This model assumes the latent transect-level abundance distribution is Poisson and that the detection processes 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 ( $\text{km}^2$ ) 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 host-plant abundance and 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 did a regression using the *lm* function in *R*.



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



**Figure 3.** Photograph of a transect for distance sampling at Blacktail Butte (BTB). The two undergraduate researchers are recording host-plant abundance.

## ◆ RESULTS

Using data from the one visit out of the two to each population in which our census was higher, our estimates of adult *L. idas* population density using distance sampling were: 4813 butterflies per square km (standard error [se] 1511) at GNP, 4600 at BTB (se 1483), 6196 at BNP (se 1727), 4672 at BCR (se 1442), 3868 at PSP (se 1626), 4285 at USL (se 1234), 5010 at MRF (se 2023), 5144 at HNV (se 1668), and 4033 at SKI (se 1207). Our estimated population sizes ranged from 366.7 to 5291.4 butterflies (Table 1). We

were unable to estimate the population size for RNV, as adult butterflies started flying much later than expected. When comparing estimates between 2013 and 2014, we observed that GNP and BTB stayed about the same, BNP increased, and BCR decreased (Table 1). The range of host-plant abundance across sites was 0.21 to 0.92, with the highest abundance at BNP and the lowest at MRF (Table 1). To collapse all 19 bioclim weather variables to one variable, we did a PCA and used the first principal component (PC1), which represented 52.6% of the variance in the original dataset. This climate variable ranged from 0 to 5.69 to -3.91 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).

**Table 1.** Population names with abbreviations, population size estimates via distance sampling in 2013 and 2014, average host-plant abundance, and a representation of long term climate at each population.

Population	2013 size	2014 size	Ave. host-plant abundance	Climate PC score
Blacktail Butte (BTB)	1838.7	1978.5	0.5	-1.5
Bull Creek (BCR)	2382	1241.7	0.5	-3.8
Bunsen Peak (BNP)	633.9	1273.2	0.9	1.2
Garnet Peak (GNP)	1119.9	1024.5	0.4	3.6
Hayden Valley (HNV)	NA	5291.4	0.3	1.1
Mt. Randolph (MRF)	NA	977.7	0.2	-1.5
Periodic Springs (PSP)	NA	366.7	0.6	-3.9
Rendezvous Mountain (RNV)	NA	NA	0.3	5.7
Ski Lake (SKI)	NA	1348.8	0.6	1.6
Upper Slide Lake (USL)	NA	1708.2	0.5	-2.4

Last, we found no relationship between 2014 population sizes and long-term climate and average host-plant abundance. Our p-values were 0.4648 (host-plant) and 0.2052 (climate), both of which are well above 0.05.

## ◆ DISCUSSION

Because we have just begun this long-term study and we have not yet sequenced the DNA from the sampled butterflies, we cannot yet make any conclusions about the rates or causes of molecular evolution in these study populations. But we have already learned a few things from the distance sampling surveys and analyses over the past two years. Based on our moderate population size estimates we predict that both genetic drift and selection are important drivers of evolution in this system (Lynch 2007). The comparison of population size estimates in 2013 and 2014 are potentially interesting and could reflect demographic variability between years. The difference in habitat (i.e., host-plant) and climate across populations highlight the spatial variation in this study system. It is possible we would have seen a significant relationship between population size and weather specifically recorded from 2013 and 2014, but we currently don't have these data; we only have the bioclim data that is an average of the weather from 1950-2000.

We will continue this study during the 2015 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 plan to begin DNA sequencing of the collected *L. idas* in 2015.

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