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Forecasting eco-evolutionary dynamics in the Northern Blue butterfly (*Lycaeides idas*)

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Abstract Natural selection can drive rapid evolutionary change, particularly in human-altered habitats. Rapid adaptation to global change requires standing genetic variation for ecologically important traits, but at present little is known about how much relevant genetic variation most populations possess. With this in mind, we began a long term study of genome-wide molecular evolution in a series of natural butterfly populations in the Greater Yellowstone Area (GYA) in 2012 to quantify the contribution of environment-dependent natural selection to evolution in these butterfly populations, and determine whether selection varies enough across space and time to maintain variation that could facilitate adaptation to global change. In 2018, we visited 11 focal populations to collect samples for DNA, estimate population sizes (using distance sampling and mark-release-recapture methods), and survey arthropod communities at the sites. Our analyses are ongoing, and this is a preliminary report, but thus far we have found that census population sizes are much higher than contemporary effective population sizes (though these metrics are highly correlated), and that both are independent of genetic diversity levels. These results are consistent with the hypothesis that selection plays a central role in eco-evolutionary dynamics in this system.

Introduction

Ever since Darwin, we have recognized that ecological interactions can drive evolutionary change (e.g., Siepielski et al., 2017). However, evolution has often been deemed too slow to have an appreciable effect on ecological dynamics, at least at the timescales most ecologists have been interested in (e.g., a few decades or generations). Accumulating evidence that rapid adaptation can affect population demography, predator-prey cycles, community composition and ecosystem processes has cast doubt on this assumption (e.g., Fussmann et al., 2007; Farkas et al., 2013; Hendry, 2016; Rudman et al., 2017). It is now apparent that evolution can affect the probability of population and species persistence in new or altered environments (Stockwell et al., 2003; Munday et al., 2013). This includes the possibility of adaptation to novel climates, as well as the potential negative evolutionary consequences of habitat fragmentation and population decline. Rapid adaptation to global change requires standing genetic variation for ecologically important traits, but at present little is known about how much relevant genetic variation most populations possess (Bay et al., 2017). This gap in our knowledge hinders our ability to forecast ecological and evolutionary dynamics for most species, and more generally to reliably predict the community and ecosystem-level consequences of global change. We believe that studies of evolution in action in natural populations are needed to fill this gap in our knowledge.

Long-term studies of wild populations have already

shown 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 in the biotic environment cause the optimal trait value to change over time (e.g., Reznick et al., 1997; Grant and Grant, 2002; Nosil et al., 2018). 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 is allowing us to quantify the contribution of environment-dependent natural selection to evolution in these butterfly populations 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 years ago (Vila et al., 2011). *Lycaeides idas* hybridizes with a second species, *L. melissa*, in the GYA (Gompert et al., 2010, 2012, 2014). *Lycaeides idas* is a holarctic species that is found in Alaska, Canada, and the central and northern Rocky Mountains of the contiguous USA (Scott, 1986). *Lycaeides 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*, *Lupinus* and *Hedysarum*; Gompert et al., 2010). We chose *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).

Figure 1. Photograph of a male *L. idas* at site GNP in the Gallatin Range, MT in 2018 (taken by L. Lucas).

This study will address the following specific questions: (i) How do shifts in the biotic and abiotic environment affect ecological and evolutionary dynamics in *L. idas*, and specifically do such shifts cause selection to fluctuate in direction over time such that genetic variation in preserved? (ii) Do GYA *L. idas* populations harbor sufficient standing genetic variation to adapt to new climate and weather patterns? and (iii) Can we accurately predict (forecast) ecological and evolutionary dynamics in this system? This report documents our results for the first seven years of this long-term study (with an emphasis on the the

most recent year, 2018). 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. Starting in 2013 (year 2), we collected *L. idas* from ten populations (most years) and used distance sampling to estimate population sizes at these populations (in some years, this was only done with a subset of populations). Starting in 2016 (year 5), we began sampling insect communities on the host plants of *L. idas* (we have been updating and refining this method annually). In 2018 (year 7), we collected our standard data, that is collections for DNA, distance sampling and insect communities at the 10 sites, added an eleventh site (BLD), and piloted an alternative mark-release-recapture method to estimate the population size at one site (BTB). We added this last component to the project to assess the robustness of our distance-sampling population size estimates with an independent approach. Herein, we present a summary of our results up to this point. We anticipate the first peer-reviewed publication from this project in 2019.

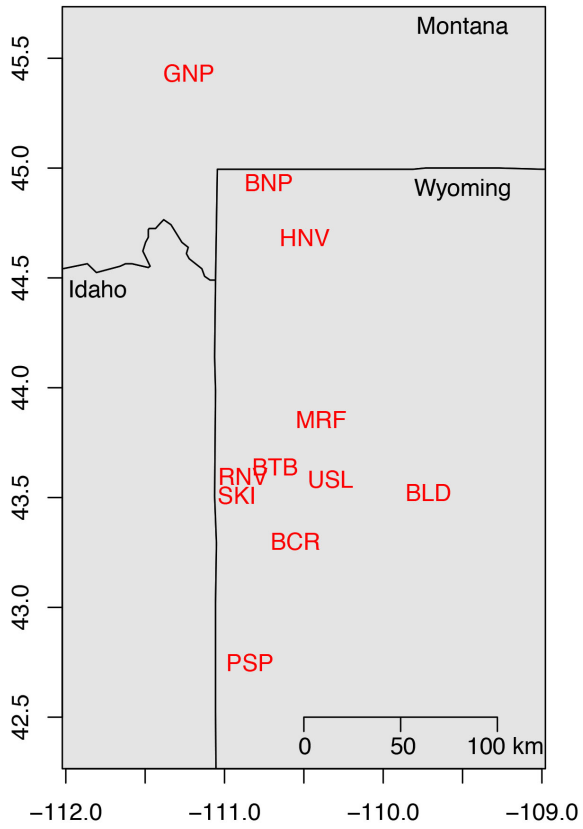

Figure 2. Map of the eleven *L. idas* populations involved in this long term study.

Methods

Field collections

In 2018, we collected 575 specimens from the eleven populations involved in this study, between July 9-July 30 (Figure 2, Table 1). Specifically, we collected 44 males and 16 females from Bull Creek (BCR), 29 males and 21 females from Bald Mt. (BLD), 42 males and 8 females from Bunsen Peak (BNP), 33 males and 17 females from Blacktail Butte (BTB), 35 males and 17 females from Garnet Peak (GNP), 36 males and 13 females from Hayden Valley (HNV), 39 males and 15 females from Mt. Randolf (MRF), 39 males and 11 females from Periodic Springs (PSP), 34 males and 16 females from Rendezvous Mountain (RNV), 35 males and 17 females from Ski Lake (SKI), and 41 males and 17 females from Upper Slide Lake (USL). BNP and HNV are within Yellowstone National Park, and BTB and RNV are in Grand Teton National Park. Butterfly samples are stored at -80°C until DNA extraction.

Population size estimates

In 2018, we used a distance sampling protocol to estimate *L. idas* adult population sizes at each of our focal sites. 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. 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, we walked an approximately 100-meter transect and: 1) counted the *L. idas* we saw along the way, recorded the sex and measured their distance on and from the transect line, and 2) quantified the abundance of butterfly host plant. We recorded a 0, 1 or 2 to denote whether there were no butterfly host-plants, less than 50% of the

Pop	2013 size	2014 size	2016 size	2017 size	2018 size	2018 host-plant	Climate PC score
BTB	1838.7	1978.5	2763.3	1155.3	1844.8	0.71	-0.7
BCR	2382	1241.7	NA	NA	NA	0.5	-2.9
BNP	633.9	1273.2	NA	NA	1199.1	0.97	1.0
GNP	1119.9	1024.5	NA	343.0	462.2	0.55	2.5
HNV	NA	5291.4	NA	NA	NA	0.40	1.4
MRF	NA	977.7	NA	NA	NA	0.16	-0.9
PSP	NA	366.6	NA	354.2	NA	0.30	-3.4
RNV	NA	NA	NA	NA	NA	NA	5.8
SKI	NA	1348.8	1242.2	694.4	3271.1	0.44	1.9
USL	NA	1708.2	2927.1	NA	995.1	0.50	-1.9
BLD	NA	NA	NA	NA	1500.7	0.64	-2.9

Table 1. Comparison of *L. idas* demographics and environmental conditions over time. Specifically, population abbreviations ("pop"), population size estimates via distance sampling in 2013-14 and 2016-18, average host-plant abundance between 0-2 (as estimated in 2018; "host plant"), and a representation of long term climate at each population (PC1 represented 41.9% of the variance in the original dataset).

ground cover was host-plants, or more than 50% of the ground cover was host-plants within a meter of each transect line, respectively (Table 1). The host-plant species recorded depended on the population: *Astragalus miser* (BCR, BLD, BTB, MRF, HNV, BNP, GNP, SKI, USL), *Astragalus bisulcatus* (USL), *Lupinus* sp. (PSP) or *Hedysarum* sp. (RNV, SKI). We only performed distance sampling between 10:00 am and 3:00 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.

On July 19, 2018, we obtained a mark-release-recapture estimate of population size at BTB to compare with the estimate from the distance sampling method (conducted July 18). We captured 64 adult *L. idas* on July 18. We made a mark on the hindwing with a permanent marker, and then released each butterfly (as in Auckland et al., 2004). We returned the following day and captured 50 adults to check for markings. This short time between release and recapture minimizes birth, mortality and movement of butterflies into and out of the site. We estimated the census population size by fitting custom hierarchical Bayesian models in JAGS.

Environmental covariates/sources of selection

As an initial assessment of 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

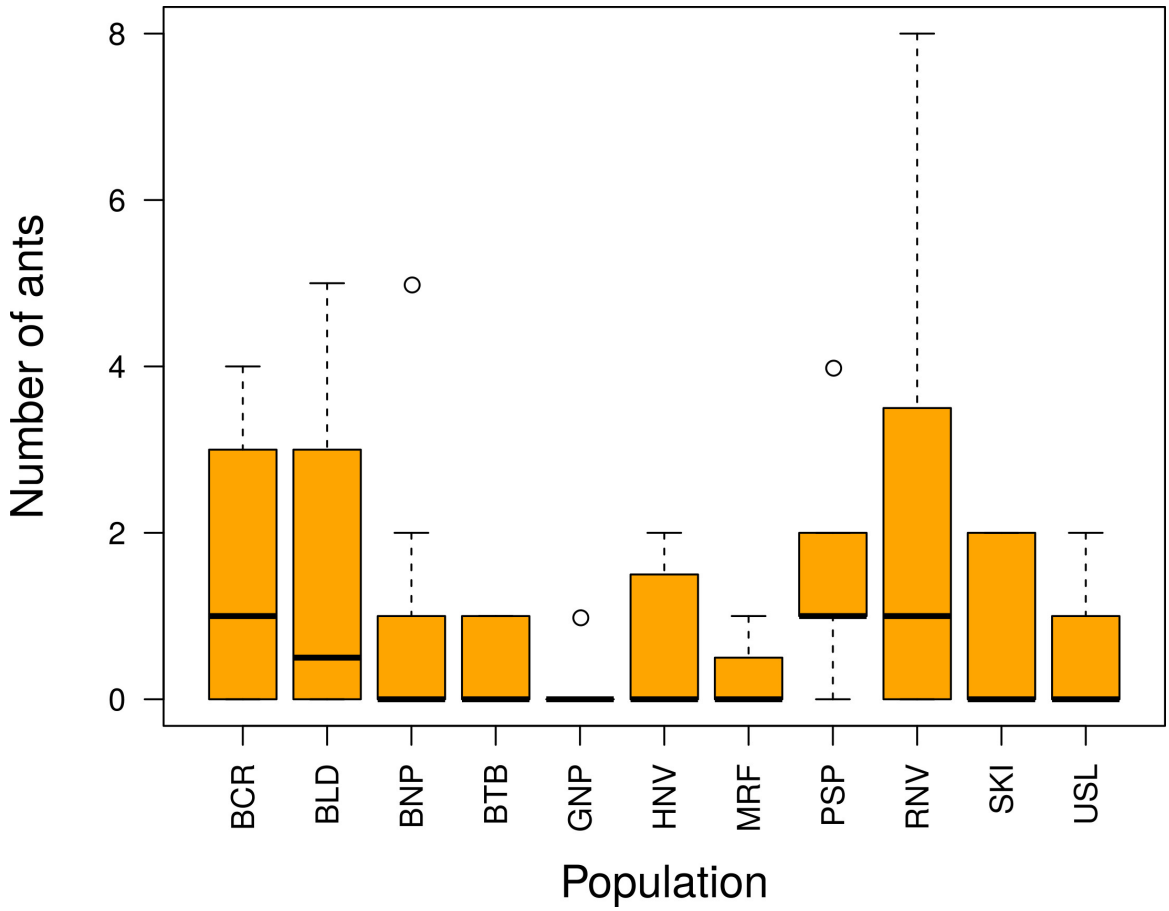

Figure 3. Boxplots depicting the numbers of ants in each of the sweep net samples for each site. Thick lines denote medians (across sweeps) and boxes indicate the 1st and 3rd quartiles.

via a principal component analysis (PCA) using the *prcomp* function in R.

We collected animal community samples from approximately three sweeps of *L. idas* host plant with a sweep net. The sweeps were conducted near the start of the transect used during distance sampling, at each of the eleven sites (10 at BTB, 5 at PSP, 5 at BCR, 9 at USL, 7 at GNP, 7 at BNP, 8 at HNV, 4 at MRF, 9 at SKI, 4 at BLD, 7 at BNP, and 7 at RNV). Samples were kept frozen until processed after the field season. Specimens in each sample were counted and classified as: ants, spiders, parasitoid wasps, hemipteran, and other. Specimens are frozen for future classification, if necessary. Random-effect ANOVAs were used to quantify variation in ants (mutualists with *Lycaeides* caterpillars) and possible predators/parasites (spiders, parasitoid wasps and hemipterans) among the sites. We fit these models with the *lmer* function in the R package *lme4*.

Genetic data and analyses

We completed a genotyping-by-sequencing (GBS) survey of genetic variation in *Lycaeides* (as in Gompert et al., 2014) from samples collected from ten populations sampled from 2013-2017 (samples from 2018 are being processed now). In brief, DNA sequences were aligned to our draft *Lycaeides* genome, and single nucleotide polymorphisms (SNPs) were identified using a Bayesian variant calling method implemented in GATK. We then used our own custom Bayesian models and C++ software to estimate SNP allele frequencies for each genetic locus, population and generation. Genetic change in these sites across years was used to estimate contemporary

variance in effective populations sizes (as in Gompert, 2016). This uses a Bayesian bootstrap approach with inferences based on the magnitude of allele frequency changes across the genome over time (bigger changes imply higher rates of genetic drift and thus a lower contemporary effective population size). Overall diversity levels (which are indicative of long-term effective population sizes, i.e., over the past tens of thousands of years) were estimated from the genetic data as well.

Preliminary Results

Population size estimates

In Table 1, we include our population size estimates from distance sampling in summers 2013-2014 and 2016-2018, as well as average host-plant abundance collected during 2018. For data collected in 2018, *L. idas* abundances were high enough at six of the eleven sites to fit models to the data and thus obtain population size estimates. When comparing the new 2018 estimates to previous years' data, we did not observe the same trend across all populations. The estimate for BTB was about average (like we saw in 2013), the BNP estimate was on the higher end (like we saw in 2014), the GNP estimate was on the lower end (like in 2017), the SKI estimate was the highest yet, and the USL estimate was the lowest to date. The range of host-plant abundance across sites was 0.16 to 0.97, with the highest abundance at BNP and the lowest at MRF (Table 1).

The analysis of the mark-release-recapture data from BTB yielded an estimated population size of 1,582 adult *L. idas* on the day of the visit (median = 1,187, 95% credible intervals = 462-5,076). Multiplied by three to account adult lifespan and the length of the flight season, the total population size point estimate is 4,746. This estimate is about 2.5 times higher than estimate from the distance sampling method, 1,845 *L. idas* (Table 1).

Environmental covariates/sources of selection

The climate variable ranged from -3.4 to 5.8 across sites. Negative numbers represent hotter and drier climates, whereas positive values represent colder and wetter climates. We found that PSP (-3.4), BCR (-2.9) and BLD (-2.9) were the hottest/driest. The coldest and wettest were RNV at 5.8 and GNP at 2.5 (Table 1).

Sweep net, insect community surveys from 2018 yielded 67 ants, 27 spiders, 22 parasitoid wasps and 409 hemipterans. 17.9% of the variation in ant abundance was partitioned among populations, and 28.8% of the variation in the abundance of putative caterpillar predators/parasites (i.e., spiders, parasitoid wasps and hemipterans) was partitioned among populations. The highest levels of ant abundance were observed at RNV (our high elevation site; 2.3 ants per sample) followed by BCR and BLD (1.6 and 1.5 ants per sample, respectively; Figure 3). Very few ants were collected from GNP and MRF (< 0.25 per sample). Predators/parasites were most abundant at BCR, BTB and USL (> 9 per sample), and least abundant at BNP and GNP (< 3 per sample; these are our northernmost sites). We failed to detect a relationship between ant and predator/parasite abundance across samples (Pearson r = -0.1, P = 0.417).

Genetic data and analyses

Contemporary estimates of variance effective population size for each site ranged from 174-373 (m = 265) (Figure 4). These estimates varied much more among populations than did genetic diversity estimates (coefficient of variation = 0.248 vs. 0.034 for expected heterozygosity), and the two variables were uncorrelated (Pearson r = 0.02, P = 0.95). These results suggest that diversity does not reflect drift-mutation equilibrium, and that alternative hypotheses, such as widespread fluctuating selection, warrant consideration. In contrast, estimates of contemporary effective population size and mean census sizes were positively correlated (Pearson r = 0.78, P = 0.02; Figure 4), suggesting that smaller populations are currently experiencing higher rates of evolution by genetic drift.

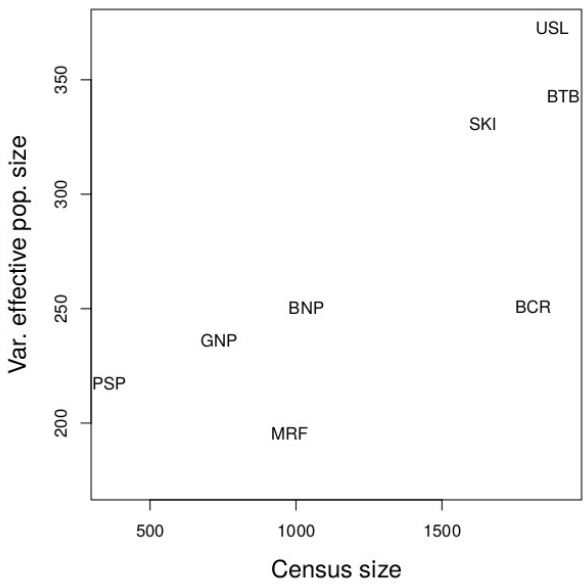

Figure 4. Estimates of variance (contemporary) effective population sizes correlate positively with census size for *L. idas*. Variance effective population sizes are based on genome-wide changes in allele frequencies between 2012-2017 and are given as 2 Ne (as these are diploid organisms), and thus denote the number of gene copies.

Preliminary Conclusions

L. idas census population sizes are about an order of magnitude larger than contemporary variance effective population sizes. This is not unexpected, but does mean that genetic drift has a greater effect on evolutionary dynamics in these populations than would be surmised based solely on the large census population sizes. The lower values for contemporary effective population sizes imply that successful breeding or contributions to the next generation are quite uneven among individual butterflies (i.e., some butterflies might have many offspring that make it into the next generation whereas others have few or none). The lower variance effective population sizes could result from natural selection, that is non-random variation in which butterflies contribute most to the next generation. The disconnect between contemporary variance effective population sizes and levels of genetic diversity within populations is consistent with this hypothesis. Selection could arise because of interactions between *L. idas* and the abiotic (e.g., weather/climate) or biotic (e.g., predators/parasites) environment; the variation in climate and arthropod communities documented in this report could thus give rise to variation in the nature and magnitude of selection over space and time. Finally, on a technical note, our results from this year's mark-release-recapture experiment at BTB suggest that our distance-sampling based estimates of population sizes could be underestimates. Additional work is needed to further test this.

Future Work

We will continue this study during the 2019 summer field season. We plan to collect *L. idas* samples and animal community samples (approximately 12 samples per site) at all eleven sites. We will perform mark-recapture-release at approximately three sites (BTB, USL, SKI).

Additional work from the 2018 field season is also ongoing. We will use methods designed to detect environment-dependent natural selection on genetic loci based on population genetic time-series data, that is, from data on allele frequencies at many loci in multiple populations samples across multiple generations. In this context, evidence of selection at the genetic-level is equivalent to evidence of standing genetic variation for environment-dependent Darwinian fitness (Darwinian fitness is a composite metric of survival and reproductive output). Estimates of genetic variation for climate adaptation, including the identities and frequencies of the specific alleles (genetic variants) involved, will then be used to parameterize models to predict future eco-evolutionary dynamics (as suggested by Bay et al., 2017).

We will use our own Bayesian Hidden Markov model approach and software (spatpg; Gompert, 2016) to test for environment-dependent selection based on the existing genetic data and the ecological and environmental covariates described above (including the data from the 2018 field season). Briefly, these methods work by asking whether consistent relationships exist between patterns of allele frequency change across sites and generations and patterns of environmental variation. We are particularly interested in re-

lationships with temperature, precipitation, and census population size. Posterior distributions of selection coefficients and coefficients describing the effect of environmental variables on selection will be examined to quantify the prevalence of environment-dependent selection across the genome. Posterior predictive checking and cross-validation will be used to assess the adequacy and accuracy of the model.

Genetic loci associated with body size, host-plant use and wing pattern have been identified (Gompert et al., 2015; Lucas et al., 2018); by scoring these same loci in the new samples we will be able to quantify standing genetic variation for these ecologically important traits. Multivariate ordination methods (e.g., PCA) and general linear models will be used to quantify and test associations among the census population size metrics, effective population sizes, and measures of standing genetic variation. Finally, analyses described in the previous paragraph will be repeated with genetic loci tied to these traits in an attempt to link sources of selection with the traits and genes under selection.

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