



Elucidating larval host preference in the endemic Hayden's ringlet butterfly (*Coenonympha haydenii*)

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Introduction

The Hayden's ringlet, *Coenonympha haydenii*, is an endemic species of butterfly that has recently undergone a notable decline in abundance. (Debinski 2013). Despite this decline, the basic life history of this species, including what host plant they feed upon, remains unknown.

- If their host is rare or patchy in distribution, habitat fragmentation and subsequent loss of gene flow may be placing the Hayden's ringlet at risk for further population decline.
- There is reason to believe that host specialization may be driving *C. haydenii*'s range restriction.
- Determining *C. haydenii*'s host plant, and the degree to which they are specialized on a particular host, is thus of crucial importance to the understanding the conservation risk faced by the Hayden's ringlet.



Figure 1. Hayden's ringlet, (*Coenonympha haydenii*.) Photo credit A. Springer.

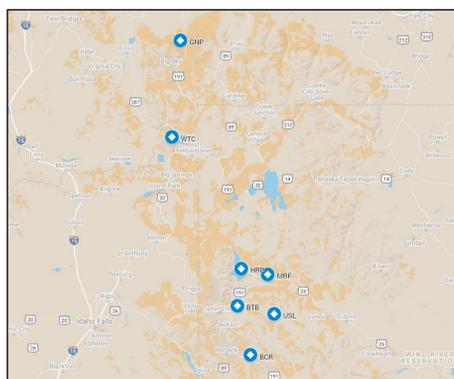


Figure 2. *C. Haydenii* collection sites

GNP- Garnet Point
 WTC-Watkins Creek
 HRP- Hermitage Point
 MRF- Mt. Randolph
 BTB-Blacktail Butte
 USL- Upper Slide Lake
 BCR- Bull Creek

Methods

1. Female *C. haydenii* specimens were collected from seven sites across Montana and Wyoming.
2. Specimens were placed in oviposition cups and eggs were acquired from each female.
3. Neonate larvae were placed in a petri dish equidistant from excised cuttings of three potential host species (Carex, Harebell, and Kentucky Bluegrass).
4. Images of the host samples were taken before and after herbivory. ImageJ software was used to quantify the area of each leaf, and the difference between the before and after images were calculated. The percentage eaten was then calculated for each sample.



Figure 3. An image of the grass cuttings after the herbivory assay

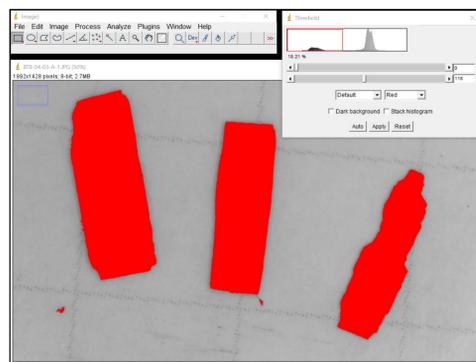
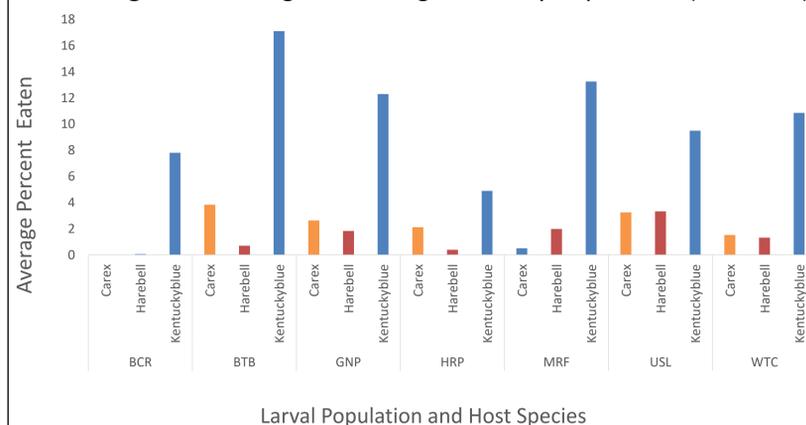


Figure 4. Using the Threshold tool in ImageJ to detect the total area of the leaves

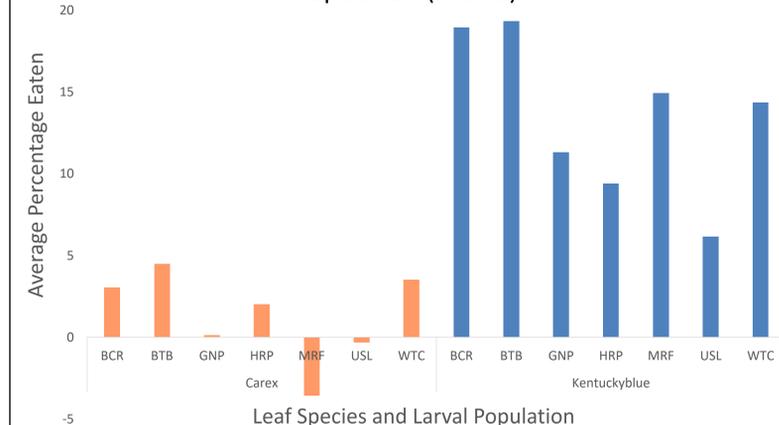
Results

Figure 5. Average Percentage Eaten by Population (Unscaled)



Because ImageJ was not able to detect leaf area to a small enough degree, some samples areas were larger in the after image than the before image. These samples represented measurement error rather than actual herbivory. To account for this, these sample differences were set to zero, and percentage eaten was averaged for each population and leaf species (see Figure 5).

Figure 6. Average Percentage Eaten by Population (Scaled)



Harebell (*Campanula rotundifolia*) samples were included in the larval assay as a control group where herbivory should theoretically be non-existent. The Harebell herbivory was used as a scaling factor to adjust the herbivory data for the other leaf species (see Figure 6). Negative percentage eaten indicates that some error remained. This graph shows how population herbivory varied within the two remaining leaves.

Conclusions and Future Directions

From this basic analysis, we have established that there is a strong larval preference for Kentucky Bluegrass, but the degree of herbivory varies across populations. We have also recognized the limitations of ImageJ when it comes to working with small scale herbivory.

We intend to conduct further statistical analysis using the data we have produced to illuminate additional patterns in the larval host preference. We also intend to use population genomic data to discover associations that may exist between genotypes and larval host preference.

References

Debinski, D., J. Caruthers, D. Cook, J. Crowley, and H. Wickham. 2013. Gradient-based habitat affinities predict species vulnerability to drought. *Ecology* 94: 1036-1045.

Acknowledgements

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