How does a lady butterfly choose where to lay her eggs?

The Case of the Baltimore Checkerspot Butterfly

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May 2015

Background

Life History.

The Baltimore checkerspot (*Euphydryas phaeton*) is a North American butterfly species that occupies territory ranging from Canada into the eastern United States (its southern limit lies in the mountains of Virginia and North Carolina). The flight period for the adult butterflies varies by latitude; adults fly in July in Massachusetts and live for approximately 1-3 weeks. Female butterflies lay clusters of eggs (up to several hundred) under the leaves of one of their two primary host plants, white turtlehead (*Chelone glabra*) and English plantain (*Plantago lanceolata*). The eggs hatch into larvae after a period of around twenty days. The newly hatched larvae begin to feed and form a communal nest on the leaves of their primary hosts. The larvae stop feeding in mid- to late-August; they will then thicken and enter diapause (a stage of suspended development) within their communal nests. In October, the larvae will move down from their diapause nest to the ground below and roll up in the debris with other larvae for the remainder of the winter. The larvae emerge beginning as early as March and start to feed again, diversifying to eat their "secondary" hosts. After a period of growth, the larvae form chrysalides and pupate; emerging as adults after a period of approximately two weeks.

Hosts Plants

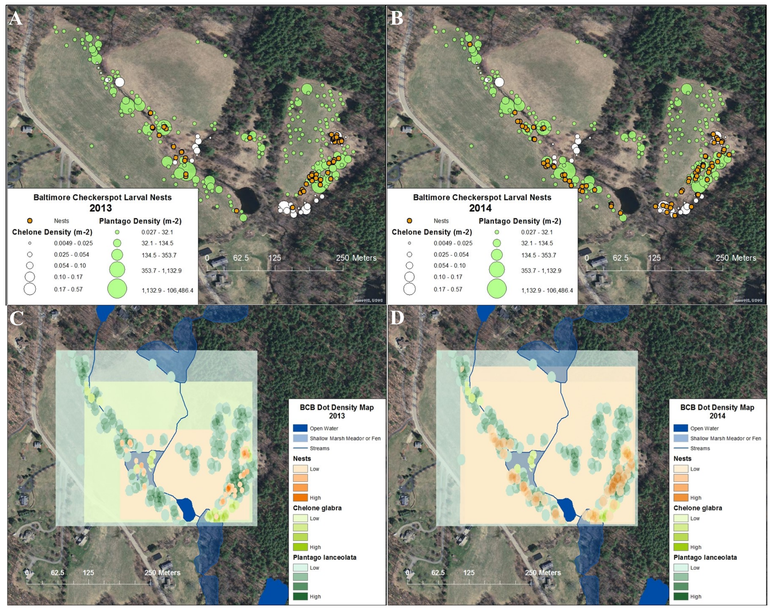
Baltimore checkerspot butterflies (BCB) native host plant is white turtlehead, an herbaceous, perennial plant found in freshwater wetlands. They sequester two types of iridoid glycosides, catalpol and aucubin, found in white turtlehead which makes them both unpalatable and emetic to birds. In their northern range, BCBs have adopted English plantain, a weedy herbaceous perennial, as a primary host plant. While English plantain also contains iridoid glycosides, the larvae raised on this plant are partially palatable to birds. BCB populations that adopt English plantain have a larger range, but are less defended and more prone to crashes.

Objectives:

A population of Baltimore checkerspot butterflies has been monitored in Harvard, Massachusetts by Dr. Elizabeth Crone (Associate Professor, Department of Biology, Tufts University) and her collaborators for the past three years (2012-present). They have compiled Global Positioning System (GPS) coordinates for BCB larval nests and their primary host plants. The goal of this project is to map and analyze the GPS coordinates to determine the following:

- **Question 1:** Is there a relationship between the location of Baltimore checkerspot larval nests and their two primary host plants?
- **Question 2:** Is there a relationship between the location of Baltimore checkerspot larval nests and local water sources?

The ability to identify what, if any, relationship exists will allow us to make informed guesses about the choices adult Baltimore checkerspot butterflies make when selecting where to lay their eggs on.



Methods:

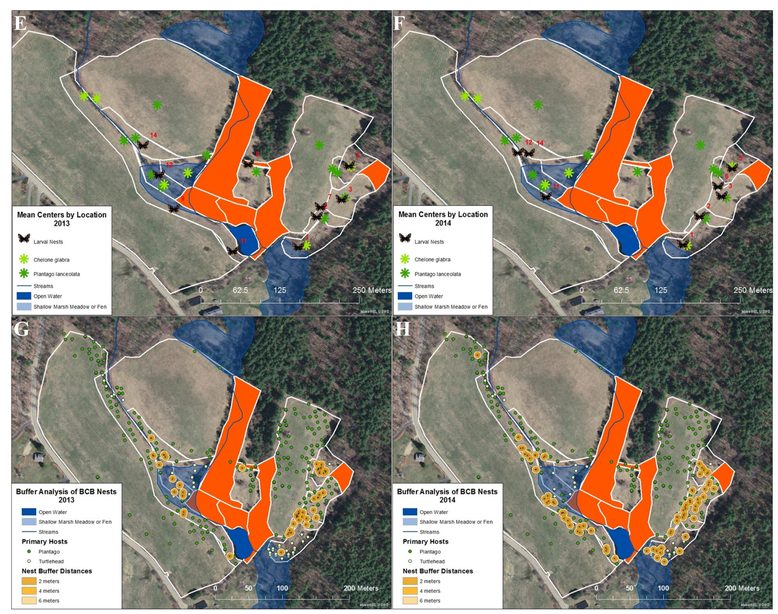
Data Collection

A. BCB Larval Nests: In both 2013 and 2014, the research site in Harvard, Massachusetts was surveyed in its entirety at minimum five times to locate and determine the coordinates of the BCB larval nests. The GPS coordinates for both 2013 and 2014 do not represent the total number of larval nests for each respective year, but most likely do provide an accurate assessment of their distribution.

B. BCB Primary Host: In 2013, Dr. Crone and her collaborators measured the three closest white turtlehead and English plantain plants within a 5x5 meter quadrat at 673 survey points. From that data, the relative abundance was estimated at each survey point..

Furthermore, the actual locations of the BCB primary host plants was estimated using the GPS coordinates of the survey points and the distance of the plants to the survey points.

GIS Analysis. The BCB larval nests were both non-randomly clustered in 2013 (Nearest Neighbor Ratio (NNR)=0.30, z-score = -10.7, $p<0.05$***) and 2014 (NNR=0.36, z-score = -10.7, $p<0.05$***). As the BCB larval nests are non-randomly distributed, the following tools were used for both qualitative and quantitative assessment in ArcGIS: Point Density, Buffer and Buffer Selection, and Mean Center (see Maps A-N).



Results:

Primary Hosts

- English plantain and white turtlehead are in discrete locations from one another (see **Maps A-B**).
- The BCB larval nests are located in areas with either dense populations of white turtlehead or English plantain (see **Maps A-H**, **Table 1**).

Table 1. Nearby BCB Primary Host Plants

Larval Host Plant	Buffer	Average # of Chelone glabra	Average # of Plantago lanceolata
Chelone	2 m	8	0
	4 m	10	2
	6 m	7	0
Plantago	2 m	0	22
	4 m	0	27
	6 m	0	36

Water Sources

- Approximately one third of the BCB larval nests are located within close proximity to one of three types of water sources (i.e. stream, open water, and shallow marsh, meadow, or fen) (see **Maps I-N**, **Table 2**).
- Approximately two thirds of the BCB larval nests are not located directly near one of the three types of water sources (i.e. 10m from a stream and/or 20m from open water and/or shallow marsh, meadow, or fen).

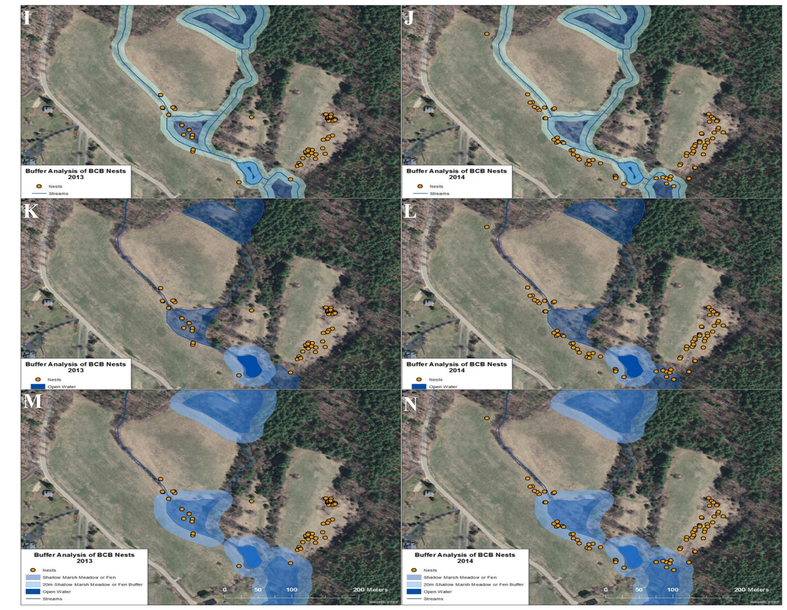


Table 2. Percentage of BCB Larval Nests Close to Water

Water Source	Average % of Nests
Stream	21.4
Open Water	4.06
Shallow Marsh, Meadow, or Fen	32.7
All	36.4

Discussion:

The location of the Baltimore checkerspot larval nests and the location and abundance of their primary hosts and the location of nearby water sources are related. However, it is not possible to conclude that they are necessarily correlated, or the extent to which one influences the other, due to several limitations imposed by the data and data collection methods used in the analysis. The major limitations include: (1) the dataset is confined to only two years, (2) the primary hosts were surveyed only in 2013 and abundance was treated as static, (3) the GPS coordinates of the host plants was estimated, (4) the location of the BCB larval nests could not be weighted, and (5) the distance to the water source is not necessarily the best measurement to determine the influence on the location and abundance of BCB larval nests.

Additional field research will be necessary to further our understanding of the relative importance of primary host abundance and water on the distribution of BCB larval nests. In 2015, I will plan to collect the following data to further analyze **Question 1**: (1) exact GPS coordinates of primary host plants using quadrat sampling, (2) plant density estimates for each designated area in the field site and for each larval nest, and (3) the number of caterpillars in each nest. For **Question 2**, I will take the following measurements: (1) average soil water potential for each area, (2) average water acidity for each area, and (3) average water content of the BCB larval primary host plants.

References:

1. Brian Gene Scholtens, "Host Plants and Habitats of the Baltimore Checkerspot Butterfly, Euphydryas Phaeton (Lepidoptera: Nymphalidae), in the Great Lakes Region," Great Lakes Entomologist 24, no. 4 (1991): 207–17.
2. Brian Gene Scholtens, "Prealighting Host Plant Location in the Baltimore Checkerspot Butterfly, Euphydryas Phaeton, and Its Implications for Host Range Evolution in Butterflies.," (Thesis, 1990), http://deepblue.lib.umich.edu/handle/2027.42/104172.
3. M. Deane Bowers, Nancy E. Stamp, and Sharon K. Collinge, "Early Stage of Host Range Expansion by a Specialist Herbivore, Euphydryas Phaeton (Nymphalidae)," Ecology 73, no. 2 (April 1, 1992): 526–36, doi:10.2307/1940758.
4. M. Deane Bowers and Leif Richardson, "Use of Two Oviposition Plants in Populations of Euphydryas phaeton Drury (Nymphalidae)," Journal of the Lepidopterists' Society 67, no. 4 (2013): 1–3.
5. NE Stamp, "New Oviposition Plant for Euphydryas Phaeton (Nymphalidae)," Journal of the Lepidopterists' Society 33, no. 3 (1979): 203–4.