

How Environmental Conditions and Changing Landscapes Influence the Survival and Reproduction of a Rare Butterfly, *Pieris virginiensis* (Pieridae)

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HOW ENVIRONMENTAL CONDITIONS AND CHANGING LANDSCAPES INFLUENCE THE SURVIVAL AND REPRODUCTION OF A RARE BUTTERFLY, *PIERIS VIRGINIENSIS* (PIERIDAE)**Additional key words:** climate change, novel host use, *Alliaria petiolata*

Pieris virginiensis Edwards, the West Virginia White butterfly, is a rare, univoltine butterfly native to riparian areas of mature forests in North America, from Wisconsin to Vermont, and as far south as northern Georgia and Alabama (Finnell & Lehn 2007). *Pieris virginiensis* has been considered in decline due to forest disturbance via logging, fragmentation, deer grazing pressure, and plant invasion (Finnell & Lehn 2007). It is considered rare, but has not yet been evaluated by the International Union for Conservation of Nature's Red List, and there are no long-term studies of *P. virginiensis* populations to confirm anecdotal observations of continual decline (IUCN 2012). Although there are butterfly monitoring organizations, *P. virginiensis* is frequently overlooked as it flies early in the spring in forested areas, which are not major sources of butterfly diversity and are not often regularly monitored.

Pieris virginiensis primarily uses the spring ephemeral mustard, *Cardamine diphylla* as its larval host plant, but also occasionally uses *Arabis laevigata*, a spring ephemeral biennial mustard. Sparsely distributed, *A. laevigata* is not an ideal host, but it is the primary host of *P. virginiensis* in a site in Marengo, OH, where *C. diphylla* is rare. An alternative host, *C. concatenata*, can be used but is not preferred due to its small size and early senescence (Shuey & Peacock 1989).

Courant et al. (1994) and Porter (1994) observed *P. virginiensis* females ovipositing on *Alliaria petiolata*, an invasive, shade-tolerant biennial mustard that is most likely toxic to emerging offspring. Bowden (1971) had no larvae survive after consumption of *A. petiolata*, and Porter (1994) had moderate mortality of larvae before his experiment ceased at the second instar. Several chemical constituents of *A. petiolata* leaves have been shown to deter feeding and reduce survival of first and fourth instars of *P. oleracea*, although *P. oleracea* populations that have been exposed to *A. petiolata* for 60–100 generations may be adapting to its chemical arsenal (Renwick et al. 2001, Keeler & Chew 2008). If *A. petiolata* is similarly toxic to young *P. virginiensis* caterpillars, adults may be wasting eggs on the plant. If *A. petiolata* deters feeding in older *P. virginiensis* caterpillars, caterpillars searching for a new host plant

after consuming their previous host may starve before reaching an appropriate native food source (Cappuccino & Kareiva 1985, Porter 1994).

Shuey and Peacock (1989) examined a population of *P. virginiensis* reproducing entirely on the alternative hosts, *A. laevigata* and *C. concatenata*. The study site is surrounded by agricultural fields, adjacent to Alum Creek in Morrow Co., Ohio. They examined plants in three locations along a roughly 150 meter section of woodland; a ridge above a shale embankment, the shale embankment, and a riparian area. They found that of the two hosts, *A. laevigata* was strongly preferred, perhaps because *A. laevigata* senesces later, increasing time available for larval development. In addition, more eggs were laid on the south-facing shale embankment than in the other two locations, perhaps because higher temperatures there increased caterpillar development. However, these authors conducted their study before the conversion of nearby agricultural areas to fallow fields, a surge in the abundance of deer, and the introduction of *A. petiolata* to the region (Shuey & Peacock 1989, Porter 1994, Stinson et al. 2006, Finnell & Lehn 2007, Ripple et al. 2010). Each of these subsequent changes to the habitat may have influenced the survival and reproduction of this isolated population of *P. virginiensis*.

Deer, whose populations in Ohio have steadily increased, may negatively influence the presence or quality of nectar sources and host plants for this rare butterfly (Ripple et al. 2010). *Pieris virginiensis* adults feed on a variety of nectar sources, including members of Claytonia, Trillium and Viola, as well as on flowers of their larval host plants (Bess 2005). Increased deer browsing may change the plant community, and in turn, alter habitat quality for *P. virginiensis*, although the effects of deer on butterflies are complex and life-history dependent (Feber et al. 2001).

Introduction of the invasive *A. petiolata* may have also had profound effects at Shuey and Peacock's study site. Poor oviposition choices could severely reduce this site's population that, in 1988, had only 14.8 % of eggs survive to fourth instar even on its native hosts (Shuey & Peacock 1989). *Alliaria petiolata* may also host potential egg/caterpillar predators, such as spiders or predatory ants, reducing survival of *P. virginiensis* on a potentially

novel host. In addition, *A. petiolata* is known to negatively influence plants around it through direct competition and allelopathy, reducing the frequency or quality of nearby nectar or host plants (Stinson et al. 2006).

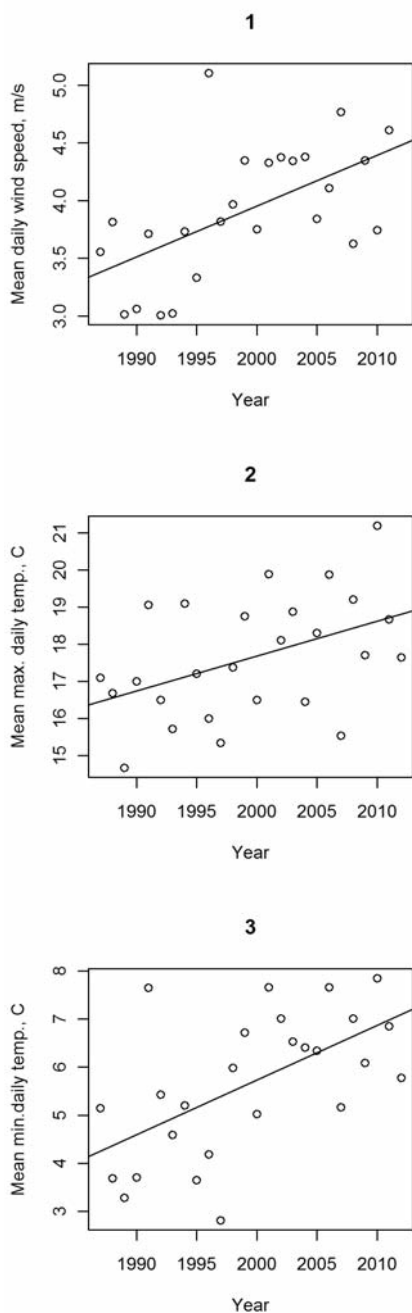


Fig. 1. FIGS. 1-3. (1) Mean daily wind speed (m/s) in April ($p < 0.01$); (2) mean daily maximum temperature (Celsius) in April ($p < 0.05$); and (3) mean daily minimum temperature (Celsius) in April ($p < 0.01$) at the Port Columbus International Airport weather station, Columbus, OH, 1987-2012, approximately 40 km. from the research location.

Finally, aberrant weather and long-term changing climatic conditions may influence *P. virginiensis* populations. Although many butterfly species are expected to increase under warming temperatures, butterflies that have strict habitat requirements or exist at the edge of their range may be at risk for population reduction and eventual extinction (Forister & Shapiro 2003, Forister & Fordyce 2011). *Pieris virginiensis* flies best in winds under 25 km/h and in temperatures between 19-30° Celsius (Cappuccino & Kareiva 1985, SD pers. obs.). To complicate matters, it is often the previous year's weather that has the most effect on butterfly population in the following year (Roy et al. 2001).

We investigated *P. virginiensis* survival and reproduction over two field seasons (2011-2012) in a habitat which previously hosted a robust population of *P. virginiensis*, to answer the following questions: Does successful reproduction occur at this location? Does *P. virginiensis* differentially use *A. petiolata* and *A. laevigata*? How frequently does non-caterpillar damage (e.g. deer herbivory) occur to host plants? What is the frequency of potential predators on all possible host plants? Have climatic conditions relevant to suitable flying conditions changed over time at this site, and have aberrant weather patterns been noted?

Methods. The study site, in Morrow County, Ohio, is a privately owned forest fragment bordering Alum Creek adjacent to two fallow fields, which was originally surveyed by Shuey and Peacock (1989). On April 21, 2011, we surveyed the site and found 5 flying adult *P. virginiensis* individuals, of which two were collected for identification and further study in the laboratory. This was the only occasion that we observed flying adults in 2011, but this confirmed that *P. virginiensis* still persisted in this location.

In 2011, mimicking Shuey and Peacock (1989), we systematically searched for and tagged flowering stalks of both the native *A. laevigata* ($n=64$) and the invasive *A. petiolata* ($n=54$) on April 21, 2012, and returned twice to score plants (May 5 and May 11) for the presence of *P. virginiensis* eggs or caterpillars, potential predators (ants, spiders), and herbivore damage (deer or other). We chose not to survey *C. concatenata* because it was a minor host in 1987. Plants were examined at the same ridge and shale embankment zones studied by Shuey and Peacock (1989), but were not systematically examined in the lowland-areas, as only one egg was found during their study in the lowland zone. Casual observations in the lowland zone revealed no eggs or caterpillars. During tagging and scoring events after the initial site visit, we searched visually for flying *P. virginiensis* adults. We only conducted search events on

days appropriate for butterfly flight (temperature above 10° Celsius, wind speed under 25 km/hr) to maximize our chances of witnessing oviposition events.

In 2012, during a warmer spring than in 2011, flowering stalks of both the native, *A. laevigata* (n = 113.6 ± 26.85 plants searched per visit) and the invasive, *A. petiolata* (n = 95 ± 34.53 plants searched per visit) were tagged (March 30) and scored weekly (April 6, 13, 20, 27; May 4) using methods identical to 2011. In addition to these tagged plants, any unmarked plants found during repeated random searching were scored, but unmarked. During tagging and scoring events, we searched visually for flying *P. virginiensis* adults, but could confirm none, as the individuals seen may have been *P. rapae* adults.

The 2011–2012 scoring data were converted to presence/absence values and fit to one of several binomial regressions in R (R Development Core Team 2011). Year (2011 or 2012) and host plant (*Arabis* or *Alliaria*) were used as predictors for the presence or absence of deer damage, other herbivorous damage, and potential predators.

In 2011, the captured adult butterflies were kept together in a 0.216 m³ enclosure and allowed to feed from a 10% (v/v) sugar:water solution, and placed on a 16:8 hr light/dark cycle under fluorescent lights. These butterflies were given the choice of individual flowering *C. diphylla* (collected from Pennsylvania), *C. concatenata* (collected from Dayton, OH), or *A. petiolata* (collected from Dayton, OH) as oviposition substrate. We examined each plant daily for eggs until the butterflies died.

In 2011, emerging caterpillars (n=4) were allowed to hatch and feed on *C. diphylla*, *A. laevigata* (collected from Yellow Springs, OH), or *A. petiolata*. All four caterpillars were initially fed on *C. diphylla*, but were divided evenly and transferred to either *A. laevigata* or *A. petiolata* at the 4th instar for a no-choice survival test.

In addition to these field-collected variables, we examined weather data from the Port Columbus International Airport Weather Station (about 40 km from research location) to evaluate if there were any

trends in weather during the month of April (*P. virginiensis* flight season) between 1987, the year preceding the Shuey and Peacock (1989) study, and 2012. We analyzed climatic variables from 1987 to present using simple linear regression. All statistical analyses were completed in R 2.15 (R Development Core Team 2011).

2011 Results. Despite multiple visits to the study site, we recovered no *Pieris virginiensis* eggs, no caterpillars, and found little damage that could be attributed to caterpillar herbivory (Table 1). Furthermore, we witnessed no flying adults after April 21, 2011. In addition to these direct observations, indirect observations of host-plant conditions suggested no *Pieris*- related herbivory, although there was occasional incidence of leaf or stem damage from deer (*Arabis*: 5.47%, *Alliaria*: 4.63%) or other organisms (*Arabis*: 5.47%, *Alliaria*: 12.96%). Ants and spiders (*Arabis*: 4.69%, *Alliaria*: 17.5%) were observed on both study species.

When captured adult butterflies were given the choice between three potential host plants, all eleven eggs were oviposited on *A. petiolata*, the invasive mustard. These data were pooled, as the adult butterflies were not separated. The four surviving caterpillars readily consumed native mustard tissue, but those placed on *A. petiolata* only consumed a small amount of tissue, then would enter a quiescent state during which they refused to eat *A. petiolata*, but would resume eating when placed on *C. diphylla*.

2012 Results. In 2012, we began our search in March when unusually warm weather facilitated early plant and butterfly emergence. We found one egg on an *Arabis* plant, but saw no confirmed *P. virginiensis* butterflies, and occasionally witnessed *P. rapae* individuals flying through the woodlands. The egg was not removed for identification, and a week later, although there was minor herbivory to the *Arabis* plant where the egg was found, no larva was recovered (Table 1). In addition, there was one pierid caterpillar recovered, from a second-year (flowering) *Alliaria* individual, however, the caterpillar was small and we

TABLE 1. Number of eggs located on plants in Morrow Co., OH, in 1988 (Shuey and Peacock 1989), 2011, and 2012.

| | <i>C. concatenata</i> ¹ | <i>A. laevigata</i> | <i>A. petiolata</i> |
|------|------------------------------------|---------------------|---------------------|
| 1988 | 21 (n=68) | 102 (n=52) | - |
| 2011 | - | 0 (n=64) | 0 (n=54) |
| 2012 | - | 1 (n=114) | 0 (n=81) |

¹ Cardamine concatenata was not searched in 2011 and 2012 due to low incidence of egg deposition in 1988. Numbers are as follows: Eggs found (n=total plants searched).

were unable to confirm its identity as either *P. virginienensis* or *P. rapae*. The incidence of leaf or stem damage from deer (*Arabis*: 2.29%, *Alliaria*: 0.63%) or other organisms (*Arabis*: 8.27%, *Alliaria*: 3.58%), as well as ants and spiders (*Arabis*: 7.75%, *Alliaria*: 9.68%) was low on both study plant species.

Combined scoring for 2011 and 2012. The presence of deer damage was affected by both host plant species and year, with a model:

$$(1) \text{Deer Damage} = -1.0529 * \text{Year} - 1.0066 * \text{Plant Species} - 2.7648 + \text{Error}$$

All factors in the model were significant, and it was more likely for us to find deer-browsed *Arabis* plants than *Alliaria* plants, and more deer damage in 2011 than 2012 ($p < 0.05$ for Year, Plant).

The presence of other herbivorous damage was predicted by host plant species, but not year, with *Arabis* having a higher incidence of damage being present ($p < 0.01$):

$$(2) \text{Other Damage} = -0.6712 * \text{Plant Species} - 2.2026 + \text{Error}$$

Finally, predator presence could not be predicted by either host plant species or year.

Weather Analysis. Linear regressions across all years (1987–2012) indicated significant increases through time in average wind speed, maximum temperature, and minimum temperature in the month of April (Figures 1–3).

Discussion. In this study, we investigated the survival and reproductive success of *P. virginienensis* at a site in Ohio last evaluated in 1988 (Shuey & Peacock 1989). It is clear that at this site, *P. virginienensis* is not successfully using either a native host, *A. laevigata*, or an invasive host, *A. petiolata*. We found only one egg on over 150 plants repeatedly searched in two years at this location (Shuey and Peacock 1989). This suggests that *P. virginienensis* at this site may, in the near future, face local extinction.

It is possible that sometime in the intervening 24 years *P. virginienensis* may have attempted to shift to *A. petiolata*. In a scenario where *A. petiolata* was the only novel introduction to this location, we would perhaps conclude that *A. petiolata* had a detrimental effect, but the clear decline in this population could be due to any number of other factors, including changes in nectar source or host plant quality, deer damage, or climate. We found evidence of low to moderate damage from both deer and other sources, as well as a significant presence of potential predators (ants and spiders).

Although there were no differences in predator presence by host plant species, *A. laevigata* plants had a higher incidence of both deer browsing and other, non-caterpillar herbivory than *A. petiolata*.

We believe aberrant weather in 2011 and 2012 caused two recent years of failed *P. virginienensis* reproduction at this location. If it is too cool, wet, or windy, the univoltine *P. virginienensis* cannot fly or reproduce. These recent unusual weather patterns may soon become a chronic issue for this butterfly under predictions of global climate change. Evidence from other butterfly population studies indicates that although some butterflies benefit from warming global temperatures, others may suffer (Sparks & Yates 1997, Roy et al. 2001, Forister & Shapiro 2003). Severe weather could have particularly strong effects on butterfly populations at the edge of their acceptable weather ranges. Furthermore, butterflies with low population numbers are more at risk for local extinction events when faced with multiple “bad” years and the lack of carry-over pupae (Forister & Fordyce 2011).

Climate data demonstrate a linear increase in temperature and wind speed over the last 23 years that may have influenced these butterflies (Figures 1–3). Cappuccino and Kareiva (1985) showed that *P. virginienensis* has a difficult time flying in strong wind speeds, or in cool weather. Many days in an adult *P. virginienensis* lifespan were not ideal for flight in the 1980s, and although warming springs may provide more favorable flying weather, an increase in windiness may ameliorate any benefits of climate change for *P. virginienensis* (Cappuccino & Kareiva 1985, Doak et al. 2006). The spring of 2011 was remarkable for rainy, poor weather in Columbus, OH, with a record of 18.1 cm of precipitation in April. The spring of 2012 had excessively warm temperatures that facilitated early plant and butterfly emergence. As the probability of extreme or unusual climate events increases, we expect further disruptions in *P. virginienensis* populations across its range.

Alliaria petiolata may further contribute to *P. virginienensis* decline by serving as a population sink, however, despite the observations made by Courant et al. (1994) and Porter (1994), no one has yet determined how frequently this occurs and the risk it poses for *P. virginienensis*. Our limited lab data suggest that *P. virginienensis* adults will oviposit on *A. petiolata*, but caterpillars refuse to feed on *A. petiolata* in the fourth instar. While we are unable to confirm the role of *A. petiolata* in *P. virginienensis* decline at this site, we believe that severe or chronic weather anomalies, like the cool and wet spring of 2011 may negatively influence butterfly populations, as was seen in other studies. In

addition, selective herbivory of nectar and larval host plants by deer may directly and indirectly contribute to *P. virginiensis* decline. Future studies will include more observations at this location, as well as expansion into other locations to investigate the direct and indirect impacts of deer, predators, weather and climate patterns, and *A. petiolata* on the *P. virginiensis* life cycle.

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