

2018

Range-Wide Variation in Common Milkweed Traits and its Effect on Larvae of the Monarch Butterfly

David De La Mater

William & Mary - Arts & Sciences, dsdelamater@gmail.com

Follow this and additional works at: <https://scholarworks.wm.edu/etd>



Part of the [Biology Commons](#), and the [Ecology and Evolutionary Biology Commons](#)

Recommended Citation

De La Mater, David, "Range-Wide Variation in Common Milkweed Traits and its Effect on Larvae of the Monarch Butterfly" (2018). *Dissertations, Theses, and Masters Projects*. William & Mary. Paper 1550153884.

<https://doi.org/10.1002/ajb2.1630>

This Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

Range-Wide Variation in Common Milkweed Traits
and Its Effect on Larvae of the Monarch Butterfly

David S. De La Mater III

Ballston Spa, New York

Bachelor of Science, State University of New York at Plattsburgh, 2016

A Thesis presented to the Graduate Faculty of The College of William & Mary in
Candidacy for the Degree of Master of Science

Department of Biology

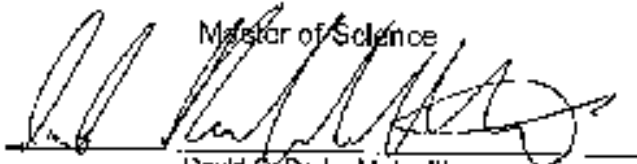
College of William & Mary
August, 2018

© Copyright by David S. De La Mater III 2018

APPROVAL PAGE

This thesis is submitted in partial fulfillment of
the requirements for the degree of

Master of Science




David S. De La Mater III

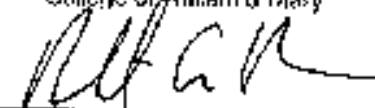
Approved by the Committee July 2018

 for Harmon Dalglish

Committee Chair
Assistant Professor Harmon Dalglish, Biology
College of William & Mary



Assistant Professor Joshua Puzay, Biology
College of William & Mary



Director Robert Rose, Center for Geospatial Analysis
College of William & Mary

ABSTRACT

Plants play an important role in structuring ecological communities from the bottom up through interactions with herbivores, and environmental variation can affect these interactions. We use the interaction between common milkweed (*Asclepias syriaca*) and the monarch butterfly (*Danaus plexippus*) to examine 1) the role of environmental variation in dictating plants traits, and 2) how those variations affect herbivores. We quantified intraspecific trait variation in 53 natural common milkweed populations, then remeasured these traits when population representatives were regrown in a common garden to control for environmental variation. We then measured growth, performance, and survival of monarch larvae feeding on these same plants. Our findings indicate distinct spatial patterns in traits throughout the range of *A. syriaca*, but these patterns dissipate when genets are regrown in a common environment. When monarch larvae are raised on these milkweeds, those fed on plants from the Northeast gain more weight than those fed on plants from the Northcentral and Southcentral regions. These results can better inform monarch conservation efforts; current conservation efforts have been focused on milkweed restoration in the Midwest, but an increased focus on milkweed restoration in the Northeast may be beneficial. Furthermore, we demonstrated plasticity in specific plant traits in response to environmental change, which could have theoretical implications in light of current and projected changes in climate.

TABLE OF CONTENTS

Acknowledgements	ii
List of Tables	iii
List of Figures	iv
Chapter 1. Milkweed Variation	1
Chapter 2. Monarch Response	30
Appendix	47
References	57

ACKNOWLEDGEMENTS

This writer wishes to express his appreciation to Dr. Harmony Dagleish, under whose guidance this investigation was conducted, for her patience, guidance and criticism throughout the investigation. The author is also indebted to Drs. Joshua Puzey and Robert Rose for their careful reading and criticism of the manuscript.

LIST OF TABLES

1. Table 1: Results of a general linear mixed model, with plant traits as response variables, latitude and longitude as fixed effects, and population as a random effect.	19
2. Table 2: Results of linear regression analyses of monarch larvae measurements and specific plant traits.	39
3. Table S1: Results of ANOVA analyses of environmental variables between regions.	50
4. Table S2: Results of linear regression analyses of environmental variables with latitude and longitude.	50
5. Table S3: Results of ANOVA analyses of plant traits between regions.	52
6. Table S4: Results of ANOVA analyses of plant traits measured at each timepoint.	53
7. Table S5: Results of linear regression analyses of plant traits measured on individuals in the field and in the greenhouse.	54
8. Table S6: Results of ANOVA analyses of monarch measurements when fed milkweed from different regions.	55
9. Table S7: Results of general linear mixed model, with monarch measurements as response variables, latitude and longitude of plant food source location as fixed effects, and plant food population as a random effect.	56

LIST OF FIGURES

1. Figure 1: Map of all sample sites and region partitioning.	10
2. Figure 2: Boxplot diagram of principal component axes of environmental variables and their variation between regions.	14
3. Figure 3: Scatterplots with fitted linear regressions for milkweed trait variation along latitudinal and longitudinal gradients.	18
4. Figure 4: Comparison of milkweed trait values of individuals as measured in the field and at 12 months in a common garden.	22
5. Figure 5: Scatterplots with fitted linear regressions for monarch measurements and milkweed traits.	39
6. Figure S1: Biplot of the first two principal component axes of environmental variables.	51
7. Figure S2: Scatterplots with fitted linear regressions for environmental variation along latitudinal and longitudinal axes.	51
8. Figure S3: Biplot of first two principal component axes of milkweed traits as measured in the field.	52
9. Figure S4: Line graph of plant traits changing values over time in a common garden.	53
10. Figure S5: Biplots of plant traits at each timepoint.	54
11. Figure S6: Boxplot diagram of monarch weight gain variation between plant food source regions.	55
12. Figure S7: Correlation matrix of plant traits measured at 12 months in a common garden and monarch measurements.	56

CHAPTER 1: MILKWEED VARIATION

Introduction

Heritable intraspecific variation of traits is a fundamental basis of evolution by natural selection. Intraspecific variation is often the result of environmental heterogeneity, which may or may not produce ecotypes of a species. Ecotypes are populations or groups of populations which are made up of individuals that occupy different geographic regions or different environments that have observably different phenotypes which are maintained when grown in uniform conditions, yet are still capable of interbreeding with no loss of fitness or fertility in offspring (Turesson 1922a; Turesson 1922b; Stebbins 1950). Alternatively, intraspecific variation may be a manifestation of phenotypic plasticity in a species, with patterns of phenotypes existing as a reflection of patterns of environmental variation (Turesson 1922a; Clausen et al. 1948; Núñez-Farfán & Schlichting 2001; Stebbins 1950; Turesson 1922b; Nicotra et al. 2010). In the case of true ecotypes, to what extent they are actually discrete units of populations and not separate segments of a broader gradient of phenotypes is often not clear due to the common practice of sampling few, far apart locations, which may provide an illusion of discrete ecotypic units (Núñez-Farfán & Schlichting 2001; Clausen et al. 1948; Stebbins 1950). These gradients of phenotypic values may occur directionally along environmental gradients in what are called “clines” (Huxley 1938; Huxley 1939). The logistic difficulty of sampling

many locations along environmental gradients is likely a major contributing factor to this confusion.

In order to determine if populations are an ecotype, one must grow suspected ecotypes in uniform conditions in a “common garden experiment” (Clausen et al. 1948; Núñez-Farfán & Schlichting 2001; Turesson 1922a; Oleksyn et al. 1998). If the phenotypic differences observed in natural populations persist in a common garden, they are genetically controlled and represent ecotypes. If these phenotypic differences do not persist in a common garden, then they are determined by the environment and the populations are not ecotypes. Common garden experiments were instrumental to the modern evolutionary synthesis of the 20th century (Stebbins 1950; Núñez-Farfán & Schlichting 2001; Clausen et al. 1948), and can be a valuable way to investigate the role that environmental variation plays in dictating plant traits.

Further investigation into the role of environmental variation in driving intraspecific trait variation is newly important in light of current and projected changes in climate: global temperatures will increase, temperature and precipitation patterns will change, sea levels will rise, snow melt will occur earlier and growing seasons will lengthen (IPCC 2014; Karl et al. 2009). These environmental changes will occur heterogeneously across the biosphere creating heterogeneous changes in physiology, range, phenology, and productivity of plants at the individual and population level resulting in changes in the

composition and net productivity at the ecosystem level (Melillo et al. 1993; Walther et al. 2002; Root et al. 2003; Thuiller et al. 2005). Phenotypic plasticity is a crucial mechanism through which plants can adapt to changing environmental conditions, but to what extent plasticity can mitigate effects of environmental variation on plants is not clear (Nicolson et al. 2010). It is important for us to understand how environmental changes will affect ecosystems so that we can respond to and possibly mitigate such environmental effects on ecosystems and their services. Because these effects initially act at the individual and population level, further investigation into the mechanistic effects of environmental variation on the traits of producers will allow us to better understand how environmentally-driven trait changes will manifest at the population and ecosystem level.

Trait-based studies are advantageous because trait measurements can be used to compare both within and between species, as well as between studies (Webb et al. 2010). In studies of individuals occupying different environments, quantitative trait measurements can be related to quantitative environmental variables in order to provide possible explanations for intraspecific variation that would otherwise be dismissed as noise in the data (McGill et al. 2006). Because trait measurements can be used to compare species and studies, this approach provides the potential for broad applicability of conclusions (Webb et al. 2010). A trait-based quantitative approach to examining the effect of environment on plant traits also lends itself to a predictive modeling framework, opening up future possibilities of using environmental variables to predict vital rates and population

dynamics, or to provide general predictions for policymakers (McGill et al. 2006). In order to lay the groundwork for such studies, a large-scale trait-based sampling effort would first need to be conducted on a species that is widespread enough to occur through broad environmental gradients and common enough to be easily located and sampled, with traits that are easily measured. Combining a large-scale sampling effort with a common garden experiment would elucidate the role of genetics and environment in generating range-wide patterns of plant traits.

Common milkweed (*Asclepias syriaca*) is an abundant, clonal, perennial herb, native to eastern US and Canada, that can be often found growing in patches in the middle of a field or clearing, on the side of roads or highways, or on the edge of agricultural fields (Figure 1). Some key traits that we can measure in common milkweed are carbon, nitrogen (and carbon-nitrogen ratio, or "C/N"), chlorophyll, photochemical reflectance index ("PRI," a measure of photosynthetic compounds other than chlorophyll), cardiac glycosides ("cardenolides," toxic secondary metabolites used as a chemical defense against herbivory), latex (a sticky sap that serves as a mechanical defense against herbivory), cellulose, lignin, leaf mass per area ("LMA"), normalized difference water index ("NDWI," a measure of how much water a leaf contains), number of leaves, plant height, and growth rate (both of leaves and of height). Some of these traits are easily measured in the field (height, number of leaves, growth rates, and latex), but most of them are traditionally difficult to measure since they are chemical and structural properties

of leaves, and thus not easily observed and require destructive sampling. However, recent strides have been made in the field of spectroscopy, which have provided us with effective tools and methods of quickly and accurately estimating foliar chemical and structural properties at a fraction of the time, cost, and labor that traditional chemical analyses would take (Couture et al. 2015; John J. Couture et al. 2013; Foley et al. 1998; Asner & Martin 2008; Serbin et al. 2014). *Asclepias syriaca* is also a species of conservation concern due to its role as a crucial host plant for larvae of the eastern migrating monarch (*Danaus plexippus*), which has been declining over the past 30 years (Brower et al. 2012; Flockhart et al. 2015). This connection to conservation interests has resulted in a substantial body of literature. However, much of this literature focuses on common milkweed's physiology, its loss from the landscape, its secondary metabolites, and its interactions with various organisms (Pleasants 2016; Pleasants & Oberhauser 2013; Malcolm & Zalucki 1996; Hunter et al. 1996; Malcolm 1994; John J. Couture et al. 2013; Couture et al. 2010; Couture et al. 2015; Vannette et al. 2013; Agrawal 2005; Hartzler 2010; Van Zandt & Agrawal 2004; Agrawal et al. 2014; Erwin et al. 2014; Malcolm et al. 1989; Bingham & Agrawal 2010a; Züst & Agrawal 2016; Wyatt et al. 1993; Woodson et al. 1954). Few studies address the issue intraspecific variation in common milkweed; whether there is significant variation in common milkweed across its vast range, whether variation occurs discretely in response to geographic barriers or continuously along environmental gradients, and whether purported variation is

heritable (i.e. genetically controlled ecotypes) or driven by environment. This paper attempts to address those concerns.

Much of what is known about intraspecific variation in common milkweed and the relative importance of environment in driving it comes from a series of common garden experiments conducted by Woods et al. in 2012. Woods et al. conducted a common garden experiment in which they planted seed collected from 22 populations spanning across the Eastern latitudinal expanse of *A. syriaca* range into three common gardens representing range center and northern and southern range edges. They measured traits of plants growing in common gardens and discovered significant heritable clines in common milkweed growth and defense traits that were well explained by variation in precipitation and latitude. They found that latex, root-to-shoot ratio, root buds, and early season height increased with latitude while shoot biomass decreased with latitude; in other words, northern plants invested more in defense and below-ground biomass. Their results indicate that common milkweed shows heritable intraspecific variation in growth and defense strategies that may indicate adaptation to differing environmental conditions throughout its range. In addition, Woods et al. (2012) found that cardenolides exhibited a marginally significant positive relationship with latitude, but only in the first year of growth – in the second year of growth, this trend was no longer detected. An increase in cardenolide concentration with latitude has been reported in previous studies of natural populations, but has only weak evidence (Malcolm 1994; Hunter et al. 1996). This pattern is particularly

interesting because it runs opposite of what we would expect from theories regarding latitudinal patterns in herbivory and defense, and runs opposite of genus-wide patterns in *Asclepias*: species from regions closer to the equator are more toxic than their congeners from higher latitudes (Rasmann & Agrawal 2011b).

In this study, we undertook the largest sampling effort of common milkweed natural populations to date: we sampled 53 populations covering the entire native range of common milkweed in the US; from Maine to Alabama, from North Dakota to Oklahoma (Figure 1). We aimed to uncover patterns of variation in plant traits among geographic regions and along environmental gradients. Our broad questions at the start of this study were:

1) What are the patterns of trait variation in *A. syriaca* throughout its entire range? Is there significant intraspecific variation in important traits, or are traits relatively uniform throughout the range? Does variation occur discretely in response to geographic barriers or does variation occur continuously, forming trait clines throughout the range?

We predicted more growth and a higher growth rate in the south and we expected to find a pattern of increasing defense in the north, aiming to resolve the issue of whether cardenolide concentrations do indeed increase along a latitudinal gradient (Hunter et al. 1996; Malcolm 1994; Woods et al. 2012). In an

attempt to link studies on regional and temporal patterns of monarch population dynamics (Stenoien et al. 2015; Flockhart et al. 2015; Inamine et al. 2016) with milkweed trait variation and possible novel explanations for patterns in trait variation as a result of glacial refugia (Soltis et al. 2006), we predicted that we'd find significant variation in traits between distinct geographic regions bounded by the Appalachian Mountains and the Mississippi River.

2) Do ecotypes of common milkweed exist? In other words, is observed phenotypic variation controlled genetically or by environmental variables?

We predicted that trait variation would be largely environmentally controlled, but that important growth and defense traits (such as C/N, height, cardenolides, and latex) would be genetically controlled.

Methods

Field Collection. Throughout the summer of 2016 (June, July, and August), 53 populations of common milkweed were sampled, spanning the entire North American range of *A. syriaca* (Figure 1). For each population, two ramets were chosen for destructive sampling on opposite sides of the population in order to minimize likelihood that they were the same genet. Three more ramets were haphazardly chosen at roughly equal intervals between the two destructive harvest ramets, for a total of five ramets sampled per population. For each ramet, we measured several traits. Observable physical traits consisted of apical height

(cm) and number of leaves. We measured latex content by pre-massing filter paper inside of microcentrifuge tubes, then cutting one centimeter off of one topmost fully expanded leaf and draining the exuded latex onto the filter paper until it stopped flowing, and re-massing the filter paper and microcentrifuge tube. The difference in mass gave a measurement for latex exudation, which is a measure that has been used in other studies, and has been shown to be indicative of herbivore resistance (Bingham & Agrawal 2010b; Van Zandt & Agrawal 2004; Woods et al. 2012). Foliar traits were estimated by spectroscopy, using a portable spectroradiometer (HR-1024i, Spectra Vista Corporation, Poughkeepsie, NY, USA), and replicating methods outlined in Couture et al. (2013) and Couture et al. (2015). Two leaves, just below the uppermost fully expanded leaves, were scanned two times each. From these spectra, we calculated foliar nitrogen (N; percent dry mass), carbon (C; percent dry mass), lignin (percent dry mass), chlorophyll (g/m^2), cellulose (percent dry mass), leaf mass per area (LMA; g/m^2), normalized difference water index (NDWI; a unitless ratio), and cardiac glycoside (cardenolide) concentration ($\mu\text{g/mg}$). We derived C/N ratios from C and N measurements, and we derived growth rates – height per growing degree day (height/GDD) and number of leaves per growing degree day (leaves/GDD) – by dividing measurements by the total number of growing degree days that had occurred at a population location at the time of sampling (method of growing degree calculations are explained below). Rootstock was collected from the two destructive harvest ramets at opposite ends of the population and soil was collected from below the sampled roots for analysis

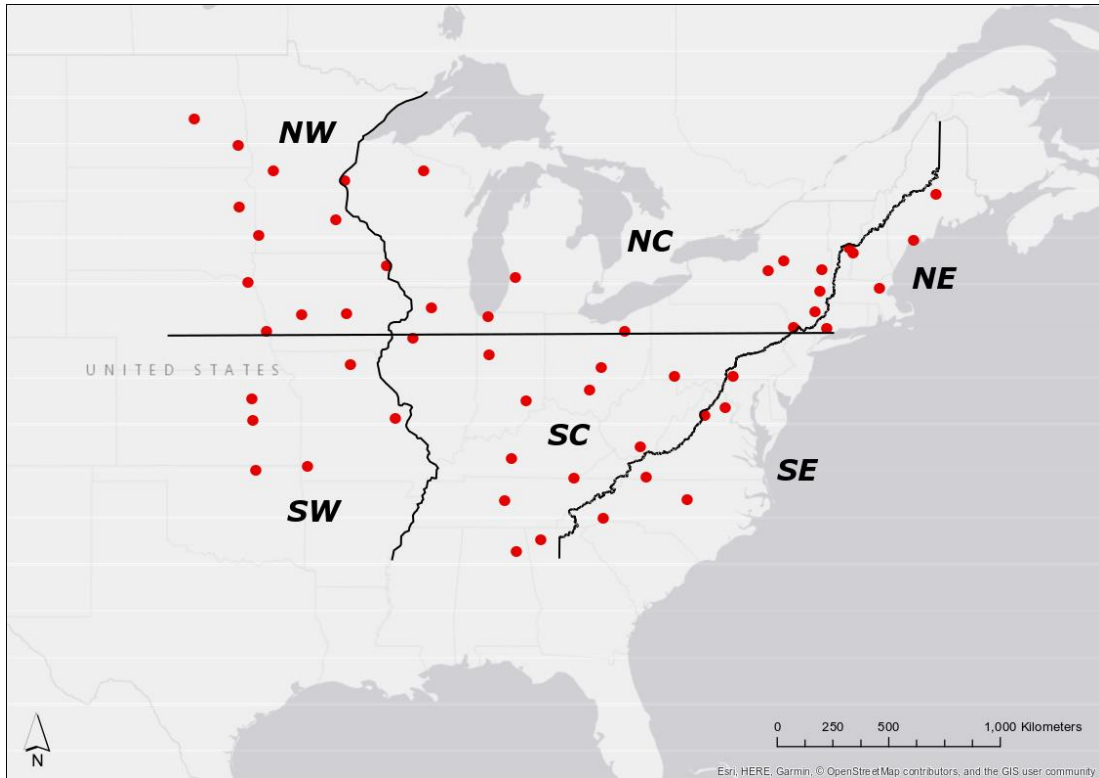


Figure 1: Map of all sample sites ($N = 53$) and region partitioning. Regions were created partly based on geographic barriers purported to be important drivers of variation in other species (Soltis et al. 2006) and partly based on regions used in studies of monarch population dynamics (Flockhart et al. 2015; Ries et al. 2015; Stenoien et al. 2015; Inamine et al. 2016). Regions are *Northwest (NW*; north of 41°N and west of the Mississippi River; $n = 11$), *Northcentral (NC*; north of 41°N, east of the Mississippi River, and west of the Appalachian Mountains; $n = 12$), *Northeast (NE*; north of 41°N and east of the Appalachian Mountains; $n = 6$), *Southwest (SW*; south of 41°N and west of the Mississippi River; $n = 6$), *Southcentral (SC*; south of 41°N, east of the Mississippi River, and west of the Appalachian Mountains; $n = 12$), and *Southeast (SE*; south of 41°N and east of the Appalachian Mountains; $n = 6$).

(described below). Root stock was transported on ice in a cooler and replanted in a greenhouse at the College of William & Mary, in Williamsburg, Virginia. We measured herbivory on a plant-by-plant basis, but considered it an *environmental* variable, as it is not a trait exhibited by the plant. Herbivory was quantified on each leaf by assigning it a score on a scale of 0-6 according to percent of leaf tissue removed (0=intact, 1=1-5% removed, 2=6-24% removed, 3=25-50% removed, 4=51-75% removed, 5=76-99% removed, 6=only the petiole remains).

Environmental Data. Weather and climate data were obtained from the PRISM Climate Group (Oregon State University). These data are derived from the Parameter-elevation Relationships on Independent Slopes Model (PRISM), which provides estimates of weather and climate data for specific geographic locations (Daly et al. 2008). This allowed us to use weather and climate estimates for the exact geographic location of our milkweed populations, rather than rely on data from the nearest weather stations, which were often too far away and do not report consistent weather and climate measurements between stations. More about this model, and the PRISM Climate Group, can be found at their website (<http://prism.oregonstate.edu>). The data obtained were 2016 cumulative precipitation (from 15 January – 15 July), 2016 daily minimum temperature (from 15 January – 15 July), 2016 daily maximum temperature (from 15 January – 15 July), 30-year average annual cumulative precipitation (1981 – 2010), 30-year average daily minimum temperature, 30-year average daily maximum temperature, and 30-year average daily mean temperature. From these 2016 estimates, we were able to derive estimates of the number of growing degree days (GDD) that had occurred at each population at the time of sampling (from 15 January until the exact date of sampling).

Percent sand, silt, and clay was determined from soil sampled from beneath collected root stock, using a simple soil textural analysis method, as described by Kettler et al. (2001). Percent soil dry weight that is carbon, hydrogen, and nitrogen was determined using an organic elemental analyzer (2400 series

elemental analyzer, PerkinElmer). Percent soil dry weight that is phosphorous was determined using an ashing and acid hydrolysis method as described by Chambers and Fourqurean (1991).

Greenhouse Common Garden. Plants were regrown from rootstock in a common greenhouse environment at the College of William & Mary. Rootstock was planted in 6 gallon (23 liter), 14 inch (36 cm) pots, using Fafard 52 Mix, a perennial crop soil (Sun Gro Horticulture, Agawam, MA, USA). Scotts slow-release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA) was added per manufacturer guidelines. A constant day/night cycle (18 light / 6 dark) was maintained throughout the seasons through the use of grow lights. Humidity was maintained at 75% and temperature was maintained at approximately 25° C. Insecticidal soap was used regularly for the first 8 months to prevent spider mite, thrip, and aphid infestation (Woodstream Corporation, Lititz, PA, USA). Plants were watered liberally as needed.

At 6 months (December 2016) and again at 12 months (June 2017), all traits were remeasured. Immediately after the 6-month measurement, plants were cut back to rootstock and allowed to resprout.

Data Analysis. All analyses were done in R (version 3.4.3). Our 53 populations were divided into six regions (Figure 1). These regions were used to test our *a priori* hypothesis about how we expected discrete ecotypes of common milkweed to emerge. This method of region partitioning represents our attempt at

synthesizing plant glacial refugia theory (Soltis et al. 2006) with studies on monarch population dynamics in an attempt to link milkweed trait variation into the discussion of monarch population dynamics. Studies on monarch population dynamics often divide regions based on an East-West Appalachian divide and a North-South divide near 40°N latitude; these regions marking distinct stages of the annual monarch migration (Flockhart et al. 2015; Stenoien et al. 2015; Ries et al. 2015; Inamine et al. 2016; Nail et al. 2015). We used principal component (PC) analyses to reduce dimensionality in our data, as many environmental variables were found to be correlated with one another, as were many plant traits. ANOVAs were conducted between geographic regions for each environmental variable and on the first two environmental PC axes. Simple linear regressions were used to look for spatial correlation of environmental variables with latitude and longitude, which revealed that latitude and longitude were good proxy measurements of most environmental variables (Figure S2; Table S1). ANOVAs were used to look for discrete patterns of trait variation between regions. In order to look for continuous patterns of trait variation across the landscape, general linear mixed models (GLMMs) were used with individual plant traits as response variables, latitude and longitude as fixed effects, and population as a random effect.

Plant measurements were compared among populations at three timepoints (field measurements, 6 months in a common garden, and 12 months in a common garden). When comparing field measurements to common garden

measurements, we should note that we incurred a dramatic loss in sample size (and therefore a loss in statistical power) between field and common garden measurements due to the logistics of transplanting root stock to our common garden (from an n of 265 to an n of 63). We compared our full field measurement dataset to an artificially limited dataset and determined that the same patterns exist in our limited dataset that we observed in our full dataset, with similar r^2 values but with slightly less significance. The limited dataset shows the same patterns as our full dataset, but less clearly. While field measurements of the limited dataset demonstrate a less pronounced difference between field

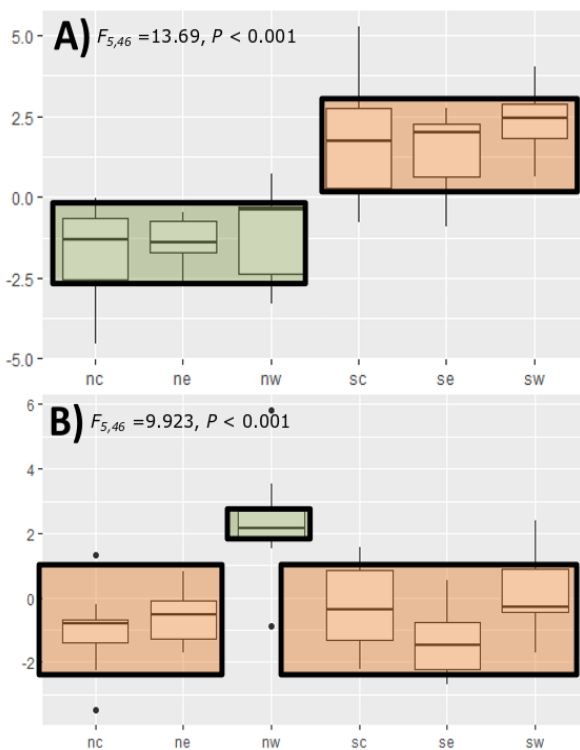


Figure 2: The first principal component axis is driven by temperature variables, and differentiates the northern and southern regions (A). The second principal component axis is driven by soil and precipitation variables, and differentiates the Northwest region from all other regions (B).

measurements and common garden measurements, overall trends are maintained. A more detailed description of this issue, and further justification for using our full dataset, can be found in the supplement.

Results

Range-wide environmental variation.

53.8 percent of the variation among sites in the 15 environmental variables we measured was described by the first two axes of the principal component (PC) analysis.

The first PC axis was well representative of temperature, with the most important variables being (in order of decreasing importance): 2016 average daily mean temperature, cumulative 2016 growing degree days at time of sampling, and 30-year average daily mean temperature. The second PC axis was well representative of soil structure and precipitation, with the most important variables being (in order of decreasing importance): soil percent clay, soil hydrogen (percent dry mass), 30-year average cumulative precipitation, soil nitrogen (percent dry mass), 2016 cumulative precipitation, and soil percent sand.

ANOVA analyses indicated significant variation between regions in all but 3 of the environmental variables that we had measured: soil hydrogen (percent dry mass), soil carbon (percent dry mass), and soil nitrogen (percent dry mass). ANOVA analyses on the scores of the first two PC axes indicated significant differences in these axes among two or more regions: PC1 (temperature axis) differed between North and South (Figure 2A, $F_{5,46} = 13.69$, $P < 0.001$) while PC2 (soil / precipitation axis) was different only in the Northwest (Figure 2B). Discrete analyses of individual environmental variables can be found in Supplemental Table 1. A biplot of PC1 and PC2 shows distinct separation of regions in trait space (Figure S1).

Latitude and longitude were good representatives of continuous environmental variation across the landscape. Similar to our analysis of discrete variation,

simple linear regression analyses indicated that latitude and longitude explained continuous variation in all environmental variables *except* for soil hydrogen, soil carbon, and soil nitrogen (Table S2; Figure S2); these were the same variables that were unexplained in our discrete analysis (Table S1). PC1 (temperature axis) was found to be negatively correlated with latitude ($r^2 = 0.74$, $P < 0.001$, $\beta_1 = -0.55$), indicating higher within-year and 30-year average temperatures in the South and lower within-year and 30-year average temperatures in the North. PC2 (soil / precipitation axis) was found to be positively correlated with latitude ($r^2 = 0.14$, $P < 0.01$, $\beta_1 = 0.22$) and negatively correlated with longitude ($r^2 = 0.25$, $P < 0.001$, $\beta_1 = -0.10$), indicating that the Northwest has lower within-year and 30-year average precipitation, and soil that contains a lower percent sand and higher percent clay while containing more nitrogen and hydrogen.

Biogeographic variation in plant traits. 56.4 percent of the variation among sites in the 15 plant traits we measured was described by the first two axes of the principal component (PC) analysis. The first PC axis was driven mostly by (in order of decreasing importance): C/N, height/GDD, nitrogen, LMA, leaves/GDD, and PRI (the “nitrogen axis”), while the second PC axis was driven mostly by (in order of decreasing importance): chlorophyll, carbon, cellulose, lignin, and cardenolides (the “carbon axis”). Along the carbon axis, cardenolides and foliar cellulose and carbon were in opposite directions, indicating a trade-off between defensive secondary metabolites and structural foliar compounds (Figure S3).

ANOVA analyses on each individual plant trait indicated significant variation between two or more regions for only five of fifteen measured traits (Table S3): cellulose, LMA, NDWI, leaves/GDD, and height/GDD. Post-hoc analyses indicated that of these five traits, all *except* cellulose showed significant variation between the Southwest and Northcentral regions; cellulose differed significantly between the Northwest and Northcentral regions. ANOVA analyses on principal component scores of the first two PC axes indicated that no significant variation existed in principal component scores between regions (Table S3). A biplot of PC1 and PC2 showed significant overlapping in trait space of all regions (Figure S3).

Although we found little support for discrete variation among regions, latitude and longitude explained the continuous plant trait variation we observed across the landscape. General linear mixed models (GLMMs) indicated that biogeographic variation in plant traits was often well explained by latitude or longitude, with variation in all but 4 plant traits (the *exceptions* being chlorophyll, PRI, latex, and lignin) explained by either latitude or longitude (Table 1). PC1 (the nitrogen axis) was found to be negatively correlated with longitude ($r^2 = 0.055$, $P = 0.045$, $\beta_1 = -0.0578$) while PC2 (the carbon axis) was found to be positively correlated with latitude ($r^2 = 0.204$, $P < 0.001$, $\beta_1 = 0.259$). As longitude increases (moving from west to east), nitrogen increases, PC1 (nitrogen axis) scores decrease, cellulose decreases, and C/N ratios decrease (Table 1; Figure 3). As latitude increases (moving from south to north), cardenolides, leaves/GDD, height/GDD, and PC2

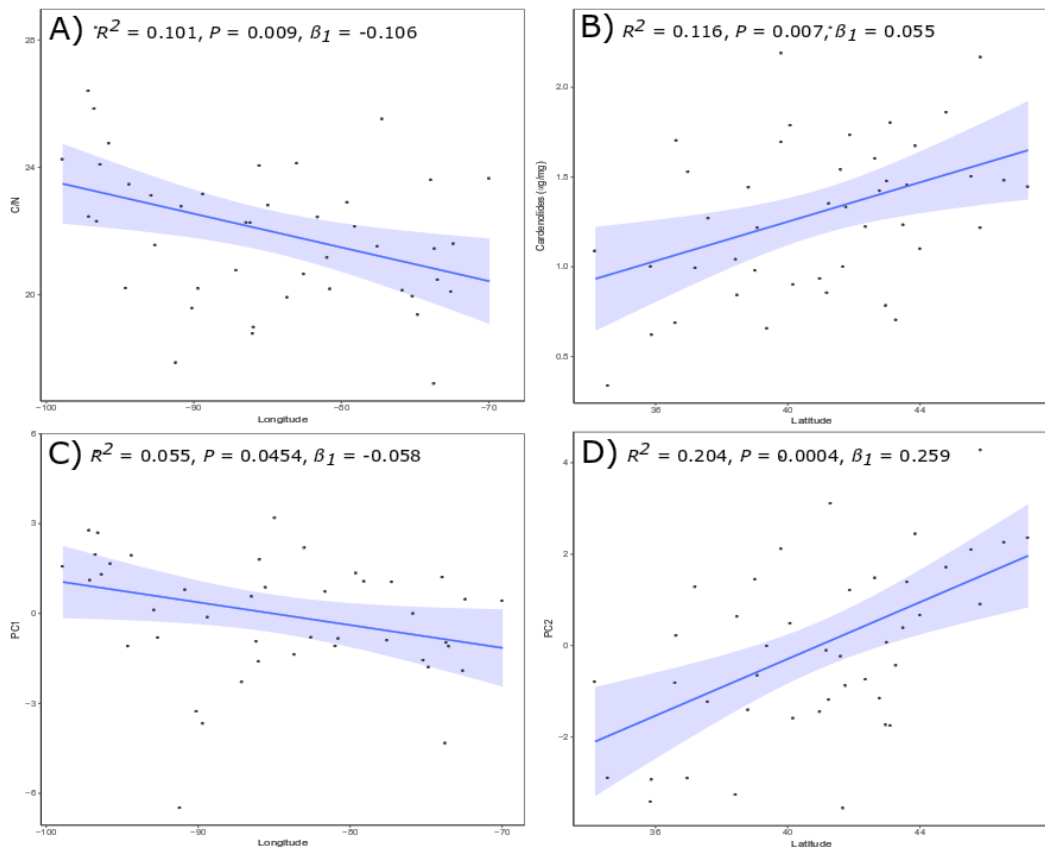


Figure 3: C/N decreases as longitude increase (A), cardenolides increase as latitude increases (B), PC1 scores decrease as longitude increases (C), and PC2 scores increase as latitude increases (D). Gray shading around regression lines represents a 95% confidence interval.

(carbon axis) scores increase, while carbon, LMA, NDWI, number of leaves, and apical height decrease (Table 1; Figure 3).

Traits in a common environment. When plants were resprouted from rootstock in a greenhouse and grown in a common environment for 12 months, 14 out of 15 traits changed over time (Table S4; Figure S4). Carbon, nitrogen, C/N, and height/GDD shifted values between measures taken in the field and measures taken at 6 months in a common garden, but remained the same between 6

Variable	Latitude (Field; Full)			Longitude (Field; Full)			Latitude (6 months)			Longitude (6 months)			Latitude (12 months)			Longitude (12 months)		
	R ²	P	Coefficient	R ²	P	Coefficient	R ²	P	Coefficient	R ²	P	Coefficient	R ²	P	Coefficient	R ²	P	Coefficient
Carbon	0.095287	0.02491	-0.05922	0.052564	0.1001	-0.01685	0.001029	0.813	0.008489	0.02842	0.21	-0.01561	0.017347	0.333	0.03493	0.025123	0.243	0.01555
C:N	0.006067	0.5355	0.06778	0.101445	0.008578	-0.1059	0.020802	0.2845	0.1513	0.002965	0.68751	-0.02	0.000183	0.92398	-0.01109	0.024574	0.2745	-0.04736
Nitrogen	0.008142	0.4687	-0.007652	0.084023	0.01652	0.009397	0.01102	0.437135	-0.01942	0.016113	0.347	0.008221	0.000213	0.917485	0.002036	0.050183	0.111	0.011528
Chlorophyll	0.050128	0.0539	-0.004032	0.00038	0.8694	0.0001346	0.023739	0.253	0.004619	0.056732	0.0745	-0.002498	0.020879	0.291	0.003638	0.075368	0.0407	0.002555
PRI	0.02135	0.2031	-0.000953	0.002434	0.67	0.0001232	0.008291	0.501	0.0007335	0.086205	0.0267	-0.000827	0.014889	0.398	0.0005227	6.50E-06	0.986	4.03E-06
Cardenolide	0.115965	0.006524	0.05475	0.000323	0.8905	0.001109	0.06528	0.0552	-0.05163	0.022595	0.264	0.010636	0.004325	0.6419	-0.0145	0.000235	0.9157	0.00125
Latex	0.036168	0.106	0.002289	0.011757	0.357	-0.000487	0.023618	0.286	-0.000714	0.000683	0.864	-4.20E-05	0.00698	0.541	-0.000514	0.000447	0.877	-4.81E-05
Cellulose	0.033224	0.1692	-0.07798	0.145465	0.002774	-0.06232	0.030693	0.193	-0.1426	0.052644	0.086106	-0.06535	0.042133	0.129	-0.10738	0.034693	0.177	-0.03607
Lignin	0.047362	0.06458	-0.13789	0.008618	0.4379	0.02254	0.019534	0.29986	-0.08143	0.008418	0.4973	-0.01871	0.014661	0.3741	-0.08168	0.020888	0.287993	0.03606
LMA	0.064585	0.04399	-1.1741	0.05907	0.05456	-0.4299	0.017127	0.332	1.415	0.099593	0.0168	1.1932	0.001493	0.777	0.3497	0.018409	0.3188	0.4542
NDWI	0.090561	0.01193	-0.000888	0.012022	0.3746	-0.000124	0.009849	0.4627	-0.000455	0.031639	0.186	-0.000285	0.013933	0.3913	-0.000323	0.014638	0.386	-0.000123
Leaves	0.089507	0.00146	-0.5912	0.000797	0.774608	0.02065	0.024752	0.2271	-1.0595	0.00228	0.715	-0.1133	0.00379	0.653	-0.8329	0.016178	0.3731	0.5915
lvs/GDD	0.186601	4.60E-06	0.001159	0.000274	0.8734	-1.65E-05	0.024752	0.2271	-0.000543	0.00228	0.715	-5.80E-05	0.00379	0.653	-0.000358	0.016178	0.3731	2.54E-04
Height	0.048701	0.0352	-2.14	0.013344	0.277697	0.4142	0.334225	2.48E-06	-5.733	0.087435	0.0307	-1.0319	0.007707	0.523	-1.181	0.00177	0.773	0.1949
Ht/GDD	0.123826	0.000991	0.004977	0.006012	0.4893	0.0004061	0.334225	2.48E-06	-0.002936	0.087435	0.0307	-0.000528	0.007707	0.523	-0.000508	0.00177	0.773	8.38E-05
PC1	0.000283	0.894	-0.01071	0.055084	0.0454	-0.05778	0.040152	0.135	-0.11764	0.136164	0.00476	-0.07576	0.006414	0.583	0.05389	0.003142	0.712	-0.01394
PC2	0.204169	0.000429	0.25914	0.025448	0.237	0.03556	0.031574	0.186	0.09991	0.010743	0.443	-0.0204	3.42E-07	0.997	0.0003523	0.028093	0.217	-0.03734

Table 1: Results of a general linear mixed model on field measurements of all individuals ($n = 265$), on measurements of individuals at 6 months in a common garden ($n = 63$), and on measurements of individuals at 12 months in a common garden ($n = 63$), with plant traits as response variables, latitude and longitude as fixed effects, and population included as a random effect. Orange highlighting indicates statistical significance ($P < 0.05$). Differences between field measurements and measurements of individuals in a common garden are attributable to a change of trait values over time. GLMMs on our field dataset indicated that latitude and longitude accurately explain variation in 11 plant traits across the landscape (carbon, C/N, nitrogen, cardenolides, cellulose, LMA, NDWI, leaves, leaves/GDD, height, and height/GDD). Variation in PC2 was accurately explained by latitude, while variation in PC1 was explained by longitude. After 6 months in a common garden, latitude and longitude accurately explain variation in only 4 plant traits across the landscape (PRI, LMA, height, and height/GDD), while variation in PC1 was accurately explained by longitude. After 12 months in a common garden, only one trait showed a spatial pattern: chlorophyll was significantly correlated with longitude.

months and 12 months. PRI and lignin did not change between field measurements and 6-month common garden measurements, but *did* change between 6 months and 12 months. Chlorophyll, cardenolides, cellulose, LMA, and leaves per GDD changed between field measurements and 6-month common garden measurements as well as between 6 months and 12 months. Latex did not change at all.

When we compare field measurements with common garden measurements, we can see that over time, relative importance of traits in PC axes shifted, and trait relationships with latitude and longitude were lost. Between field measurements and 6 months in a common garden, the nitrogen axis no longer appears and is replaced by the carbon axis as the first PC axis. The new second PC axis was driven mostly by (in order of decreasing importance): height, LMA, and leaves (Figure S5). At 12 months in a common garden, the nitrogen axis reappears as the first PC, and the carbon axis shifts back to the second PC (Figure S5). While chlorophyll had always been an important trait in the carbon axis at all three timepoints, it becomes newly important in the nitrogen axis when it reemerges as the first PC at 12 months in a common garden – chlorophyll in fact is shown to be important for both PC1 and PC2 at 12 months in a common garden. A more in depth explanation can be found in the supplement, describing how the relative importance of specific traits in PC axes shifted over time in a common garden.

When plant traits were measured after 6 months in a common garden, GLMM results indicate that latitude and longitude accurately explained variation in only 4 plant traits across the landscape (PRI, LMA, height, and height/GDD; Table 1). Both PRI and LMA were significantly correlated with longitude, while height and height/GDD were correlated with both latitude and longitude. PC1 was significantly correlated with longitude. After a total of 12 months in a common garden, GLMM results indicate that only one trait showed a spatial pattern: chlorophyll was significantly correlated with longitude (Table 1). Out of the 11 traits that showed spatial patterns in field measurements, none remained after 12 months in a common garden, but one trait (chlorophyll) exhibited a *newly* significant pattern (Table 1). Patterns that were present in field measurements in carbon, C/N, nitrogen, cardenolides, cellulose, LMA, NDWI, leaves, and leaves/GDD did not persist after 6 months in a common garden. Patterns that were present in field measurements in height and height/GDD persisted after 6 months in a common garden, but not after 12 months in a common garden. PRI, LMA, height, and height/GDD all showed significant relationships with longitude after 6 months in a common garden despite no such patterns being shown in field measurements – however, none of those patterns persisted after 12 months in a common garden. Chlorophyll was not shown to have any spatial patterns in either field measurements or at 6 months in a common garden, but was a significant relationship with longitude was observed after 12 months in a common garden. PC1 retained its relationship with longitude after 6 months in a common

garden but did not after 12 months in a common garden. PC2 showed no spatial patterns after 6 months in a common garden.

Role of environment vs. genetics in traits. When trait measurements in a common garden (at 6 months and at 12 months) were regressed against field measurements, we expected that linear models of traits that are driven by environmental variation would have slopes close to zero and have large P values. In other words, if a trait is environmentally controlled, its field values would have no correspondence to its common garden values (Figure 4A) (Oleksyn et al. 1998). When 6 month common garden measurements and 12

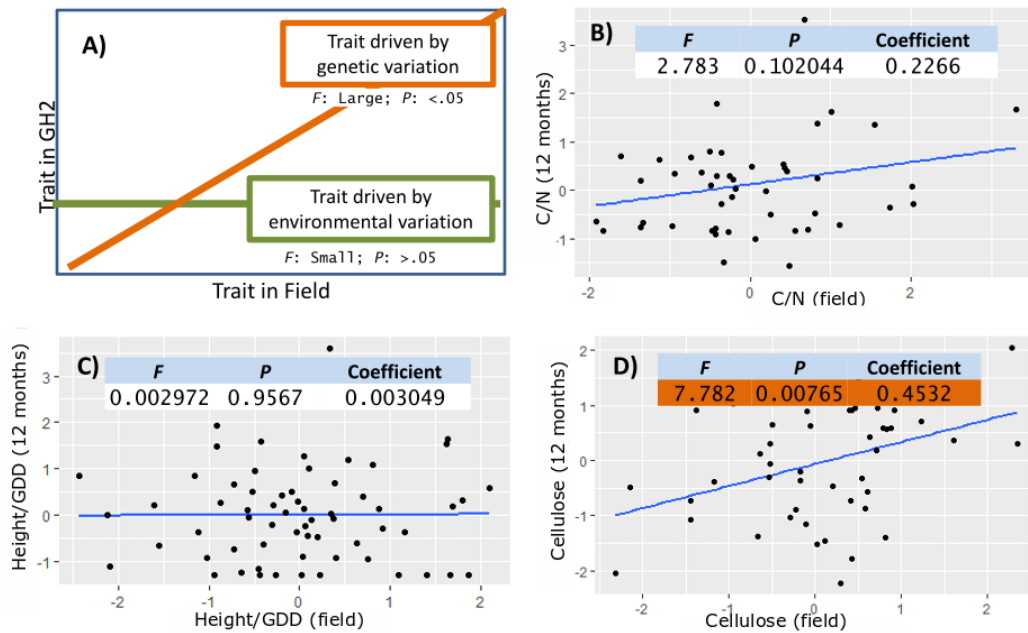


Figure 4: Comparing trait values of individuals as measured in the field and at 12 months in a common garden may indicate if a trait is more heavily controlled by environment or genetics (A). C/N (B) and Height/GDD (C) show evidence of environmental control of those traits, while Cellulose (D) shows evidence of genetic control.

month common garden measurements were compared against field measurements, all but 2 traits showed no relationship with field measurements, indicating that nearly all of the traits that we measured are environmentally controlled. Latex in field measurements was found to be weakly predictive of 6 month common garden measurements, while cellulose in field measurements was found to be strongly predictive of 12 month common garden measurements (Table S4; Figure 4).

Discussion

Our results indicate that common milkweed exhibits many biogeographic patterns of trait variation, and that these patterns manifest as continuous gradients across the landscape that are well approximated by latitude and longitude. When grown in a common garden for 12 months, plant traits changed significantly and lost their relationships with latitude and longitude (as well as any patterns of between-region variation). Most traits showed no relationship between field measurements and common garden measurements, indicating that most traits are environmentally controlled. Latex and chlorophyll were the exceptions, and may be genetically controlled.

Because plant trait variation across the range is best described as occurring continuously along latitudinal and longitudinal axes, we can reject our initial hypothesis that trait variation in *A. syriaca* exists discretely between regions as a result of glacial refugia (genetic analyses may be able to shed more light on this).

Because latitude and longitude are here being used as proxies for environmental variation across the landscape, these observed patterns in plant trait variation indicate that 1) environmental variables occur in directional patterns that are well described by latitude and longitude, and 2) plant traits are, for the most part, influenced by these environmental variables, which creates similar directional patterns in plant traits along these same latitudinal and longitudinal gradients. There are distinct directional environmental gradients throughout the range of common milkweed, and these underlying environmental gradients drive plant trait gradients along these same latitudinal and longitudinal axes, producing clines in plant trait values through the range of common milkweed. While environmental variables likely drive plant traits and not vice-versa, it should be noted that there are some environmental variables that are likely influenced by plant traits – namely herbivory (included here as a *biotic* environmental variable, as it is not a plant trait) and soil composition. Much of the variation in plant traits in natural populations was well described by two axes in trait space: the “nitrogen axis” and the “carbon axis.” The nitrogen axis was found to be negatively correlated with longitude (decrease closer to the East coast) and primarily driven by the plant traits C/N, height/GDD, nitrogen, LMA, leaves/GDD, and PRI. The carbon axis was found to be positively correlated with latitude (increase in the North) and primarily driven by chlorophyll, carbon, cellulose, lignin, and cardenolides. Because C/N was found to be on the nitrogen axis and orthogonal to the carbon axis, this indicates that C/N is driven primarily by variation in nitrogen and not by variation in carbon. C/N is an important plant trait for herbivores (a higher C/N

equating to a higher quality food source for herbivores), and knowing that it is controlled by nitrogen is a valuable insight when considering potential ramifications of fertilizer runoff / nutrient loading, especially near agricultural areas, which are areas that common milkweed tends to grow. Cardenolides and foliar cellulose and carbon varied in opposite directions in trait space, indicating a trade-off between defensive secondary metabolites and structural foliar compounds (Figure S3). Cardenolides and cellulose are carbon-rich compounds, and investment of elemental carbon in one may result in a reduced investment in the other. Why foliar carbon (which is derived from % dry mass of leaf material that is carbon) would increase with cellulose but not with cardenolides is unclear.

Nitrogen was higher in the East and lower in the West. Nitrogen was found to be positively correlated with PRI and negatively correlated with C/N. Carbon was lower in the North and increased in the South. Cardenolides were positively correlated with latitude (increased defense in the North). This latitudinal trend in cardenolide concentrations in common milkweed is something that had been reported in the literature but had not been fully resolved; previous studies of natural populations had weak evidence (Malcolm 1994; Hunter et al. 1996), while a common garden study using seeds from natural populations to grow new genets in a common garden found the pattern in the first year but not the second year (Woods et al. 2012). However, it has been found that the genus *Asclepias* shows an opposite pattern; species from regions closer to the equator are more toxic than their congeners from higher latitudes (Rasmann & Agrawal 2011b).

With our study featuring the most complete coverage of the species range, and including the most samples of natural populations, it seems reasonable to conclude that this pattern is indeed real, at least in natural populations. This would indicate that natural populations of *A. syriaca* show a pattern of toxicity that is opposite of their genus. Our best guess to explain this phenomenon is that repeated herbivory pressure from migrating monarchs moving north through the range of common milkweed every year creates stronger herbivory pressure in the north due to the exponential nature of monarch population growth as they move north. This pattern of increased herbivory in the north is opposite of the typical patterns of greater herbivory at the equator, which may have resulted in common milkweed, which is a main host plant of monarch larvae and one which overlaps significantly with the monarch breeding range, developing increased resistance in the north in response to increased herbivory pressure year after year.

As latitude increases, cardenolides and growth rate (leaves/GDD and height/GDD) were found to increase while carbon and total growth (height and number of leaves) were found to decrease, which indicates that northern plants are smaller and grow more efficiently, but are also more toxic. As longitude increases, C/N and cellulose decrease while nitrogen decreases. While foliar carbon content was not found to significantly vary directionally with longitude, it should be noted that cellulose is a structural compound that contains a high amount of carbon, and that foliar carbon content was found to be highly correlated with foliar cellulose content ($r^2 = 0.62$, $P < 0.001$, $\beta_1 = 0.35$).

Carbon/nitrogen ratios (C/N) are often used as a measure of food quality for herbivores; a lower C/N indicates higher quality food (Rasmann et al. 2009). C/N is the inverse of nitrogen, and thus was negatively correlated with longitude ($r^2 = 0.101$, $P < 0.01$, $\beta_1 = -0.106$). This would indicate that plants in the east may represent a higher quality food source for herbivores than plants in the west. Taken together, these results indicate that plants in the Northeast may be of a higher quality food source, and are better defended (i.e. more toxic), than plants in the Southwest. This observation supports existing theory that the more valuable the plant tissue, the better defended it will be (Rasmann et al. 2009; Bingham & Agrawal 2010b; Coley et al. 1985; Coley 1983).

Over the span of one year, plant traits changed and these biogeographic patterns with latitude and longitude were lost. We showed that common milkweed is capable of relatively rapid adjustment of phenotype inside a single generation – nearly all traits demonstrate the potential for plasticity (except for latex). This suggests that given novel environmental conditions, common milkweed will rapidly change in response, which may provide context for how environmental changes brought on by climate change or other anthropogenic disturbances (e.g. nutrient loading, land cover / land use change, etc.) might affect this species and how they may plastically adjust their phenotype, which is an important insight for conservation efforts (Nicotra et al. 2010).

At 6 months in a common garden, only the relationship between latitude and plant height remained. At 12 months in the greenhouse, no biogeographic trends that were observed in the field could be detected, but chlorophyll was significantly correlated with longitude. This indicated that the patterns of plant trait variation in natural populations, along latitudinal and longitudinal gradients, are driven by environmental variation along those axes. Plant traits in common milkweed appear to be mostly dictated by environment; variation observed among natural populations is a reflection of the variation between the environments they occupy. When this underlying environmental variation is removed, plants adapt and no longer exhibit the variation in trait values that they had in their natural environment. Plant traits no longer track latitude and longitude because the link of environmental variation is no longer there. Why chlorophyll becomes significantly correlated with longitude after 12 months in a common garden is unclear, though it could be merely a statistical anomaly. When we compared plant traits of individuals at 6 months and at 12 months in a common garden to their values when measured in the field, only cellulose at latex showed significant correlation between field and common garden values, indicating that these traits may be more genetically controlled than environmentally controlled.

The largest sampling effort of natural populations to date, this study can be used as a frame of reference moving forward in studies dealing with common milkweed traits, and may provide some needed unifying context to studies that

are carried out in different parts of the range. Additionally, we have confirmed the existence of a latitudinal pattern of common milkweed toxicity that runs opposite of the genus-wide pattern of increased toxicity towards the equator, which is something that has previously been unresolved in the literature. These patterns of trait variation are likely primarily driven by environmental variation, but cellulose and latex may be traits that are primarily genetically controlled. Finally, our results may provide valuable information for current efforts to conserve the monarch butterfly through milkweed conservation and restoration projects; milkweed from the Northeast appears to be of a higher nutritional quality (low C/N) and better defended (higher cardenolide concentrations). If monarchs are fed on common milkweed from the Northeast, they *may* benefit from the increased nutritional quality as well as the added protection of higher cardenolides (which monarchs use to defend themselves against predation). If this is the case, placing added emphasis on milkweed conservation in the Northeast may be a valuable use of limited resources. A next step in this research is to be able to link variation at the producer level to the next trophic level. We determined that in common milkweed, there exist biogeographic patterns of traits, and that these traits are capable of plastically adapting to novel environments, but do those patterns of trait variation affect herbivores? Do changes in trait values at the plant level manifest at the next trophic level to influence growth, performance, or survival? These questions could be asked within the monarch-milkweed model interaction in order to answer these fundamental questions while providing context for a declining species.

CHAPTER 2: MONARCH RESPONSE

Introduction

The monarch butterfly (*Danaus plexippus*) is well known for its annual multi-generational migration between Mexico, The U.S., and Canada. The monarch's 80% decline over the past 30 years has created widespread public concern for its conservation (Flockhart et al. 2015; Brower et al. 2012). This concern has prompted extensive research on monarch demography, life history strategies, migration patterns, and its interactions with its most important host plant, common milkweed (*Asclepias syriaca*) (Malcolm et al. 1989; Inamine et al. 2016; Pleasants 2016). However, there has been insufficient research into how intraspecific variation in common milkweed affects monarch larvae. In 2012, Woods et al. conducted a common garden experiment in which they planted seed collected from 22 populations across the range and showed that both the latitude and precipitation (30-year averages) of the seed collection sites were negatively correlated with monarch performance (measured by total wet mass), though the relationship with latitude was reported as only marginally significant ($0.1 > P > 0.05$). In 2015, Couture et al. used seed collected from 5 populations in Wisconsin and Michigan – 3 northern populations and 2 southern populations – to assess the effects of elevated temperature and water stress (both independently and interactively) on milkweed and the cascading effects of those treatments on monarch larvae through their food. They found that larvae grew larger when fed plants that were exposed to increased elevated temperatures,

plants that were exposed to water stress, plants that were exposed to both treatments (compared to larvae fed control plants), and plants from southern populations (as opposed to northern populations). They also found evidence that monarch growth is positively impacted by increases in plant foliar nitrogen content, while monarch growth is negatively impacted by increases in plant water content and carbon-nitrogen ratio (C/N). Taken together, these two studies indicate that there may be clines in the *quality* of common milkweed as a food source for monarch larvae, but more work is needed to fully resolve this issue.

Section 1 detailed our findings regarding intraspecific variation in common milkweed and the role that environment plays in driving that variation. A large amount of variation exists in common milkweed traits throughout its range. Most of these traits, however, were found to be environmentally controlled, with at least two traits (latex and cellulose) that may be genetically controlled. Given the results of our common garden experiment, it is unlikely that ecotypes of common milkweed exist, either as discrete groups of populations or as a continuous gradient of heritable phenotypic differences. This section explores what impacts, if any, intraspecific variation in common milkweed has on the growth, performance, and survival of monarch larvae. We wanted to know: 1) Is monarch growth, performance, or survival affected by variation in specific milkweed traits? If that is the case, then how do those differences manifest or play out? 2) Despite our inability to detect ecotypes of common milkweed, does the origin of a milkweed plant affect the growth, performance, or survival of monarch larvae in

such a way that it would indicate distinct ecotypes in common milkweed? In other words, could ecotypic differentiation be so subtle that we were unable to detect it with our study design but still significant enough to be experienced by monarch larvae? 3) Given insights from 1 & 2, are there parts of the common milkweed native range with the “best” milkweed, in terms of food source for monarch larvae? Our results from Section 1 indicate that milkweed from the Northeast may have a higher nutritional quality (lower C/N) and greater toxicity (higher cardenolide concentrations).

The monarch butterfly (*Danaus plexippus*) is a Nymphalid butterfly with larvae that feed exclusively on plants of the genus *Asclepias*, or the milkweeds. Larvae grow rapidly and undergo 5 instar phases over the span of 2 weeks before entering their chrysalis phase. While in the chrysalis for about 2 weeks, the body reforms as an adult butterfly. After eclosion, the breeding adult will live for 2-6 weeks. Adult females will deposit eggs exclusively on milkweed species (Opler et al. 1992). There are four main American populations of *Danaus plexippus*: a non-migratory Florida population, a non-migratory Mexican population, a Western migrating population, and an Eastern migrating population. There are also pockets of small populations throughout the Caribbean and South America, and even in Australia (Zalucki & Rochester 2004). The Western migrating population overwinters on the southwest coast of California, and moves northeast in the spring to breed in states west of the Rocky Mountains, and in British Columbia, Canada. The largest population, in terms of both size and range, is the Eastern

migrating population (Oberhauser et al. 2008; USDA NRCS 2015), and it is members of this population that we will focus on further (and which hereafter may be referred to simply as “monarchs”).

The entire Eastern migrating population overwinters in a small cluster of mountain tops in central Mexico, between the State of Michoacán and the State of Mexico (West of Mexico City). The monarchs wait out the winter in a state of reproductive diapause (non-breeding) as they cluster on branches of the Oyamel fir tree (*Abies religiosa*), which is the dominant tree on these mountaintops. This habitat and this tree are essential for the monarch’s survival through the winter. The high elevation of the mountain range keeps the butterflies cool enough that they do not expend much energy on metabolic processes, while being just warm enough for them to survive. The tree itself has thermoregulative properties and forms microclimates in its stands; it keeps the monarchs cooler than ambient temperature during the day (which prevents them from expending too much energy on metabolic processes), and warmer than ambient temperature during the night (which prevents them from freezing). The needles of the fir trees also collect moisture from the air, which the monarchs use as a source of drinking water (Oberhauser et al. 2008; USDA NRCS 2015).

In March of every year, the overwintering monarchs break diapause, move north, and start reproducing in northern Mexico and southern United States. Adults lay their eggs exclusively on milkweed species. There are 4-5 breeding generations

of monarchs that move north and east through their breeding habitat of eastern United States and Canada. These breeding adults live for 2-6 weeks while feeding on flower nectar. The final generation at the end of the breeding season (generation 4 or 5) enters reproductive diapause and migrates south to the overwintering site in Mexico. These non-breeding adults can live far longer than the breeding adults (up to 8 months if needed), and are the same generation that moves north the next spring (Oberhauser et al. 2008; USDA NRCS 2015).

The eastern migrating population of the monarch butterfly has declined by an alarming 80% in past three decades, with a projected quasi-extinction probability of 11-57% within 20 years (Flockhart et al. 2015; Brower et al. 2012). Some main causes of monarch decline are overwintering habitat loss, climate change or extreme weather events, loss of nectaring sources along the monarch migration routes, and loss of breeding habitat, specifically common milkweed (*Asclepias syriaca*) (Semmens et al. 2016; Inamine et al. 2016; Malcolm et al. 1993; Brower et al. 2012; Thogmartin et al. 2017; Pleasants & Oberhauser 2013; Pleasants 2016).

Since monarchs cluster very densely in such a small and specific habitat, conservation of the overwintering site has been of great concern. The Monarch Butterfly Biosphere Reservation (MBBR) was established by the Mexican president in 1980, and was meant to protect the monarch's overwintering habitat from destruction or disturbance. Illegal logging activity within, and subsistence

farming in buffer areas around the reserve, have contributed to the decline of the overwintering habitat over the years. Between 1971 and 1984, there were 1.7% annual rates of forest decline, and between 1984 and 1999, there were 2.4% rates of annual forest decline in and around the reserve (Brower et al. 2002). Between 2012 and 2012 alone, 2057 hectares were illegally logged (Vidal et al. 2014). The “milkweed limitation hypothesis” attributes monarch decline to milkweed decline driven by the use of herbicide-resistant crops and glyphosate herbicide, such as “Roundup” (Inamine et al. 2016; Stenoien et al. 2015; Pleasants & Oberhauser 2013; Pleasants 2016). Recently, the importance of milkweed has been disputed, and survival during migration has been suggested as a more important driver of monarch decline (Inamine et al. 2016). Despite disagreement over the cause of monarch decline, an estimated 1.6 billion additional milkweed stems would be needed to meet monarch conservation goals (Pleasants 2016). Already, US Fish and Wildlife and other conservation organizations have begun milkweed restoration projects (Lee 2015; Fritsher 2015; USDA NRCS 2015; Oberhauser et al. 2008); however, we know little about how milkweed variation affects monarch growth, performance, and survival, which is necessary information needed to support and direct these efforts (Woods et al. 2012; Couture et al. 2015).

Methods

Larvae bioassay. In the summer of 2017, we conducted a monarch feeding trial with our plants from across the range of *A. syriaca*. We performed two rounds of

feeding trials with 63 plants and 126 larvae (two replicates of 63 plants, 1 larva per plant per replicate, for a total of 126 larvae). This occurred after the plants had adapted to a common environment over the period of one year, thus eliminating any effects of environmental variation on larval growth, performance, and survival. Each plant was randomly assigned to a larva *a priori*, and larvae were fed on leaf tissue from only their assigned plant. This entire trial was performed two times, and the average measurement of those two trials was used in data analysis. We obtained monarch eggs from our on-site captive breeding population. Eggs were placed on a leaf from their assigned plant and closely monitored in a growth chamber until they hatched. Larvae were kept separate by growing them in individual containers. The growth chamber was kept at optimal conditions for rearing monarch larvae (Couture et al. 2015; Hughes et al. 1990; Hughes et al. 1993). Upon their hatching, we measured the mass and length of first instar larvae. Larvae were provided *ad libitum* access to water and leaf tissue (from their assigned plant) for one week. We recorded the mass of all leaf tissue entering and exiting the larvae containers in order to determine how much leaf tissue was consumed. After one week, we remeasured the length and mass of the larvae. Larval growth was determined by the amount of length gained and the amount weight gained. Performance was determined by two measurements of efficiency: efficiency of *ingestion* (which is a ratio of the amount of weight gained divided by the amount of plant tissue consumed), and efficiency of *digestion* (which is a ratio of the amount of weight gained divided by the difference between the amount of plant tissue consumed and the amount of frass

excreted); a higher ratio (for either) indicates a more efficient (or better performing) larva (Hughes et al. 1990). Survival was determined by whether or not the larvae survived for the entire trial (binary).

Statistical Analyses. All analyses were done in R (version 3.4.3). We used the same region divisions that we did to investigate regional variation in milkweed traits (Figure 1; Figure 2). ANOVAs were used to look for discrete patterns of monarch larvae trait variation when fed plants from different regions. General linear mixed models (GLMMs) were used to test for linear relationships between monarch measurements and the latitude or longitude of the collection site from which the plant they were fed came from. Because it has been suggested that patterns in herbivory may occur not on a latitudinal or longitudinal gradient, but instead based on absolute distance from plant range center (Alexander et al. 2007), we also tested for relationships between monarch measurements and distance from range center. We used monarch larvae measurements as response variables, latitude, longitude, and distance from range center as fixed effects, and milkweed population as a random effect, in order to look for continuous patterns of monarch larvae measurement variation when fed plants from along the aforementioned spatial gradients. Simple linear regressions were used to look for linear relationships between monarch larvae measurements and specific plant traits.

Results

ANOVA analyses on each individual monarch variable measured indicated significant variation between two or more regions for only one measured variable: weight gain (Table S6). Post-hoc analyses indicated that weight gain showed significant variation between the Northeast region and the Northcentral and Southcentral regions (Figure S6). It should be noted, however, that these two regions (Northcentral and Southcentral) each have a single extreme value that lies within the range of values similar to those for the Northeast region. Results of our general linear mixed models (GLMMs) showed no significant trends in monarch measurements with latitude, longitude, or distance from range center (Table S7). We found that while many monarch variables showed nearly statistically significant relationships with distance from range center, no models cleared our significance threshold of $P < 0.05$. Simple linear regressions indicated that several milkweed traits had linear relationships with two monarch growth variables: increases in plant chlorophyll and nitrogen resulted in increases in monarch weight gain, increases in plant NDWI and C/N resulted in decreases in monarch weight gain, and increases in plant height resulted in decreases in monarch length gain (Figure 5; Table 2; Figure S7). Comparing plant PC axes to monarch measurements revealed that only PC1 (the nitrogen axis) influenced a single monarch variable: food eaten had a negative relationship with PC1 ($r^2 = 0.298$, $P = 0.001$, $\beta_1 = -29.53$). Although we expected to find negative linear relationships between monarch growth and cardenolide concentrations, we found none. However, looking back to our plant carbon and nitrogen PC axes for 12

Relationship	R^2	P	Coefficient
Monarch weight ~ Plant chlorophyll	0.1017	0.03475	34.91
Monarch weight ~ Plant nitrogen	0.2974	0.000418	9.739
Monarch weight ~ Plant C/N	0.1526	0.0117	-1.1947
Monarch weight ~ Plant NDWI	0.1445	0.01396	-351.333
Monarch length ~ Plant height	0.1364	0.01662	-0.03566

Table 2: Results of linear regression analyses of monarch larvae measurements with specific plant traits ($n = 45$). Several milkweed traits had correlative relationships with two monarch growth variables: increases in *chlorophyll* and foliar *nitrogen* resulted in increases in monarch *weight gain*, increases in *NDWI* and *C/N* resulted in decreases in monarch *weight gain*, and increases in plant *height* resulted in decreases in monarch *length gain*. Only statistically significant results are shown.

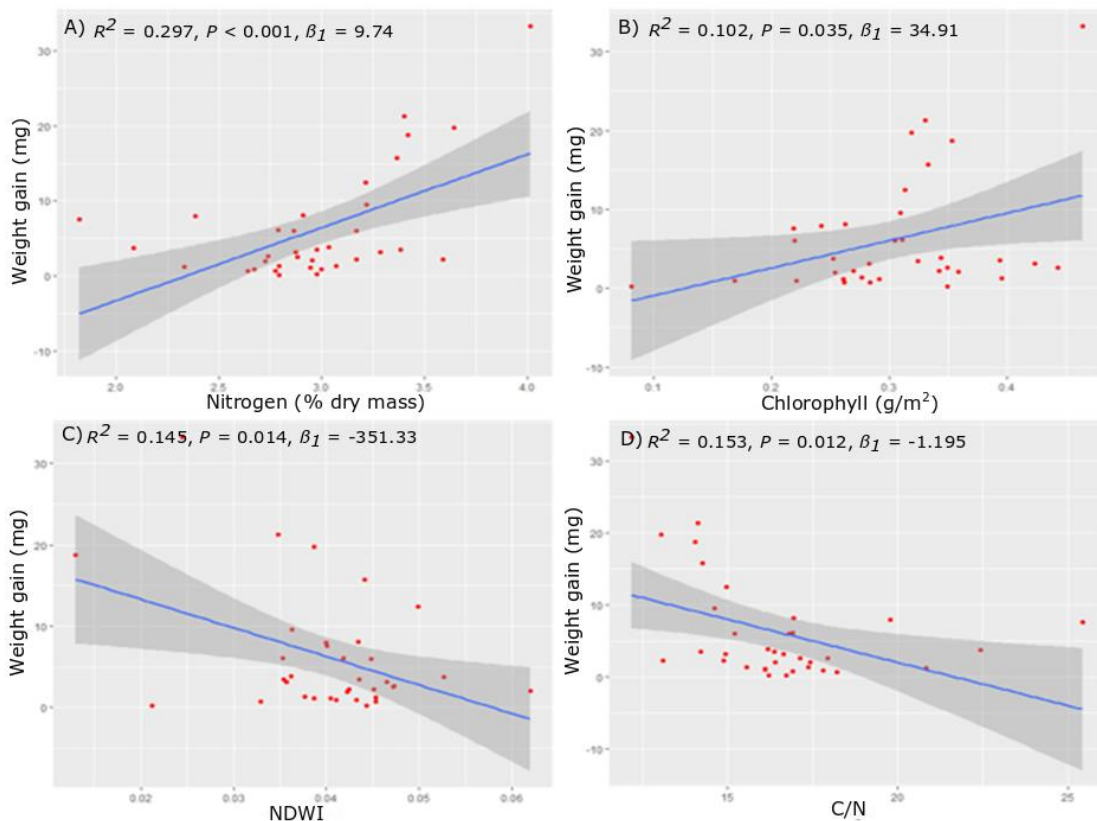


Figure 5: Monarch weight gain increases with milkweed nitrogen (A) and chlorophyll (B), and decreases with milkweed NDWI (C) and C/N (D) ($n = 45$). Gray shading around regression lines represents a 95% confidence interval.

month common garden measurements from Section 1 (Figure S5), we know that we should expect cardenolides to exhibit a negative relationship with chlorophyll content, which we did observe ($r^2 = 0.453$, $P < 0.0001$, $\beta_1 = -0.077$).

While no significant continuous biogeographic trends in larvae measurements were detected along latitudinal, longitudinal, or distance from range center gradients, some biologically interesting spatial patterns were observed along a distance from range center gradient (but did not clear our significance threshold of $P < 0.05$). Specifically, GLMM results revealed that distance from range center had a marginally positive influence on monarch weight gained ($r^2 = 0.0676$, $P = 0.0847$, $\beta_1 = 0.574$), length gained ($r^2 = 0.0804$, $P = 0.0631$, $\beta_1 = 0.265$), and efficiency of ingestion ($r^2 = 0.1183$, $P = 0.0735$, $\beta_1 = 0.005$) (Table 2).

Discussion

Although no biogeographic trends were detected in monarch growth, performance, or survival, some marginal patterns in monarch measurements were observed along a distance from range center gradient. This should be specifically investigated in a larger study; if range center patterns in plant-herbivore dynamics can be uncovered, it would help to develop our understanding of those dynamics and biogeographic theory generally.

Between-region ANOVAs revealed that larvae raised on plants from the Northeast gained significantly more weight than those fed on plants from the

Northcentral and Southcentral regions. It is important to note that both the Northcentral and Southcentral regions each have a single extreme value that lies within the range of values similar to those for the Northeast region, so it is not out of the realm of possibility that with additional replications of this feeding trial, more points would land within that range and that the difference between those regions and the Northeast region could become insignificant. When plants were fed to larvae, we could detect no continuous biogeographic trends or interregional variation in plant trait values except for a longitudinal cline in foliar chlorophyll. This indicates that larvae were experiencing some variation in the plants that we were unable to detect with our current study design. Again, a larger study may provide the statistical power to more clearly determine whether this pattern is a genuine representation of natural phenomena.

Simple linear regression analyses on the relationship between monarch measurements and plant trait values at time of feeding reveal several linear relationships between important milkweed traits and measurements of monarch growth. Monarch weight gain was positively impacted by increased chlorophyll and nitrogen, and negatively impacted by increased NDWI and C/N, while length gain was negatively impacted by plant height (Figure 5; Table S8; Figure S7). These results support previous findings by Woods et al. (2012) and Couture et al. (2015); while we could not replicate their findings that latitude of collection site influenced monarch growth, we did find that plant water content (NDWI) was negatively correlated with monarch growth, which supports findings from both

studies. Our results also support specific findings in Couture et al. (2015): that monarch growth is positively impacted by increased foliar nitrogen while being negatively impacted by increased NDWI and C/N.

We showed that monarch growth increased as plant nitrogen increased and C/N decreased. In our field measurements of plant traits from Section 1, we learned that as longitude increased, nitrogen increased and C/N decreased. Interpreted together, these results would indicate that we would expect better monarch growth when eating plants from the East. We showed that monarch growth increased as plant NDWI and height decreased. In our field measurements of plant traits, we found that as latitude increased, both NDWI and height decreased. Interpreted together, these results would indicate that we would expect better monarch growth when eating plants from the North. If increasing latitude and increasing longitude should both result in higher quality food for monarchs, one might guess that the Northeast would represent the “best” food source for monarch larvae, an assumption which is supported by the results of our ANOVA analyses on the effect of plant origin on monarch weight gain (Table S6; Figure S6).

Cardenolides and latex are two important defensive measures milkweed employs to protect against herbivory. It is not surprising that latex did not have an impact on monarch growth, performance, or survival, because we fed monarchs leaf clippings, which removed the flow of latex from mechanically inhibiting monarch

feeding. The effect of latex on monarch development, and the effect of stopping latex flow, has been well studied (Zalucki & Brower 1992; Zalucki et al. 2001; Woods et al. 2012). Using leaf clippings was part of a decision we made to be able to obtain higher resolution monarch growth and performance data – which is information that is missing from the current literature – at the potential expense of giving up the ability to observe the effects of latex. In order to collect information on the amount of food consumed and the efficiency of ingestion and digestion, we needed to be able to measure all plant biomass going into and coming out of larvae containers, which would have been impossible if monarchs were fed on intact plants. While it is not surprising that latex had no observable effect on monarch growth, performance, or survival, it is surprising that cardenolides had no effect. While it is generally accepted that increased cardenolide concentrations are associated with decreased monarch survival, whether cardenolide concentrations (or cardenolide toxicity) and latex are intertwined is surprisingly unresolved (Rasmann & Agrawal 2011b; Rasmann et al. 2009; Cohen 1983; Vickerman & Boer 2002; Zalucki & Brower 1992; Agrawal 2005; Rasmann et al. 2011; Rasmann & Agrawal 2011a; Seiber et al. 1982). While we found no relationship between cardenolide concentrations and monarch growth, performance, or survival, we did find that an increase in plant chlorophyll concentrations was associated with an increase in monarch weight gain. Considering that cardenolide concentrations had a strong negative relationship with chlorophyll concentrations, it may be that a negative relationship between cardenolides and monarch growth existed but that we were unable to detect it.

Because we measured plant traits *before* the feeding trial, when plants were fully intact, it is possible that our trait measurements are not fully indicative of what larvae were eating. Further bioassays using intact plants, spectroscopy, and latex measurements would be needed to resolve this issue.

In terms of specific conservation implications, our results indicate that monarchs thrive best on common milkweed from the Northeast, and that plants are capable of having differential effects on monarchs even after adapting to a new environment for up to one year. Considering current and ongoing milkweed conservation efforts in light of these conclusions, it may be beneficial to focus more intently on conservation of monarch breeding habitat in the Northeast – a region which is often overshadowed by the Midwest when discussing monarch conservation and milkweed restoration. This might include endeavors as involved as explicit restoration and replanting efforts, or as simple as easing roadside and highway median mowing.

Previous studies have shown that milkweed *abundance* may not be the main driver of monarch loss because the bulk of within-year monarch population decline does not occur until *after* the breeding season (Inamine et al. 2016). Our conclusions do not refute those findings, but instead provide a caveat: there may be latent effects of milkweed *quality* on monarchs. In other words, perhaps there are enough milkweed stems on the landscape to produce a sufficiently large population of monarch adults at the end of the breeding season, but those adults

may be either more or less robust for the long migration ahead of them depending on where they grew up.

Some studies predict that climate change will result in conditions that will facilitate a northward shift in monarch range, further into Canada (Zalucki & Rochester 2004; Bataalden et al. 2007), but it is unclear if milkweed (i.e. monarch breeding habitat) will be able to make the same northward shift, meaning that it is unclear if the potential expansion in monarch range will be realized. If monarch ranges do shift northwards, however, there may be an increased reliance on milkweed from the North, which may result in more monarchs feeding on milkweed from the Northeast as larvae, which could result in increased growth, and monarchs that are more robust for their southern migration. Recent findings using herbaria records indicate that common milkweed may have historically increased in response to anthropogenic disturbance and land-use change (Boyle et al., *In Prep*). Given that within-species variation in common milkweed traits affects monarch growth, it is fair to assume that between-species variation in *Asclepias* traits also affects monarch growth. If the proportion of *Asclepias* species has shifted over the years, the *quality* of food available to monarchs on the landscape may have changed as well. In other words, as the composition of the *Asclepias* community throughout the monarch breeding range has shifted, monarch growth (and subsequently migration success) may have shifted in response.

Although our results indicate that monarch larvae perform differentially depending on milkweed traits (and possibly milkweed source), we do not know if those results carry over into the larvae's adult stage. The adult is an important stage in terms of monarch population trends, as breeding and migration happen in this stage. It may be fair to assume that greater growth in larval form will result in larger adults, with more fat reserves and longer wingspans, and therefore more successful migrants, but we cannot be sure until those experiments are conducted. Further research testing the effects of intraspecific variation in milkweed traits (and possibly source) on monarch adult traits would be beneficial.

APPENDIX (SUPPLEMENT)

Relative importance of traits in PC axes shifts over time in a common

garden. In field measurements, 56.4 percent of the variation among sites in the 15 plant traits we measured was described by the first two axes of the principal component (PC) analysis. The first PC axis was driven mostly by (in order of decreasing importance): *C/N*, *height per GDD*, *nitrogen*, *LMA*, *leaves per GDD*, and *PRI* (the “nitrogen axis”), while the second PC axis was driven mostly by (in order of decreasing importance): *chlorophyll*, *carbon*, *cellulose*, *lignin*, and *cardenolides* (the “carbon axis”). Along the carbon axis, *cardenolides* and foliar *cellulose* and *carbon* were in opposite directions, indicating a trade-off between defensive secondary metabolites and structural foliar compounds (Figure S3). At 6 months in a common garden, 43.8 percent of the variation among sites in the 15 plant traits we measured was described by the first two axes of the principal component (PC) analysis. The first PC axis was driven mostly by (in order of decreasing importance): *lignin*, *chlorophyll*, *carbon*, *cellulose*, *NDWI*, and *cardenolides* while the second PC axis was driven mostly by (in order of decreasing importance): *LMA*, *height*, and *leaves* (Figure S5). After 12 months in a common garden, 57.2 percent of the variation among sites in the 15 plant traits we measured was described by the first two axes of the principal component (PC) analysis. The first PC axis was driven mostly by (in order of decreasing importance): *leaves*, *PRI*, *height*, *chlorophyll*, *nitrogen*, and *C:N*, while the second PC axis was driven mostly by (in order of decreasing importance): *carbon*, *LMA*, *lignin*, *chlorophyll*, *cardenolides*, and *cellulose* (Figure S5).

Loss of statistical power in common garden measurements. We measured 5 individuals in the field but only transplanted 2 out of those 5 to a common garden. Additionally, some plants did not survive the transplant. This has resulted in a dramatic decrease in sample size and therefore a decrease in statistical power, as we went from an n of 265 to an n of 63. To determine if this would affect our comparisons of field measurements with common garden measurements, we limited our dataset to only consider individual plants that were transplanted and survived to the final timepoint (12 months in a common garden) and compared the spatial patterns and principal component compositions of that limited dataset to those of our full dataset.

In field measurements, in our limited data set, 51.3 percent of the variation among sites in the 15 plant traits we measured was described by the first two axes of the principal component (PC) analysis (as opposed to 56.4% in the full dataset). The first PC axis was driven mostly by (in order of decreasing importance): *chlorophyll*, *nitrogen*, *C/N*, *PRI*, and *lignin* (the “nitrogen axis”), while the second PC axis was driven mostly by (in order of decreasing importance): *carbon*, *LMA*, *cellulose*, *NDWI*, *height per GDD*, *cardenolides*, and *lignin* (the “carbon axis”) (Figure S5A). This is relatively unchanged from the full dataset (Figure S3).

As opposed to 11 traits in our full dataset, GLMMs on our limited dataset indicated that latitude and longitude accurately explain variation in only 6 plant traits across the landscape (*C/N*, *cardenolides*, *cellulose*, *leaves*, *leaves/GDD*, and *height/GDD*). Variation in PC2 was still accurately explained by latitude, but variation in PC1 was no longer explained by longitude. If we compare the full dataset and limited dataset r^2 and P values for the models that were no longer significant, we can see that r^2 values are very similar and that while P values do not meet our significance threshold of 0.05, they are all still below 0.1 for all individual traits (but not for PC1). In other words, these patterns still exist, but we are less confident in them.

It is unlikely that these relationships and patterns ceased to exist in our limited dataset, but rather that we lacked the statistical power to detect them. In other words, the full dataset is a more accurate representation of natural phenomena, resulting in more realistic models. While comparisons using the limited dataset demonstrate a less pronounced difference between field measurements and common garden measurements, the overall trend is maintained: the patterns and relationships observed in field measurements shift and dissipate over time in a common garden, with the exception of chlorophyll (Table 1). For these reasons, we chose to illustrate differences between the full dataset and common garden measurements in our main manuscript, as we believe that it is more true to natural phenomena and more conveys our central points.

Supplemental Tables and Figures.

Variable	$F_{5,47}$	P	Range
Herbivory	5.351	0.000566	0.01 - 1.02 (units: see Methods)
Herb per GDD	2.95	0.0214	1×10^{-5} - 9.8×10^{-4}
Soil % Sand	8.13	1.46E-05	4 - 92 (% composition)
Soil % Silt	5.452	0.000508	7 - 85 (% composition)
Soil % Clay	8.453	9.85E-06	1 - 33 (% composition)
Soil Nitrogen	0.89	0.496	0.1 - 0.7 (% dry mass)
Soil Carbon	0.336	0.888	1 - 13 (% dry mass)
Soil Phosphorus	5.087	0.000855	0.03 - 0.4 (% dry mass)
Soil Hydrogen	2.365	0.0543	0.2 - 1.4 (% dry mass)
Elevation	3.06	0.018	17 - 848 (m)
GDD (2016)	9.96	1.53E-06	408 - 1264
Precipitation (2016)	12.48	9.90E-08	193 - 700 (mm)
Mean Temp (2016)	13.2	4.80E-08	3 - 15 (°C)
Precipitation (30yr)	15.14	7.48E-09	486 - 1462 (mm)
Mean Temp (30yr)	15.77	4.24E-09	4.5 - 15.6 (°C)

Variable	$F_{5,46}$	P	Proportion of variance
PC1	13.69	3.39E-08	0.307
PC2	9.923	1.75e-06	0.231

Table S1: Results of ANOVA analyses of environmental variables between geographic regions. Orange highlighting denotes statistical significance ($P < 0.05$).

Variable	Latitude			Longitude		
	R^2	P	Coefficient	R^2	P	Coefficient
Herbivory	0.1897	0.0006563	-0.031557	0.0004393	0.8816	0.0005395
Herb per GDD	0.05915	0.04391	-1.95E-05	0.003946	0.655	1.63E-06
Soil % Sand	0.04866	0.06325	2.063	0.1545	0.002304	1.1948
Soil % Silt	0.05798	0.04722	-1.8354	0.09051	0.01719	-0.7939
Soil % Clay	0.01079	0.4637	-0.2271	0.2398	0.0001363	-0.40088
Soil Nitrogen	0.003921	0.6592	-0.002512	0.008968	0.5043	-0.00138
Soil Phosphorus	0.04603	0.06872	0.004595	0.1404	0.003614	0.0026014
Soil Carbon	0.00573	0.2607	-0.10072	7.88E-05	0.9502	-0.002045
Soil Hydrogen	0.01863	0.3346	-0.01171	0.01327	0.4161	-0.00359
Elevation	0.0006347	0.3142	-7.794	0.08477	0.01952	-6.541
GDD (2016)	0.6746	3.01E-14	-56.851	0.0008084	0.8398	-0.724
Precip (2016)	0.4676	1.00E-08	-26.64	0.2153	0.0002755	6.8373
Temp (2016)	0.841	2.20E-16	-0.82984	5.30E-05	0.9588	-0.002429
Precip (30yr)	0.4631	1.25E-08	-47.26	0.4172	1.06E-07	16.616
Temp (30yr)	0.908	2.20E-16	-0.8414	0.0003664	0.8918	-0.00624
PC1	0.7364	2.64E-16	-0.55244	0.004874	0.6229	-0.01627
PC2	0.1381	0.003881	0.21922	0.2536	8.41E-05	-0.10477

Table S2: Results of linear regression analyses of environmental variables with latitude and longitude. Latitude and longitude accurately represent variation in all but 3 variables across the landscape. Orange highlighting denotes statistical significance ($P < 0.05$).

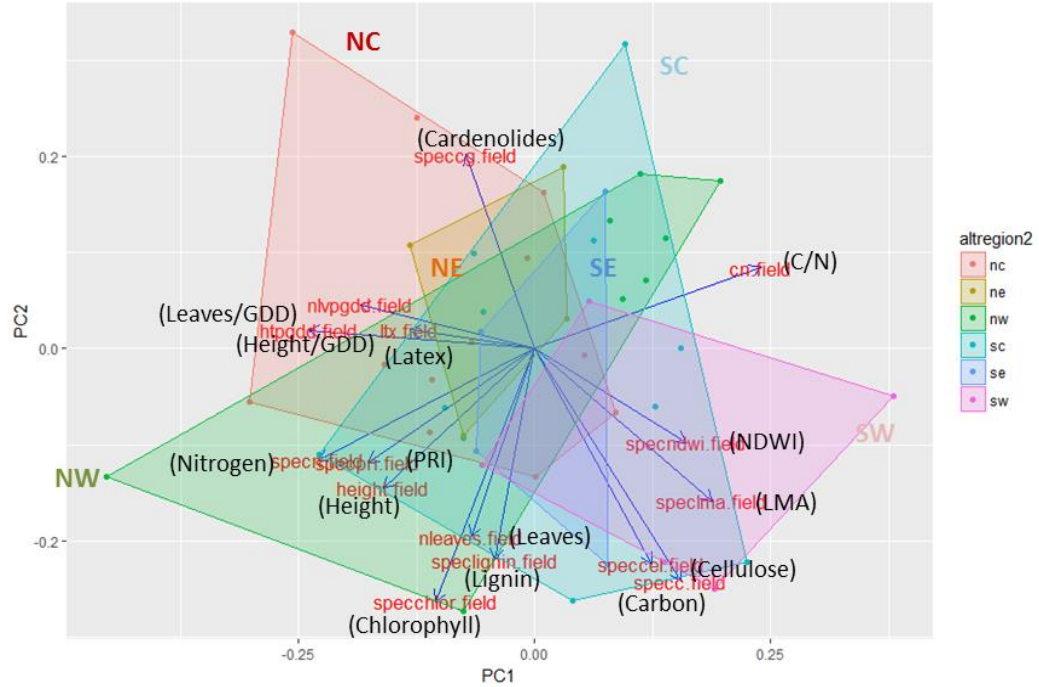


Figure S3: Biplot of PC1 and PC2 of plant trait values. Each point represents a population average (n = 53). PC1 is driven mostly by nitrogen, C/N, growth rate, and PRI . PC2 is driven mostly by cellulose, carbon, chlorophyll, lignin, and cardenolides.

Variable	$F_{5,38}$	P	Range
Carbon	1.913	0.115	46.78759 - 49.45389
C:N	1.847	0.127	17.20994 - 28.40669
Nitrogen	1.344	0.267	1.751217 - 2.767846
Chlorophyll	0.993	0.435	0.2300900 0.3822808
PRI	1.034	0.412	-0.04906711 - 0.02234348
Cardenolides	1.153	0.35	0.3399998 - 2.3358476
Latex	0.881	0.501	0.00000 - 0.20904
Cellulose	2.765	0.0317	18.43381 - 24.64484
Lignin	0.088	0.994	3.319232 - 10.201720
LMA	2.623	0.0393	23.04656 - 76.84884
NDWI	2.59	0.0413	0.05952115 - 0.10115041
Leaves	1.198	0.325	
Lvs/GDD	4.192	0.00312	0.009386632 - 0.043150631
Height	0.882	0.501	
Ht/GDD	2.597	0.0374	0.0273507 - 0.2574125

Variable	$F_{5,38}$	P	Proportion of variance explained
PC1	1.904	0.116	0.3075
PC2	1.102	0.375	0.2563

Table S3: Results of ANOVA analyses of population averages of plant traits between geographic regions. Orange highlighting denotes statistical significance ($P < 0.05$).

Variable	$F_{2,129}$	P	Range
Carbon	15.98	6.33E-07	44.83250 - 49.91925
C:N	65.08	<2e-16	9.020624 - 30.672772
Nitrogen	49.7	<2e-16	1.600284 - 4.970000
Chlorophyll	5.368	0.00576	0.07725 - 0.51055
PRI	19.76	3.25E-08	-0.07693411 - 0.04057500
Cardenolides	45.35	1.21E-15	0.266694 - 3.890000
Latex	1.547	0.217	0.000 - 0.126
Cellulose	35.84	4.17E-13	14.48750 - 25.00731
Lignin	10.26	7.33E-05	4.030778 - 13.412500
LMA	7.832	0.000616	6.8950 - 130.7825
NDWI	233.6	<2e-16	-0.0192000 - 0.1147334
Leaves	29.58	2.66E-11	0 - 175
Lvs/GDD	21.52	8.58E-09	0.00000000 - 0.07521059
Height	5.175	0.00689	0 - 203
Ht/GDD	138.5	<2e-16	0.0000000 - 0.2079099
PC1	1.176	0.312	-6.684809 - 3.855278
PC2	0.094	0.911	-5.535651 - 4.194960

Table S4: Results of ANOVA analyses of plant traits measured on individuals at three timepoints (TP0: field measurements; TP1: 6 months common garden; TP2: 12 months common garden). Orange highlighting denotes statistical significance ($P < 0.05$).

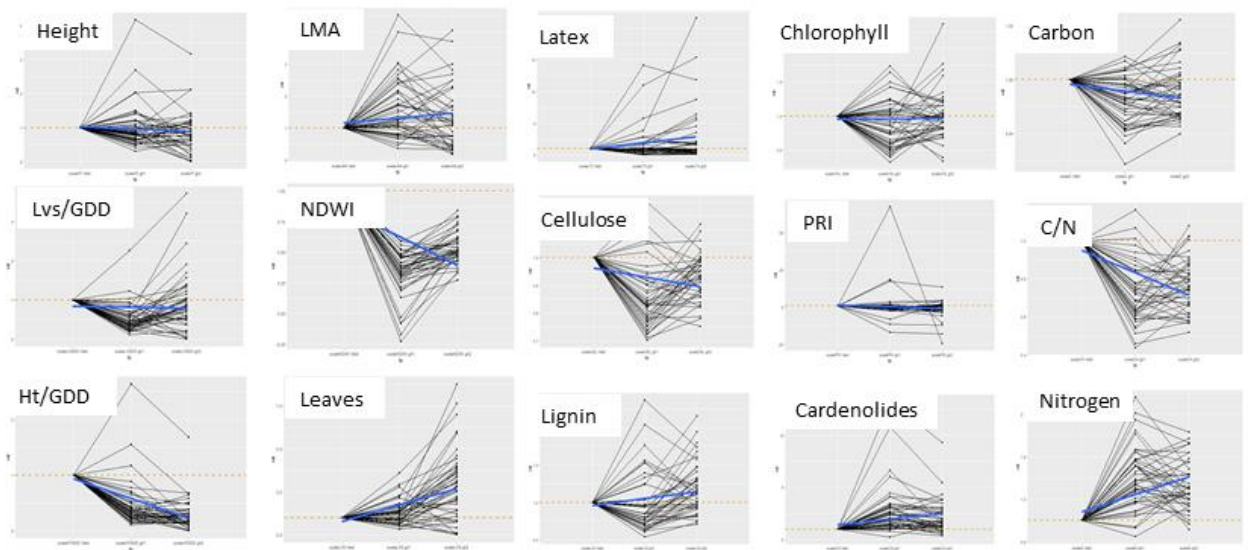


Figure S4: Plant traits (scaled to values in field measurements) change over time in a common garden, measured in the field, at 6 months in a common garden, and at 12 months in a common garden. All traits except for *latex* changed significantly over time.

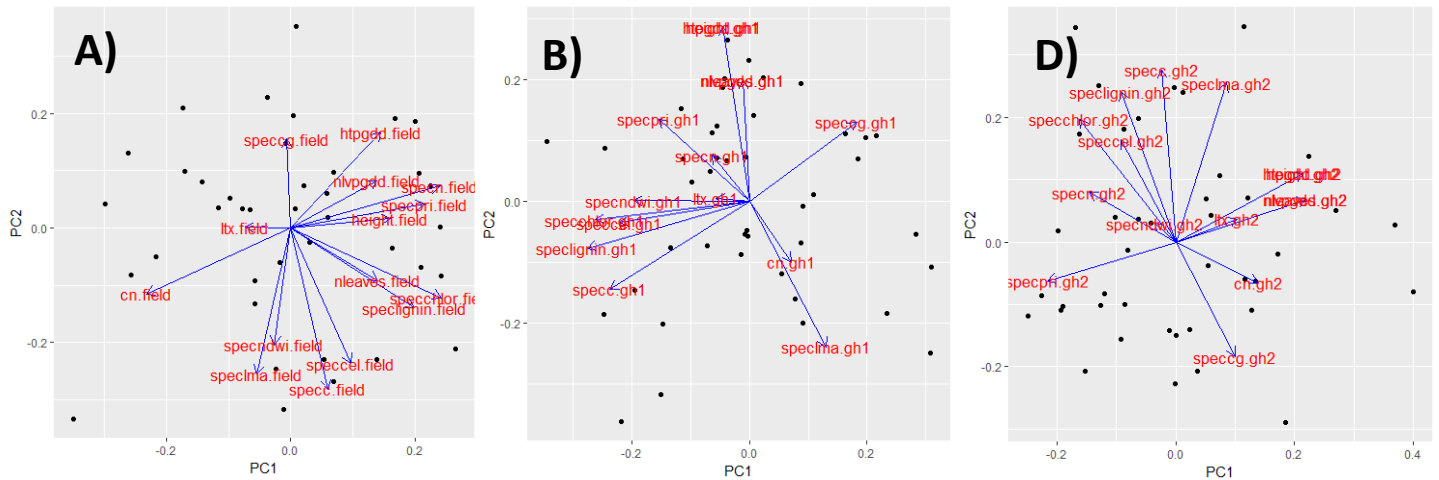


Figure S5: Biplot of PC1 and PC2 of trait measurements on individuals in the field (A), after 6 months in a common garden (B), and after 12 months in a common garden (C). Each point represents a population average ($n = 45$). PC1 for field measurements (A) is driven mostly by nitrogen, C/N, chlorophyll, lignin, and PRI, while PC2 is driven mostly by carbon, LMA, and cellulose. PC1 for 6 month common garden measurements (B) is driven mostly by lignin, chlorophyll, carbon, cellulose, NDWI, cardenolides, and PRI, while PC2 is driven mostly by growth rate, LMA, and carbon. PC1 for 12 month common garden measurements (C) is driven mostly by PRI, leaves, height, and growth rate, while PC2 is driven mostly by carbon, chlorophyll, cardenolides, cellulose, lignin, and LMA.

Variable	GH1 ~ Field				GH2 ~ Field			
	R^2	F	P	Coefficient	R^2	F	P	Coefficient
Carbon	0.009891	0.4795	0.492	0.1178	0.009805	0.4555	0.503	-0.1204
C:N	0.00846	0.4096	0.52523	0.1182	0.05706	2.783	0.102044	0.2266
Nitrogen	0.002334	0.1123	0.738981	0.1123	0.06157	3.018	0.089029	0.4086
Chlorophyll	2.82E-05	0.001356	0.97078	0.008274	0.00865	0.4014	0.5295	0.12883
PRI	0.004951	0.2388	0.6273	-0.073105	0.02675	1.265	0.267	0.088435
Cardenolides	0.05126	2.593	0.114	-0.2705	0.0007584	0.03491	0.853	-0.04021
Latex	0.07887	4.709	0.03434	0.24449	0.003675	0.1918	0.663	0.07462
Cellulose	0.02845	1.406	0.2416	0.2841	0.1447	7.782	0.00765	0.4532
Lignin	0.006757	0.3266	0.57	0.07541	0.05374	2.612	0.113	0.2847
LMA	0.01141	0.5542	0.46	-0.1904	0.06372	3.131	0.0835	-0.4277
NDWI	0.01671	0.8157	0.371	0.1762	0.01345	0.6271	0.432484	0.09053
Leaves	0.0003577	0.02075	0.886	0.06662	0.001242	0.07463	0.78565	-0.2442
Lvs/GDD	0.001901	0.1105	0.7408	0.06094	0.01514	0.9223	0.341	0.27721
Height	0.02299	1.365	0.247	0.167	0.002599	0.159	0.6915	-0.0755
Ht/GDD	0.03568	2.146	0.148	-0.074376	4.87E-05	0.002972	0.9567	0.003049
PC1	0.003827	0.1614	0.69	-0.04176	0.01524	0.6498	0.425	0.125
PC2	0.003158	0.1331	0.7171	-0.04942	0.0007426	0.03121	0.861	-0.02728

Table S5: Results of a simple linear regression of trait values of individuals as measured in the field against trait values as measured at either 6 months in common garden or 12 months in common garden. A large F value and a P value < 0.05 indicate that the linear model has a slope that is statistically different from zero, which itself suggests that a trait is “genetically controlled”. Latex at TP1 and cellulose at TP2 show significance. Orange highlighting denotes statistical significance ($P < 0.05$).

Variable	$F_{5,27}$	P	Range
Food eaten	1.29	0.297	0.0000 452.6491 (mg dry mass)
Weight gained	2.567	0.0485	0.0000 - 33.2197893 (mg dry mass)
Length gained	0.877	0.509	0.0000 - 13.8 (mm)
Efficiency Dig.	1.585	0.224	0.0000 - 0.73979653
Efficiency Ing.	2.1	0.11	0.0000 - 0.202457753 (mg dry mass)
Lifespan	0.878	0.506	4.00 - 7.00 (days)
Survival	1.771	0.145	0 - 1

Table S6: Results of ANOVA analyses of monarch measurements when fed milkweed from different regions ($n = 45$). Weight gain showed significant variation between the Northeast region and Northcentral and Southeast regions. It should be noted, however, that these two regions (Northcentral and Southcentral) each have a single extreme value that lies within the range of values similar to those for the Northeast region. Orange highlighting denotes statistical significance ($P < 0.05$).

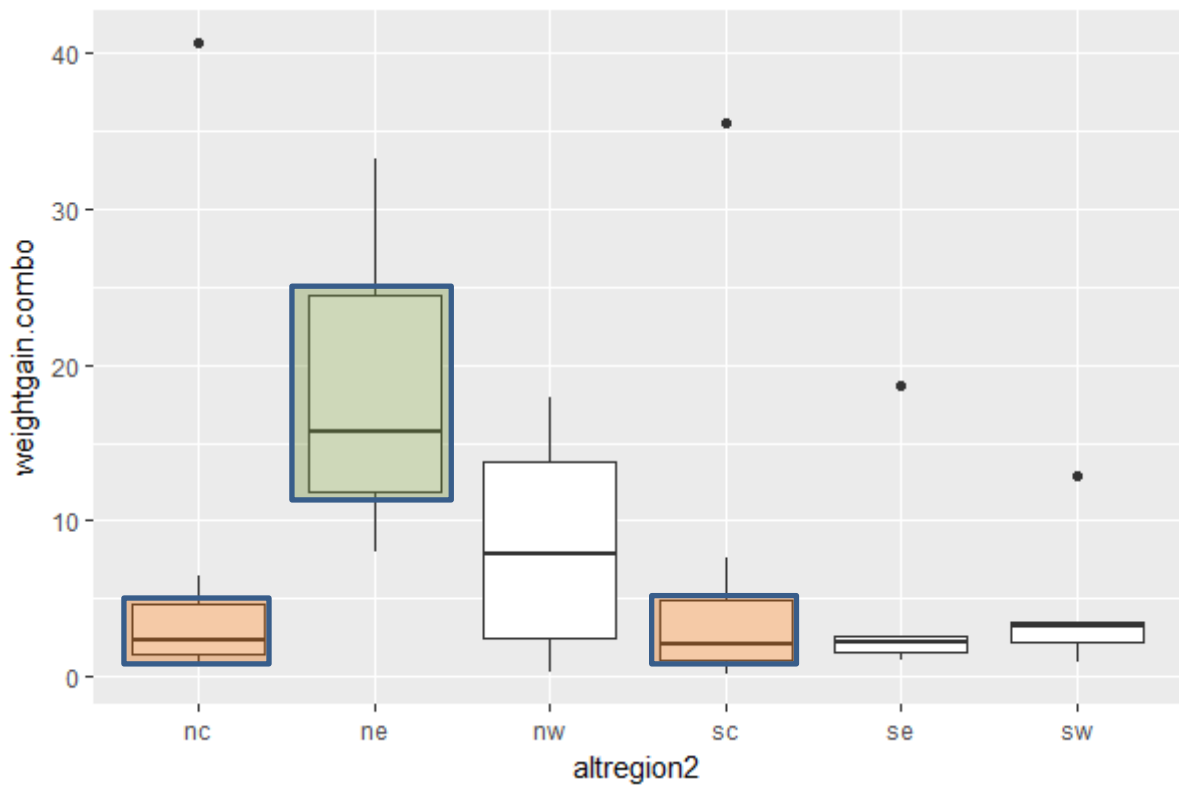


Figure S6: ANOVAs indicate a significant difference (see Table S6) in monarch weight gain when fed plants from the Northeast versus plants from the Southcentral or Northcentral regions ($n = 45$). It should be noted, however, that these two regions (Northcentral and Southcentral) each have a single extreme value that lies within the range of values similar to those for the Northeast region.

Variable	Latitude			Longitude			Dif. from range center		
	R ²	P	Coefficient	R ²	P	Coefficient	R ²	P	Coefficient
Food eaten	0.036275	0.224	-9.484	0.028896	0.279	-2.896	0.006609	0.606	2.737
Weight gained	0.000713	0.862	0.08081	0.034527	0.2218	0.2201	0.067626	0.0847	0.5736
Length gained	4.35E-05	0.966	0.008454	0.003355	0.713	0.02911	0.08043	0.0631	0.2653
Efficiency Dig.	0.058206	0.246	0.011127	0.072952	0.192	0.005895	0.111554	0.103	0.012
Efficiency Ing.	0.030549	0.374	0.003074	0.004055	0.748	4.83E-04	0.118335	0.0735	0.004523
Lifespan	0.004689	0.623525	-0.02217	0.025228	0.254	0.01871	0.026579	0.241	-0.03719
Survival	0.011217	0.4373	-0.0121	0.034261	0.1721	0.007703	0.031942	0.188	-0.01439

Table S7: Results of a general linear mixed model on monarch larvae measurements of individuals fed common milkweed that had been collected from across the range and kept in a common garden for 12 months ($n = 63$). We used monarch larvae measurements as response variables, latitude, longitude, and distance from range center as fixed effects, and population included as a random effect, in order to look for continuous patterns of monarch larvae measurement variation when fed plants from along the aforementioned spatial gradients. No models produced statistically significant results.

Plant traits and monarch larvae measurements

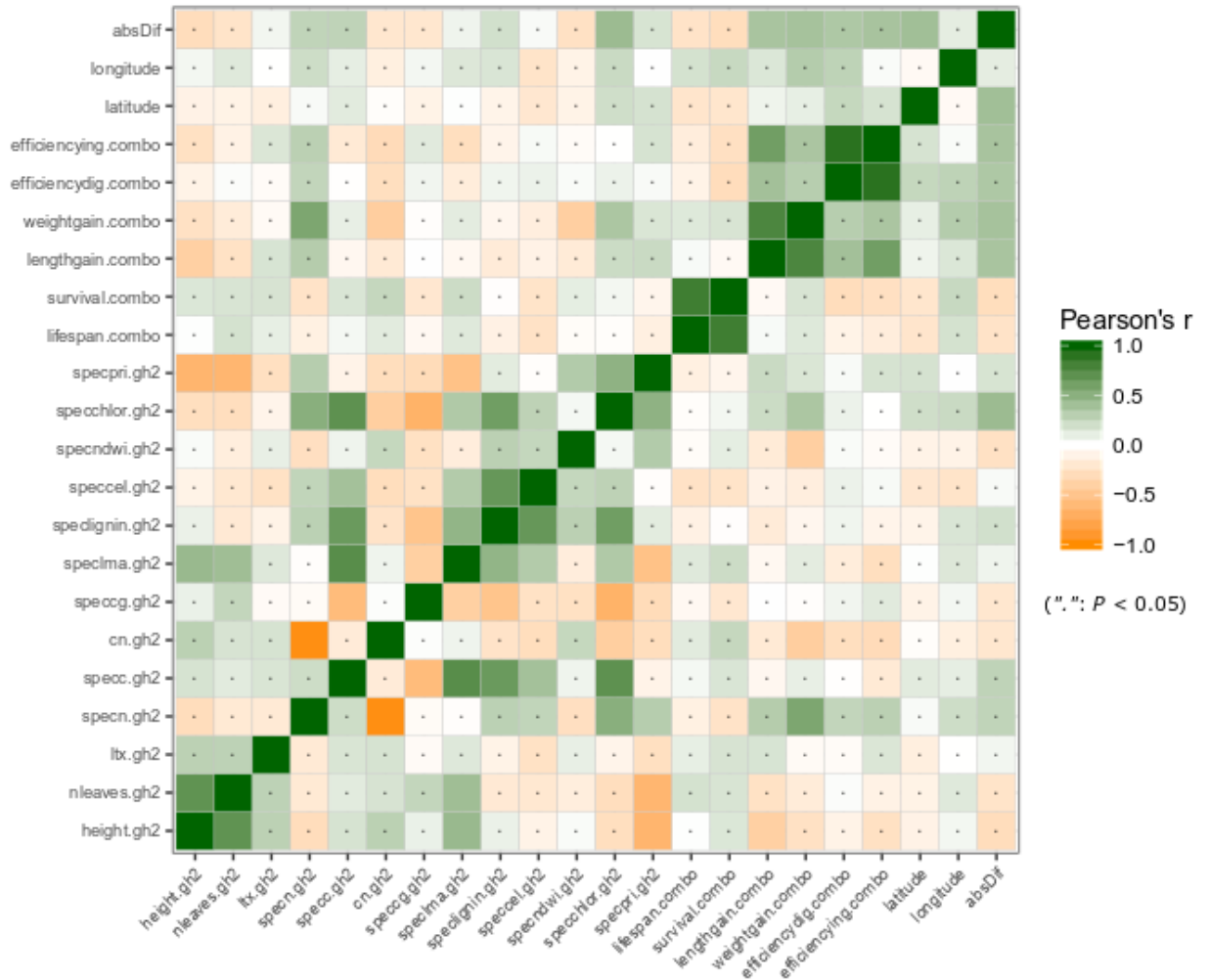


Figure S7: A correlation matrix of plant traits at time of feeding (12 months' time growing in a common garden) and monarch measurements ($n = 45$).

REFERENCES

- Agrawal, A.A., 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*, pp.651–667.
- Agrawal, A.A., Patrick, E.T. & Hastings, A.P., 2014. Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere*, 5(10), pp.126.
- Alexander, H.M. et al., 2007. Is there reduction in disease and pre-dispersal seed predation at the border of a host plant's range? Field and herbarium studies of *Carex blanda*. *Journal of Ecology*, 95(3), pp.446–457.
- Asner, G.P. & Martin, R.E., 2008. Spectral and chemical analysis of tropical forests: Scaling from leaf to canopy levels. *Remote Sensing of Environment*, 112(10), pp.3958–3970.
- Batalden, R. V., Oberhauser, K. & Peterson, A.T., 2007. Ecological Niches in Sequential Generations of Eastern North American Monarch Butterflies (Lepidoptera: Danaidae): The Ecology of Migration and Likely Climate Change Implications.
- Bingham, R.A. & Agrawal, A.A., 2010a. Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. *Journal of Ecology*, 98(5), pp.1014–1022.
- Bingham, R.A. & Agrawal, A.A., 2010b. Specificity and trade-offs in the induced plant defence of common milkweed *Asclepias syriaca* to two lepidopteran herbivores. *Journal of Ecology*, 98(5), pp.1014–1022.
- Brower, Lincoln P. et al., 2012. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? *Insect Conservation and Diversity*, 5(2), pp.95–100.
- Brower, L.P. et al., 2002. Quantitative Changes in Forest Quality in a Principal Overwintering Area of the Monarch Butterfly in Mexico, 1971-1999. *Conservation Biology*, 16(2), pp.346–359.
- Chambers, R.M. & Fourqurean, J.W., 1991. Alternative criteria for assessing nutrient limitation of a wetland macrophyte (*Peltandra virginica* (L.) Kunth). *Aquatic Botany*, 40(4), pp.305–320.
- Clausen, J., Keck, D.D. & Hiesey, W.M., 1948. *Experimental Studies on the Nature of Species. III: Environmental Responses of Climate Races of Achillea*, Carnegie Institution, Washington.
- Cohen, J.A., 1983. *Chemical interactions among milkweed plants (asclepiadaceae) and lepidopteran herbivores*.
- Coley, P.D., 1983. Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecological Monographs*, 53(2), pp.209–234.
- Coley, P.D., Bryant, J.P. & Chapin, F.S., 1985. Resource Availability and Plant Antiherbivore Defense. *Science*, 230(4728).
- Couture, J.J., Serbin, S.P. & Townsend, P.A., 2015. Elevated temperature and periodic water stress alter growth and quality of common milkweed (*Asclepias syriaca*) and monarch (*Danaus plexippus*) larval performance. *Arthropod-Plant Interactions*, 9(2), pp.149–161.

- Couture, J.J., Serbin, S.P. & Townsend, P.A., 2013. Spectroscopic sensitivity of real-time, rapidly induced phytochemical change in response to damage. *New Phytologist*, 198, pp.311–319.
- Couture, J.J., Serbin, S.P. & Townsend, P.A., 2013. Spectroscopic sensitivity of real-time, rapidly induced phytochemical change in response to damage. *New Phytologist*, 198(1), pp.311–319.
- Couture, J.J., Servi, J.S. & Lindroth, R.L., 2010. Increased nitrogen availability influences predator–prey interactions by altering host-plant quality. *Chemoecology*, 20(4), pp.277–284.
- Daly, C. et al., 2008. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *International Journal of Climatology*, 28(15), pp.2031–2064.
- Erwin, A.C. et al., 2014. Above-ground herbivory by red milkweed beetles facilitates above- and below-ground conspecific insects and reduces fruit production in common milkweed K. Whitney, ed. *Journal of Ecology*, 102(4), pp.1038–1047.
- Flockhart, D.T.T. et al., 2015. Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies T. Coulson, ed. *Journal of Animal Ecology*, 84(1), pp.155–165.
- Foley, W.J. et al., 1998. Ecological applications of near infrared reflectance spectroscopy - a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia*, 116(3), pp.293–305.
- Fritsher, J., 2015. USDA launches new conservation effort to aid monarch butterflies | NRCS.
- Hartzler, R.G., 2010. *Reduction in common milkweed (Asclepias syriaca) occurrence in Iowa cropland from 1999 to 2009*.
- Hughes, P.R. et al., 1990. The effects of consuming ozone-injured milkweed leaves on feeding, growth, and development of monarch butterflies.
- Hughes, P.R., Radke, C.D. & Renwick, A.A., 1993. A Simple, Low-Input Method for Continuous Laboratory Rearing of The Monarch Butterfly (Lepidoptera: Danaidae) for Research. *American Entomologist*, 39(2), pp.109–112.
- Hunter, M.D., Malcolm, S.B. & Hartley, S.E., 1996. Population-level variation in plant secondary chemistry, and the population biology of herbivores. *Chemoecology*, 7(1), pp.45–56.
- Huxley, J.S., 1939. Clines: An auxiliary method in taxonomy. *Bijdragen tot de Dierkunde*, 27, pp.491–520.
- Huxley, J.S., 1938. SPECIES FORMATION AND GEOGRAPHICAL ISOLATION. *Proceedings of the Linnean Society of London*, 150(4), pp.253–264.
- Inamine, H. et al., 2016. Linking the continental migratory cycle of the monarch butterfly to understand its population decline. *Oikos*, 125(8), pp.1081–1091.
- IPCC, 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, IPCC.

- Karl, T.R., Melillo, J.M. & Peterson, T.C., 2009. *Global Climate Change Impacts in the United States: A State of Knowledge Report from the U.S. Global Climate Change Research Program*, Cambridge University Press.
- Kettler, T.A., Doran, J.W. & Gilbert, T.L., 2001. Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analyses. *Soil Science Society of America Journal*, 65(3), p.849.
- Lee, J.J., 2015. Imperiled Monarch Butterflies Get \$3.2 Million From U.S. Government. *National Geographic*.
- Malcolm, S., Cockrell, B. & Brower, L.P., 1993. Biology and conservation of the monarch butterfly.
- Malcolm, S.B., 1994. Milkweeds, monarch butterflies and the ecological significance of cardenolides. *Chemoecology*, 5–6(3–4), pp.101–117.
- Malcolm, S.B., Cockrell, B.J. & Brower, L.P., 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. *Journal of Chemical Ecology*, 15(3), pp.819–853.
- Malcolm, S.B. & Zalucki, M.P., 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. *Entomologia Experimentalis et Applicata*, 80(1), pp.193–196.
- McGill, B.J. et al., 2006. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution*, 21(4), pp.178–185.
- Melillo, J.M. et al., 1993. Global climate change and terrestrial net primary production. *Nature*, 363(6426), pp.234–240.
- Nail, K.R., Stenoien, C. & Oberhauser, K.S., 2015. Immature Monarch Survival: Effects of Site Characteristics, Density, and Time. *Annals of the Entomological Society of America*, 108(5).
- Nicotra, A.B. et al., 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, 15(12), pp.684–692.
- Núñez-Farfán, J. & Schlichting, C.D., 2001. Evolution in Changing Environments: The “Synthetic” Work of Clausen, Keck, and Hiesey. *The Quarterly Review of Biology*, 76, pp.433–457.
- Oberhauser, K. et al., 2008. *North American monarch conservation plan*,
- Oleksyn, J., Tjoelker, M.G. & Reich, P.B., 1998. Adaptation to changing environment in Scots pine populations across a latitudinal gradient. *Silva Fennica*, 32(2), pp.129–140.
- Opler, P.A., Peterson, R.T. & Malikul, V., 1992. *A Field Guide to Eastern Butterflies (Peterson Field Guides): Paul A. Opler, Roger Tory Peterson, Vichai Malikul*, New York: Houghton Mifflin Company.
- Pleasants, J., 2016. Milkweed restoration in the Midwest for monarch butterfly recovery: estimates of milkweeds lost, milkweeds remaining and milkweeds that must be added to increase the monarch population S. R. Leather & A. Stewart, eds. *Insect Conservation and Diversity*.
- Pleasants, J.M. & Oberhauser, K.S., 2013. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conservation and Diversity*, 6(2), pp.135–144.
- PRISM Climate Group, O.S.U., 2004. PRISM Climate Group, Oregon State University. <http://prism.oregonstate.edu>.

- Rasmann, S. et al., 2011. Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. *Journal of Ecology*, 99(1), pp.16–25.
- Rasmann, S. & Agrawal, A.A., 2011a. Evolution of specialization: a phylogenetic study of host range in the red milkweed beetle (*Tetraopes tetraophthalmus*). *The American naturalist*, 177(6), pp.728–37.
- Rasmann, S. & Agrawal, A.A., 2011b. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters*, 14(5), pp.476–483.
- Rasmann, S., Johnson, M.D. & Agrawal, A.A., 2009. Induced Responses to Herbivory and Jasmonate in Three Milkweed Species. *Journal of Chemical Ecology*, 35(11), pp.1326–1334.
- Ries, L., Taron, D.J. & Rend N-salinas, E., 2015. The Disconnect Between Summer and Winter Monarch Trends for the Eastern Migratory Population: Possible Links to Differing Drivers. *Ann. Entomol. Soc. Am*, 108(5), pp.691–699.
- Root, T.L. et al., 2003. Fingerprints of global warming on wild animals and plants. *Nature*, 421(6918), pp.57–60.
- Seiber, J.N., Nelson, C.J. & Lee, S.M., 1982. Cardenolides in the latex and leaves of seven asclepias species and calotropis procera. , 21(9), pp.2343–2348.
- Semmens, B.X. et al., 2016. Quasi-extinction risk and population targets for the Eastern, migratory population of monarch butterflies (*Danaus plexippus*). *Scientific Reports*, 6, p.23265.
- Serbin, S.P. et al., 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications*, 24(7), pp.1651–1669.
- Soltis, D.E. et al., 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, 15(14), pp.4261–4293.
- Stebbins, G.L. (George L., 1950. *Variation and evolution in plants.*, New York: Columbia University Press.
- Stenoien, C., Nail, K.R. & Oberhauser, K.S., 2015. Habitat Productivity and Temporal Patterns of Monarch Butterfly Egg Densities in the Eastern United States. *Annals of the Entomological Society of America*.
- Thogmartin, W.E. et al., 2017. Restoring monarch butterfly habitat in the Midwestern US: ‘all hands on deck.’ *Environmental Research Letters*, 12(7), p.074005.
- Thuiller, W. et al., 2005. Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 102(23), pp.8245–50.
- Turesson, G., 1922a. The genotypical response of the plant species to the habitat. *Hereditas*, 3(3), pp.211–350.
- Turesson, G., 1922b. The species and the variety as ecological units. *Hereditas*, 3(1), pp.100–113.
- USDA NRCS, 2015. *Monarch Butterfly Habitat Development Project*.

- Vannette, R.L., Hunter, M.D. & Rasmann, S., 2013. Arbuscular mycorrhizal fungi alter above- and below-ground chemical defense expression differentially among *Asclepias* species. *Frontiers in Plant Science*, 4, p.361.
- Vickerman, D.B. & Boer, G., 2002. Maintenance of narrow diet breadth in the monarch butterfly caterpillar: response to various plant species and chemicals. *Entomologia Experimentalis et Applicata*, 104(2–3), pp.255–269.
- Vidal, O., J, L.-G. & E, R.-S., 2014. Trends in Deforestation and Forest Degradation after a Decade of Monitoring in the Monarch Butterfly Biosphere Reserve in Mexico. *Conservation Biology*, 28(1), pp.177–186.
- Walther, G.-R. et al., 2002. Ecological responses to recent climate change. *Nature*, 416(6879), pp.389–395.
- Webb, C.T. et al., 2010. A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology Letters*, 13(3), pp.267–283.
- Woods, E.C. et al., 2012. Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs*, 82(2), pp.149–168.
- Woodson, R.E., Froeschner, E.H. & Heinze, A.A., 1954. The North American species of *Asclepias* L. Source: *Annals of the Missouri Botanical Garden Missouri Botanical Garden*, 41(1), pp.1–211.
- Wyatt, R. et al., 1993. Range Extension Southward in Common Milkweed, *Asclepias syriaca* Range extension southward in common milkweed, *Asclepias syriaca* L.1. Source: *Bulletin of the Torrey Botanical Club*, 120(1202), pp.177–179.
- Zalucki, M.P. & Brower, L.P., 1992. Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology*, 3(2), pp.81–93.
- Zalucki, M.P., Brower, L.P. & Alonso-M, A., 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology*, 26(2), pp.212–224.
- Zalucki, M.P. & Rochester, W.A., 2004. Spatial and temporal population dynamics of monarchs downunder: Lessons for North America. , pp.219–228.
- Van Zandt, P.A. & Agrawal, A.A., 2004. Specificity of induced plant responses to specialist herbivores of the common milkweed *Asclepias syriaca*. *Oikos*, 104(2), pp.401–409.
- Züst, T. & Agrawal, A.A., 2016. Population growth and sequestration of plant toxins along a gradient of specialization in four aphid species on the common milkweed *Asclepias syriaca* K. P. Lee, ed. *Functional Ecology*, 30(4), pp.547–556.