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Limitations of Host Plant use in Two Andean Altinote (Nymphalidae, Heliconiinea, Acraeini), Butterflies, from a Tritrophic Perspective

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LIMITATIONS OF HOST PLANT USE IN TWO ANDEAN *ALTINOTE* (NYMPHALIDAE, HELICONIINEA, ACRAEINI), BUTTERFLIES, FROM A TRITROPHIC PERSPECTIVE.

A thesis submitted in partial fulfillment of the requirements for the

degree of Master of Science

BY

KAREN M PEDERSEN
B.S., Wright State University, 2009

2015
Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

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Date

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Karen M Pedersen ENTITLED Limitations of Host Plant Use in Two Andean Altinote (Nymphalidae, Heliconiinea, Acraeini), Butterflies, from a Tritrophic Perspective BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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Abstract

Pedersen, Karen M., M.S. Department of Biological Sciences, Wright State University, 2015. Limitations of host plant use in two Andean *Altinote* (Nymphalidae, Heliconiinea, Acreaini) Butterflies, from a Tritrophic perspective.

Despite the clear advantages of generalist feeding, many insect herbivores feed on a relatively small number of available host plants with in phylogenetically restricted groups. To better understand patterns of host plant use I used the sister species *Altinote stratonice* and *Altinote dicaeus* and their overlapping but distinct host plant range. I measured physiological effects of plants by using development time, pupal mass, and survival. To determine the importance of enemies I quantified rates of parasitism and rates of predation. Finally I measured host plant frequency, and host plant abundance. I found that survival of *A. dicaeus* and *A. stratonice* was reduced on low quality host plants. Additionally host plant use by *A. stratonice* was correlated with host plant abundance and host plant use by *A. dicaeus* was correlated host plant size. Overall patterns of host plant use appeared to be driven by bottom up forces even when enemies present a clear threat.

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Introduction

All organisms have a restricted set of biotic and abiotic conditions in which they can survive. In this sense all organisms are specialists (Price et al. 2011, Forister et al. 2012). Nowhere is this more clearly illustrated than in the herbivorous insects. The adaptations of insects to plants and plants to insects has resulted from millions of years of co-evolution, in which both the plant and the insect attempt to maximize their own fitness, often at the cost of the other. A temporary upper hand may be gained by the plant or the insect, but it is often short lived. As a result, many plants have showy, nutrient rich flowers for pollinators and an arsenal of physical and chemical defenses to deter herbivores. These modifications have increased both specialization and interdependence between plants and insects (Ehrlich and Raven 1964, Schoonhoven et al. 2005, Price et al. 2011).

Species interactions and feeding specializations are important ecologically and practically (Futuyma and Agrawal 2009). Understanding species interactions in ecosystems informs conservation efforts because it helps us to identify and target keystone species (Memmott 1992, Fisher 1998). Conservation efforts also benefit from the use of specialists as biological control agents for invasive species. Invasive species cost millions of dollars in lost revenue each year. Expanding our knowledge of host use will also help us to better control agricultural pests. We may be able to control pest herbivores with parasitoids and predators and weeds with herbivores (McFadyen 1998, Pimental et al. 2004). Furthermore, more than half of the worlds described species are involved in plant-herbivore-parasitoid interactions (Hawkins 1994). In herbivorous insect systems, parasitoids are sometimes responsible for more deaths than predators and pathogens combined (Hawkins et al. 1997). Host use by herbivorous insects and parasitoids is often highly specialized relative to other feeding guilds (Mitter et al. 1988, Price et al. 2011). Understanding what drives host specialization in these feeding guilds will improve our understanding of the majority of species interactions.

Co-evolutionary physiological adaptations of plants and insects go a long way toward explaining the observed narrow host breadth of most insect herbivores, but they do not completely explain host plant specialization in insect herbivores (Craig and Itami 2008). In recent years, we have seen mounting evidence that predators and parasitoids also influence herbivore-plant relationships (Singer and Stireman 2003, Murphy and Berenbaum 2004, Oppenheim and Gould 2002). For example, parasitoids can favor the use of lower quality host plants that provide refuge from parasitoids that often use host plant volatiles to find hosts. Lower quality host plants might increase rates of mortality and development time of herbivores, but they may also provide refuge from parasitoids (Murphy and Berenbaum 2004, Rodrigues et al. 2010). Enemies can also influence the parts of the plant an herbivore feeds upon. For example, parasitoids can select for feeding inside fruits or other protected areas (Oppenheim and Gould 2002). Parasitoids can also influence host plant selection in some insect species with wide host plant ranges. For example, certain woollybear caterpillars (Erebidae) change their food-plant preferences in response to parasitism (Singer et al. 2003, Singer et al. 2004).

Here, I assess the ecological and physiological determinates of host plant use in two closely related, co-occurring nymphalid butterfly species, *Altinote dicaeus* and *Altinote stratonice*, with overlapping yet distinct host plant ranges. First I examine the issue of host plant use and specialization in herbivorous insects and how it is assessed and briefly review some of the major hypotheses that have been put forward to explain host plant use in this group. I then present methods and results from my studies designed to elucidate the underlying causes of host use in this species pair. including performance assays on alternate hosts in the absence of enemies, examination of rates of predation and parasitism on alternate host plants, and assessment of host plant use relative to plant availability. Finally I discuss what these results indicate about the forces that shape patterns of host plant use in this system and the broader implications for other insect herbivores.

All Insects Should Be Generalists

Intuitively one would expect shifts that broaden diet breadth to be advantageous. An expanded host range should increase fitness by increasing the available resources relative to individuals or populations with a narrower host range. A large host plant range may also allow for a larger geographic range and reduce dependence on particular host plant species, which should make populations less prone to extinction (Yotoko et al. 2005,Biesmeijer et al. 2006, Hardy and Otto 2014). Generalists should experience reduced inter- and intraspecific competition because food should be more widely available. Some generalists exploit a large number of plants, typically feeding on high quality host plants, only switching to toxic plants after being parasitized, thus increasing survival after an event of parasitism (Singer et al. 2004). Generalists may have greater genetic variability that permits them to exploit a larger number of plants and maintain larger population sizes, which should decrease the likelihood of extinction by increasing the likelihood of adaptation (Kelley et al. 2000). Despite the apparent advantages of broad host ranges, relatively generalist herbivores are comparatively rare; most herbivorous insects are relatively specialized feeding on only one plant family (Strong et al. 1984, Forister et al. 2015). So, a natural question is: Why do most insect herbivores feed on such a limited number of plants?

To answer this question the terms generalist and specialist must be defined. The terms are often defined by convenience. Specialization in insect herbivores has been defined in a myriad of ways, including feeding on one plant species, feeding on a single plant genus, feeding on only one plant family or herbivore taxa feeding on three or fewer plant families. In these cases generalist is usually used to refer to everything that is not a specialist (Bernays and Graham 1988, Yotoko et al. 2005, Hardy and Otto 2014). This means that comparing results between studies can be extremely difficult. Generalists and specialists exist on a continuum, which at one extreme are, specialists consuming but a single food plant species (or even just a particular part of one host plant species) and on the other, food mixers consuming plants from 50+ families (Singer 2008, Forister et al. 2015). The term generalist often refers to a generalist relative to the other insect herbivores studied, but not relative to all insect herbivores. While these comparisons are useful within studies, they make it hard to interpret the results of several studies simultaneously. For this reason, the model proposed by Jorge et al. (2014) which numerically assigns values to the degree of generalization and specialization may allow more useful comparisons among studies.

Insects can also be specialists at multiple levels. First the whole species is specialized if the number of host families, genera or species is relatively small. Second, some populations may use a smaller number of host families or genera relative to other populations of the same species. Third, the individual insect may be functionally specialized, only using one host (Singer 2008). Here I will use generalist to refer to insects feeding on more than one family of plants and specialist to indicate that the host range is less than or equal to one plant family. I may refer to an insect as relatively generalized if that insect feeds on more plants species or more plant genera than the insect I am comparing it to.

Hypotheses to explain insect host range

Although numerous hypotheses have been proposed to explain the general narrow host ranges of phytophagous insects and the patterns of host use in particular groups (Mayhew 1997, Awmakc and Leather 2002, Singer 2008), most of these conform to two primary arguments: the physiological efficiency hypothesis and enemy avoidance. In addition to these main hypotheses, there are a few others including the neurological constraints hypothesis (Bernays 1998) and the optimal foraging hypothesis (Forister et al. 2009). Another hypothesis that has been only rarely addressed in the literature, but which I explore here, is that the abundance of a host plant or its size also determines patterns of host plant use.

Physiological Efficiency

The physiological efficiency hypothesis predicts that the narrow host plant use observed in most herbivorous insects is driven by digestive adaptations to circumvent specific plant toxins (Cornell and Hawkins 2003, Price et al 2011). Plant toxins deter generalized herbivory because non-adapted herbivores have may be killed by or have trouble digesting chemically defended plants. Feeding on toxic or low quality host plants results in decreasing fitness, as measured by increased time to development, lower pupal mass, decreased immune function, and decreased fecundity (Erickson and Feeney 1974, Levin 1976, Blau et al. 1978, Cornell and Hawkins 2003). Specializing on a plant species or a group of chemically similar plants should allow insects to evolve adaptations that increase the efficiency of biomass conversion by mitigating the negative effects of plant defensive compounds. To circumvent toxic plant chemicals, insects may avoid feeding on extremely toxic parts of their host plant, reduce the permeability of their guts to toxic chemicals, develop enzymes to detoxify toxic compounds, or sequester toxins for defense against predators (Lill and Marquis 2001, Singer

2008, Opitz and Müller 2009). While the Physiological Efficiency Hypothesis may explain some of the narrowness in host plant use, it does not provide a full explanation, because the preference by a female for a host plant, does not always match larval performance on that plant (Craig and Itami 2008, Forister et al*.* 2009, Davis and Cipollini 2014).

Enemy Avoidance

While there is clearly much support for the physiological efficiency hypothesis, it does not explain all patterns of host plant use. Avoiding enemies may in some instances provide a better explanation. Sometimes host shifting can provide this protection. Host shifting can occur when an oviposition "mistake" provides some fitness advantage. In most documented cases of host shifts, new host plant associations are often chemically similar to old host plants (Forister et al*.* 2009), however shifts to chemically different host plants also occur (Brown and Francini 1990, Silvia-Brandao et al. 2008). New host plants can be advantageous if they provide better nutrition, reduced competition, increased fecundity, and/or escape from enemies. A new host plant that provides the same level of fitness as the old host plant would also be an advantage because the new host plant increases available resources. The result of a host shift is either a wider host plant range or a complete shift to the new host. A special kind of host shift is one that results in "enemy-free" or "enemy-reduced space", a niche that provides refuge from enemies at some physiological cost (Jeffries and Lawton 1983).

Use of plants that provide refuge from predators (Camara 1997a) and parasitoids (Oppenheim and Gould 2002) can explain the use of otherwise suboptimal host plants and drive host plant shifts. These shifts may confer what is referred to as enemy free space if the following requirements are met: (1) Enemies must reduce fitness, (2) the new habitat must provide some protection from enemies, and (3) if natural enemies were absent from the old habitat, the new habitat would represent a fitness cost (Jeffries and Lawton 1983, Murphy and Berenbaum 2004). Mediation of enemy-free space is not limited to the use of a new host plant (spatially mediated enemy-free space). Behaviorally mediated enemy-free space, e.g. feeding in protected areas like inside fruits (Oppenheim and Gould 2002), and chemically mediated enemy-free space, e.g., feeding on a plant that is toxic to enemies (Singer et al. 2004), also protects herbivorous insects from predators and parasitoids, and can favor host plant specialization. Enemy-free space refers to, by definition, a new habitat, but a temporary diet expansion does not mean that colonizing enemy-free space cannot lead to later specialization in the new habitat.

Resource Availability

For any insect herbivore, plant abundance and size, may shape patterns of host plant use for a variety of reasons. Abundant plants and large plants are easier to locate, and using a plant that can be found quickly would limit the female's exposure to predators. Using an abundant plant could limit competition for resources or allow an insect to migrate to a neighboring compatible host plant if needed. An example of this is the fall web worm (*Hyphantria cunea)* where the frequency of host plant use is predicted by plant abundance (Mason et al. 2011). Even though females of this species only have a single clutch, they use plant relative to abundance and not quality plants they encounter to oviposit on (Mason et al. 2011). However, not all systems appear to be sensitive to local host plant abundance, and herbivores may be highly sensitive to both plant quality and parasitoid pressure (Stiling and Moon 2005). This variation may in part be due to different life history characteristics of the studied insects. For example, solitary insects may not require as much resources as gregarious insects.

Study System

In this study, I evaluate the aforementioned hypotheses for host specificity and assess the bottom-up and top-down ecological factors that determine host plant use and specificity in co-occurring and closely related species of Andean butterflies that differ in host use: *Altinote dicaeus albofasciata* (Hewitson, 1869) and *Altinote stratonice aereta* (Jordan, 1913) (Nymphalidae, Heliconiinea, Acraeini). These two butterfly species occur commonly from 1,100 m in elevations up to 1,800 m in the Andes Mountains of Columbia, and Ecuador. Additionally *A. dicaeus* is found in northern Peru and *A. stratonice* in Venezuela and Costa Rica. As with many tropical species, occurrence records are at best spotty, so ranges are likely a bit larger than reported here. Adults of *A. dicaeus* and *A. stratonice* are both morphologically distinct from one another and sexually dimorphic (fig. 1). *Altinote dicaeus* and *A. stratonice* are gregarious from the time they leave the egg to the fourth instar. The eggs of *A. stratonice* change from a cream color after they are placed to a red color, however while the eggs of *A. dicaeus* never turn red they do change from a bright white to a cream color as the larvae near eclosion (fig. 2). After eclosion larvae are about 1 mm in length. The two species appear very similar until the second instar when *A. stratonice* begins to develop a lighter coloration, and by the third instar the white patterning appears on *A. stratonice* while *A. dicaeus* remains solid black (fig. 2). At around the third instar larvae appear to migrate in smaller groups to other plants. How far they migrate and if they continue to use their natal host plant remains an open question. However groups of younger larvae often have older caterpillars as companions so it is possible that they join groups of younger caterpillars. By the fifth instar most caterpillars have wandered off on their own to pupate. They will do this on almost any available structure, including the walls and supports of the research station but also commonly on other plants both host and non-host alike. As their aposematic coloration suggests, both *A. dicaeus* and *A. stratonice* larvae and adults are chemically protected with cyanogenic glycosides. Cyanogenic glycosides are synthesized *de novo* and have not been found in the larval host plants of *A. dicaeus* and *A. stratonice* (Brown and Francini 1990). Even though cyanogenic glycosides are not found in the host plants of *A. dicaeus* and *A. stratonice* that does not mean that the plants are without defenses. Pyrrolizidine Alkaloids (PAs) have been found in other plants in the tribe Liabeae (Brown and Francini 1990).

Figure 1 Dorsal view of *Altinote dicaeus* and *Altinote stratonice* males and females. The size and color of the wing patches varies significantly within species. **A)** *A. dicaeus* males, have distinctive pink to red bands. **B)** Adult male *A. stratonice* have a small black dot contained within their distinctive yellow patches. **C)** Female *A. dicaeus* are characterized by their cream colored vertical bands. **D)** *A. stratonice* females are characterized by a continuous but jagged band of black in the middle of the yellow to orange color patches on their forewings.

Figure 2 Third instar *A. dicaeus* (Top) and *A. stratonice* (Bottom) caterpillars and mature eggs. The exact coloration of *A. stratonice* is extremely variable ranging from faint discontinuous yellow lines to the bold cream pattern above.

I used the plants I encountered *A. dicaeus* or *A. stratonice* on most often for my experiments. At my study site at the Yanayacu Biological Research Station in Ecuador, *A. dicaeus* larvae were found primarily on *Erato polymnioides* and less often on *Munnozia hastifolia*. In contrast *A. stratonice* was found most often on *Munnozia pinnatipartita,* and less often on *M. hastifolia*. Thus they exhibit distinct, but overlapping patterns of host use. I classify the host plant used most often as the primary host plant and the host plant used less often as the secondary host plant. It is noteworthy that I personally observed *A. dicaeus* on both *M. pinnatipartita* and on an unidentified *Munnozia* species. *A. dicaeus* was observed on two plant genera, *Erato*, and *Munnozia*. In contrast, *A. stratonice* was only ever observed on plants in the genus *Munnozia*. Given this we can say that *A. dicaeus* has a broader range of locally acceptable host plants (fig. 3). It is worth noting that there are records of *A. dicaeus* and *A. stratonice* on other plant taxa. However, it appears that this is often the result of fifth instar wandering to locate a pupation site or an individual migrating and not a food plant choice. In some cases there do appear to be valid alternate host plant records of *A. stratonice* in other geographic locations for example *Liabum* spp. in Columbia and Venezuela (Brown and Francini 1990).

Comparing co-occurring, closely related butterflies with overlapping host plant ranges is an ideal system in which to evaluate hypotheses for the ecological determinants of host plant use and host specificity because it controls to some extent for confounding variables such as phylogenetic history and variation in habitat. I used *A. dicaeus* and *A. stratonice* to evaluate the predictive power of the physiological efficiency hypothesis, the enemy avoidance hypothesis and the food availability hypothesis in explaining patterns of host use and host specificity.

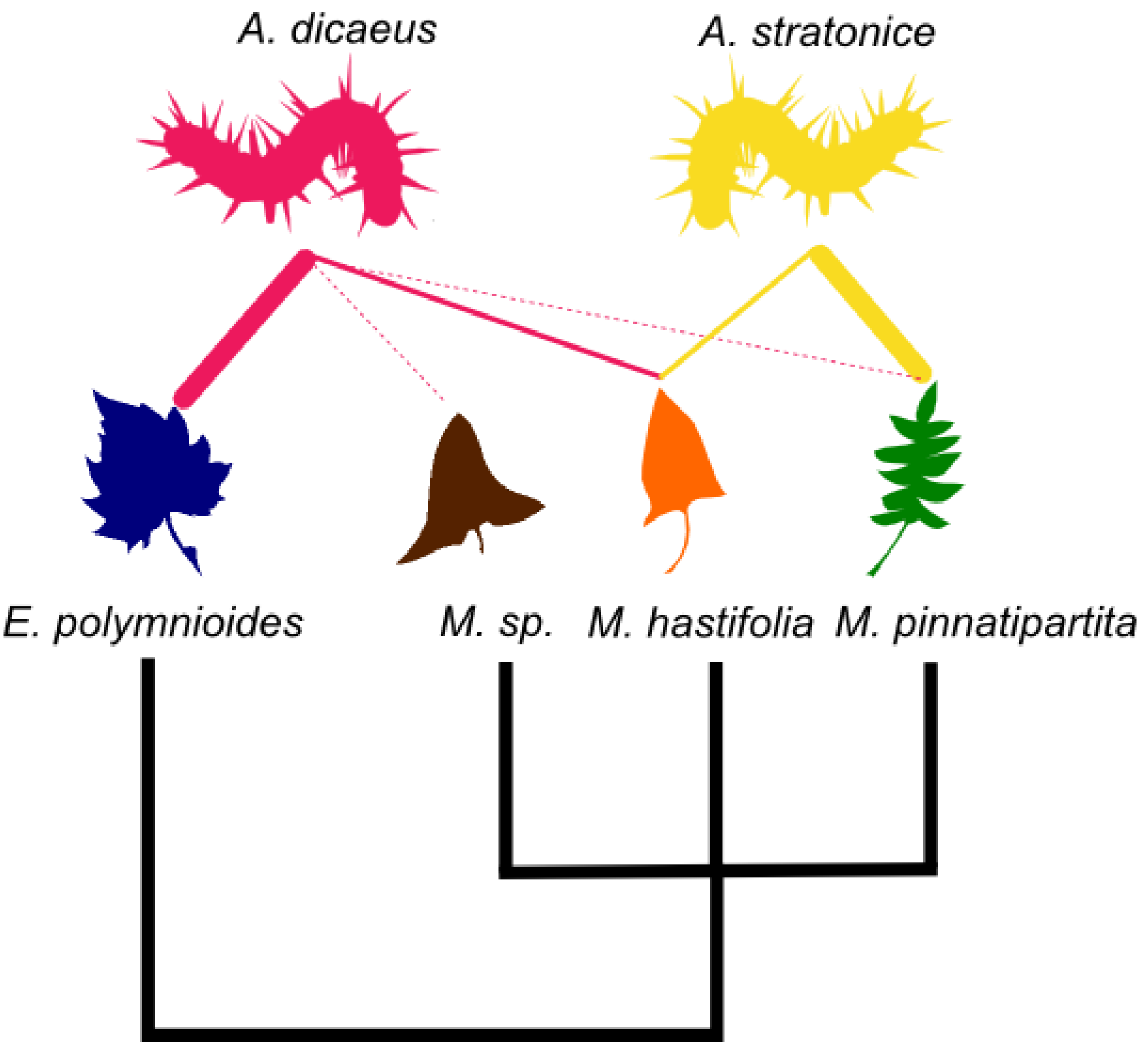


Figure 3 Major host-plant relationships of *A. dicaeus* and *A. stratonice.* A depiction of the host plant relationships between caterpillars and host plants. The thickness of the lines represents the proportion of caterpillars observed on each host plant species, dashed lines represent recorded, but rarely observed host plant associations.

Specific Hypotheses

Below, I outline predictions generated by each of the hypotheses discussed above to explain host plant use in *A. dicaeus* and *A. stratonice*.

Physiological Efficiency

If patterns of host plant use are driven by selection for adaptations to overcome secondary plant chemicals or to overcome nutritional deficiencies, then I would expect to see a significant decrease in performance on secondary host plants. Specifically I would predict slower growth, longer time to pupation, and lower survival on secondary host plants. In addition if there is a reduction in performance on one host plant I would expect females to place smaller clutches on that plant. Finally if one host plant increases performance relative to the other I would expect the plant with increased performance to have a higher protein concentration.

Enemy Avoidance

If patterns of host plant use are driven by enemies I would expect lower rates of parasitism and predation on the primary host plant than other hosts and for caterpillars to exhibit higher concentrations of cyanogenic glycosides on the primary host plant, thus increasing their ability to defend against enemies.

Food Availability

If patterns of host plant use are driven by plant abundance, I would expect to see more abundant plants used more often in proportion to their abundance. Plant size may also influence patterns of host plant use. If patterns of host plant use are driven by plant size, I would expect plants to be used in proportion to their size.

Methods

Study Site

All collections and experiments took place within 5 km of the Yanayacu Biological Research Station Cosanga, Napo Province, Ecuador (36°00'00.0"S

77°53'00.0"W

, 2100 m in elevation) in Andean cloud forest. Rapid plant growth necessitates frequent clearing of the access road leading to the Yanayacu Biological Research Station. This creates the disturbed habitat favored by *E. polymnioides*, *M. hastifolia* and *M. pinnatipartita*. In addition to cloud forest surrounding the station there are also several cattle farms with pasture grass along the same road. Farmers often make a special effort to clear *M. pinnatipartita* from their land because of its apparent toxicity to pasture grass. *M. pinnatipartita* also facilitates regrowth of native plants (Paoletti et al. 2012). This is consistent with my observations that cattle pasture is poor habitat for *M. pinnatipartita*, *M. hastifolia* and *E. polymnioides*, and by extension *A. dicaeus* and *A. stratonice*.

Caterpillar Surveys

Caterpillar surveys were initially conducted to evaluate *in situ* patterns of host plant use by caterpillars. Data was also used to determine if my data were consistent with the data from the Caterpillars and Parasitoids of the Eastern Ecuadorian Andes (CAPEA) project, which has been rearing caterpillars from the area surrounding the Yanayacu Biological Research Station for more than ten years methods described in Dyer et al. (2007). My collections began December 2012 and ended in March 2013. *A. dicaeus* and *A. stratonice* caterpillars were collected from along the access road in a haphazard fashion. After collection, caterpillars were reared in an open sided shed in one gallon plastic bags. The number of caterpillar groups found on each host plant was used to determine patterns of host plant use.

Clutch size by host plant

Plant use by *A. dicaeus* and *A. stratonice*, was also evaluated using egg clutches. I photographed all egg clutches I encountered starting December 2012 and ending May 2013, and again December 2013 through January 2014 and counted the number of eggs in each clutch. During this time I located 47 clutches, 21 *A. dicaeus* clutches, 13 on E*. polymnioides*, and eight on *M. hastifolia*, 26 clutches of A. stratonice 20 on *M. pinnatipartita* and 6 *M. hastifolia*. The average number of eggs per clutch was calculated and a two tailed t-test was used to determine if *A. dicaeus* and *A. stratonice* placed the same number of eggs on both of their commonly used host plants. To reliably identify *Altinote* species at the egg stage I waited for eggs to develop, because mature *A. dicaeus* and *A. stratonice* eggs are different colors. Mature *A. stratonice* eggs are red and mature *A. dicaeus* eggs are creamy white.

I compared the patterns of host plant use I observed in both caterpillars and eggs to the data collected by the CAPEA. The host plant relationships the CAPEA project observed were very similar to the relationships I observed.

Physiological Efficiency

Rearings

To test whether *A. dicaeus* and *A. stratonice* perform better on their primary host plants than their secondary host plants, I collected eggs, first, and second instar larvae of both *A. dicaeus* and *A. stratonice*. Collections began 19 December 2012 and ended May 2013, and rearing continued until June 2013. Due to the high mortality of eggs and first instar larvae under the rearing conditions, only the results of second instar larvae were analyzed. Caterpillars were reared in groups because they could not survive alone in early instars. Groups of ten caterpillars from the same clutch were placed on freshly collected leaves from *E. polymnioides*, *M. hastifolia* or *M. pinnatipartita*. The caterpillars and leaves were then placed in plastic bags and hung up. The rearing shed was open sided, allowing the temperature to fluctuate with ambient temperature. Inside the shed, the temperature was noticeably lower than the caterpillars experience *in* situ on sunny days and these lower temperatures may have slowed development times. In total I reared 22 clutches of *A. dicaeus* and *A. stratonice* in this manner. However only 11 clutches were included in statistical analyses because, 11 clutches experienced 100% mortality under rearing conditions. For statistical analysis I used six clutches of *A. stratonice* (180 caterpillars), three clutches collected from *M. pinnatipartita* and three clutches collected from *M. hastifolia*. I used five clutches of *A. dicaeus* (150 caterpillars), three clutches collected from *E. polymnioides*, and two clutches collected from *M. hastifolia*. The survival data was used to estimate a Kaplan-Meier curve and significance was assessed using a log-rank test to determine if survival was host plant dependent.

Growth rates were not calculated because removing caterpillars from leaves to get an accurate mass often resulted in the death of that caterpillar even when a small paint brush was used. Length of individual caterpillars was measured to the nearest mm and an average was taken for the group. However these measurements proved unreliable and were not included in statistical analysis.

When pupae were sufficiently sclerotized they were removed from the leaf, and weighed. The approximant number of days to pupation and the mass of the pupae were compared between host plant treatments with two tailed t-tests.

Crude Protein

To test whether protein concentrations were higher in primary host plants than secondary host plants leaves were collected from *E. polymnioides*, *M. hastifolia*, and *M. pinnatipartita* along the access road to Yanayacu Biological

Research Station and from the surrounding area. Leaves were collected from 32 plants: 8 *E. polymnioides*, 14 *M. hastifolia*, and 10 *M. pinnatipartita* from January through May 2013 and then again from December 2013 through January 2014. Leaves were collected from plants with egg clutches and plants of an equivalent age nearby. Because I was interested in the protein concentration of leaves where caterpillars were likely to feed I was careful to collect leaves females had placed eggs on or leaves of comparable age to the leaves females had placed eggs on. After collection leaves were placed in small ziplock sandwich bags with a generous amount of silica gel to dry. The analysis of protein concentrations was done at Wright State University, Dayton, Ohio.

Protein Extractions

In a cold room at 2°C-11°C, I crushed 0.25 – 0.5 g of dry leaf material in 3 ml of neutral pH sodium phosphate buffer and centrifuged the samples for 15 minutes. The supernatant was then transferred to a second tube and frozen before the protein concentration was measured (Bollag and Edelstein 1991).

Protein Concentration

To create a standard curve for comparison a series of albumin protein dilutions were made as follows: 3, 1.5, 0.75, 0.38, 0.19, 0.094, 0.047, 0.00 mg/ml. This series was use to interpret the protein concentrations in the plant samples. Samples of extracted leaf protein were thawed and placed on ice while the Bio-Rad Dye Reagent warmed to room temperature. The Bio-Rad Dye Reagent was diluted to 1:2.5 ratio with water. Each sample was loaded into a microplate in triplicate and read in a Photospectrometer. A mean was taken from the replicates of each sample and then placed into the linear equation generated using the standard curve.

Enemies

Parasitoid Rearing

Caterpillars collected for host-use surveys were reared and used to estimate rates of parasitism. After collection caterpillars were reared on the same plant species on which they were found in the field. If found together, caterpillars were reared together. Caterpillars were cleaned twice a week and given fresh leaves. To maintain a hygienic environment, some large groups had to be placed in more than one bag at around the third instar. To minimize infection by potential pathogens, plastic bags were placed overnight in a 5% bleach solution before reuse. Removal of first and second instars from plant material often results in mortality. For this reason the part of the leaf with the first or second instar caterpillars was transferred with them into the clean bags with fresh leaves.

The fate (i.e., dead, pupa or parasitoid) was recorded for each caterpillar. Parasitoids of three families emerged from *A. dicaeus* and *A. stratonice* caterpillars: Tachinidae, Ichneumonidae, and Braconidae. Caterpillars were counted as dead if a body was found and no parasitoid located, or occasionally, if a caterpillar was missing and may have been eaten by the others. Caterpillars had to successfully pupate to be considered a pupa. Pupa were weighed when they were sclerotized enough to be handled. Voucher specimens of adult male and female butterflies were placed in Dr. J.O. Stireman's collection at Wright State University, Dayton, Ohio.

Frequency of parasitism were calculated from the rearings that I conducted and from the data collected by the CAPEA project. A χ^2 test was used to determine if rates of parasitism were different on different host plants for both *A. dicaeus* and *A. stratonice*. A logit regression was run on data from both species to test if overall parasitism frequency differed by host plant. Dead caterpillars were excluded from statistical analysis of rates of parasitism because the reason the caterpillar died was unknown and they could have been a parasitized. Data from the CAPEA project was only included if the identity of the caterpillar could be verified with a photo. *A. dicaeus* and *A. stratonice* look very similar to each other, however later instar *A. stratonice* caterpillars have a white pattern on their dorsum while *A. dicaeus* remains solid black. The white patterning on *A. stratonice* is highly variable and may be very subtle or very bold (fig. 2).

Cyanogenic Glycoside Testing

I tested 3rd instar caterpillars for cyanogenic glycosides to see if different host plants increase or decrease the caterpillar's ability to synthesize cyanogenic glycosides *de novo*. Lower levels of cyanogenic glycosides make caterpillars more vulnerable to enemies, particularly generalist predators (Gleadow and Woodrow 2002).

Sodium Picrate Paper

To make sodium picrate paper a slurry was created using 5 g sodium bicarbonate, 50 ml of 10% picric acid and 50 ml of distilled water. One by ten cm strips of filter paper were submerged in the solution and then placed on newspaper and allowed to dry overnight. The prepared indicator paper was stored in a sealed plastic bottle.

Standard Curve

I made an initial 20.0 mg/L solution of potassium cyanide and water. This solution was than diluted by halves to make 10.0, 5.0, 2.5, 1.25, 0.625, and 0 mg/L solutions. One ml of each dilution was placed in a test tube and a strip of sodium picrate paper added. It was then allowed to sit for eight hours at ~20^{o}C. The test strips were then photographed on a white background with a Macbeth Color Checker Card.

Testing for Cyanogenic Glycosides

Ten third instar larvae of each species were collected from their primary and secondary host plants. Each caterpillar was placed in a tube with 1 ml of distilled water and crushed. A strip of sodium picrate paper was then placed in the test tube and the time recorded. Controls were made using 1 ml distilled water and a second negative control was made using a crushed caterpillar of *Dysschema* sp. (Erebidae: Arctiinae), of approximately the same size. *Dysschema* sp. was used because I found it feeding on both *E. polymnioides* and *M. hastifolia* in large gregarious groups, thus it is ecologically similar to *A.*

dicaeus. Because the complete color change can take several hours the picrate paper was left in the test tubes with the caterpillar extract in a room where the temperature measured 20°C. Temperatures higher than 20°C have been shown to alter the coloration of sodium picrate paper (Egan et al. 1998). Photographs of test strips were taken on a white background with a Macbeth Color Checker Card. The photographs of the test strips were first run through imageJ using the Chart_White_Balance plug-in to white balance. The colors were then visually compared to those of the standard curve, also white balanced, to approximate cyanogenic glycoside concentrations.

Predation

To test if predation pressure of each butterfly species differed between their primary and secondary host plants I followed caterpillar clutches in the field and assessed rates of disappearance. For both *A. dicaeus* and *A. stratonice* I located 20 clutches of 1st, 2nd or 3rd instar caterpillars. Ten clutches on their primary host plant and ten clutches on their secondary host plant, for a total of 40 initial clutches. Each clutch was marked by attaching pink or orange flagging to the host plant and assigning it an ID. Then both sides of the leaf were photographed to capture as many caterpillars as possible. Later the number of caterpillars in the group was counted using the photographs. This method may not capture all caterpillars, because caterpillars moved while I photographed the clutch. However, it is more accurate than trying to count large groups of moving individuals by hand in the field. Plants were visited six times over the course of two weeks. At first the interval between visits was three days but this was clearly too infrequent because many clutches went missing, so the interval was reduced to two days and then one day between visits. For the first three periods of data collection missing clutches were replaced by comparable clutches. It rapidly became clear that third instar caterpillars migrated frequently. Data from those clutches is considered to be unreliable and not indicative of predation. All third instar clutches were excluded from statistical analysis. Additional data was excluded from clutches where the number of caterpillars increased dramatically between visits, because those data clearly did not represent rates of predation. All told data was collected from 55 clutches but only 34 clutches were included in statistical analysis. A total of 1377 *A. dicaeus* and 1365 *A. stratonice* caterpillars, were used to create a Kaplan-Meier survival curve to compare survival of *A. stratonice* and *A. dicaeus* caterpillars on *E. polymnioides*, *M. Hastifolia*, and *M. pinnatipartita*. I conducted a Log Rank Test to see if there was a statisically significant difference between the survival cuves of *A. dicaeus* and *A. stratonice* on their primary versus secondary hostplants

Food Availability

To test whether patterns of host plant use are associated with host plant abundance or host plants size I surveyed and collected plant stems along the access road leading to Yanayacu Biological Research Station.

Plant Distribution Surveys

Plant distribution was surveyed from along the access road leading to Yanayacu Biological Research Station for 7.4 km, starting from where the access road meets the main road (Amazónica/E45). This represents the area where I collected the majority of my plants and caterpillars. Portions of the road were surveyed on three separate days, one side on 16 and 17 December, 2013 and the second side on 31 December 2013. Approximately every 100 m, a quadrat was sampled. Plants were sampled by recording which species of host plant were present if any and then counting the number of ramets inside a one m^2 quadrat. If caterpillars or eggs were present the species was recorded. To determine the location of the quadrat I closed my eyes and threw the quadrat toward the side of the road. I then sampled inside the quadrat. To estimate the distance between quadrats I used one stride to estimate 1 m. However my stride is ~ 0.85 m and thus slightly shorter than 1 m resulting in 174 quadrats instead of the expected 148 (7.4 km sampled every 100 m = 74 quadrats x two sides of the road = 148 quadrats). These data were used to calculate plant frequency (the number of quadrats in which a species appears), plant abundance and rates of plant occupancy. Finally an index of dispersion was used to test for an aggregated plant distribution.

Leaf area / plant

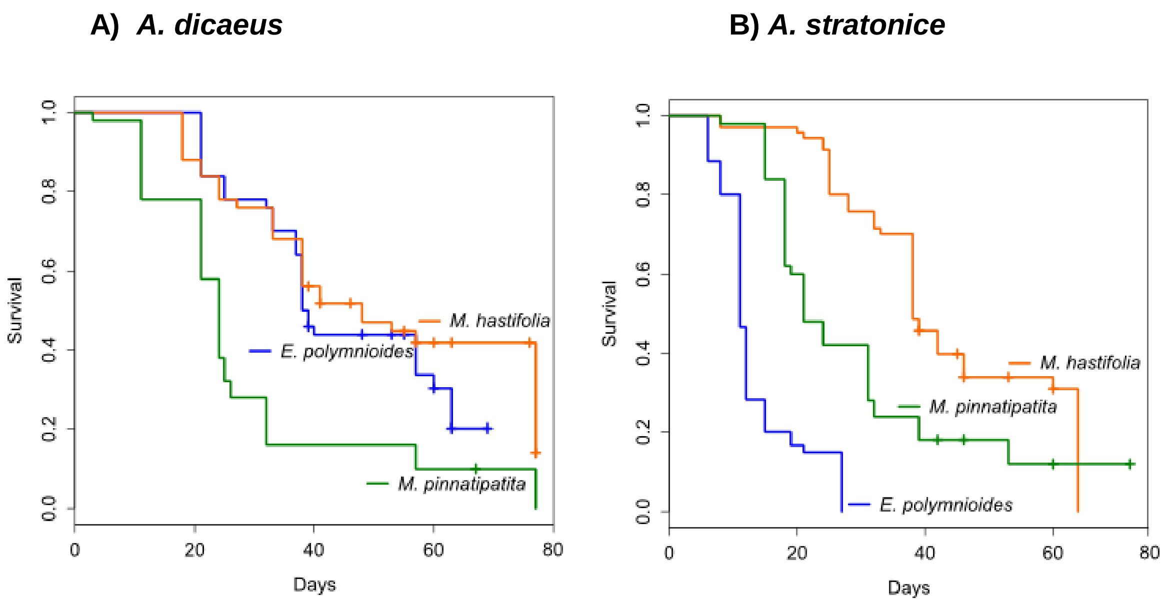
To quantify the observed variation in plant size, I haphazardly gathered ten ramets each from *E. polymnioides*, *M. hastifolia*, and *M. pinnatipartita* from the road side. The ramets were collected and taken back to the field station and photographedon a white bed sheet. I then used imageJ to calculate leaf area of each leaf on an individual ramet. The leaf areas for all leaves on a ramet were summed and an average area per leaf per ramet was calculated for *E. polymnioides*, *M. hastifolia* and, *M. pinnatipartita*. To determine if leaf area per ramet varied significantly among plant species an ANOVA was used. Because the leaves of *E. polymnioides* wilted during the walk back to the station the average is considered an underestimate. These data were used in conjunction with the plant surveys to calculate plant abundance (the number of ramets in a given m^2) and the leaf area in a given m^2.

Results

Physiological efficiency

Survival

Under the experimental rearing conditions, survival of larvae was very low for both *A. dicaeus* and *A. stratonice.* A total of 22 caterpillar clutches were reared, but half of the clutches experienced 100% mortality and were excluded from statistical analysis. Survival was further reduced by unsuitable host plants (fig. 4a). *A. dicaeus* experienced the lowest rate of survival on *M. pinnatipartita* (2%), and approximately equal rates of survival on *E. polymnioides* (34%) and *M. hastifolia* (40%) under experimental conditions. *A. stratonice* experienced 100% mortality on *E. polymnioides*, moderate survival on *M. pinnatipartita* (13%) and the highest rate of survival (20%) on *M. hastifolia* under rearing conditions (fig. 4b).


A) ***A. dicaeus***

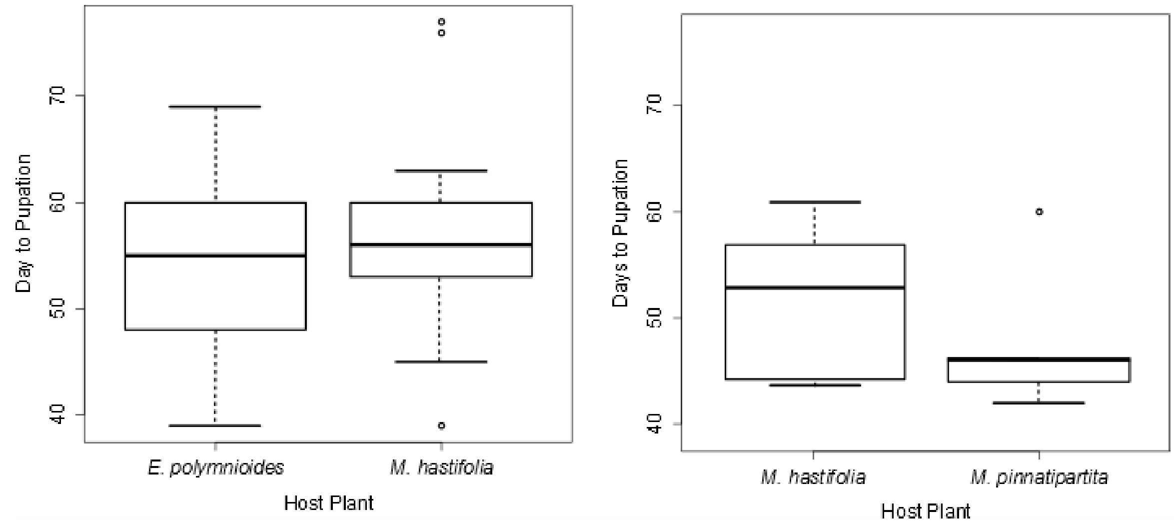
B) ***A. stratonice***

Figure 4 Caterpillar survival under experimental rearing conditions Kaplan Meier estimators of caterpillar survival on *E. polymnioides*, *M. hastifolia* and *M. pinnatipartita*. A "+" is used to represent an individual that pupated and survived longer than the study's duration. **A)** Survival of *A. dicaeus* (N= 150) differed significantly across host plants (P <<0.0001). **B)** *A. stratonice* (N = 180) survival also varies significantly across host plants (P << 0.0001).

Development Time and Pupal Mass

Of the 330 caterpillars followed, 53 survived to pupation. Of these, 36 pupa were *A. dicaeus* and 17 were *A. stratonice*. Of the five groups of *A. dicaeus* caterpillars that were placed on *M. hastifolia*, four produced pupae (19 total pupae). Of the five groups of *A. dicaeus* caterpillars placed on *E. polymnioides*, four produced pupae (17 total pupae). *A. dicaeus* pupated once on *M. pinnatipartita* but was not included in statistical analysis of development time or pupal mass because a sample size of one is unlikely to produce reliable results. Six groups of *A. stratonice* caterpillars were placed on *M. hastifolia*, and three of these produced pupae (11 total pupae), and of the six groups of *A.*

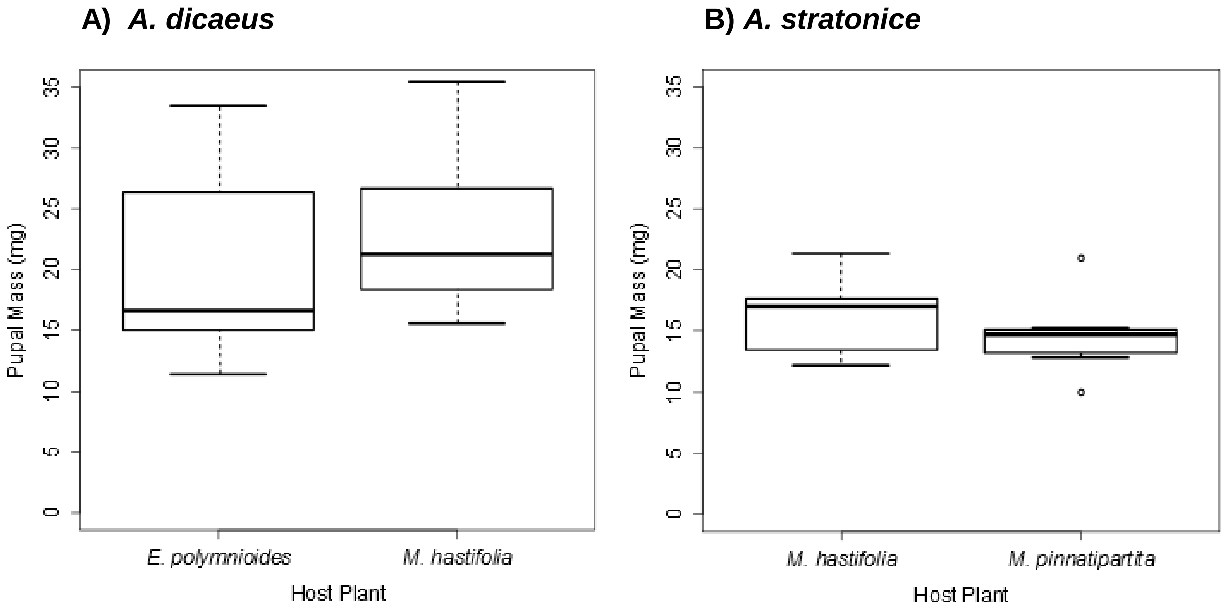
stratonice caterpillars placed on *M. pinnatipartita*, four produced pupae (6 total pupae). None of the *A. stratonice* larva survived to pupation on *E. polymnioides*. The mean pupal mass of *A. dicaeus* caterpillars on *E. polymnioides* (22.4 mg) was not statistically significantly different from the mean pupal mass (20.4 mg) of those on *M. hastifolia* (T = -0.891, df = 34, P = 0.3792; fig. 5). The larval development time for *A. dicaeus* appears approximately the same on both *E. polymnioides* and *M. hastifolia,* with means of 55 days and 58 days respectively (T = -1.1381, df = 34, P = 0.263) (fig. 3). *A. stratonice* caterpillars took longer to pupate on *M. hastifolia* (mean 51 days) than on *M. pinnatipartita* (mean of 47 days) but the difference is not statistically significant (T = 1.3038, df = 17, P = 0.2097) (fig. 5). There was no difference in the mean pupal mass on either *M. hastifolia* 16.3 mg or *M. pinnatipartita* 15.3 mg (T = 0.752, df = 17, P = 0.4623) (fig. 6). While I did measure caterpillar length the measurements proved unreliable and are therefore not included.



A) ***A. dicaeus***

B) ***A. stratonice***

Figure 5 Mean and range of larval development. **A)** *A. dicaeus* (P = 0.263). **B)** *A. stratonice* (P = 0.2097).


A) ***A. dicaeus***

B) ***A. stratonice***

Figure 6 The mean and range of pupal mass for caterpillar raised on alternate plant diets. **A)** *A. dicaeus* (P = 0.3792). **B)** *A. stratonice* caterpillars (P = 0.4623).

Caterpillar Survival by Clutch

An examination of the survival of particular clutches on different host plants supports the notion that caterpillars of both species survive best on the host plants on which they were placed by their mother, although limited sample size prevents statistical inference. In three out of four *A. dicaeus* caterpillar cltuches examined, survival was higher on the plant from which they were collected in the field than it was on either of the two alternate host plants. In all cases *A. dicaeus* preformed better on both *E. polymnioides* and *M. hastifolia* than it did on *M. pinnatipartita* (fig. 7a). In four out of five *A. stratonice* clutches examined, caterpillars performed better on their original host plant than they did on the alternate host plants. As mentioned earlier, there was no survival on *E. polymnioides* (fig. 7b).

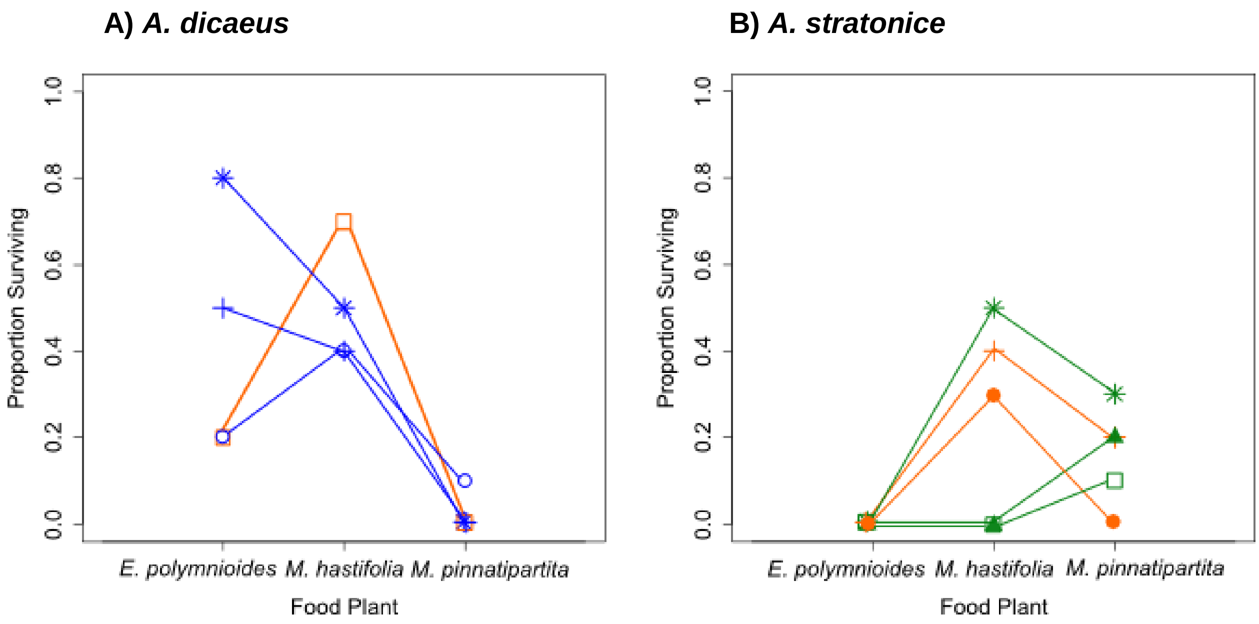
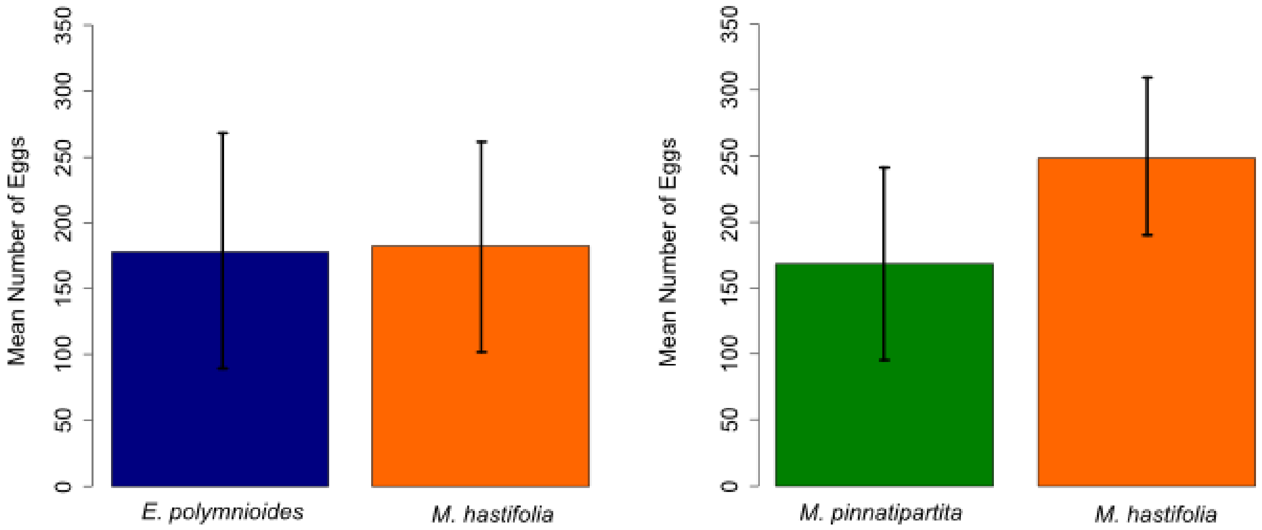


Figure 7 Proportion of each family surviving on each host plant. Caterpillar families collected from *E. polymnioides* plants are in blue, caterpillar families collected from *M. hastifolia* are in orange and caterpillar families collected from *M. pinnatipartita* are in green. Each was assigned its own symbol.

Clutch size

A total of 21 *A. dicaeus* clutches were observed, 13 on *E. polymnioides* and eight on *M. hastifolia*. A total of 26 *A. stratonice* clutches were observed six on *M. hastifolia* and 20 on *M. pinnatipartita*. *A. dicaeus*’s clutch size is not statistically different (T = -0.1214, df = 16.357, P = 0.9049) between host plants, *E. polymnioides* (mean 178 ± 90) and on *M. hastifolia* (mean 182 ± 80). In contrast, *A. stratonice* has almost 46 % larger clutches on *M. hastifolia* (mean 248 ± 59) than on *M. pinnatipartita* (mean 169 ± 73) and clutch size is significantly different (T = 2.7186, df = 9.97, P = 0.02166) (fig. 8).



A) ***A. dicaeus*** **B)** ***A. stratonice***

Figure 8. Mean (± stdev) number of eggs per clutch female butterflies placed on each host plant in the field. **A)** *A. dicaeus* (P = 0.9049). **B)** *A. stratonice* mean clutch size differs significantly (P = 0.02166).

Host Plant Protein Content

A total of 32 plants were assayed for protein content: eight *E. polymnioides*, 14 *M. hastifolia* and 10 *M. pinnatipartita*. Mean protein concentrations are not statistically different (F-value = 2.34, P = 0.114). Crude protein was found to be highest in *E. polymnioides*, 0.021 protein (mg)/ leaf (mg), followed by *M. pinnatipartita* 0.015 protein (mg)/ leaf (mg) and lowest on *M. hastifolia* 0.014 protein (mg)/ leaf (mg). Differences between plant species are not statistically significant (fig. 9).

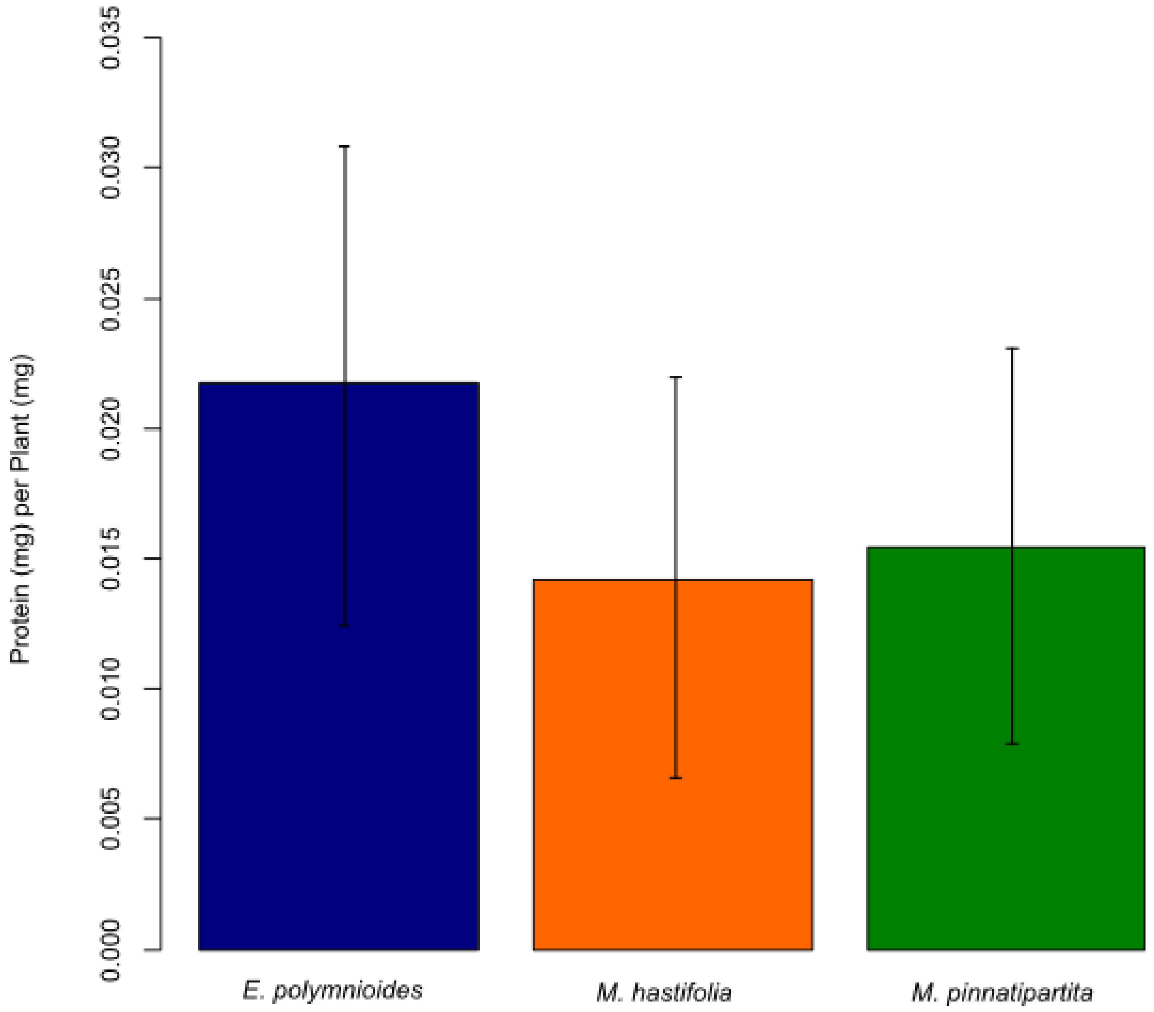


Figure 9 The mean protein concentrations in protein (mg) / plant (mg) ± stdev of the three commonly used host plants. Differences are not significant (P = 0.114).

Enemies

De novo synthesis of Cyanogenic Glycosides

Each caterpillar species, irrespective of host plant appears to be equally well defended. The concentration of cyanogenic glycosides was found to fall between 0.625 and 1.25 mg/L for all 20 *Altinote* caterpillars. The *Dysschema sp.* caterpillars used as negative controls did not produce cyanogenic glycosides (i.e., 0 mg/L). While there may be differences between species or among host plants in ability to synthesize cyanogenic glycosides, I did not find any evidence of this using the sodium picrate paper assay.

Parasitoids

I calculated frequency of parasitism using the 875 caterpillars I collected and 474 caterpillars from the CAPEA project for a total of 1349 caterpillars. Data was collected from 686 *A. dicaeus* caterpillars, 565 from *E. polymnioides*, 117 from *M. hastifolia* and three from *M. pinnatipartita*. Of the 545 *A. stratonice* caterpillars collected 469 were from *M. pinnatipartita* and 76 were from *M. hastifolia*. The reason caterpillars died was unknown and could have been the result of unsuccessful parasitoid so they were excluded. Out of 473 caterpillars that either survived to pupation or produced a parasitoid, 131 parasitoids were reared. The results of a logit regression found both butterfly species and host plant where significant indicators of the frequency of parasitism (table 1). Each event of parasitism was treated as separate because one clutch of caterpillars could produce parasitoids from three parasitoid families. All parasitoids reared from *A. dicaeus* and *A. stratonice* were from one of the following families Tachinidae, Ichneumonidae, or Braconidae. *A. dicaeus* and *A. stratonice* appear to experience somewhat lower rates of parasitism when feeding on *M. hastifolia*, than on their primary host plants (fig. 8).The rate of parasitism for *A. dicaeus* drops on *M. hastifolia* from 18% parasitism to 7% parasitism these differences are statistically significant for *A. dicaeus* (χ^2 = 9.1715, df = 1, P = 0.002458). The rates of parasitism are much higher on *A. stratonice.* Overall the trend is similar for *A. stratonice,* which has 37% parasitism on *M. pinnatipartita,* but drops to

28% parasitism on *M. hastifolia*. This difference is not statistically significant (χ^2 = 1.7748, df = 1, P = 0.1828) (fig. 10).

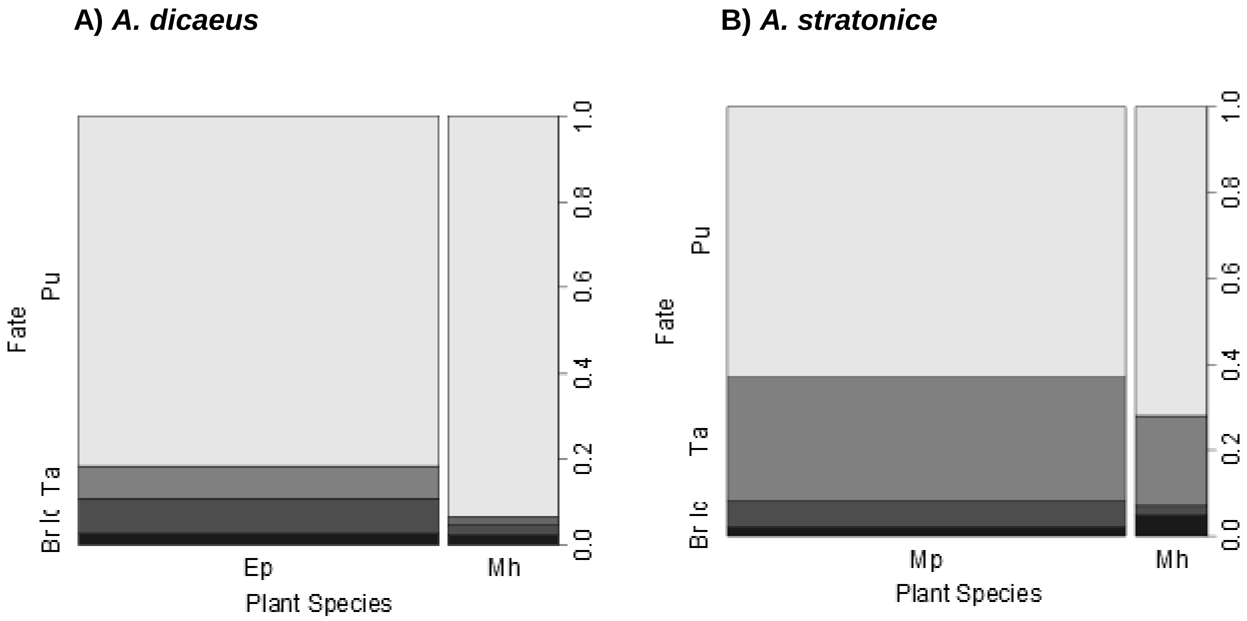


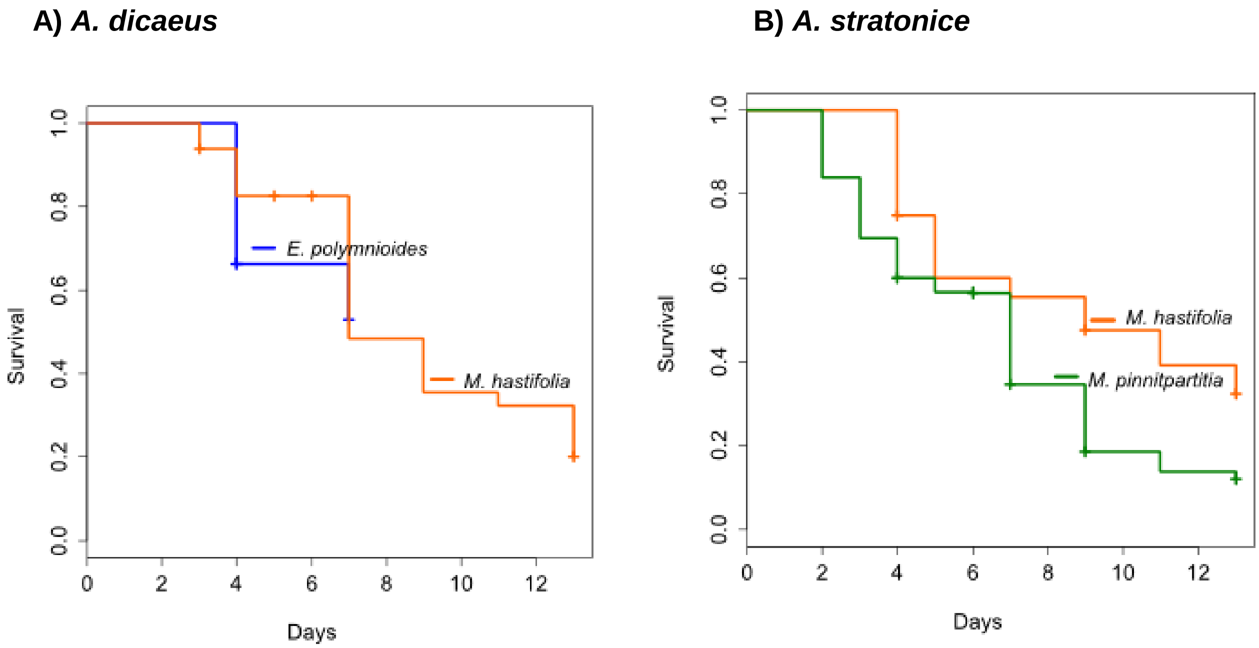
Figure 10 The proportion of caterpillars that either pupated or were parasitized relative to the host plant they were collected on. Pupa (Pu), Tachinidae (Ta), Ichneumonidae (Ic), Braconidae (Br). *A. dicaeus* N = 192, P = 0.002458 *A. stratonice* N = 281 rates of parasitism are not significantly different with inspecies P = 0.1828. Data I collected and data from Dyer et al. (2015).

Table 1 Results of a logistic regression comparing the effects of Lepidoptera species and host plant on the frequency of parasitism.

	Df	**Residual**	**P**
Host Plant	2	24.536	0.0000047
Lep.Sp.	1	12.008	0.0005297

Predation

Judging from rates of larval disappearance in followed clutches, *A. dicaeus* appears to be equally vulnerable to predation on both *E. polymnioides* and *M. hastifolia* hosts. Disappearances could occur for a number of reasons, death from enemies or migration. The predation data on *E. polymnioides* is truncated earlier than the data for *M. hastifolia,* because I initially included more third instar *A. dicaeus* larva on *E. polymnioides* so a larger number of the original clutches were excluded than *A. dicaeus* clutches on *M. hastifolia*. *Altinote stratonice* appears to experience significantly lower rates of predation on *M. hastifolia* than it does on *M. pinnatipartita* (fig. 11).



A) *A. dicaeus*

B) *A. stratonice*

Figure 11 Survivorship plots of *A. dicaeus* and *A. stratonice* clutches *in situ*. **A)** *A. dicaeus* (P = 0.702). **B** *A. stratonice* (P < 0.001).

Host Plant Size and Density

Among the host plant species examined size varied considerably. *Erato polymnioides* has the largest mean leaf area per ramet 0.45 m^2/ramet ± 0.14 m^2 followed by *M. pinnatipartita* with 0.15 m^2/ramet ± 0.0097 m^2, and *M. hastifolia* has the smallest leaf area per ramet with 0.07 m^2/ramet ± 0.024 m^2. The results of an ANOVA found the three means are not equal to each other (F = 5.72, df = 2, P = 0.00849) (fig. 10a). Not only was host plant size variable but so was host plant frequency. Frequency was defined as the number of times a plant species appears in a given number of sample points. Both *Munnozia* species are rhizomatous so it is difficult to distinguish between individual plants. For this reason the number of times a plant appeared in a quadrat was used to calculate the frequency and not the number of plants in a given area. *M. hastifolia* occurred most frequently appearing in 28% (49) of the quadrats, *M. pinnatipartita* appeared in 14% (25) of the quadrats and *E. polymnioides* was encountered least often in 12% (21) of the quadrats (fig. 12b).

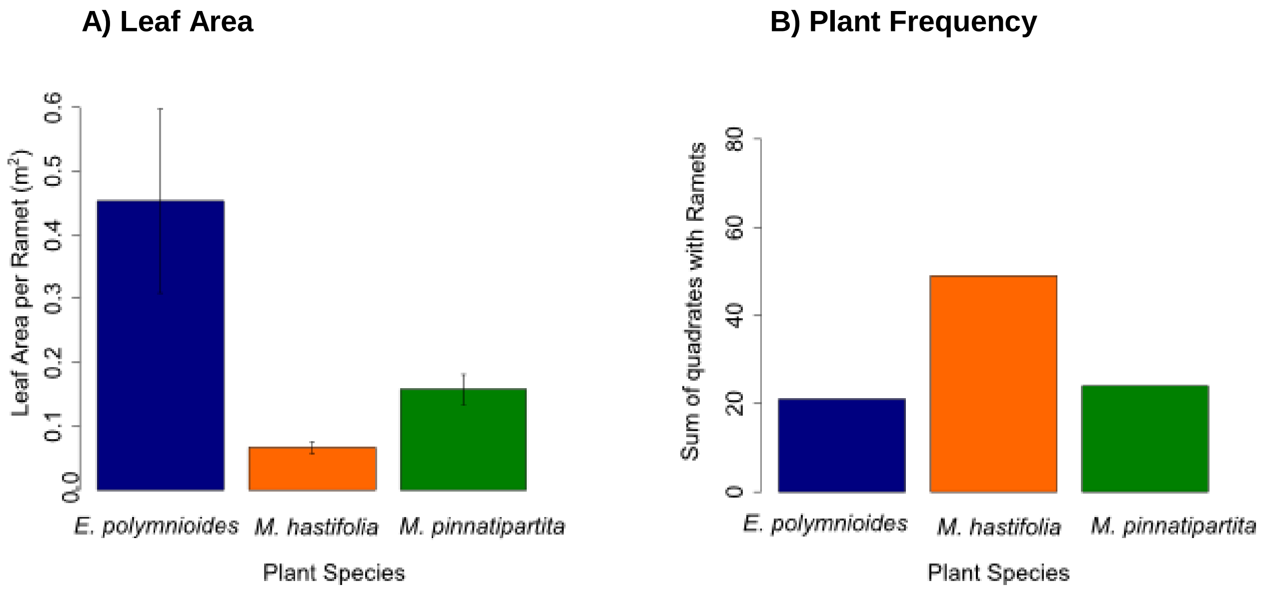


Figure 12. Size and number of plants. **A)** The mean leaf area per plant ± SEM (P = 0.00849). **B)** The frequency of plant occurrence out of the 174 sampled quadrats, *E. polymnioides* n = 21, *M. hastifolia* n = 49, *M. pinnatipartita* n = 24.

To estimate plant abundance, the number of ramets of each plant species (*E. polymnioides* = 62 ramets, *M. hastifolia* = 216 ramets and *M. pinnatipartita* = 262 ramets) was divided by the number of sampled quadrats (N = 174). *M. pinnatipartita* is the most abundant species with 1.5 ramets/m^2, followed closely by *M. hastifolia* 1.24 ramets/m^2, the least abundant species is *E. polymnioides* 0.36 ramets/m^2 (fig. 13a). All plant species have an aggregate distribution, *E. polymnioides* (I = 6.242458, χ^2 = 1079.9453, P << 0.00001), had the most aggregated distribution followed by *M. pinnatipartita* (I = 5.057487, χ^2 = 874.9453, P << 0.00001), and lastly *M. hastifolia* (I = 3.357495, χ^2 = 580.8467, P << 0.00001).

Despite or perhaps because *E. polymnioides* is both the least frequent and least abundant plant, it also experiences the highest rate of occupancy, with 19% of ramets occupied, followed by *M. hastifolia,* with 4% of ramets occupied, and finally *M. pinnatipartita* with 1% of ramets occupied (fig. 35b).

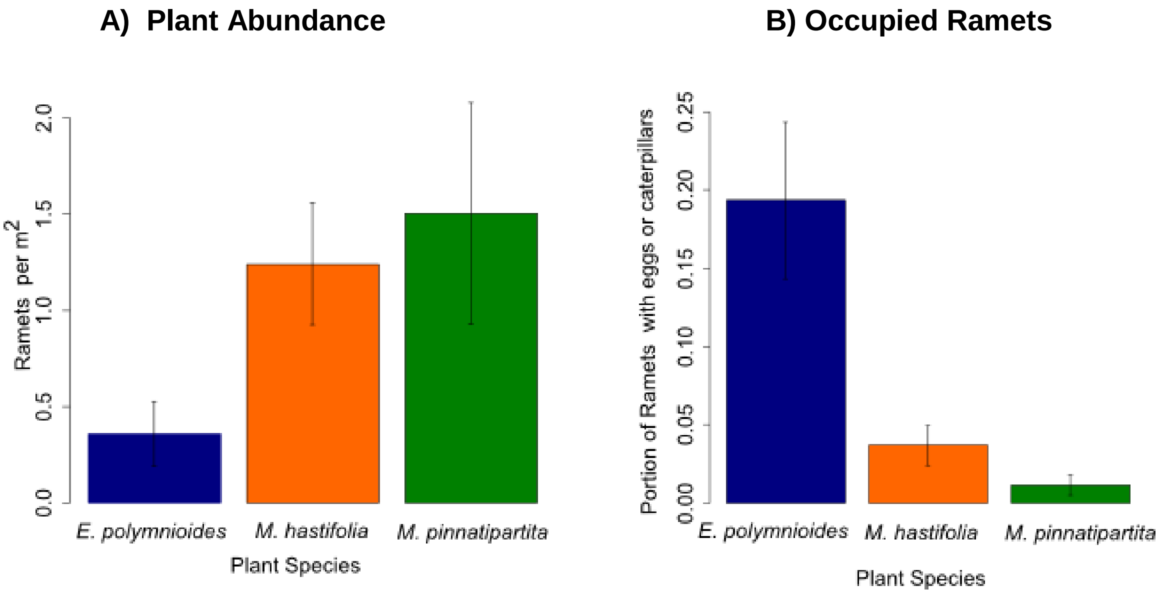


Figure 13 Histograms of host plant abundance and plant occupancy by *Altinote* caterpillars and eggs. **A)** The total number of ramets surveyed (Ep = 62, Mh = 216, Mp = 262 respectively) divided by the number of quadrates was used to sample abundance (N=174 quadrats) ± SEM. **B)** The number of ramets with caterpillars or eggs divided by the number of ramets of that plant species ± SE (Ep = 16 plants, Mh = 8 plants, Mp = 3 plants).

The leaf area in a given meter squared may be the most relevant estimator of host plant availability. Given that *M. pinnatipartita* has about four times the number of total ramets and a mean leaf area more than double *M. hastifolia* (0.236 m^2 leaf/m^2), it is not surprising that it has the largest leaf area in an average meter, followed by *E. polymnioides* (0.161 m^2 leaf/m^2) and lastly by *M. hastifolia* (0.082 m^2 leaf/m^2) (fig. 14).

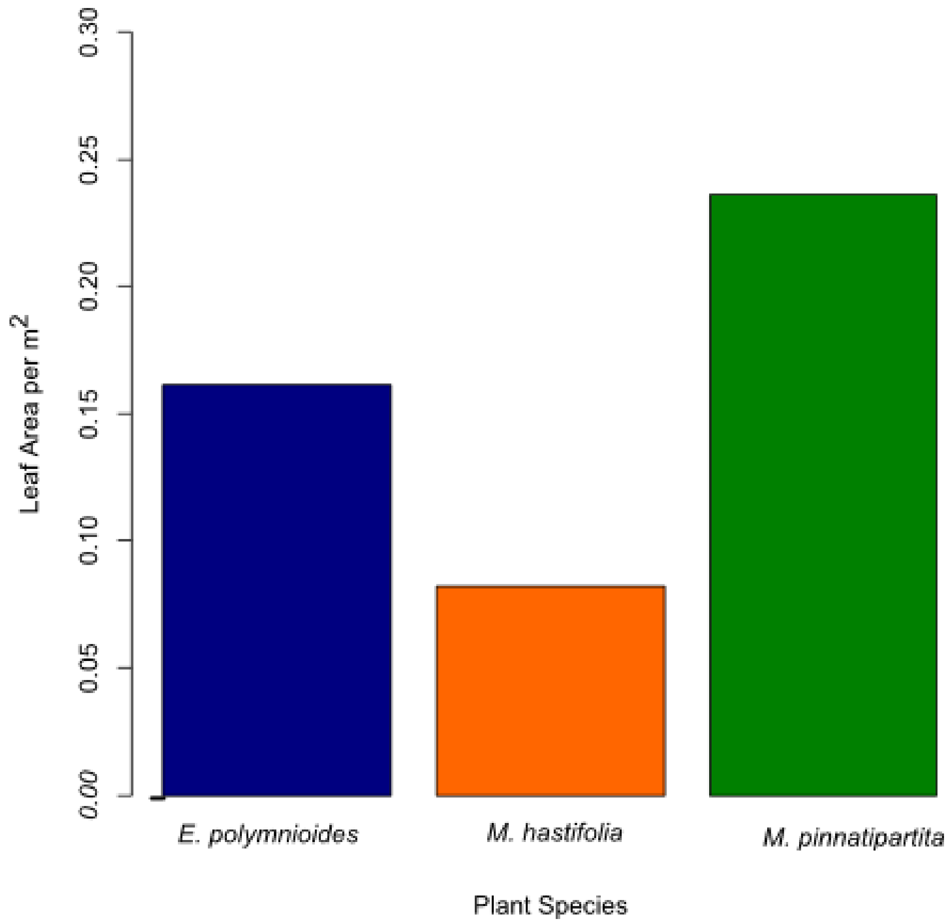


Figure 14 Mean leaf area of each host plant in the landscape.

Of the 540 ramets searched, I found caterpillars or eggs on 23 ramets. There were seven plants occupied by *A. stratonice* and 16 plants occupied by *A. dicaeus*. The relative frequency of plant encounters appears to be associated with host plant use by *A. stratonice* (fig. 15a) and plant size appears to be a good predictor of *A. dicaeus* host plant use (fig. 15b).

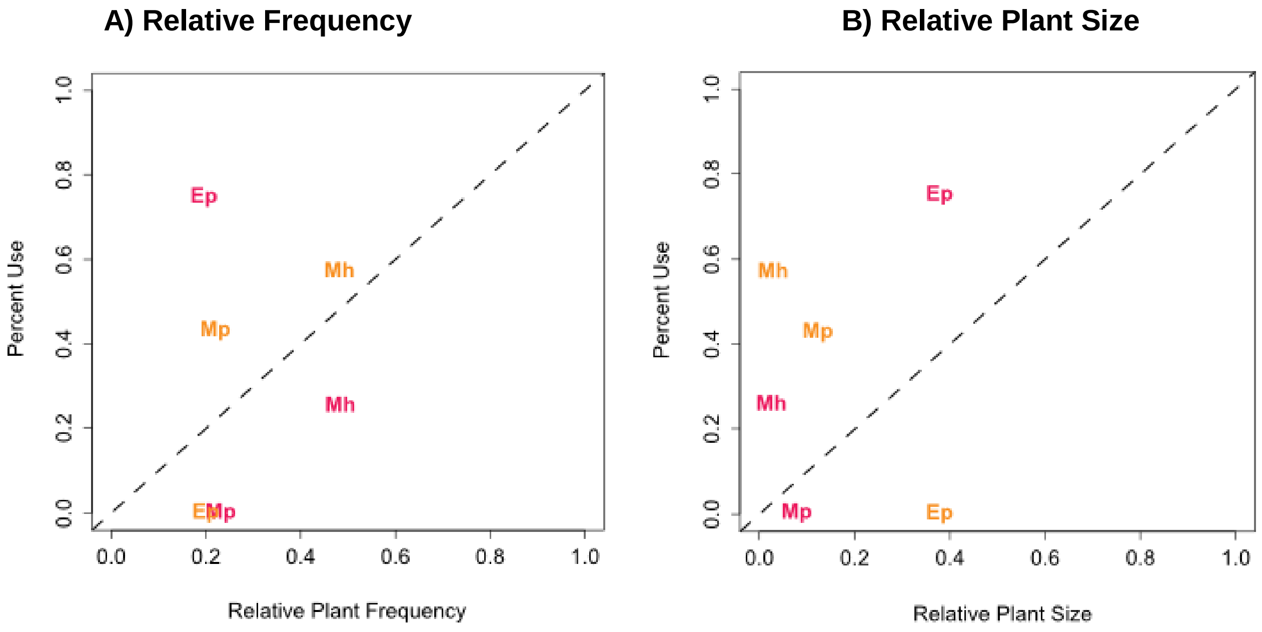


Figure 15 Figures depict host plant use by *A. dicaeus* and *A. stratonice* by relative frequency and relative plant size. Each plant species is given an abbreviation (Ep = *E. polymnioides*, Mh = *M. hastifolia*, and Mp = *M. pinnatipartita*). Each caterpillar species is given its own color, pink = *A. dicaeus* and yellow orange = *A. stratonice*. The percent use in both **A** and **B** was calculated by taking the total number of caterpillars of each species on each plant. **A)** The dotted line represents expected use if females select plants simply according to their frequency. **B)** The dashed line represents expected host plant use if plant size predicts patterns of host plant use.

Discussion

Low rates of survival on unused or rarely used host plants is consistent with the physiological efficiency hypothesis, which states that caterpillar should use host plants that provide the highest quality food and highest rates of survival, fastest development times, highest pupal mass and highest rate of fecundity. In this system the physiological efficiency hypothesis would predict that *A. stratonice* would use *M. hastifolia* more often than *M. pinnatipartita*, and *A. dicaeus* would use *E. polymnioides* and *M. hastifolia* equally however this pattern was not observed.

In recent years the enemy avoidance hypothesis has gained a lot of support in the literature. Unlike the physiological efficiency hypothesis, the enemy avoidance hypothesis allows herbivores to feed on suboptimal host plants if the plant provides protection from enemies and increases overall survival. The results of this study were not consistent with the enemy avoidance hypothesis, because while *M. hastifoli*a does appear to offer *A. stratonice* some protection from enemies it does not appear to drive host plant use. Host plant use appears to be associated to some extent with host plant abundance (*A. stratonice*) and host plant size (*A. dicaeus*). This indicates that bottom up forces both in plant suitability and plant availability are important predictors of host plant use in *A. dicaeus* and *A. stratonice*.

Physiological Efficiency

All rates of survival under rearing conditions were very low. Two reasons why rates of survival might have been artificially low are, 1) gregarious caterpillars often need relatively large groups to attained optimal survival even under laboratory conditions (Fordyce 2003, Allen 2010) and, 2) gregarious animals may experience high rates of pathogen transmission (Hochberg 1991, Brown et al. 2001). Groups of caterpillars in these experiments may have been too small to support proper development, and this may have been exacerbated by rearing caterpillars in relatively small plastic bags. Even with low rates of larval survival under rearing conditions, host plant taxon clearly played a role in overall larval survival, as it did in a similar study by Reader and Hochuli (2003) on

Doratifera casta (Lepidoptera: Limacodidae). Similar strong impacts of host plant on larval survival have been found in many systems (e.g., Strong et al. 1984, Reader and Hochuli 2003, Price et al. 2011, Davis and Cipollini 2014).

The overlapping but disparate host plant use observed in *A. dicaeus* and *A. stratonice* can be partially explained by the physiological efficiency hypothesis. While *E. polymnioides*, *M. hastifolia*, and *M. pinnatipartita* all belong to the same tribe (Funk et al. 2012), they do not provide *A. dicaeus* and *A. stratonice* with equally suitable sources of food. *A. dicaeus* appears to perform just as well on *E. polymnioides* as it does on *M. hastifolia* and very poorly on *M. pinnatipartita*. Poor performance on *M. pinnatipartita* explains why it is rarely used as a host plant by *A. dicaeus*. Similarly the poor performance of *A. stratonice* on *E. polymnioides* probably explains why it is not a suitable host plant. As caterpillars ate all plants offered to them it is unlikely that difference in performance on different host plants are due to absent feeding stimulants. Clearly the physical properties of *E. polymnioides* and *M. pinnatipartita* must be different, or both *A. dicaeus* and *A. stratonice* would be able to use the plants interchangeably. We surmise that even though both *A. dicaeus* and *A. stratonice* can use *M. hastifolia*, it must have different chemical or physical properties from both *E. polymnioides* and *M. pinnatipartita* because otherwise it would be a suitable host plant for either *A. dicaeus* or *A. stratonice* but not for both. Because protein content did not differ significantly between species, and there are no obvious nutritional differences, this suggests that the plants are defended in different, species specific ways. This would be consistent with the physiological efficiency hypothesis, which presumes that adaptations for specific plants may be required to overcome the arsenal of species-specific plant defenses, and these defenses drive host plant specialization. Future research should include categorization of plant defenses in each of these species.

One possible difference among host plant species in defense is in the type and concentration of pyrrolizidine alkaloids (PAs), toxins found in many related asteraceous species (Brown and Francini 1990, Silva-Brandâo 2008). Previous research has focused primarily on the toxic effects PAs have on vertebrates, because there have been cases where livestock and humans have died after consuming food contaminated with PAs. In vertebrates PAs cause liver disease, jaundice, photosensitization, copper poisoning and ammonia intoxication. The symptoms of PA toxicity are often delayed until near death (Rizk 1990).

The causes of PA toxicity in invertebrates are less well understood partly because the deterrent effects of PAs are so strong that many nonadapted insects and predators will vehemently reject leaves or prey containing PAs. When *Philosamia ricini,* a generalist silkworm, was fed leaves treated with PAs, growth was slower than on untreated leaves. This result indicates that PAs make plants suboptimal hosts for unspecialized herbivores (Narberhaus et al. 2005). While other species in the tribe Liabeae tested positive for PAs, *E. polymnioides*, *M. hastifolia* or *M. pinnatipartita* have not yet been tested for the presence of PAs (Brown and Francini 1990, Rizk 1990, Silva-Brandâo 2008).

To determine which plant defenses differ and how they differ, further investigation is required. Further evidence that *A. stratonice* perceives *M.*

hastifolia and *M. pinnatipartita* as being of different quality can be found by comparing clutch sizes on the different plants. *A. stratonice* females place larger clutches on *M. hastifolia* than on *M. pinnatipartita*. The question then becomes: are these butterflies actively placing larger clutches on host plants where survival is highest, or is there genetic variation within *A. stratonice*? If there is genetic variation, it would appear a butterfly that prefers *M. pinnatipartita* as a host plant also produces smaller clutches.

Given that we observed increased survival on mother-selected host plants, genetic variation may underlie this variation in host use. Genetic variation and resource partitioning is not without precedent in Lepidoptera. A cryptic species complex among *Leptidea sinapis* and *Leptidea reali* butterflies found preference for either forest or prairie habitat was responsible for host plant use and not larval performance (Friberg and Wiklund 2008). However, it is possible that conditioning occurs and changes gene expression to favor performance on one plant species over others (Després et al. 2007). By artificially switching host plants I may have inadvertently reduced survival. However this does not explain the larger clutch sizes on *M. hastifolia*.

Tritrophic interactions

Sometimes plants offer protection from enemies that can outweigh bottom-up forces associated with varying plant quality or defense, and avoiding enemies can be the strongest driver in the occurrence or frequency of host plant use (Singer and Stireman 2003, Murphy and Berenbaum 2004, Rodrigues et al.

2010). Solitary caterpillars may be more vulnerable to enemies, than large groups which may be able to swamp predators, or mount coordinated defenses (Reader and Hochuli 2003, Allen 2010). However, in at least five observed events of parasitism in the field, I did not see evidence that the coordinated defense (fig. 16) by *Altinote* caterpillars deterred parasitoids (fig. 17), which is consistent with the high rates of parasitism I measured. High rates of parasitism appear to be common in chemically defended caterpillars (Gentry and Dyer 2003) and gregarious caterpillars (Stireman and Singer 2003). Both chemical and physical defenses of aposematic caterpillars maybe more affective against vertebrate and invertebrate predators than parasitoids (Bowers 1993 and Dyer 1995, 1997). I did not observe any events of predation possibly because predators are more active at night or possibly because the combined physical defenses (hairs, and coordinated defensive behavior), and chemical defenses (cyanogenic glycosides) provide *Altinote* butterflies with an effective defense against most predators.

In the present system, enemy avoidance does not appear to explain host plant use. In both caterpillar species, the rate of parasitism appears to be slightly (but not statistically significantly) lower on *M. hastifolia* which is used as a secondary host plant by both *A. dicaeus* and *A. stratonice*. If enemies were important in determining host plant use in the *Altinote* butterflies, the apparent trend towards reduced parasitism on *M. hastifolia* would be expected to increase the use of *M. hastifolia* as a host plant relative to either *E. polymnioides* or *M. pinnatipartita.* Also it is noteworthy that *A. stratonice,* which is locally more specialized than *A. dicaeus,* experienced much higher rates of parasitism. Herbivores that are closely tied to a host plant may experience higher rates of parasitism because parasitoids may have adaptions which allow them to use plant volatiles to locate caterpillars (Fatouros et al. 2012, Ichiki et al. 2012). Plants are larger and easier to find than small caterpillars so it seems likely that many enemies may use plants to locate hosts or prey. The more closely tied a caterpillar species is to their host plant, the easier it would be to locate that caterpillar species using plant volatiles.

When the net effect of enemies was measured *in situ, M. hastifolia* appeared to provide *A. stratonice* with more protection for enemies than *M. pinnatipartita*. However the increased protection from enemies does not result from higher levels of *de novo* production of cyanogenic glycosides on either *M. pinnatipartita* for *A. stratonice* nor *E. polymnioides* for *A. dicaeus*. While enemies are clearly an important source of mortality, selection pressures from enemies alone does not appear to drive the observed patterns of host plant use.

An alternate explanation for the observed increase in survival experienced by *A. stratonice* on *M. hastifolia* is larger clutch size. Large clutches may provide increased protection against predators as observed by Reader and Hochuli (2003) using *Doratifera casta* (Lepidoptera: Limacodidae). *In situ* experiments using small, medium and large groups both exposed to and sheltered from enemies found higher mortality due to enemies in small groups than in medium or large groups when compared to caterpillars sheltered from enemies. The lower rates of predation observed on *M. hastifolia* may be a function of group size and not host plant *per se*.

Figure 16 Group defensive posture of third instar *A. dicaeus*. In addition to the raised heads some caterpillars appear to have a drop of liquid held in their mandibles, possibly regurgitate. The caterpillars with their heads on the leaf are scraping their mandibles on the leaf creating a rasping noise.

Figure 17 Three events of parasitism from three families, **A)** Ichneumonidae, **B)** Braconidae, and **C)** Tachinidae.

Resource Availability

Host plant abundance and patch size are important forces limiting host plant use by some insect herbivores (McLain 1981, Strong et al. 1984, Lee 1988, Krauss et al. 2004, Mason et al. 2011). Large resource patches formed by either monocultures or simple polycultures have been shown to increase the density of specialist insect herbivores (Strong et al. 1984, Lee 1988). For example in the European corn borer (*Ostrinia nubilalis,* Lepidoptera, Pyralidae) oviposition is positively correlated with host plant densities (Lee 1988). In polycultures, herbivore densities are often higher on single plants than expected (Strong et al. 1984) and this may be part of the reason a greater densities of *A. stratonice* caterpillars were found on *M. hastifolia* which is more often near other plants thanon *M. pinnatipartita* when plants were surveyed. In contrast when caterpillars were surveyed more were found on *M. pinnatipartita* possibly because caterpillar densities may be higher within the spectacularly large monocultures formed by *M. pinnatipartita*.

Concentrated resources appear to be advantageous for specialist herbivores. Large plants may be thought of conceptually as large patches because they both offer a particular resource in a concentrated manner forming a resource island. Both large plants and large patches might conceivably offer a variety of advantages to insect herbivores. A large plant or patch may provide more resources which may be of particular importance to gregarious caterpillars, a large plant or patch may make it harder for predators to locate prey because they need to expend more energy searching a larger area, and large plants or patches may be easier for females to find, because they may give off more volatiles and be easier to see (Allen 2010). Large plants may offer additional benefits, like avoiding grazing by herbivores so an insect is less likely to be consumed accidentally with the leaves (Gish et al. 2012). Large plants may also have greater access to sun and be able to repair damage inflicted by and insect herbivore more quickly, there by continuing to provide the insect with shelter and food (Allen 2010).

Gregarious caterpillars may be more likely to depend on larger plants if plants are spaced further apart, forming resource islands, because it may be difficult to find another plant if the original plant is completely consumed or dies for other reasons. Additionally in some species of gregarious caterpillars, females only lay a single clutch of eggs; in those cases selecting a “good” host plant that provides sufficient resources is particularly important. However in the case of *A. dicaeus,* plant size appears to be more important than plant quality, because when given the option of using a more frequent plant providing equivalent survival and pupal mass, *A. dicaeus* uses the larger, less common plant. Field observations suggest that large individuals of the plant *E. polymnioides* are used more frequently by multiple females than smaller plants. Similarly Benson et al. (1975) suggested that the gregarious *Heliconius hewitsoni* feeds only on large *Distephana coccine* plants.

In some gregariously feeding species, group sizes might be large enough to warrant selection for either large patches of plants or large plants. In the case of *A. dicaeus,* there were often one or more groups of different ages on the same plant indicating either clutches laid by different females, or different oviposition events by the same female. Female *H. hewitsoni* will both oviposit gregariously with other females and return to the same host plant to deposit additional eggs

(Reed 2003). This behavior is observed in *H. hewitsoni* even though food is plentiful. Host plants of *A. dicaeus* are not in short supply, and in the case of all three host plant species, the majority of plants have neither caterpillars nor eggs on them. So, it appears that something other than food scarcity is driving host plant use. While using the same host plant for multiple cohorts might be driven by some of the benefits of large group size, there are alternate hypotheses that are worthy of investigation such as amount of sun, possible variation in host plant quality, and plant apparency

Plant Distribution

Using plants proportional to their frequency indicates that either females oviposit on the first plant they come across or repeated encounters influence host acceptance. Females may not look for the "best plant" if they are time limited, resources are rare, or they are patchily distributed (Doak et al. 2006). Such a strategy is consistent with observed host use in *A. stratonice* but not in *A. dicaeus*. This may help to explain why *A. stratonice* is reported to also use another host, *Liabum* sp. in Columbia (Brown and Francini 1990). Although I did not observe local use of a *Liabum* sp.*,* there is a small population of plants in the genus *Liabum* sp. near my study location. In contrast, *A. dicaeus* has been reported to dominate herbivore assemblages on *E. polymnioides* at another site in southern Ecuador at La Reserva Biológica San Francisco (Bonder et al. 2012). Several websites also report *A. stratonice* and *A. dicaeus* feeding on a variety of genera including *Eupatorium*, *Vernonia*, and *Mikania*, however they did not cite sources, and I was unable to verify those claims.

The use of plants relative to their abundance by *A. stratonice* is similar to the pattern of host plant use by the fall web worm (*Hyphantira cunea*), which uses abundant plants more often regardless of variation in quality (Mason et al. 2011). This seems particularly strange given that the fall webworm only produces one clutch, and therefore her reproductive success depends upon one plant. While the number of clutches an *A. stratonice* female produces is unknown, it is unlikely that she would be able to produce very many clutches of 100+ eggs, and thus her reproductive potential may be limited by host plant quality. *Hyphantira cunea* has a spectacularly wide range of acceptable host plants, and by comparison, both species of *Altinote* are specialized.

Finally, in a few studies of closely related butterfly species with overlapping host plant ranges have largely focused on partitioning as a result of interspecies competition for resources (Emmel and Emmel 1969, Queiroz 2002, Friberg and Wiklund 2009). Friberg and Wiklund (2009) measured female host plant preference and larval performance of *Leptidea sinapis* and *Leptidea reali* on seven host plants and found females preferred to use a relatively small number of good quality, locally available host plants. Oviposition preference appears to correlate more closely with habitat than with larval survival. It is possible that *A. dicaeus* and *A. stratonice* experience *in situ* fitness costs associated with their overlapping host plant use, which in turn reduces the use of an otherwise suitable host.

Conclusion

While mortality caused by enemies may vary by host plant in these two *Altinote* species, it does not appear to drive host plant use. Instead, bottom up forces appear to be more important in limiting host plant use. This may be true for many gregarious caterpillars that are relatively well defended against enemies but may be more susceptible to plant availability, quality and defenses (Stamp and Bowers 1990, Reader and Hochuli 2003, Mason et al. 2011). Even though *M. hastifolia* appears to provide *A. stratonice* with protection from enemies and may provide *A. dicaeus* with protection from parasitoids, selection pressures from enemies does not appear to be strong enough to increase the use of *M. hastifolia* by either species. A similar trend was seen in the temperate and much more generalized fall webworm (Mason et al. 2011). Selection pressures from bottom-up forces may be more important in driving host plant use by well defended gregarious caterpillars than those from the top down. However further investigation of genetic differentiation, and the effects of intraspecific competition, is required. These should be paired with additional longer term studies of predation, plant abundance and caterpillar host plant use, and finally more in-depth analysis of plant and caterpillar defenses.

References

Allen P E. (2010) Group Size effects on survivorship and adult development in the gregarious larvae of *Euselasia chrysippe* (Lepidoptera, Riodinidae). *Insect Society*, 57:199-204.

Ankes S, Niemüller D, Moll S, Hänsch R, and Ober D. (2004) Polyphyletic Origin of Pyrrolizidine Alkaloids within the Asteraceae. Evidence from Differential Tissue Expression of Homospermidine Synthase1. *Plant Physiology*, 136:4037-4047.

Awmack S, and Leather S R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual review of entomology*, 47:817-844.

Benson W W, Brown K S, and Gilbert L E. (1975) Coevolution of Plants and Herbivores: Passion Flower Butterflies *Evolution*, 29:659-680.

Berenbaum M R, and Feeny P P. (2008) Chemical Mediation of Host-Plant Specialization: The Papilionid Paradigm. University of California Press, Los Angeles, California.

Bernays E A and Funk D J. (1999) Specialists Make Faster Decisions than Generalists: Experiments with Aphids. *The Royal Society*, 266:151-156.

Bernays E, and Graham M. (1988) On the Evolution of Host Specificity in Phytophagous Arthropods. *Ecology*, 69:886-892.

Bernays, E. A. (1998) The value of being a resource specialist: behavioral support for a neural hypothesis. *The American Naturalist*, 151:451-464.

Blau P A, Feeny P, Contardo L, and Robson D S. (1978) Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. *Science*, 200:1296-1298.

Brown K S, and Francini R B. (1990) Evolutionary Strategies of Chemical Defense in Aposematic Butterflies: Cyanogenesis in Asteracea-feeding American Acraeinea. *Chemoecology*, 1:52-56.

Brown C R, Komar N, Quick S B, Sethi R A, Panella N A, Brown M B, and Pfeffer M. (2001) Arbovirus infection increases with group size. *Proceedings of the Royal Society of London B: Biological Sciences*, 268:1833-1840.

Biesmeijer J C, Roberts S P M, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers A. P, Potts S G, Kleukers R, Thomas C D, Settele J and Kunin W E. (2006) Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science*, 313:313351-313354.

Bollag D M, Edelstein S J. (1990) Protein Methods Wiley-Liss, Inc. New York, New York.

Bodner F, Strutzenberger P, Brehm G, and Fiedler K. (2012) Species richness and host specificity among caterpillar ensembles on shrubs in the Andes of southern Ecuador. *Neotropical entomology*, 41:375-385.

Bowers, M. D. (1993) Aposematic caterpillars: life-styles of the warningly colored and unpalatable. Pages 331–371 Caterpillars: ecological and evolutionary constraints on foraging. Routledge, Chapman, and Hall, New York, New York, USA.

Camara M D. (1997) Predator Responses to Sequestered Plant Toxins in Buckeye Caterpillars: Are Tritrophic Interactions Locally Variable? *Journal of Chemical Ecology,* 23:2093-2106.

Cornell H V, and Hawkins B A. (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. *The American Naturalist*, 161:507-522.

Craig T P, and Itami J K. (2008) Evolution of Preference and Performance Relationships. University of California Press, Los Angeles, California.

Crutterwell McFadyen R E. (1998) Biological Control of Weeds. *Annual Review of Entomology*, 43:369-393.

Davis S L, and Cipollini D. (2014) Do mothers always know best? Oviposition mistakes and resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic host. *Biological Invasions*, 16:1941-1950.

Despres L, David J P, and Gallet C. (2007) The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution*, 22:298-307.

Doak P, Kareiva P, and Kingsolver J. (2006). Fitness consequences of choosy oviposition for a time-limited butterfly. *Ecology*, 87:395-408.

Dyer L A. (1995) Tasty generalists and nasty specialists? A comparative study of antipredator mechanisms in tropical lepidopteran larvae. *Ecology* 76:1483–1496.

Dyer L A. (1997) Effectiveness of caterpillar defenses against three species of invertebrate predators. *Journal of Research on the Lepidoptera* 34:48–68.

Dyer L A, Singer, M S, Lill J T, Stireman J O, Gentry G L, Marquis R J, Ricklefs R E, Greeney H F, Wagner D L, Morais H C, Diniz I R, Kursar T A, and Coley P D. (2007) Host specificity of Lepidoptera in tropical and temperate forests. *Nature*, 448:696-699.

Dyer L A, Miller J S, Rab Green S B, Gentry G L, Greeney H F, and T W Walla. (17, March, 2015). Caterpillars and parasitoids of the Eastern Andes in Ecuador. http://www.caterpillars.org

Dicke M. (2000) Chemical Ecology of Host-plant Selection by Herbivorous Arthropods: A Multitrophic Perspective. *Biochemical Systematics and Ecology*, 28:601-617

Egan S V, Yeoh H H, and Bradbury J H. (1998) Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. *Journal of the Science of Food and Agriculture*, 76:39-48.

Egan S P and Funk D J. (2006) Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa The *Royal Society,* 273:843-848.

Ehrlich P R and Raven P H. (1964) Butterflies and Plants: a Study in Coevolution. *Evolution*, 18:586-608.

Emmel Thomas C. and Emmel John F. (1969) Selection and Host Overlap in Two Desert Papilio Butterflies. *Ecology* 50:158–159.

Erickson J M, and Feeny P. (1974) Sinigrin: a chemical barrier to the black swallowtail butterfly, *Papilio polyxenes*. *Ecology*, 55:103-111.

Fatouros N E, Lucas-Barbosa D, Weldegeris B T, Pashalidou F G, van Loon J J A, Dicke M, Harvey J A, Gols R, Huigens M E. (2012) Plant Volatiles induced by herbivore egg deposition affect insects of different tropic levels. *PLOS one* 7:1-13.

Fisher B L. (1998) Insect behavior and ecology in conservation: preserving functional species interactions. *Annals of the Entomological Society of America*, 91:155-158.

Francini R B, Barbosa E P, and Fretias A V L. (2011) Immature Stages of *Actinote zikani* (Nymphalidae:Heliconiinae), a Critically Endangered Butterfly from Southeastern Brazil. *Tropical Lepidoptera Research*, 21:20-26.

Francini B R and Freitas A V L. (2003) Aggregated oviposition in *Actinote pellenea pellenea* Hübner (Lepidoptera: Nymphalidae). *Journal of Research on the Lepidoptera* 42:74-78.

Friberg M, and Wiklund C. (2009) Host plant preference and performance of the sibling species of butterflies *Leptidea sinapis* and *Leptidea reali*: a test of the trade-off hypothesis for food specialization. *Oecologia*, 159:127-137.

Fordyce J A. (2003) Aggregative feeding of pipevine swallowtail larvae enhances hostplant suitability. *Oecologia*, 135:250-257.

Forister M L, Nice C C, Fordyce J A, Z Gompert. (2009) Host range evolution is not driven by the optimization of larval performance: the case of *Lycaeides melissa* (Lepidoptera:Lycaenidae) and the colonization of alfalfa. *Oecologia* 160:551–561.

Forister M L, Dyer L A, Singer M S, Stireman J O, and Lill J T. (2012) Revisiting the Evolution of Ecological Specialization, with Emphasis on Insect-plant Interactions. *Ecology*, 93:981-991

Forister, M. L., Novotny, V., Panorska, A. K., Baje, L., Basset, Y., Butterill, P. T, Cizel L, Coley P D, Dem F, Diniz I R, Drodz P, Fox M, Glassmire A E, Hazen R, Hrcek J, Jahner J P, Kaman O, Kozubowski T J, Kursar T A, Lewis O T, Lill J, Marquis R J, Miller S E, Morais H C, Murakami M, Nickel H, Pardikes N A, Ricklefs R E, Singer M S, Smilanich A M, Stireman J O, Villamarin-Cortez S, Vodka S, Volf M, Wagner D L, Walla T, Weiblen G D. and Dyer L A. (2015) The global distribution of diet breadth in insect herbivores. *Proceedings of the National Academy of Sciences*, 112:442-447.

Funk V A, Kelloff C, and Chan R. (2012) Phylogeny and biogeography of the tribe Liabeae (Compositae subfamily Cichorioideae). *Taxon*, 61:437-455.

Futuyma D J, and Agrawal A A. (2009) Macroevolution and the biological diversity of plants and herbivores. *Proceedings of the National Academy of Sciences*, 106:18054-18061.

Gleadow R M, and Woodrow I E. (2002) Mini-Review: constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of chemical ecology*, 28:1301-1313.

Greeney H F and Stireman J O. (2001) Emergence of Parasitic Flies from Adult Actinote Diceus (Nymphalidae:Acraeinae) in Ecuador. *Journal of Lepidoptera Research*, 33:79-80.

Gentry G H, and Dyer L A. (2003) On the Conditional Nature of the Neotropical Caterpillar Defenses against their Natural Enemies. *Ecology*, 83: 3108–3119.

Gish M, Dafni A, and Inbar, M. (2012). Young Aphids Avoid Erroneous Dropping when Evading Mammalian Herbivores by Combining Input from Two Sensory Modalities. *PLoS ONE*, 7:e32706- e32706.

Hardy N B, and Otto S P. (2014) Specialization and generalization in the diversification of phytophagous insects: tests of the musical chairs and oscillation hypotheses. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1795), 20132960.

Hawkins BA (1994) Pattern and Process in Host-Parasitoid Interactions. Cambridge
University Press, Cambridge, UK.

Hawkins B A, Cornell H V, and Hochberg M E. (1997) Predators, Parasitoids, and Pathogens as Mortality Agents in Phytophagous Insect Populations. *Evolution*, 78:2145-2152.

Hochberg, M. E. (1991). Viruses as costs to gregarious feeding behaviour in the Lepidoptera. *Oikos*, 61:291-296.

Ichiki R T, Ho G T T, Wajnberg E, Kairnoh Y, Tabata J, and Nakamura S. (2012) Different uses of plant semiochemicals in host location strategies of the two tachinid parasitoids. *Springer* 99:687-694.

Janz N. (2005) The Relationship Between Habitat Selection and Preference for Adult and Larval Food Resources in the Polyphagous Butterfly *Vanessa cardui* (Lepidoptera: Nymphalidae). *Journal of Insect Behavior,*18:767-780.

Jeffries M J, and Lawton J H. (1984) Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, 23:269-286.

Jorge L R, Prado P I, Almeida-Neto M, and Lewinsohn T M. (2014) An integrated framework to improve the concept of resource specialization. *Ecology letters*, 17:1341-1350.

Krauss J, Steffan-Dewenter I, and Tscharntke T. (2004) Landscape occupancy and local population size depends on host plant distribution in the butterfly *Cupido minimus*. *Biological Conservation*, 120:355-361.

Kelley S T, Farrell B D, and Mitton J B. (2000) Effects of specialization on genetic differentiation in sister species of bark beetles. *Heredity*, 84:218-227.

Lampert E C, Dyer L A, and Bowers M D. (2011) Chemical Defense Across Three Trophic Levels: *Catalpa bignonioides*, the Caterpillar *Ceratomia catalpae*, and its Endoparasitoid *Cotesia congregate. Journal of Chemical Ecology*, 37:1063–1070.

Lawrence W S. (1990) The effects of group size and host species on development and survivorship of a gregarious caterpillar *Halisidota caryae* (Lepidoptera: Arctiidae). *Ecological Entomology*, 15:53-62.

Lee D A. (1988) Moth Density and Oviposition Patters of European Corn Borer, Ostrina nuilalis (Lepidoptera: Pyraliadae), in Alberta. *Environmental Entomology*, 2:220-224.

Lewis A C. (1986) Constraints and Flower Choice in *Pieris rapae*. *Science*, 232:863-865.

Levin, D. A. (1976) The chemical defenses of plants to pathogens and herbivores. *Annual review of Ecology and Systematics*, 7:121-159.

Lill J T, and Marquis R J. (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. *Oecologia* 126:418–428.

Mason P A, Wilkes S R, Lill J T, Singer M S. (2011) Abundance Trumps Quality: Bi-trophic Performance and Parasitism Risk Fail to Explain Host Use in the Fall Webworm. *Oikos*, 120:1509-1518.

Mayhew P J. (1997) Adaptive patterns of host-plant selection by phytophagous insects. *Oikos*, 79:417-428.

McFadyen R E C. (1998) Biological control of weeds. *Annual review of entomology*, 43:369-393.

McLain, D. K. (1981) Resource partitioning by three species of hemipteran herbivores on the basis of host plant density. *Oecologia*, 48:414-417.

Miller J Y and Miller L D. (1978) Notes on Mexican Actinote (Nymphalidae:Acraeinae) and Their Relatives, with a Description of a New Subspecies. *Journal of the Lepidopterists' Society*, 32:261-272.

Mitter C, Farrell B, and Wiegmann B. (1988) The Phylogenetic Study of Adaptive Zones: Has Phytophagy Promoted Insect Diversification? *American Naturalist*, 132:107-128.

Memmott J. (1999) The structure of a plant-pollinator food web. *Ecol. Lett*. 2:276–280.

Murphy S M, and Berebaum M R. (2004) Enemy-Free Space Maintains Swallowtail Butterfly Host Shift. *PNAS*,101:18048-18052.

Narberhaus I, Zintgraf V, and Dobler S. (2005) Pyrrolizidine alkaloids on three trophic levels–evidence for toxic and deterrent effects on phytophages and predators. *Chemoecology*, 15:121-125.

Nosil P. (2002) Transition Rates Between Specialization and Generalization in Phytophagous Insects. *Evolution,* 56:1701–1706.

Opitz S E and Müller C. (2009) Plant Chemistry and Insect Sequesteration. *Chemoecology*, 19:117-154.

Oppenheim S J and Gould F. (2002) Behavioral Adaptations Increase the values of Enemy-free Space for *Heliothis subflexa*, a specialist herbivore. *Evolution*, 56:679-689.

Paoletti M G, Rascio N, Moro I, Brugnerotto E, Onore G, Tapia C, Bricchi C, and Pruski, J F. (2012) Ethnobiology of Munnozia pinnatipartita (Compositae: Liabeae), an Intriguing Weed of Dairy Farms and Its Use as an Herbicide to Convert Pastures into Cloud Forest in the Otonga Reserve in Highland Ecuador. *Journal of Sustainable Agriculture*, 36:69-82.

Pimentel D, McNair S, Janecka J, Wight J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, and Tsomondo T. (2001) Economic and Environmental Threats of Alien Plant, Animal, and Microbe Invasions. *ScienceDirect,* 84:1-20*.*

Price P W, Denno R F, Eubanks M D, Finke D L, and Kaplan I. (2011) Insect Ecology: Behavior, Populations and Communities. Cambridge University Press, New York.

Queiroz J M. (2002) Host plant use among closely related Anaea butterfly species (Lepidoptera, Nymphalidae, Charaxinae). *Brazilian journal of biology*, 62:657-663.

Raubenheimer D. (1988) Cyanoglycoside Gynocardin from Acaea horta (L) (Lepidoptera: Acaeinae) Possible Implications for Evolution of Acraeine Host Choice. *Journal of Chemical Ecology*, 15:2177-2189.

Reader T, and Hochuli D F (2003). Understanding gregariousness in a larval Lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology*, 28:729-737.

Reed R D. (2003) Gregarious Oviposition and Clutch Size Adjustment by a Heliconius Butterfly. *Biotropica*, 35:555-559.

Rizk, A. (1990) Naturally Occurring Pyrrolizidine Alkaloids. CRP Press, Inc. Boca Raton Florida.

Rodrigues, D., Kaminski, L. A., Freitas, A. V., & Oliveira, P. S. (2010). Trade-offs underlying polyphagy in a facultative ant-tended florivorous butterfly: the role of host plant quality and enemy-free space. *Oecologia*, 163:719-728.

Rossini C,Hoebeke E R, Iyengar V K, Conner W E, Eisner M, and Eisner T. (2000) Alkaloid content of parasitoids reared from pupae of an alkaloid-sequestering arctiid moth (*Utetheisa ornatrix*). *Entomological News*, 111:287-290.

Schoonhoven L M, van Loon J A J, and Dicke M. (2005) Insect-Plant Biology: Chapter 2, Herbivorous Insects: Something for Everyone. Oxford University Press, New York, New York.

Scott J A. (1986) The Butterflies of North America: A Natural History and Field Guide. Stanford University Press, Stanford, California.

Segura D F, Viscarret M M, Ovruski S M , and Cladera J L. (2012) Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* to host and host-habitat volatile cues. *Entomologia Experimentalis et Applicata,* 143:164–176.

Silva-Brandano K L, Wahlberg N, Francini B R, Azeredo-Espin A M L, Brown K S Jr, Paluch M, Lees D C, and Freita V L. (2008) Phylogenetic Relationships of Butterflies of the TribeAcraeini (Lepidoptera, Nymphalidae, Heliconiinae)and the Evolution of Host Plant Use. *Molecular Phylogenetics and Evolution,* 46:515–531.

Sime K R. (2002) Experimental Studies of the Host-Finding Behavior of *Trogus pennator*,A Parasitoid of Swallowtail Butterflies. *Journal of Chemical Ecology*, 28:7, 1377-1392.

Singer M S, Carrie Y, Theuring C, and Hartmann T. (2004) Disentangling Food Quality from Resistance against Parasitoids: Diet Choice by a Generalist Caterpillar. *The American Naturalist*, 164:423-429.

Singer M S, and Stireman J O. (2003) Does Anti-parasitoid Defense Explain Host-plant Selection by a Polyphagous Caterpillar? *Oikos*, 14:554-562.

Singer M S, Rodrigues D, Stireman J O, and Carrière Y. (2004) Roles of food quality and enemy-free space in host use by a generalist insect herbivore. *Ecology*, 85:2747-2753.

Singer M S. (2008) Evolutionary Ecology of Polyphagy. University of California Press, Los Angeles, California.

Stamp N E and Bowers M D. (1990) Variation in Food Quality and Temperature Constrain Foraging of Gregarious Caterpillars *Ecology*, 71:1031-1039.

Stiling P, and Moon D C. (2005) Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. *Oecologia*, 142:413-420.

Stireman J O and Singer M S. (2003) Determinants of Parasitiod-Host Associations: Insights from a Natural Tachinid-Lepidopteran Community, *Ecology,* 84:296-310.

Stireman J O, O'Hara J E, and Wood D M. (2006) Tachinidae: Evolution, Behavior, and Ecology. *Annual Review of Entomology*, 51:525-555.

Strauss S Y, Conner J K and Rush S L. (1996) Foliar Herbivory Affects Floral Characters and Plant Attractiveness to Pollinators: Implications for Male and Female Plant Fitness. *The American Naturalist*, 147:1098-1107.

Strong, D. R., Lawton, J. H., & Southwood, S. R. (1984). Insects on plants. Community patterns and mechanisms. Blackwell Scientific Publications.

Velez1 P D, Montoya H H V, Wolff M. (2011) Immature Stages and Natural History of the Andean Butterfly *Altinote ozomene* (Nymphalidae: Heliconiinae: Acraeini). *Zoologia*, 28:593–602.

Yotoko K S, Prado P I, Russo C A, and Solferini V N. (2005) Testing the trend towards specialization in herbivore–host plant associations using a molecular phylogeny of Tomoplagia (Diptera: Tephritidae). *Molecular phylogenetics and evolution*, 35:701-711.