Ecological interactions between *Euphydryas editha* larvae and their host plants

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Abstract

Ecological interactions between *Euphydryas editha* larvae and their host plants

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I examined ecological interactions between larvae of *Euphydryas editha* (Lepidoptera: Nymphalidae) and their host plants. These caterpillars, and the plants they eat, provide an intriguing system for studying several aspects of basic and applied ecology. In various chapters I focus on plant-mediated indirect effects, multi-trophic chemical interactions, ontogenetic niche shifts, the ecology and conservation of early-instar caterpillars, and the management and recovery of rare species.

Euphydryas editha larvae are oligophagous herbivores, specializing on a few related host plant species. Two hosts I focus on are in the genus *Castilleja*, and the third host is *Plantago lanceolata,* an exotic species which *E. editha* recently incorporated into its diet. The plants *E. editha* specializes on produce iridoid glycosides, secondary compounds which are deterrent to many organisms, but which *Euphydryas* and some other specialists co-opt, sometimes accumulating them at high concentrations to defend against predators.

Members of the genus *Castilleja* are hemiparasites; they form connections to other plants' roots and extract resources from them. Therefore, *Castilleja* traits could depend on interactions with host plants, creating an indirect interaction pathway in which the plants *Castilleja* parasitizes affect herbivores (*E. editha*) by modifying the quantity or quality of food available to them. I grew *C. levisecta* with six different hosts, as well as without a host, while *E.*

editha larvae fed on it. *Castilleja* size and leaf N concentrations depended on the host it parasitized, and larger, more N-rich plants resulted in larger *E. editha* larvae with higher survival rates. The ratio of two iridoid glycosides the larvae sequestered also depended on the identity of the host used by *Castilleja.* This work shows that hemiparasitic plant traits can mediate strong indirect interactions.

In a field study, I compared outcomes for *E. editha* ssp. *taylori* larvae as they fed on *C. levisecta, C. hispida,* and *P. lanceolata*. This subspecies of *E. editha* is endangered, and inhabits grasslands in the Pacific Northwest. Managers involved in recovery efforts need information about the suitability of its host plants. Therefore, I placed clusters of *E. e. taylori* eggs on each species, and tracked larval survival from instar to instar. I also measured larval phenology, mass, and sequestration of iridoid glycosides. I tracked the senescence rates, pigmentation, and leaf nutrition (C:N ratios) for plants in each host species, and measured several environmental variables that could influence them.

I found that survival depended on the host species that was eaten; it was highest on *P. lanceolata,* intermediate on *C. hispida*, and considerably lower on *C. levisecta.* Importantly, the factors influencing survival depended strongly both on the plant species larvae ate and their larval instar, with different predictors of survival for different instars. The overall differences in survival were mostly because of a disparity in survival during second instar. Larvae feeding on *C. levisecta* were less likely to survive from hatching to second instar, and from second to third instar, when plants were senescing, but this did not occur when they fed on the other two species. Group size was important to larvae feeding on *P. lanceolata* (but not on either *Castilleja* species); they were more likely to survive from second to third instar, and developed to fourth instar faster, when they were members of larger sibling groups. Survival from third to fourth

instar was higher than for previous stages, and was not related to any of the variables that were measured. These findings related to larval survival show the importance of assessing survival instar by instar, as well as the importance of measuring outcomes for early-instar caterpillars.

Larval mass was unaffected by any of the variables that were measured. Contrary to expectations, environmental variables like slope, aspect, and vegetation structure had no discernable effects on mass or development rate of the larvae. However, larvae that reached fourth instar earlier spent much more time feeding before entering diapause, suggesting butterflies that fly earlier (whose larvae consequently develop earlier) could have higher reproductive success. Environmental variables in this study had no measurable direct effects on larvae, but they could still influence them by changing the quality of their host plants: senescence of *C. levisecta* was faster in dry microsites than mesic ones, indicating plants growing in mesic microsites could be more phenologically compatible with *E. e. taylori*.

There were also strong differences in the amounts of iridoid glycosides larvae were able to sequester from their hosts. They sequestered the compounds aucubin and catalpol from *P. lanceolata*, and when they fed on either *Castilleja* species, they sequestered these two compounds plus two others, macfadienoside and (putatively) methyl shanzhiside. The overall amounts sequestered from *C. levisecta* were lower than for the other two species, and may be low enough to leave them undefended against predators.

In summary, I found that several outcomes for *E. editha* larvae are attributable to differences that occur within and among their various host plants. These differences can be attributable to innate species characteristics, but also to intraspecific differences caused by parasitic interactions and environmental factors. In this system, differences in host plants

strongly influenced mass, growth rate, survival, and secondary chemical sequestration by the herbivore *E. editha*.

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Chapter 1: Background and overview

This dissertation is about species interactions, specifically those between plants and herbivorous insects. Each chapter is an experimental or semi-experimental study investigating how variability in plant phenology, nutrition, or secondary chemistry affects outcomes for larvae of the herbivore, *Euphydryas editha* (Edith's checkerspot; Lepidoptera: Nymphalidae). Some of the chapters are oriented towards advancing ecological theory, while others are designed to answer practical questions related to conservation. Hopefully each of them does a little of both.

Background

Members of the genus *Euphydryas* and their relatives have figured prominently in the development of ecology and evolutionary biology during the 20th century. *Euphydryas editha* has been used as a model organism for understanding population biology (summarized in Ehrlich and Hanski 2004), and evolution of host plant preference (Singer 1971, Singer 1982, Radtkey and Singer 1995, Singer and Thomas 1996). Both *E. editha* and a European relative, *Melitaea cinxia,* were used as model systems to develop and refine the metapopulation concept (Harrison et al. 1988, Hanski 1999). *Euphydryas phaeton, E. editha,* and *E. anicia,* as well as another Nymphalid, *Junonia coenia,* have contributed to our understanding of chemical ecology, since they sequester iridoid glycosides from their hosts and advertise their unpalatability to predators with warning coloration (Bowers 1980, Bowers 1981, Bowers 1983, Gardner and Stermitz 1988, Bowers and Collinge 1992, Bowers 1993).

Euphydryas editha is comprised of several subspecies, distributed across Western North America (Ehrlich & Hanski 2004). One could argue that the characteristics making this organism a compelling study system—dietary specialization, and dense, sedentary populations that are prone to explosive growth and rapid crashes—have also made some subspecies vulnerable to extinction. *Euphydryas e. taylori, quino,* and *bayensis* (alternately called *editha*) have all declined and are now of conservation concern (USFWS 1998, USFWS 2003, USFWS 2013).

This dissertation focuses on *E. e. taylori,* which is endemic to grasslands of the Pacific Northwest and was listed as endangered in 2013 (USFWS 2013). Declines are generally attributed to habitat loss,

invasion by exotic grasses and shrubs, forest encroachment, and to accompanying declines in their larval hosts (Schultz et al. 2011). While *E. editha* has been studied extensively, particularly in California, it evolves quickly and its subspecies and populations are notoriously variable, so recovery efforts for *E. e. taylori* are currently held up by knowledge gaps.

At the present time, *E. e. taylori* is at an interesting and ambiguous point in its host plant affiliations (Schultz et al. 2011, Severns and Breed 2014, Dunwiddie et al. 2016). Historically, we speculate that many populations fed on *Castilleja hispida* (Orobanchaceae). There is also evidence that some populations used *Castilleja levisecta*, although whether this was a primary host is not known. It can also use *Veronica scutellata* (Plantaginaceae), although the populations we studied have little opportunity to interact with this species and it is not dealt with in this dissertation.

Like some of its relatives, *E. e. taylori* adopted the exotic *Plantago lanceolata* (Plantaginaceae) into its diet at some point in the 20th century, and some populations now rely primarily, or exclusively, on this novel host (Dunwiddie et al. 2016). In the South Puget Sound region, at one point only a single *E. e. taylori* population remained, and it used *P. lanceolata* almost exclusively until managers planted more *C. hispida* at the site (M. Linders, personal communication). Managers also translocated *E. e. taylori* to several additional sites that contained both *P. lanceolata* and *C. hispida*.

Land managers currently establish and augment populations of both *P. lanceolata* and *C. hispida* as part of recovery efforts. The two taxa are often used by the butterfly interchangeably. *Castilleja levisecta* did not figure into recovery efforts until recently—it is a federally threatened species, and like *E. e. taylori*, was at one point restricted to a single population in the South Puget Sound. However, successful recovery efforts have established (or re-established) large populations at sites occupied by *E. e. taylori*, as well as sites where *E. e. taylori* is likely to be reintroduced in coming years. The two *Castilleja* species hybridize and introgress easily, meaning sites designated for recovery of *C. levisecta* cannot be augmented with *C. hispida* and thus those sites' suitability for reintroducing *E. e. taylori* is questionable. Therefore, for recovery efforts for either taxa to proceed, more information is needed about the

performance of *E. e. taylori* on each of the three hosts. See Dunwiddie et al. (2016) for a detailed account of recovery efforts for both taxa and how they interact.

Overview

Each chapter in this dissertation examines relationships between early-instar *E. editha* larvae and their host plants. Differences among the three hosts (*Castilleja hispida*, *Castilleja levisecta*, and *Plantago lanceolata*) are examined, but I also demonstrate effects of variation within each species. Plant species are often perceived as being monolithic, but characteristics within a species can vary for a number of different reasons. They vary with genotype, but also across environmental gradients, and over a plant's lifespan. *Castilleja* spp. are hemiparasitic, so they can also vary depending on their relationships with the plants they parasitize. These types of variation, and their effects on interactions with *E. editha*, are a common conceptual thread appearing in each chapter of this dissertation.

In Chapter 2, I show how hemiparasitic plants can mediate interactions between their host plants and herbivores. Depending on the host plant being parasitized, *C. levisecta* differs in size, leaf N, and possibly iridoid glycoside chemistry. These differences affect mass gain, survival, and sequestration of secondary chemicals by *E. editha*. This study shows that parasitic plants are important mediators of indirect interactions, and that outcomes for *E. editha* can depend not just on *Castilleja*, but also on the host plants it parasitizes.

Chapters 3-6 show how variability within and among the three host species used by *E. e. taylori* affects larvae during their early development. I conducted a field study, introduced in Chapter 3, to compare outcomes for caterpillars feeding on each of the three host species. I measured how differences in phenology and nutrition within and among the three species affected survival for Taylor's checkerspot. I quantified mortality during each pre-diapause larval instar, allowing me to pinpoint different sources of mortality for each ontogenetic stage on each host plant. Survival was highest on *P. lanceolata*, intermediate on *C. hispida*, and lowest on *C. levisecta*, and the strongest differences in survival occurred during second instar, and the most important source of larval mortality was senescence of *C. levisecta*.

Chapter 4 builds on the previous chapter by testing how differences within and among host plants affect mass and development time for *E. e. taylori* larvae. Remarkably, I found host plants had no effects on mass, development time, or fourth instar duration of larvae. I considered both the species identity of the hosts as well as within-species differences; both of these had strongly influenced larval survival. However, on all three hosts, larvae that reached fourth instar sooner spent much more time feeding before entering diapause. This could have important implications for development and reproductive success of later life stages.

In Chapter 5, I tested several environmental variables to see if they predicted outcomes for caterpillars, and the extent to which they control the phenology and nutrition of their host plants. I found that topography and vegetation structure had no discernible effects on larval survival, growth, or development time. Host plant quality, in contrast, varied at least weakly; *C. levisecta* in wetter sites with more nutrient availability senesced more slowly.

Finally, in Chapter 6 I begin to characterize the chemical interactions that occur between Taylor's checkerspot and its host plants. Checkerspot butterflies specialize on plants that produce iridoid glycosides (Bowers 1983). These compounds taste bitter to humans and are deterrent to some generalist herbivores, but some specialists, including *E. editha,* sequester them to co-opt and use as a defense against predators (Bowers 1980, Bowers 1981, Bowers 1983). This chapter documents the iridoid glycoside compounds sequestered from each host by *E. e. taylori*. Depending on the host that caterpillars ate, there were strong differences in the compositions and overall amounts of iridoid glycosides sequestered.

A note to the reader about structure and formatting

These chapters are presented in manuscript form so they can be adapted for submission to peer-reviewed journals. Therefore, information in the introductions and methods sections will be somewhat redundant (especially in later chapters), and formatting varies from chapter to chapter.

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Chapter 2: Hemiparasites can transmit indirect effects from their host plants to herbivores

Abstract

Plants can mediate indirect interactions between other organisms. These plant-mediated indirect effects are often overlooked, but have important influences on ecosystems. Previous research has shown that parasitic plants can serve as intermediaries between their hosts and other organisms, but these relationships are poorly understood. In particular, the relative importance of the various traits that could mediate these interactions is unexplored.

We studied the role of the root hemiparasite, *Castilleja levisecta* (Orobanchaceae), as a mediator of interactions between the host plants it parasitizes and the lepidopteran herbivore *Euphydryas editha* (Nymphalidae), whose caterpillars feed on *Castilleja* and sequester iridoid glycosides from it. We tested whether the hemiparasite's size, leaf N concentration, and iridoid glycoside concentrations were influenced by the identity of its host plant, and then whether changes to these three traits influenced outcomes for the herbivore.

We found that the hemiparasite's size and leaf N depended on the host it parasitized, and these traits in turn affected survival and mass of *E. editha*. We also found preliminary evidence that host identity influenced iridoid glycoside sequestration by the herbivore. *Euphydryas editha* survival increased with stem length of the hemiparasite (used as a proxy for biomass). Caterpillar mass increased with leaf N, and subsequently, caterpillars with greater mass were more likely to survive during diapause. Mean iridoid glycoside concentrations in caterpillars ranged from 1-12% depending on the host being parasitized by *Castilleja*. This study demonstrates that root parasitism can result in strong indirect effects on higher trophic levels, influencing organisms' survival, growth, and chemical interactions.

Key Words: Hemiparasite, plant-mediated indirect effect, iridoid glycoside, herbivore, tri-trophic interaction

Introduction

Indirect interactions among organisms are ubiquitous in natural systems, and can have strong ecological and evolutionary consequences (Wooton 1994, Walsh 2012). Much attention has been given to the top-down role of carnivores in trophic cascades, where for example, their interactions with herbivores change outcomes for plant communities (e.g., Beschta and Ripple 2009). Tri-trophic interactions among plants, herbivores, and parasitoids (e.g., Kessler and Baldwin 2001) are similarly well-studied. Both of these examples are typically mediated by animals. Plant-mediated indirect effects, in contrast, attract less attention but can also strongly affect ecosystems (Utsumi et al. 2010, Yoshimoto and Nishida 2008). For example, plants can mediate strong interactions when multiple herbivores share a food source (Denno et al. 1995, Ali and Agrawal 2014). Similarly, they provide indirect interaction pathways between above- and belowground communities (Pangesti et al. 2013). Plants that are parasites, such as those in family Orobanchaceae, interact with both the host plants they parasitize and with other organisms, including herbivores. Therefore, they provide a particularly interesting and pertinent system for understanding plant-mediated indirect effects.

In this study we focus on the role of hemiparasitic plants as mediators of indirect effects. Hemiparasites perform photosynthesis, but also can form parasitic xylem connections (haustoria) with the roots of some neighboring plants, siphoning water, carbon, nutrients, and secondary chemicals (Kuijt 1969). While parasitic connections often occur below ground and are thus easily overlooked, parasitic plants are relatively common: they make up about 1% of all plant species (including 20 families) and occur in a wide range of Earth's ecosystems (Heide-Jorgensen 2008; Westwood et al. 2010). Parasitic plants can have impressively strong effects in agricultural systems (e.g., Frost et al. 1997) and on less-managed systems. For example, they can increase plant diversity by suppressing competitively dominant species, and change nutrient cycling rates and community structure through their litter inputs (Marvier 1998; Quested et al. 2003; Watson 2009; Fisher et al. 2013). Consequently, some hemiparasites are increasingly

considered to be keystone species (Houston and Wolff 2012; Rowntree et al. 2014; Hartley et al. 2015)—although these effects may be context-dependent (Schmidt 2016).

Many hemiparasites can attack a range of host plants (Press and Phoenix 2005). Consequently, the identity of the host plant being parasitized can have strong effects on the hemiparasite's reproduction, growth, and defensive chemistry (Marvier 1996, Matthies 1997, Marko and Stermitz 1997). It follows that these trait changes could have implications for herbivores feeding on the hemiparasite, even though the herbivore does not interact directly with the hemiparasite's hosts. This general pattern has been demonstrated in some studies (e.g., Marvier 1996; Adler et al. 2001; Schädler et al. 2005; Rowntree et al. 2014), but we know little about the traits that mediate this effect.

In this study we tested for an indirect effect in which the identity of the hemiparasite's host influences herbivores by changing the hemiparasite's traits and consequently its value as a food source. We tested three mechanisms that we expected to underlie this pattern; namely, changes to the quantity, quality, and secondary chemistry of hemiparasite tissues that the herbivore feeds on. For each trait, we tested its effects on herbivore mass, survival, and chemical interactions with the hemiparasite. First, we expected hosts to affect hemiparasite size, which determines the quantity of food available to the herbivore. We expected survival and mass of the herbivore to improve when it fed on larger plants. Second, we expected hosts to affect the amount of nitrogen secured by the hemiparasite, controlling its nutritional quality to the herbivore. We expected the herbivore's mass and survival to increase when it fed on hemiparasites with greater leaf N concentrations. Third, we expected hosts to affect the hemiparasite's secondary metabolism, resulting in changes in its defensive chemistry. To our knowledge, this possibility has not been explored to date, although it was hypothesized by Schädler et al. (2005). Since the herbivore we tested sequesters secondary metabolites, we expected chemical concentrations in the herbivore to mirror those in the hemiparasite.

Finally, the degree of physical connectedness between hosts and hemiparasites should influence the hemiparasite's ability to secure resources (e.g., Rowntree et al. 2014). Therefore, we also tested whether the number of haustorial connections between the hemiparasite and its hosts affected the three mechanisms described above. To address our hypotheses, we examined relationships between the hemiparasite *Castilleja levisecta* Greenm. (Orobanchaceae), six of its hosts, and the specialist herbivore, *Euphydryas editha* Boisduval (Nymphalidae), which feeds on this hemiparasite.

Methods

Study system

We tested for effects of six different host species on the hemiparasite *Castilleja levisecta* and the herbivore *Euphydryas editha*, which feeds on *C. levisecta*. All of these organisms occur in native grasslands in the Pacific Northwest USA. *Castilleja levisecta* is federally listed as threatened in the USA (FWS 2000), but has been the target of focused recovery efforts, and is now locally common in relict and restored native grasslands in Western Washington (Dunwiddie et al. 2016). *Castilleja* and several related genera produce iridoid glycosides (IGs hereafter), bitter secondary compounds thought to deter generalist herbivores (Bowers 1983, Marko and Stermitz 1997). However, members of the lepidopteran genus *Euphydryas* specialize on plants that produce these compounds, sequestering them as a defense against predators (Bowers 1980, 1983; Bowers and Williams 1995).

Euphydryas editha is distributed across western North America and is comprised of several subspecies with varied morphology, life history, and host plant associations (Ehrlich and Hanski 2004). The subspecies *E. e. taylori* shares roughly the same distributional range as *C. levisecta*, and is federally endangered in the USA (FWS 2013). It specializes on *Castilleja* and related genera, and has been observed feeding on *C. levisecta* in the field. We used a closely related subspecies, *E. e. colonia*, as a surrogate for *E. e. taylori* so that insights from this study would be relevant to conservation workers involved in recovery efforts without causing negative impacts on *E. e. taylori* populations. *Euphydryas e.*

colonia inhabits subalpine meadows in the Pacific Northwest USA, and has been used as a surrogate for *E. e. taylori* in other studies (Schultz et al. 2016). We obtained eggs from nine *E. e. colonia* females, which we collected from Quartz Mountain, Washington, USA (WA, 47.077 ° N, 121.077 °W, 1920 m elevation).

Experimental methods

The experiment took place in a greenhouse at the Center for Urban Horticulture, University of Washington, Seattle, Washington, USA. We grew *C. levisecta* in pots, paired with one of six hosts or a control consisting of two *C. levisecta* plants. We have never observed *C. levisecta* parasitizing other members of its own species, so this treatment controlled for the number of plants per pot while eliminating parasitic interactions. Hosts were *Achillea millefolium* (Asteraceae), *Eriophyllum lanatum* (Asteraceae), *Deschampsia caespitosa* (Poaceae), *Festuca roemeri* (Poaceae), *Lupinus lepidus* (Fabaceae), and *Plantago lanceolata* (Plantaginaceae; also a host for *Euphydryas* (Stamp 1979)). We chose these species because they represent different functional groups (forb, grass, and legume), they overlap in range and habitat with *C. levisecta*, and they or their close relatives were known to be parasitized. Seeds for all plant species came from grasslands in western Washington, USA. We refer to all taxa in this study hereafter by their genus.

All plants were grown from seed for 2-4 weeks before being transferred to 2.5 L pots. In each pot, a host plant seedling was planted directly adjacent to a *Castilleja* seedling to facilitate root contact and encourage parasitism. Seedlings were selected randomly from a larger pool. All plants were grown in Sunshine #4 growth medium, uniformly watered every ~3 days, and treated once with liquid 7-4-10 fertilizer (30 mL diluted in 30 L water).

In total, we included 63 pots in the experiment, nine replicates per host, although one *Plantago* replicate was removed from the study because of accidental damage (n = 62 pots). We grew the potted host-hemiparasite combinations for seven weeks, then placed them in screened insect enclosures (Butterfly

Farm ™, Nasco, Fort Atkinson, WI, USA). Enclosure dimensions were 42 x 42 x 76 cm. Since *Plantago* is also fed on by *Euphydryas,* we installed a mesh barrier that limited access so larvae could feed only on *Castilleja*. For control pots with two *Castilleja* plants, we used a similar barrier to restrict larval feeding to one randomly selected plant.

Euphydryas eggs were hatched and reared in the lab. Once the caterpillars reached second instar, we released five individuals on the *Castilleja* in each pot and allowed them to feed until they entered diapause (a period of prolonged inactivity lasting approximately 8 months for this taxa). We assigned one matriline to each replicate per treatment to account for potential genetic differences within *Euphydryas*. We chose to release five individuals per plant because it allowed for gregarious feeding, and because it approximated the densities of larvae we have observed in the field feeding on a single plant (Haan, personal observation). Pre-diapause larvae in this system are often restricted to the individual plant where their eggs are laid until third instar, and since host plants are often scattered, the fate of the larvae can be wholly tied to a single plant in some cases.

It would have been ideal to install egg clusters so they hatched directly onto *Castilleja*, but we waited until they reached second instar for two reasons. First, *Euphydryas* lay eggs in clusters of ~20 or more; since our experiment contained only a single *Castilleja* individual in each replicate, introducing an entire egg cluster to a single plant would cause larvae to starve (potted plants were somewhat smaller than those observed in the field). Second, first-instar larvae feed gregariously in a tight web and are prone to desiccation if they are isolated from the group. We reared newly hatched larvae on *Plantago*, because there were not enough *Castilleja* plants to feed to larvae in the lab, and because *Castilleja* leaves desiccate quickly when removed from the plant.

Euphydryas caterpillars fed on *Castilleja* during their second and third instar. They entered diapause at the beginning of the fourth instar, about three weeks after being released on the plants. After they entered

diapause, we measured three hemiparasite traits that could mediate indirect effects on the caterpillars: food quantity, food quality, and defensive chemistry. To assess food quantity, we measured the total length of all stems for each hemiparasite, which is a proxy for the amount of leaf material available for larvae to feed on (we also measured aboveground mass, but chose to focus on stem length instead because mass would be impacted by the rate of feeding by larvae and thus could not be attributed as directly to host plant effects). As a measure of leaf nutritional quality, we measured leaf N concentration using a CHN analyzer (2400 Model, Perkin Elmer Co., Waltham, MA). Ten plants were excluded because they did not have enough tissue for the analysis. All leaves from each plant were dried and ground, with a 20 mg sample extracted and used to measure leaf N. We also disassembled each pot and quantified categorically the number of haustorial connections between plants (categories: none, 1-5, 6-10, 11-20, 21-50, 51-100, >100).

To assess plant defensive chemistry we used gas chromatography (GC) to measure the concentrations of aucubin and catalpol, two iridoid glycosides produced by *C. levisecta* (N. Haan and M.D. Bowers, *unpublished data*) and known to be sequestered by *Euphydryas* larvae. Two plants were excluded from this analysis because not enough tissue was present. IG methods followed Bowers and Stamp (1997) and Bowers (2003). Leaf material was oven dried (50°C for 48 h) and ground to a fine powder, with a 25 mg aliquot taken for extraction in 95% methanol for 24 h. The solid material was filtered out and methanol evaporated. After adding the internal standard phenyl-β-D-glycopyranoside (PBG) at 0.500 mg/mL, each sample was partitioned with ether to remove hydrophobic compounds. The ether layer was removed and the water layer (containing iridoid glycosides) was evaporated. The residue was suspended in 1.0 mL methanol, and a 100 µL aliquot removed for analysis. The methanol was evaporated and the remaining residue derivatized using Tri-Syl-Z (Thermo-Fisher Chemical Company) in pyridine before injection into an Agilent 7890A gas chromatograph equipped with a DB-1 column (30 m, 0.320 mm, 0.25 µm particle size) and using flame-ionization detection. Amounts of aucubin and catalpol were quantified using ChemStation B-03-01 software.

We tallied surviving caterpillars and weighed them to the nearest 0.1 mg before placing them in a dry, sheltered outdoor compound for diapause. We placed the individuals from each replicate in a 150 mL ventilated plastic tub and placed the tubs under inverted terra cotta pots. These methods follow standard captive rearing procedures for *E. e. taylori* (Barclay et al. 2009). We re-assessed survival in late January, at approximately the midpoint of their diapause period, because we thought the size differences evident at the start of diapause might differentially affect subsequent survival. Iridoid glycoside concentrations were quantified for a subset of the replicates (2-5 per treatment), with whole larvae crushed and iridoid glycosides extracted and analyzed using the methods described above. We only tested a subset of the larvae (i.e., not all replicates) so that other individuals could be preserved for future study.

Data analysis

First, we assessed the effects of host identity, and number of haustoria, on hemiparasite traits. Analyses were carried out in R 3.2.5 (R Foundation for Statistical Computing 2016). An alpha level of 0.05 was used for all tests. We used Pearson's Chi-square test to assess whether the degree of haustorial connectedness (classified categorically) differed among host plant treatments. We used univariate two-way ANOVAs to determine if host plant treatment and/or the number of haustoria (classified categorically) affected *Castilleja* stem length, leaf N, or leaf iridoid glycosides. Stem length and leaf N were natural log transformed, and leaf iridoid glycosides were arcsine square root transformed, prior to analysis to correct for normality. Significant ANOVA results were followed with Tukey HSD tests to detect pairwise differences.

Next we determined which hemiparasite traits (stem length, leaf N, or leaf IGs) affected caterpillar survival, mass, or IGs. Survival at the beginning of diapause, and again midway thorough diapause, were analyzed using binomial distributions in generalized linear mixed models (GLMMs, function *glmer*) with package *lme4* (Bates et al. 2015). The mass of larvae that survived to diapause, and IGs of those surviving

to mid-diapause were analyzed using linear mixed models (LMMs; function *lmer*). Mass was natural log transformed, and iridoid glycoside concentrations were arcsine square root transformed to improve normality. Each hemiparasite trait was transformed as described above, then converted to a z-score so that all variables could be expressed on the same scale (although transformation was not necessary, we did this so the variables would be expressed uniformly across all analyses). In each model, we specified traits as fixed effects and larval maternal line as a random effect. For each response variable, we considered models with all possible combinations of hemiparasite traits and selected the best model based on AICc. When multiple models had similar explanatory power, with ΔAICc less than 2 from the minimum (Burnham and Anderson 2002), we calculated full model averaged parameters using the *MuMIn* package (Bartón 2015; model comparisons shown in Table S1).

Multiple variables in this study served both explanatory and response roles, forming potentially complex chains of causal relationships. Therefore, in addition to testing for relationships in isolation as above, we used piecewise structural equation modeling (Shipley 2009; hereafter SEM) with package *piecewiseSEM* 1.2.0 (Lefcheck 2016) to perform a confirmatory analysis. In contrast to traditional SEM, piecewise SEM uses local estimation, which accommodates the mixed effects and non-normal response variables present in this dataset.

The individual models comprising the SEM took the same forms as described above, with the same fixed and random effects and data transformations. Due to limited sample size, we omitted data on larval IGs from this analysis and tested them separately (see below). Since piecewise SEMs do not accommodate categorical explanatory variables, we reclassified haustoria counts to continuous data, using the lower bound of each category, and assessed their effects on hemiparasite traits using linear models. Host identity was reduced to a binary variable based on results from Tukey tests described above—since *Achillea* differed most consistently from the other hosts in terms of its effects on *Castilleja* (and on *Euphydryas*, see Results), we compared replicates grown with *Achillea* to those with other hosts. We

acknowledge that this approach is somewhat circular; however we reclassified this variable in other similar ways (e.g., based on the number of haustoria formed) and found the effects of doing so were relatively small and resulted in models with similar structure (see Supplement S2). Coefficients for links originating from this variable were estimated using range standardization following Grace and Bollen (2005).

We began the SEM analysis with an initial model containing all the hypothesized direct interaction links which we had tested individually (Figure 3, top). For simplicity, we only included larval survival to mid-diapause instead of both survival metrics. We refined the initial model by dropping links which were unsupported by the individual analyses we had completed and/or resulted in coefficients with an associated p-value below 0.10 in the SEM (these two criteria were in agreement). We then added any missing links with an associated p-value below 0.10. We used Shipley's d-separation test, which produces a C statistic and X^2-based p-value to assess model fit, and compared AICc from the final model to that of the initial one (Figure 3).

Finally, since larval IGs were excluded from the SEM, we analyzed them separately to determine if, generally, an indirect effect occurred—i.e., whether they varied as a function of *Castilleja* host plant and/or haustorial connectedness. We used LMMs to determine if larval iridoid glycosides varied with *Castilleja* host identity, haustoria, or both. We used the same data transformations as described previously, and tested host identity, haustoria count, and their combination as fixed effects and larval maternal line as a random effect. In each case we selected the best model using AICc, and tested it against one containing only the maternal line random effect using a likelihood ratio test. We also used LMMs and GLMMs, respectively, and followed them with likelihood ratio tests to determine whether mass or survival of *Euphydryas* larvae varied by *Castilleja* host, in order to develop the pairwise contrasts shown in Figure 2. Contrasts were calculated using the lsmeans package (Lenth 2016). Data transformations and random effects were the same as described above.

Results

Effects of hosts on hemiparasite traits

The number of haustoria formed between hemiparasites and their hosts differed strongly among host treatments ($X^2_{[36]} = 76.54$, $p < 0.001$; Table 1). Stem length and leaf N of *Castilleja* varied depending on both the host species being parasitized and the degree of connectedness to the host (Fig 1; Table 2). Stem length varied seven-fold among host treatments; for example, the mean (±se) stem length of *Castilleja* was 107 cm (±25) when paired with *Achillea* compared to just 14 cm (±3) when grown without a host. Hemiparasites forming more haustorial connections were notably larger than those with few or none detected; those with more than ten connections had a mean (±se) stem length of 90 cm (±10) compared to just 22 cm (±5) among those with less than ten connections.

Host species identity also strongly affected *Castilleja* leaf N concentration, which ranged from a mean (±se) of 4.4% (±0.25) when parasitizing *Achillea* to 2.5% (±0.08) when parasitizing *Deschampsia*. Host identity and haustoria had an interactive effect on leaf N, indicating that the level of connectedness had differential effects on leaf N depending on the host species in question. For example, *Castilleja* parasitizing *Plantago* appeared to increase in N when haustoria were evident, while the number of haustoria appeared to have no effect for those parasitizing *Deschampsia* or *Eriophyllum.*

Castilleja produced low levels of the IGs aucubin and catalpol (mean total = 0.20% of dry weight, ±0.04). Iridoid glycoside levels varied nearly significantly with host plant treatment, and appeared unaffected by numbers of haustoria present (Table 2). In every case but one, aucubin was the only compound detected; catalpol was either absent or occurred at undetectably low levels. Other species of *Castilleja* produce a variety of other IGs (e.g., Mead and Stermitz, 1993); however, if other IGs were present their levels were low enough to escape detection.

Effects of hemiparasite traits on the herbivore

Outcomes for *Euphydryas* varied as a function of hemiparasite traits (Figure 2; Table 3). Survival to diapause, and to mid-diapause, were best explained by *Castilleja* stem length, with more individuals surviving on larger plants. Caterpillar mass, on the other hand, was positively associated with *Castilleja* leaf N. Larval IG levels (and the ratio between the two compounds) could not be accounted for by hemiparasite traits—no model we tested had comparable explanatory power to that containing only the matriline of the larvae, although IGs in larvae appeared to decrease when they fed on larger *Castilleja.* While our focus is on trait-based causal pathways, *Euphydryas* survival and mass also varied by *Castilleja* host treatment (Figure 3; Survival $X^2_{[6]} = 31.62$, $p < 0.001$; Mass $X^2_{[6]} = 25.21$, $p < 0.001$).

Structural Equation Model

The significant relationships we identified using individual models were confirmed by the SEM (Figure 4). The final model decreased AICc by 34.78 from the initial one, and resulted in a good overall model fit (Fisher's $C = 23.37_{[20]}$, $p = 0.271$). This analysis also identified two relationships that we had not tested with individual analyses: a positive relationship between leaf N and leaf IGs, and a very strong positive relationship between mass of larvae going into diapause and their subsequent survival to mid-diapause.

Larval IGs

Total IG concentrations in the caterpillars were not strongly related to *Castilleja* host identity or the number of haustoria formed; models including either of these predictors

failed to decrease the model AICc below that with the random effect alone and were not significant (for host species, the better predictor, $X^2_{[5]} = 9.297$, $p = 0.10$). However, larval IG concentrations ranged from a mean (±se) of 1.0% (±0.6) dry weight when *Castilleja* parasitized *Achillea* to up to 12.1% (±5.0) when it parasitized *Plantago*, suggesting differences could be detected with larger sample size (Fig 5). Larvae contained both aucubin and catalpol; the overall mean concentrations of aucubin and catalpol in larvae

were 1.8% (±0.5), and 2.4% (±1.0) dry weight, respectively. The ratio of aucubin to catalpol varied with the identity of the plant being parasitized by *Castilleja* ($X^2_{[5]}$=11.06, p=0.050), although including this effect in the model improved AICc only weakly compared to that with only the random effect (ΔAICc = 0.58).

Discussion

Host effects across trophic levels

This study assessed how a hemiparasite's host plants can change its traits, which in turn influence outcomes for herbivores feeding on the hemiparasite. We found that relationships with host plants strongly influenced both the size and leaf N concentrations of the hemiparasite *Castilleja*. These two traits then influenced survival and mass of *Euphydryas* larvae as they fed on *Castilleja*.

Both the identity of *Castilleja* hosts, and the number of haustoria formed, influenced outcomes for *Castilleja* (and by extension, *Euphydryas*). *Castilleja* stem length increased drastically when more haustoria were formed, and also varied depending on the host species being parasitized. Leaf N levels were not controlled closely by the number of haustoria formed, but were very high when *Castilleja* parasitized *Achillea*. Rowntree et al. (2014) studied interactions between hemiparasitic *Rhinanthus minor*, its host plants, and aphid herbivores. *Rhinanthus* biomass varied depending on the host it parasitized, and also increased with haustoria number. Consistent with our findings, in this system aphid herbivores benefitted from larger host plants. However, in contrast to our findings, leaf N in *R. minor* had no detectable influence on aphids (at least in terms of population size). The traits mediating these types of interactions probably vary depending on the system in question and the nutritional requirements and feeding strategy of the herbivore.

In our study, caterpillar survival improved when they fed on larger plants, and also benefitted indirectly from plants with more leaf N. Caterpillars feeding on plants with high leaf N levels grew larger, and then

were subsequently more likely to survive to mid-diapause (Fig 4). The SEM coefficient from *Castilleja* stem length to *Euphydryas* survival was 0.40, while the product of the indirect path from leaf N to survival was 0.38. This indicates these two traits were of similar importance, although they affected survival through different pathways. Also, differences in survival were much more pronounced after three months of diapause than at the beginning of diapause, suggesting diet quality during early instars has strong legacy effects on survival through the diapause phase.

Importantly, *Castilleja* and *Euphydryas* did not always mirror one another in their responses to different host species (Figure 1; Figure 3). Hemiparasites grown with *Achillea* were large and rich in N, while those grown with other hosts were mostly indistinguishable from those with no host at all. The caterpillars, on the other hand, were more varied in their responses. For example, they gained more mass than average when feeding on *Castilleja* paired with *Plantago*, despite the poor performance of *Castilleja* in this treatment (*Plantago* is itself a host for *Euphydryas*, we prevented larvae from feeding on it in this experiment). Similarly, larvae survived and grew larger than average when feeding on plants grown with *Festuca*, despite *Festuca* being a relatively poor host for *Castilleja.* We offer no specific explanation for these patterns, but they certainly suggest that the processes operating in this system are not straightforward and are probably mediated by other hemiparasite traits that were not measured. Our analysis showed strong links from hemiparasite size and leaf N to outcomes for the larvae, but other host-specific mechanisms could also be at play. For example, one possibility is the presence of host-derived secondary compounds in the hemiparasite (Stermitz et al. 1989; Stermitz and Pomeroy 1992; Adler 2001). While we did not test for this in our study, *Lupinus* in particular could have provisioned *Castilleja* with alkaloids, with toxic effects on larvae (e.g., Adler and Wink 2001).

Iridoid glycosides

Neither host identity nor the degree of connectedness to hosts was a significant predictor of IG levels in *Castilleja*, although effects of host identity were nearly significant ($p = .054$). Several studies have shown

that secondary compounds can be transferred from host plants to hemiparasites (Gardner and Stermitz 1988, Stermitz et al. 1989; Stermitz and Pomeroy 1992; Adler 2001), but to our knowledge ours is the first to test whether a hemiparasite's host can influence its endogenous secondary chemistry. IG levels in plants can vary strongly in response to factors like nutrient availability (Darrow and Bowers 1999; Jamieson et al. 2012; Prudic et al. 2005) and mycorrhizal colonization (Bennett et al. 2009). Since hemiparasite hosts influence their resource acquisition and nutrient economy, we had expected them to influence iridoid glycoside levels as well.

IG levels in this study were quite low compared to those in a field setting; *C. levisecta* growing in the field can contain up to 9.6% IGs including both aucubin and catalpol (as well as other IGs), with aucubin being somewhat more abundant (unpublished data). Younger plants often contain less IGs than older ones (Fuchs and Bowers 2004; Quintero and Bowers 2012). Therefore, it is likely that greenhouse conditions and the relative youth of plants used in this study resulted in reduced levels of these compounds across all of the plants and masked any effects hosts could have had.

Since *Plantago* produces aucubin and catalpol, and these compounds are present in their root tissues (Quintero and Bowers 2012), it could be possible for *Castilleja* to acquire these compounds from *Plantago* in addition to producing them itself (Stermitz et al. 1993). *Plantago* hosts in this study contained both aucubin and catalpol (average total iridoid glycosides 1.08%; data not shown); interestingly, *Castilleja* paired with *Plantago* had the highest levels out of all the treatments, although this difference was only marginally significant. Future studies could examine in more detail whether iridoid glycosides can be transferred from hosts to hemiparasites.

Iridoid glycoside concentrations in *Castilleja* and *Euphydryas* were very different from one another. While IG concentrations were low in *Castilleja,* they were considerably higher in *Euphydryas,* and occurred in concentrations consistent with those found for related checkerspots (Gardner and Stermitz

1988) and other butterflies specializing on these compounds (Bowers and Collinge 1992; Bowers and Stamp 1997). Other studies with IG sequestering lepidopterans have shown that larvae accumulate substantially higher levels of IGs than those found in their diet (e.g., Gardner and Stermitz 1988; Bowers and Collinge 1992). While we focus on the IGs aucubin and catalpol, note that there may be other IGs in *Castilleja*; most *Castilleja* species examined for IGs contain several different compounds (e.g., Arslanian et al. 1985; Stermitz et al. 1986; Gardner and Stermitz, 1988). In this study, levels of these other IGs may have been too low to detect.

The relative amounts of aucubin and catalpol also differed strikingly in *Castilleja* and *Euphydryas*. *Castilleja* in this study contained almost exclusively aucubin, with catalpol either absent or undetectable in nearly every plant. In contrast, *Euphydryas* contained large amounts of both aucubin and catalpol. This is consistent with previous findings; herbivores sequestering these two compounds often contain disproportionately high levels of catalpol compared to their food plants (Dobler et al. 2011). This has been hypothesized to occur either because esters of aucubin or catalpol are converted to catalpol in the gut (Gardner and Stermitz 1988), or because herbivores sequester or degrade these two compounds at different rates resulting in relatively high levels of catalpol (Belofsky et al. 1989; Bowers and Collinge 1992). One caveat to our conclusions related to iridoid glycosides is that larvae in our study were fed a diet of *P. lanceolata* for a few days just after hatching. This occurred because all of the *Castilleja* available to us was required for the experimental setup, and *P. lanceolata* is much more abundant, easy to grow, and slower to desiccate when fed to larvae in a lab setting. The inclusion of *P. lanceolata* in the larval diet, for however brief a period, could have changed the relative concentrations of iridoid glycosides reflected in the larvae. However, we believe this had minimal impact on our results, since larvae consumed larger quantities of *Castilleja* and older larvae fed at a much higher rate than those that had just hatched.

We not only found that catalpol was disproportionately abundant in larvae, but also found the ratio of aucubin to catalpol in larvae differed depending on the host plant being parasitized by *Castilleja* ($p = 0.050$). One explanation for this is that *Castilleja* paired with some hosts contained catalpol esters that can be metabolized by larvae into catalpol (see Gardner and Stermitz, 1988). Another explanation is that larvae associated with different hosts underwent physiological adjustments related to diet quality and then converted, sequestered, or metabolized these compounds at different rates. Regardless, it represents yet another means by which hemiparasites can transmit indirect effects. The concentrations of aucubin and catalpol in lepidopteran larvae have important implications for their interactions with predators (Bowers 1980, Bowers 1983, Dobler et al. 2011). Therefore, while our experiment did not explicitly include predators, the bottom-up effects of host plants we detected would have implications for a fourth trophic level as well.

Conclusions

The organisms used in this study are of particular interest because of conservation concerns. *Castilleja levisecta* is federally threatened, and *Euphydryas editha taylori* is endangered (FWS 2000; FWS 2013), and important knowledge gaps about their interactions persist. Both species are targets of active recovery efforts, which include aggressive seeding of *Castilleja* spp. and host plants (Dunwiddie et al. 2016). Understanding whether *Castilleja* host plants influence outcomes for caterpillars in this context could have implications for management and recovery of these two species, especially when designing seed mixes. For instance, seed mixes including *Achillea* alongside *Castilleja* could not only improve *Castilleja* establishment and growth, but also improve its suitability as a food source for *Euphydryas.*

In addition to practical applications, this study advances our understanding of plant-mediated indirect interactions and the ecology of parasitic plants, both of which are poorly understood. We showed that a hemiparasite's relationship to its host plants can strongly influence outcomes not just for the hemiparasite but also for herbivores feeding on it. Hosts affect multiple hemiparasite traits, influencing both the

quantity and nutritional quality of food available. We also showed that while host identity may not directly change a hemiparasite's secondary metabolism, it can still affect chemical interactions between the herbivore and hemiparasite. It has been increasingly recognized that hemiparasites can affect ecosystem patterns and processes, particularly by altering competitive dynamics among other species and changing nutrient cycling rates (Marvier 1998; Quested et al. 2003; Watson 2009; Fisher et al. 2013). This study reinforces the importance of another ecological role for hemiparasites—that of mediators for interactions between other organisms.

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Table 2.1. Numbers of haustorial connections established between *Castilleja* and its various hosts. For each host, the category or categories with the highest count is in bold.

	Number of haustoria detected							
Host	**0**	**1-5**	**6-10**	**11-20**	**21-50**	**51-100**	**>100**	***Total***
Achillea	0	1	1	1	**2**	**2**	**2**	9
Castilleja (none)	**9**	0	0	0	0	0	0	9
Deschampsia	**3**	**3**	1	2	0	0	0	9
Eriophyllum	1	0	0	0	**4**	2	2	9
Festuca	2	2	**4**	1	0	0	0	9
Lupinus	**4**	3	1	0	0	1	0	9
Plantago	**4**	**4**	0	0	0	0	0	8
Total	23	13	7	4	6	5	4	62

Table 2.2. Results of two-way ANOVAs evaluating the effects of host identity and haustorial connectedness on hemiparasite traits (Significance levels: **.** < 0.1, * < .05; ** <.01; *** < .001).

Hemiparasite trait	***Factor***	***df***	***F***	***p-value***
Stem length	host identity	6	9.711	<.001***
	haustoria	6	3.736	.005**
	host:haustoria	12	1.417	.202
	residuals	37		
Leaf N	host identity	6	10.993	<.001***
	haustoria	6	0.729	.630
	host:haustoria	9	2.948	.012*
	residuals	30		
Iridoid glycosides	host identity	6	2.323	.054 **.**
	haustoria	6	1.894	.109
	host:haustoria	11	0.787	.651
	residuals	36		

Table 2.3. Effects of *Castilleja* traits on outcomes for *Euphydryas*. Models describing survival contain full model-averaged parameters, since more than one model had an AICc score within two of the minimum. Model comparisons can be found in Table S1 in supplementary materials (significance levels: * < .05; ** <.01; *** < .001)

Herbivore response	***Model type***	***Fixed effects in best model***	***Estimate***	***SE***	***Test statistic***	***p***
Survival to diapause	GLMM	intercept	1.659	0.205	Z = 8.073	<0.001***
		stem length	0.462	0.198	Z = 2.334	0.020*
		iridoid glycosides	0.116	0.216	Z = 0.537	0.591
		leaf N	0.027	0.103	Z = 0.262	0.793
Survival to mid-diapause	GLMM	intercept	-0.602	0.200	Z = 2.933	0.003**
		stem length	0.641	0.156	Z = 4.102	<0.001***
		iridoid glycosides	0.085	0.143	Z = 0.593	0.553
		leaf N	0.017	0.079	Z = 0.211	0.833
Mass	LMM	intercept	2.120	0.064	T = 32.894	<0.001***
		leaf N	0.219	0.065	T = 3.358	0.002**
Iridoid glycosides (total concentration)	LMM	(none)				
Aucubin to catalpol ratio	LMM	(none)				

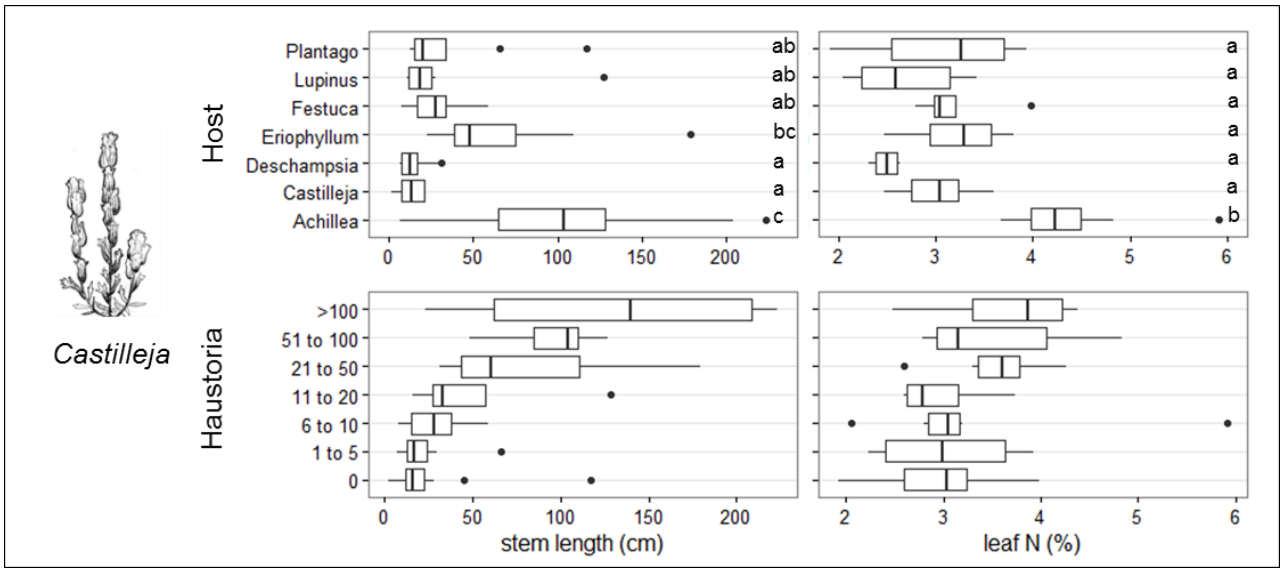

Figure 2.1. Effects of seven host species treatments and number of haustoria on *Castilleja* size and leaf N. For significant results, treatments within each plot that do not share a letter differ significantly from each other. (Although the overall effect of haustoria on stem length was significant, the levels of this variable were unbalanced and consequently no pairwise differences were detected).

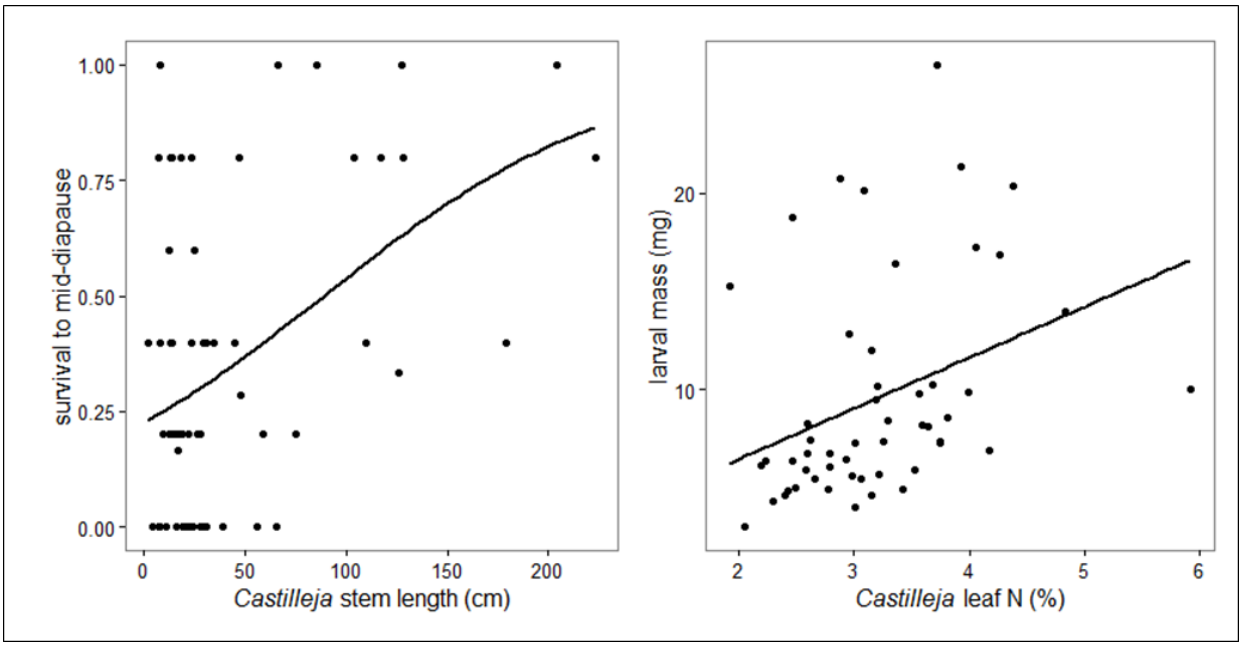

Figure 2.2. Effects of hemiparasite (*Castilleja*) traits on outcomes for the herbivore (*Euphydryas*; details in Table 3). Left: *Euphydryas* survival to mid-diapause was positively associated with *Castilleja* stem length. Right: *Euphydryas* mass increased with *Castilleja* leaf N.

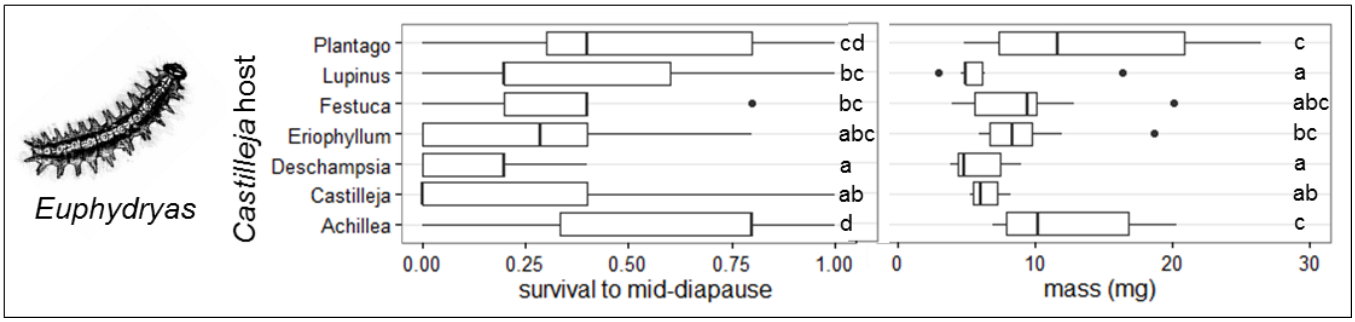

Figure 2.3. Mass and survival of *Euphydryas* caterpillars fed on *Castilleja* paired with each of seven species. Treatments within each plot that do not share a letter differ significantly from each other.

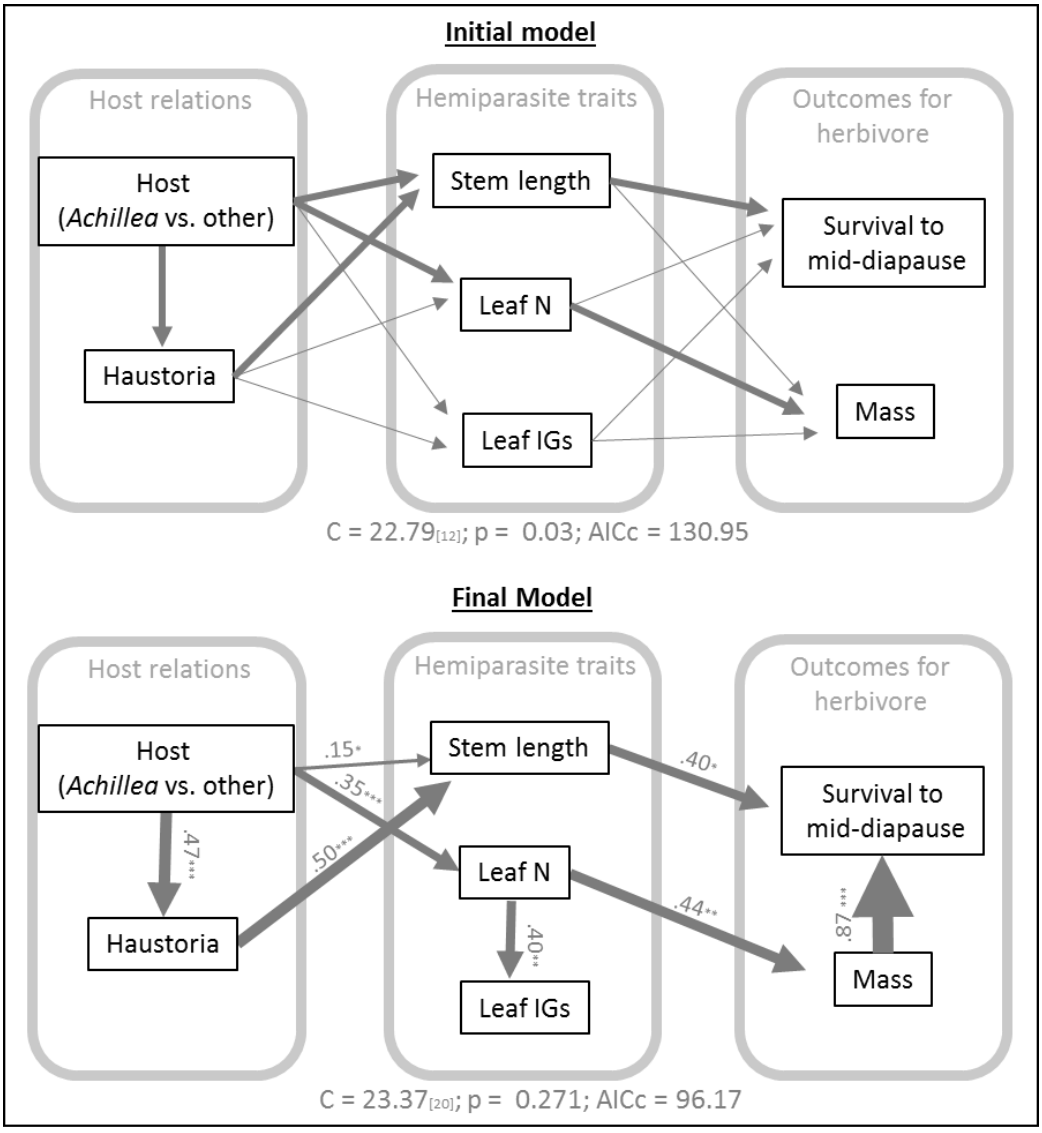

Figure 2.4. Structural equation model showing effects of hosts on hemiparasite traits and outcomes for herbivores. The top panel shows the initial hypothesized model, which corresponds to the non-structural analyses we conducted. Bold arrows in the top panel represent relationships that were significant when we analyzed individual relationships, although all links were included in the initial SEM. The bottom panel shows the final model. Arrow thickness is proportional to the coefficient for each pairwise relationship. Note that all coefficients are positive. (Significance levels: * < .05; ** <.01; *** < .001).

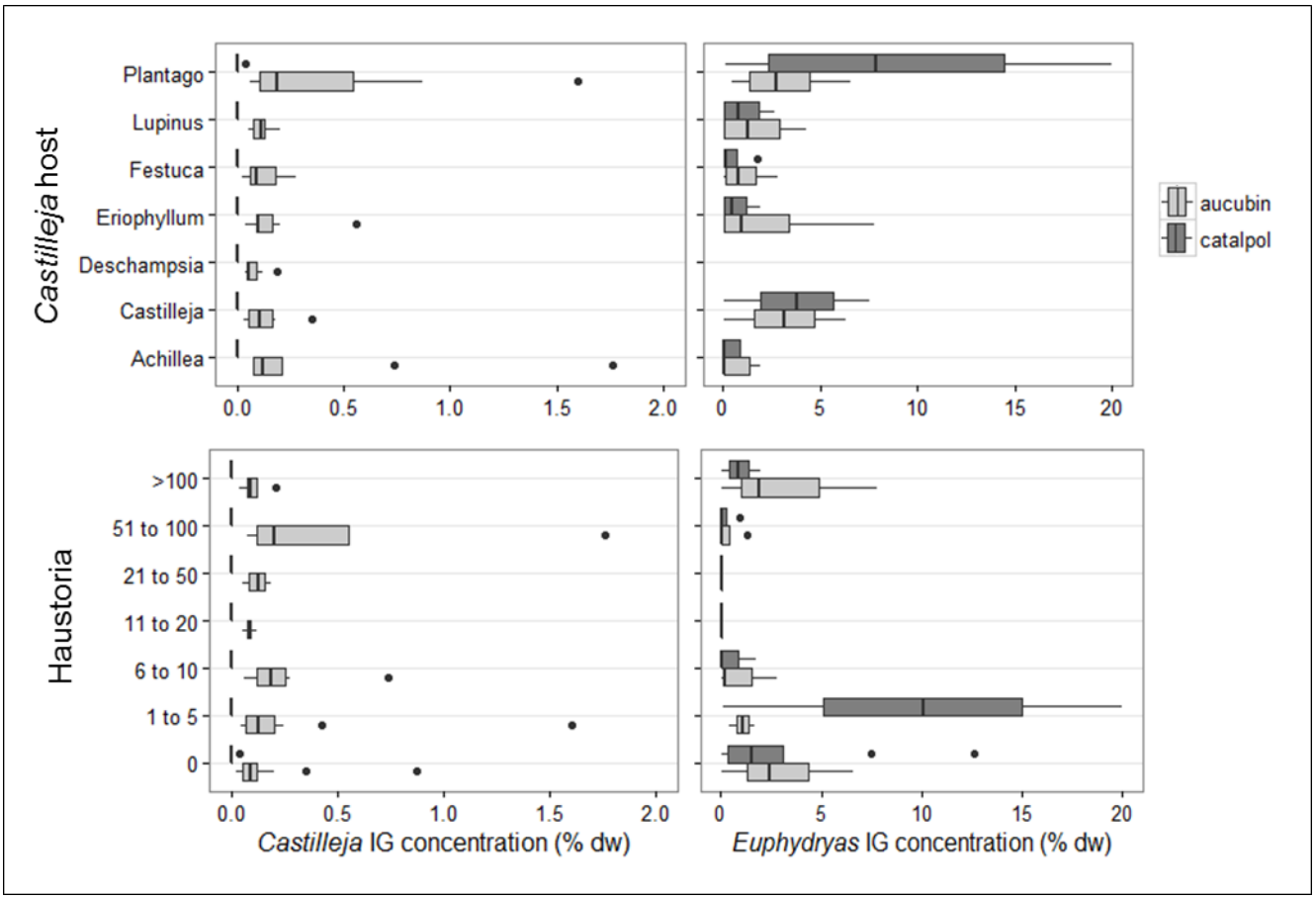

Figure 2.5. Iridoid glycoside levels in *Castilleja* (left) and *Euphydryas* (right). Note that axis scales for the two organisms differ.

Chapter 2 Supplement

S1. Model comparisons testing effects of hemiparasite traits on the herbivore

Table S2.1. Model comparison testing effects of hemiparasite traits on survival, mass and iridoid glycoside concentrations of caterpillars. This table shows the four best candidate models ranked by AICc. When needed (i.e., for models of survival), we averaged models with and AICc within two of the minimum.

Herbivore response	*Candidate models*	*ΔAICc*	*Weight*
Survival to diapause	**matriline + stem length**	0	0.341
	matriline + stem length + leaf IGs	0.42	0.276
	matriline + stem length + leaf N	1.78	0.140
	matriline + stem length + leaf IGs + leaf N	2.79	0.084
Survival to mid-diapause	**matriline + stem length**	0	0.397
	matriline + stem length + leaf IGs	0.25	0.351
	matriline + stem length + leaf N	1.99	0.147
	matriline + stem length + leaf IGs + leaf N	2.69	0.104
Mass	**matriline + leaf N**	0	0.587
	matriline + stem length	3.26	0.115
	matriline + leaf N + stem length	3.78	0.088
	matriline + random effect only	4.38	0.066
Iridoid glycosides (total concentration)	**matriline**	0	0.864
	matriline + stem length	4.61	0.086
	matriline + leaf N	6.67	0.031
	matriline + leaf IGs	8.11	0.015
Aucubin to catalpol ratio	**matriline**	0	0.520
	matriline + stem length	2.64	0.139
	matriline + leaf IGs	2.86	0.124
	matriline + leaf N	2.91	0.121

S2. Structural equation model

For the SEM described in the text, we reclassified the host plant treatment based on the results of Tukey tests. Since *Achillea* differed most strongly from the other hosts in terms of its effects on *Castilleja*, we compared the *Achillea* host plant treatment to all others. Another way to characterize the effects of host plant would be based on the number of haustoria developed. Counts were highest for *Castilleja* paired with *Eriophyllum* and *Achillea*, so here we recoded the host plant treatment as 1 for either of these species and 0 for all others. The resulting initial and final model, following the same model refinement rules described in the main text, are shown below in Figure S1.

The initial model is a poor fit, and is similar to the one in the main text. The final model is quite similar to the one in the main text, although it is a poorer fit. The right-hand side of the model, showing effects of *Castilleja* traits on outcomes for the caterpillars, is unchanged. There is now a non-significant link from stem length to leaf N, indicating a weak positive correlation between these two variables (the algorithm suggested a causative link from stem length to leaf N, but the relationship is probably more likely to occur in the other direction). Adding or removing this non-significant link had negligible effects on the overall model fit. Links from hosts to hemiparasite traits differ slightly in this model from the one in the main text, but do not challenge the basic conclusions drawn.

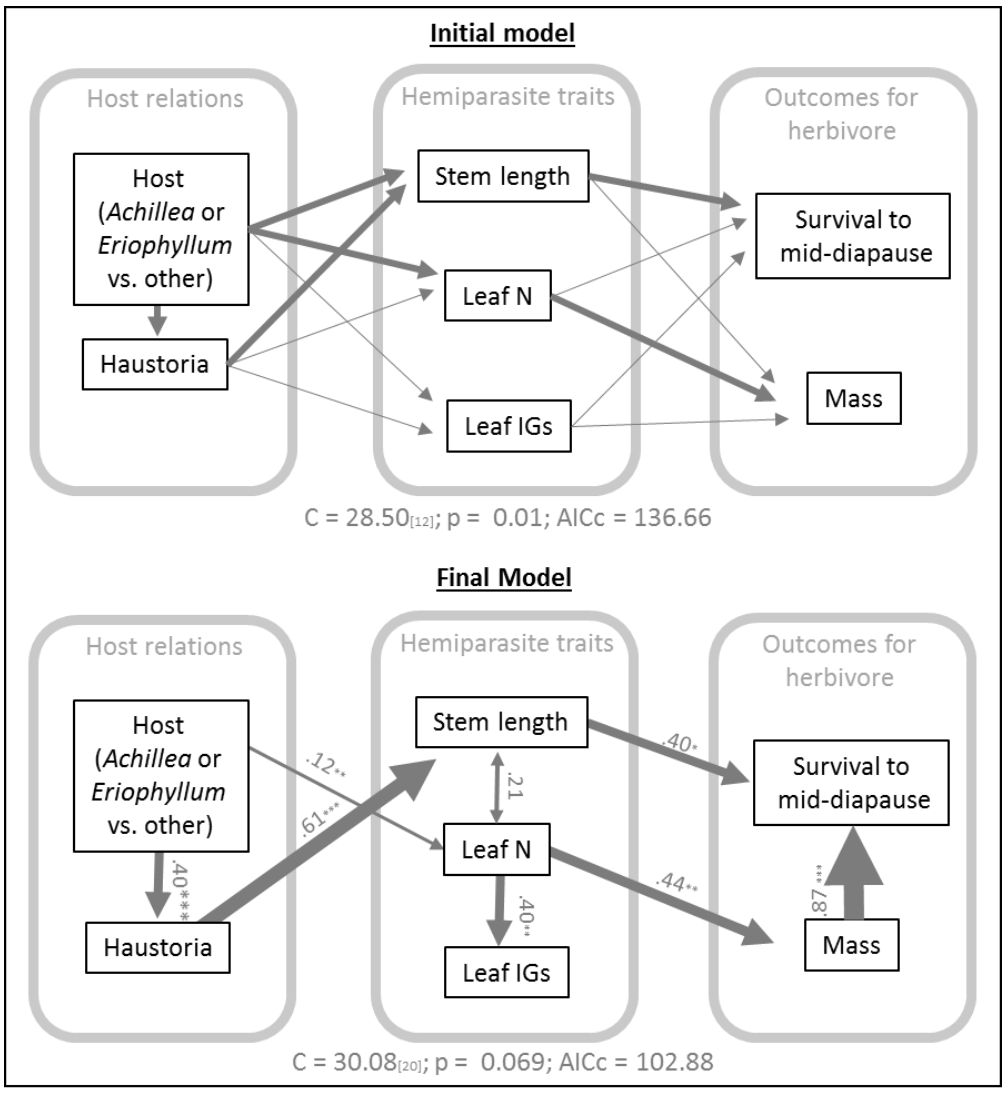

Figure S2.1. Alternate structural equation model showing effects of hosts on hemiparasite traits and outcomes for herbivores. The top panel shows the initial hypothesized model, which corresponds to the non-structural analyses we conducted. Bold arrows in the top panel represent relationships that were significant when we analyzed individual relationships, although all links were included in the initial SEM. The bottom panel shows the final model. Arrow thickness is proportional to the coefficient for each pairwise relationship. Note that all coefficients are positive. (Significance levels: * < .05; ** < .01; ***<.001).

Chapter 3: Stage-specific effects of host plants on larvae of an endangered butterfly

Abstract

I examined the relationship between early instar larvae of an endangered oligophagous butterfly, *Euphydryas editha* ssp. *taylori* (Taylor's checkerspot) and three host plants: *Castilleja levisecta, Castilleja hispida,* and *Plantago lanceolata*. I placed 126 egg clusters (total = 4130 eggs) on all three hosts in the field and quantified survival from hatching to second instar, from second to third instar, and from third to fourth instar. Within the groups feeding on each host species, I tested if host plant characteristics (degree of senescence, and anthocyanin pigmentation) affected survival; I also tested for effects of oviposition timing and larval group size on survival since these vary unavoidably and could affect outcomes for larvae differently on different hosts.

Survival rates generally increased for later instars, and differed depending on the host species being consumed. Survival was lowest on *C. levisecta*, intermediate on *C. hispida,* and highest on *P. lanceolata,* and the largest differences in survival occurred during the transition from second to third instar. Importantly, predictors of survival changed depending on the instar being considered. For caterpillars feeding on *C. levisecta,* survival to both second and third instar were limited if plants were senescing. *Plantago lanceolata* did not senesce during larval feeding, and survival of second-instar larvae was very high if they belonged to larger sibling groups. For caterpillars feeding on *C. hispida*, survival of first-instar larvae decreased when eggs were laid later in the flight period. None of the variables I measured strongly affected survival from third to fourth instar on any of the host plants, contrasting with earlier instars. This study illustrates that host plant suitability can vary strongly both within and among species, with effects differing depending on caterpillars' ontogeny, even during early instars.

Introduction

Suitable habitat for specialist herbivores is limited to places where their host plants grow. It can be limited even further because, within a host species or population, only some of the individual plants are actually suitable food for the herbivore. Others can be nutritionally, chemically, or phenologically unsuitable (Thomas 1984, Weiss et al. 1988, Harvey and Gols 2011, Wetzel et al. 2016). A similar principle applies to oligophagous herbivores, which interact with a small number of host species: in addition to within-species variation, depending on the context, some host species are probably more suitable than others (e.g., Ohsaki and Sato 1994, Parry and Goyer 2004, Ansari et al. 2012, Robertson et al 2015).

For these reasons, conservation efforts for specialist butterflies key in on caterpillars and their relationships with host plants. Managers work to establish or augment host plant populations, and may also reintroduce or translocate butterflies to them (e.g., Kuussaari et al. 2015, Dunwiddie et al. 2016). Ideally, managers should be equipped to distinguish between high- and low-quality host plants (Thomas et al. 2011). Otherwise, conservation efforts that produce or utilize low-quality host plants will be at best, inefficient, and at worst, counterproductive.

Caterpillars change dramatically as they grow, and considering these changes can enrich our understanding of host plant suitability. Ontogenetic niche shifts are ubiquitous—organisms' morphology, environmental tolerances, trophic interactions, and behavior change over the course of their lifespan (Werner and Gilliam 1984, Olson 1996, Berger et al. 2011). Lepidopterans undergo ontogenetic niche shifts not just when they transition to adulthood, but also within the larval stage. Their body size, feeding strategy, and behavior shift as they grow, changing their interactions with host plants (Reavey 1993, Hochuli 2001, Zalucki et al. 2002). Therefore, host plant characteristics affecting neonate larvae might be quite different from those that matter for older, larger individuals.

Investigating the intersection of these two concepts—host plant variability and caterpillar ontogeny—should yield detailed and useful information that can be applied to conservation. Therefore, I tested the effects of host plant variation, both within and among species, on survival of *Euphydryas editha*

ssp. *taylori* (Taylor's checkerspot; Nymphalidae), an endangered oligophagous butterfly. I placed clusters of eggs on three different host species in the field, and tested how differences within and among them affected survival rates for each larval colony at different stages of growth. *Euphydryas e. taylori* is the focus of coordinated recovery efforts, but thus far we know little about its relationship to its host plants (Dunwiddie et al. 2016). Therefore, the practical purpose of this study was to provide managers with useful information about how differences within and among host species affect survival of caterpillars at different points in their ontogeny.

Adult *E. e. taylori* lays eggs on *Castilleja hispida* (harsh paintbrush, Orobanchaceae), *Plantago lanceolata* (lanceleaf plantain, Plantaginaceae), and at least occasionally, *Castilleja levisecta* (golden paintbrush, Orobanchaceae; see 'Study System' below). All three species are perennial. Eggs are laid in clusters on host plants each spring, and caterpillars feed for usually four instars before entering diapause in early summer. In this study I focus on the four prediapause instars, as managers perceive these to be a critical part of the life cycle during which survival is most closely tied to host plant quality. Each of these instars corresponds to a fairly distinct developmental stage with increasing body size, mobility, and independence from siblings (Reavey 1993). Therefore I assessed survival rates separately from hatching to second instar, from second to third, and from third to fourth instar.

I tested two host plant characteristics I expected to affect larval survival, and which appeared to vary both within and among host species (Figure 1). First, many individuals of both *Castilleja* species senesce quickly while larvae feed, meaning the quality and quantity of food available probably diminishes quickly on some plants. Anecdotal observations suggest *P. lanceolata* differs from *Castilleja* spp. and remains green. Host plant senescence can be a critical mortality source for other *E. editha* subspecies using different hosts (Singer 1972, Weiss et al. 1988, Hellman et al. 2002), but its importance for *E. e. taylori* has never been tested.

I predicted that larval survival would decrease on senescent plants with necrotic leaf tissue. I expected early instars to be more sensitive to this mortality source, since third- and fourth-instar larvae can disperse if plants senesce, and their larger mandibles could be better equipped to cut through tissues

of varying toughness (e.g., Khalsa et al. 1979). Since eggs are laid throughout a multi-week flight period, caterpillar development is staggered and plant senescence effects are necessarily confounded with hatch date. Also, generally, eggs laid by older butterflies can be smaller and can result in decreased caterpillar performance (Begon and Parker 1986, Pöykkö and Mänttäri 2012). Therefore, I also tested for effects of oviposition date on caterpillar survival in order to distinguish between these effects and those that are more directly related to host plant senescence.

Second, both species of *Castilleja* express a wide range of pigmentation from bright green to deep crimson (Figure 1; *P. lanceolata* does not usually vary noticeably in this respect during early-instar feeding, pers. obs.). Many plants produce and accumulate anthocyanins, which give them a red or purple appearance, generally for photo-protection in stressful circumstances like nutrient and water limitation (reviews by Steyn et al. 2002, Close and Beadle 2003). Field observations suggested that deeply pigmented plants occurred more often in unproductive microsites, so I suspected these plants could be nutrient stressed and of lower nutritional value to larvae. Thus I expected heavy anthocyanin pigmentation to be an indicator of high C:N ratios in *Castilleja*, and expected caterpillar survival to decrease on these plants. There is little evidence that anthocyanins themselves affect herbivores (Close and Beadle 2003); rather, here I explore their use as a simple visual indicator of leaf nutrition that could be useful to managers.

Finally, I considered the influence of colony size, which varies in every population and could influence survival differently on different hosts. For Lepidoptera that lay eggs in clusters, larger sibling groups can confer advantages like higher feeding rates, protection from predators, and improved thermoregulation (Seymour 1974, Stamp 1980, Clark and Faeth 1997), increasing their odds of survival. On the other hand, large colony size could become a liability if host plants are senescent and siblings must compete for disappearing food. Similarly, large colony size should be of no utility to later-instar larvae that forage individually. Therefore, I expected group size to generally be a positive predictor of survival to second and third instar, but to become negative if host plants were senescent.

Methods

Study system

Euphydryas editha taylori (Taylor's checkerspot) is a subspecies of Edith's checkerspot, and is endemic to grasslands in the Pacific Northwest US. It was once locally common, but declined precipitously in the late 20th century and is currently known to occur on only ten sites. It was recently federally listed as endangered (USFWS 2013), and is the focus of active recovery efforts which include habitat enhancements with host plants and nectar sources, and a captive rearing and release program.

This study focuses on the relationship between *E. e. taylori* and three host species: *Plantago lanceolata, Castilleja hispida,* and *Castilleja levisecta.* Butterflies oviposit on all three species, and in lab experiments did not distinguish between the *Castilleja* species, preferring both of them over *P. lanceolata* (Buckingham et al. 2016). *Plantago lanceolata,* an exotic species, naturalized in this ecosystem in the early 20th century, has become very common, and is now the primary host plant used by several *E. e. taylori* populations (Dunwiddie et al. 2016). *Castilleja hispida* is assumed by managers to be the primary historical host for *E. e. taylori,* and is fairly common locally due to habitat enhancement efforts. We know little about the suitability of the third species, *C. levisecta,* as a host plant for *E. e. taylori*; *C. levisecta* is quite rare and federally listed as threatened (USFWS 1997). It was recently re-established in checkerspot-occupied sites, and *E. e. taylori* now oviposit on it at least occasionally (pers. obs.). Observations and records suggest *C. levisecta* was historically a larval host plant, and the two taxa were sympatric at several sites before becoming isolated from each other in the late 20th century. Land managers have established large *C. levisecta* populations at several sites; these sites could also be used for *E. e. taylori* recovery if *C. levisecta* is a suitable host (Dunwiddie et al. 2016). This is the first test of its suitability for prediapause larvae in a field setting.

Size and behavior of larval *E. e. taylori* shift during each of the four pre-diapause instars (pers. obs.). During the first two instars, they feed with their sibling group in a communal web, and are almost always restricted to the plant where their eggs were laid. First instar larvae usually move only a few centimeters and rarely leave the confines of their web, while second instar individuals use the web

intermittently but often forage across the extent of their host plant and, rarely, adjacent host plants. During third instar they usually disperse from their original host to feed on surrounding plants. They often disperse in groups, but occasionally feed singly. Fourth-instar individuals are the most independent and mobile, and tend to disperse up to a meter while grazing before entering diapause in the leaf litter. They re-emerge in early spring the following year to resume feeding, pupate, and reach adulthood, although in adverse conditions some individuals may re-enter diapause (Ehrlich and Hanski 2004).

Experimental setup

This study took place in three grassland preserves located near the southern extent of Puget Sound, Washington, USA; Glacial Heritage Preserve (46.87^0 N, 123.04^0 W), West Rocky Prairie (46.89^0 N, 122.87^0 W), and Tenalquot Prairie (46.90^0 N, 122.73^0 W). I monitored outcomes for caterpillars which were placed in 110 unique plots. Each plot contained a patch of at least five plants from one of the three host species. I required at least five plants per plot because this was a rule of thumb used by conservation workers to ensure a patch of plants would contain enough food to support a larval colony. Of the 110 plots, 34 were used only in 2015, 76 were used only in 2016, and 16 were used in both years, resulting in 126 total observations across the two years. All *Castilleja* populations at these sites were introduced by land managers at least two years before the study. Some *P. lanceolata* had colonized the sites on their own, but these populations were also augmented by managers. I seeded 36 additional plots in 2014, 12 with each species; 19 of these established successfully and were included in the study in 2016.

Of the 126 plot observations, 49 contained *P. lanceolata*, 40 *C. hispida*, and 37 *C. levisecta*. The number of plots per species was unbalanced because quantities were limited by the availability of extant plants growing near each other in similar environments (and by establishment rates in the plots that were seeded for this study), and I wanted to avoid confounding the host species treatment with environmental conditions that could influence outcomes for larvae and be mistakenly attributed to host species interactions. Therefore, in 2015 I preferentially established plots in areas where two or three of the species co-occurred whenever possible. In 2016, shifts in plant populations allowed for most plots be arranged in

blocks containing two or three species growing directly adjacent to one another. In 2016, 11 plots were located singly, while 66 were paired with one other species and 15 were in three-species blocks. I surrounded each patch of plants with an enclosure to restrict larvae to their assigned host species and to discourage larger herbivores from browsing (Figure 2). The area of each enclosed plot was $1.57m^2$.

I harvested eggs from 53 gravid female butterflies (18 in 2015 and 35 in 2016) collected from the last naturally occurring *E. e. taylori* population in the South Puget Sound region, an active artillery range at Joint Base Lewis McChord (47.02^0 N, 122.57^0 W). Butterflies were housed at the Oregon Zoo (Portland, Oregon), Mission Creek Correctional Center for Women (Belfair, Washington) and the University of Washington Center for Urban Horticulture (Seattle, Washington), where they oviposited on potted *P. lanceolata* and *C. levisecta*. Methods for housing butterflies and obtaining eggs followed the captive rearing procedures developed for *E. e. taylori* recovery efforts (Barclay et al. 2009). Eggs were laid between April 28 and May 13; median oviposition date was May 5 in 2015 and May 3 in 2016. I used egg clusters containing between 16 and 67 eggs (mean = 33, SE = 0.94). The oviposition date and number of eggs that appeared viable in each egg cluster were recorded.

After each cluster was laid, I removed it and a fragment of the surrounding leaf from the plant. I transported eggs to the field, and established one egg cluster in each plot. In each case I attached the leaf fragment with its egg cluster to a medium or large plant near the center of the plot using paperclips. In 2015, eggs were placed in the field several days prior to hatching, and several clusters disappeared or were eaten by predators (unpublished data; in this study I only report on eggs that hatched successfully). Therefore, in 2016 I incubated eggs in the lab and introduced them to host plants as they were hatching. I also made additional efforts to exclude predators. I pounded PVC cylinders (d = 20 cm, h = 8 cm) into the ground so they surrounded the plant where eggs were released, and coated the outside of each cylinder in Tanglefoot™ (Scotts Company LLC) to prevent terrestrial arthropods from accessing the eggs or larvae. I removed the cylinders when larvae entered second instar, before they were capable of dispersing and encountering the barrier. In all I tracked 126 egg clusters, which contained a total of 4,130 eggs.

Assessing survival

I visited each plot to survey larvae every 3-6 days in 2015, and every 1-3 days in 2016, until all surviving individuals had entered diapause (approximately 8 weeks). On each visit I recorded the number of larvae present as well as their instar phenology. After they began to disperse, I counted them by starting with the plant where they had originally been released, then systematically examining the surrounding host plants and terrain in each plot. The survey at each plot lasted three minutes. Since each colony was counted multiple times during each instar, I used the highest count for each stage as the most accurate estimate of how many larvae were present. If more larvae were detected for a later instar than had been detected in the previous one, I retroactively adjusted the number from the previous instar and assumed a 100% survival rate between instars. Therefore, since detection was imperfect, estimates of survival are conservative. I do not report on first instar larvae because counting them accurately is not feasible without destroying their web, which would affect their fitness.

Host plants

I quantified senescence by recording the amount of necrotic tissue on the plants at least once per week. On each plant where I had placed eggs, I visually estimated the percent of the plant's leaf tissue (or for *Castilleja* spp., leaf and bract) that had turned brown and necrotic, rounded to the nearest 10%. For *Castilleja* spp., I recorded the degree of anthocyanin pigmentation during each visit using a simple visual color assessment on a 1-10 scale. Therefore I did not measure anthocyanins directly, and other pigments could also be present.

Larvae in instars three and four usually disperse and feed on a number of plants near the one where they hatched. Therefore, to assess the plot-wide status of food plants, I also took weekly measurements from five plants in each plot. For plots with more than five host plants, I sampled systematically by selecting one individual from each 'quarter' of the plot as well as one from the center, and selected individuals that were representative of the range of plant sizes that occurred in that plot. These plants were monitored in the same manner as above.

To measure leaf C:N ratios, I collected leaf samples from a subset of the plots, as well as from some surrounding plots where eggs had not been placed (these plots were interspersed with those containing larvae, but either did not have eggs installed in them or the eggs disappeared prior to hatching). I avoided collecting leaves from some plots where larvae were feeding if I judged that removing tissues would deplete their food sources below an acceptable level. Samples were collected in 2014 (during which time plots were established in the same areas of each preserve but no larvae were released), 2015, and 2016. I removed one intermediate-aged leaf from every plant in each plot on dates when larvae were predominantly in second or third instar, dried and ground the samples, and measured C and N with a CHN analyzer (2400 Model, Perkin Elmer Co., Waltham, MA) (N = 32 for *C. hispida*; N = 49 for *C. levisecta*).

Statistical methods

I used R 3.2.5 for all analyses (R Core Development Team 2016). First I tested if larval survival varied depending on the host species being eaten. I built binomial generalized linear mixed models (GLMMs) using the package *lme4* (Bates et al. 2015). I tested for differences in survival rates from hatching to second instar, from second to third instar, and from third to fourth instar. Since some of the plots were grouped in blocks, I included block as a random effect. I included year and site as fixed effects in every model to account for differences in survival that could be attributed to them.

We compared models including only year, site, and block to those that also contained host species identity, based on QAICc (Libreton et al. 1992, Anderson et al. 1994), and treated models with ΔQAICc <2 as having similar explanatory power (Burnham and Anderson 2002). I used QAICc, rather than AIC, throughout this study to avoid overfitting in the context of overdispersion and low sample size (ĉ was defined as model deviance divided by the residual degrees of freedom for the full model). Results were followed with pairwise contrasts using the lsmeans package (Lenth 2014). After interpreting some of our initial findings, I also followed this same statistical protocol to determine if the amount of necrotic tissues differed among the host species when caterpillars were entering second instar.

Next, within each host species I tested whether plant traits and colony characteristics influenced larval survival at each stage. In each case I modeled survival as a function of host necrosis level, host anthocyanins, larval group size, and/or oviposition date, using generalized linear models (GLMs; three host species x three stage transitions = nine sets of models). In each case I also tested for an interaction between tissue necrosis and group size, since I had hypothesized that group size would have opposite effects if plants had senesced. I did not include block as a random effect for these models, because within each species, each observation for each year was from a unique block. I converted continuous explanatory variables to z-scores prior to analysis.

I compared models with every combination of the covariates based on QAICc, with the variables year and site included in every model. I considered models with a QAICc value within two of the minimum to have similar predictive value (Burnham and Anderson 2002), and when more than one model was within two of the minimum QAICc, I averaged the models using the *model.avg* function in package *MuMIn* (Bartón 2015). I report full averages, in which coefficients for covariates were set to zero in models where they were absent.

Caterpillar development was staggered depending on when eggs had been laid, so I regressed each sibling group's survival to each stage against plant characteristics corresponding to the date when that group advanced to the instar in question (usually within 1-2 days). For example, for each group, survival to second instar was regressed against the degree of leaf necrosis on that group's host corresponding to the date when they were entering second instar. For models concerning survival to second and third instar, host plant measurements were from the individual plant where eggs were placed, while for models testing survival from third to fourth instar, values for host plants were taken as the average of the five plants per plot that were sampled throughout the season, since larvae in this stage usually interact with several plants.

Finally, I used GLMs to test if anthocyanin pigmentation increased with C:N ratios for both *Castilleja* species. I compared models containing only year and site to those that also contained species identity, C:N ratios, and their interactions (in case the relationship differed between the two species). I

averaged models within two of the minimum QAICc as above, and interpreted coefficients from the full averaged model.

Results

Predictors of survival

Survival rates depended on the host species that was eaten (Table 1; Figure 3). Overall, 6% of eggs placed on *C. levisecta* produced fourth-instar larvae, compared to 20% of eggs placed on *P. lanceolata* and 17% on *C. hispida* (raw numbers, averaged across years and sites). The largest disparity in survival occurred during the transition from second to third instar. On *C. levisecta,* 54% of colonies produced at least one surviving fourth-instar larva, compared to 78% of colonies on *C. hispida* and 81% of those on *P. lanceolata.*

Predictors of survival for larvae differed by life stage but also depended on the host species being consumed (Tables 2-3; Figure 4). From hatching to second instar, larvae feeding on *C. levisecta* were much less likely to survive on senescent host plants with necrotic tissues, and also showed a slight (non-significant) decrease in survival if eggs were laid later in the season. Larvae feeding on *C. hispida* were unaffected by host plant characteristics or colony size, but were less likely to survive to second instar if their eggs were laid later in the flight period. Survival to second instar for larvae feeding on *P. lanceolata* was not significantly affected by any of the variables I measured. Survival decreased slightly for later oviposition dates and increased slightly with group size, but these terms were not significant in the averaged model, and the single best model included only the terms for year and site.

Factors affecting survival to third instar differed from those that mattered to the previous stage (Tables 2-3). None of the factors strongly affected survival on *C. hispida*: the single best model describing survival from second to third instar included only the year and site terms. The averaged model suggested that survival increased very slightly on anthocyanic plants, and decreased slightly with group size, although these terms were not significant. Individuals feeding on *P. lanceolata* were very likely to survive to third instar if they were members of a larger sibling group, but were unaffected by the host

plant variables I measured. For caterpillars feeding on *C. levisecta*, survival from second to third instar was influenced by a number of variables. First, as in the previous stage, survival decreased when they fed on necrotic host plants. It also decreased for larger sibling groups (although not significantly), which is opposite the trend I observed for those feeding on *P. lanceolata.* Finally, larvae on *C. levisecta* were slightly more likely to survive if they were from eggs laid later in the flight season.

Survival from third to fourth instar, in contrast to the previous stages, was not explained well by any of the predictor variables I measured (Tables 2-3). This held true across all three of the host species; in each case, the single best model of survival included none of the variables I tested beyond year and site.

Differences among host plants

The amount of plant necrosis, assessed when caterpillars were entering second instar, differed among the three host species. *Plantago lanceolata* was less senescent than the other two species. When I considered only the individual plants where eggs were deployed, I found no difference between the two *Castilleja* species after accounting for block, site, and year. In contrast, when I considered the average of the five plants that were monitored in each plot (not just the one with caterpillars present), *C. hispida* was on average slightly more senescent than *C. levisecta* (14% necrotic tissue compared to 11%; $p = 0.012$; Figure S2).

Heavily anthocyanic plants from both *Castilleja* species had higher C:N ratios than those that were mostly green in appearance (Figure 5), although this indicator had little to no effect on caterpillar survival. The averaged model included significant effects of host species identity and C:N ratios, but not their interactions (Table S5).

Discussion

The factors controlling caterpillar survival depended on the hosts being consumed, but also changed depending on the developmental stage of the caterpillars. Survival was lowest during earlier

instars, and steadily increased for later ones. Caterpillars feeding on *C. levisecta* died in both first and second instar when they fed on senescent plants. Caterpillars feeding on *C. hispida* did not respond strongly to plant senescence, but suffered reduced survival to second instar if they hatched from eggs laid later in the flight period. Caterpillars on *P. lanceolata*, unlike those that fed on *Castilleja* spp., were very likely to survive to third instar if they belonged to a large group in second instar. Finally, survival from third to fourth instar was relatively high on all three species and not closely related to the variables I measured.

By considering host plant suitability and caterpillar ontogeny jointly, I was able to pinpoint some of the factors limit caterpillar survival at different points in their development, and to identify practical differences in host plant suitability. Host plant characteristics and insect ontogeny probably interact for most specialist herbivores; therefore, research and conservation efforts should incorporate them more explicitly.

This study also underscores the importance of understanding factors that affect early-instar larvae. Events affecting neonate larvae can dictate survival and fitness, but ironically, these stages are also the most likely to be ignored by ecologists (Zalucki et al. 2002). After hatching, caterpillars are forced to contend with plant defenses and challenging microclimates, all while they are relatively immobile. In our study, survival from hatching to second instar was the lowest of any of the stages I examined, with only around a quarter of individuals surviving. This is on the low end of the range described in a review of early-instar Lepidoptera studies (Zalucki et al. 2002); among taxa that lay eggs in clusters, an average of 40% of hatching first instar caterpillars survive to second.

Plant senescence and oviposition timing

Some of the *C. levisecta* with caterpillars feeding on them senesced quickly during the study period, and first- and second-instar caterpillars died as a result (Table 3, Figure 4). This illustrates that early instar *E. e. taylori* larvae are vulnerable to phenological mismatches with hosts in some contexts, as has been documented in other *E. editha* systems (Singer 1972, Weiss et al. 1988, Cushman et al. 1994).

Caterpillars on *Castilleja hispida* were less impacted by senescence, although if they hatched later they were still less likely to survive. If eggs had been laid later or if plants had senesced faster, which could happen in some years, senescence would be a much stronger mortality source for caterpillars on *Castilleja.* In this context, *P. lanceolata* would provide an important phenological refuge since it persists well into the dry season.

It is worth noting that the range of oviposition dates I tested was narrower than occurs in the field. Eggs in our study were laid between April 28 and May 13 (median May 5 in 2015; May 3 in 2016). In 2016, for example, wild butterflies in nearby populations flew as early as April 11, peaking around April 20; the last individuals were seen on May 17 (Linders 2016). Site access restrictions prevented us from collecting butterflies during the earliest portion of the flight period. Therefore, fates of eggs laid earlier in the flight period could differ from those reported here. The data I collected, as well as other studies in similar systems (Weiss et al. 1988, Cushman et al. 1994), suggest survival would probably be higher for these individuals.

Counter to expectations, larval survival from second to third instar on *C. levisecta* increased slightly when eggs were produced later in the flight period (although the pattern was weak; Figure 4). One explanation for this pattern is that larvae from non-senescent plants survived from hatching to second instar in larger numbers. Then these larger groups, when faced with increasingly senescent plants during second instar, could have faced more competition as their food source declined. Regardless, it is important to consider that group size in this system is a feedback mechanism. Outcomes for later instars are not independent of those for earlier ones, since conditions determining survival rates for early instars also determine group size for later ones.

Importantly, *C. levisecta* does not inherently senesce faster than *C. hispida.* Six *C. levisecta* plants in our study senesced very quickly while larvae were in first instar (Figure 3), but senescence rates did not differ between the two species after taking block, year, and site into account. In fact, when I considered all five plants that were monitored in each plot, *C. hispida* senesced slightly faster than *C. levisecta* (Figure S2). However, I note that second instar larvae died on senescent *C. levisecta* but not *C.*

hispida, (Table 3, Figure S1), even though both species were similarly senescent at that point in time—it is possible that remaining tissues on senescent *C. hispida* are more suitable food for caterpillars than those on *C. levisecta*, even if the plants are similarly necrotic.

Larvae died on *C. levisecta* plants that that were only slightly brown (e.g., 10%), despite the fact that most of their tissues were still alive and available for larvae to feed on. Here it is important to consider leaf ontogeny more fully. I measured the amount of necrotic leaf tissue, but necrosis is the final step in leaf senescence, and is preceded by a number of processes that would cause a decline in leaf nutrition. During senescence, cell contents are systematically disassembled and nutrients are re-allocated elsewhere in the plant, especially to developing seeds, meaning leaf N and water content become limited before the onset of necrosis (reviews: Thomas et al. 1980, Lim et al. 2007). I measured necrosis because it was non-invasive, it was a simple metric that is reproducible by conservation workers, and because I had frequently observed larvae feeding on partly necrotic *Castilleja* in the field. However, prior to necrosis, caterpillars are probably adversely affected by these nutritional changes, even if the plants have not begun to wither (Slansky 1993).

Anecdotally, I noticed that once larvae reached second instar and could move quickly around their host plant, on both *Castilleja* species they fed preferentially on flowers and leaf bracts at the stem apex, i.e., the newest growth on the plant. I also noticed that they appeared to graze selectively, often consuming only small portions of each leaf when feeding on older tissues. In contrast, when feeding on *P. lanceolata* they were more sedentary, often consuming large portions of a single leaf as a group before moving to an adjacent one (pers. obs.).

Comparison to similar systems

Plant senescence and oviposition timing govern population dynamics in other *E. editha* systems. For some *E. editha bayensis* populations (California, USA), only the butterflies that eclose early contribute offspring that survive to diapause, because caterpillars that develop later die when their host plants senescence (Singer 1972, Weiss et al. 1988, Cushman et al. 1994). Post-diapause and pupal

development (and therefore eclosion) are faster on warm slopes, but host senescence is slowed on cooler ones; therefore topographic diversity allows some butterflies to develop early on warm slopes, while cool slopes delay host plant senescence (Weiss et al. 1988).

Similar processes are probably at work for *E. e. taylori*, but with less pronounced effects (at least during the years of our study). Lowland grasslands in Washington have less topographic relief and a milder summer drought than those in California, and all three host species are medium-sized perennials, whereas *E. e. bayensis* often use *Plantago erecta*, a tiny annual (Ehrlich and Hanski 2004). These factors probably attenuate the senescence process and buffer its effects on survival. Still, our findings suggest topographic diversity could increase the number of early-flying individuals with access to the highest-quality host plants. Chapter 4 of this dissertation explores the effects of topography and other environmental factors on host plant senescence and pre-diapause survival; the effects of topography on post-diapause larvae in this system are in need of study.

Anthocyanins

I found that anthocyanic *Castilleja* plants of both species had high C:N ratios (Fig 5), but plant color was not an important predictor of caterpillar survival compared to the effects of plant senescence, oviposition timing, and colony size. Since anthocyanins can to some degree indicate poor leaf nutrition, there could be sub-lethal effects on larvae. Effects of this and other predictors on caterpillar mass and development time are explored in Chapter 4 of this dissertation.

Colony size

When caterpillars fed on *P. lanceolata*, large second instar colonies enjoyed very high survival to third instar. In contrast, for those feeding on either *Castilleja* species, survival decreased somewhat for larger groups (Figures 1, S1). This was consistent with our hypothesis related to group size: caterpillars on the more senescent host plants may have faced intraspecific competition, while those on non-senescent *P. lanceolata* enjoyed the benefits of larger colony size (Seymour 1974, Stamp 1980, Clark and Faeth

1997). This suggests the different hosts could be applying conflicting selective pressures on egg clutch size; this should be a focus of future research.

As a caveat, the pattern I observed for larvae on *P. lanceolata* could be attributable to more than just density (i.e., genetically fit sibling groups that survived to second instar at higher rates would also be more likely to survive to third), but this possibility would have stronger support if the same pattern had been observed on the other species as well. Finally, I used clusters ranging from 16 to 67 eggs, but note that clutch sizes can range from one to well over a hundred eggs, and different patterns could emerge if the whole spectrum were considered.

Variation among years and sites

Survival at most stages also varied among the three sites and between the two years. I analyzed data from all years and sites together – partly because of small sample size in 2015 and partly because even when survival rates were variable, their rank order on the three hosts was fairly consistent across years and sites (Tables S1-S4). Of course, results from this study should be applied with the understanding that outcomes for larvae probably differ from year to year and will almost certainly differ among sites and populations. *E. editha* is notorious for variability among subspecies, populations, and through time (Ehrlich and Hanski 2004). Also, outcomes for pre- and post-diapause larvae on different host species do not necessarily match (Brown et al. 2017), so host species effects on other parts of the life cycle also need to be considered.

Adaptation, host plant suitability, and E. e. taylori *recovery*

In recent decades *E. e. taylori* in the South Puget Sound region came to rely entirely on *P. lanceolata* as their range retracted and populations of other host species declined. However, they were known to feed on *C. hispida* until very recently and quickly reincorporated it into their diet when it reappeared after restoration efforts (M. Linders, pers. comm). The relationship to *C. levisecta* is more mysterious, since the two taxa were isolated from each other for at least a few decades and their

relationship prior to separating is poorly documented (Dunwiddie et al. 2016). Our findings that survival was best on *P. lanceolata,* intermediate on *C. hispida,* and lower on *C. levisecta* align with the degree of affiliation *E. e. taylori* has had with each of these hosts in recent decades.

Adaptive changes toward *P. lanceolata* could have occurred in recent decades, or this species could simply fall within the historical diet breadth of *E. e. taylori*. Either way, it can provide a phenological refuge when other hosts senesce too quickly. Recovery efforts utilizing *C. levisecta* should proceed with caution; although I determined it is clearly within the prediapause diet breadth of *E. e. taylori*¸ if it attracts ovipositing butterflies but results in lower survival to diapause, it could reduce population growth rates of reintroduced butterfly populations. On the other hand, *E. editha* can quickly adapt to new hosts over short time periods (Ehrlich & Hanski 2004, Singer et al. 2008). In this case, the benefits of adaptation toward *C. levisecta* would need to be weighed against the possibilities of genetic bottlenecks and other risks.

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Table 3.1. Models describing effects of host species identity on survival from stage to stage. For each transition I compared a model including year, site, and block to one that also included a host species term (block was a random effect, while the others were fixed). Model-estimated mean survival rates and results of pairwise contrasts are included in Figure 3.

Instar transition	**Candidate model**	**ΔQAICc**	**w**
Egg to second	year + site + block + host species	0	0.534
N = 126	year + site + block	0.27	0.466
Second to third	year + site + block + host species	0	1
N = 110	year + site + block	30.46	0
Third to fourth	year + site + block + host species	0	0.516
N = 100	year + site + block	0.13	0.484
Overall survival	year + site + block + host species	0	1
egg to fourth	year + site + block	25.62	0
N = 126			

Table 3.2. Model comparisons testing predictors of *E. e. taylori* survival for each instar transition. I show all models with a QAICc within two of the minimum, which were averaged when necessary to develop the coefficients shown in Table 3. The simplest models I considered were those including only year and site.

Instar transition	**Host species**	**Candidate models**	**ΔQAICc**	**w**
Hatching to second	*C. hispida* N = 40	year + site + oviposition date	0	0.347
	C. levisecta N = 37	year + site + plant senescence	0	0.364
		year + site + plant senescence + oviposition date	1.75	0.164
	P. lanceolata N = 49	year + site	0	0.385
		year + site + oviposition date	1.54	0.178
		year + site + group size	1.82	0.155
Second to third	*C. hispida* N = 37	year + site	0	0.261
		year + site + anthocyanins	1.02	0.157
		year + site + group size	1.06	0.153
	C. levisecta N = 29	year + site + senescence + oviposition date + group size	0	0.330
		year + site + senescence + oviposition date	0.65	0.239
		year + site + senescence + oviposition date + anthocyanins	1.79	0.135
	P. lanceolata N = 44	year + site + group size	0	0.608
Third to fourth	*C. hispida* N = 34	year + site	0	0.233
		year + site + senescence	0.11	0.220
		year + site + oviposition date	1.32	0.120
	C. levisecta N = 25	year + site		
	P. lanceolata N = 41	year + site	0	0.402
		year + site + oviposition date	1.25	0.215

Table 3.3. Parameters and pairwise contrasts from GLMs examining the effects of host plant and colony characteristics on caterpillar survival. These are averaged parameters from the models listed in Table 2. Values for intercepts have been reverse-transformed from the logit scale, so they can be interpreted as estimates of survival rates. Since covariates were converted to z-scores before analysis, slopes can be interpreted as the change in expected survival on the logit scale when the predictor value changes by one standard deviation, and intercepts are the estimated survival rate at the mean value for the predictor variable in question. Covariates with slopes differing significantly from zero are in bold.

	Host species	**Parameter**	**estimate**	**SE**	**z**	**p**
 Hatching to second instar	*C. hispida*	intercept	0.232	0.152	-7.904	<0.001***
		oviposition date	**-0.536**	**0.077**	**-6.973**	**<0.001*** **
	C. levisecta	intercept	0.158	0.238	-6.975	<0.001***
		plant senescence	**-2.465**	**0.429**	**5.742**	**<0.001*** **
		oviposition date	-0.088	0.143	0.618	0.537
	P. lanceolata	intercept	0.243	0.179	-6.352	<0.001***
		oviposition date	-0.046	0.085	0.543	0.587
		group size	0.032	0.066	0.487	0.626
 Second to third instar	*C. hispida*	intercept	0.339	0.313	2.130	0.033*
		anthocyanins	0.103	0.180	0.571	0.568
		group size	-0.079	0.139	0.567	0.571
	C. levisecta	intercept	0.327	0.746	0.968	0.333
		group size	-0.2734	0.313	0.873	0.382
		plant senescence	**-1.470**	**0.350**	**4.207**	**<0.001*** **
		oviposition date	**0.771**	**0.177**	**4.345**	**<0.001*** **
	P. lanceolata	intercept	0.316	0.333	-2.314	0.021*
		group size	**1.021**	**0.134**	**7.648**	**<0.001*** **
 Third to fourth instar	*C. hispida*	intercept	0.697	0.470	1.704	0.088
		senescence	-0.174	0.232	0.747	0.455
		oviposition date	-0.115	0.240	0.480	0.631
	C. levisecta	intercept	0.924	1.056	2.365	0.018*
	P. lanceolata	intercept	0.819	0.666	2.265	0.023*
		oviposition date	-0.094	0.142	0.664	0.507

Figure 3.1. Two types of variation in plant quality measured in this study. All three plants are *Castilleja hispida.* Plants show a range of pigmentation from green to purple (left, center), and some plants senesce during the larval feeding period (right). Larvae in each photo are in fourth instar.

Figure 3.2. Enclosures used to restrict caterpillars to a single host plant species.

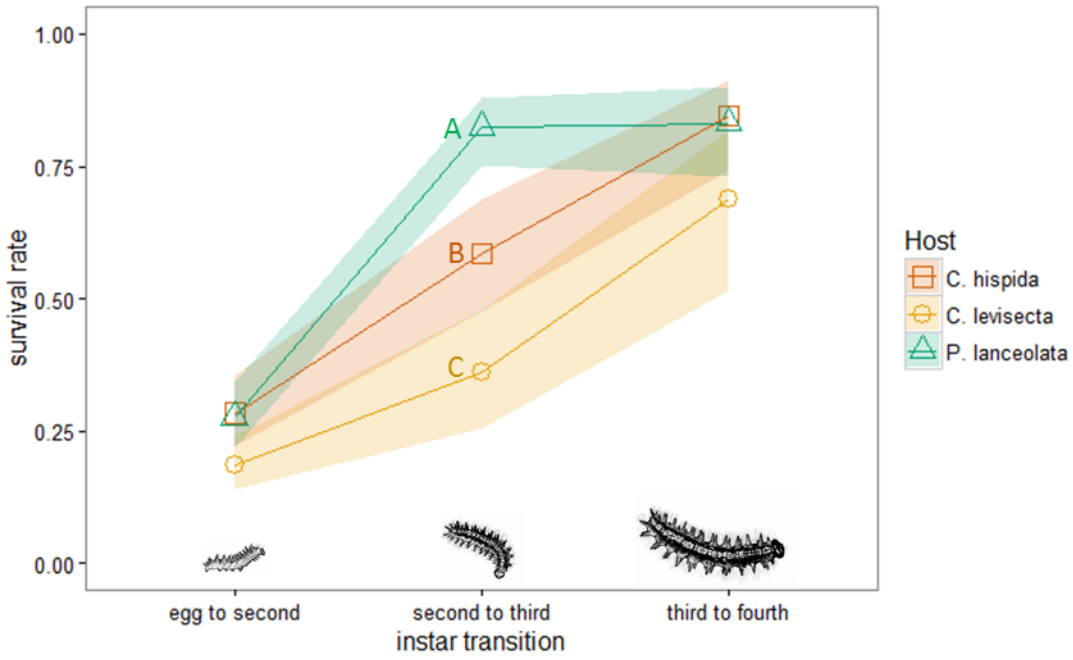


Figure 3.3. Model-estimated survival rates from hatching to second instar, from second to third instar, and from third to fourth instar on larvae feeding on each host. Letters indicate significant differences detected with pairwise contrasts, and shaded areas represent 95% confidence intervals around each mean.

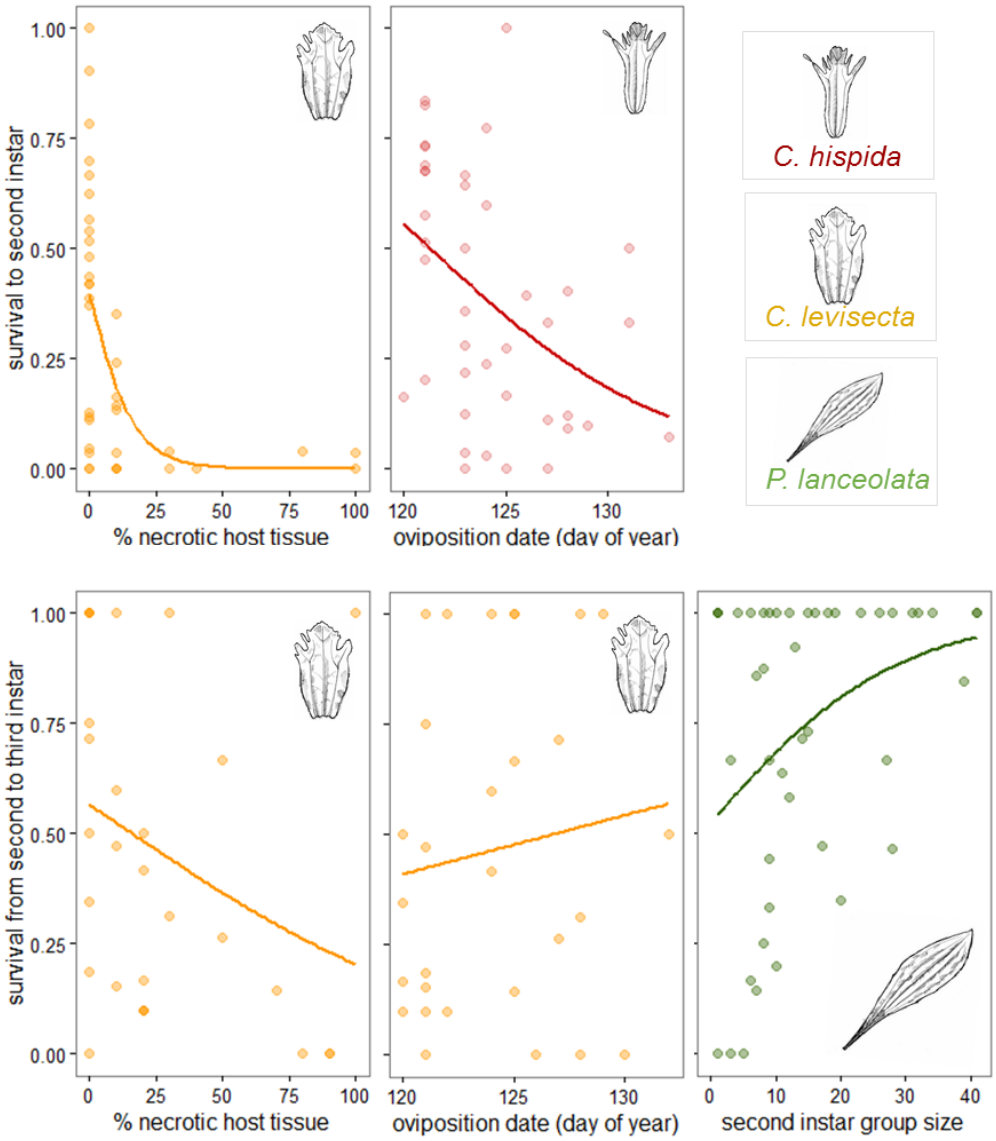


Figure 3.4. Factors most strongly predicting *E. e. taylori* survival from hatching to second instar (top) and from second to third instar (bottom). Curves are GLMs fitted to raw data. Plots show variables that were identified as predictors of survival during model selection and had slopes that differed significantly from zero after model averaging.

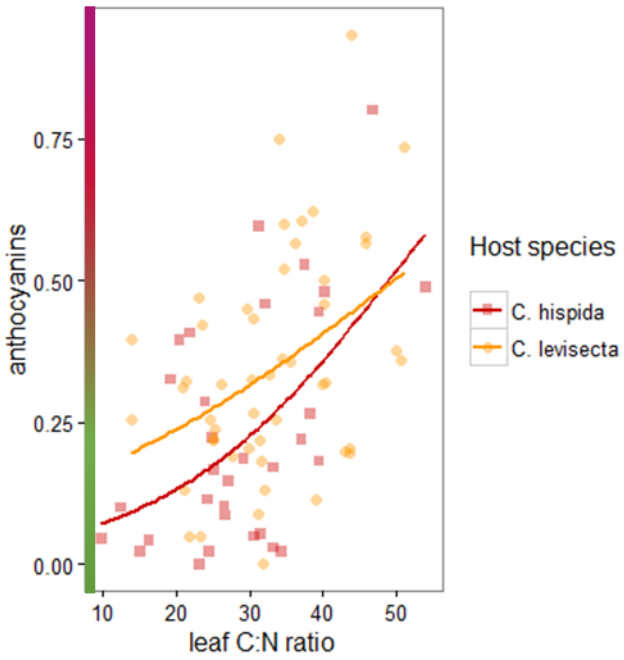


Figure 3.5. Association between leaf C:N ratios and prevalence of anthocyanins in leaves for both *Castilleja* species. Curves are based on GLMs with raw data.

Chapter 3 Supplement

Tables S3.1-S3.4 show mean *E. e. taylori* survival, broken down by year and site. These numbers are based on raw data, in contrast to the fitted values shown in the manuscript. 'Colony persistence' refers to the proportion of colonies which were present in the previous stage that persisted to the stage in question (i.e., at least one individual survived), while 'mean survival' refers to the average proportion of individuals surviving within each sibling group. I also show results from pairwise contrasts of GLMs testing effects of year and site on survival rates within each sibling group. Within each transition and host species, years and sites not sharing a letter differ significantly from one another. Table S5 shows results from model selection and model averaging related to leaf C:N ratios and anthocyanin levels.

Table S3.1. Survival from hatching to second instar, broken down by year and site.

	Host		N	Colony persistence	Mean survival	SE	Contrast group
Egg to second instar 	*C. hispida* (N = 40) 	Site					
		Glacial Heritage	15	0.867	0.325	0.078	A
		West Rocky	13	1.000	0.442	0.075	B
		Tenalquot	12	0.917	0.403	0.079	AB
		Year					
		2015	13	0.846	0.232	0.076	A
		2016	27	0.962	0.461	0.050	B
	C. levisecta (N = 37) 	Site					
		Glacial Heritage	19	0.842	0.352	0.071	C
		West Rocky	10	0.600	0.143	0.082	A
		Tenalquot	8	0.875	0.290	0.089	B
		Year					
		2015	11	0.636	0.165	0.085	A
		2016	26	0.846	0.332	0.056	B
	P. lanceolata (N = 49) 	Site					
		Glacial Heritage	18	0.889	0.458	0.064	
		West Rocky	12	0.916	0.410	0.086	
		Tenalquot	19	0.895	0.303	0.060	
		Year					
		2015	10	0.800	0.185	0.065	A
		2016	39	0.923	0.437	0.044	B

Table S3.2. Survival from second to third instar, broken down by year and site.

	Host		N	Colony persistence	Mean survival	SE	Contrast group
Second to third instar	*C. hispida*	Site					
	(N = 37)	Glacial Heritage	13	0.846	0.583	0.110	A
		West Rocky	13	1.000	0.756	0.083	B
		Tenalquot	11	0.909	0.614	0.098	B
		Year					
		2015	11	0.727	0.474	0.122	A
		2016	26	1.000	0.729	0.057	B
	C. levisecta	Site					
	(N = 29)	Glacial Heritage	16	0.875	0.502	0.099	A
		West Rocky	6	0.667	0.387	0.196	A
		Tenalquot	7	1.000	0.449	0.093	A
		Year					
		2015	7	0.571	0.288	0.140	A
		2016	22	0.955	0.522	0.078	A
	P. lanceolata	Site					
	(N = 44)	Glacial Heritage	16	1.000	0.689	0.079	A
		West Rocky	11	0.909	0.734	0.092	B
		Tenalquot	17	0.882	0.758	0.095	B
		Year					
		2015	8	0.750	0.473	0.141	A
		2016	36	0.972	0.784	0.050	B

Table S3.3. Survival from third to fourth instar, broken down by year and site.

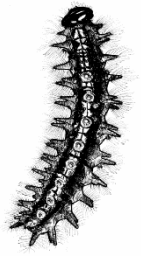
	Host		N	Colony persistence	Mean survival	SE	Contrast group
Third to fourth instar	*C. hispida*	Site					
	(N = 34)	Glacial Heritage	11	0.909	0.558	0.106	A
		West Rocky	13	0.923	0.764	0.087	B
		Tenalquot	10	0.900	0.657	0.133	B
		Year					
		2015	8	0.875	0.729	0.141	A
		2016	26	0.923	0.646	0.069	A
	C. levisecta	Site					
	(N = 25)	Glacial Heritage	14	0.786	0.417	0.101	A
		West Rocky	4	0.500	0.500	0.289	A
		Tenalquot	7	1.000	0.881	0.079	B
		Year					
		2015	4	0.750	0.750	0.250	B
		2016	21	0.809	0.521	0.088	A
	P. lanceolata	Site					
	(N = 41)	Glacial Heritage	16	1.000	0.531	0.073	A
		West Rocky	10	1.000	0.739	0.098	B
		Tenalquot	15	0.933	0.727	0.078	B
		Year					
		2015	6	1.000	0.875	0.125	B
		2016	35	0.971	0.616	0.020	A

Table S3.4. Overall survival from hatching to fourth instar, broken down by year and site.

	Host		N	Colony persistence	Mean survival	SE	Contrast group
Overall survival from hatching to fourth instar	*C. hispida* (N = 40) 	Site					
		Glacial Heritage	15	0.667	0.094	0.031	A
		West Rocky	13	0.923	0.234	0.051	C
		Tenalquot	12	0.750	0.168	0.055	B
		Year					
		2015	13	0.538	0.092	0.041	A
		2016	27	0.889	0.196	0.033	B
	C. levisecta (N = 37) 	Site					
		Glacial Heritage	19	0.579	0.046	0.014	B
		West Rocky	10	0.200	0.019	0.014	A
		Tenalquot	8	0.875	0.114	0.057	C
		Year					
		2015	11	0.273	0.076	0.023	A
		2016	26	0.654	0.100	0.020	A
	P. lanceolata (N = 49) 	Site					
		Glacial Heritage	18	0.889	0.157	0.035	A
		West Rocky	12	0.833	0.247	0.082	B
		Tenalquot	19	0.737	0.188	0.048	A
		Year					
		2015	10	0.600	0.069	0.029	A
		2016	39	0.872	0.223	0.036	B

Table S3.5. Model selection and model-averaged coefficients describing the effect of leaf C:N ratios on the appearance of anthocyanin pigments in both *Castilleja* species. Under 'model selection' I show models within two of the minimum QAICc.

Model selection

Candidate model	**ΔQAICc**	**w**
year + site + host species + C:N ratio	0	0.603
year + site + host species + C:N ratio + host species : C:N ratio	1.83	0.241

Model-averaged coefficients

Parameter	**Estimate**	**SE**	**Z**	***p***
intercept	0.065	0.222	12.070	<0.001***
C:N ratio	0.058	0.006	8.687	<0.001***
Host species			2.084	0.037*
C:N ratio : host species			2.917	0.558

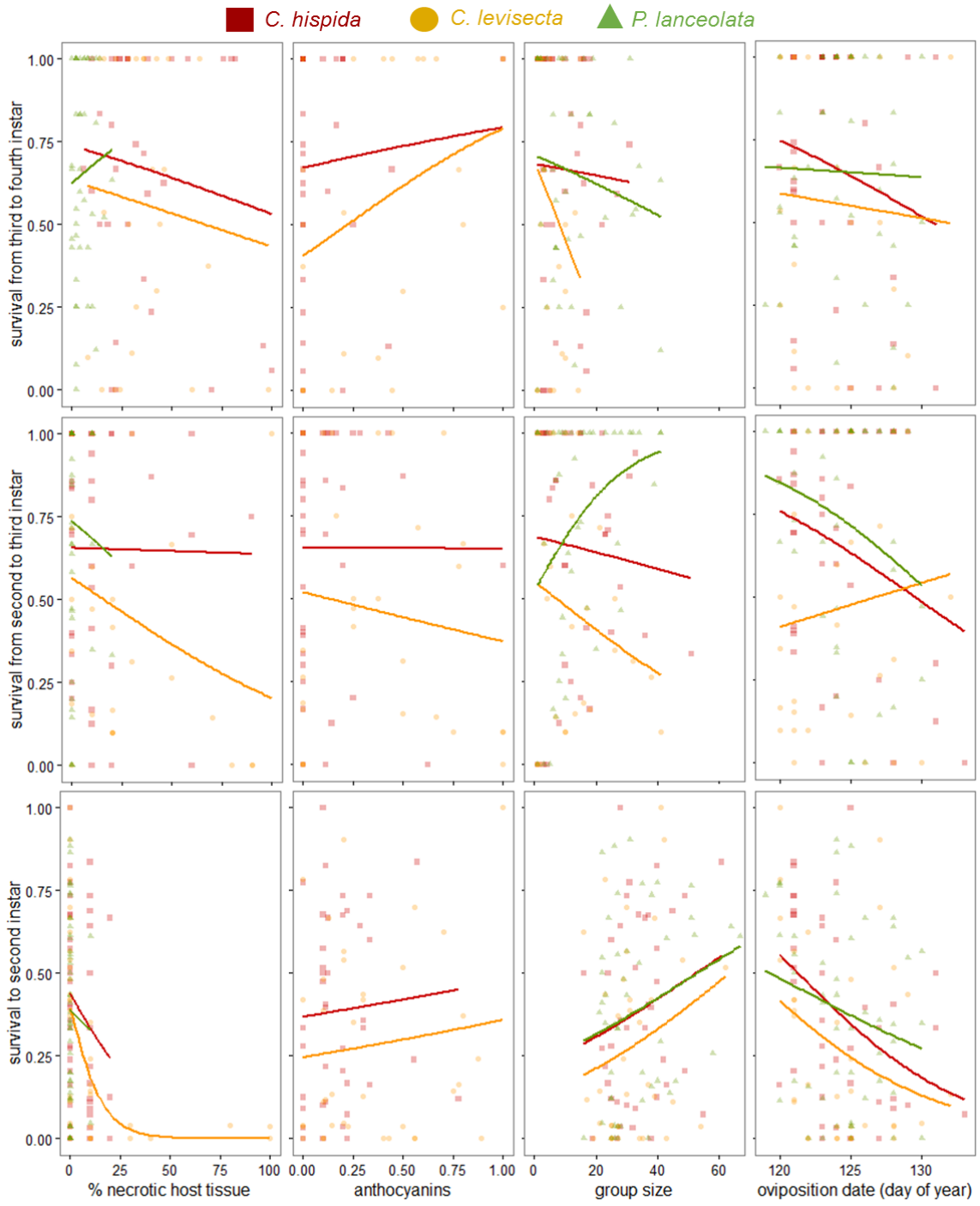


Figure S3.1. Relationships between each explanatory variable and caterpillar survival to each stage. Curves are GLMs fitted to raw data.

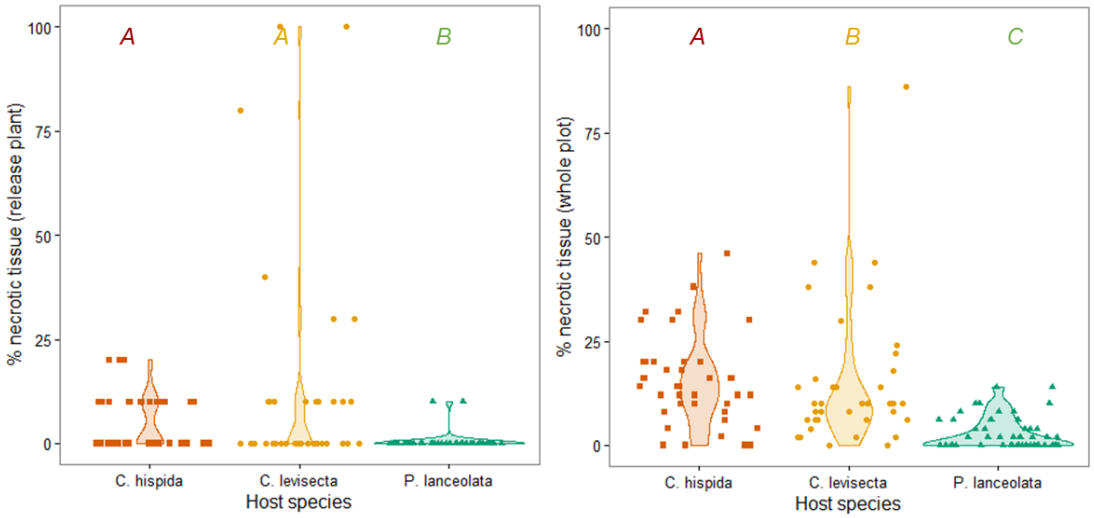


Figure S3.2. Amount of necrotic tissue on host plants when larvae transitioned to second instar. Left: the individual plants where eggs were deployed and larvae fed until usually third instar. Right: average values for the five plants monitored in each plot.

Chapter 4: Host plant effects on mass and development rate of *Euphydryas editha taylori*

Abstract

I compared effects of different host plants on several fitness-related outcomes for early instar larvae of an endangered butterfly, *Euphydryas editha taylori*. Specifically, I tested whether larval mass, development rate, or duration of the feeding period before entering diapause were affected by differences within and among host plant species. I placed eggs on three plant species in the field, each of which is a host for *E. e. taylori*: *Castilleja hispida, Castilleja levisecta*, and *Plantago lanceolata.* I measured the amount of time it took larvae to develop to fourth instar, at which time they are capable of entering diapause. I weighed larvae when they reached fourth instar, and also measured the amount of time they remained active during this stage before entering diapause. I tested whether any of these outcomes responded to differences in host plant species identity, senescence, degree of anthocyanin pigmentation, or C:N ratio. I also assessed effects of larval group size and oviposition date on these responses. Neither host plant species identity nor host plant characteristics had any discernable effect on larval mass, development time, or fourth instar duration. However, when larvae fed on *Plantago lanceolata*, larger groups developed to fourth instar faster. On all three hosts, larvae that reached fourth instar sooner spent much more time actively feeding in fourth instar before they entered diapause. This could have important implications for survival during the diapause phase and possibly for the amount of time required to reach adulthood afterwards.

Introduction

Food plant quality influences outcomes for oligophagous Lepidoptera, and effects can be especially strong during early instars (e.g., Zalucki et al. 2001, Zalucki et al. 2002, Harvey and

Gols 2011). For the endangered butterfly *Euphydryas editha* ssp. *taylori*, host plants strongly affect survival (see Chapter 3 of this dissertation), but could also influence other fitness-related metrics for surviving individuals, like mass and development rate. Therefore the goal of this study was to determine how mass and development of *E. e. taylori* are influenced by differences within and among the host plant species they eat.

Insect mass often correlates positively with fitness, and for insects that grow only during the larval phase, larval mass ultimately determines pupal and adult mass (Peters 1983, Kingsolver and Huey 2008, Speight et al. 2008). Adult mass, in turn, often controls fecundity since larger female individuals carry more eggs (Hŏnek 1993). Thus, mass gain during the larval stage influences reproductive success later in life.

Development rate can also have implications for fitness. First, individual herbivores that take longer to develop have been hypothesized to accumulate more predation risk (slow growth, high mortality hypothesis; Clancy and Price 1987), although evidence for this has been mixed (Benrey and Denno 1997, Lill and Marquis 2001, Cornelissen and Stilling 2006). Second, and probably more importantly, prediapause *E. editha* larvae often feed on rapidly-senescing host plants (Singer 1972, Weiss et al. 1988, Chapter 3 of this dissertation). Caterpillars that develop faster should have an advantage because their food plants are of higher quality on average.

Some of the implications of early-instar mass gain and development rate should come to bear during the diapause phase. Many insects spend the majority of their lifespan in diapause; for *E. editha*, this period spans both summer and winter, meaning larvae must tolerate not just the hottest and driest parts of the year, but also the coldest and wettest. Survival during diapause is variable; Moore (1989) found that diapause survival for *E. editha* in southern California (in captivity, with no predation) ranged from 52-81%, depending on the host species consumed and

on the year in question. In Chapter 2 of this dissertation, survival of *E. editha colonia* to mid-diapause was 44%. Average mass per diapausing individual in each colony ranged from 3-27 mg, a factor of nearly ten, and larger individuals were much more likely to survive during diapause.

The timing of diapause onset could also have important implications for the diapause phase, especially through its influence on mass gain. Larvae of *E. e. taylori* feed for four instars, then enter diapause sometime during the fourth instar. Reaching fourth instar is an important milestone because at this stage larvae are more mobile, and if they run out of food, they can enter diapause and resume feeding in the spring. (Some *E. editha* larvae can enter diapause in third instar, but I have not observed this for *E. e. taylori*). But while reaching fourth instar is important, the timing of events that follow could also affect diapause survival and post-diapause development. Caterpillar mass can increase by one or two orders of magnitude within some instars (Reavey 1993), meaning an individual that that lingers to feed for a few extra days before diapause could accumulate much more mass during that time than it did during all of the preceding instars combined. In contrast, one that enters diapause quickly after reaching fourth instar will be smaller and have gathered less resources. This could have important effects on diapause survival.

The factors that trigger the onset of diapause should govern the amount of time larvae spend feeding before they start to become dormant. For insects with obligate diapause, its onset is often tied to day length, and can sometimes be secondarily affected by temperature (Taylor et al. 1995, Speight et al. 2008). However, for some Lepidoptera it can also be influenced by relationships with host plants, including host plant phenology and species identity (Usua 1973, Scheltes 1976, Hunter and McNeil 1997). Factors that influence the onset of diapause for *E.*

editha have not been studied to my knowledge, but it would be reasonable to assume that host plant identity and quality could play a role.

I tested several hypotheses related to mass gain, development rate, and the duration of fourth instar for *E. e. taylori*, all of which could have implications for diapause survival and reproductive success. I used data collected from the experiment described in Chapter 3, and my hypotheses roughly parallel those described there (in particular, descriptions of differences in host plant quality and colony characteristics are presented there in detail, and are not repeated here). I tested for effects of three host species, as well as differences that occur within and among each species. The three plant species used as hosts were *Castilleja hispida, Castilleja levisecta*, and *Plantago lanceolata.*

I expected larval mass and development time to depend on the host species being eaten. I expected mass to be lower, and development time to attenuate, on senescent or anthocyanic hosts. Larval mass can depend strongly on leaf N (see Chapter 2), so I expected it to decrease when they fed on plants with high C:N ratios. I also expected colony characteristics to affect mass and development time: larvae from eggs laid later in the flight season, or those belonging to smaller groups, should be smaller and slower to develop.

I predicted that larvae arriving in fourth instar earlier would spend more time feeding before entering diapause. Larval development is necessarily staggered because eggs are laid at different times, but managers have observed anecdotally that diapause onset is more synchronous (suggesting it is at least partially tied to day length and/or temperature). This could be an important mechanism by which the earliest-developing larvae are afforded additional time to feed before diapause—either because their eggs were laid early, or because the larvae developed faster.

Finally, I also expected fourth instar duration to be affected to some extent by host plants: *P. lanceolata* does not senesce as quickly as either of the *Castilleja* species, so fourth instar duration could be longer for larvae feeding on this species. For larvae feeding on *Castilleja* spp., I also expected caterpillars should remain feeding in the fourth instar for longer if their host plants were less senescent.

Methods

Experimental setup

The design for this experiment is described in detail in Chapter 3 of this dissertation. Briefly, I established plots, each of which contained a patch of one of the three host species eaten by *E. e. taylori*. Plots were located at three grassland sites in Western Washington, USA: Glacial Heritage Preserve (46.87^0 N, 123.04^0 W), West Rocky Prairie (46.89^0 N, 122.87^0 W), and Tenalquot Prairie (46.90^0 N, 122.73^0 W). Each site is managed as habitat for *E. e. taylori*. I installed one cluster of eggs on a host plant near the center of each plot. In total I installed and monitored 126 egg clusters (4130 eggs); 34 egg clusters were installed 2015, and 92 in 2016. Larvae from each group fed on their host plants for four instars before entering diapause.

Data collection

Measurements related to host plants and larval colony characteristics are described in detail in Chapter 3. Briefly, eggs were laid by wild *E. e. taylori* individuals which had been brought into captivity. I recorded the number of eggs in each egg cluster, as well as the date on which the eggs were laid. Host plants in each plot were assessed for the degree of senescence (quantified as the percent of their tissues that had turned brown, rounded to the nearest 10%), and the degree to which they were flushed with anthocyanins (0-10 scale). Here I report the average values from five plants per plot, when the larvae in that plot were entering fourth instar. I also

assessed the C:N ratio for leaves in a subset of the plots. I removed one intermediate-aged leaf from each plant in the plot and measured its C:N ratio with a CHN analyzer (2400 Model, Perkin Elmer Co., Waltham, MA). I did not collect from some plots where I judged vegetation was sparse enough that removing leaf material would limit the quantity of food available to the larvae; therefore, leaf C:N ratios were measured for 52 plots.

To assess development time, I monitored larvae and recorded the number of days that elapsed from when eggs were laid until they reached fourth instar. Development time was assessed for larvae in both 2015 and 2016. I also measured mass at fourth instar; as soon as larvae from a given group entered fourth instar, I collected one individual from that group and weighed it. In each case I chose an individual that was representative of typical larval size observed for that plot (although in most plots there were no discernable differences). I assessed mass for larvae only in 2016, as those in 2015 were not collected immediately at the onset of fourth instar and mass accumulates quickly. In all, 69 caterpillars (one per plot) were collected and weighed; 21 fed on *C. hispida*, 16 on *C. levisecta*, and 32 on *P. lanceolata*. Sample size was necessarily unbalanced because survival rates differed depending on the host being eaten (Chapter 3, this dissertation). Caterpillars from six additional plots survived to fourth instar, but were observed by field technicians when I was not present to collect them.

I also measured the duration of fourth instar before larvae entered diapause. After larvae entered fourth instar in each plot, I monitored them every 1-3 days to determine if they were still active, and recorded when they disappeared and presumably entered diapause. Larvae diapause inside of grass tussocks, in leaf litter, or sometimes below the soil surface (unpublished data), so it is not feasible to distinguish in the field between fourth instar larvae that enter diapause and those that may have died. Here I assume that all disappearing fourth-instar larvae were entering

diapause rather than dying; therefore, dying caterpillars could have shortened my estimates of fourth instar duration. I assessed fourth instar duration for larvae only in 2016, as larvae were collected prior to diapause in 2015. I also omitted six plots in 2016 with only one fourth-instar survivor, as that individual was collected to measure its mass. Finally, I omitted one plot that was not re-surveyed after a single individual was detected at the very end of the field season (total = 67 fourth instar colonies monitored).

Statistical analysis

All analyses were conducted in R 3.2.5 (R Core Development Team, 2016). First, I used Linear Mixed Models (LMMs) with package lme4 (Bates et al. 2015) to determine if caterpillar mass depended on the host species they ate. Mass was log transformed to improve normality. I compared a null model with only site (fixed effect) and block (random effect) to one that also contained host species identity, based on AICc. (In the rest of this chapter, I will refer to models containing only site, block, and/or year as 'null' models.)

Next, I tested for within-species differences for each host, that could have affected caterpillar mass. I used the same model selection procedure as is described in detail in Chapter 3, although I grouped the two *Castilleja* species together because of limited sample size. For caterpillars feeding on *P. lanceolata,* I compared a null model including only site to models that also included the level of plant necrosis, third instar group size, an interaction between necrosis and group size, and oviposition date. These are the same factors I tested in the previous chapter. I compared models with every combination of these variables on the basis of AICc. Here and elsewhere, I report full model-averaged parameters in cases where more than one model fell within two of the minimum AICc (Burnham and Anderson 2002). Model averaging used the R package *MuMIn* (Bartón 2015).

I analyzed plots with the two *Castilleja* species together, and compared a null model to those that also contained species identity, necrosis, anthocyanins, group size, and oviposition date. I tested for an interaction between necrosis and density as above, but also tested for an interaction between host species and each variable, in case their effects differed depending on whether larvae fed on *C. hispida* or *C. levisecta*. I compared models containing every combination of these variables, based on AICc, averaging models when necessary.

I also tested whether the C:N ratio in host plants affected mass of the caterpillars on any of the hosts. I tested this variable separately from the others because data were from a small subset of the plots. I tested whether leaf C:N ratios, or their interaction with host identity, predicted larval mass using LMMs and compared them to a null model as described above.

Next I tested for differences in the number of days required for larvae to reach fourth instar. I used LMMs and LMs in exactly the same manner described above, but with development time as a response variable instead of mass, and with year as an additional fixed effect since these data were collected in both 2015 and 2016. Development time was log transformed to correct for normality.

Finally, I tested for variables controlling the amount of time larvae spent in fourth instar. To test whether the amount of time depended on the host species larvae ate, I used LMMs to compare a model with host species to a null model, as above. Next, I tested whether host plant senescence affected the amount of time larvae spent in fourth instar before entering diapause. For larvae that fed on *P. lanceolata,* I used LMs to determine if fourth instar duration decreased on senescent host plants, and if it was longer for individuals that reached fourth instar sooner. I also tested for an interaction between these two factors, since senescent plants could change the effect of arriving in fourth instar early. I grouped data for both *Castilleja* species together, as above,

and tested for an interaction between species identity and the factors of interest in case they differed between the two species.

Results

Development time to fourth instar

The amount of time required to reach fourth instar did not differ by host species (AICc after adding host species increased by 14.45). Development from oviposition to fourth instar took an average of 42 days on *C. hispida*, 40 days on *C. levisecta*, and 41 days on *P. lanceolata* (Figure 1, left). Development times did not differ among the three sites either (40 days on average at Glacial Heritage; 41 at Tenalquot and West Rocky). The largest difference was between years; in 2015 development occurred over 44 days while in 2016 it took only 40 days on average.

Plant C:N ratio had no bearing on development time; adding it to the model worsened its explanatory power (ΔAICc = 12.97). Additionally, for *Castilleja* spp., none of the plant or colony characteristics predicted development time to fourth instar better than the null model (ΔAICc = 8.34). The only significant effect was for caterpillars feeding on *P. lanceolata*: development time on this species decreased for larger sibling groups ($t = -3.921$, $p < 0.001$; Figure 1, right).

Mass at fourth instar

Like development time, caterpillar mass at the beginning of fourth instar did not depend on the plant they ate. During model selection, adding host species identity resulted in a poorer fit than that of the null model (ΔAICc = 5.03). Mean mass (±SE) of caterpillars was 20.04 mg (±2.17) when they fed on *C. hispida*, 16.34 mg (±1.07) on *C. levisecta*, and 18.28 mg (±1.21) on *P. lanceolata* (Figure 2), but differences within species were greater than those among them.

None of the variables related to host plant or colony characteristics had strong effects on mass of the caterpillars, and this held true across all three host species. The model selection and averaging procedure for *P. lanceolata* included oviposition date and colony size as predictors of mass, but slopes for these predictors were close to zero and neither was significant ($p = 0.608$ and 0.767, respectively). For *Castilleja* spp., no model outperformed the null model (ΔAICc = 2.19). Similarly, when testing for effects of plant C:N ratios on caterpillar mass, no model outperformed the null model (ΔAICc = 10.25).

Duration of fourth instar before diapause

The amount of time spent in fourth instar did not vary strongly by host species, although the model with host species slightly outperformed the null model (ΔAICc = 1.34; Figure 3, left). Instead, the amount of time larvae spent in fourth instar depended very strongly on the date on which they reached it. Larvae reaching fourth instar on or before June 13 (the 165th day of the year in Figure 3, center) fed for an average of ten days, while those arriving after this date fed for an average of only four. This pattern was nearly identical across all three hosts. For larvae feeding on *Castilleja*, there was also an interactive effect of plant necrosis and host species on fourth instar duration (Figure 3, right; Table 1). On *C. hispida*, larvae spent less time feeding in fourth instar when their hosts were necrotic, but on *C. levisecta*, they appear to have done the opposite.

Discussion

Mass and development time

Surprisingly, I found that neither mass gain, development time, nor fourth instar duration depended on the host species the caterpillars ate. This stands in stark contrast to the host-based differences in survival described in Chapter 3. Remarkably, mass and development time were

also unresponsive to host plants' senescence status, anthocyanins, and leaf C:N ratios. This suggests that while some of these factors determined how many larvae survive, those individuals that did survive grew and developed similarly, at least to the beginning of fourth instar. Caterpillars that would have had lower fourth instar mass might have died in earlier instars—i.e., host plant quality was expressed in terms of mortality, but not mass of the survivors. Leaf age and stoichiometry are generally important predictors of growth for caterpillars and other herbivores (Slansky 1993, Elser 2000), so the insensitivity of *E. e. taylori* growth to these variables is puzzling. Caterpillars can undertake compensatory feeding when they eat lower-quality leaves, increasing consumption rates (Fajer 1989). This could have occurred here, resulting in comparable mass in later instars even if food quality differed.

There was only one instance in which caterpillar development rate to fourth instar was predicted by a variable I measured. For those caterpillars that ate *P. lanceolata*, development accelerated for larger groups: the largest groups matured around ten days faster than the smallest ones. For Lepidoptera that feed gregariously, larger groups are thought to thermoregulate more effectively (Seymour 1974) and can develop faster (Long 1953). Temperature and nutritional characteristics of a plant can interact to determine insect development rates (see review by Clissold and Simpson 2015), which could account for why this pattern only held for larvae that fed on *P. lanceolata.*

Note that this positive effect of group size is in addition to the finding in Chapter 3 that larger sibling groups were more likely to survive to third instar. Importantly, in both cases, the pattern applied only to larvae feeding on *P. lanceolata*. Therefore, in contrast to *Castilleja* spp., *P. lanceolata* may be exerting selective pressure for larger egg clutch size. However, I did not manipulate group size experimentally: if the same outside factor (e.g., genotype) influenced both

group size and development time, the observed relationship could be misleading. Additional research on selective pressure for group size should manipulate group size experimentally.

Fourth instar duration and its implications

The date when caterpillars arrived in fourth instar dictated how much time they spent feeding before beginning diapause: those that entered fourth instar early sometimes fed for 10 or 15 days, while those developing later fed for as little as a day or two. This disparity could have very important implications for caterpillar success during diapause. Larvae at the beginning of fourth instar usually weighed 10-25 mg (Figure 2), but fourth-instar larvae that fed for several days sometimes weighed 60 mg or more (unpublished data).

The driving influence on fourth instar duration was mostly oviposition timing, since the amount of time required to reach fourth instar was usually uniform (the notable exception being the effect of group size when larvae fed on *P. lanceolata*). Butterflies that laid eggs earlier allowed their offspring to feed longer in fourth instar before entering diapause. This could represent a strong reproductive advantage conferred to butterflies that emerge and lay eggs early in the flight period. Future work will assess survival during diapause and whether it increases with fourth instar duration.

Surprisingly, duration of fourth instar did not depend on the host species being consumed. Therefore, although *P. lanceolata* usually remained phenologically available, larvae stopped feeding regardless of the identity of their host, apparently in response to other cues like day length. Fourth instar duration did, however, respond to *Castilleja* senescence—albeit unpredictably. The feeding period before diapause was shorter when larvae fed on senescent *C. hispida,* but appeared to lengthen when they fed on senescent *C. levisecta*. This is counterintuitive. A speculative explanation is that larvae on *C. hispida,* which is generally a

higher-quality host than *C. levisecta* (Chapter 3), could have entered diapause facultatively when their food declined because they had already fed enough. In contrast, those on senescent *C. levisecta* could have been required to continue feeding on their marginal food source for longer; an inefficient process because fresh tissues were harder to find.

Conclusion

Differences within and among host plant species failed to influence *E. e. taylori* mass gain, development time, or fourth instar duration—despite the large differences in survival documented in Chapter 3. However, this work uncovered a potentially important driver of diapause survival and adult fecundity: larvae whose eggs were laid early, or who developed quickly, were able to feed in fourth instar for several additional days before entering diapause. Future work should assess whether these individuals are more likely to survive through diapause, and whether they reach adulthood faster. If so, this could represent an important reproductive advantage for adults that emerge earlier, and could be important for understanding population dynamics of *E. e. taylori*.

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Table 4.1. Parameters describing the amount of time larvae spent in fourth instar. Significant predictor variables are in bold.

Host species	**# models**	**Parameter**	**estimate**	**SE**	**Test statistic**	**p**
P. lanceolata	1	intercept	9.055	0.919	t = 9.851	<0.001
		necrosis	-	-	-	-
		fourth instar date	**-3.016**	**0.571**	**t = -5.283**	**<0.001**
		fourth instar date : necrosis	-	-	-	-
Castilleja spp.	2	intercept	7.787	0.664	z = 11.720	<0.001
		necrosis	**-1.576**	**0.528**	**z = 2.983**	**0.003**
		fourth instar date	**-1.9758**	**0.5701**	**z = 3.466**	**<0.001**
		fourth instar date : necrosis	-	-	-	-
		host species : necrosis	**2.686**	**1.352**	**z = 1.986**	**0.047**
		host species : fourth instar date	-0.1214	0.6564	z = 0.185	0.853

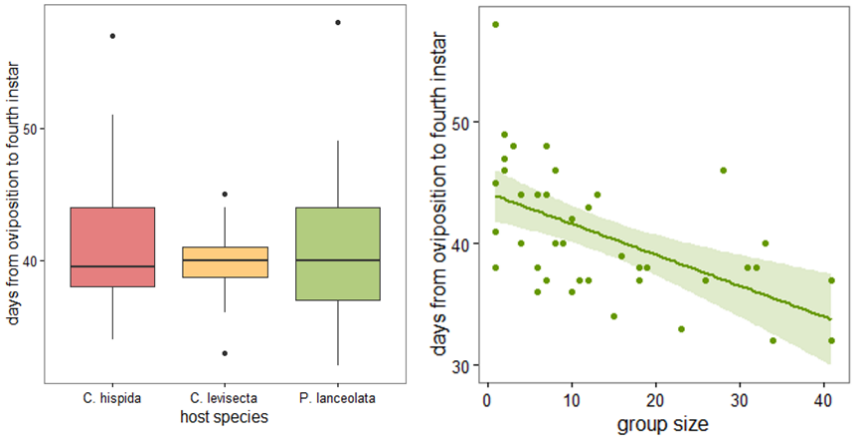

Figure 4.1. Host plant effects on development rate to fourth instar. Left: number of days from oviposition date until caterpillars reached fourth instar on each host species. Right: on *P. lanceolata*, development time accelerated for larger groups. The statistical analysis and plot on the right focus on group size in third instar, but slopes were very similar regardless of the instar considered.

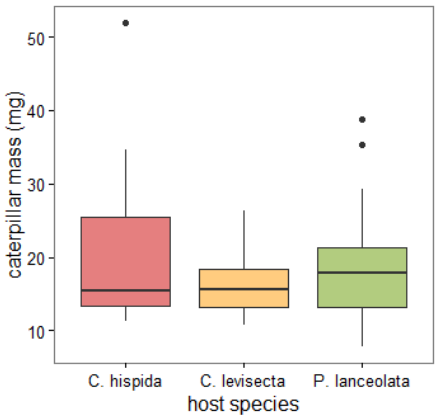

Figure 4.2. Mass of *E. e. taylori* caterpillars just after entering fourth instar.

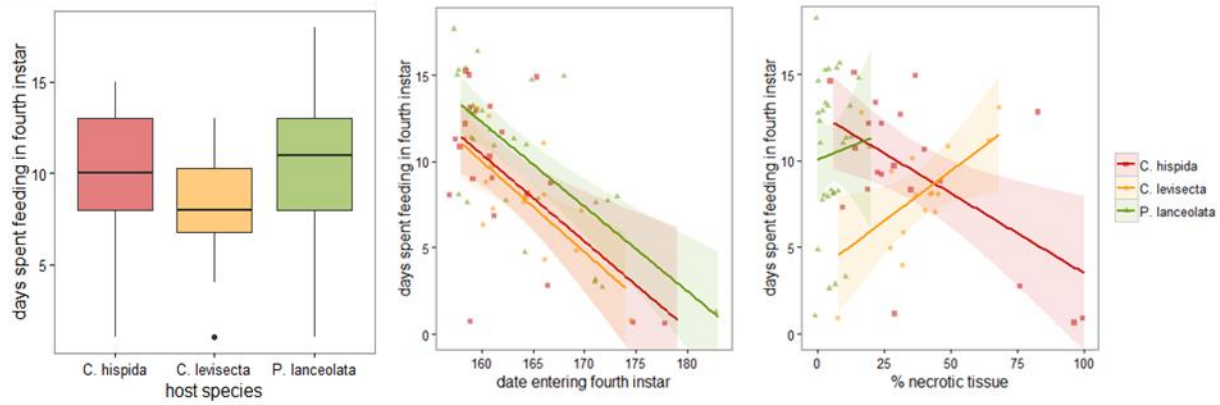

Figure 4.3. Predictors of fourth instar duration. Left: Fourth instar duration on each of the three hosts. Center: When larvae reached fourth instar earlier, they spent more time feeding before diapause. Right: varied relationships between host plant necrosis and amount of time spent in fourth instar. Plantago is shown here for reference although it was not included in the analysis.

Chapter 5: Environmental controls on early-instar *Euphydryas editha taylori* larvae and their host plants

Abstract

I tested for effects of several environmental variables on survival, mass, and development time of early instar *E. e. taylori* larvae. I also tested for environmental controls on the phenology and nutritional suitability of their host plants. I tested for effects of heat load (calculated from slope and aspect) and vegetation structure on larvae, since these variables should influence their thermal environment. I tested for effects of the same variables, plus several additional variables related to soil moisture and nutrients, to see if they influenced the timing of senescence, degree of anthocyanin pigmentation, or C:N ratio of host plants. The three host plants tested were *Castilleja hispida*, *Castilleja levisecta,* and *Plantago lanceolata*. Larvae were unaffected by the variables I measured; there was no effect on survival, mass gain, or development rates. Host plants were affected more strongly; senescence of *C. levisecta* was accelerated slightly in dry soils and those with low nutrient availability. Anthocyanin pigmentation also increased for both *Castilleja* species when they grew in unproductive areas with sparse vegetation. Finally, C:N ratios for *C. levisecta* were much higher when the plants grew in sparsely vegetated, unproductive areas. This work shows that topography and vegetation structure, at least on the spatial scale that I measured them, had almost no direct effects on outcomes for *E. e. taylori* larvae. However, soil and vegetation characteristics may affect them indirectly, by modifying the nutritional quality and phenology of their host plants.

Introduction

The fates of early instar caterpillars depend on their interactions with host plants, but other environmental factors influence them as well. Some environmental effects exert direct controls: for example, temperature is one of the most important factors dictating caterpillar growth rates and foraging behavior (Scriber and Slansky 1981, Casey 1993, Stamp 1993). Since the microsites caterpillars inhabit can fall across a spectrum of vegetation densities, slopes, and aspects, the caterpillars may experience different thermal environments depending on their position in the landscape (e.g., Weiss et al. 1988). Other environmental factors—for example, soil moisture or nutrient contents—could influence caterpillars as well, because differences in soil resource availability can affect foliar nutrients (e.g., Hobbie and Gough 2002). The purpose of this study was to identify environmental variables affecting early-instar larvae of the endangered butterfly, *Euphydryas editha* ssp. *taylori.* I explored ways topography and vegetation structure could affect larvae directly, as well as ways these variables and additional soil characteristics could influence them indirectly by modifying their host plants.

To date, studies of topographic and thermal effects on *E. editha* larvae have mostly focused on the development rate of late-instar larvae (instars 5 and 6), and have been concerned with two *E. editha* subspecies in California, USA (*E. e. bayensis* and *E. e. quino*). In late winter, when caterpillars emerge from diapause, topographic differences in insolation dictate caterpillars' feeding and development rates. Those that feed on warm slopes develop faster, resulting in adults that fly earlier. These are usually the only individuals to contribute eggs that survive to diapause; often, most if not all of the larvae from eggs laid later in the season perish because their host plants senesce quickly (Singer 1972, Weiss et al. 1988, Cushman et al. 1994). Thus, topographic effects on larval development can have important implications for population

growth rates. Post-diapause development has not been studied directly for *E. e. taylori*, but anecdotal observations, and patterns of prediapause mortality, suggest similar processes could affect this system.

Early instar larvae (instars 1 to 4, before diapause) could also be affected by the thermal effects of topography and vegetation structure, but this possibility has not been addressed. Early instar larvae of *E. e. taylori* feed in late spring and early summer; therefore temperatures are higher than for late-instar larvae, but still may range both above and below the thermal optimum while larvae develop. This should influence feeding and development rates (e.g., Sherman and Watt 1973, Casey 1976). At extremes, high temperatures could also cause mortality—prediapause larvae feed just centimeters from the soil surface, which can be in excess of 70^0C at some sites (unpublished data).

Environmental factors could also exert important influences on *E. e. taylori* caterpillars by modifying their host plants' phenology or nutritional quality. These caterpillars often feed on rapidly-senescing host plants (see Chapter 3). Plant senescence is genetically pre-programmed, often coinciding with fruit and seed maturation, but environmental stresses like drought and nutrient limitation can accelerate it (Thomas and Stoddart 1980, Lim et al. 2007). Therefore, the timing of senescence could differ across environmental gradients. The nutritional value of their host plants could also vary with soil nutrient availability, since a plant's nutrient contents in part reflect the resources present in the soil.

In this study I tested whether survival, mass, or development time of prediapause *E. e. taylori* larvae were affected by thermal heat load (calculated from slope and aspect), and also whether these outcomes for larvae varied depending on surrounding vegetation density and structure. I also tested for environmental effects on the senescence phenology, C:N ratios, and

degree of anthocyanin pigmentation for three of their host plant species: *Castilleja hispida*, *Castilleja levisecta*, and *Plantago lanceolata*.

Methods

Detailed descriptions of the experimental layout can be found in Chapter 3, as can descriptions of methods for assessing caterpillar survival, plant senescence, anthocyanins, and C:N ratios. Methods for measuring caterpillar mass at fourth instar, development time to fourth instar, and time spent in fourth instar are described in Chapter 4.

When assessing host plant senescence, anthocyanins, and C:N ratios, measurements occurred at each of the plots where larvae were released in 2015 and 2016 (described in Chapters 3 and 4), plus several additional plots in the same areas that were monitored in 2014 and 2015 but where larvae were not released. In total, there were 217 plot observations for the host plant measurements; 69 plots with *C. hispida*, 66 with *C. levisecta*, and 82 with *P. lanceolata*. Of these, 126 were the plots where larvae were installed in 2015 or 2016.

When assessing the degree of plant senescence, I used data collected during the first week of June, when larvae were typically in second or third instar and strongly affected by differences among their host plants (Chapter 3). Plant samples for C:N ratios were collected at approximately the same time, from a subset of the plots. For anthocyanins, I use data collected during the week of May 15, when many of the eggs were hatching. I chose this date because it preceded plant senescence and most larval feeding. If purple tissues senesced faster, or if larvae fed preferentially some tissues over others, the amount of anthocyanic tissue remaining would be quantified less accurately. In Chapters 3 and 4 plant measurements were staggered in time to correspond to different developmental stages of *E. e. taylori* in each plot. In contrast, here for

each response I use plant measurements that were collected within a day or two of each other, order to make comparisons among plots and species.

Environmental measurements

I measured slope, aspect, and vegetation structure in each plot. I measured slope and aspect using a compass and gradometer, and calculated the thermal heat load using methods described by McCune and Keon (2002, Equation three). I measured leaf area index (LAI) at each plot using an AccuPAR LP-80 PAR/LAI meter. LAI is the amount of leaf area present relative to ground area, so I used it to approximate the amount of shading (and transpirative cooling) in each plot. I also quantified the percent cover of open ground in each plot (bare soil + rock + moss). This latter metric further differentiated between plots with accumulated thatch (mostly dead grass) and those with open, bare substrates which are relatively flat, dark, and tend to heat up more in the sun. Open ground was not very closely correlated with LAI ($r = -0.15$).

I also collected data on several environmental variables that could affect plant senescence rates and nutrient acquisition. Since soil moisture could influence plant senescence rates, I measured volumetric soil water content at every plot, approximately weekly, during the larval feeding period using a Delta-T HH2 theta probe. Each week I took four measurements from four points around the edge of each plot, then calculated the overall average soil moisture for each plot during the larval feeding period.

Several other soil characteristics could influence both leaf nutrition and senescence phenology. Therefore I collected soil samples and measured the proportion gravel (diameter > 2mm) in each sample using a sieve. Soil P, K, S, Ca, Mg and Na, as well as pH, cation exchange capacity, and organic matter content were measured by A & L Western Laboratories (Portland, OR). Soil N was not measured because it fluctuates more quickly than most of these other

nutrients. Soil samples were collected to a depth of 10 cm. For the plots arranged in blocks in 2016, soil measurements were applied to all the plots in that block. Similarly, soils were collected only once from each plot and assumed to be stable from year to year.

Statistical analysis

All analyses were conducted in R version 3.2.5 (R Core Development Team, 2016). First, I used generalized linear models (GLMs) to test whether heat load index, LAI, or open ground influenced caterpillar survival from hatching to second instar, from second to third, and from third to fourth instar, paralleling the approach described in Chapter 3. In each case, I compared models with every combination of the explanatory variables to a null model containing host species and year. I included interaction terms between open ground and heat load, and LAI and heat load, since plots with sparse vegetation or patches of open ground should heat up disproportionately on warm slopes. Finally, I also included interaction terms between host species and each of the variables, since environmental effects could differ depending on the host species being used. Explanatory variables were converted to z-scores before analysis. I did not include block or site in any of the models, in contrast to those in Chapters 3 and 4, because these variables account for the environmental differences among the plots, which are of primary interest for this analysis. Models were ranked based on QAIC ($\hat{c}$ = model deviance divided by the residual degrees of freedom).

I used linear models (LMs) to test effects of these same environmental variables on caterpillar mass, development time to fourth instar, and time spent in fourth instar, paralleling the approach taken in Chapter 4. The model selection procedure was the same as for caterpillar survival. Mass and development time were log transformed to improve normality. Models were compared based on AICc. Both this and the previous analysis omitted two plots that were burned

before all of the measurements could be taken. They also omitted the same plots as were omitted in Chapter 4, where a small number of larvae were not collected for logistical reasons, or were not tracked during fourth instar because the last surviving individual had been collected and returned to the lab.

Next, I assessed the effects of environmental variables on host plant characteristics. Some of the environmental variables were correlated with one another, so I used principal components analysis (PCA) to distill them into a smaller set of uncorrelated synthetic variables (Figure 1). All explanatory variables were converted to z-scores for this analysis. I also adjusted for inter-annual differences in LAI, soil moisture, and open ground by calculating the difference between the average value for each year and the overall mean for that variable, and subtracting that number from the value from each individual plot. I did this to maximize differences among plots and minimize differences among years, which could be due to turnover in field technicians or inter-annual differences in weather patterns. I also removed data from six plots, located at the far southern edge of the study area at Glacial Heritage Preserve. Soil nutrients and pH were much higher here than in any of the other plots, so I treated them as outliers (pH was between 6 and 7 in these plots, whereas no other plots had a pH > 5.5, median = 5.0). These areas were under tree cover until recently, which could account for the differences in soil attributes.

The first axis (PC1) described 29.2% of the variation in the data, and the second (PC2) described an additional 17.1% (Figure 1). The first axis mostly described nutrient availability. Plots with higher scores had higher pH, higher cation exchange capacity, and contained more micronutrients. The second axis correlated with productivity and vegetation density. Plots with high scores along this axis had high LAI, less gravel, and somewhat more soil P, in contrast to gravelly plots with more open ground.

I used binomial GLMs to test for environmental effects on the degree of plant senescence and anthocyanin pigmentation, and LMs to test for effects on C:N ratios. I tested the three species separately, since each could respond to different cues. I did not test for effects on senescence or anthocyanins in *P. lanceolata*, since it does not vary much in this regard and these characteristics had no effect on larvae feeding on this species (see Chapters 3 and 4). I used PC1 and PC2 as explanatory variables, but also heat load and soil moisture. I included these two variables separately because they were of primary interest as causes of plant senescence, and they were not represented strongly by the PCA (especially soil moisture). For each analysis, I compared models including just the 'year' variable to every combination of models including PC1, PC2, their interaction, soil moisture, and heat load. The block and site variables were excluded as in the analysis of larval responses. When required, for each set of models I averaged all models within two of the minimum information criterion to develop model-averaged coefficients (package *MuMIn*, Bartón 2015). I used the full average to calculate coefficients.

Results

Topography and vegetation structure did not influence caterpillar survival to any stage, nor their mass, development time, or fourth instar duration. In the averaged models, none of the effects had slopes differing significantly from zero. Plant characteristics, in contrast, were at least in part influenced by the environmental variables I measured (Table 1, Figure 2). Senescence of *C. levisecta* was somewhat more severe in dry soils, and for both *Castilleja* species, anthocyanin pigments correlated negatively with PC2: plants growing in productive plots with dense vegetation (and more soil P) were less flushed with anthocyanins than those on open, gravelly plots. Leaf C:N ratios in *C. levisecta* were strongly negatively correlated with PC2. That is,

nitrogen-rich plants grew in productive, densely vegetated plots, while those growing in sparse, open plots were very low in N.

Discussion

Caterpillars were remarkably insensitive to changes in topography and vegetation structure among the plots. I had expected both development rate and survival to be sensitive to topography and vegetation structure in this system, but on the spatial scale that I measured them, they were not. The plots differed substantially in heat load and vegetation structure; heat load index ranged from 0.79 (e.g., a 10^0 slope facing north) to 0.96 (e.g., an 18^0 slope facing south; mean = 0.88, SE < 0.01), and open ground ranged from 0% to 60% cover (mean = 17, SE = 1.15). LAI ranged from 0.13 to 1.65 among the plots where larvae were released (mean = 0.55, SE = 0.02). These variables could probably influence outcomes for larvae at some critical threshold, but the ranges that were expressed in this study were apparently within the acceptable range for prediapause larvae. Relationships with host plants, which strongly influenced survival (Chapter 3), may supersede the effects of slope, aspect, and vegetation structure for prediapause larvae.

The insensitivity of pre-diapause larvae to environmental variables contrasts with what is known about the post-diapause stage (at least that of other subspecies), where development rates are governed closely by topography and its thermal effects (Weiss et al. 1988). Pre-diapause larvae feed during a warmer time of year, so we would not expect to observe the stark pattern that can be characteristic of post-diapause larvae (which feed in late winter). However, the absence of a pattern here is striking, given that temperatures usually affect insect foraging, development rates, and size (Casey 1993, Stamp 1993, Kingsolver and Huey 2008).

It may be that finer-grain thermal patterns override the broad physical characteristics I measured. These could include differences between the base and apex of individual host plants, the sun-exposed vs. shady sides of leaves, or the specifics of webbing architecture during first and second instar. These types of small-scale differences are beyond the scope of my study, but investigating them in detail for *E. e. taylori* could be fruitful.

Environmental factors did not have obvious direct effects on larvae, but they did have some effects on some important host plant characteristics. First, *C. levisecta* were slightly more senescent in areas with dry soils and low nutrients (and senescence rates for this species, in turn, influence early-instar survival; see Chapter 3). However, this relationship was weak, and no relationship was detected for *C. hispida*. Plants growing next to one another were often at very different stages of senescence, indicating that other processes are at work beyond broad environmental determinants of senescence. The drivers of senescence in *Castilleja* have not been investigated to my knowledge, but these two species may be more strongly governed by internal processes related to flowering or fruit maturation, or by environmental controls on their growth earlier in the season.

Castilleja levisecta plants were also more senescent in plots with low PC1 scores; that is, in acidic plots with less nutrients and lower cation exchange capacity. However, this pattern only occurred in plots at West Rocky prairie—the other two sites showed no particular trend (Figure 2). I did not include a term for site during statistical analysis, but I show fit lines from each site in Figure 2 to determine if within-site patterns corroborate the overall pattern. Based on these curves, we can conclude that generally, the patterns were consistent across sites, except for the relationship between PC1 and *C. levisecta* senescence. Plants belonging to both *Castilleja* species were less anthocyanic in plots with higher PC2 scores, that is, productive plots with

dense vegetation. However, anthocyanins were not strong predictors of outcomes for larvae (Chapter 3).

C. levisecta C:N ratios decreased substantially in productive, densely vegetated plots (those with high PC2 scores). This was the strongest pattern observed in this study (Figure 2). The average C:N ratio for terrestrial plants is around 36 (sd = 23; Elser et al. 2000), meaning *C. levisecta* in productive plots are nutrient dense, with ratios closer to 20. In contrast, some of those in sparse, unproductive plots had ratios closer to 40, and would require larvae to consume twice as much plant material to accumulate an equivalent amount of N.

In summary, *E. e. taylori* larvae appear remarkably insensitive to broad environmental differences among areas where their host plants are found. Nutritional quality and phenology of *E. e. taylori* host plants vary more strongly along environmental gradients, which could affect outcomes for larvae. Future work should focus on environmental effects on larvae at fine spatial scales, as well as on topographic and thermal effects on larvae when they reach later instars.

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Table 5.1. Model-averaged parameters describing environmental effects on plant senescence, anthocyanins, and C:N ratios of each of the three host species. Significant coefficients are bolded. Dashes are for variables not included in any of the averaged models for that species.

Response	Species	# models	Parameter	estimate	SE	z	p
Senescence	*C. levisecta*	3	intercept	0.127	0.061	31.270	<0.001
			PC1	**-0.148**	**0.022**	**6.826**	**<0.001**
			PC2	0.010	0.021	0.473	0.636
			PC1:PC2	-	-	-	-
			soil moisture	**-0.298**	**0.043**	**6.966**	**<0.001**
			heat load	0.020	0.043	0.466	0.641
	C. hispida	5	intercept	0.154	0.059	29.121	<0.001
			PC1	-0.017	0.029	0.596	0.551
			PC2	-0.005	0.017	0.323	0.747
			PC1:PC2	-	-	-	-
			soil moisture	-0.067	0.080	0.838	0.402
			heat load	-	-	-	-
Anthocyanins	*C. levisecta*	1	intercept	0.415	0.041	-8.270	<0.001
			PC1	-	-	-	-
			PC2	**-0.189**	**0.017**	**11.394**	**<0.001**
			PC1:PC2	-	-	-	-
			soil moisture	-	-	-	-
			heat load	0.020	0.037	0.553	0.580
	C. hispida	3	intercept	0.449	0.050	4.030	<0.001
			PC1	-0.077	0.073	1.052	0.293
			PC2	**-0.211**	**0.046**	**4.569**	**<0.001**
			PC1:PC2	-0.064	0.069	0.936	0.349
			soil moisture	0.027	0.054	0.500	0.617
			heat load	-0.046	0.081	0.590	0.569
C:N ratio	*C. levisecta*	4	intercept	32.380	1.925	16.817	<0.001
			PC1	0.329	0.567	0.579	0.562
			PC2	**-2.836**	**0.675**	**4.204**	**<0.001**
			PC1:PC2	0.439	0.599	0.734	0.463
			soil moisture	-	-	-	-
			heat load	-	-	-	-
	C. hispida	2	intercept	29.155	3.197	9.119	<0.001
			PC1	-	-	-	-
			PC2	-2.106	1.726	1.220	0.222
			PC1:PC2	-	-	-	-
			soil moisture	-	-	-	-
			heat load	0.653	1.502	0.435	0.664
	P. lanceolata	2	intercept	29.764	1.074	27.716	<0.001
			PC1	0.117	0.244	0.482	0.630
			PC2	-0.398	0.527	0.756	0.450
			PC1:PC2	-	-	-	-
			soil moisture	-	-	-	-
			heat load	-0.595	0.640	0.930	0.352

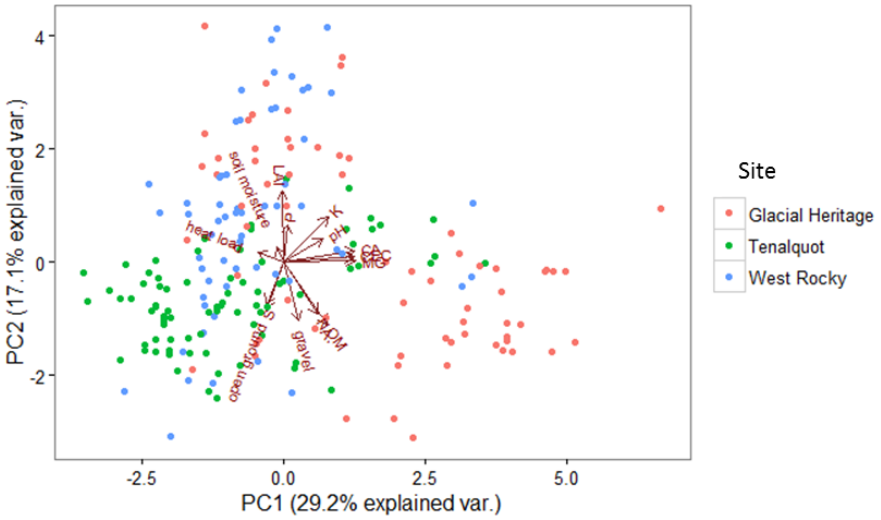

Figure 5.1. Biplot of the first two principal components describing correlations between environmental variables that were collected. Each point represents a plot, and arrows are sized in proportion to loadings for each variable. Points are color coded by site for reference.

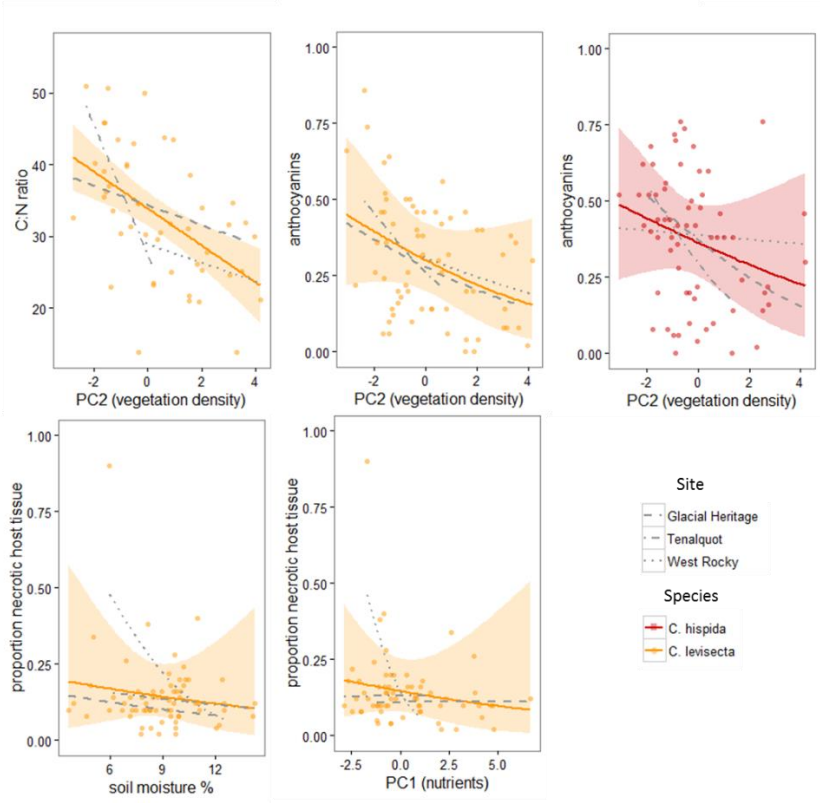

Figure 5.2. Environmental variables influencing various plant characteristics.

Chapter 5 supplement

Exploring thermal characteristics of plots

To provide an illustration of thermal differences among plots and learn about thermal characteristics of different substrates in the plots, I also photographed some of them using a camera equipped with infrared thermal imaging (Flir Systems, Inc.). Thermal imaging demonstrated anecdotally how the substrates present in each plot influenced thermal environments. Figure A1 shows two plots with different vegetation structure at Tenalquot prairie, in late morning on 14 July 2016. Air temperature was 20^{0}C. Note that plants were 30-40^{0}C, while open ground was often in excess of 60^{0}C. Weiss et al. (1988) suggest optimal growth for *E. editha* may occur between 30 and 35^0.

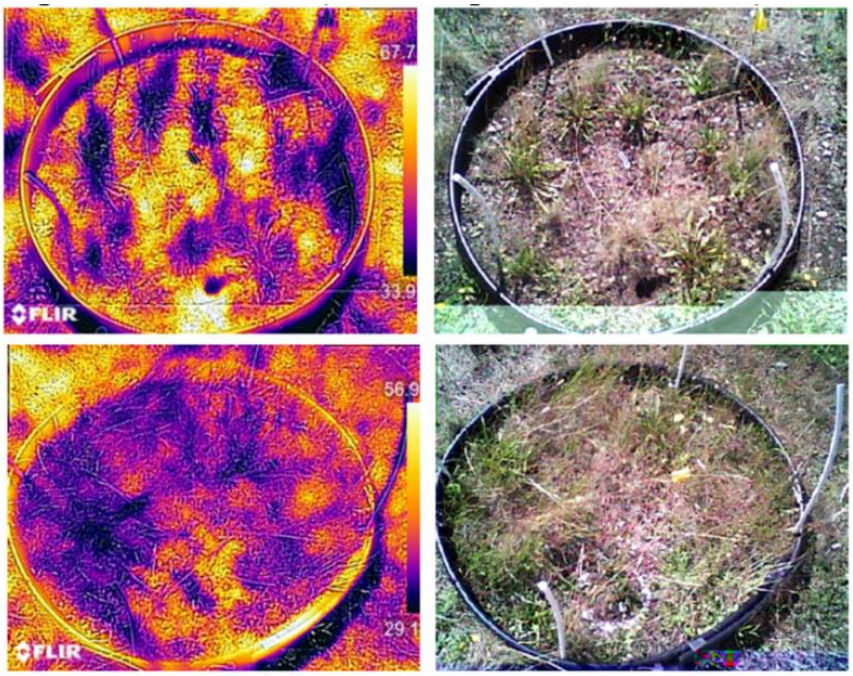

Figure S5.1. Left: thermal images of two contrasting plots at Tenalquot prairie. Thermal images on the left side correspond to photographs on the right. Temperature guides are on the right side of each thermal image.

Overall differences in E. e. taylori host species senescence, anthocyanins, and C:N ratios

The host plants described in this (and other) chapters differ in their senescence phenology, degree of anthocyanin pigmentation, and C:N ratios. Here, I document overall differences among *C. hispida, C. levisecta,* and *P. lanceolata* in terms of these three variables. Methods for data collection are described in Chapter 3. This analysis focuses on 217 plots, which were monitored in 2014, 2015, and 2016. 126 of these contained eggs and larvae in 2015 and 2016.

Statistical analysis

I tested for overall differences among the three host species in terms of their C:N ratios, senescence, and anthocyanins. I used LMMs to test C:N ratios, and GLMMs for the other two variables. In each case I compared a null model containing year, site, and block (random effect) to one that also contained host species identity, and followed significant results with pairwise contrasts using package *lsmeans* (Lenth 2015). Field assessments of plants were made weekly throughout the larval feeding period; when testing for differences I used senescence values from the first week of June and color assessments from the middle of May, which is consistent with the analyses described earlier in this chapter.

Results

The degree of senescence, measured as the amount of necrosis evident on leaf tissues, differed strongly among the three hosts during the larval feeding period (ΔQAICc = 43.99; Figure B1, top). However, while both *Castilleja* species differed from *P. lanceolata* during the first week of June ($p < 0.001$ in both cases), they did not differ from one another at this time ($p = 0.320$). When I ran the same statistical test on data from two weeks later, the two *Castilleja* species differed significantly from each other (with *C. levisecta* being more senescent), but this

would probably not affect larvae as they would mostly have reached fourth instar or diapause by this point in time.

Castilleja hispida plants were slightly more flushed with anthocyanins than *C. levisecta* after taking year, site, and block into account although the difference was much less dramatic than that for senescence (ΔQAICc = 4.72), and visual differences between the two species appear minimal (Figure B1, bottom). Finally, C:N ratios were higher on average for *C. levisecta* than the other two species (Figure B2), indicating larvae would have to eat more *C. levisecta* than the other two species to accrue an equivalent amount of N.

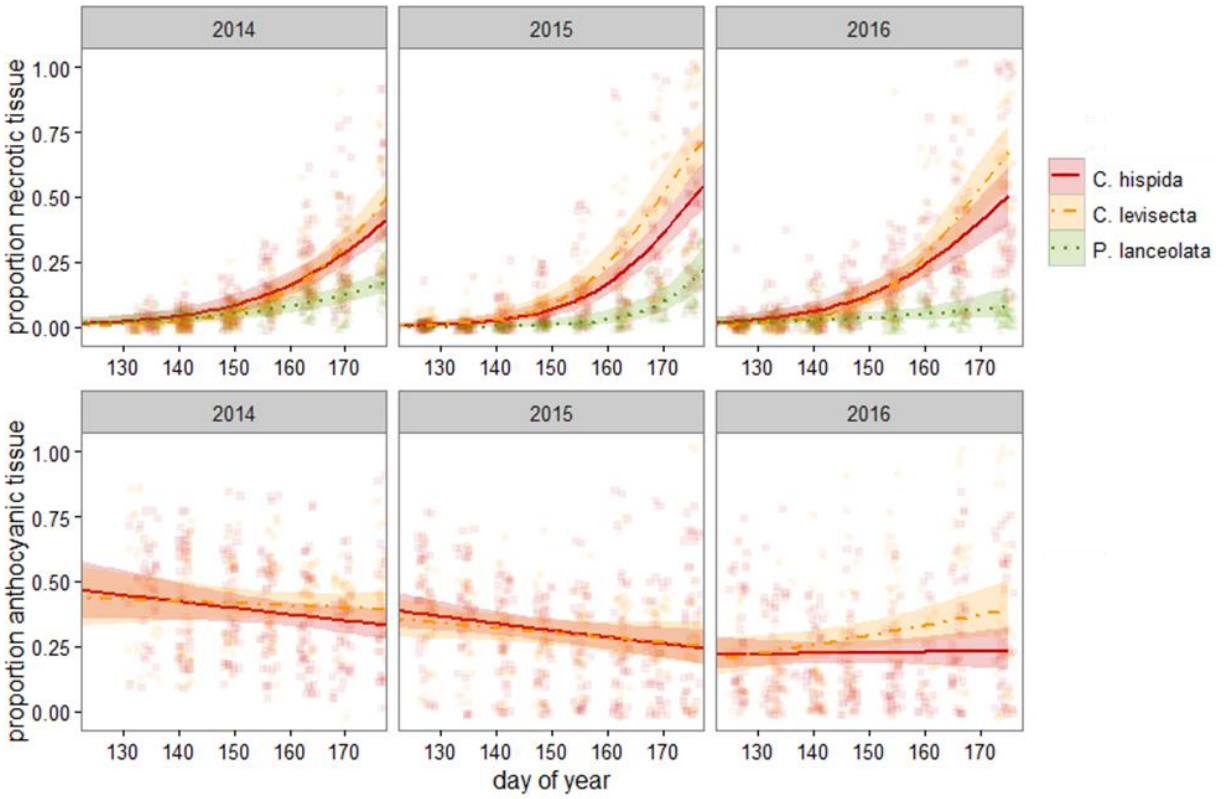

Figure S5.2. Plant senescence (top) and pigment levels (bottom) through time. For reference, eggs are often laid in late April or early May (day ~120), and larvae usually enter fourth instar in mid-June (e.g., day ~165). As a note, even when *P. lanceolata* accumulated necrotic leaves, it usually continued to produce new ones as well.

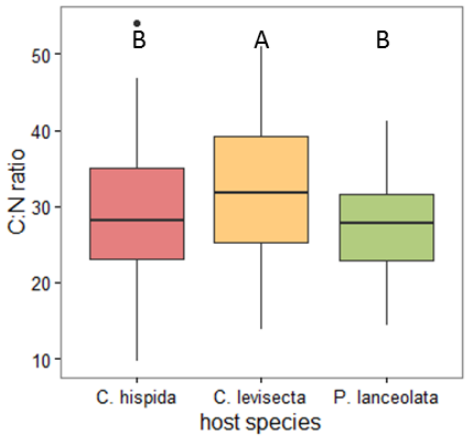

Figure S5.3. C:N ratios of each of the three host species. Those not sharing a letter differed significantly.

References for supplement

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Chapter 6: Sequestered chemical defense in an endangered butterfly and its potential implications for recovery efforts

Abstract

Some specialist herbivores sequester secondary compounds from their host plants, accumulating them as a defense against predators. The identities and concentrations of the compounds they acquire depend on the host plants they eat. I tested whether sequestration of iridoid glycosides by larvae of *Euphydryas editha* ssp. *taylori* (Taylor's checkerspot) depended on the host plant species it ate. This taxon is endangered and subject to active recovery efforts. To a degree, its host plant interactions are determined by land managers, since they typically restore or augment host plant populations and choose where to found new butterfly populations. Consequently, more information is needed about the relative suitability of three host species it utilizes, and whether larvae are able to sequester iridoid glycosides from them. Some of the hosts senesce while larvae feed on them, so I also tested whether sequestration was altered when larvae fed on senescent plants.

We placed eggs on three different hosts in the field—*Castilleja hispida, Castilleja levisecta,* and *Plantago lanceolata*—then collected larvae after four instars of feeding and measured the iridoid glycosides they had sequestered. Larvae that ate *C. hispida* and *P. lanceolata* sequestered moderate amounts; mean total concentration (% dry weight ±SE) was 3.42% (±0.52) for caterpillars on *C. hispida* and 2.31% (±0.37) for those on *P. lanceolata.* Concentrations were markedly lower for those that fed on *C. levisecta* (0.78% ±0.25), calling into question whether larvae feeding on this species accumulate amounts that are adequate to defend against predators. Larvae that ate *P. lanceolata* sequestered aucubin and catalpol; while those that ate the other two species sequestered these two compounds but also macfadienoside

and a fourth compound which was tentatively identified as methyl shanzhiside. Each of the host plant species differed from the others in the proportions of these compounds that they imparted to larvae. Neither the total amounts larvae sequestered, nor the relative abundance of the various individual compounds, differed when larvae fed on senescent hosts compared to non-senescent hosts.

Introduction

Some specialist herbivores co-opt secondary chemical compounds from their host plants, and use them as their own defense against predators (Duffey 1980, Bowers 1993). Butterflies from multiple species in the genus *Euphydryas* feed on plants in families Plantaginaceae, Orobanchaceae, Scrophulariaceae, and a few others, which produce an assortment of iridoid glycoside (IG) compounds (Jensen et al. 1975). Larvae are able to sequester a subset of the array of compounds the plants produce; they accumulate them in much greater concentrations than are found in their hosts, and as a result are unpalatable to predators (Duffey 1980, Bowers 1980). Birds, ants, centipedes, spiders, and other predators have been shown to avoid prey with IGs, or to suffer consequences if they do consume them (Bowers 1980, Bowers 1981, Bowers and Farley 1990, Theodoratus and Bowers 1999, Baden and Dobler 2009, Opitz et al. 2010, Baden et al. 2011).

Euphydryas editha is made up of several subspecies that occur across Western North America. Like other members of its genus, *E. editha* is aposematic and relies on IG sequestration: rather than hiding from predators, individuals are unpalatable and display warning coloration as both larvae and adults (Bowers 1981, Bowers 1993). Some subspecies (e.g., *E. e.*

bayensis, E. e. quino, E. e. taylori) are in danger of extinction and are the focus of recovery efforts.

Each *E. editha* subspecies typically interacts with a small number of host plant taxa (Ehrlich and Hanski 2004). Since they can feed on more than one species, both the overall amounts, and individual identities, of the compounds they sequester should depend on what they eat (Bowers 1980, Gardner and Stermitz 1988, Bowers 1991, Bowers and Williams 1995). This is an important consideration for recovering these taxa: a butterfly population that cannot sequester IGs from its host will be more vulnerable to predation, particularly if predators stop associating their warning coloration with unpalatability.

In this study I examined the ability of prediapause *E. e. taylori* (Taylor's checkerspot) to sequester IGs from its various larval host plants. This butterfly was listed as endangered in 2013 (USFWS 2013), and managers are captive-rearing caterpillars in order to re-establish populations that went extinct and to augment existing ones. This taxon primarily uses three host species in the region where our study occurred. One of them, *Castilleja hispida*, was likely a historical host, and continues to be used with intermediate frequency. Increasingly, butterflies have also come to lay eggs on *Plantago lanceolata*, an exotic species that naturalized in their grassland habitat and is now common. This species is now used extensively alongside *C. hispida*. Finally, the butterflies also use *Castilleja levisecta*, although to a lesser degree. Evidence summarized by Dunwiddie et al. (2016) suggests some populations used it historically, but as both taxa declined they eventually stopped occupying the same sites and, as a result, did not interact for at least a few decades.

Castilleja levisecta is a threatened species with its own recovery program, and as a result of land managers' efforts, it is now locally common at several sites where *E. e. taylori* either has

been or will be reintroduced. This species has also been successfully introduced at sites with extant *E. e. taylori* populations. In both cases, butterflies use it occasionally, and at one site in Oregon, they began using it quite frequently in 2015 and more so in 2016 (T. Kaye, personal communication).

Managers are considering whether to found new *E. e. taylori* populations on sites with *C. levisecta,* and also whether they should introduce *C. levisecta* in areas which are designated as critical butterfly habitat. Some *C. levisecta* populations now number in the tens of thousands, and it would be ideal to combine recovery efforts for both taxa rather than operating them in isolation, since only a few habitat remnants are available (Dunwiddie et al. 2016).

Therefore, I tested whether larvae of *E. e. taylori* were able to sequester IGs from *C. hispida, C. levisecta,* and *P. lanceolata* in the field. I expected both the amounts of IGs they sequestered, and the composition of individual chemicals they took in, to depend on the host they ate. Additionally, both *Castilleja* species senesce while larvae are feeding on them, becoming increasingly withered and necrotic. IG concentrations can increase throughout the growing season (Stamp and Bowers 1994, Fuchs and Bowers 2004, Quintero and Bowers 2012,) but may also decrease with leaf age (Bowers and Stamp 1992). Whether larvae are able to acquire IGs from very senescent leaves has not been explored to our knowledge. Therefore, I also tested whether IG sequestration differed for larvae feeding on more senescent plants of either *Castilleja* species.

Methods

This study was conducted at three grassland sites in Western Washington, USA: Glacial Heritage Preserve (46.87^0 N, 123.04^0 W), West Rocky Prairie (46.89^0 N, 122.87^0 W), and Tenalquot Prairie (46.90^0 N, 122.73^0 W). All three sites are managed for *E. e. taylori*; a population

was recently founded at Glacial Heritage, and the other two sites will likely receive reintroductions in coming years. All three host species (*Castilleja hispida, Castilleja levisecta*, and *Plantago lanceolata*) grow at all three sites.

We established plots centered on patches of each host species at each site (see Chapter 3 of this dissertation for details). I then placed egg clusters, produced by captive *E. e. taylori*, on each host (27 on *C. hispida*, 26 on *C. levisecta*, and 39 on *P. lanceolata*). Larvae were allowed to feed in the plots until they reached fourth instar, at which time I collected one individual from each surviving colony (N = 20 for *C. hispida*, 16 for *C. levisecta*, and 32 for *P. lanceolata*). In each case I chose an individual that was representative of typical larval size observed for that plot (although in most plots there were no discernable differences). I collected them at the beginning of fourth instar because they enter diapause shortly after this point.

We assessed host plant senescence by quantifying the amount of brown, necrotic tissue on the plants. Estimates were made to the nearest 10%, and averaged across five plants in each plot, since third instar larvae usually disperse from their oviposition plant and feed on several adjacent ones. When more than five plants occurred in a plot, I sampled them systematically by marking one plant from each quarter of the plot and selecting plants that were representative of the size distribution that was present. Plants were assessed just after larvae entered third instar, since this stage is when they would have done the most feeding and should have the largest impact on sequestration.

After they entered fourth instar, larvae were returned to the lab, starved for 48h to reduce the amount of plant material in their gut, and weighed. IGs were analyzed using gas chromatography to determine the identities and amounts of IGs they had sequestered. IG methods followed Bowers and Stamp (1997) and Bowers (2003). Caterpillars were frozen, then

ground whole and extracted in 95% methanol for 24 h. The solid material was filtered out and methanol evaporated. After adding the internal standard phenyl-β-D-glycopyranoside (PBG) at 0.500 mg/mL, each sample was partitioned with ether to remove hydrophobic compounds. The ether layer was removed and the water layer (containing iridoid glycosides) was evaporated. The residue was suspended in 1.0 mL methanol, and a 100 µL aliquot removed for analysis. The methanol was evaporated and the remaining residue derivatized using Tri-Syl-Z (Thermo-Fisher Chemical Company) in pyridine before injection into an Agilent 7890A gas chromatograph equipped with a DB-1 column (30 m, 0.320 mm, 0.25 µm particle size) and using flame-ionization detection. Amounts of aucubin, catalpol, and macfadienoside were quantified using ChemStation B-03-01 software. A fourth IG was present, but a standard was not available to identify it unequivocally. Its amount was estimated with a conversion factor based on the internal standard, PBG, since the original amount of this compound in each sample was known. Thus my estimates of this fourth compound are not exact, and based on the assumption that it behaves identically to PBG.

I used R 3.2.4 for all analyses (R Core Development Team 2016). I used linear mixed models with package *lme4* (Bates et al. 2015) to test if the overall amounts (mg) and concentrations (% dry weight) of IGs in the larvae differed depending on the host species they ate. In each case I compared a model containing only site (fixed effect) and block (random effect) to a model that included these terms plus host species identity. Significant results were followed with pairwise contrasts using package *lsmeans* (Lenth 2015). IG concentrations were arcsine square root transformed to improve normality. I tested for effects of plant senescence on IG levels in the larvae using a separate linear model for each host species.

Next I used a multivariate approach to determine if the composition of individual IG compounds in larvae depended on the host species being used, and/or the degree of senescence among the hosts. I used multivariate permutational ANOVA (PERMANOVA), with the adonis2 function in the vegan package (Oksanen et al. 2016). Values were relativized by the total IG amount in each larva so they would express the proportion of each compound that was present relative to the total. I used a Bray-Curtis distance measure, and used 10,000 permutations to develop a pseudo-F statistic. Significant results for categorical variables were followed with pairwise contrasts. The model included site, host species, host necrosis level, and an interaction term between host species and necrosis because the effects could differ by host. This approach used sequential sums of squares, so site was listed as the first term in the model to account for differences that might be attributed to it.

Finally, I used NMDS to visualize differences in chemical composition among the larvae, with the *metaMDS* function in *vegan* (Oksanen et al. 2016). Data were standardized in the same way for this analysis as for the PERMANOVA above, and used a Bray-Curtis distance measure and random starting configuration. The model was run 60 times with 400 iterations each, and used a two-dimensional solution. The final stress value was 0.093.

Results

Caterpillars on the three hosts sequestered different amounts of IGs. This was true of both the overall amounts they sequestered (in mg; $p < 0.001$), and the concentrations of the compounds ($p < 0.001$; Table 1). Caterpillars sequestered the most IGs when they fed on *C. hispida*, intermediate amounts when they ate *P. lanceolata,* and least when they fed on *C. levisecta* (Figure 1). Mean IG concentration (% dry weight ±SE) was 3.42% (±0.52) for caterpillars on *C. hispida,* 2.31% (±0.37) for those on *P. lanceolata,* and 0.78% (±0.25) for those that fed on *C.*

levisecta. Two caterpillars on *C. levisecta* contained undetectably low IG levels; the highest single concentration I observed was 8.6%, for an individual that fed on *C. hispida.*

There were four IG compounds detected in the larvae: aucubin, catalpol, macfadienoside, and another whose identity has not yet verified unequivocally (see Discussion). The chemical composition found in the larvae (i.e., the relative amounts of these compounds) differed strongly depending on the host species that was eaten (Table 3). Pairwise contrasts indicated that larvae feeding on all three species differed from each other in this respect (Figure 1).

Larvae that fed on *C. hispida* contained aucubin, catalpol, large amounts of macfadienoside, and moderate amounts of the unidentified IG. On average, macfadienoside concentrations were about five times those of aucubin or catalpol, and around 2.5 times those of the unknown compound. Larvae feeding on *C. levisecta* contained these same four compounds in at least some cases, but on average contained more of aucubin and the unknown compound than the other two. Out of sixteen larvae that fed on *C. levisecta*, 6 had undetectable levels of aucubin, 8 had undetectable levels of catalpol, 9 had undetectable levels of macfadienoside, and 7 had undetectable levels of the unknown compound. Caterpillars that fed on *P. lanceolata* contained predominantly aucubin and catalpol. I also found low concentrations of macfadienoside and the unknown compound in some larvae that fed on *P. lanceolata* (one individual contained both compounds, and five contained the unknown compound). NMDS plots provide a visual illustration of IG compositions in the larvae (Figure 2; see Discussion for details).

Finally, the amounts of IGs larvae sequestered had no detectable relationship to the senescence status of the plants they fed on (Table 2). Similarly, the relative amounts of the four compounds were not affected by plants' senescence status or its interaction with species identity (Table 3).

Discussion

This study confirms that *E. e. taylori* sequesters IGs from its host plants, suggesting it may be unpalatable to predators, consistent with other *E. editha* (Bowers 1981, Schultz et al. 2016) and with *Euphydryas* in general (Bowers 1980, Bowers 1981, Bowers 1983). It also shows how the sequestration of secondary chemicals hinges on the host plant species being eaten. Since *E. e. taylori* is endangered, it inhabits heavily managed or restored sites, and the hosts it interacts with are largely selected by managers: they decide which species to plant, where to plant them, and where to start new butterfly populations—consequently, they also influence the IG profiles of caterpillars and butterflies in those populations, and potentially their interactions with predators. Generally, successful conservation efforts must ensure that larvae feed on suitable host plants (Thomas et al. 2011). Here, we see that management decisions involving host plants could have ramifications for chemical interactions with predators.

Iridoid glycoside concentrations and identities

Overall IG concentrations in the larvae were similar to, if somewhat lower than, those found for other *Euphydryas.* Adult *E. anicia* can contain 1-10% total IGs (Gardner and Stermitz 1988; *Euphydryas* can retain IGs into adulthood). Seventh-instar *E. phaeton* can contain 15% IGs (Bowers 1993). Concentrations are often highly variable among individuals (e.g., Gardner and Stermitz 1988), and change from instar to instar. For example, Bowers and Stamp (1992) found that for *Junonia coenia*, which feeds on *P. lanceolata*, IG concentrations more than doubled between fourth and fifth instar, and actual amounts they took up increased by a factor of almost seven. Importantly, this study focuses only on IG levels in pre-diapause caterpillars. Therefore, the levels found in other life stages of *E. e. taylori* could be completely different from those I found in these larvae, which were in early fourth instar.

One of the compounds that was measured could not be identified unequivocally, but three lines of evidence suggests it is methyl shanzhiside. This compound is known to occur in *C. hispida* (Mead and Stermitz 1993), and the retention time in the gas chromatograph matched that of methyl shanzhiside relative to the other three compounds (Gardner and Stermitz 1988). It is also one of relatively few IGs that can be sequestered by Nymphalid butterflies (*Euphdryas anicia* in Gardner and Stermitz 1988; *Chlosyne leanira* in Mead et al. 1993). Therefore I refer to it tentatively as methyl shanzhiside, with recognition that additional data could support or refute this.

Each host species imbued larvae with a different chemical composition: those that fed on *P. lanceolata* contained aucubin and catalpol; those that fed on *C. hispida* contained all four compounds but were especially rich in macfadienoside; and those that fed on *C. levisecta* contained the same four compounds, but at very low concentrations and with less catalpol and macfadienoside. The two *Castilleja* species can hybridize (Kaye and Blakeley-Smith 2008) and may have introgressed, which could account for why the same four compounds appear in larvae feeding on both of them. Note that when considering total IG amounts and concentrations in larvae, we assume that each individual compound is equally deterrent to predators, which may not be true. The IG composition in larvae that fed on *C. levisecta* also differs here from that found in a greenhouse study (Chapter 2); this is discussed in Chapter 6.

Larvae that fed on *P. lanceolata* contained aucubin and catalpol, but in some instances also contained the two other compounds, which are not produced by *P. lanceolata*. This could have occurred because larvae fed on *Castilleja* germinants which I failed to detect. Some plots also contained *Plectritis congesta* (Caprifoliaceae) and *Collinsia* spp. (Plantaginaceae), both of which are occasionally grazed on (but usually by fifth- and sixth-instar larvae only; pers. obs.).

To my knowledge, IGs that could occur in these taxa have not been documented. I recorded plant community composition in each plot, though, and only some of the plots in question contained *P. congesta* or *Collinsia* (unpublished data), suggesting the compounds probably came from *Castilleja.*

The distinct profile of caterpillars that fed on each species is analogous to findings for *Danaus plexippus* and the cardenolides it sequesters from its hosts in the genus *Asclepias,* in which profiles found in butterflies give evidence of their larval host plant affiliation (Brower et al. 1984, Seiber et al. 1986). In our study I found obvious differences between larvae that fed on either *Castilleja* species and *P. lanceolata*; larvae from the two *Castilleja* species also differed significantly from one another, but differences were less dramatic since they usually contained same four compounds but in different ratios.

The NMDS plots in Figure 2 provide additional insights into differences among larvae that fed on each species. Each point represents a larva that was sampled, and distances between the points are proportional to their multivariate (Bray-Curtis) differences from each other. Larvae that ate *P. lanceolata* are clustered on the right, and those that ate either *Castilleja* species are in the center and left, with some overlap but also some differences (note that *C. levisecta* point in the bottom left of the plot is actually three points, superimposed on one another because they had identical compositions). Generally, points on the right side of the NMDS plots were dominated by aucubin and catalpol, with a greater proportion of aucubin for points in the upper right and more catalpol for those in the bottom right. Larvae that ate *P. lanceolata* are clustered fairly tightly in this area (except for one that presumably ate *Castilleja* sp.). Larvae that were dominated by macfadienoside and/or methyl shanzhiside populate the upper left portion of the

plots; finally, those with more methyl shanzhiside (and often less macfadienoside) are closer to the bottom of the plot.

Effects of senescence

Plant senescence, as I measured it, had no effect on IG levels in the larvae. This is somewhat surprising, since IGs are known to vary with both leaf age and plant age (Bowers and Stamp 1992, Stamp and Bowers 1994, Fuchs and Bowers 2004, Quintero and Bowers 2012). However, third instar larvae are mobile and able to forage selectively, and they could have focused their feeding in a way that provided consistent IG uptake even if host plants were changing. Plant senescence also had no effect on the composition of compounds found in the larvae: the ratios of different IGs larvae sequestered were unaffected by deteriorating plants.

Low IG levels in larvae feeding on C. levisecta

Most larvae feeding on *C. levisecta* contained very low levels of IGs. *Castilleja levisecta* plants growing in field conditions can contain variable amounts of these compounds; some plants I tested preliminarily contained up to 8% aucubin + catalpol (unpublished data; other compounds, if present, were not quantified). Those grown in the greenhouse contained low levels of aucubin (usually < 1%; Chapter 2, this dissertation). Therefore, it is possible or even likely that IGs were present in *C. levisecta*, but were not sequestered for some reason.

Implications for predation risk and conservation

Predators vary in their sensitivity to IGs. Some ant species avoid concentrations as low as 0.003% (Opitz et al 2010), although others appear insensitive to levels up to 0.74% (Baden et al. 2011). Centipedes are deterred by 2% IG concentrations, but earwigs are not (Baden and Dobler 2009). In our study, larvae feeding on *C. levisecta* contained an average of 0.78% dry weight IGs, and the median value was even lower – 0.34%. Therefore the low IG concentrations I

observed in larvae that ate *C. levisecta* could leave them relatively undefended at this stage in their life cycle.

Euphydryas editha taylori relies on aposematism rather than predator avoidance or cryptic appearance. Therefore, in essence, some individuals feeding on *C. levisecta* in our study were bluffing, relying on automimicry (Brower et al. 1967) but without a strong chemical defense. Variable chemical defense appears to be the rule, rather than the exception, both within and among aposematic herbivore species (Bowers 1988). However, warning coloration without adequate chemical defense should eventually have negative consequences. First, some predators may be able to pick out poorly-defended individuals (e.g., Brown 1984). Second, more generally, poorly-defended individuals should erode the aposematic mimicry ring: if the level of defense in a population is too variable, predators that rely on learning (e.g., Bowers 1980) may not associate the warning coloration with unpalatability, thereby penetrating the defense and consuming more individuals. In an evolutionary context, I speculate this would drive oviposition preference away from *C. levisecta,* or select for increased IG uptake by larvae feeding on this species. But in the short term, the conservation implications may be untenable. More information is needed on whether *E. e. taylori* sequester larger amounts of IGs at later life stages—for example, if they are mostly sequestered (or applicable as a predator deterrent) in the post-diapause stage, differences in concentrations for fourth instar larvae could be relatively unimportant.

One of the remaining *E. e. taylori* populations in Oregon has begun to use *C. levisecta* heavily at a site where it was planted (T. Kaye, personal communication), and other populations use *C. levisecta* at least occasionally in Washington. Land managers are also considering whether to release *E. e. taylori* onto sites with abundant *C. levisecta*, since the available habitat for recovering both taxa is limited, and increasingly the same organizations and personnel are

involved in coordinated recovery for both taxa (Dunwiddie et al. 2016). Unfortunately, our findings suggest that encouraging interactions between *E. e. taylori* and *C. levisecta* may not be an ecologically sound approach—at least in the short term, for early instars, and for the population that was studied. *Euphydryas editha* are notoriously variable (Ehrlich and Hanski 2004), so it is very possible that other populations are able to sequester IGs in adequate amounts, and also that new populations introduced onto *C. levisecta* would adapt quickly.

Future work

In the short term, a logical next step is to test whether larvae in other populations are able to sequester IGs from *C. levisecta,* as well as whether the differences I detected are true for *E. e. taylori* at other life stages. The IGs from the plants in this study are currently being quantified, which will hopefully shed light on why they were sequestered less from *C. levisecta* than from the other two species. The simplest explanation would be if IGs were less prevalent in *C. levisecta* than in the other species. It is also possible that other nutritional difficulties inhibited caterpillars' ability to uptake the IGs even if they were present. If the latter appears true, it would merit more investigation.

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Table 6.1. ANOVA tables describing effects of host species identity (and site) on the amounts (top) and concentrations (bottom) of IGs sequestered by *E. e. taylori.* Satterthwaite approximation was used for denominator degrees of freedom.

Response	**Factor**	**SS**	**MS**	**df**	**F**	**p**
Iridoid glycoside amount (mg)	host plant	1.647	0.823	2, 46.540	12.063	<0.001***
	site	0.107	0.053	2, 40.455	0.782	0.464
Iridoid glycoside concentration (% dry weight)	host plant	0.070	0.035	2, 46.508	9.335	<0.001***
	site	0.000	0.000	2, 40.655	0.056	0.946

Table 6.2. Relationships between the degree of host plant senescence (i.e., amount of necrosis) and the amounts of IGs larvae were able to sequester from them.

Host species	***parameter***	***estimate***	***SE***	***t***	***p***
C. hispida	(intercept)	0.756	0.146	5.185	<0.001
	senescence	-0.003	0.006	-0.537	0.598
C. levisecta	(intercept)	0.348	0.097	3.580	0.003
	senescence	-0.005	0.004	-1.239	0.236
P. lanceolata	(intercept)	0.452	0.085	5.317	<0.001
	senescence	0.011	0.014	0.780	0.441

Table 6.3. PERMANOVA results testing for effects of host species, site, and plant senescence on IG composition in the larvae.

Factor	***df***	***SS***	***Pseudo-F***	***p***
site	2	0.077	1.267	0.273
host species	2	1.425	23.431	<0.001***
senescence	1	0.005	0.171	0.886
host species : senescence	2	0.074	1.212	0.295
Residuals	60	1.824		

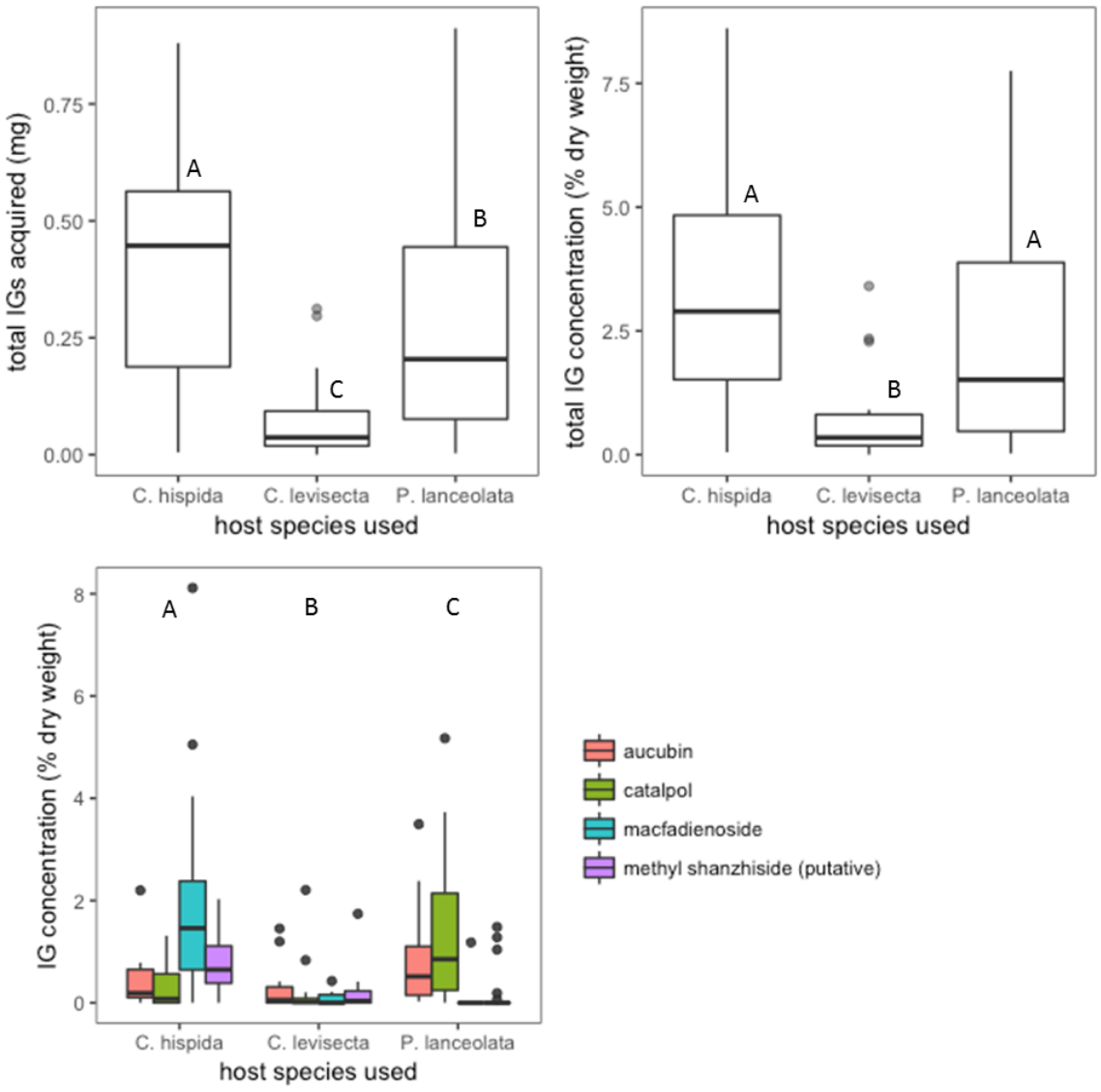

Figure 6.1. Iridoid glycosides sequestered by larvae from each of three plant species. Top left: absolute amounts of iridoid glycosides. Top right: the same data, expressed as concentrations (% dry weight). Bottom: concentrations of each of the four compounds I detected. Letters show results from pairwise contrasts.

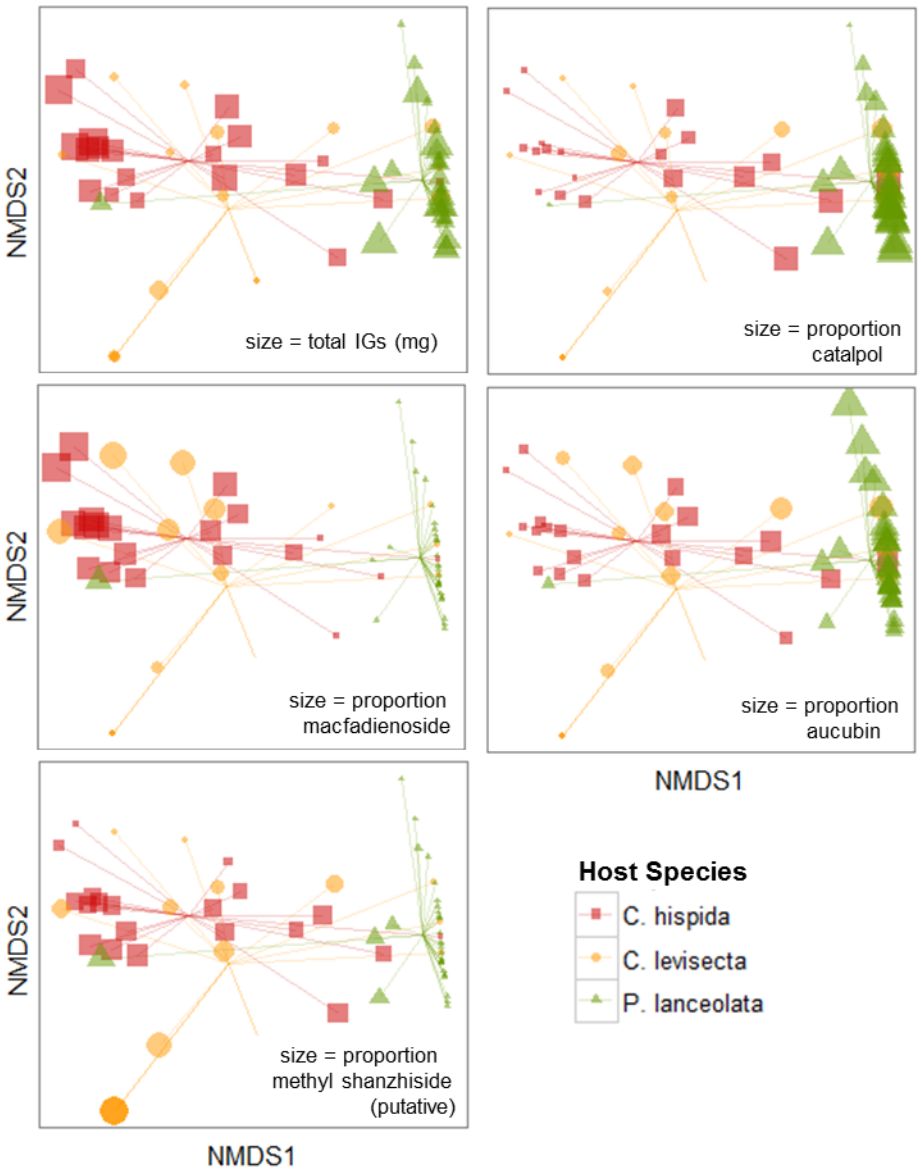

Figure 6.2. NMDS plots showing the composition of IGs in *E. e. taylori* larvae. Each point represents a larva from one of the plots, and color and shape correspond to the host species that was eaten. Lines connect points from each host species to that group's centroid. Each plot is from the same NMDS axes, but in each case points are sized in proportion to a different variable to show how larvae from each plot differed in composition. The darker colored yellow point in the bottom left plot represents three larvae that had identical IG composition—methyl shanzhiside was the only detectable compound they contained. The line segment with no point represents two plots where larvae contained no detectable IGs.

Chapter 7: Synthesis and new questions raised by this research

The studies in Chapters 2-6 describe ecological interactions between *Euphydryas editha* and its host plants. Some of the findings enhance our general knowledge of plant-insect interactions, and of the biology and ecology of hemiparasitic plants. Others address more applied questions. Through experimental manipulation, but also detailed observation, I was able to document several aspects of the early-instar larval life history and interactions of *E. editha* ssp. *taylori*.

Summary of findings

Here is a distillation of some of the broad findings which are described in Chapters 2-6 and should be of general interest to ecologists:

First, hemiparasitic plants can transmit strong indirect effects (Chapter 2). In the system I studied, both the size and nitrogen concentrations in *C. levisecta* depended on the hosts it parasitized (both their identities and the number of parasitic connections formed), and these traits in turn controlled *E. editha* mass and survival as it fed on *C. levisecta*. A hemiparasite's host can also affect uptake of secondary chemicals by herbivores that feed on the hemiparasite; in this system, the ratio of the iridoid glycosides aucubin and catalpol found in *E. editha* depended on the host species being parasitized by *C. levisecta*. This work corroborates other work on hemiparasite-mediated interactions, but also identifies some of the specific plant traits that drive the interaction in this and potentially other systems, which had not been explored in much detail until now.

Next, by explicitly considering organismal ontogeny, we can gain a much more detailed and accurate understanding of species' interactions (Chapter 3). For herbivores, host plant quality is variable, and differences in nutrition, phenology, and secondary chemistry can affect their interactions. However, the factors that influence the herbivore, and the magnitude of their effect, are likely to change as the herbivore develops.

I found that mortality sources for early instar *E. e. taylori* larvae depended on differences both within and among the host species they ate, but also changed depending on larval instar. Different factors affected larvae at different stages, and sensitivity to these factors also changed (mostly decreased) as they advanced to later prediapause instars. Caterpillars undergo changes in terms of their feeding strategy and dispersal ability, both within and among instars. In many ways they are ideal organisms for studying ontogenetic niche shifts, and although ecologists studying Lepidoptera generally recognize that these shifts occur, it could be fruitful to incorporate them more explicitly into ecological studies.

Finally, chemical interactions between specialist herbivores and their hosts can affect conservation decisions (Chapter 6). It is well known that herbivores sequestering plant secondary compounds acquire different amounts and identities of compounds from different hosts. Therefore it is not surprising that that *E. e. taylori* sequestered different amounts and compositions of iridoid glycosides depending on the plants it ate. But fascinatingly, when larvae fed on *C. levisecta*, they sequestered substantially less iridoid glycosides, and the resulting concentrations in their bodies were low enough that at least some individuals were likely insufficiently guarded against predators—at least during early instars. This could have implications for conservation efforts, because managers involved with recovery of both taxa are unsure of whether to prioritize *C. levisecta* as a host plant for *E. e. taylori.* The finding that early

instar larvae sequester only low levels of iridoid glycosides from *C. levisecta* casts some doubt on its usefulness as a larval host, since *E. e. taylori* relies on aposematism, rather than crypsis, to decrease predation risk. More information is needed about whether this pattern applies to post-diapause caterpillars, and to other populations.

Synthesis and new questions for future study

Hemiparasite interactions

Hemiparasite traits like size and leaf N can mediate strong indirect effects. Future work could identify how, specifically, parasitic relationships influence these traits. Which resources does *Castilleja* acquire from its hosts? How does the amount of water, nitrogen, or carbon it acquires change with the identity of its host? To what extent is resource provisioning by a host controlled by the number of haustorial connections that are formed? For example, if *Castilleja* formed the same number of connections on two species, would resource acquisition and the resulting *Castilleja* traits still differ depending on the identity of the host? Some of these questions could be answered by experimentally manipulating haustoria formation, and by using stable isotopes to quantify the actual fluxes of resources passing from hosts to hemiparasites. Ecophysiological measurements that assess how hemiparasites and hosts influence each other's photosynthesis and conductance rates could also provide new insights into their interactions.

Many studies of hemiparasite-host interactions use potted plants in greenhouses, because the plants are easier manipulate in this environment. However, hemiparasites in the field encounter roots from many taxa simultaneously, and generalists probably affiliate with multiple hosts at once. Future study could test the effects of the host community, rather than single hosts, on hemiparasites and their herbivores. Plant communities with different levels of richness,

evenness, or functional diversity could influence hemiparasites (and indirectly, their herbivores) through different levels of resource provisioning and competition. Experimental manipulations along these lines in the field or in mesocosms would show how the patterns that have been discovered in greenhouse conditions translate to field settings.

Phenology of E. editha *and its hosts*

I found that survival of early-instar *E. e. taylori* larvae can be limited by host plant senescence in some cases, and that survival can be higher for eggs that are laid earlier (Chapter 3). I did not study post-diapause larvae, but in other *E. editha* populations, development rate during this stage is of critical importance and determines survival rates for the successive generation: individuals on sunny, warm slopes develop faster, and in some years they are the only butterflies to lay eggs that survive, because pre-diapause hosts senesce quickly (Weiss et al. 1988).

The *E. e. taylori* system I studied involves perennial host plants, in contrast to the annual *Plantago erecta* studied by Weiss and colleagues (1988). It also occurs mostly on sites with less topographic relief, so the phenological constraints may be less dramatic for *E. e. taylori* than for *E. e. bayensis*. Still, in our system post-diapause larvae develop in late winter and spring, when skies are mostly overcast; they spend much of their time basking, and disperse several meters to position themselves on south-facing slopes (pers. obs.). Future research should focus on the interplay between topographic diversity, post-diapause dispersal and development rate, and the population-level implications of these factors. Applied work could also assess whether the spatial distribution of host plants (which is mostly controlled by land managers) is compatible with larval thermoregulatory and dispersal behavior.

In Chapters 3 and 4 I focused on the amount of necrotic tissue on host plants, and used it as an indicator of how senescent they were. However, necrosis marks the end of the senescence process, and usually occurs after nutrients have been reallocated from leaves and water content has dropped. Older leaves are tougher, and deficient in both water and nitrogen (Slansky 1993), but I did not measure their declining quality except to note when they visibly senescent and becoming necrotic. I found that neonate larvae died when they ate plants that were only 10-20% necrotic (i.e., when most of the food on the plant was still available). This strongly suggests that deteriorating plant quality, before the tissues actually die, is what actually affects the larvae. Follow-up lab trials could manipulate the age of the food plants larvae are fed, and quantify how changes in water, N, and iridoid glycosides affect larval survival and mass gain. This would be especially important for first and second instar larvae, who are usually trapped on a single plant.

I also found that when larvae reached fourth instar earlier, they spent much more time feeding before entering diapause (Chapter 4), and these individuals grew quite large (personal observation). As a result they were probably larger and better provisioned with resources when they entered diapause, and might be able to develop to adulthood more quickly if they have a head start when emerging from diapause. Since development time required to reach fourth instar was mostly uniform, the date that larvae arrived in fourth instar depended on when their eggs were laid (although when feeding on *P. lanceolata*, it also depended on group size). This means that early flying butterflies could produce larvae that are not just more likely to survive, but also to fly and lay eggs earlier themselves. This interesting possibility is another area for potential future study, since it could have population-level implications.

Determining population growth rate

My research focused on host plant interactions during just a fraction of the *E. editha* life cycle, but ideally, host plant relations should also be assessed in terms of how they affect population growth rates. *Euphydryas editha* and its relatives have been model organisms for many of the concepts associated with population biology and metapopulation ecology (Hanski 1999, Ehrlich and Hanski 2004). Comparing survival (for all life stages) and fecundity among larvae on different host plants would allow us to calculate population growth rates and understand how they are affected by host plant use. For instance, are rates of increase less than one when larvae feed on *C. levisecta*? Could excessive population growth rates on *P. lanceolata* lead to food plant depletion and subsequent crashes?

Some of this work was recently completed with *Euphydryas phaeton*, comparing population growth rates on *P. lanceolata* to those on the ancestral host *Chelone glabra* (Brown et al. 2017). They found that prediapause and overwinter survival were higher on *P. lanceolata*, post-diapause survival was higher on *C. glabra*, and the overall the population growth rate was higher on *P. lanceolata.* Thus, one could reach differing, and potentially misleading, conclusions about host plant suitability if one life stage is studied to the exclusion of others. Similar work should therefore be done with *E. e. taylori*, quantifying diapause and post-diapause survival rates and adult fecundity. Toward this end, I am currently quantifying survival through diapause.

Evolution of host plant adaptation in E. editha

Some *E. e. taylori* populations may have historically been monophagous, while others could have used multiple hosts simultaneously. Currently, some populations feed on just one of the three host species, while others interact with two or three host species simultaneously

because management efforts introduce multiple hosts. How oviposition preference and feeding strategies sort themselves out remains to be seen.

Different host plant species can apply different selective pressures on herbivores. Therefore, the three host plants in my study system could be applying conflicting selective pressures on *E. e. taylori*. This has been documented for other *E. editha* subspecies. In a system studied by McBride and Singer (2010), populations feeding on different hosts (*Pedicularis semibarbata* and *Collinsia torreyi*) developed contrasting strategies in terms of egg clutch size, oviposition height, and foraging height. Hybrids between the two *E. editha* populations perform poorly because they have intermediate strategies that are maladaptive on either host—this is an example of extrinsic postzygotic isolation, i.e., the discouragement of hybridization between the two populations, and a potential driver of speciation. It is interesting to consider the possibility of a similar process occurring for *E. e. taylori* and its hosts.

For practical purposes, it would be helpful to know whether feeding on *P. lanceolata* results in selection for traits that are maladaptive on the other hosts. As evidence of different host species applying differing selective pressures, in Chapters 3 and 4, I found inverse-density dependent effects when larvae fed on *P. lanceolata,* but not on either *Castilleja* species. When they fed on *P. lanceolata*, larger colonies of second instar larvae were much more likely to survive to third instar, and larger groups also developed to fourth instar faster than smaller ones. This suggests that when *E. e. taylori* feed on *P. lanceolata*, selection could be occurring for larger egg clusters. There is some evidence that larger group size could be maladaptive when larvae feed on *Castilleja*; in the study area I used, *P. lanceolata* grow in denser patches while *Castilleja* patches were usually sparser and contained less plants per unit area (unpublished data).

This, combined with differences in senescence, suggests selective pressure related to egg clutch size could differ by host.

In general, larvae performed as well or better on *P. lanceolata* than on *C. hispida*, and markedly better on either of these species than on *C. levisecta.* Survival was slightly lower on *C. hispida*, but there was no obvious evidence that pre-diapause larvae have lost their adaptations to this species. The superior performance on *P. lanceolata* could be the result of recent selection, as the butterflies used in this study originated from a population that uses almost exclusively *P. lanceolata*. Poor performance on *C. levisecta* could be because *E. e. taylori* lost adaptations to this host in past decades, since the two taxa have had little opportunity to interact. It could also be because the two taxa never interacted extensively (despite evidence to the contrary). It is also possible that *E. e. taylori* populations that were particularly adapted to feeding on *C. levisecta* are now extinct. Detailed comparisons among *E. e. taylori* populations could shed light on how they could adapt to each of these species, and the genetic and evolutionary consequences of switching between them or using more than one simultaneously.

Iridoid glycosides

Finally, our findings related to iridoid glycoside sequestration by *E. editha* raise a number of interesting questions. When we grew *C. levisecta* in the greenhouse (Chapter 2), the only compound we found was aucubin, except for one plant that also contained a small amount of catalpol. Aucubin concentrations were quite low (mean 0.20% dry weight), and other compounds, if produced, were at undetectably low levels. Despite this, *E. e. colonia* larvae sequestered relatively high amounts of aucubin and catalpol (4.2% dry weight; if they sequestered other iridoid glycosides, they were at levels too low to detect).

In contrast, when *E. e. taylori* fed on *C. levisecta* in the field they sequestered more individual compounds (aucubin, catalpol, macfadienoside, and probably methyl shanzhiside), but at much lower concentrations than *E. e. colonia* (mean 0.78% dry weight) (Chapter 5). This difference is puzzling; it could be because of differences between the two *E. editha* subspecies, although this appears to be refuted because *E. e. taylori* sequestered the same four compounds in greater amounts from *C. hispida*. The difference could also be because plants grown in the field and greenhouse express different compounds. We are currently working to identify and quantify iridoid glycosides in the plants measured in Chapters 3-6, which could help us account for some of these differences.

When we quantify the iridoid glycosides from plants in Chapters 3-6, we will also be able to determine whether the environmental variables that were measured (Chapter 4) shaped iridoid glycoside concentrations in the plants, and then whether these differences were reflected in the larvae. This will provide insight into how environmental gradients occurring across the landscape influence plant secondary chemistry and whether they have bottom-up effects on higher trophic levels.

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Appendix A: Anecdotal observations and pilot studies

Here is a summary of pilot studies and observations which were not included elsewhere in this dissertation but would still benefit from being summarized and recorded.

Oviposition trials with golden paintbrush

In lab oviposition trials, Taylor's checkerspot prefers to lay eggs on either paintbrush species over plantain (Buckingham et al. 2016). As a follow-up to this work, I tested oviposition preference in the lab. I enclosed females in pots that contained golden paintbrush and plantain side by side, and allowed them to choose where to lay their eggs. I left each butterfly in its pot for up to 24 hours, but placed them in a new pot if I noticed they had laid eggs. In total, I tested preference of 21 butterflies with a total of 58 trials in which they chose plantain, golden paintbrush, both species, or abstained from laying.

Butterflies chose to lay eggs on only plantain in 20 of the trials, and laid on only golden paintbrush in 19 of them. In ten of the trials, eggs were laid on both species, and in nine, no eggs were laid. No statistical analysis has been run, but this pilot study obviously detected no difference in preference. Among butterflies that laid multiple clusters during the trial, none of them laid exclusively on one species or the other, suggesting individuals (from Range 76) are uniform in their lack of post-alighting oviposition preference.

Pilot release of post-diapause larvae on golden paintbrush

With Peter Dunwiddie, Jon Bakker, Mary Linders, and Cheryl Fimbel

In spring 2013 we undertook a pilot research project to explore post-diapause Taylor's checkerspot larval use of golden paintbrush in the field. We used four study plots; two contained golden paintbrush and were located in the Prairie Habitat Restoration plots, while two were located in extant prairie a few hundred meters away and contained plantain. Within each set of plots, one had been burned the previous year, while the other had not. We released 105 larvae onto golden paintbrush or plantain in each of the four plots (5 larvae each per 21 plants; 420 larvae total).

We revisited them four times over the next month, and counted the number of larvae remaining in each plot. On average, we encountered 32% of the larvae we released. Neither food-plant species nor burn treatment appeared to affect the number of larvae we found. However, larvae released on golden paintbrush appeared to be more likely to remain on the original plant to which they were released, rather than exploring and settling on adjacent plants. Larvae at the burned plantain plot disappeared midway through the study, and we suspect they were eaten by robins which were abundant at the plot. We encountered several adult butterflies in the PHR plots, confirming that post-diapause Taylor's checkerspot larvae can survive on golden paintbrush and reach adulthood.

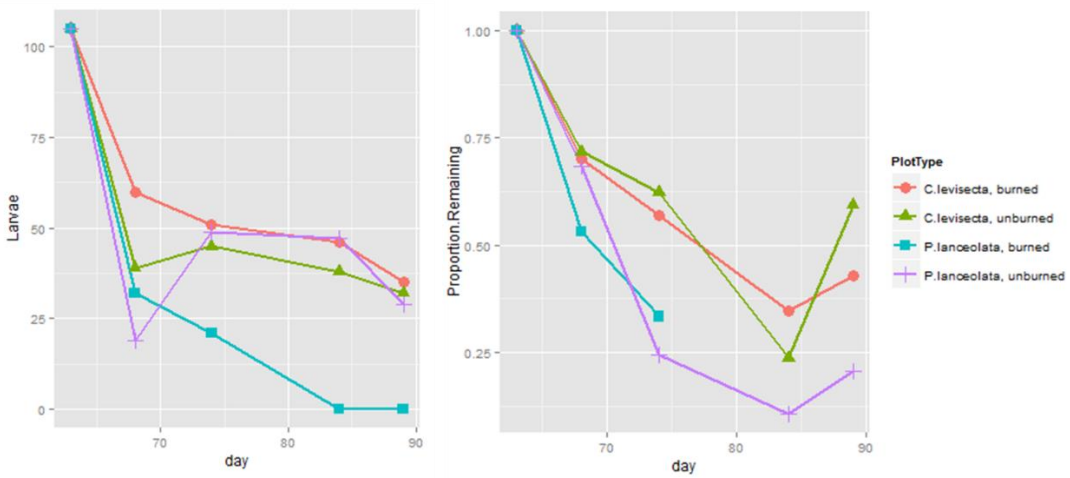


Figure A1. Survival (left) and movement patterns (right) of Taylor's checkerspot larvae on golden paintbrush and plantain. The x-axis represents the day of the year (March 1 = day 60). The plot on the left shows the number of caterpillars we detected during each survey; the plot on the right shows the proportion of those caterpillars that were found on the same plant where they had originally been released.

Identifying diapause habitat
With Susan Waters

In November 2016, we informally searched 40 plots at the three sites where fourth-instar larvae had fed and presumably entered diapause (See Chapter 3). The goal was to identify the substrates and microhabitats that might be used for diapause. We searched one quarter of each plot, combing through as much debris and dead vegetation as possible (we did not remove soil and therefore would not have detected individuals diapausing underground). We found 20 larvae: 17 were housed in Roemer's fescue bunches, 1-4 cm above the soil surface. Two were between scales of Douglas-fir cones, and one was in a tall oat-grass bunch. Almost all of them were diapausing singly, rather than in groups.

This finding, though anecdotal, has implications for management. First, many larvae diapause above ground, where they are much more likely to be affected by controlled burns and herbicide applications. Second, Roemer's fescue may provide important diapause habitat, and could be an important component of high-quality habitat for Taylor's checkerspot.

Predation on pre-diapause larvae
During the study described in Chapter 3, I recorded whenever I saw evidence of predation on pre-diapause larvae. The identities of predators are mostly unknown, as are the magnitude of their potential effects. On two occasions, I found larval nests that were empty (but had been occupied 1-3 days previously), and contained a molted exoskeleton from a European earwig (*Forficula auricularia*). I did not witness the interaction, if there was one, but it suggests that earwigs are potential predators. These larvae were in first or second instar. I also saw a fourth-instar individual, dispersing across the soil surface, which was attacked and carried off by several thatch ants (*Formica obscuripes*).

Testing for indirect effects of host plants on larvae in the field

In Chapter 2 I found that larval survival and mass were affected indirectly by the hosts *Castilleja* parasitized. In the greenhouse, I found that *Achillea millefolium* had the strongest positive effect on larval mass and survival. Here, in an exploratory effort, I tested if the same pattern could be detected among these organisms when they grew in the field.

I documented plant composition for the plots that were monitored in this study, and tested whether the presence of *A. millefolium* correlated with increased caterpillar survival when they fed on either *Castilleja* species. I used plots where larvae fed on *P. lanceolata* as a control: if positive effects of *A. millefolium* were transmitted by parasitism, they should occur when larvae fed on *Castilleja* spp. but not *P. lanceolata.* If the pattern occurred for all three species, then it must be attributable to other factors.

I did not collect spatial data on the physical proximity of *A. millefolium* to *Castilleja* (only whether it was present in each plot) and did not verify if parasitism of this species actually occurred. Therefore, this effort was purely exploratory: absence of a pattern could mean the indirect effect does not occur in field conditions, or equally could mean that *A. millefolium* was not being parasitized.

I used GLMs to test whether survival to second, third, or fourth instar, differed when *A. millefolium* was present in the plot, and LMs to test the effects of mass. I tested each species separately, and compared models with site and year to those that also contained presence/absence of *A. millefolium* in each plot.

Results and discussion

Achillea millefolium was present in 37 of the plots. I found no association with larval mass. Survival to various stages differed when *A. millefolium* was present, but the association was positive in some cases and negative in others (Table 3). Furthermore, in instances where it was positive (e.g., survival to second instar on *C. levisecta*), it was similarly positive on *P. lanceolata*, suggesting any differences in survival associated with the presence of *A. millefolium* did not have to do with its parasitic relationship to *C. levisecta.*

Table A1. Associations between *A. millefolium* presence and outcomes for *E. e. taylori.*

		Host species		
		C. hispida	**C. levisecta**	***P. lanceolata***
Response	*Survival to second instar*	NS	+***	+***
	Survival from second to third instar	NS	-***	+*
	Survival from third to fourth instar	-.	NS	-***
	Mass at fourth instar	NS	NS	NS

"NS" = not significant, "+" = positive effect, "-" = negative effect
Significance levels: . p < 0.1; * p < 0.05, **p < 0.01, ***p < 0.001

Appendix B: Pre-diapause instar guide for *E. editha taylori*

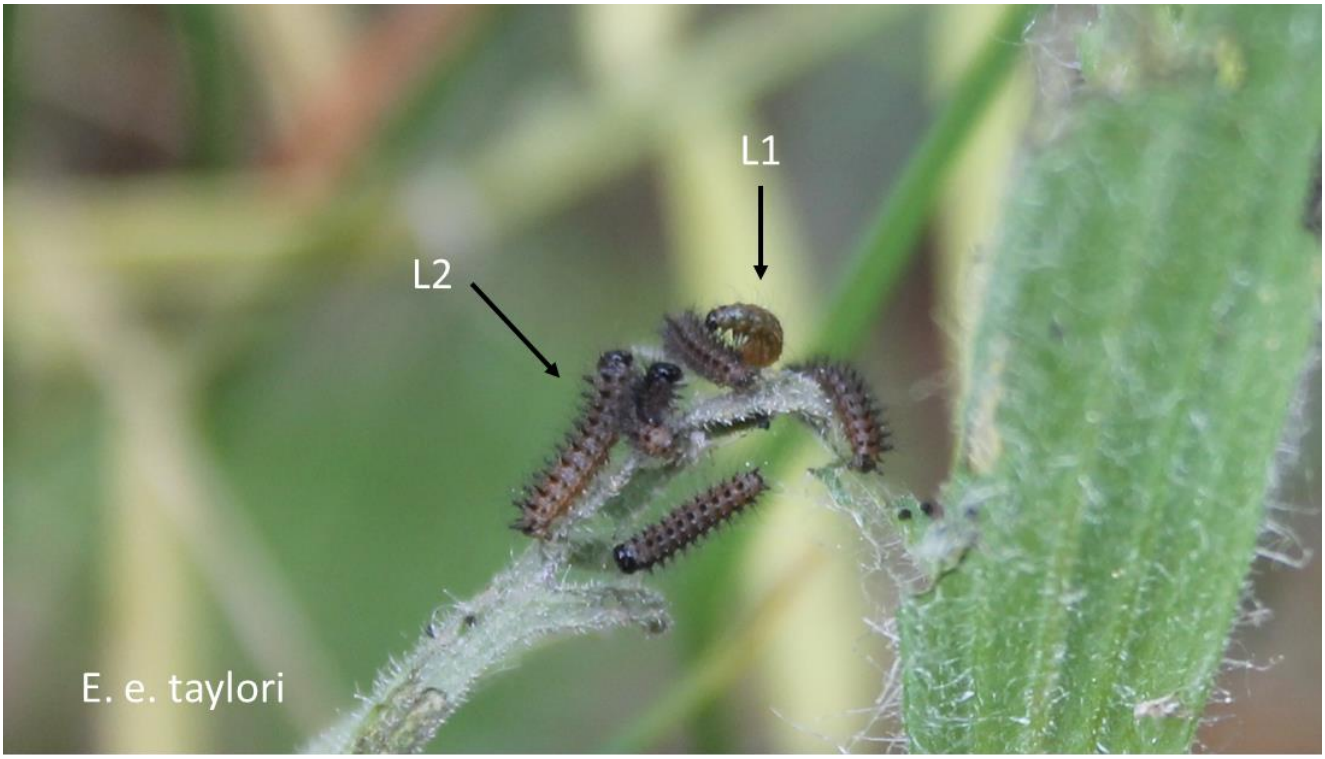
This is a brief guide for determining the instar status of *E. e. taylori*, adapted from a resource I gave to field technicians. The focus is on the first four instars. Photos from the field are *E. e. taylori*, and those from the lab are *E. e. taylori* or *E. e. colonia*. I use "L1" to refer to first instar, "L2" for second, etc. All photos by N. Haan.

First instar

L1 are very small with tan coloration and a black head capsule. Their bristles are made up of setae arranged singly (they are clustered on for all other instars) and are usually hard to see. Late L1 can develop dark spots at the base of setae. They usually stay concentrated in a small area on the plant and spend most of their time in and around webbing, although the extent of the webs is variable.

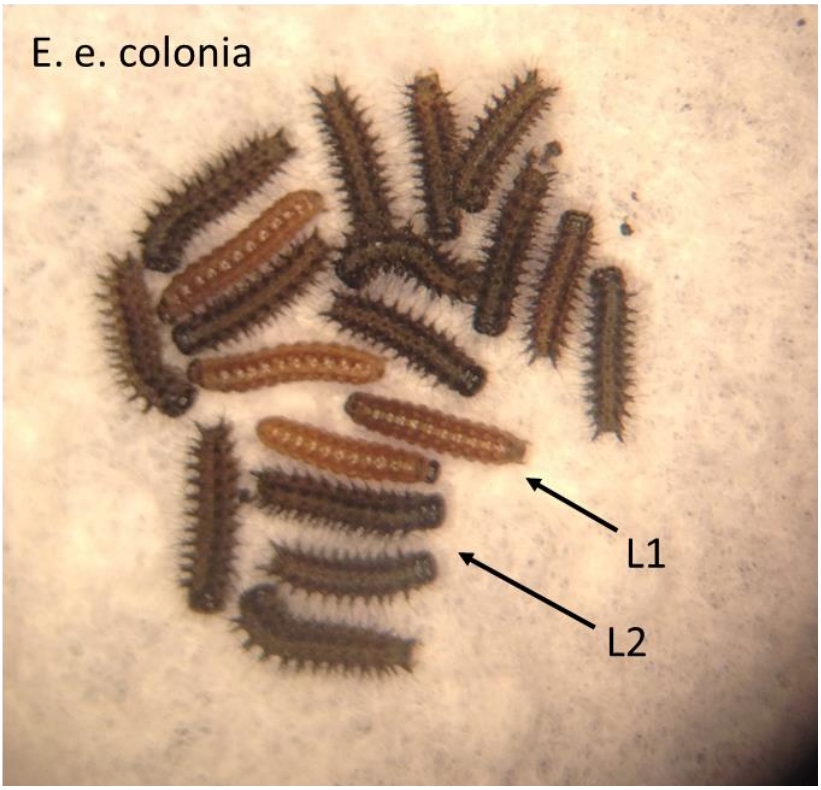
(First instar)

Molt from first to second instar



Second instar caterpillars are darker in color (brown, but not black) and setae are grouped on cone-shaped pegs. There are usually 6-10 bristles per peg.

Note the increase in head capsule size.



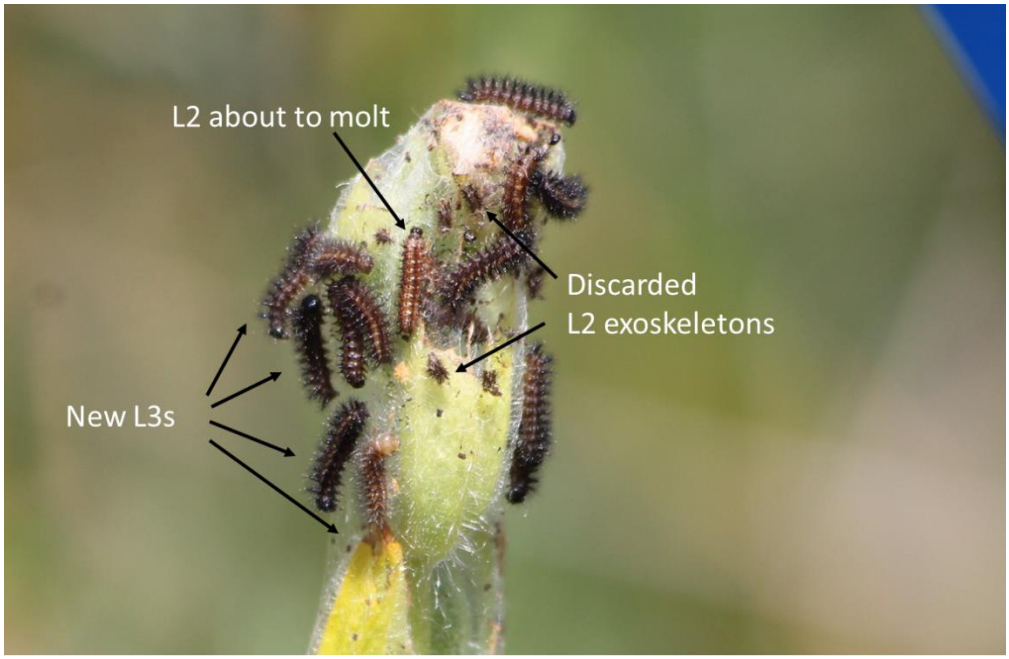
Second instar

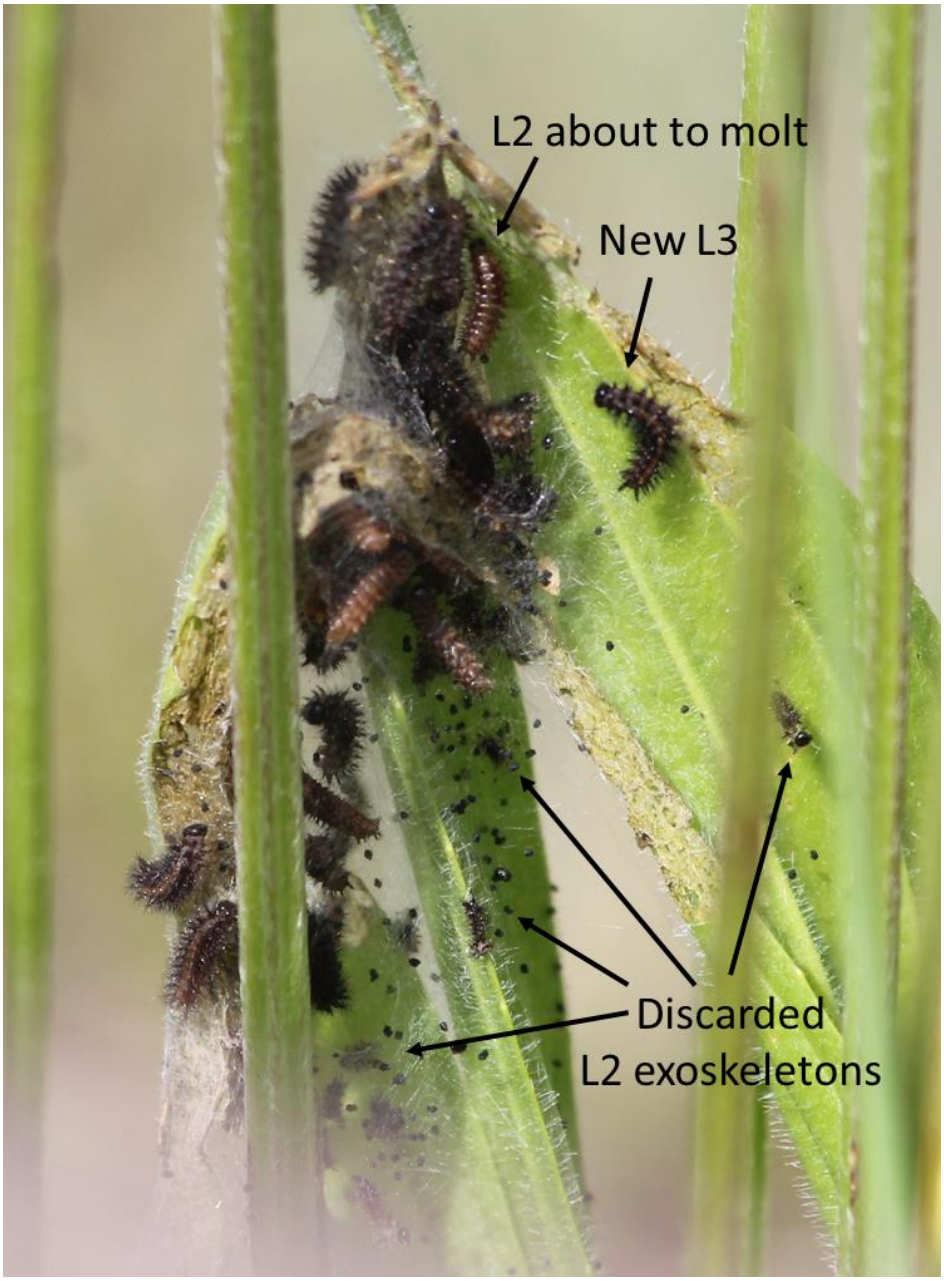
Second instar larvae feed in groups and usually spend time in webbing, but also can be found on any part of their oviposition host. If they run out of suitable food they are able to disperse at least 15-20 cm from the plant where their eggs were laid.

Molt from second to third instar

L3 are darker in color than L2, and often look mostly black. Orange dorsal spots start to become visible, and some gray mottling can show up. As they grow, dark coloration can be diluted giving them a translucent appearance similar to that of L2, but late L3 are much longer than late L2—usually approaching 1cm in length. Setae are packed into denser bristles (>10 per bristle)

At this stage they usually disperse from the plant where eggs were laid and can travel up to a meter, and sometimes spend time on other vegetation.

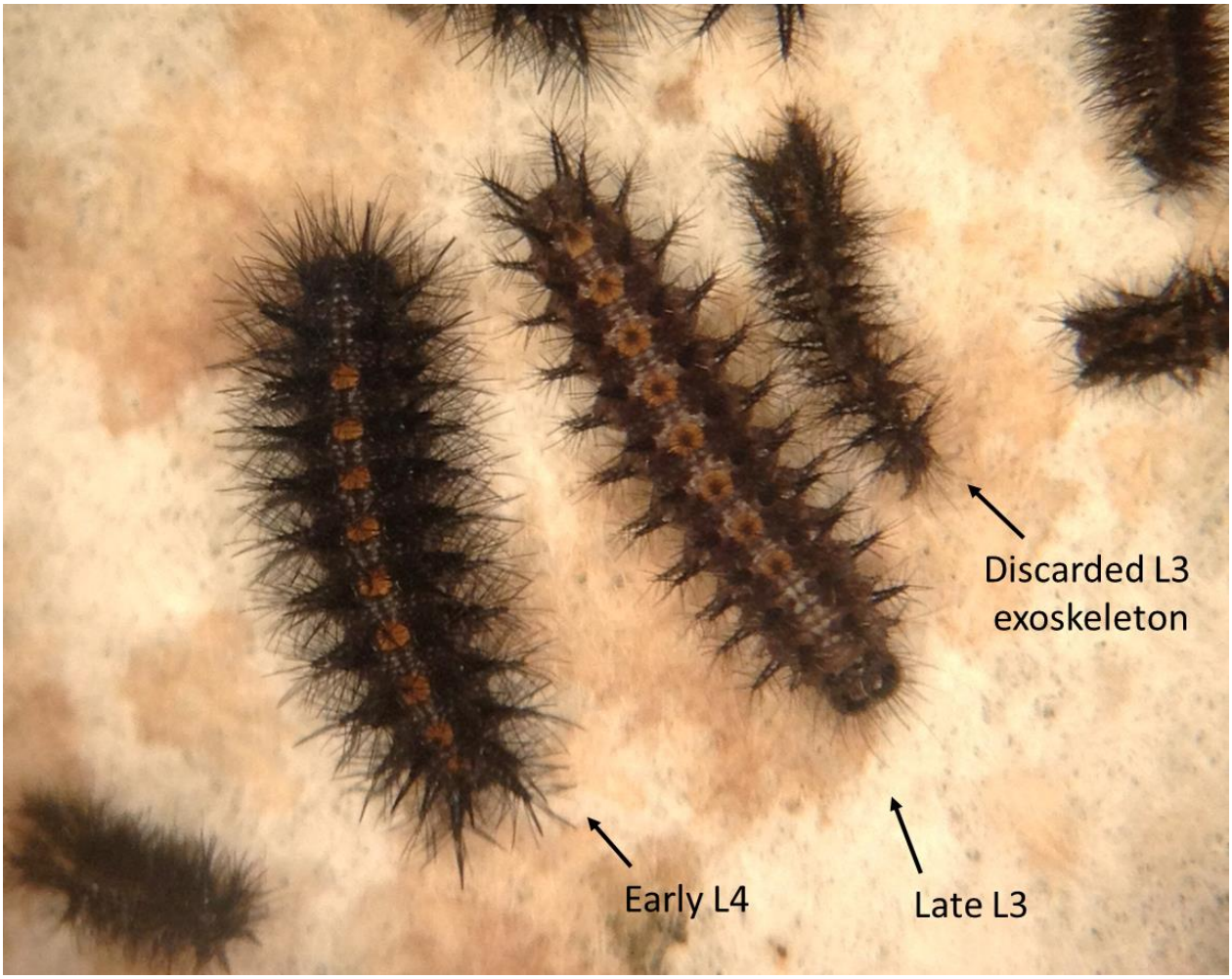



L2 about to molt
New L3
Discarded
L2 exoskeletons


E. e. colonia under a microscope
L3
L3, just molted
L2 exoskeleton

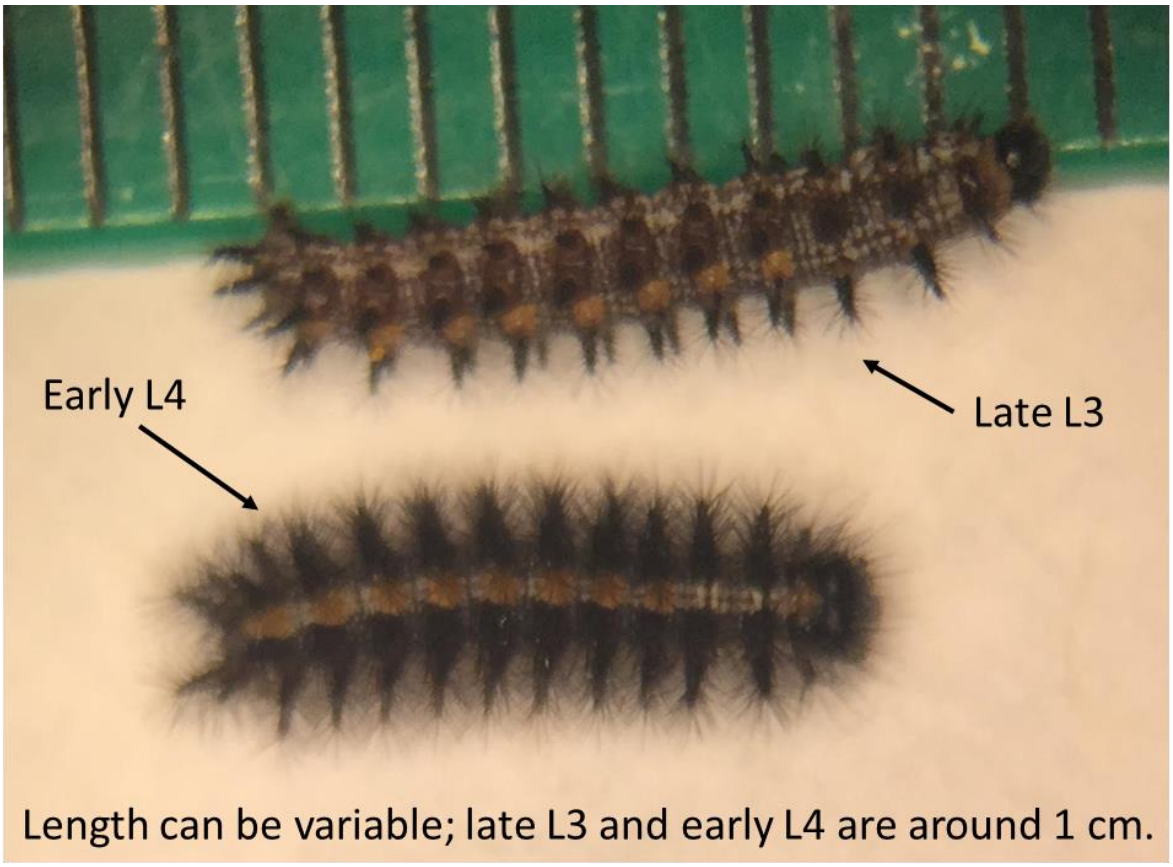
(third instar)

Molt from third to fourth instar

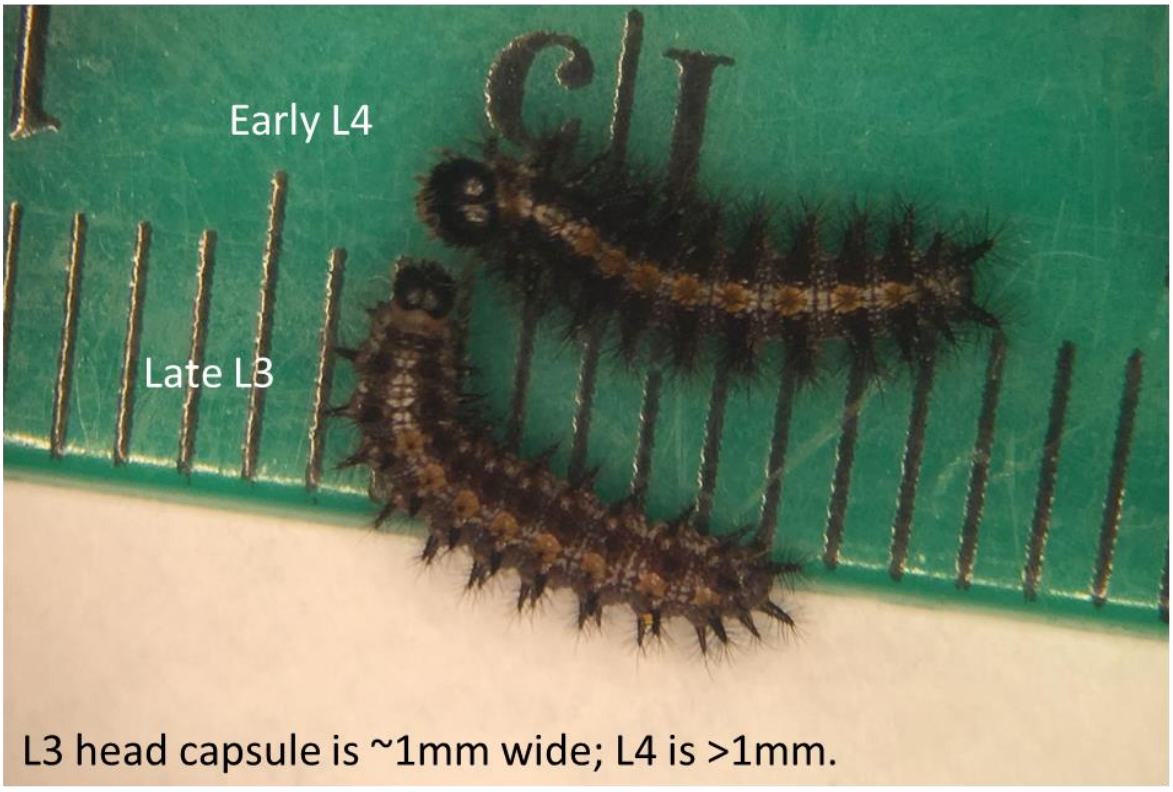


L4 begin at a length of around 1cm, then sometimes double in length during this stage. Bristles are longer and denser. Orange spots are usually prominent at this stage, as is gray mottling. As they get larger, the spots and grey pattern tend to become more prominent.

L4 disperse quickly and can typically move up to a meter. Sometimes they are grouped with siblings, but often forage independently. They do not appear to use webbing at this stage, and also travel and bask on other vegetation and on the soil surface.



E.e. taylori in the lab



(fourth instar)

(fourth instar)

(fourth instar)

(fourth instar)

Appendix C: Additional photos of the study system and experiments

All photos by N. Haan unless otherwise specified

Figure C1. Dorsal view of adult *E. e. taylori*

Figure C2. Ventral view of adult *E. e. taylori*

Figure C3. *Plantago lanceolata*

Figure C4. *Castilleja hispida*

Figure C5. *Castilleja levisecta*

Figure C6. A likely *C. hispida* x *levisecta* hybrid at West Rocky Prairie.

Figure C7. *Castilleja hispida*, *Castilleja levisecta*, and *C. hispida* x *levisecta* hybrids at West Rocky Prairie.

Figure C8. *E. e. taylori* eggs on *P. lanceolata*

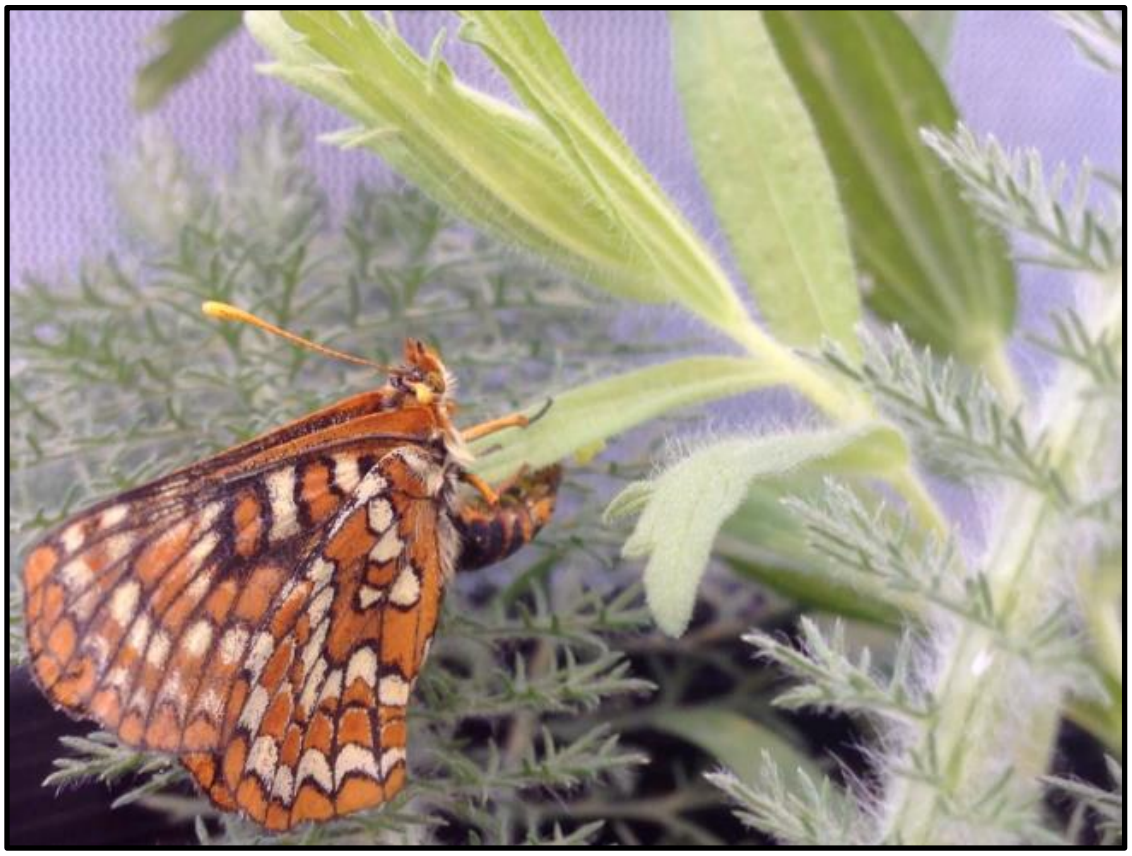


Figure C9. *E. e. colonia* ovipositing on *C. levisecta* in captivity (see Chapter 2)

Figure C10. Potted *C. levisecta* growing with *Eriophyllum lanatum* (Chapter 2)

Figure C11. Caged pots containing *C. levisecta*, various host species, and five *E. e. colonia* larvae (Chapter 2)

Figure C12. *C. levisecta* haustoria formed on the root of *Achillea millefolium.* Photo: Raona Mecka

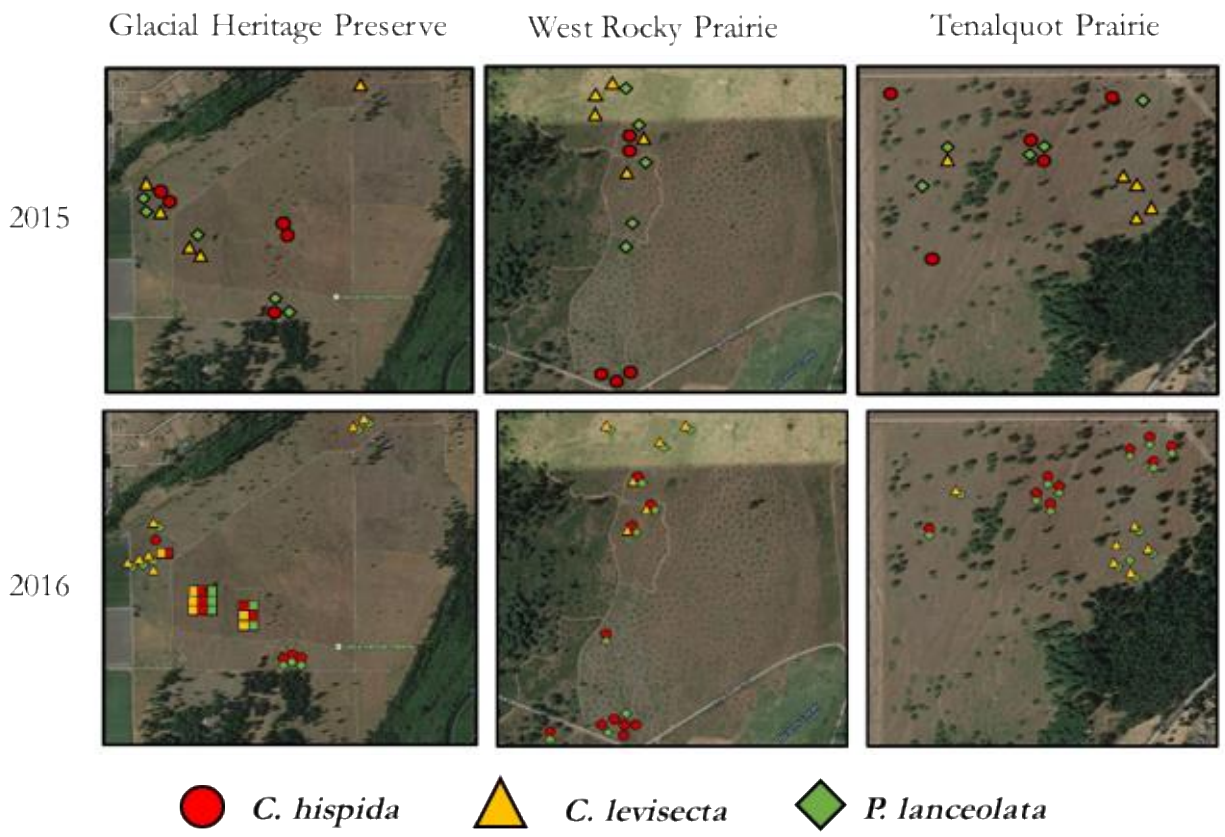


Figure C13. Locations of plots used in the studies described in Chapters 3-6. The square plots in the bottom left panel were seeded in 2014 and 2015, while all others were extant plants.

Figure C14. Plots under construction. Each caged plot contained a patch of five or more of one of the three host species (Chapters 3-6)

Figure C15. Cages used to restrict caterpillars to their assigned host species and exclude predators (Chapters 3-6)

Figure C16. Egg installation. Left: installing eggs on a host plant with paddle forceps. Top right: PVC cylinders (covered with Tanglefoot) used to exclude insect predators from neonate larvae. Bottom center and bottom right: plants with egg clusters and hatchling larvae. Installation sites were created with paperclips to secure the eggs and provide shelter from rain (Chapters 3-6).

Figure C17. Time series of a single *C. hispida* plant at Glacial Heritage preserve. The photos were taken approximately weekly for a month, beginning in mid-May and ending mid-June (Chapters 3-6).

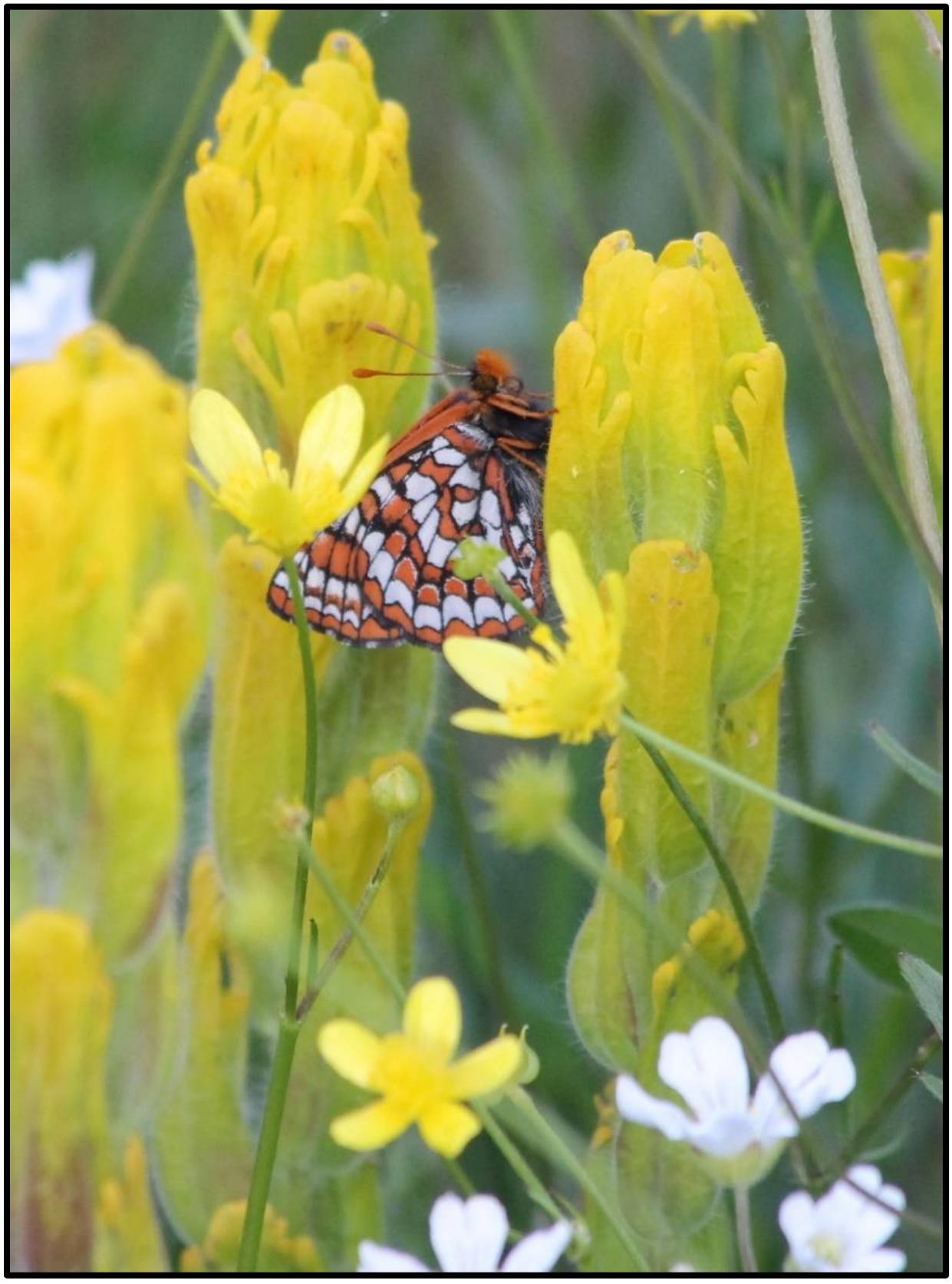


Figure C18. *E. e. taylori* resting on *C. levisecta* at Glacial Heritage Preserve.