

EXPLORING TRADEOFFS OF ALTERNATIVE LIFE HISTORY STRATEGIES IN
TWO TEXAS BUTTERFLY SPECIES

by

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DEDICATION

I would like to dedicate this work to all the future Latinx scientists, and to my four siblings Avana Bree, Abram Three, Amaya Cree and Alain Zee.

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ABSTRACT

The evolution of gregarious feeding is an intriguing problem in ecology. It occurs in many phytophagous insects and typically coincides with eggs laid in large clutches. Despite many benefits to gregarious feeding, including accelerated larval growth rates, not all species feed gregariously suggesting disadvantages to gregariousness. To investigate the advantages and disadvantages, I studied a system of two sympatric, congeneric butterfly taxa that employ drastically different oviposition and larval feeding strategies. The Emperor butterflies both lay eggs on Hackberry trees (*Celtis laevigata*, *C. reticulata*); the Hackberry Emperor (*Asterocampa celtis*) lays a single egg and caterpillars feed alone, while the Tawny Emperor (*A. clyton*) lays a large clutch and caterpillars feed gregariously. To explore whether gregarious feeding drives clutch size differentiation while simultaneously filling in natural history information on *Asterocampa* butterflies this research addressed the following questions: 1) Is there a difference in emergence in terms of relative abundance of *Asterocampa* between sexes or species? 2) Do *Asterocampa* species have an oviposition preference between *Celtis* (Hackberry) host plants? 3) Do *Asterocampa* larvae experience enhanced performance in gregarious feeding groups? I predicted that the advantage of gregarious feeding would be realized for *A. clyton*, where females lay large clusters of eggs. Fruit baited traps were used to capture females. Phenological variation in relative abundance was analyzed with partial correlation coefficients. Female oviposition preference was assessed with a choice experiment with leaves of both host trees. Caterpillar group sizes were manipulated

across the two host plant species. The group sizes approximated ranges from nature. Caterpillar performance was measured by average weight gained and proportion of caterpillars reaching their second instar. Oviposition preference was quantified using a Bayesian hierarchical model. Caterpillar performance was analyzed using an ANOVA in R. There were no differences in relative abundance across time. Females of both butterfly species preferred to oviposit on *C. laevigata*. I found significant differences in weight gained between group size treatments and caterpillar species but not between host species. I also found a significant difference in proportion reaching second instar between group size treatments, caterpillar species and host species. My results indicate a disadvantage to being gregarious and demonstrate that solitary feeding can enhance caterpillar performance; thus, the predicted benefits of gregariousness for caterpillar performance were not observed. The advantages of gregarious feeding were not realized in terms of caterpillar performance but might be related to defense against natural enemies.

I. EXPLORING TRADEOFFS OF ALTERNATIVE STRATEGIES

Plant Insect Interactions

The interaction between plants and their herbivores is the most common association on the planet (Thompson, 1988; Jaenike, 1990; Forister et al., 2015). These interactions can range from exploitative to mutualistic; however, since most of these interactions can be detrimental to plants, their association can escalate into an evolutionary arms race (Berenbaum and Zangerl, 1998; Law et al. 2001; Augustine and Kingsolver, 2017). Plant-insect interactions are the products of coevolution as herbivorous insects adapted to specific host plant defenses and plants evolved new defenses against the insects (Ehrlich and Raven, 1964; Berenbaum, 1983; Agrawal, 1998). The evolutionary arms race between plants and insects has been focus of research in evolutionary ecology because of the diversity it generates.

An essential component of the evolutionary arms race is the production of plant defenses against herbivory. These defenses probably included mechanical defenses, such as thorns or trichomes, or novel secondary chemicals, that were effective deterrents against herbivorous insects because they changed host plant suitability as food and allowed an evolutionary radiation of host plants into new places as they escaped herbivory. This was followed by herbivorous insects developing successful ways to circumvent specific host defenses, allowing herbivorous insects to exploit their host plant resources (Ehrlich and Raven, 1965; Benson et al. 1975; Berenbaum, 1983). There are

many cases where evolutionary arms races can be witnessed and this phenomenon has been studied in a wide variety of systems (Benson et al., 1975; Benson, 1978; Berenbaum; 1983; Thompson, 1988; Agrawal, 1998).

Plant-insect interactions are thus important model systems to understand the selective forces behind the adaptations that arise from evolutionary arms races (Benson et al., 1975; Seastedt and Crossley, 1984). Herein I investigate the impacts plant-insect interactions have on insect's life history strategies using a series of experiments focusing on the oviposition strategies of adult butterflies and on the feeding strategies of their larvae. These experiments were designed to explore the basis of different clutch size and feeding groups between two closely related species.

Plant Defense

From the plant's side of the evolutionary arms race, numerous forms of resistance to insect herbivores have evolved which can be classified as chemical or mechanical, which are either constitutive or inducible. Constitutive defenses are always active and because of this the host plant incurs a continuous energetic cost (Westra et al., 2015). Plants or plant parts more likely to be attacked by herbivores often have constitutive defenses, while plants less likely to be attacked use inducible defenses (Wittsock and Gershenson, 2002). Inducible defenses are turned on in response to an attack on the host plant (Benson et al., 1975).

The most vulnerable hosts and parts of host plants typically have more constitutive defenses in place (Zangerl and Rutledge, 1996). Some examples of

constitutive mechanical defenses include trichomes, thorns, tough leaves, tree gum, and latex; these defenses reduce the herbivore's ability to access vulnerable host plant tissues (Benson et al., 1975; Young, 1987; Agren and Schemske, 1993; Agrawal, 1998; Speight et al., 1999; Karban et al., 2000). The localization of silica to more vulnerable parts of the leaf effectively defend the host plant by toughening the leaf tissue (Zanger and Rutledge, 1996; Benrey and Denno, 1997; Fordyce, 2003). Waxy leaves can indirectly make herbivores more vulnerable to predators by reducing herbivore ability to remain on the host, and is also a direct defense against leaf mining (Price et al. 1980; Eigenbrode and Espelie. 1995; Speight et al. 1999). Mechanical defenses can also contain constitutive chemical responses, for example, as herbivorous insects cut through mechanical defenses, like glandular trichomes, sticky substances or deadly toxins can be encountered preventing them from reaching the vulnerable tissue (Speight et al., 1999).

Constitutive chemical defenses can be found in the most vulnerable parts of host plants, such as the seeds and fruits (Speight et al., 1999). Varying by host, these vulnerable parts of the host plant contain toxins that are detrimental to herbivorous insects in ways ranging from preventing effective nutrient absorption to injuring the herbivore (Wittsock and Gershenzon, 2002). For example, furanocoumarins are phototoxic because the double-bonded furan ring reacts with UV light allowing it to crosslink the herbivore's DNA and interfere with transcription (Berenbaum, 1978; Berenbaum 1983; Berenbaum and Zangerl, 1998). Plants can compartmentalize their toxins in specialized vacuoles to avoid auto-toxicity (Berenbaum, 1983; Speight et al.,

1999). Often by combining different compounds, host plants are able to release complex poisons against herbivores to debilitate or even kill them, thus plants with unique chemical defenses will be chemically defended from most insects (Price et al., 1980; Wittsock and Gershenzon, 2002).

Induced defenses are those elicited in response to herbivory. Following the initial damage by herbivorous insects, an induced response, which can be mechanical or chemical, is activated against the herbivores (Benson et al., 1975; Agren and Schemske, 1993; Raffa et al., 1998; Fordyce and Agrawal, 2001; Fordyce, 2003). Increased trichome density on new leaves grown after an herbivory event is an example of an induced mechanical defense (Agrawal, 1998). Likewise, thorns on branches that have been grazed can increase in length and sharpness to prevent further herbivore damage (Young, 1987). Certain compounds can interfere with digestive efficiency of the herbivores and prolong their development which is dangerous to the herbivorous insect because prolonged development increases their risk of natural enemy attack (Price et al., 1980). Effective induced chemical responses against herbivory include compounds such as saponins and alkaloids that reduce the host plant's palatability and are often toxic or mutagenic to herbivores (Gibbs, 1974; Benson et al., 1975). Direct induced responses such as these detrimentally impact herbivore development and/or reduce the quality of the host plant (Havill and Raffa, 2000).

Tritrophic Interactions

Indirect induced responses such as releasing volatile organic compounds in response to herbivory can enhance the foraging success of the herbivore's natural enemies (Price et al., 1980; De Moraes et al., 1998; Speight et al., 1999; Kessler and Baldwin, 2001; Fordyce, 2003); therefore, a third trophic level can be regarded as part of the host plant's suite of defenses (Price et al., 1980; Turlings et al. 1995; Havill and Raffa, 2000). The origin of these intricate tritrophic interactions is still not entirely understood. It is possible that the resulting tritrophic cascade began when plants released a chemical response to attract the natural enemies or that the natural enemies have evolved the ability to sense the chemical cue of the host plant and effectively prey upon plant herbivores (Turlings et al., 1995; Schmitz et al., 1997; Speight et al. 1999).

Natural enemies, such as predators and parasitoids, can affect the community structure of insects on their host plants, for example, an attack by a natural enemy can obliterate a group of feeding larvae and give the host plant a reprieve from herbivory (Jaenike, 1990; Stamp and Bowers, 1990). Natural enemies can reduce herbivory in two main ways: predators can directly prevent host plant injury by consuming the herbivores feeding on them, and predators can indirectly protect the host plant when their presence induces a behavioral change in the herbivorous insect's grazing (Turlings, et al., 1995; Schmitz et al., 1997). Parasites and other natural enemies are often suppressors of herbivory; they can circumvent damage, like full defoliation, to host plants by killing the herbivorous insects consuming plants (Turlings et al. 1995; De Souza Tavares et al.,

2012). Herbivorous insects have been known to shift foraging time and thus reduce their feeding in the presence of predators (Schmitz et al., 1997). When herbivorous insects shift their behavior in the presence of natural enemies, the reduction in feeding time (which translates to a reduction in energy intake) can increase the herbivorous insect's risk of starvation and their exposure to natural enemies (Schmitz, et al., 1997).

By residing in the host plant some predators can provide protection by lowering herbivory with their presence (Hölldobler and Wilson, 1990; Turlings, et al. 1995; Speight et al. 1999). Hosts plants can produce structures specifically designed for natural enemies to reside in as shelters (Turlings et al., 1995). These shelters can produce a mutualistic benefit for host plants and herbivore natural enemies (Turlings et al., 1995). One example of this mutualistic behavior is the ant-Acacia symbiosis; ants find shelter in Acacia thorns as well as a food source from Beltion bodies (modified leaf tips) while the host plant is directly protected from herbivorous insects by the ant's presence on patrols (Janzen, 1966; Carroll and Janzen, 1973; Rischet al., 1977; Hölldobler and Wilson, 1990; Turlings et al. 1995). Because of their influence on herbivore foraging capabilities natural enemies can play a central role in determining the diet range of herbivorous insects (Ehrlich et al.,1988).

Insects Overcoming Defenses

Herbivorous insects are shaped by both the plants they eat and the natural enemies that eat them, thus, insects feeding on their host plants have evolved mechanisms to overcome host plant defenses and evade natural enemies (Fordyce and Agrawal, 2001).

Foraging patterns of herbivorous insects are influenced by the quality of their host plant and by the lethality of their natural enemies (Heinrich, 1979; Slansky and Rodriguez, 1987; Stamp and Bowers, 1990). Due to the wide variation in chemical, mechanical, and phenological characteristics of plants, and because the main task of herbivorous larvae is to eat, herbivorous insects are more likely than not to have specific adaptations that overcome their host plant's defenses to increase their foraging efficiency (Benson et al., 1975; Benson, 1978; Heinrich, 1979; Jaenike, 1990). Herbivorous insects can respond cooperatively or individually to increase their survival against natural enemies (Turlings et al., 1995).

From avoidance to sequestration, herbivorous insects have evolved many ways to overcome host plant chemical defenses. Lepidopteran larvae can avoid intoxication from host plant's chemical defenses by cutting or 'trenching' upstream of their feeding site or induce galls to provide an inhabitation with altered chemical composition (Tune and Dussourd, 2000; Wittsock and Gershenozen, 2002; Straka et al., 2010). For example, specialist herbivores attacking milkweeds often trench to block the flow of latex to their feeding site (Dussourd and Eisner, 1987; Ehrlich et al. 1988). Umbellifer feeding herbivores can avoid the phototoxicity of furanocoumarins by rolling leaves to shield themselves while they eat (Berenbaum, 1983). The above methods protect the herbivore from the host's chemical defenses through toxicity avoidance.

Some herbivorous insects can alter their own biology by turning on enzymatic pathways to detoxify the chemical compounds they consume. Some of these pathways

involve enzymes (often triggered by p450 genes) that can metabolize mutagens (Nebert and Gonzalez, 1987). For example, many umbellifer feeding specialists can overcome the phototoxicity of coumarins through Cytochrome p450 mediated metabolism (Berenbaum and Zangerl, 1998). These detoxification pathways are one method herbivorous insects can employ to reduce the efficiency of the host plant's chemical defense (Lindroth and Bloomer 1991; Broadway, 1995; Fordyce and Agrawal, 2001).

Another way herbivorous insects can overcome host plant defensive compounds is by sequestering those toxins to the cuticle of their body, this method serves the dual purpose of also rendering the herbivorous insect toxic to their natural enemies (Price et al., 1980; Fordyce and Agrawal, 2001). Lepidopteran larvae have been known to sequester the toxins of their host plants, thus creating a paradox by turning the host plant's toxin into their own defense against predators and parasites (Brower, 1958; Rothschild and Schoonhoven, 1970; Fordyce and Agrawal, 2001). Herbivorous insects can consume plants that are toxic to their natural enemies, by sequestering these toxins and employing aposematic coloring, herbivores can sometimes benefit from predators learning to avoid them (Sillén-Tullberg, 1988; Prokopy and Roitberg, 2001).

Herbivorous insects can overcome host plant mechanical defenses in many ways. One might expect larvae to avoid areas of the host plant with the most defenses, but large groups of caterpillars are often seen on these parts of the plant because they have the highest quality resources (Zangerl and Rutledge, 1996). The foraging behavior of the large herbivore groups are more effective at overcoming plant mechanical defenses thus

enhancing the herbivore's feeding success (Agren and Schemske, 1993; Agrawal, 1998; Agrawal, 1999; Fordyce and Agrawal, 2001; Fordyce, 2003; Fordyce and Nice, 2004; Westra et al., 2015). Herbivorous insects are also known to overcome trichomes by spinning silk to tie them up (Speight et al., 1999).

To increase their foraging efficiency herbivorous insects have also evolved many adaptations to stay on their host plant (Juniper and Southwood, 1986; Speight et al., 1999). Modified tarsi allow herbivorous insects to maneuver through trichomes and other host plant mechanical defenses (Speight et al., 1999). Silking allows herbivorous insects to anchor themselves to their host plant, larvae can climb their silk back if they are dislodged from the leaf; likewise rolling leaves closed can both keep them on their host and hide them from their natural enemies (Stamp, 1980; Stamp, 1984; Speight et al., 1999). Leaf galling insects modify their host plant tissue into a favorable habitat that simultaneously protects them from their natural enemies (Speight et al., 1999; Straka et al., 2010; Nability et al., 2013). Adaptations such as these allow the insects to increase their feeding efficiency by securing them to their host.

Natural enemies are a leading contributor to herbivorous insect's mortality (Price et al., 1980; Speight, 1999). To reduce their mortality, herbivorous insects can defend themselves from predators and parasites at multiple life stages. For example, an egg cluster's design can garner the greatest protection for the eggs by reducing the amount of surface area exposed to mortality causes such as natural enemies or desiccation (Stamp 1980; Friedlander, 1986). Variation in clutch size can reduce the effectiveness of egg

parasitism by natural enemies; single eggs are easier for females to hide from natural enemies, while larger clutches can protect a greater number of individual eggs (Stamp, 1980; Friedlander, 1986). Lepidopterans are also known to vary the hardness or thickness of egg chorion to impede drilling by natural enemies (Stamp, 1980; Gross 1993; Jervis, et al. 2001). Typically, when eggs are clustered together at oviposition, larvae can more readily locate each other and by remaining together create a gregarious feeding group (Stamp, 1980; Clark and Faeth, 1997; Fordyce and Nice, 2004). Gregarious feeding is often observed as a strategy employed by herbivorous insects to protect themselves from natural enemies (Stamp, 1980; Prokopy & Roitberg, 2001). There are several other benefits that arise from feeding gregariously, but not all herbivorous insects employ this strategy. The diversity of herbivorous insect feeding styles highlights a gap in our knowledge on the tradeoffs of gregarious feeding.

Gregarious Feeding

Gregarious (aggregative) feeding occurs when organisms feed as a group. This strategy, used to overcome plant defenses and evade natural enemies, has evolved independently at least 23 times in Lepidopteran families (Stamp, 1980; Sillén-Tullberg, 1988; Prokopy & Roitberg, 2001). The evolution of aggregative feeding in herbivorous insects is linked to multiple larval benefits that include: overwhelming the host plant defenses, a faster development time, higher survivorship through dilution or cooperative defenses, enhanced aposematism, and improved thermoregulation (Stamp, 1980; Sillén-Tullberg, 1988; Clark and Faeth, 1997; Benrey and Denno, 1997; Denno and Benrey,

1997; Bryant et al., 2000; Fordyce and Nice, 2004; Fordyce and Agrawal, 2001; Fordyce, 2005; Allen, 2010; McClure and Despland, 2011; Fiorentino et al., 2014).

First instar larvae are typically more vulnerable to predators than older instars, but feeding in gregarious groups can allow them to more successfully overcome host plant defenses as well as experience enhanced evasion from predators either through dilution or cooperative defense (Fordyce and Agrawal, 2001). It is well documented that gregarious groups of caterpillars can more successfully overwhelm host plant defenses and thus forage more adeptly (Agren and Schemske, 1993; Agrawal, 1998; Agrawal, 1999; Fordyce and Agrawal, 2001; Fordyce, 2003; Fordyce and Nice, 2004; Westra et al., 2015). This increase in herbivore forage efficiency leads to higher per capita nutrient intake. Therefore, gregarious feeding might improve larval performance and lead to an accelerated growth rate (weight gain, faster time to next instar) which according to the slow growth-high mortality hypothesis (longer development in herbivorous insects results in more exposure to natural enemies and consequently increases larval mortality) will reduce the herbivore's window of vulnerability to natural enemies (Clancy and Price, 1987; Agrawal 1999; Reader and Hochuli, 2003).

Insects feeding gregariously can also avoid being eaten through dilution effects, where being in a bigger group reduces the individual odds of natural enemy notice (Gross, 1993). Herbivorous insects feeding in a group can employ cooperative defenses to fend off predators and parasites either by silking to create protective clonal webs, or more active defenses such as thrashing, squirming, or whipping their head (Stamp, 1980;

Stamp, 1984; Gross, 1993; Fordyce and Agrawal, 2001). Aposematic coloring can be enhanced in gregarious groups and reduces larval predation risk (Sillén-Tullberg, 1988). Aposematism could have originated from the increased noticeability that coincides with being in a gregarious group (Stamp, 1980; Sillén-Tullberg, 1988). Enhanced thermoregulation is also an important benefit, especially to ectotherms because group feeding extends the temperature range in which feeding can occur and increases larval survival (Heinrich, 1979; Stamp, 1980; Bryant, et al., 2010).

However, not all species feed in aggregations, suggesting there are some costs to this strategy (Prokopy and Roitberg, 2001; Campbell and Stastny, 2015). For example, exploitative competition can occur between siblings in feeding aggregations (Codella and Raffa, 1995; Denno et al., 1995; Prokopy and Roitberg, 2001). The increased transmission of pathogens is another negative side effect of being in a gregarious group (Hochberg, 1991; Jaenike, 1990; Prokopy and Roitberg, 2001). Another example of an adverse effect of group feeding is the increased apparency to natural enemies which results from host plant overcrowding (Codella and Raffa, 1995; Denno et al. 1995; Prokopy and Roitberg, 2001). Since aggregative feeding can increase the feeding efficiency of herbivorous insects the likelihood of host plant defoliation is increased and once their food source has been depleted larvae will have to move to a new foraging location. On their journey to a new foraging location larvae are more visible to natural enemies (Stamp, 1984; Prokopy and Roitberg, 2001). The tradeoff relationship of the

detrimental effect of these costs compared to the advantageous effects of the above-mentioned benefits is still not well known.

Study System

The focus of this research is two closely related species: *Asterocampa celtis* and *A. clyton* that differ dramatically in clutch size and the size of feeding groups (Stamp, 1980; Friedlander, 1987). These species are sympatric throughout that portion of their geographic range in South-Central Texas where they utilize the same two host plants (Hackberry trees, *Celtis laevigata* and *C. reticulata*) during the same season but employ opposing oviposition and larval feeding strategies (Stamp, 1980; Brock and Kaufman, 2006). Both *Asterocampa* species are multi-voltine and lay eggs from May to November, with at least 3 generations a year in South-Central Texas (Brock and Kaufman, 2006).

Hackberry Emperors (*Asterocampa celtis*) are notable by a broken bar on the top of their forewing along with bold eyespots in the submargin of their wing (Friedlander, 1987; Brock and Kaufman 2006; Fig. 1d-e). *A. celtis* hindwings have yellow eyespots dotted with blue in the center (Friedlander, 1987; Brock & Kaufman, 2006; Fig. 1f). Tawny Emperors (*Asterocampa clyton*) are distinguished by two solid bars on their forewing and lack the sub-marginal black spots on their wings (Friedlander, 1987; Brock & Kaufman, 2006; Fig. 1a-b). *A. clyton* hindwings have eyespots like those of *A. celtis* but are smaller and have a more subdued coloration (Friedlander 1987; Fig. 1c).

The Hackberry Emperor (*A. celtis*) and the Tawny Emperor (*A. clyton*) both oviposit on Hackberry trees (*Celtis* sp.) but they produce different clutch sizes (Friedlander, 1987). Female *A. celtis* lay eggs singly (Stamp, 1980; Friedlander, 1987; Fig. 2a-b), while *A. clyton* females lay eggs in multi layered egg masses that form tightly stacked pyramid-shaped clusters of 50-500 eggs (Edwards, 1884; Stamp, 1980; Friedlander, 1986; Friedlander, 1987). These striking differences are illustrated in Fig. 2c. Caterpillars of *A. celtis* do not aggregate as early instars, but are sometimes seen feeding on the same leaf, without bodily contact (Stamp, 1980; Friedlander, 1987). In contrast, *A. clyton* larvae feed gregariously with body contact for the first three instars following eclosion from the pyramid shaped egg clusters (Edwards, 1884; Stamp, 1980; Friedlander, 1987). Although common in the southwestern United States, little is known of the natural history of the several dozen species of Emperor butterflies in the genus *Asterocampa* (Family: Nymphalidae, Subfamily: Apaturini), (Friedlander, 1987).

Butterflies in the Apaturini subfamily are mainly associated with Ulmaceae [now Cannabaceae] host plants, particularly *Celtis* (Ehrlich and Raven, 1964; Whittemore and Townsend, 2007). Hackberry trees, (Family: Cannabaceae), are fast growing deciduous trees that occur across North America and throughout the Texas Hill Country but are especially abundant throughout the Edwards Plateau (Barnes et al., 2000; Gilman and Watson, 2005). Hackberries are important shade species and are drought, heat, and flood tolerant (Redlin and Herman, 1987; Barnes et al., 2000; Whittemore and Townsend,

2007). Furthermore, Hackberry trees (*Celtis*) are constructive in preventing erosion (Whittemore and Townsend, 2007).

North American *Celtis* were previously believed to hybridize but recent evidence suggests that although their natural ranges overlap broadly in Central Texas, little to no hybridization occurs between *C. laevigata* and *C. reticulata* (Whittemore, 2005). The bright-yellow green *C. laevigata* leaves, which are thin and flexible, often have a more glabrous leaf surface and sometimes contain trichomes on the major leaf veins (Buck and Bidlack, 1998; Whittemore, 2005; Fig. 3a). In comparison, the dark green *C. reticulata* leaves typically are rougher due to trichomes on the lower surface of the leaf facing away from the stem (Buck and Bidlack, 1998; Whittemore, 2005; Fig. 3b). Importantly, these differences in leaf morphology which aide in plant identification could potentially be involved in driving the differences in *Asterocampa* oviposition and larval feeding behaviors. Leaf morphology could contribute to differences in host plant quality that could determine to female's oviposition preference. These Hackberry species potentially differ in the nutritional quality which could influence oviposition preference if *Asterocampa* females oviposit on the host plant using the egg deposition strategy that gives their offspring the highest foraging efficiency. Any differences in foraging efficiency based on plant quality could favor gregarious feeding.

Research Questions and Objectives

This research used two butterfly species in the genus *Asterocampa* to address the knowledge gap regarding the costs and benefits of aggregative behavior and improve our understanding of clutch size differentiation between related species. This system is ideal for exploring the question of whether aggregative feeding is a driver of the clutch size differentiation among sympatric species because potential confounding effects such as different host plant use, and allopatry are not applicable. Species of *Asterocampa* exhibit extreme clutch size variation, which translates into variation in larval feeding group sizes. To explore whether gregarious feeding drives clutch size differentiation and fill in natural history information on *Asterocampa* butterflies this research addresses the following questions: 1) Is there a difference in the timing of emergence measured by temporal patterns of relative abundance between *Asterocampa* species or sexes within species? If so, then the issue of whether asynchrony of emergence times translates into differences in host plant quality that females oviposit onto and larvae eat becomes an important dimension in addressing alternative strategies of egg deposition. 2) Do *Asterocampa* species have an oviposition preference between *Celtis* (Hackberry) host plants? 3) Do *Asterocampa* larvae experience enhanced growth and survivorship in gregarious feeding groups?

II. METHODS

Experimental Methods

The objectives of this study were to understand any differences in temporal patterns of relative abundance between the species (or sexes between the species), if clutch size relates to oviposition preference for host plant, and how gregarious feeding effects caterpillar performance. These objectives are critical components of understanding the adaptive significance of between species clutch size variation because differences in emergence times can equate to differences in host plant quality, differences in host plant preference in oviposition can explain differences in clutch size, and differences in caterpillar performance due to feeding groups can elucidate the adaptive significance of different feeding strategies. Fruit-baited traps were used to capture foraging *Asterocampa* females and used to monitor the patterns of relative abundance of *Asterocampa* species from April to August 2017. To investigate the evolution of clutch size differentiation female *Asterocampa* oviposition preference was assayed. To explore the impacts of gregarious feeding on caterpillar performance, group sizes were manipulated on each of the two host plant species. The details of each experiment are described below.

Trapping

Traps were used to survey to estimate the relative abundance of *A. celtis* and *A. clyton*, and quantify phenological differences in the flight period, and sex ratios between species. Based on the fruit-feeding Nymphalid preference for rotting fruit (DeVries,

1988; Krenn et al., 2001), baited traps were used at three sites in South-Central Texas: Lockhart (Caldwell Co. Tx.), Martindale (Caldwell Co. Tx.), and Freeman Ranch (Hays Co. Tx.) to capture female *A. celtis* and *A. clyton*. The traps at Martindale and Lockhart sites were placed in riparian environments. Traps in Freeman Ranch were in Juniper-Oak woodland environments which lack permanent streams (Barnes et al., 2000). The traps were constructed by attaching a 30 cm by 30 cm plywood board to a cylinder of tulle with hooks and wire, following the design of DeVries et al. (2012) and were hung in Hackberry trees (*Celtis* sp.) about a meter off the ground (Fig. 4 a-c). The plywood board acted as the feeding platform and was baited with fermented bananas which was replenished every other day. Traps were checked daily and the number and sex of each species caught was recorded. Over the course of the experiment (5 months) 235 *Asterocampa* females were captured. Traps in woodland environments of Freeman Ranch caught 79 *A. celtis* (32 females, 47 males) and a total of 25 *A. clyton* (7 females, 18 males). Traps in riparian environments of Lockhart and Martindale captured 210 *A. celtis* (89 females, 121 males) and 295 *A. clyton* (107 females, 188 males). All captured female *Asterocampa* were returned to the lab in glassine envelopes to assay oviposition preference and provide eggs for subsequent feeding experiments. The numbers of *A. celtis* and *A. clyton* as well as the sex ratios were computed and compared between months. The response variable, relative abundance over time, was calculated by dividing the number of *Asterocampa* caught divided by the total number of trap days that month.

Abundance over time between species was compared using a partial correlation analysis in R (Kim, 2015).

Oviposition Preference

To examine whether clutch size differences are based on leaf morphology, female *Asterocampa* oviposition preference between two Hackberry tree species (*Celtis laevigata* and *C. reticulata*) was recorded. Oviposition preference was assessed by running choice tests and counting the number of eggs laid by *Asterocampa* females in oviposition arenas (Fig. 5a-b) on *Celtis laevigata* and *C. reticulata*. Oviposition arenas were created by first submerging a 5-cm block of Oasis floral foam into an 8-oz. solo cup with water. When moistened, Oasis floral foam is designed to prolong the life of cutting arrangements. Two holes were then cut out of the bottom of a 12-oz. solo cup for the cuttings of *C. reticulata* and *C. laevigata* to go through the Oasis block, this method kept the cuttings alive the longest. Every two days arenas were restocked with fresh cuttings. The leaves of both species were cut from Hackberry trees in the field and placed in water until they were brought back to the lab to prevent desiccation. To standardize leaf age and quality only ‘summer wood’ (cutting with a greenish-grey soft wood stem) and with no evidence of herbivory or predators were used in oviposition trials. *Asterocampa* females were placed on these leaves in their oviposition arenas and then a 7.6 by 7.6 cm tulle square was secured on the top of the cup with a rubber band. To nourish females, the tulle was brushed with fruit-punch Gatorade© daily. Oviposition arenas were placed on a table under 60-watt lights with a 12 h light and dark cycle. The arenas were monitored daily

for eggs. Within 24 hours of oviposition eggs were counted on each leaf. The data for oviposition preference of female *Asterocampa* were then analyzed using a hierarchical Bayesian analysis using the bayesPref package in R (Gompert and Fordyce, 2012).

Group Size Experiment

Eggs laid during oviposition preference assays were collected and enclosed in petri dishes for 4 to 7 days until they hatched. To test whether larval performance was related to feeding group size, treatments consisting of artificial group sizes were created for both *A. celtis* and *A. clyton*. Newly hatched neonates were assembled into the group size treatments per species and stored in 3-oz. capped cups until deployed. The group sizes were 1 (solitary), 10 (small group), 20 (medium group), and 50 (large group) included the minimum clutch size of *A. clyton* and the maximum clutch size of *A. celtis* (Friedlander, 1987). These artificial gregarious groups were deployed onto native Hackberry trees in the study area where they foraged for a predetermined number of degree days. A degree day (DD) is a measurement of thermal units across time; calculated daily based on minimum and maximum temperatures, degree days standardize the physiological time ectotherms are developing (Murray, 2008). To establish a DD range for this study, a subset of neonates were allowed to forage until they reached their second instar. From these data, the DD for the time it took neonates in each replicate to reach their second instar was averaged, giving an average DD of 82 °C. Approximately 20 replicates of each group size treatments were left to forage until the sum of each replicate's DD's exceeded 60 °C, at which time they were returned to the lab to be

weighed. Baseline neonate weights are three orders of magnitude less than the final weights making the individual variances between neonates trivial, so we can assume the baseline weights of neonates of both species are equal. Because of this we could weigh caterpillars at the end of the experiment to get the weight gained. The response variables to ascertain enhanced performance were weight gain, proportion of neonates at 2nd instar, and proportion of survivors. I focused on the first two performance measures because they are both relevant fitness proxies since they are markers of faster development which is often associated with more effectively escaping predation (Stamp, 1980; Allen, 2010; Fiorentino et al. 2014; Fordyce and Nice, 2004).

Replicates of all treatments were placed in mesh drawstring bags which enclosed healthy leaves. To establish whether *Asterocampa* neonate's performance differed between Hackberry trees species, larvae were bagged on leaves of both *C. laevigata* and *C. reticulata*. As in the preference trials only branches containing greenish-summer wood and soft light green leaves with little to no previous herbivory were used. Once identified, a piece of cotton ball was wrapped around the distal end of the Hackberry stem to deter neonate escape and prevent natural enemy entrance and larvae egress (Fig. 6a-b).

Neonates were painted onto Hackberry leaves to ensure a connection with host plant material. Neonates were allowed to forage until the desired number of DD were met. As soon as the criteria of DD > 60 °C, was met all the surviving caterpillars/replicate were returned to the lab and weighed on a balance (Mettler Toledo). The number of survivors, the average weight of the surviving larvae and the number of larvae at their second instar

per group was recorded. After weighing all larvae were returned to the field and placed onto leaves of the host plants. The data for average weight gain, the number of larvae reaching second instar, and the number of survivors were transformed by dividing them by DD. The transformed data were then analyzed using an ANOVA in R.

III. RESULTS

Relative Abundance of Sexes and Species

A partial correlation analysis was run to determine the relationship between *A. celtis* and *A. clyton* whilst controlling for the month. I found there was difference in the relative abundance between *Asterocampa* species across the months, but there was not a significant difference between *Asterocampa* in their abundance (Pearson: $r(4)=0.61$, $p=0.39$; Fig 7a). There was also a difference in the relative abundance of sexes across the months, but there was not a significant difference between males and females in their abundance (Pearson: $r(4)=0.73$, $p=0.27$; Fig 7b).

Oviposition Preference

A total of 72 of the 235 captured *Asterocampa* females laid eggs. Oviposition preference data were collected from 58 *A. celtis* females and 14 *A. clyton* females. There was evidence from the hierarchical Bayesian Analysis of host plant preference among both *Asterocampa* species. *A. celtis* showed high preference for *Celtis laevigata* (preference and 95% credible interval: 0.753 (0.643, 0.759), meaning about 75% of the time female *A. celtis* preferred to lay on *C. laevigata* leaves (Fig. 8a). *A. clyton* also showed high preference for *C. laevigata* (preference and 95% credible interval: 0.725

(0.664, 0.779), meaning about 73% of the time female *A. clyton* preferred to lay on *C. laevigata* leaves (Fig. 8b). Although both species preferred *C. laevigata*, there was more variation in individual preference among *A. celtis* females (Fig. 8 a-b). This variation may come from the sample size disparity or from *A. celtis* females making more choices by laying eggs more times than *A. clyton* females.

Group Size Experiment

Throughout the course of this experiment 321 group size replicates were deployed on Hackberry trees (Table 1). I found a significant difference in weight gained between the group size treatments of *Asterocampa* larvae. Larvae feeding in aggregative groups of 10 gained significantly less weight than other groups across both species on both host plants (ANOVA: $F=5.94$, $DF=3$, $p=0.0006$; Fig. 9a). There was also a significant difference in weight gained between species of *Asterocampa* larvae; *A. celtis* larvae gained significantly more weight than *A. clyton* larvae across all group size treatments and both host plant species (ANOVA: $F=85.79$, $DF=1$, $p < 2e-16$; Fig. 9d). However, both *Asterocampa* larvae across both host plant species did not show a significant difference in weight gained between host species (ANOVA: $F=1.65$, $DF=1$, $p=0.20$; Fig. 9g).

I found a significant difference in the proportion reaching second instar in group size treatments of *Asterocampa* larvae; solitary larvae across both species and host plants reached their second instar significantly faster than larvae in the aggregative groups (ANOVA: $F=6.85$, $DF=3$, $p=0.0002$; Fig. 9b). Moreover, there was a significant

difference in the proportion reaching second instar between species of *Asterocampa*; *A. celtis* in all feeding groups and on both host plants reached their second instar significantly faster than *A. clyton* (ANOVA: $F=28.7$, $DF=1$, $p=1.67e-07$; Fig. 9e). As well, there was also a significant difference in the proportion reaching second instar in *Asterocampa* larvae based on host species; larvae reached their second instar faster on *Celtis reticulata* leaves (ANOVA: $F=8.46$, $DF=1$, $p=0.0039$; Fig. 9h).

I did not find a significant difference in the proportion of survivors between group size treatments (ANOVA: $F=1.98$, $DF=3$, $p=0.117$; Fig. 9c). There was however, a significant difference in the proportion of survivors between *Asterocampa* species; more *A. celtis* larvae survived than *A. clyton* larvae (ANOVA: $F=32.43$, $DF=1$, $p=2.32e-08$; Fig. 9f). There was not a significant difference in the proportion of survivors based on host plant species (ANOVA: $F= 0.651$, $DF=1$, $p=0.420$).

IV. DISCUSSION

Interactions with plants are a driving evolutionary force shaping the life history traits of many host specific insects. This is likely a result of the evolutionary arms race that arises when plants defend themselves against herbivory and insects develop strategies to overcome plant defenses (Berenbaum and Zangerl, 1998; Law et al. 2001; Augustine and Kingsolver, 2017). One such strategy employed by herbivorous insects is gregarious (or aggregative) feeding which typically arises from eggs clustered at oviposition which lead to siblings being able to more readily locate one another and remain together as they forage (Stamp, 1980; Clark and Faeth, 1997; Fordyce and Nice,

2004). Despite the various benefits to feeding gregariously it is not ubiquitous among herbivorous insects and the trait varies among closely related species within clades suggesting there might be tradeoffs between the alternative strategies of gregarious feeding and solitary feeding (Prokopy and Roitberg, 2001; Campbell and Stastny, 2015). To explore the advantages and disadvantages of gregarious feeding, I focused on two sympatric *Asterocampa* butterfly species which differed dramatically in their oviposition strategy. Investigation of phenological variation did not detect differences in relative abundance in either *Asterocampa* species or sex across the season. Quantification of female preference for oviposition substrate showed a marked preference for *C. laevigata* as their host plant for both butterfly species. Aggregative feeding experiments revealed there was a benefit to solitary feeding in both the solitary feeding and the gregarious feeding species. This result is of particular interest as it runs counter to expectations. This investigation of *Asterocampa* biology revealed details on the patterns of life history variation in these butterflies and unexpected results with respect to the evolution and ecology of aggregative feeding.

The phenology experiments found no differences in relative abundances. Although males in many Lepidopteran species have been known to emerge before females to maximize their mating (Blumer, 1982; Fagerström and Wiklund, 1982), no difference in emergence times, in terms of relative abundance by month, were found between sexes in *Asterocampa*. There were also no differences in relative abundance between *Asterocampa* species based on month. The findings make the different

oviposition and larval feeding strategies in *Asterocampa* even more of a mystery; if *A. celtis* and *A. clyton* are equally relatively abundant throughout the same times then presumably the quality of the host plant is the same for both, so the driver of the differences in gregarious feeding and oviposition strategies is probably not the host plants.

Asterocampa use the same host plant in very different ways; *A. celtis* lay a single egg and *A. clyton* lay a large pyramid of eggs. The choice test between *Celtis laevigata* and *C. reticulata* as oviposition substrate established that despite their alternative oviposition strategies, *A. celtis* and *A. clyton* both preferred *C. laevigata* as their oviposition substrate. Despite the similar preferences between species I noted greater variation in individual female preference in *A. celtis*. This variation could be linked to the amount of eggs females have to lay, if both species have the same amount of eggs to lay then female *A. celtis* are making more choices because they only lay a single egg at a time compared to the fewer choices given the large number of eggs *A. clyton* females lay at one time.

The results of the group size manipulation experiments indicate gregarious feeding did not enhance caterpillar performance. Although *A. celtis* gained more weight and reached their 2nd instar at a higher proportion than *A. clyton* there was no interaction effect between species and group size. The main effect of host plant did not influence the response variables of weight gained or proportion of survivors but a greater proportion of caterpillars reached their 2nd instar when feeding on *C. reticulata*. The response variable

of weight gained showed that groups of 10 performed more poorly than solitary feeders and larger gregarious groups. The response variables of proportion at 2nd instar showed a seeming advantage to solitary feeding. The response variable of proportion of survivors did not show any effect in terms of group size. The findings from the gregarious feeding experiments seem to be a contradiction to the slow growth-high mortality hypothesis which predicts that to reduce the window of vulnerability larvae will gain weight more quickly. Thus, the advantages of gregarious feeding are not realized in terms of caterpillar performance but might be related to defense against natural enemies. Perhaps gregarious feeding is a secondary effect of female oviposition strategies to evade egg parasitoids. Future work should be done exploring the tradeoffs between clutch size strategies, especially thinking about the single egg strategy of escaping egg parasitism in space versus the pyramid strategy of escaping egg parasitism through sacrifice.

Figures

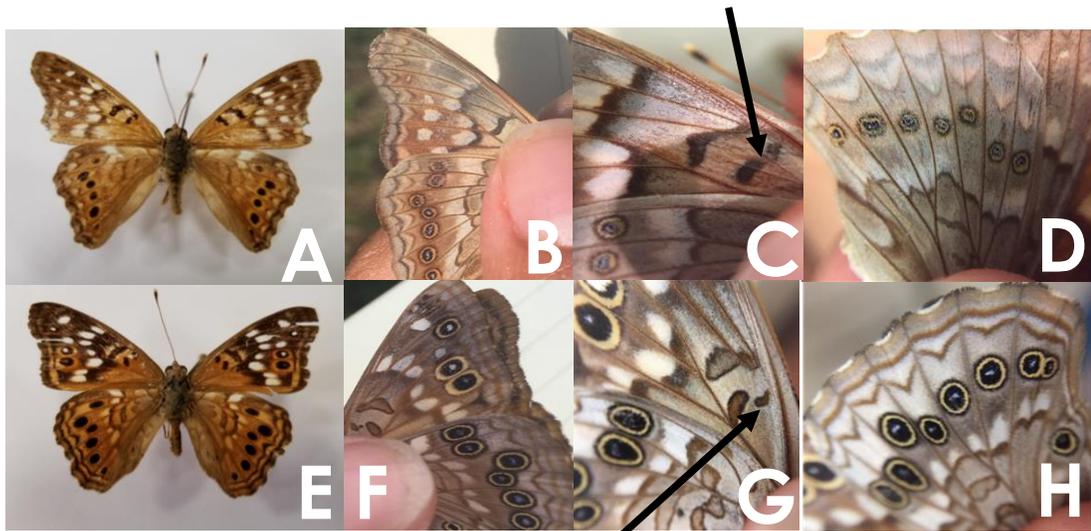


Figure 1-Asterocampa butterflies: A) Dorsal view of *A. clyton* wings, white eyespots and two solid bars are the distinguishing features. B) *A. clyton* distinguished by light brown (tawny) coloring, two solid bars on the forewing and small eyespots on the hindwing. C) Close up of *A. clyton* showing the two solid forewing bars (denoted by black arrow). D) Close up of *A. clyton* hindwing, eyespots are small blue dots surrounded by a yellow ring. E) Dorsal view of *A. celtis* wings, black around their white eyespots and a broken bar are the distinguishing features. F) *A. celtis* distinguished by brownish-gray coloring, a broken and a solid bar on the forewing and medium sized eyespots on the hindwing. G) Close up of *A. celtis* showing the solid and broken bars of the forewing (denoted by black arrow). H) Close up of *A. celtis* hindwing, eyespots are larger than *A. clyton*'s with a bigger dark spot surrounding the blue, and a yellow ring around both.

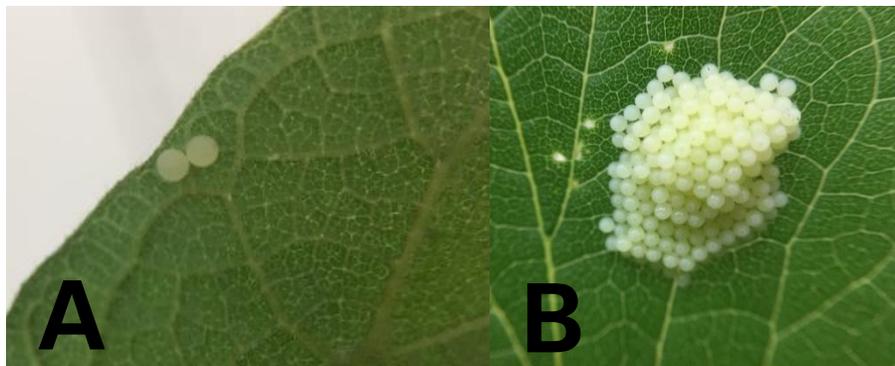


Figure 2- Clutch Differentiation of Asterocampa eggs: A) Small clutch of eggs laid by *A. celtis*. B) Large clutch of eggs laid by *A. clyton*.

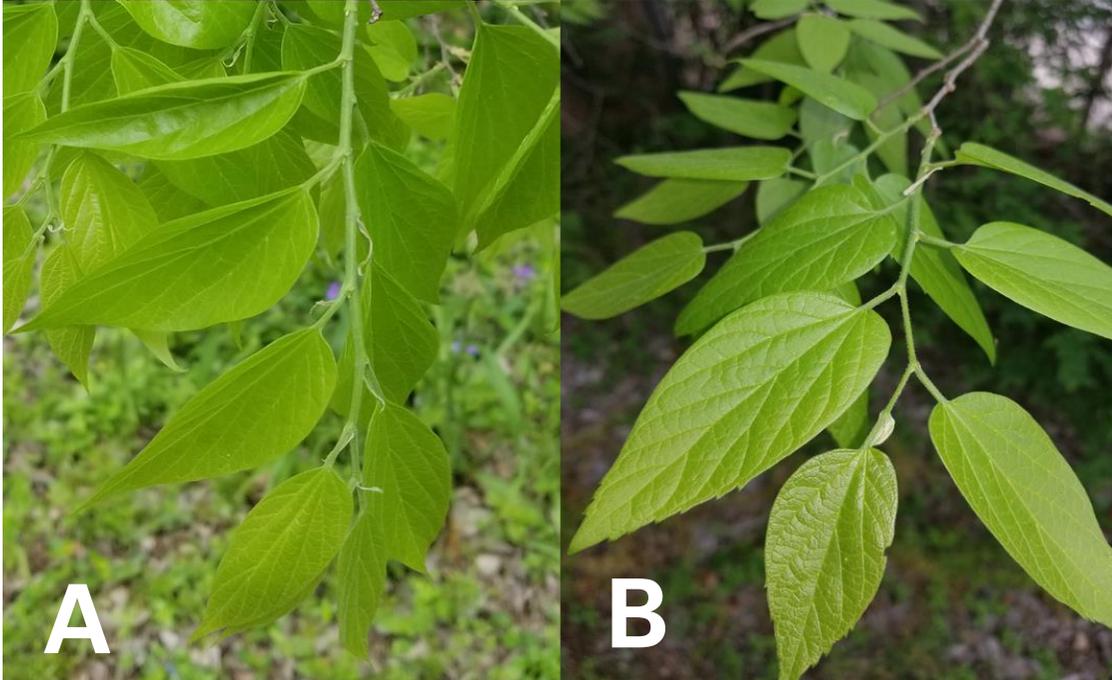


Figure 3- Hackberry Tree (*Celtis*) leaves: A) *C. laevigata* leaves are non-serrated on the edges and are softer and smoother than *C. reticulata* leaves. B) *C. reticulata* leaves have serrated edges and a rough sand paper texture.

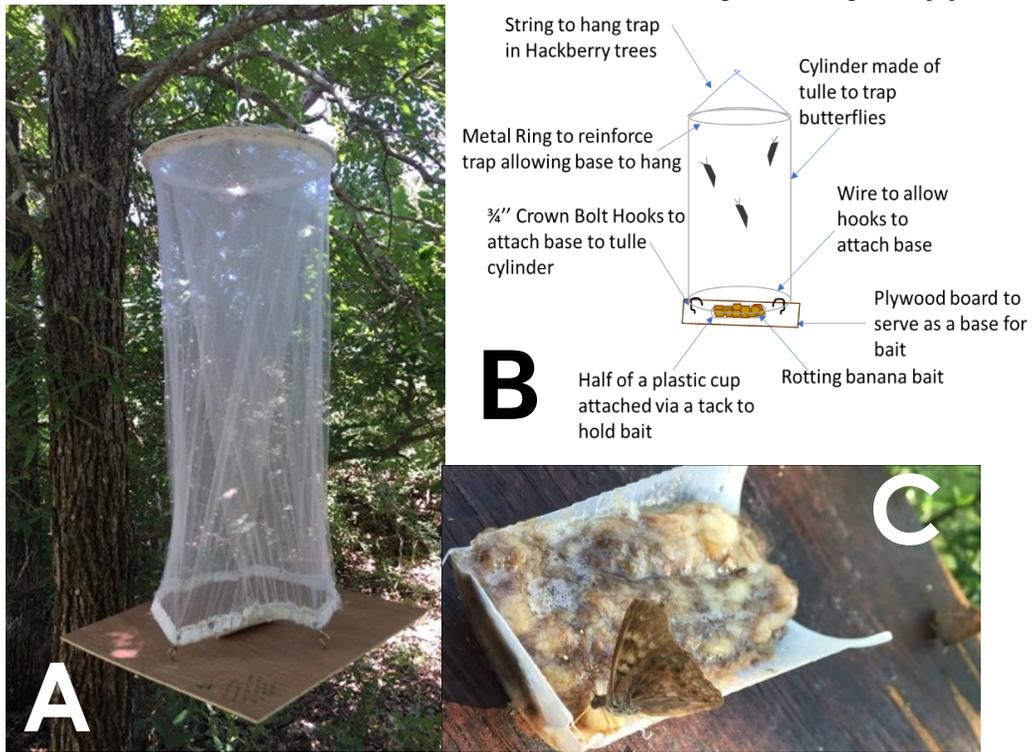
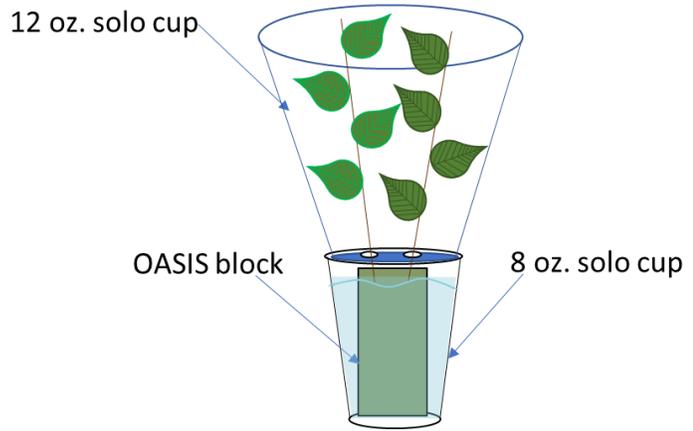


Figure 4- Fruit Baited Traps for Surveys and Collecting Female *Asterocampa*: A) Fruit baited trap set out in the field at Freeman Ranch. B) Labeled diagram of the parts of the fruit baited traps. C) *A. clyton* male feeding on rotten banana bait.



A

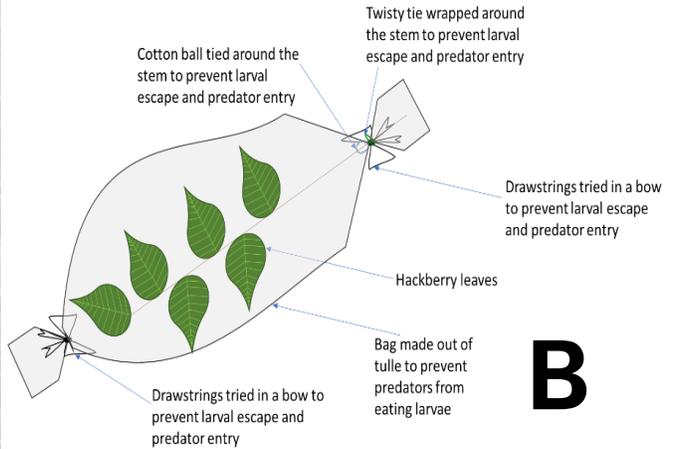


B

Figure 5- Oviposition Arenas for *Asterocampa* Preference Assays: A) Oviposition Arena in the lab. B) Labeled diagram of an oviposition arena.



A



B

Figure 6-Mesh Drawstring Bags for Gregarious Feeding Experiments: A) Mesh Drawstring Bag out in the field. B) Labeled diagram of a mesh drawstring bag.

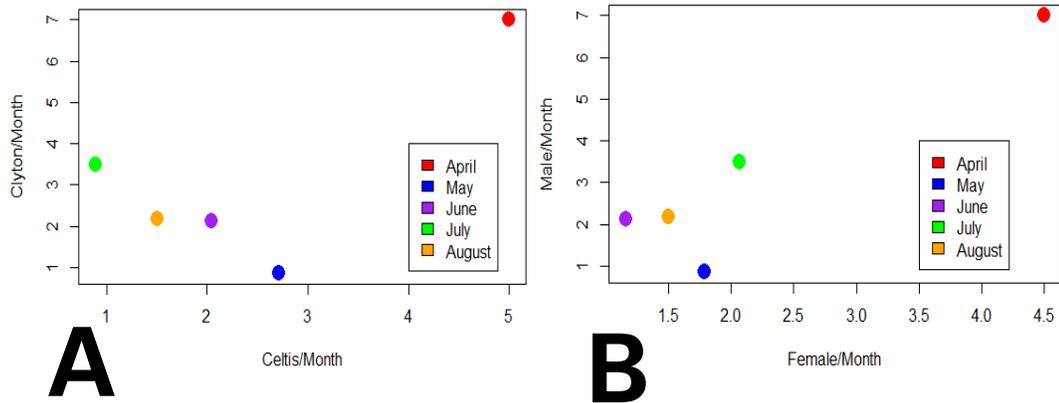


Figure 7-Partial Correlation Analysis on Relative Abundance: A) Partial Correlation Graph of Relative Abundance by Species per Month. B) Partial Correlation Graph of Relative Abundance by Sexes per Month. For both graphs: The red dot is the month of April. The blue dot is the month of May. The purple dot is the month of June. The green dot is the month of July. The orange dot is the month of August.

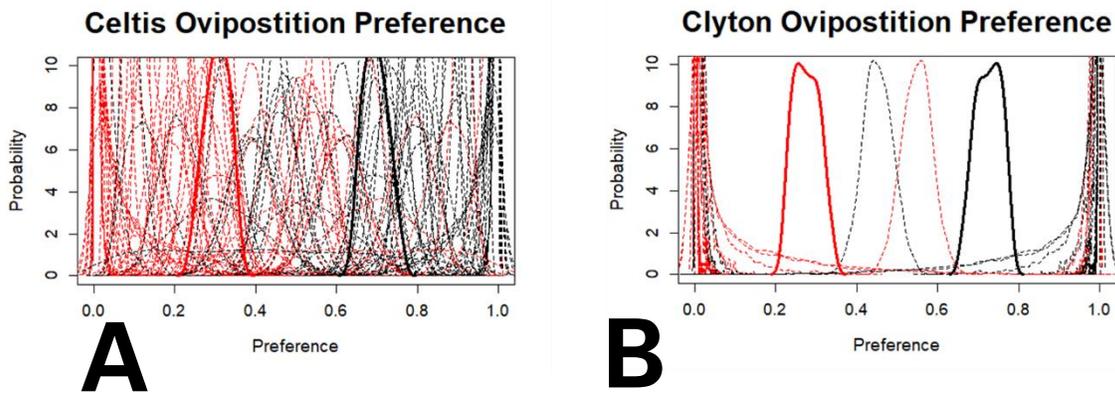


Figure 8- Graphs of *Asterocampa* Oviposition Preference for *Celtis* host: A) *Asterocampa celtis* female oviposition preference. Of the 58 females sampled there was about a 75% preference for *C. laevigata* leaves (black) and a 25% preference for *C. reticulata* leaves (red). B) *A. clyton* female oviposition preference. Of the 14 females sampled there was a 73% preference for *C. laevigata* leaves (black) and a 27% preference for *C. reticulata* leaves (red). For both plots, solid lines represent population (species) level preference, and dashed lines represent individual level preference.

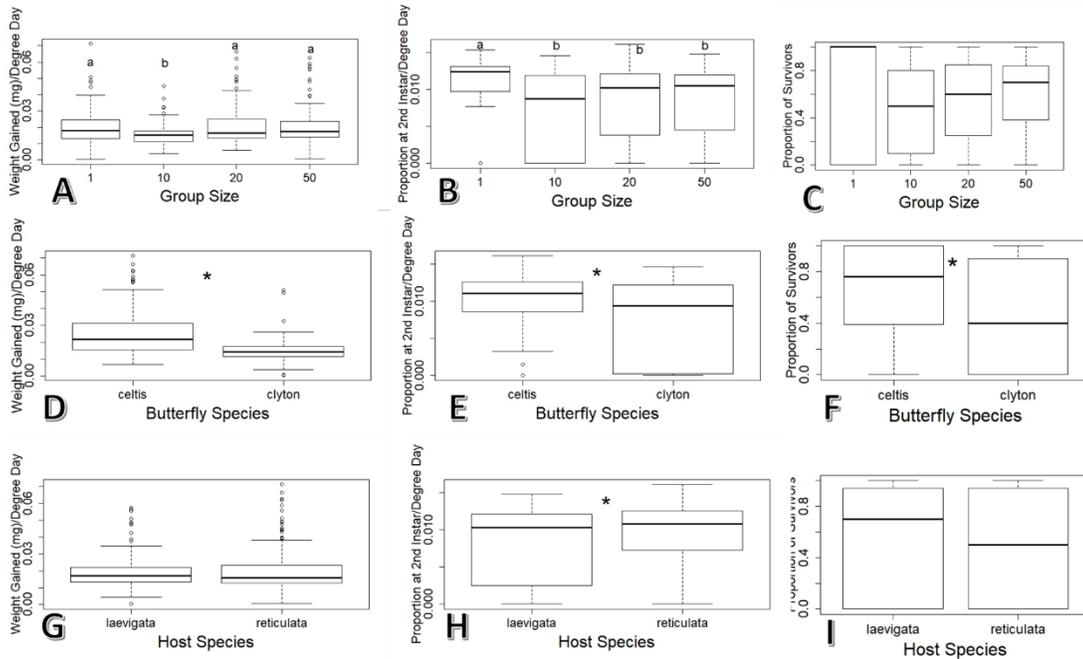


Figure 9-Box Plots of ANOVA Results: A) Bar graph comparing weight gained by Group Size. There was a significant difference in weight gain between groups of 10 and the rest of the groups. B) Bar graph comparing proportion at second instar by Group Size. There was a significant difference in the proportion of larvae at their second instar. Singleton larvae had a significantly higher proportion reach their second instar compared to other groups. While larvae in groups of 10 had a significantly lower proportion reach their second instar compared to other groups. C) Bar graph comparing the proportion of survivors by Group Size. More *Asterocampa* survived when feeding singly than in other feeding groups. D) Bar graph comparing weight gained by *Asterocampa* species. *A. celtis* gained significantly more weight than *A. clyton*. E) Bar graph comparing proportion at second instar by *Asterocampa* species. *A. celtis* had a significantly higher proportion of larvae reach their second instar compared to *A. clyton* larvae. F) Bar graph comparing the proportion of survivors based on *Asterocampa* species. More *A. celtis* larvae survived than *A. clyton* larvae. G) Bar graph comparing weight gained by *Celtis* host species. There was no significant difference in weight gain between *C. laevigata* and *C. reticulata* leaves. H) Bar graph comparing proportion at second instar by *Celtis* host species. There was a significant difference in the proportion of larvae at their second instar. *Asterocampa* larvae on *C. reticulata* leaves had a higher proportion of larvae at their second instar compared to *Asterocampa* larvae on *C. laevigata*. I) Bar graph comparing the proportion of survivors by *Celtis* host species. There was no difference in survivorship between host plant species.

Table 1: Number of Replicates Per Treatment for Gregarious Feeding Experiment					
Treatment	Number of Replicates	Average Degree Day (°C)	Average Weight Gained (mg)/Degree Day (°C)	Average Proportion at Second Instar/Degree Day (°C)	Average Proportion of Survivors
<i>A.celtis</i> on <i>C. laevigata</i> feeding alone	20	83.48	0.024	0.01	0.759
<i>A.celtis</i> on <i>C. laevigata</i> feeding in a group of 10	22	83.98	0.018	0.008	0.562
<i>A.celtis</i> on <i>C. laevigata</i> feeding in a group of 20	19	84.82	0.026	0.01	0.718
<i>A.celtis</i> on <i>C. laevigata</i> feeding in a group of 50	21	80.21	0.026	0.009	0.712
<i>A.celtis</i> on <i>C. reticulata</i> feeding alone	20	78.72	0.028	0.012	0.571
<i>A.celtis</i> on <i>C. reticulata</i> feeding in a group of 10	19	82.86	0.019	0.008	0.5
<i>A.celtis</i> on <i>C. reticulata</i> feeding in a group of 20	20	82.83	0.031	0.011	0.667
<i>A.celtis</i> on <i>C. reticulata</i> feeding in a group of 50	20	81.97	0.031	0.011	0.744
<i>A.clyton</i> on <i>C. laevigata</i> feeding alone	20	89.27	0.017	0.007	0.432
<i>A. clyton</i> on <i>C. laevigata</i> feeding in a group of 10	20	81.41	0.013	0.005	0.388
<i>A. clyton</i> on <i>C. laevigata</i> feeding in a group of 20	20	81.42	0.015	0.006	0.481
<i>A. clyton</i> on <i>C. laevigata</i> feeding in a group of 50	20	79.39	0.016	0.006	0.43
<i>A. clyton</i> on <i>C. reticulata</i> feeding alone	20	83.14	0.014	0.012	0.4
<i>A. clyton</i> on <i>C. reticulata</i> feeding in a group of 10	20	80.56	0.013	0.007	0.448
<i>A. clyton</i> on <i>C. reticulata</i> feeding in a group of 20	21	79.61	0.016	0.007	0.46
<i>A. clyton</i> on <i>C. reticulata</i> feeding in a group of 50	19	83.24	0.014	0.008	0.494

LITERATURE CITED

- Agrawal, A.A. 1998. Induced responses to herbivory and increased plant performance. *Science*. 279, 1201-1202.
- Agrawal, A.A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. *Ecology*. 80, 1713-1723.
- Agren, J., Schemske, D.W. 1993. The cost of defense against herbivores: An experimental study of trichome production in *Brassica rapa*. *The American Naturalist*. 141, 338-350.
- Allen, P.E. 2010. Group size effects on survivorship and adult development in the gregarious larvae of *Euselasia chrysippe*. *Insect. Soc.* 57, 199-204.
- Augustine, K.E., Kingsolver, J.G. 2017. Biogeography and phenology of oviposition preference and larval performance of *Pieris virginiensis* butterflies on native and invasive host plants. *Biol Invasions*. 1-10.
- Barnes, P.W., Liang, S.Y., Jessup, K.E., Ruiseco, L.E., Phillips, P.L., Reagan, S.J. 2000. Soils, topography and vegetation of the Freeman Ranch. *Freeman Ranch Publication Series 1*, 1-29.
- Benson, W.W., Brown, K.S., Gilbert, L.E. 1975. Coevolution of plants and herbivores: Passion flower butterflies. *Evolution*. 29, 659-680.
- Benson, W.W. 1978. Resource partitioning in passion vine butterflies. *Evolution*. 32, 493-518.
- Berenbaum, M. 1978. Toxicity of a furanocoumarin to armyworms: A case of biosynthetic escape from insect herbivores. *Science*. 201, 532-534.
- Berenbaum, M. 1983. Coumarins and caterpillars: A case for coevolution. *Evolution*. 37, 163-179.
- Berenbaum, M.R., Zangerl, A.R. 1998. Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl. Acad. Sci.* 95, 13743-13748.
- Benrey, B., Denno, R.F. 1997. The slow-growth—high-mortality hypothesis: A test using the cabbage butterfly. *Ecology*. 78, 987-999.
- Blumer, M.G. 1983. Models for the evolution of protandry in insects. *Theoretical Population Biology*. 23, 314-322.
- Brock, J.P., Kaufman, K. Kaufman Field Guide to Butterflies of North America. Houghton Mifflin Harcourt, 2006.
- Broadway, R.M. 1995. Are insects resistant to plant proteinase inhibitors? *Journal of Insect Physiology*. 41, 107-116.
- Brower, J.V.Z. 1958. Experimental studies of mimicry in some North American butterflies: Part II. *Battus philenor* and *Papilio troilus*, *P. polyxenes* and *P. glaucus*. *Evolution*. 12, 123-136.

- Buck, G.W., Bidlack, J.E. 1998. Identification of *Quercus* and *Celtis* species using morphological and electrophoretic data. *Proc. Okla. Acad. Sci.* 78, 22-23.
- Bryant, S.R., Thomas, C.D., Bale, J.S. 2000. Thermal ecology of gregarious and solitary nettle-feeding nymphalid butterfly larvae. *Oecologia*. 122, 1-10.
- Campbell, S.A., Stastny, M. Benefits of gregarious feeding by aposematic caterpillars depend on group age structure. *Oecologia*. 177, 715-721.
- Carroll, C.R., Janzen, D.H., 1973. Ecology of foraging by ants. *Annu. Rev. Ecol. Syst.* 4, 231-257.
- Codella, S.G., Raffa, K.F. 1995. Contributions of female oviposition patterns and larval behavior to group defense in conifer sawflies (Hymenoptera: Diprionidae). *Oecologia*. 103, 24-33.
- Clancy K.M., Price, P.W. 1987. Rapid Herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology*. 68, 733-737.
- Clark, B.R., Faeth, S.H. 1997. The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*. 22, 408-415.
- De Moraes, C.M., Lewis, W.J., Pare, P.W., Albron, H.T., Tumlinson, J.H. 1998. Herbivore-infested plants selectively attract parasitoids. *Letters to Nature*. 393, 570-573.
- Denno, R.F., Benrey, B. 1997. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. *Ecological Entomology*. 22, 133-141.
- Denno, R.F., McClure, M.S., Ott, J.R. 1995. Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annu. Rev. Entomol.* 40, 297-331.
- De Souza Tavares, W., Salgado-Neto, G., Legaspi, J.C., DeSouza Ramalho, F., Serrão, J.E., Zanuncio, J.C. 2012. Biological and ecological consequences of *Diolcogaster* sp (Hymenoptera: Braconidae) parasitizing *Agaraea minuta* (Lepidoptera: Arctiidae) and the effects on two *Costus* (Costaceae) plant species in Brazil. *Florida Entomologist*. 95, 966-970.
- De Vris, P.J. 1988. Stratification of fruit-feeding nymphalid butterflies in a Costa Rican rainforest. *Journal of Research on Lepidoptera*. 26, 98-108.
- De Vris, P.J., Alexander, L.G., Chacon, I.A., Fordyce, J.A. 2012. Similarity and difference among rainforest fruit-feeding butterfly communities in Central and South America. *Journal of Animal Ecology*. 81, 472-482.
- Dussourd, D.E., Eisner, T. 1987. Vein-cutting behavior: insect counterploy to the latex defense of plants. *Science*. 237, 898.
- Edwards, W.H. 1884. Revised catalogue of the diurnal Lepidoptera of America north of Mexico. *Transactions of the American Entomological Society and Proceedings of the Entomological Section of the Academy of Natural Sciences*. 11, 245-337.

- Ehrlich, P.R., Raven, P.H. 1964. Butterflies and plants: A study on coevolution. *Evolution*. 18, 586-608.
- Ehrlich, P.R., Murphy, D.D. 1988. Plant chemistry and host range in insect herbivores. *Ecology*. 69, 908-909.
- Eigenbrode, S.D., Espelie, K.E., Effects of plant epicuticular lipids on insect herbivores. *Annu. Rev. Entomol.* 40, 171-194.
- Erwin, T.L. 1982. Tropical forests: Their richness in Coleoptera and other Arthropod species. *The Coleopterists Bulletin*. 36, 74-75.
- Fagerström, T., Wiklund, C. 1982. Why do males emerge before females? Protandry as a mating strategy in male and female butterflies. *Oecologia*. 52, 164-166.
- Fiorentino, V.L., Murphy, S.M., Stoepler, T.M., Lill, J.T. 2014. Facilitative effects of group feeding on performance of the saddleback caterpillar (Lepidoptera: Limacodidae). *Entomological Society of America*. 43, 131-138.
- Fordyce, J.A., Agrawal, A.A. 2001. The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. *Journal of Animal Ecology*. 70. 997-1005.
- Fordyce, J.A. 2003. Aggregative feeding of pipevine swallowtail larvae enhances host plant suitability. *Oecologia*. 135, 250-257.
- Fordyce, J.A., Nice, C.C. 2004. Geographic variation in clutch size and a realized benefit of aggregative feeding. *Evolution*. 58, 447-450.
- Fordyce, J.A. 2005. Clutch size plasticity in the Lepidoptera. Pg.125-144 In *Insects and phenotypic plasticity*. Enfield: Science Publishers.
- Forister, M.L., Novotny, V., Panorska, A.K., Baje, L., Basset, Y., Butterill, P.T., Cizek, L., Coley, P.D., Dem, F., Diniz, I. R., Drozd, P., Fox, M., Glassmire, R.H., Hreck, J., Jahner, J.P., Kaman, O., Kozubowski, T.J., Kursar, T.A., Lewis, O.T., Lill, J., Marquis, R.J., Miller, S.E., Morais, H.C., Murakami, M., Nickel, H., Pardikes, N.A., Ricklefs, R.E., Singer, M.S., Smilanich, A.M., Stireman, J.O., Villamarín-Cortez, S., Vodka, S., Volf, M., Wagner, D.L., Walla, T., Weiblen, G.D., Dyer, L.A. 2015. The global distribution of diet breadth in insect herbivores. *PNAS*. 112, 442-447.
- Friedlander, T.P. 1986. Egg mass design relative to surface-parasitizing parasitoids, with notes on *Asterocampa clyton* (Lepidoptera: Nymphalidae). *Journal of Research on the Lepidoptera*. 24, 250-257.
- Friedlander, T.P. 1987. Taxonomy, phylogeny and biogeography of *Asterocampa Röber* 1916 (Lepidoptera, Nymphalidae, Apaturinae). *Journal of Research on the Lepidoptera*. 25, 215-338.
- Gibbs, R.D. 1974. Chemotaxonomy of flowering plants. Vols. I-IV. Montreal & London: McGill-Queen's Univ. Press.

- Gilman, E.F., Watson, D.G. 2014. *Celtis lavigata*: Sugarberry. IFAS-University of Florida.
- Gompert, Z., Fordyce, J.A. (2012). bayespref: Hierarchical Bayesian analysis of ecological count data. R package version 1.0. <https://CRAN.R-project.org/package=bayespref>
- Gross, P. 1993. Insect behavioral and morphological defenses against parasitoids. *Annu. Rev. Entomol.* 38, 251-273.
- Havill, N.P., Raffa, K.F. 2000. Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecological Entomology.* 25, 171-179.
- Heinrich, B. 1979. Foraging strategies of caterpillars. Leaf damage and possible predator avoidance strategies. *Oecologia.* 42. 325-337.
- Hocberg, M.E. 1991. Viruses as costs to gregarious feeding behavior in Lepidoptera. *Oikos.* 61, 291-296.
- Hölldobler, B., Wilson, E.O. 1990. The Ants. Harvard Univ. Press.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annu. Rev. Ecol. Syst.* 21, 243-273.
- Janzen, D.H. 1966. Coevolution of mutualism between ants and *Acacias* in Central America. *Evolution.* 20, 249-275.
- Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A., Kidd, N.A.C. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology.* 70, 442-458.
- Juniper, B., Southwood, R. 1986. Insects and the plant surface. Cambridge: CUP.
- Karban, R., Baldwin, I.T., Baxter, K.J., Laue, G. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia.* 125, 66-71.
- Kessler, A., Baldwin, I.T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science.* 291, 2141-2144.
- Kim, S. (2015). ppcor: Partial and Semi-Partial (Part) Correlation. R package version 1.1. <https://CRAN.R-project.org/package=ppcor>
- Krenn, H.W., Zulka, K.P., Gatschnegg, T. 2001. Proboscis morphology and food preferences in nymphalid butterflies (Lepidoptera: Nymphalidae). *J. Zool. Lond.* 254, 17-26.
- Law, R., Bronstein, J.L., Ferrière, R. 2001. On mutualists and exploiters: Plant-insect coevolution in pollinating seed-parasite systems. *J. theor. Biol.* 212, 373-389.
- Lindroth, R.L., Bloomer, M.S. 1991. Biochemical ecology of the forest tent caterpillar: Responses to dietary protein and phenolic glycosides. *Oecologia.* 86, 408-413.

- McClure, M., Ralph, M., Despland, E. 2011. Group leadership depends on energetic state in a nomadic collective foraging caterpillar. *Behavioral Ecology and Sociobiology*. 65, 1573-1579.
- Nabity, P.D., Haus, M.J., Berenbaum, M.R., DeLucia, E.H. 2013. Leaf-galling phylloxera on grapes reprograms host metabolism and morphology. *PNAS*. 41, 16663-16668.
- Nebert, D.W., Conzalex, F.J. 1987. P450 genes: Structure, evolution, and regulation. *Annu. Rev. Biochem.* 56, 945-993.
- Price, P.W., Bouton, C.E., Gross, P., McPherson, A., Thompson, J.N., Weis, A.E. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*. 11, 41-65.
- Prokopy, R.J., Roitberg, B.D. 2001. Joining and avoidance behavior in nonsocial insects. *Annu. Rev. Entomol.* 46, 631-665.
- R Development Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. ISBN 3-900051-07-0. URL <http://www.R-project.org>
- Raffa, K.F., Krause, S.C., Reich, P.B. 1998. Long-term effects of defoliation on red pine suitability to insects feeding on diverse plant tissues. *Ecology*. 79, 2352-2364.
- Reader, T., Hochuli, D.F. 2003. Understanding gregariousness in larval Lepidopteran: the roles of host plant, predation, and microclimate. *Ecological Entomology*. 28, 729-737.
- Redlin, S.C., Herman, D.E. 1987. Vegetative propagation of *Celtis occidentalis* L. *PNAS*. 113-125.
- Risch, S., McClure, M., Vandermeer, J., Waltz, S. 1977. Mutualism between three species of tropical piper (Piperaceae) and their ant inhabitants. *The American Midland Naturalist*. 98, 433-444.
- Rothschild, M., Schoonhoven, L.M. 1977. Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). *Nature*. 266, 352-355.
- Schmitz, O.J., Beckerman, A.P., O'Brien, K.M., 1997. Behaviorally mediated trophic cascades: Effects of predation risk on food web interactions. *Ecology*. 78, 1388-1399.
- Seastedt, T.R., Crossley, D.A. 1984. The influence of arthropods on ecosystems. *Bioscience*. 34, 157-161.
- Sillen-Tullberg, B. 1988. Evolution of gregariousness in aposematic butterfly larvae: A phylogenetic analysis. *Evolution*. 42, 293-305.
- Speight, M.R., Hunter, M.D., Watt, A.D. 1999. Ecology of insects: Concepts and applications. Oxford: Blackwell Science Ltd.

- Stamp, N. 1980. Egg deposition patterns in butterflies: Why do some species cluster their eggs rather than deposit them singly? *The American Naturalist*. 115, 367-380.
- Stamp, N.E. 1984. Foraging behavior of Tawny Emperor caterpillars (Nymphalidae: *Asterocampa clyton*). *Journal of the Lepidopterists' Society*. 38, 186-191.
- Stamp, N.E., Bowers, M.D. 1990. Variation on food quality and temperature constrain foraging of gregarious caterpillars. *Ecology*. 71, 1031-1039.
- Straka, J.R., Hayward, A.R., Emery, R.J.N. 2010. Gall-inducing *Pachypsylla celtidis* (Psyllidae) infiltrate hackberry trees with high concentrations of phytohormones. *Journal of Plant Interactions*. 5, 197-203.
- Thompson, J.N. 1988. Coevolution and alternative hypotheses on insect/plant interactions. *Ecology*. 69, 893-895.
- Tune, R., Dussourd, D.E. 2000. Specialized generalists: constraints on host range in some plusiine caterpillars. *Oecologia*. 123, 543-549.
- Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Röse, U.S.R., Lewis, W.J., Tumlinson, J.H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl. Acad. Sci. USA*. 92, 4169-4174.
- Westra, E.R., van Houte, S., Oyesiku-Blakemore, S., Makin, B., Broniewski, J.M., Best, A., Bondy-Denomy, J., Davidson, A., Boots, M., Buckling, A. 2015. Parasite exposure drives selective evolution of constitutive versus inducible defense. *Current Biology*. 25, 1043-1049.
- Whittemore, A.T. 2005. Genetic structure, lack of introgression and taxonomic status in the *Celtis laevigata*: *Celtis reticulata* complex (Cannabaceae). *Systematic Botany*. 30, 809-817.
- Whittemore, A.T., Townsend, A.M. 2007. Hybridization of self-compatibility in *Celtis*: AFLP analysis of controlled crosses. *J. Amer. Soc. Hort. Sci.* 132, 368-373.
- Wittsock, U., Gershenzon, J., 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology*. 5, 1-8.
- Young, T.P. 1987. Increased thorn length in *Acacia depreanlobium*-an induced response to browsing. *Oecologia*. 71. 436-438.
- Zangerl, A.R., Rutledge, C.E. 1996. The probability of attack and patterns of constitutive and induced defense: A test of optimal defense theory. *The American Naturalist*. 147, 599-608.