Spring 4-23-2020

Environmental Drivers of Morphological and Physiological Adaptation in an Invasive Defoliator, Lymantria Dispar

Chelsea Jahant-Miller
SUNY College of Environmental Science and Forestry, chelseajahant@gmail.com

Recommended Citation

Jahant-Miller, Chelsea, "Environmental Drivers of Morphological and Physiological Adaptation in an Invasive Defoliator, Lymantria Dispar" (2020). *Dissertations and Theses*. 182.
https://digitalcommons.esf.edu/etds/182

ENVIRONMENTAL DRIVERS OF MORPHOLOGICAL AND PHYSIOLOGICAL ADAPTATION IN AN INVASIVE DEFOLIATOR, *LYMANTRIA DISPAR*

by

Chelsea J. Jahant-Miller

A thesis
submitted in partial fulfillment
of the requirements for the
Doctor of Philosophy Degree
State University of New York
College of Environmental Science and Forestry
Syracuse, New York
April 2020

Department of Environmental and Forestry Biology

Approved by:
Dylan Parry, Major Professor
Giorgos Mountrakis, Chair, Examining Committee
Melissa Fierke, Department Chair
S. Scott Shannon, Dean, The Graduate School

Acknowledgements

First and foremost, I am extremely grateful to my major professor, Dr Dylan Parry, for providing me the opportunity to pursue a graduate degree in his lab. He took on extra risk bringing me in as a Ph.D. student more or less fresh out of my bachelor's degree, and continued to believe in my capacity for success even when I wasn't as sure. He has been a continuous and invaluable source for research ideas, meticulous edits, and academic and scientific support and guidance, throughout. I have grown in confidence and ability as a scientist, and as a person, through my experience at ESF and under Dylan's guidance. Thank you for giving me the time and resources I needed to grow.

I owe special thanks to the members of my committee. To Dr Kristine Grayson's continuous positivity, support, and research guidance, and for the opportunity to collaborate and spend time in the Grayson lab. Thank you to Dr Melissa Fierke who is a passionate and positive force in science, teaching, leadership, and family, and is a stellar role model for women in science. Through his suite of candidacy questions, Dr Dave Althoff pushed the limits of my understanding of evolutionary theory, and played no minor role in shaping the way I conceptualized an entire data chapter. Finally, thank you Dr Colin Beier for taking the time to act as my defense examiner, and for teaching my favorite class at ESF. Thanks also to my funding sources, especially the Henrietta and John Simeone Fellowship in Forest Entomology and the Edna Bailey Sussman Foundation.

Very special thanks to the members of the Grayson lab for their many hours of help, and my deepest gratitude to Lily Thompson who provided expert support during the egg phenology experiment. I am thankful to Dr Patrick Tobin for his input and collaboration on the wing length project. Thanks to the many people who provided resources, analyses, advice, and friendly reviews throughout this process. The success of this research depended on undergrad technicians, including Seiara Goetz, Emma Livingston, Chapin Czarnecki, Shianne Lindsay, Emily Booth, Marina Wittman, and Noah Garwood. I am grateful for the comradery and support of all of my fellow EFB graduate students, and graduate and undergraduate TAs. Most special thanks to Rea Manderino who as she describes it, is the yin to my yang. She has been an unparalleled source of knowledge and encouragement, and I am so grateful to have shared this journey with her.

I am deeply grateful for the continuous support of family, friends, and colleagues throughout – without these people, I surely would not have made it. First, I am deeply grateful for the support of Dr Ray Barbehenn who opened the door for me to the world of gypsy moth research, then stood behind me as I chose to pursue it further. My warmest thanks to Teresa and Jeff Given, their children, and their dog Lily, for welcoming me into their home in Richmond, VA. The Given family has a special place in my heart. Thank you to all of my many Syracuse roommates and upstairs neighbors, especially Emily Burdo Gray and Da'jon James, who were essential to my sanity during my first year, and to Elena Juodisius, Sarah Caltabiano, and Maryssa Schlough. To my best friend David Landskroener who has provided much appreciated support in the form of birb memes and adventures, I say, "Thanks. You?".

Most importantly, to my family. My brother Adam who I know will always have my back. My grandparents who instilled in me early the idea that girls who look under rocks discover cool things. My mom for her endless love and support, and for showing me that women who work hard go far, and to my dad whom I believe has all the answers, and always, always has time to listen. Finally, my utmost gratitude and love to Rand Michaels who has weathered the lion's share of my stress and anxiety. He makes the light at the end of this tunnel even brighter.

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Appendices

Abstract

C. J. Jahant-Miller. Environmental drivers of morphological and physiological adaptation in an invasive defoliator, *Lymantria dispar*. 195 Pages, 17 Figures, 2020. APA style guide used.

Climatic gradients result in life-history tradeoffs across diverse taxa. Studying range-expanding invasive species can offer insight to environmental conditions that drive adaptive responses. Insects, which have rapid generations and high fecundity, can adapt rapidly to novel or changing environmental conditions, making them ideal model organisms for studying evolution *in situ*. European gypsy moth was introduced to North America in 1869 near Boston, Massachusetts, USA, and has since expanded its range to occupy 12˚ of latitude and diverse thermal regimes. I investigated environmental drivers of adaptation across the latitudinal-climatic range of gypsy moth in North America.

I linked variation in wing length, a proxy for body size, of pheromone-trapped males to metrics of habitat quality including forest composition, phenology, and population density. I found support for previously reported seasonal phenology-linked decline in wing length in both endemic and outbreaking gypsy moth populations. Furthermore, I found significant differences in wing length between males from forests of different quality by using discrete forest quality estimates, but not using continuous metrics of forest quality. Mean wing length of males from outbreak populations was smaller on than those from endemic populations.

Male flight capacity is an important aspect of fitness, and has important implications for mate-finding and establishment success during range expansion. Using fixed-arm flight mills, I found that body size is the most important predictor for total flight distance and maximum speed, and forewing aspect and relative thorax mass also had a significant effect on flight capacity.

Using a reciprocal transplant study, I found evidence for adaptive shifts in hatch timing occurring in populations from across the latitudinal range of gypsy moth, which may serve to reduce asynchrony with budburst of preferred host tree species at latitudinal range margins. I demonstrated that fitness costs of hatching ahead of or behind red oak budburst may provide sufficient selective pressures to drive the change observed in natural populations.

The results herein may inform current gypsy moth management priorities, and identify knowledge gaps where future research can improve our understanding of the barriers to range expansion in gypsy moth and perhaps other invasive species.

Key words: Invasive insect, forest pest, evolution, phenology, plant-insect interactions, latitudinal clines

C. J. Jahant-Miller
Candidate for the degree of Doctor of Philosophy, April 2020
Dylan Parry, Ph.D.
Department of Environmental and Forest Biology
State University of New York College of Environmental Science and Forestry,
Syracuse, New York

Chapter 1: Literature Review and Project Summary

Overview

As the frequency of biological invasions increase, so does the threat to global biodiversity, ecosystem services, and the economic cost of management and eradication efforts (Simberloff *et al*., 2013; Mollot *et al*., 2017). A major hurdle for an invading species is establishment, both initially and during range expansion (Tobin *et al*., 2009; Lockwood *et al*., 2013). Environmental and demographic stochasticity, genetic drift, and Allee effects can all act antagonistically to suppress population growth rates of nascent invading populations, limit expansion, and even cause extinction (Taylor & Hastings, 2005; Johnson *et al*., 2006; Tobin *et al*., 2007; Tobin *et al*., 2009). On the other hand, beneficial adaptation in invasive populations can lead to increased fitness, higher population growth rates, and range expansion (Kanarek & Webb, 2010; Dlugosch *et al*., 2015).

Due to rapid generations and high fecundity, insects are capable of adapting quickly to changing environments, especially in response to climate (e.g., Lee, 2002; Colautti & Lau, 2015; Lombardo et al., 2017; Banahene et al., 2017). Additionally, as poikilotherms, insects are highly sensitive to variation in temperature; thus, much of the existing literature on adaptation in invasive insects is related to climate (e.g., Hill *et al*., 2011; Lombardo *et al*., 2017) and phenology, which has important fitness effects, particularly for species relying on ephemeral resources (Ivashov *et al*., 2002; van Asch & Visser, 2007; Parry *et al*., 1998; Banahene *et al*., 2018; Keena & Shi, 2019). Understanding conditions and processes facilitating invasions can improve surveillance and management, and limit harmful impacts of current and future invasions.

Invasion of gypsy moth (*Lymantria* dispar L.) into North America is perhaps the most well-studied biological invasion in North America and has a massive database of literature gathered over more than a century. Since the late 1800’s, the range of the invasive gypsy moth has expanded to encompass 12˚ of latitude, covering large climatic and ecological gradients (Grayson & Johnson, 2018). Understanding environmental characteristics driving gypsy moth adaption and facilitating range expansion can enhance future gypsy moth management efforts and inform modern predictions for range limits. Furthermore, our granular understanding of the rate of invasion and range extent, coupled with data on environmental characteristics, demography, and evidence of adaptive shifts accompanied by genetic analyses suggests gypsy moth could be a model organism for improving our understanding of obstacles to establishment success and range expansions during invasions. The importance of these implications will increase in magnitude as human-mediated climate change and biological invasions continue to alter biodiversity and ecosystem processes.

Invasion ecology

Modern global connectivity has vastly accelerated the rate at which species are moved around the planet relative to historical rates of natural long-distance dispersal (Riccardi, 2007; Hulme, 2009). Invasive species threaten ecological processes by altering trophic interactions and ecosystem properties (e.g., productivity, nutrient cycling, decomposition), reducing species richness, and ultimately threatening biodiversity (Vittousek, 1986; Mack & D'Antonio, 1998; White *et al*., 2006; McGeoch *et al*., 2010; Simberloff *et al*., 2013; Mollot *et al*., 2017). Pimentel *et al*. (2001) estimated there were >120,000 species of plants, animals, and microbes moved to nonnative habitats in only six of the world's geopolitical regions (United States, United Kingdom, Brazil, India, Australia, South Africa), at a global economic cost upwards of $300 billion (Pimentel *et al*., 2000; Hulme, 2009; Pimentel, 2014). The increased attention and call to action garnered by biological invasions over time is mirrored in the relevant scientific literature (Gurevitch *et al*., 2011).

Many invasions are the result of relatively few individuals being introduced to a novel habitat. These incipient populations are susceptible to founder effects, which reduce genetic diversity relative to native range populations and may decrease the ability to adapt to novel environments (Prentis *et al*., 2008). Many founding populations may be limited by Allee effects (Roman & Darling, 2007; Dlugosch & Parker, 2008; Kanarek & Webb, 2010), a density dependent relationship where species functions such as mate finding, aggregation, or cooperative feeding are less successful at low densities, resulting in negative population growth below a density threshold (Allee, 1927; Allee, 1938; Stephens *et al*., 1999). These can be a major barrier to establishment and range expansion during invasions and can result in failure to establish, increased lag times, and reduced invasion rate (Taylor & Hastings, 2005; Johnson *et al*., 2006; Tobin *et al*., 2007). Some invasive species can overcome these limitations by reproducing asexually or rapidly or through increased propagule pressure (e.g., multiple introductions, or introductions of more individuals), or though random beneficial adaptation (Ellstrand & Elam, 1993; Lee, 2002; Frankham, 2005; Alleaume-Benharira *et al*., 2006; Gilchrist & Meats, 2010). Individuals immigrating to founder populations from established ones can increase mating success and gene flow, resulting in greater genetic diversity and fitness, and can ultimately facilitate positive population growth (Kirkpatrick & Barton, 1997; Alleaume-Benharira *et al*., 2006). However, high levels of gene flow to founder populations may swamp out beneficial adaptation to local conditions if immigration events become too frequent (García-Ramos & Kirkpatrick, 1997).

Numerous examples of adaptation in invasive species exist, both in response to local conditions (e.g., Gomi & Takeda, 1996; Kollman & Banuelos, 2004; Maron *et al*., 2004), and for greater dispersal ability (Simmons & Thomas, 2004; Phillips *et al*., 2006; Dytham, 2009). For example, cane toads *Rhinella marina* (Linneaus), which were introduced to Australia in 1935 in an attempt to control beetle pests of sugarcane, have increased dispersal ability with greater leg length, and populations near the invasion front have longer legs than those behind the invasion front (Phillips *et al*., 2006). Similar selection for increased flight-related morphology at range margins has been reported for speckled wood butterfly *Pararge aegeria* (Linneaus), and for wing-dimorphic bush crickets, *Conocephalus* spp. and *Metriotera* spp. (Hughes *et al*., 2003; Simmons and Thomas, 2004). Greater allocation towards dispersal ability can occur in populations that undergo frequent extinction recolonization events (Forsman *et al*., 2010).

Gradients in environmental characteristics, especially relating to climate and phenology, can result in measurable variation in insect life history traits (Denno and Dingle, 1981; Addo-Bediako *et al*., 2000). For example, populations of several invasive insects sampled across a latitudinal gradient varied significantly in thermal tolerances (e.g., Masaki, 1965; Jing and Kang, 2003; Thompson *et al*., 2017; Banahene *et al*., 2018; Lombardo *et al*., 2017). Climate-driven clines have also been noted for fecundity and egg provisioning, as was demonstrated for spruce budworm *Choristoneura fumiferana* (Clemens) forest tent caterpillar *Malacosoma disstria* Hübner, and the butterfly *Lycaena hippothoe* (Hoffmannsegg) (Harvey, 1983; Parry *et al*.,2001; Fischer & Fielder, 2001), and diapause regulation (e.g., Japanese Emma field crickets *Teleogryllus emma* (Ohmaki & Matsura) (Masaki, 1965).

Due to fast generational turnover and high fecundity in insects, environmentally-driven adaptation can occur fairly rapidly and has been observed in some invasive insects. For example, the invasive European fly *Drosophila subobscura* Collin has undergone rapid latitudinally-linked evolution for several traits including wing size and resistance to desiccation (Glichrest *et al*., 2004; Balanya *et al*., 2006*;* Gilchirst *et al*., 2008). Similarly, there is evidence that northern populations of hemlock wooly adelgid *Adelges tsugae* Annand are more capable of surviving exposure to colder temperatures than southern populations (Lombardo *et al*., 2017).

The gypsy moth as a model system for invasions

Gypsy moth *Lymantria dispar* (L.) (Erebidae: Lepidoptera) is a univoltine defoliating Palearctic moth species capable of feeding on the leaves of a wide range of deciduous and coniferous tree and shrub species. In its native European range, preferred hosts vary by region, but include species of *Quercus*, *Carpinus*, *Alnus*, *Prunus*, *Populus*, *Gleditsia*, *Tilia*, *Corylus*, and *Robinia* (Fuester *et al.*, 1981). Gypsy moth in North America feed on > 300 species of woody plants, but prefer *Quercus*, *Salix*, *Populus*, and *Betula* (Liebhold *et al*., 1995). The Asian subspecies of gypsy moth has a greater host range than European gypsy moth, and feed more readily on conifer species than the European subspecies (Johns *et al*., 2015).

Lymantria dispar is divided into three subspecies based on geography and female flight capability (Pogue and Shaefer, 2007). Asian and Japanese gypsy moth are commonly grouped together and referred to simply as Asian gypsy moth, though there is sufficient genetic evidence in support of three distinct subspecies (Wu *et al*., 2015). Males of the European subspecies are mottled brown and capable of flight, whereas females are white with black markings, and are not capable of ascending flight. Compared to the European gypsy moth, female Asian and Japanese gypsy moth have greater wing area and reduced abdominal mass, and have strong flight capability (Shi *et al*., 2015). Female *L. dispar* have little if any selectivity during oviposition and indiscriminate deposition of egg masses on available substrates greatly increases the risk of the insect becoming invasive (Bigsby *et al*., 2011). Additionally, flighted Asian gypsy moth females are attracted to lights, especially at well-lit ports where they deposit eggs on ships (Walsh, 1993; Schaffer and Strothkamp, 2014).

Greater flight capability coupled with wider breadth of diet gives Asian gypsy moth higher invasive potential relative to European gypsy moth, which is reflected by monitoring and eradication efforts on the western coast of North America where frequent importation of Asian products are likely to introduce the potential invader (Hajek & Tobin, 2009). Canada and New Zealand have also eradicated populations of introduced Asian gypsy moth (Gray, 2010). Concern for potential invasion by European and Asian gypsy moth in other countries is ongoing (Matsuki *et al*., 2001; Pitt *et al*., 2007). Recent genetic research suggests there has been introgression of Asian gypsy moth genome into some North American populations (Wu *et al*., 2020).

European gypsy moth was introduced to North America in 1869 (Forbush and Fernald, 1896). Gypsy moth is uncommon among invasive species in that the precise place and time of its intentional introduction, and subsequent unintentional release and establishment in North America is known and well-documented (Forbush and Fernald, 1896). Historic accounts of gypsy moth indtroduction are corroborated by recent genetic analyses of North American populations which indicate that gypsy moth underwent founder effects, and are genetically distinct descendents of western European populations (Wu *et al*., 2015). Since its establishment near Boston, MA, 150 years ago, gypsy moth has expanded north into Quebec, Canada, south to the Outer Banks of North Carolina, and west to the Iron Range of Minnesota (Grayson and Johnson, 2018). The latitudinal distribution of gypsy moth across Europe and Asia lies between 30˚N and 60˚N (Doane and McManus, 1981), and at present, the gypsy moth distribution in Canada and the United States lies between 35˚N and 48˚N latitude (USDA APHIS, 2019). The potential for further spread within North America is primarily affected by host availability and climate suitability (Gray, 2004; Morin *et al*., 2005; Hlansy and Turcani, 2009). The invasive range of gypsy moth covers diverse climatic conditions and thermal regimes, which may be contributing to mismatched hatch-bud burst phenology in some populations (e.g., Foster *et al*., 2013).

In its native European territory, gypsy moth exhibits cyclical outbreaks every 8–13 years (Hlásny *et al*., 2016), whereas North American populations exhibit varying cyclicity (4–5 years or 8–10 years) depending on forest type (Johnson *et al*., 2006). Repeated defoliation events can impact the health and economic value of individual trees, and shift forest composition over time (Campbell & Sloan, 1977; Gottschalk, 1990; Favan & Wood, 1996; Jedlicka *et al*., 2004). Non-target effects of gypsy moth management can negatively affect native Lepidoptera (Wagner *et al*., 1996; Boettner *et al*., 2000; Parry, 2009), and gypsy moth defoliation events can have important trickle-down ecological consequences for trophic interactions (Gottschalk, 1990; Thurber *et al*., 1994; Elkinton *et al*., 1996; Ostfeld *et al*., 1996; Redman & Scriber, 2000; Showalter & Whitmore, 2002). Overall annual spending on gypsy moth by federal and local governments in North America has been estimated to range between $83 million and $3.2 billion depending on the year and outbreak cycle (Aukema *et al*., 2011; Bradshaw *et al*., 2016). Given the increasing threat of both biological invasions and climate change, understanding factors driving rapid adaptation to novel environmental characteristics is critical to inform ongoing management and conservation efforts.

The remainder of this chapter and the chapters that follow focus on *Lymantria dispar dispar*, thus throughout the remainder of this document the use of “gypsy moth” or “*Lymantria dispar*” refers specifically to the European subspecies *Lymantria dispar dispar*, unless otherwise noted.

Gypsy moth management in North America: Slow-the-Spread

Although early phases of gypsy moth management (1891–1923) focused on eradication, subsequent management was directed at reducing the rate of spread (Doane & McManus, 1981). Slowing the spread of gyspy moth has been shown to have significant economic benefits (Tobin, 2008; Bogich *et al*., 2008). Barrier zones are narrow detection areas along an invasion front, which are intended to detect and inform management practices for eradication of spot populations, and slow the spread of a target species (Sharov & Liebhold, 1998). Gypsy moth undergo stratified dispersal, a combination of short range random dispersal accomplished by larval ballooning and long-distance human mediated transport, making it an ideal candidate for barrier zone mediated control within the transition zone (Bigsby *et al*., 2011). A barrier zone implemented in 1923 in the Lake Champlain and Hudson River valleys (Felt, 1924; Doane & McManus, 1981), slowed further expansion west and northward but was breached in the 1940's. Subsequently, eradication and containment efforts were discontinued, allowing the invasion rate to increase to an unprecedented speed over the next two decades (McFadden & McManus, 1991).

Female gypsy moth sex pheromone was identified in the early 1960s, and successfully synthesized in 1970 (Bierl *et al*., 1970). In tests conducted by Bierl *et al*. (1970) to compare the efficacy of equal amounts of the natural sex pheomone and the synthetic pheromone Disparlure®, the natural pheromone attracted only three males while Disparlure® attracted 110 in an equal time period. The increased attractiveness of synthetic pheromone, which resulted from a minor alteration of the molecular structure compared to the natural pheromone, provided an effective basis for trapping. The advent of pheromone-baited gypsy moth traps allowed for increased efficacy in monitoring small low-density populations which were difficult to detect using traditional methods such as egg mass surveys or burlap banding (Schwalbe, 1981). In addition to detecting low-density populations, pheromone trapping is an efficient method for estimating relative population density for gypsy moth (Elkinton & Carde, 1981; Thorpe *et al*., 1993),

Following the advent of synthetic pheromone, several regional management programs (e.g., Maryland Gypsy Moth Integrated Pest Management Program and the Appalachian Integrated Pest Management program) and a national Slow-the-Spread pilot program (1993 - 1995) were implemented (Reardon, 1991). Following success of the pilot, the United States Department of Agriculture (USDA) Forest Service and USDA Animal and Plant Health Inspection Service (APHIS) funded the national Slow-the-Spread (STS) program in 1999, which implemented a 100 km wide barrier zone across the entire invasion front in Michigan, Wisconsin, Illinois, Indiana, Ohio, West Virginia, and Virginia (Sharov e*t al.*, 2002; Stills, 2007), and now also includes Minnesota and North Carolina (Slow-the-Spread, Inc., 2019). The STS barrier zone consists of an array of > 100,000 pheromone-baited traps, and is divided into action and monitoring zones. Within the action zone, populations targeted for management may be treated using *Bacillus thuringiensis kurstaki* (Btk) or mating disruption, which takes advantage of Allee effects (Tobin & Blackburn, 2007; Tobin *et al*., 2011). The Slow the Spread Foundation collects a vast quantity of empirical data on an annual basis, providing an unprecedented resource that has enabled novel research in invasion ecology and population biology (Grayson & Johnson, 2018).

Habitat quality for folivorous Lepidoptera

Abiotic

As ectotherms, insect development and survival is highly sensitive to temperature (Huey & Slatkin, 1976; Ratte, 1984). The relationship of development and survival with temperature is typically nonlinear, because development peaks at a thermal optimum and decreases on either side of that optimum (Huey & Slatkin, 1978; Tauber *et al*., 1990; Jaramillo *et al*., 2009). Suboptimal thermal conditions can increase development time, and reduce survival and fecundity (Ratte, 1984; Bale, 1987; Hodek & Hodková, 1988). Insects can tolerate seasonal extremes in temperature and precipitation by entering either a facultative or obligate diapause, which is often terminated in a temperature-dependent manner (Lee, 1991). Interest in insect thermal tolerance and ecological implications of climate change has increased in the last two decades (e.g. Volney & Fleming, 2000; Kingsolver *et al*., 2011; Paaijmans *et al*., 2013). Overall, studies suggest that shifting thermal regimes are expected to cause local extinctions, shifting range limits, and adaptation to novel climatic envelopes.

Gypsy moth development through all four life stages (egg, larva, pupa, adult) is sensitive to variation in temperature (Cassagrande *et al*., 1987; Tauber *et al*., 1990; Logan *et al*., 1991; Gray, 2004). Thermal optimum for larvae changes depending on instar, but lies between 28 and 31ºC (Logan *et al*., 1991). Knapp and Casey (1986) reported gypsy moth larvae tend to avoid direct sun exposure where conditions are hotter and drier than similar shaded locations. Gypsy moth eggs, which are laid in late summer, enter an obligate diapause during the cold winter months, then hatch in the spring after chilling requirements have been met, and sufficient warming degree days accumulated (Doane & McManus, 1981). Diapause consists of three stages (pre-diapause, diapause, post-diapause), each of which depends on different temperature regimes for development. Pre-diapause consists of approximately two weeks of embryonic development, when developmental rate is dependent on warm conditions and increases as temperatures approach 30˚C (Gray *et al*., 1991). Embryonic development slows during diapause, during which exposure to low temperatures is critical, because diapause is terminated when diapause enzymes are depleted, which requires adequate exposure to temperatures < 5˚C (Gray *et al*., 2001). Although termination of diapause occurs more quickly with exposure to colder temperatures, eggs held at warmer overwintering temperatures (10–15˚C) are still able to maintain a hatch rate of 50–80% (Tauber *et al*., 1990). Post-diapause development, which has a temperature threshold > 3˚C, was modelled using the sine-wave technique. Egg hatch occurs following post-diapause warming degree day accumulation of 282 ± 29 DD (Johnson *et al*., 1983).

In addition to temperature, other important abiotic components of habitat quality include topography, soil chemistry, and precipitation (Liebhold *et al*., 1997), although these mainly act indirectly on gypsy moth fitness via host-mediated effects (Koricheva *et al*., 1998a). In other words, abiotic factors that shape the growth, phenology, productivity, nutritional quality and other characteristics of plant hosts can be important controls on gypsy moth habitat quality. Leaf chemistry can be differentially impacted by solar radiation, and host trees in the sun or on southern slopes may allocate more resources to defensive compounds than trees on shady north-facing slopes (Bryant, 1987; Dudt & Shure, 1994). Soil has a similar indirect effect on habitat quality for gypsy moth, because variation in soil composition and chemistry affects the species composition of plant hosts as well as their nutritional quality via foliar chemistry (Hemming & Lindroth, 1999; Giertych *et al*., 2005). Precipitation has indirect effects on gypsy moth habitat quality through bottom up and top down forces. Precipitation can affect foliar quality positively and negatively through indirect effects on nutritional quality and secondary defenses (Talouk *et al*., 1990; Koricheva *et al*., 1998b). On the other hand, increased spring precipitation increases the likelihood of fungal epizootics by the pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper which can decimate larval gypsy moth populations (Weseloh *et al*., 1993; Hajek *et* al., 2015; Elkinton *et al*., 2019).

Biotic

i. Host

The quality and quantity of food available to herbivorous insects is a critical determinant of fitness (Awmack & Leather, 2002; Parry & Goyer, 2004). As sessile organisms, plants have evolved an array of defense mechanisms in response to herbivory including chemical compounds, which can be toxic (qualitative) or dose-dependent growth inhibitors (quantitative), and physical barriers (e.g., trichomes, foliar toughness; Karban & Myers, 1989; War *et al.*, 2012). The specific suite of defenses implemented by an individual plant varies within and between species, and can also vary ontogenetically and in response to herbivore damage or other stressors and stimuli (Feeny, 1970; Ali & Agrawal, 2012). An inability to compensate for plant defenses can lower the fitness of herbivorous insects; as a result, insects have developed myriad ways to counteract them including avoidance, mechanical disruption, sequestration, or metabolic detoxification (Despres *et al.*, 2007).

European gypsy moth are highly polyphagous folivores capable of feeding on > 300 species of trees and shrubs in North America, but prefer woody plants from a relatively small subset of genera such as *Quercus*, *Populus*, *Betula*, and *Salix* in North America (Hough & Pimentel, 1978; Roth *et al.*, 1994; Liebhold *et al.*, 1995). Preferred host plants for gypsy moth tend to have quantitative secondary compounds including tannins and phenolics, and digestibility reducers (e.g., cellulose, lignin), that increase proportionally as leaves mature (Feeny, 1970; Barbosa & Krischik, 1987). Gypsy moth larvae, especially late instars, have very high midgut pH, which limits protein-binding activity of tannins and enables larvae to feed on host foliage with high tannin content (Schultz & Lechowicz, 1986). Preference for, and superior performance on, oak foliage by gypsy moth has been demonstrated in controlled lab and field experiments (Hough & Pimetel, 1978; Lance & Barbosa, 1981; Raupp *et al.*, 1988; Shields *et al.*, 2003).

Gypsy moth reared on northern red oak (*Quercus rubrum*) have increased pupal mass and fecundity and reduced developmental duration and mortality compared to larvae reared on other common eastern hardwood genera such as maple and beech (Hough & Pimentel, 1978; Raupp *et al.*, 1988). In choice tests to determine host preference of first, second, and third instars, oak was always most preferred (Barbosa *et al.*, 1979). Gypsy moths also perform well on *Populus* and *Betula* spp. (Roth *et al.*, 1994) and exhibit similar fitness to those reared on oak.

Given these host preferences, forest composition can affect gypsy moth behavior and population dynamics. Larvae that begin feeding on highly acceptable hosts are less likely to seek alternate hosts via larval ballooning than those on unsuitable hosts (Capinera & Barbosa, 1976; Lance & Barbosa, 1981; Hunter & Elkinton, 2000). Given the mortality of dispersing neonates can be high (Weseloh, 1998), higher proportions of good quality hosts in a stand will tend to support higher population densities of gypsy moth. A compendium of tree and shrub species and their likelihood of being defoliated during gypsy a moth outbreak ranks *Quercus*, *Populus*, *Betula*, and *Salix* among the highest genera (Liebhold, 1995). Forest patches composed of a higher proportion of highly suitable host species are more susceptible to gypsy moth outbreak, and promote relatively faster rate of spread, than forest patches dominated by lower quality hosts (Houston *et al.*, 1977; Sharov *et al.*, 1999).

ii. Phenology

Spring feeding Lepidoptera, which depend on young leaves for successful development, are especially sensitive to positive (i.e., hatch occurs prior to leaf emergence) and negative (i.e., hatch occurs after leaf emergence) phenological mismatch between hatch and leaf emergence (Hunter, 1991; Parry *et al.*, 1998; Hunter and Elkinton, 2000; van Asch and Visser, 2007; Keena & Shi, 2019). The window for early instar larvae to feed on young spring foliage is short and therefore the phenological synchrony between hatch and host bud burst is critical (Raupp *et al*., 1988; Hunter *et al*., 2000). Newly emergent leaves are ideal food because as leaves rapidly mature in the spring, they have lower moisture content and lower nutritional value due to a higher C:N ratio (Feeny, 1970; Hough & Pimentel, 1978; Schroeder, 1986). Larvae that hatch before leaf emergence have an increased risk of starvation, while those hatching after leaf emergence have reduced developmental rate, survival, pupal mass, and fecundity, and increased risk of exposure to natural enemies (Hough and Pimentel, 1978; Parry *et al*., 1998; Hunter and Elkinton, 2000; van Asch and Visser, 2007; Keena & Shi, 2019).

Because host plants and insects have different phenological cues related to dormancy and spring emergence, asynchronies can occur. Insect diapause in many species is primarily controlled by temperature, while leaf emergence phenology for most temperate tree species is based on winter chilling, long-day photoperiod, and warmer spring temperatures (Basler & Korner, 2010; Clark *et al*., 2014). For most temperate deciduous trees, if the chilling and day length requirements for ending dormancy are not met, increased temperature will have little or no effect on the timing of bud and leaf development (Basler & Korner, 2014; Flynn & Wolkovich, 2018). Asynchronies that result from differential respnoses to spring cues in insects and their host plants have negative effects on fitness that, in turn, have important implications for insect population growth and outbreak dynamics (Hunter, 1993; Hunter & Elkinton, 2000; van Asch & Visser, 2007).

Gypsy moth hatch is regulated primarily by temperature, with little to no influence from photoperiod (Gray, 2004). The current invasive range of gypsy moth in North America encompasses diverse temperature regimes, making gypsy moth vulnerable to phenological mismatch with bud burst of preferred hosts. In Appalachian forests, gypsy moth egg hatch lags behind leaf emergence by a week on average, and up to nearly three weeks in some years (Foster *et al.*, 2013). Whether or not gypsy moth egg hatch stays relatively synchronous with host tree phenology across its latitudinal range is dependent upon its ability to adapt to variation in local climatic conditions.

There is evidence that at lower latitudes, insufficient winter chilling for trees, but not for gypsy moth, may be responsible for decoupled spring phenology in which gypsy moth hatch occurs prior to budburst (Foster *et* al., 2013). Hatching in advance of host trees at southern latitudes increases risk of starvation, and in a warm southern climate, gypsy moth larvae are highly unlikely to survive an extended period without food (Stockhoff, 1991; Keena & Shi, 2019). At the northern limits of the invasion front, thermal requirements for budburst are likely met long before those of gypsy moth. Leaf emergence occurs soon after photoperiod (day length) requirements are met (Korner & Basler, 2010; Pletsers *et al*., 2015) and may occur long before gypsy moth hatch. In the shorter and cooler growing seasons at higher latitudes, hatching late relative to leaf emergence may exacerbate climatic constraints by slowing growth even further (Logan, 1991). Under these conditions, the realized pool of suitable hosts may be considerably smaller than predicted by Liebhold *et al*. (1995), and only a subset of the highest ranked hosts may produce growth rates sufficiently fast to complete larval development, oviposit, and enter diapause before temperatures become too cold.

Consequences of temperature regime change due to range shifts or climate change can have significant ecological impacts (Harrington *et al.*, 1999; Forrest, 2016). Species that are chronically affected by climate-mediated asynchrony are likely to exhibit one of three generalized outcomes: becoming locally extinct, shifting their range, or adapting in place (Menendez, 2007). Failure to adapt to local thermal regimes and climatic variability may make gypsy moth vulnerable to phenological mismatch (and subsequent associated fitness costs) at the extremes of its North American range.

Range limits

Constraints on southern and northern gypsy moth range expansion are largely temperature dependent. The theoretical southern range limit occurs where winter temperatures are not sufficiently cold to satisfy diapause chilling requirements (Tobin *et al*., 2014b; Thompson *et al*., 2017). Conversely, the northern range limit is determined by low winter temperatures that fall below the supercooling point for gypsy moth eggs (-27˚C), at which point ice crystals form inside the eggs and cause them to rupture (Denlinger *et al*., 1992; Andreson *et al*., 2001). A study by Tobin *et al*. (2014b) found temperatures in the coastal plains of Virginia and North Carolina that exceed the thermal optimum for larval development (27˚C) are becoming more frequent due to climate change, which lowers fitness in local populations and results in range retraction. This suggests southern range limits may in reality lie farther north than predicted by models based on diapause requirements alone. In addition to extremely low winter temperatures, northern range limits may be constrained by failure of larvae to complete development within the constraints of a compressed growing season that is also characterized by relatively cool spring and summer temperatures.

Gray (2004) predicted gypsy moth establishment in the United States based on current climate and projected warming (1.5˚C increase) and gypsy moth thermal tolerances, and reported gypsy moth would eventually invade the entire continental United States except for parts of the northern Rocky Mountains, the Iron Range of Minnesota, the southern tip of Texas, and southern Florida. Modeling based on distribution of preferred hosts (i.e. those rated most preferred by Liebhold *et al*., 1995), predicted large uninhabitable areas in North Dakota, South Dakota, Nebraska, and Kansas, and smaller ones in Ohio, Indiana, and Illinois (Morin *et al.*, 2005), primarily due to large-scale agriculture and a lack of tree cover. Established populations of gypsy moth have since been detected in Lake, Cook, and St. Louis counties of Minnesota (Tobin et al. 2016), which lay outside the northern limits of the predicted range, most likely due to the insulative value of snow cover, which protects egg masses despite winter air temperatures often falling well below the documented gypsy moth supercooling point (-27˚C; Doane & McManus, 1981; Tobin *et al.*, 2007; Streiffel *et al.*, 2019).

Under current climate trends, gypsy moth's potential range is likely to continue shifting northward as temperatures warm. In addition to the documented range retraction occurring at the southern range limit, the current range is expected to expand further northward as climate warms (Hlasny & Turcani, 2009). However, the temperate deciduous forest in northeastern North America transitions to boreal forest north of the current gypsy moth range. The absence of suitable hosts is likely to limit northward expansion, unless the range of host trees responds to climate change in a similar manner (Régnière *et al.*, 2008). Indeed, gypsy moth reached the northern limits of suitable hosts in eastern North America 30 years ago and there has been little further northward spread (Régnière *et al.*, 2008).

Adaptation along latitudinal gradients

Phenotypic shifts occurring in response to variation in environmental conditions can happen as a result of phenotypically plastic responses or through genetically-driven adaptation to novel or changing conditions, which can occur rapidly in nature and in response to human-mediated environmental changes (Kettlewell, 1951; Boag & Grant, 1981; Hendry *et al.*, 2008; Hoffman & Willi, 2008; Hansen *et al.*, 2012). A striking description of rapid adaptive response to environmental conditions in Darwin's medium ground finch *Geospiza fortis* Gould reported changes in body size resulting from precipitation-meditated variation in food availability (Boag & Grant, 1981). Notably, the change in body size frequency occurred within the span of only four years. Atmospheric interaction with seasonal latitudinal variation in solar intensity, photoperiod, growing season, temperature, precipitation, and nutrient and energy availability ultimately produces latitudinal biotic gradients as populations adapt to local environmental conditions (Endler, 1977; Boag & Grant, 1981; De Frenne *et al.*, 2013; Inglezakis *et al.*, 2016). Invasions and climate change both have the potential to disrupt spatiotemporally linked ecological processes (e.g., phenology; Donnelly *et al.*, 2011). Therefore, understanding the ability and processes for adaptation to local conditions during range expansion can elucidate adaptive processes critical for species adaptation and survival in the face of the current and ongoing climate crisis (De Frenne *et al.*, 2013).

Organisms that encompass large environmental gradients often exhibit corresponding clineal variation in physiological traits. For example, two invasive annual weeds *Impatiens glandulifera* Royle and *Lythrum salicaria* Linneaus display latitudinal clines in growth and reproduction in relation to the length of the growing season: individuals from northern populations produce flowers sooner, but have reduced vegetative mass at reproduction than those from southern populations, indicating timely maturation relative to the growing season comes at the cost of reduced fecundity (Kollmann & Bañuelos, 2004; Colautti & Barret, 2013). Forest tent caterpillar, *Malacosoma disstria* Hubner, exhibit a latitudinal gradient in fecundity and egg provisioning with southern populations having higher fecundity, but smaller eggs, while northern populations have fewer, larger eggs with greater energy reserves (which may increase the odds of survival for first instar larvae that experience less predictable spring phenology than in the south; Parry *et al.*, 2001). Pupae from northern populations of a European dung fly *Scathophaga* *stercoraria* (Linneaus) were more likely to enter overwintering diapause than southern pupae, and diapause length increased with latitude and the corresponding length of winter (Demont & Blackenthorn, 2008). Similarly, gypsy moth populations from east Russia, United States (NC and NJ), and China, which fall between 40°N and 44°N, require less chilling to successfully initiate hatch than populations originating farther north (Keena, 1996; Keena, 2016).

Due to increasingly cooler and shorter growing seasons as latitude increases, there may be selection for tradeoffs in growth and reproduction in gypsy moth. Friedline *et al.* (2019) compared proxies for gypsy moth fitness (larval and pupal duration, and pupal mass) to genetic analyses and found patterns of genetic variation mirrored adaptive shifts in gypsy moth larval development time in populations sampled across the latitudinal gradient. However, May *et al.* (2018) reported no discernable difference in metabolic rate for populations of gypsy moth spanning the latitudinal range – although the number of sampled populations were small.

The current invasive North American range of gypsy moth spans 12° of latitude, encompassing diverse climatic regimes (USDA APHIS, 2019). Given the strong founder effect gypsy moth underwent at introduction (Wu *et a*l., 2015), there is heightened interest in understanding potential adaptive changes in gypsy moth physiology. The southern invasion front where populations have undergone range retraction, likely due to summer temperatures frequently exceeding thermal optima (Tobin *et al*., 2014b), is of particular interest given strong selection pressure for physiological or behavioral mechanisms to counter thermal conditions. Larvae reared under high-heat conditions in the lab exhibit reduced developmental rate, fitness, and survival (Logan, 1991; Banahene *et al*., 2018), but there is evidence for adaptation in southern populations for greater heat tolerance (Thompson *et al*., 2017; Banahene *et al*., 2018; Faske *et al*., 2019). Populations originating from the Coastal Plains region of Virginia exhibit greater heat tolerance and suffered reduced fitness consequences compared to those from the Appalachian Mountains (Thompson *et al.*, 2017; Banahene *et al.*, 2018). Genetic analyses of populations sampled along the altitudinal climate gradient suggest variation in heat tolerance is genetic in nature (Faske, 2017).

Adult behavior and fitness

Gypsy moth mating behavior

Gypsy moth are protandrous (males emerge before females), and sexually dimorphic with males mottled brown and capable of flight while females are white with dark markings and flightless due to the enormous size of their abdomens and reduced thoracic musculature compared to Asian gypsy moth (Doane & McManus, 1981; Shields *et al.*, 1997). Upon emergence, males engage in a haphazard zig-zag searching and dispersal phase until females emerge and are located (Doane & Carde, 1973; Wiklund & Fagerstrom, 1977; Doane & McManus, 1981). Calling by females via pheromone emission begins shortly after diurnal eclosion, when females intermittently protrude a pheromone-producing gland from the tip of their abdomen to release a unique sex pheromone (Doane, 1968; Richerson *et al.*, 1976; Odell, 1978). Males typically follow the pheromone plume upwind to its source.

Female attractiveness and fecundity are negatively affected by age, mating status, and ‘spewing’, in which unmated females lay partial clutches of unfertilized eggs (Richerson *et al.*, 1976; Tobin *et al.*, 2014a). Females that have ‘spewed’ are less attractive to males, and when mated, produce fewer viable offspring compared to females that have not spewed (Richerson *et al.*, 1976). Giebultowicz *et al.* (1991) reported pheromone emission is reduced both during and after mating due to pheromone-suppression induced by presence of a spermatophore in the spermatheca, which may explain why females become less attractive to males after mating (Richerson *et al*., 1976).

Gypsy moth are generally monogamous, though both males and females have been noted to mate more than once, at least in captivity (Forbush & Fernald, 1896; Prosholf & Bernon, 1994; Tobin *et al*., 2014a). Prosholf and Bernon (1994) reported male gypsy moth that mated more than twice transferred fewer sperm with each mating. If a male fails to transfer a full complement of spermatophore, consisting of sperm and a protein packet which supplements egg provisioning and female energy reserves in some Lepidoptera (Boggs & Gilbert, 1979; Loerch, 1984), the female will mate again (Proshold & Bernon, 1994). Given the method in which mates are attracted and located, and the short timespan for mating, it is unlikely that either sex is selective in mate choice (Doane & McManus, 1981; Charlton & Carde, 1990). However, Doane & Carde (1973) described male competitive behavior; when more than one male is searching at the same source, wing-contact is made and searching in one or both males immediately stops. Thus, being the first male to encounter a receptive female is highly advantageous.

Quantifying fitness in Lepidoptera

Fitness is the measure of an individual's contribution of genetic material to the next generation, or the number of offspring produced. Reproductive capacity and mate-finding ability in capital-breeding Lepidoptera (i.e., those that depend on resources accuired during larval feeding for adult metabolic needs) depend largely on environmental factors such as diet quality and quantity, and intraspecific competition for food resources (Ruohomäki, 1992; Evenden *et al*., 2015). Among outbreaking species, individuals from high density populations are more likely to have decreased body mass and wing length due to increased competition for food (Klomp, 1966; Mason *et al*., 1977; Leonard, 1981; Klemola *et al*., 2004). Conversely, larvae developing in populations at low densities with little intraspecific competition attain greater body mass and may consequently have greater dispersal capability, which would be beneficial for locating mates under low-density conditions. Ruohomäki (1992) found that for *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae), male wing length decreased as larval density increased. Evenden *et al*. (2015) found that flight propensity of field-reared individuals of an outbreaking insect *Malacosoma disstria* Hubner (Lepidoptera: Lasiocampidae) increased with wing load when reared under high density conditions, but not low density, likely as an adaptation to promote dispersal during high density conditions and reduce competition for progeny in subsequent generations.

Body size is correlated with reproductive capacity in many lepidopteran species (Rutowski *et al*., 1983; Svärd & Wiklund, 1989; Parry et al. 2001). Potential female fecundity in insects is often measured as the number of chorionated eggs present in the ovarioles of a dissected female (Tammaru *et al*., 1996; Tisdale & Sappington, 2001, Parry et al. 2001). Realized fecundity is more difficult to measure as not all eggs are fertilized and there may be differences between viability of eggs. A strong positive correlation between female body size and fecundity in capital-breeding Lepidoptera is well established (Brewer *et al*., 1985; Tammaru *et al*., 1996; Tammaru *et al*., 2002; Rhainds, 2015), including for gypsy moth (Hough & Pimentel, 1978; Faske *et al*., 2017). Many female insects maximize lifetime fecundity by mating multiple times (Arnqvist & Nillson, 2000). A female's ovigeny index (the number of eggs ready to lay at any given time divided by the female's lifetime fecundity) is inversely related to several characteristics including larval diet breadth, adult female mobility, adult lifespan, degree of polyandry, and dependency on adult resource acquisition via feeding or nuptial gifts (Jervis *et* *al*., 2005). Female gypsy moths have high ovigeny index (adult females emerge with a full egg compliment) and comply with the listed criteria (Doan & McManus, 1981).

Male reproductive potential is more difficult to measure, but correlates can be quantified by counting the number of eggs successfully fertilized after mating (e.g., Svärd and Wiklund, 1989; Bissoondath & Wiklund, 1995; Arnqvist & Nillson, 2000; Tobin *et al*., 2014a). In many lepidopterans, males pass an ejaculate (spermatophore) to the female during mating, which, in addition to sperm, contains a proteinaceous mass that may supplement energetic costs of egg production (Boggs & Gilbert, 1979; Svärd & Wiklund, 1989). Svärd and Wiklund (1989) found for both pierid and satyrid butterflies, mass of the spermatophore is correlated with adult male body mass. Bissoondath & Wiklund (1995) found that, although the mass of male pierids was correlated with spermatophore size, as body mass increased, mass of the spermatophore represented a smaller proportion of the total body mass. Additionally, smaller males were observed to provide proportionately more protein relative to the spermatophore when compared with larger males. While the spermatophore persists in the bursa copulatrix of the female for several days in other species of Lepidoptera, in gypsy moth it disintegrates quickly (Drummond, 1984). Additionally, gypsy moth copulation lasts an average of 60–73 min but ranges from 20–198 minutes, making the window for observation of the spermatophore relatively short (Forbush & Fernald, 1896). Quantifying viable eggs post-copulation relative to total female egg production eliminates the need to closely monitor copulation (Butlin & Day, 1985; Tobin *et al*., 2014a). Tobin *et al*. (2014a) estimated the effect of male and female age on reproductive success for gypsy moths by counting the number of viable and non-viable eggs produced by mating pairs.

Male flight and reproduction

For many insects, flight is a critical component of migration, dispersal, and mate-finding. Multiple studies have revealed the importance of flight capacity and behavior for insect migration and dispersal (e.g., Brown & Chippendale, 1974; McAnelly & Rankin, 1986; Han & Gatehouse, 1993). However, energy resources are limited and it has long been acknowledged that allocation of resources towards flight trades off with other life history traits (Johnson, 1969). Recent research on flight behavior of a capital breeding North American defoliator, forest tent caterpillar *M. disstria*, illustrates several facets of the ecological importance of male flight behavior. Forest tent caterpillar reared under high density conditions allocate proportionally more resources to increased wing area than those reared under low density conditions, likely as a mechanism to increase long distance dispersal and reduce competition for subsequent generations (Evenden *et al*., 2015a). Additionally, because smaller males are less likely to initiate mate-seeking behavior, mate-finding efforts of male forest tent caterpillar developing under moderate-density conditions are more successful than that of small low-quality males originating from high density conditions (Evenden *et al*., 2015b). Among noctuid moths, those with greater body size, and increased allocation to flight morphology (e.g., relative thorax mass, forewing aspect, etc) were more likely to initiate flight and flew greater distances (Jones *et al*., 2016). Immigration of relatively large males from endemic populations behind the invasion front to low density populations at or ahead of the invasion front may alleviate Allee effects in low-density gypsy moth populations (Sarov *et al*., 1995).

Flight musculature and stored energetic resources allocated for flight activity are housed in the insect thorax (Marden, 2000). For wing-dimorphic insects, in which one morph has improved flight morphology and is a strong flier, and the other has reduced flight morphology and is a weak or non-flier, examples of resource allocation trade-offs between flight and reproduction are plentiful (e.g. Guerra, 2011). Occurrence of a flight-reproduction trade off in wing-monomorphic species is less consistent, though not uncommon (e.g., Tigreros *et al*., 2019). The majority of studies assessing resource allocation trade-offs between flight and reproduction do so for female insects; for species in which female flight affects fecundity, it typically does so negatively by reducing reproductive output (Tigreros *et al*., 2019). Tradeoffs between flight and reproduction for male insects have been reported primarily for wing-dimorphic species, and tend to manifest as reduction of sperm and spermatophore output or reduced courtship ability (Langellotto *et al*., 2000; Guerra & Pollack, 2008; Guerra, 2011; Velde *et al*., 2012).

Tradeoffs between male reproduction and flight may be particularly important in sparse populations where males spend relatively more energy searching for females. Failure to find mates in isolated small populations is thought to drive their high probability of extinction (Contani *et al*., 2009; Tobin *et al*., 2009). If energy expenditure during mate-finding reduces allocation to the spermatophore (Elliott & Evenden, 2014; Wijerathna, 2016), challenges to mating success inherent in low-density populations may be exacerbated. Discrepancies between low rates of fertilization for lab reared (30%) and very low-density wild (<5%) gypsy moth populations may reflect the cost of mate-finding behaviors at low densities (Tobin *et al*., 2014a).

Flight mills have been used for more than half a century to quantify flight capacity in insects (e.g., Taylor et al. 2010; Fahrner *et al*., 2014; Evenden *et al*., 2015a; Kees *et al*., 2017; Minter *et al*., 2018; Naranjo, 2019). Flight capacity is commonly assessed in relation to physiological (e.g., lipid reserves, age, mating status, pathogen load) and morphological characteristics (e.g., body size, flight morphology), and relationships vary by species. For example, body size significantly affects mountain pine beetle *Dendroctonus ponderosae*

Hopkins, diamondback moth *Plutella xylostella* (Linneaus), and noctuid moth total flight distance (Shirai, 1995; Evenden *et al*., 2014; Jones *et al*., 2016), where increased body size corresponds to greater flight capacity, but not for large aspen tortrix *Choristoneura conflictana* (Walker), navel orangeworm *Amyelois transitella* (Walker), or Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) (Elliott & Evenden, 2009; Sappington & Burks, 2014; Lopez *et al*., 2017). No previous studies have investigated the relationship between male gypsy moth body size or flight morphology and flight capability, reproductive capacity, or the interaction between them.

Research objectives

Fitness consequences resulting from maladaptation to local biotic and abiotic conditions are a primary driver of evolution (e.g., Kettlewell, 1955; Bradshaw & Holpzafel, 2006). The focus of my dissertation research was to assess the influence of local environmental conditions on morphological components of fitness, and quantify adaptive shifts occurring in response to disparate climate conditions along the latitudinal range of gypsy moth in North America. Specifically, I aimed to 1) evaluate whther morphometrics of pheromone-trapped male gypsy moths are indicators of habitat quality, 2) quantify the influence of body size and flight-related morphology on flight capacity and behavior for gypsy moth, and 3) determine whether wide-ranging climatic conditions occurring across gypsy moth's invasive range have influenced adaptive shifts in hatch timing relative to oak leaf emergence.

In the first data chapter (Chapter 2), I used wing length measurements of trap-captured males to assess the utility in estimating parameters of habitat quality. Foliage from oaks (*Quercus* spp.) is preferred by gypsy moth and supports higher larval performance. In addition, the quality of oak foliage decreases seasonally at a rate slower than many other acceptable host species (Raupp, 1988). Therefore, I hypothesized population-based differences in male wing length, with larger males originating from oak-dominated forests and appearing earlier in the flight season, could be used to understand local habitat quality and seasonality. Gypsy moth reared under high density conditions experience intraspecific competition, resulting in a relative reduction in body size compared to those reared at low density (Leonard, 1981; Lance *et al.*, 1986). Additionally, a proportion of larvae developing in high-density populations undergo behavioral changes that result in completion of larval development at smaller body size and more quickly than expected based on temperature alone (Campbell, 1978; Lance *et al.*, 1987). Therefore, I hypothesized that wing length in outbreak populations would be smaller than in endemic populations and would follow a humped distribution with peak wing length in the middle of the flight season.

In Chapter 3, I used fixed-arm computer-linked flight mills to quantify the influence of body size and flight-related morphology and physiology on gypsy moth flight capacity and behavior. Specifically, I measured the effect of wing length (a proxy for body size), forewing aspect, relative thorax mass, and relative mass lost during flight on total flight distance and maximum flight speed. Additionally, I assessed the effect of body size on flight propensity. As capital breeders like gypsy moth do not supplement adult energetic needs by feeding (Tammaru & Haukioja, 1996; Marden, 2000), I expected flight capacity would increase with body size. Reduced flight capacity has important fitness implications, including reduced gene flow and mate-finding success in low-density populations, such as those at the invasion front, and may drive adaptive shifts for improved flight capacity.

In Chapter 4, I used a reciprocal transplant experiment to determine whether gypsy moth populations spanning the North American latitudinal range exhibit differential hatch timing, and whether adaptive shifts improve synchrony with local deciduous bud burst. Termination of gypsy moth diapause is temperature dependent (Gray *et al.*, 2001), whereas timing of budburst in deciduous trees is controlled by both temperature and photoperiod (Basler & Korner, 2010), making phenological synchrony between the two vulnerable to altered thermal regimes. Asynchronous hatch relative to budburst results in reduced fitness for spring-feeding Lepidoptera (van Asch & Visser, 2007), therefore, I hypothesized fitness consequences of hatching asynchronously with oak bud burst (either too late or too early) would result in an adaptive shift in hatch timing in populations across the latitudinal range.

In Chapter 5, I used two factorial design experiments to demonstrate the fitness costs of hatching early and late relative to the timing of leaf emergence for preferred hosts. Spring-feeding Lepidoptera are vulnerable to phenological asynchrony and hatching out of synchrony with ephemeral high-quality food sources can result in reduced survival and fitness (Parry *et al.*, 1998; Hunter & Elkinton, 2000; van Asch & Visser, 2007). I hypothesized that feeding on later-phenology foliage would result in reduced foliage compared to larvae feeding on foliage with optimal synchrony. Additionally, I hypothesized that larvae exposed to increasing temperatures without food would show shorter survival duration that larvae held without food at cooler temperatures representative of more northern spring conditions.

The final chapter summarizes my research findings within the dissertation, discusses biological and ecological implications, and suggests future research directions.

Chapter 2: Spatial and temporal changes in male gypsy moth wing morphology reflect host tree phenology and habitat quality[1]

---

[1] Jahant-Miller, C. J., Tobin, P.C., & D. Parry. (2020) *Agricultural and Forest Entomology*. (*In revision*).

Abstract

The size of adult gypsy moths, (*Lymantria dispar* L.), a capital-breeder, is correlated with environmental conditions experienced as larvae. Proxies for adult size such as wing length may provide information about habitat quality and population density.

We used male gypsy moths collected from pheromone traps at intervals through the flight season to assess phenological change in wing length. Consistent with a previous study conducted at our reference site, we found that wing length declines seasonally, likely resulting from phenological reduction in host foliage quality. This pattern was evident at our reference site over eight years, and at our experimental sites with low-density populations in three years.

We assessed forest quality using two unique metrics, basal area of red oak (*Quercus rubra*), a high quality host tree, and a composite value generated from a published ranking of tree species quality for gypsy moth. We did not find a relationship between these metrics and wing length, although we found that the mean size of males was larger in stands with oak.

Mean wing length in outbreak populations was significantly smaller reflecting density related processes such as intraspecific competition, although there was no significant seasonal effect on wing length.

Key words: *Lymantria dispar*, invasive species, habitat quality, adult morphometrics, phenology, pheromone-trapping

Introduction

Resource acquisition and allocation strategies are critical in determining the contribution of an individual to the subsequent generation (Boggs, 2009). In capital breeding Lepidoptera, all resources are acquired during the larval stage, making them useful models for studying the influence of larval environment on adult resource allocation (Tammaru & Haukioja, 1996). Resource quality can vary dramatically at relatively small spatial scales in response to biotic characteristics including population density, phenology, and intraspecific variation in host quality, and abiotic factors such as precipitation, soil quality and temperature (Raupp *et al*., 1988; DuMerle & Cornic, 1991; Shure *et al*., 1998; Jones & Evenden, 2008). Fitness costs incurred by spring-feeding Lepidoptera from reduced resource quality are reflected in measurements of phenotypic fitness proxies such as increased development time, and reduced fecundity, body size, and survival (Schroeder, 1986; Raupp *et al*.,1988; Parry *et al*., 1998; vanAsch & Visser, 2007).

Gypsy moth (*Lymantria dispar* L.) is a non-native defoliator of North American deciduous forests with an invasive range that spans from Quebec, Canada, to the Outer Banks of North Carolina, USA, and west to the Iron Range of northern Minnesota (Grayson & Johnson, 2018). Following identification of the sex pheromone in gypsy moth 50 years ago (Bierl *et al*., 1970), synthetic pheromone lures have been widely deployed along and ahead of the invasion front to inform delineation surveys, monitor changes in relative density, inform and evaluate management efforts, and estimate seasonal phenology (USDA, 2001; Tobin & Blackburn, 2007). Each year, the vast majority of trapped males are enumerated, a subset of which is subjected to genetic analyses to screen for the presence of Asian gypsy moth biotypes (Streifel *et al*., 2017), and eventually discarded. However, morphological attributes of individual males may provide ecological insight beyond simple counts and assessment of presence or absence. Forewing length in trapped males was first evaluated by Bellinger *et al*. (1990) as a potential predictor of population growth. In wild populations, male wing length was correlated with egg mass density and the relative size of egg masses in the vicinity of the traps. In another study, Carter *et al*. (1991) found that male gypsy moths originating from high-density populations were significantly smaller than those from low-density populations, likely due to competition for diminishing food resources that occurs during outbreaks. However, Tobin *et al.* (2016) recorded large numbers of males from low density populations with no history of outbreak in size ranges that Carter *et al*. (1991) classified as originating from an outbreak population. Instead, Tobin *et al*. (2016) showed that wing length was negatively correlated with seasonal flight phenology of males in low density populations and suggested that the occurrence of large males early in the flight season and smaller males later may reflect an interaction between larval development and the phenological condition of host trees.

Gypsy moths are typical capital breeders with all resources for flight and reproduction a product of the mass gained as larvae (Leonard & Doane, 1966; Hough & Pimentel, 1978). Thus, male size, and wing length, by proxy, reflect the collective ecological conditions experienced as larvae. Although gypsy moths are highly polyphagous, feeding on more than 300 species of trees, a smaller subset of those species (e.g., *Quercus* spp., *Populus* spp., *Betula* spp.) are highly preferred and provide high quality food, especially during the early instars (Barbosa & Greenblatt, 1979; Raupp *et al*., 1988; Hunter & Lechowicz, 1992; Liebhold *et al*., 1995). Gypsy moth larvae feeding on oak (*Quercus* spp.) foliage have increased survival and fitness compared to those reared on many other common host species in the northeastern US (Hough and Pimentel, 1978; Raupp *et al*., 1988). In addition to variation in host suitability among species, foliar nutritional quality for spring-feeding Lepidoptera varies intraspecifically in complex ways that reflect soil characteristics, as well timing through the growing season. In temperate forests, the nutritive value and water content of leaf tissue declines, while secondary phytochemicals and toughness increase as leaves mature (Feeny, 1970; Schroeder, 1986). Asynchrony between egg hatch and bud burst reduces survival, growth rate, pupal mass, and fitness in many lepidopterans, and increases the risk of mortality imposed by natural enemies (Hough & Pimentel, 1978; Parry *et al.*, 1998; Hunter & Elkinton, 2000). Red oak, *Q. rubra*, foliage maintains a low C:N ratio relatively late into the growing season when compared to other hosts (Hunter & Lechowicz, 1992), and thus may provide folivores a longer window for optimal growth.

Negative density-dependent effects on fitness in Lepidoptera are often attributed to intraspecific resource competition during larval stages. Gypsy moths reared in high-density conditions in both the laboratory and field are smaller than those that develop under low-density conditions (Leonard, 1981; Carter *et al.*, 1991; Lazarevic *et al.*, 2004). Although intraspecific competition for foliar resources increases development time in the first instar, behavioral and physiological changes induced during gypsy moth outbreaks reduce development time in later instars for some individuals (Leonard, 1981; Lance *et al.*, 1986), resulting in a tradeoff between survival and other components of fitness such as fecundity.

In this study, we evaluated the utility of trap-captured males as an indicator of habitat quality as measured by host tree abundance and stand composition. Specifically, we assessed seasonal changes in male wing size, and quantified male wing length across low-density populations from a range of relatively poor to good quality habitats for gypsy moth based on estimates of the proportion of forest composed of oak species and a quality index based on the relative abundance of preferred and non-preferred host tree species. Finally, we examined seasonal patterns of male wing length in two outbreaking populations to evaluate the effects of intraspecific competition for foliage on wing length.

Materials and Methods

Site selection and trapping

The low-density study was conducted in central New York (NY) where gypsy moth has been established since the 1950s. Although population size fluctuates from year to year, outbreaks have not been recorded in this area for more than 25 years. Egg mass densities were recorded from the sample plots each year and populations remained at very low levels throughout the study. Plot selection (Fig. 1) for oak-present (Table 1; n = 4 in 2015; n=5 in 2016 and 2017) and oak-absent (n=3 in 2015; n=4 in 2016; n=5 in 2017) sites was determined following extensive ground surveys of sites with low-density gypsy moth populations. In central NY, oak-dominated stands are patchily distributed and generally occur embedded in a matrix of predominantly sugar maple (*Acer saccharum* Marsh.), red maple (*A. rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.). A low-density, oak-absent reference site in Kirkville, NY, was monitored yearly from 2012–2019 for baseline estimates of phenology and to inform trap collection timing. Kirkville reference trap data represent moths captured and pooled from two traps 100 m apart in 2012 and 2013, and a single trap in each subsequent year. In 2017, two high density (outbreak) populations were chosen for trapping in Massachusetts (MA; n=3 traps) and Rhode Island (RI; n=2 traps), where gypsy moth has been established since the early 1900s, for comparison with the low-density sites.

Two continuous metrics of gypsy moth habitat quality were estimated based on forest composition. Tree surveys were conducted at each low-density site to measure diameter at breast height (DBH) for all trees with DBH > 6 cm. Tree surveys used three 20 m diameter plots, with one centered at a pheromone-baited trap (0 m), and two plots radiating in different randomly chosen cardinal directions set 50 m from the trap and 100 m from the trap. Proportion oak (PO) was estimated as the sum DBH for *Quercus* species divided by total sum DBH among all three plots for each site. To assess the impact of non-oak hosts on gypsy moth habitat quality, we developed a forest quality index (FQI), which incorporates a weighted score based on species-specific ratings of gypsy moth preference and susceptibility to defoliation during gypsy moth outbreak (Liebhold *et al.* 1995). In our calculation of FQI, the original scale in Liebhold *et al.* (1995) was reversed so that trees that are most likely to be defoliated have the highest score (e.g., red oak is 4 rather than 1). FQI was calculated for each site using the function,

$$\mathrm{FQI} = \frac{\sum_{i=1}^{n}(l_i \mathrm{DBH}_i)}{\sum_{i=1}^{n}(4\mathrm{DBH}_\mathrm{i})},$$

in which l_i is the species inverted defoliation rating based on Liebhold *et al.* (1995). A FQI value closer to one indicates higher forest quality for gypsy moth. Many of the low-density sites (both oak-absent and oak-present) had other tree species ranked as highly preferred (e.g., *Populus tremuloides*, *Ostrya virginiana*, *Tilia americana*) or moderately preferred (e.g., *Acer* spp, *Prunus serotina*) (Liebhold *et al.* (1995). The presence of these tree species resulted in relatively high FQI scores even when oak species were absent, and some oak-absent sites had higher FQI than some sites where oak was present (Table 1).

Prior to the onset of the gypsy moth flight season, a single green bucket Unitrap™ (Great Lakes IPM, Vestaberg, MI) was deployed at each site in the center of the 0 m plot, suspended from a branch ~1.5 m above the ground, and baited with disparlure impregnated string lures (Trécé Inc, Adair, OK) and a dichlorvos strip (Vaportape II: Hercon Environmental, Emigsville, PA) as the toxicant. A reference pheromone-baited gypsy moth trap was prepared and hung as described above in forest with few individual oak trees in Kirkville, NY (Table 1, Fig. 1a). The reference trap was checked daily during the ~ 8-week flight season in 2012 and 2013, and during 'early' and 'late' (first 10 – 12 day interval was designated 'early flight season', and the last 10 – 14 days 'late flight season') flight seasons during 2014 – 2019 to monitor flight phenology. In addition to showing a consistent seasonal effect on male gypsy moth wing length in a low-density population, the data were used to calibrate the timing of moth collections at the other sites. At the sites in central NY, trap contents were collected approximately every 10 – 12 days from the onset of flight until the end of the flight season. All moths were removed from the traps, counted, dried at 50˚C for 24 hrs for preservation, and stored at room temperature until measurement.

At each of the high-density sites in MA and RI, gypsy moth populations reached defoliating levels in 2015, and caused complete defoliation in 2016 and 2017 (Pasquarella *et al.*, 2018; Elkinton *et al.*, 2019). Traps at the MA (n=3) and RI (n=2) high-density sites were prepared and hung as described above for those at low-density sites, and monitored daily until the first moth was caught. Trap contents were collected every two days thereafter for the duration of the flight season. Moths were preserved in a freezer until wing measurement.

Wing length measurement

The right forewing of each moth was gently detached from the body, and the distance between the point of attachment with the thorax to the apex of the wing was measured to the nearest 0.01 mm using a digital caliper (Fig. 2). If the right forewing was damaged by trapping or in the process of wing removal, the left forewing was measured. If there were 30 or fewer moths in a trap, the wings of all moths were measured; for traps with more than 30 moths, 30 were randomly selected for measurement. Wing length data from low density trap collections were pooled by forest type and flight season to test for the effect of oak presence and seasonality on wing length, and the interaction. To test for the effect of proportion oak on wing length in low density populations, data from each oak site were pooled by site. Wing length data were pooled by trap and collection day for moths in outbreak populations. Cumulative trap catch was noted for all sites in 2017 to compare relative densities for low-density and outbreak populations; total trap capture was not enumerated in 2015 or 2016.

Statistical analyses

All statistical analyses were performed in R (version 3.6.1, R Core Development Team 2019). Wing length distributions for all samples were found to be normally distributed. Since the number of trapping sites in low-density populations varied by year, effects of forest type and phenology on wing length of pheromone trapped males in low-density sites were assessed using linear mixed effects models (package *lmerTest*; Kuznetsova *et al*., 2017) with Satterthwaite approximation of degrees of freedom due to unequal sample sizes. The proportion of variation in the response variable explained by predictors in the final models was estimated using the function *r.squaredGLMM* (package *MuMIn*; Barton & Barton, 2019).

To evaluate variation in wing length as a function of capture timing (early vs late flight season) for reference trap data collected from 2012 – 2019, linear mixed effects regression was performed with wing length as the dependent variable, early/late flight season as a categorical fixed effect, and year included as a random effect to account for inherent variation among years.

To assess the effect of flight season and forest type on wing length in low-density sites from 2015 – 2017, we used linear mixed effects regression with forest type (oak-present, oak-absent), flight season (early, late), and the interaction (season × forest type) as fixed effects, and wing length as the dependent variable. Trapping site was included as a random effect to capture variation in environmental characteristics among sites, and year was included as a random effect to account for variation between years. We used two separate linear mixed regression models to assess the relationship between wing length and each of the continuous gypsy moth habitat quality metrics: proportion of the canopy composed of oak spp. (PO) and FQI. In each model, wing length was the dependent variable, and flight season (early, late) and either PO or FQI were fixed effects. Trapping site was included as a random effect to account for variation among sites, and year was included to account for inherent variation between years. An interaction effect (season × forest type) was included in the full models to determine whether the effect of forest type on male wing length varied by season. Full and reduced models were compared using Aikake Information Criterion (AIC) to select the most parsimonious subset of predictor variables.

To assess the relationship between wing length and calendar day for moths from the RI and MA high-density populations, as well as the 2012 and 2013 reference data from Kirkville, NY, we used ordinary least squares regression with wing length as the dependent variable and calendar day as the explanatory variable. Mean wing length for low-density (NY 2012 and 2013) and outbreak (RI and MA) populations was compared using one-way ANOVA with population density as the explanatory variable. Significance level for all analyses was $\alpha = 0.05$.

Results

Trap counts for low-density sites in Central NY in 2017 totaled 7,365 moths from oak-present forests (n = 5 traps) and 2,211 moths from forests with no oak present (n = 5 traps). Total catch from the Rhode Island and Massachusetts outbreaking populations were 1,195 (n = 2 traps) and 643 (n = 3 traps) moths, respectively (Table 1). The low number of individuals in traps from outbreak sites reflect a region-wide pathogen epizootic after two successive years (2016 – 2017) of very high gypsy moth abundance (Elkinton *et al.*, 2019). The epizootic, consisting of primarily the fungal pathogen *Entomophaga maimaiga,* occurred in late instars after the stands had largely been defoliated (Pasquerella *et al.*, 2018).

Reference site

There was a strong, consistent seasonal effect on wing length apparent across eight consecutive years for the reference population in Kirkville, NY (Fig. 3). Wing length was significantly greater for early-flying males than for late-flying males (MS = 32.71, $F_{1,7} = 154.69$, $p < 0.0001$, estimated $R_2 = 0.89$), where the first 10 – 12 day interval was designated ‘early flight season’, and the last 10 – 14 days ‘late flight season’. Mean wing length for early- and late-flying males pooled across all years was 21.72 mm ± 0.18 (n = 2,627) and 18.86 mm ± 0.23 (n = 1,279), respectively.

Low-density sites

The effect of binary forest type and season on wing length was tested using mixed effect models with wing length as the dependent variable, season and forest type as fixed effects, and year and trapping site as random effects. In low-density trapping sites, there was significant seasonal reduction in wing length in ($F_{1,44} = 236.27$, $p < 0.0001$), and a significant effect of forest type ($F_{1,9} = 9.46$, $p = 0.013$; Fig. 4) with males trapped in oak-present forests being larger than males trapped in oak-absent forests. The interaction effect between season and forest type was not retained in the final model. Wing length (± SE) for males trapped in forests without oak was 21.46 mm ± 0.15 (n = 772) and 19.4 mm ± 0.21 (n = 450), for early- and late-flying males, respectively. For males caught in traps placed in oak-present forests, mean wing length was 22.19 mm ± 0.22 (n = 660) and 21.47 mm ± 0.30 (n = 444) for early- and late-flying males, respectively.

Proportion oak (PO; measured as the relative basal area of oak species in the stand) and FQI were each used as explanatory variables in two separate linear mixed effects models to estimate the effect of habitat quality as a continuous metric on male wing length. The results of model selection with FQI was $WL_{FQI} = 19.86 – 2.23(Season) + 2.50(FQI)$. The final model which used PO as an explanatory variable was $WL_{PO} = 21.96 – 2.51(Season) + 0.54(PO)$. Wing length was not significantly affected by either FQI ($F_{1,8} = 4.37$, $p = 0.068$) or PO ($F_{1,3} = 0.42$, $p = 0.57$). The effect of flight season on wing length was significant when gypsy moth habitat quality was estimated by FQI ($F_{1,40} = 192.53$, $p < 0.0001$) and by proportion oak ($F_{1,20} = 131.54$, $p < 0.0001$) with wing length decreasing through the season in both cases. Final models for both FQI and PO were selected based on AIC and did not retain the interaction effect.

Outbreak sites

In 2017, an *E. maimaiga* epizootic in southern MA and neighboring RI decimated late instars across the regional outbreak after significant defoliation had already occurred in our trapped stands (Elkinton *et al.*, 2019); thus, trapped males largely reflect the negative consequences of high population density and intraspecific competition for food they experienced as larvae earlier in the season. The number of moths trapped in outbreaking populations reflects the effects of this epizootic; the number of moths caught during early flight season is relatively high and likely represents the individuals that pupated ahead of the mortality wave, while late season capture is very low.

There were no significant seasonal effects on wing length in the RI outbreaking population ($R_2 = 0.01$, $F_{1,30} = 0.22$, $p = 0.65$) or in the MA outbreak population ($R_2 = 0.10$, $F_{1,33} = 3.73$, $p = 0.06$; Fig. 2.5). There was however a significant negative seasonal effect on wing length in the low-density reference traps in both 2012 ($R_2 = 0.95$, $F_{1,27} = 549.8$, $p < 0.001$) and 2013 ($R_2 = 0.90$, $F_{1,22} = 190.1$, $p < 0.001$). Mean wing length for low-density and outbreak populations were significantly different ($F_{1,2} = 81.12$, $p = 0.0121$) with outbreak populations having smaller mean (± SE) wing length (RI: 18.22 (0.056) mm; MA: 18.61 (0.062) mm) than low-density populations (NY 2012: 20.73 (0.079) mm; NY 2013: 20.27 (0.071) mm).

Discussion

Changes in the size of gypsy moth males, when using wing length as a proxy for body size, have historically been attributed to fluctuations in population density (Bellinger *et al.*, 1990; Carter *et al.*, 1991; Tobin *et al.*, 2016). We found, however, that there is a strong seasonal decline in male wing length in low-density gypsy moth populations where competition with conspecifics for foliage should be low. There was no evidence of any defoliation in our low-density plots in any year, and egg mass counts were predominantly zeros with an occasional single mass, far below the level where competition would be important (Liebhold *et al.*, 1993). Changes in wing length were likely influenced by seasonal phenology and the relative effects of host quality in oak-present and oak-absent stands. However, gypsy moth host quality measured as a continuous variable (e.g., FQI and PO) had no significant effect on male gypsy moth wing length. Males in outbreak populations were on average smaller than those in low-density populations. Though there was not statistical support for a seasonal trend in wing length for outbreak populations, there was a tendency for wing length to decrease through the flight season, indicating that the seasonal effect may outweigh fitness effects of high population density and epizootics.

The seasonal reduction in wing length in low density populations across variable forest compositions was significant, and apparent in all three study years, which is consistent with prior research (Tobin *et al.*, 2016). Additionally, the seasonal pattern in wing length was constant over eight years for the reference population, indicating that the pattern persists independently of interannual variation in climatic variables such as temperature and variation in the onset of bud burst in spring. At any given site, egg hatch in gypsy moth occurs over an extended period as there is considerable variation in hatch timing both within and among egg masses (Leonard, 1981; Tauber, 1990). Thus larvae from the same population that hatch at different points begin feeding on hosts that vary phenologically in quality, resulting in intraspecific variation in performance (Raupp *et al*., 1988; Hunter & Elkinton, 2000). In controlled studies assessing the fitness costs associated with delayed hatch relative to budbreak, late hatching spring-feeding Lepidoptera in numerous species take longer to develop and have reduced pupal mass and survival compared to larvae that hatch synchronously with bud break (Raupp *et al*., 1988; Parry *et al*., 1998; Hunter & Elkinton, 2000; Haukioja *et al*., 2002; Jones & Despland, 2006).

Early and mid-season oak foliage supports relatively fast gypsy moth larval development, high survival rates, large body size, and high fecundity, especially compared to mid-season foliage of other potential host trees, such as beech *F. grandifolia* or sugar maple *A. saccharum* (Raupp *et al*., 1988; Hunter & Lechowicz, 1992). Congruent with our expectation, wing length was smaller on average for males from oak-absent forests than from forests where oak species were present. However, the use of forest quality as a continuous variable (i.e., PO and FQI) failed to show significant habitat quality effects on wing length. This may be due, in part, to the inherent difficulty in detecting habitat-based differences in mosaic forests. Many ‘high quality’ sites were characterized by relatively small pockets of oak dominated, or co-dominated, forest embedded in larger areas of lower quality maple-beech dominated forest. Pheromone-baited traps can attract males from distances up to 800 m (Elkinton & Carde, 1980), and traps placed in oak forests in the present study could have lured moths from nearby lower-quality forest stands. Additionally, larvae feeding on less suitable host trees within oak-dominated plots may have obscured wing length signatures resulting from forest-type. In a controlled study, larvae reared on beech and sugar maple took only 3.6 and 8.2 days longer, respectively, than those reared on oak (Hough & Pimentel, 1978). Late instars can switch hosts when they descend diurnally and ascend to feed at night; thus, host-switching may allow partial compensation for the cost to development time incurred from feeding on low-quality hosts (Lance & Barbosa, 1982). The interval between trap collection dates in the present study (10 – 12 days) may have failed to exclude moths that fed on less suitable foliage than oak during all or a portion of larval development.

Similar seasonal reductions in wing length have been documented in other spring-feeding Lepidoptera. DuMerle and Cornic (1991) reported a reduction in wing length as the flight season progressed in a population of fir budworm, *Choristoneura murinana* (Lepidoptera: Tortricidae), and Rhainds (2015) reported a seasonal reduction in wing length for spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Neither of these studies suggested a link between reduced wing length and temporal reduction of host quality. However, Jones and Evenden (2008) reported a similar seasonal pattern in wing size for forest tent caterpillar *Malacosoma disstria* (Lepidoptera: Lasiocampidae), and suggested that the trend resulted from reduced fitness associated with asynchronous hatch. While the present study found little statistical evidence for a relationship between forest type and wing length, DuMerle and Cornic (1991) reported that mean wing length for fir budworm was greater in high-quality stands (silver fir *Abies alba*) than those dominated by low-quality host (Atlas cedar *Cedrus atlantica*), and that the seasonal decline in wing length in high-quality stands was slower than in the low-quality habitat.

In high-density gypsy moth populations, larval development is often more rapid than predicted by temperature alone (Leonard, 1981; Lance *et al.*, 1986). This change in development is likely due to a shift from primarily nocturnal feeding to feeding day and night, increased temperatures in the canopy as it becomes defoliated, and an increased occurrence of larvae that pupate after completing a reduced number of instars (Campbell, 1978; Lance *et al*., 1986). Given these physiological changes, we predicted that male moths originating from high-density populations might exhibit a humped distribution with small individuals early and late. Although males from outbreak populations were smaller on average than males from low-density populations, there was no statistical support for a seasonal effect on wing length for males trapped in high-density populations. However, an apparent seasonal trend in wing length suggests a negative relationship between wing length and calendar day reflective of the trend observed in low-density populations, which may indicate that the seasonal effect on wing length outweighs the effects of intraspecific competition.

The present study is the first to evaluate male gypsy moth wing morphometrics as an index of habitat quality as measured by host tree suitability. Although wing length tracks the phenological reduction in host quality in high and low density populations, male gypsy moth wing morphometrics may prove more useful in predicting habitat quality in more homogenous forest mosaics. Habitat quality is determined by a complex of factors, and a simplification of quality, such as measuring the quantity of a single preferred host species in the forest canopy, may not be useful. Presence of larger males caught in pheromone-baited traps has been demonstrated to correspond to larger egg masses in wild populations, leading to higher population growth (Bellinger *et al*., 1990). Thus, monitoring male wing length in susceptible forests may be useful for informing management decisions by identifying areas where population density is likely to increase in subsequent years. Future studies should assess the relationship between population growth rates and pheromone-trapped male wing length in nascent populations ahead of the invasion front to establish a threshold value for wing length which can reliably identify populations that are most likely to establish successfully.

Acknowledgments

We thank Hannah Nadel (USDA APHIS) and Lisa Tewksbury (University of Rhode Island) for collecting moths from the outbreak populations. Thanks to Seaira Goetz for her invaluable assistance in the laboratory and field. Thanks to Melissa Fierke and Colin Beier (SUNY-ESF) for constructive feedback and thorough editing of an earlier version of the manuscript. Funding was provided by USDA Forest Service McIntire Stennis Grant to DP, USDA Forest Service, Northern Research Station (Grant Number 11-JV-11242303-053) to DP, and National Science Foundation grant DEB-1556111 to PCT.

Table 2.1. Locations and characteristics for all trapping sites, and summary wing length (WL; mm) statistics for each trap by year. Moths from reference trap were captured from 2012-2019, low-density populations were trapped from 2015-2017, and outbreak populations were trapped in 2017. Total trap catch was not available for 2015 or 2016. RI had two traps at the trapping site, and MA had three traps; totals separated by semicolons for outbreak populations reflect individual traps.

Site	**Year**	**Mean ± SD WL (mm)**	**Med. WL (mm)**	**Min. WL (mm)**	**Max. WL (mm)**	**Location (°N, °W)**	**Forest Type**	**Prop. Oak**	**FQI**	**Pop. Density**	**Total Trap Catch**
Morgan Hill State Forest	*2017*	19.72 ± 1.19	19.69	17.19	22.65	42.808056, -75.997222	Oak-absent	0.0	0.733	Low-density	345
Hewitt State Forest	*2016*	20.93 ± 1.49	20.80	17.44	24.51	42.751111, -76.223333	Oak-absent	0.0	0.683	Low-density	NA
	2017	20.39 ± 1.48	20.32	16.67	23.81						724
Danby State Forest	*2016*	20.95 ± 2.06	21.32	17.08	24.18	42.315833, -76.476111	Oak-present	0.379	0.799	Low-density	NA
	2017	21.73 ± 1.66	22.09	17.05	25.51						1,224
DeRuyter State Forest	*2015*	20.33 ± 1.74	20.45	16.18	24.66	42.813056, -75.865833	Oak-absent	0.0	0.721	Low-density	NA
	2016	21.09 ± 1.52	21.15	16.01	24.29						NA
	2017	21.00 ± 1.27	21.10	17.80	23.84						339
Hammond Hill State Park	*2015*	21.56 ± 1.86	22.05	17.99	24.52	42.441111, -76.290833	Oak-present	0.776	0.944	Low-density	NA
	2016	20.57 ± 2.25	21.18	15.46	24.45						NA
	2017	20.30 ± 1.77	20.37	15.60	23.49						2.028
Heiberg Memorial Forest	*2015*	20.34 ± 1.77	20.18	16.33	24.51	42.769722, -76.084167	Oak-absent	0.0	0.776	Low-density	NA
	2016	21.16 ± 1.55	20.96	17.40	25.24						NA

	2017	21.17 ± 1.26	21.17	17.78	23.72						347
Highland State Forest	*2015*	20.27 ± 1.88	20.32	16.76	24.51	42.821389, -75.921389	Oak-absent	0.0	0.767	Low-density	NA
	2016	20.75 ± 1.40	20.66	16.48	24.08						NA
	2017	20.42 ± 1.48	20.43	16.48	24.08						456
Kettlebail State Forest	*2015*	21.12 ± 1.38	21.09	17.64	24.19	42.771111, -76.0575	Oak-present	0.596	0.899	Low-density	NA
	2016	21.88 ± 1.09	22.12	18.54	24.25						NA
	2017	21.11 ± 1.29	21.21	17.45	24.17						511
Shindagin State Forest	*2015*	22.10 ± 1.66	22.52	16.71	24.78	42.320833, -76.344444	Oak-present	0.295	0.763	Low-density	NA
	2016	20.51 ± 1.94	20.47	16.15	23.92						NA
	2017	21.63 ± 1.37	21.79	17.53	24.45						874
Yellowbarn State Forest	*2015*	22.09 ± 1.46	22.25	16.77	24.65	42.455556, -76.339722	Oak-present	0.261	0.792	Low-density	NA
	2016	21.14 ± 2.07	21.58	16.48	25.41						NA
	2017	20.66 ± 1.41	20.61	17.39	23.60						2,728
University of Rhode Island, North Woods (RI)	*2017*	18.22 ± 1.57	18.33	12.71	22.14	41.498858, -71.521585	NA	NA	NA	Outbreak	479; 539

Francis Crane Wildlife Management Area (MA)	*2017*	18.61 ± 1.52	18.42	14.11	23.42	41.63720, -70.557786	NA	NA	NA	Outbreak	254; 186; 203
Kirkville, NY	*2012*	20.73 ± 2.26	20.13	13.62	25.96	43.080624, -75.981274	Oak-absent; Reference	0.0	0.799	Low-density	
	2013	20.27 ± 1.62	20.21	14.81	24.69						
	2014	21.19 ± 1.76	21.45	15.53	23.91						
	2015	20.73 ± 1.91	21.20	17.04	23.62						
	2016	21.14 ± 1.67	21.65	16.51	24.14						
	2017	20.24 ± 1.28	20.51	17.32	22.63						
	2018	20.49 ± 1.93	20.24	16.24	24.08						
	2019	20.73 ± 1.76	21.01	16.02	25.64						

Table 2.2. ANOVA table for mixed linear effects models with wing length as the response variable, forest type (oak-present, oak-absent) and season (early, late) as fixed effects, and year and site as random effects.

Variable	**MS**	**df**	**F**	**p**	**Estimated full model R_2**
Forest type	3.12	1, 9	9.48	0.014	0.80
Season	75.07	1, 45	228.37	<0.0001	
Year				0.76	
Site				0.74	

Table 2.3. ANOVA table for mixed linear effects models with wing length as the response variable, continuous metric of gypsy moth host quality (PO, FQI) and season (early, late) as fixed effects, and year and site as random effects.

Variable	**MS**	**df**	**F**	**p**	**Estimated full model R_2**
PO	0.14	1, 3	0.42	0.57	0.78
Season	44.03	1, 20	131.55	<0.0001	
Year				0.17	
Site				0.47	
FQI	1.47	1, 8	8.39	0.068	0.79
Season	64.75	1, 40	192.53	<0.0001	
Year				0.88	
Site				0.62	

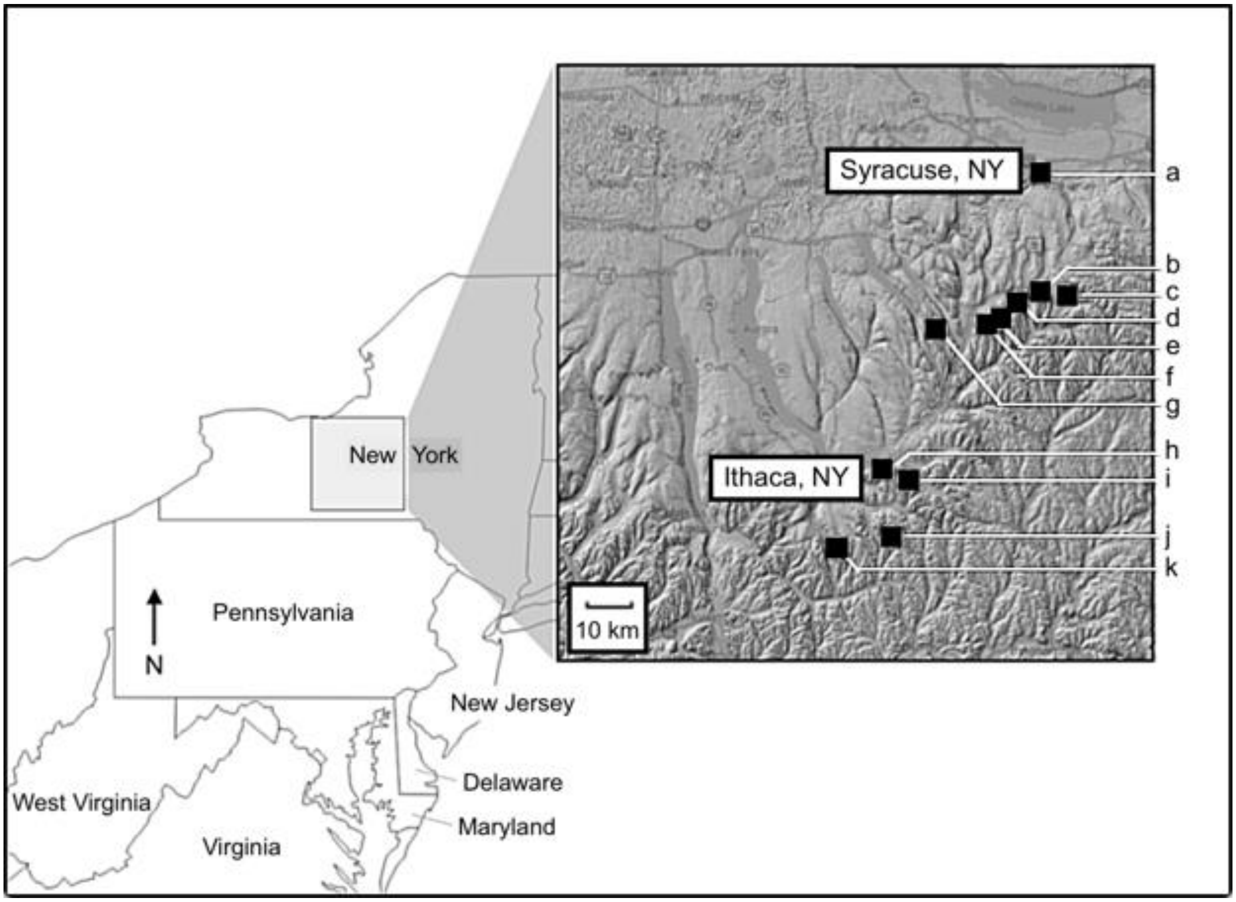


Figure 2.1 Low-density pheromone trapping sites in central New York, USA, are indicated by black squares: a) Kirkville (Reference population), b) Highland Forest County Park, c) Deruyter State Forest, d) Morgan Hill State Forest, e) Kettlebail State Forest, f) Heiberg Memorial Forest , g) Hewitt State Forest, h) Yellow Barn State Forest, i) Hammond Hill State Forest, j) Danby State Forest, k) Shindagin Hollow State Forest. The cities of Ithaca, NY, and Syracuse, NY, are shown for spatial reference.

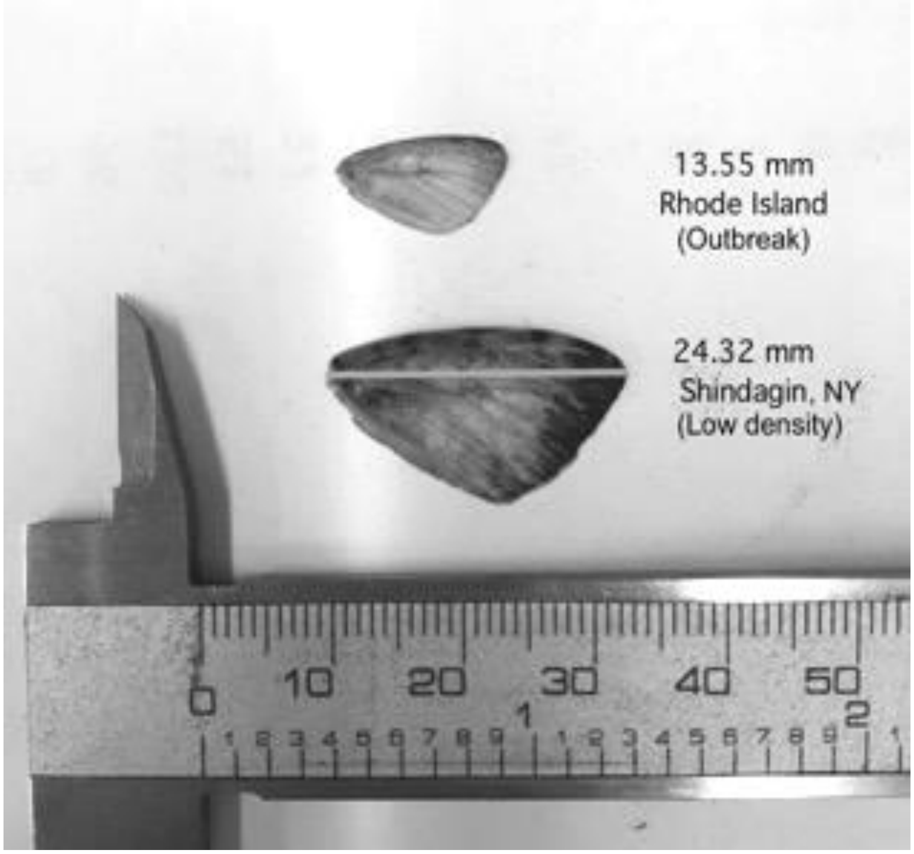


Figure 2.2 Two male gypsy moth forewings illustrate variation in size from a high density outbreak population and a low density population with no history of outbreak in 2017. The gray line across the large wing indicates the transect used for wing measurement.

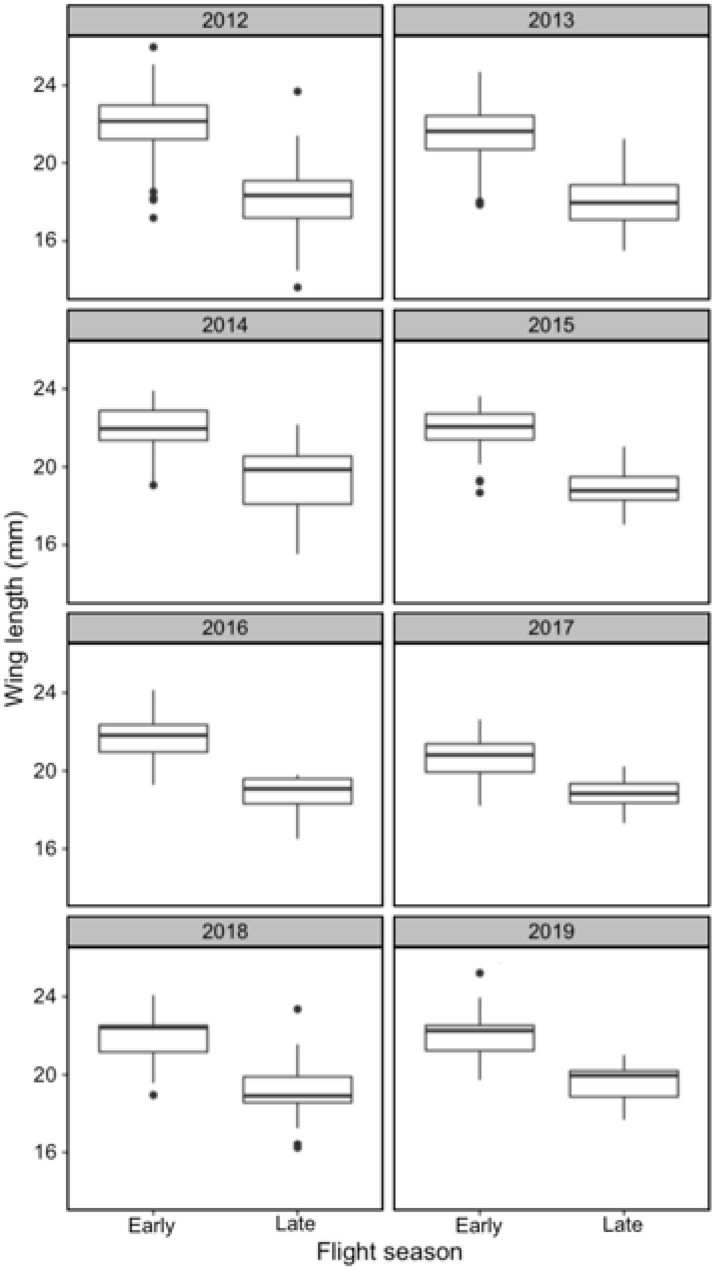


Figure 2.3. Boxplots illustrating the seasonal effect in wild-trapped male wing length during eight years (2012-2019) at the oak-absent reference site in Kirkville, NY.

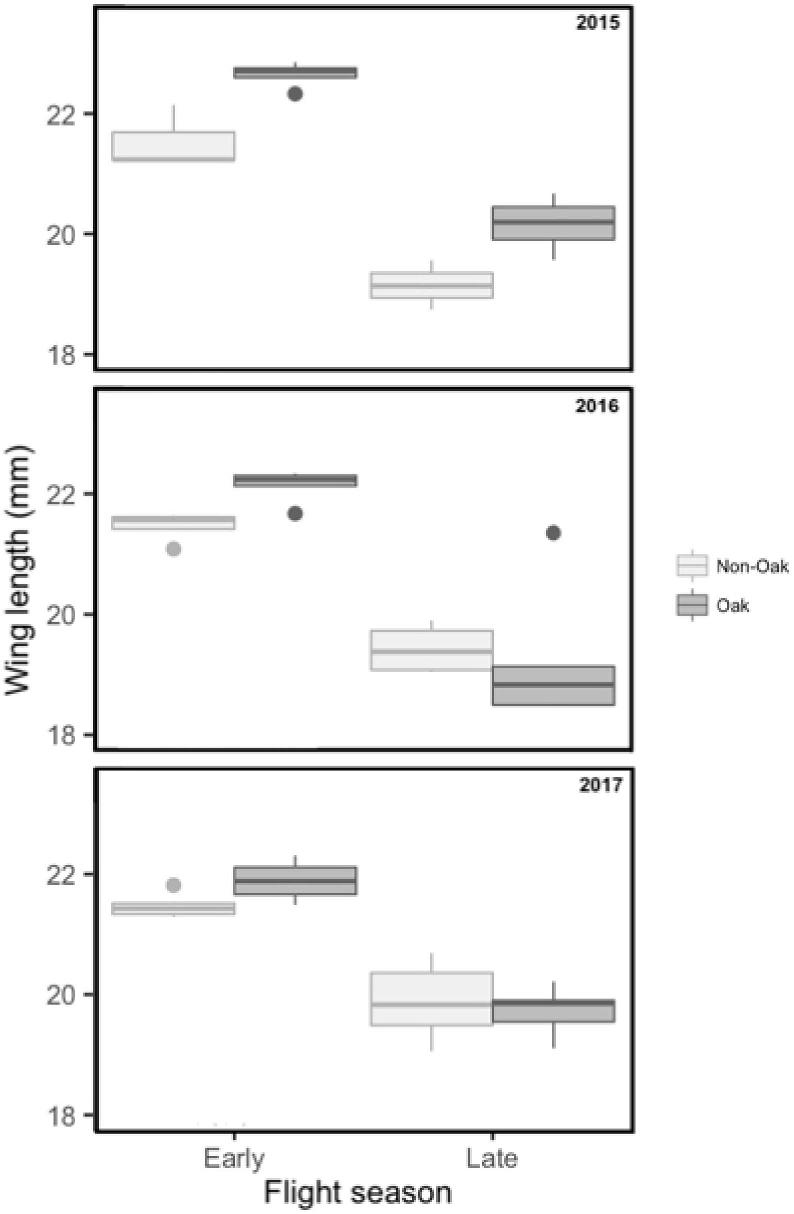


Figure 2.4. Boxplots illustrating the effect of flight season and forest type on wing length for male gypsy moths originating from low-density oak-present (n = 4 in 2015; n=5 in 2016 and 2017) and oak-absent (n=3 in 2015; n=4 in 2016; n=5 in 2017) forests in 2015, 2016, and 2017.

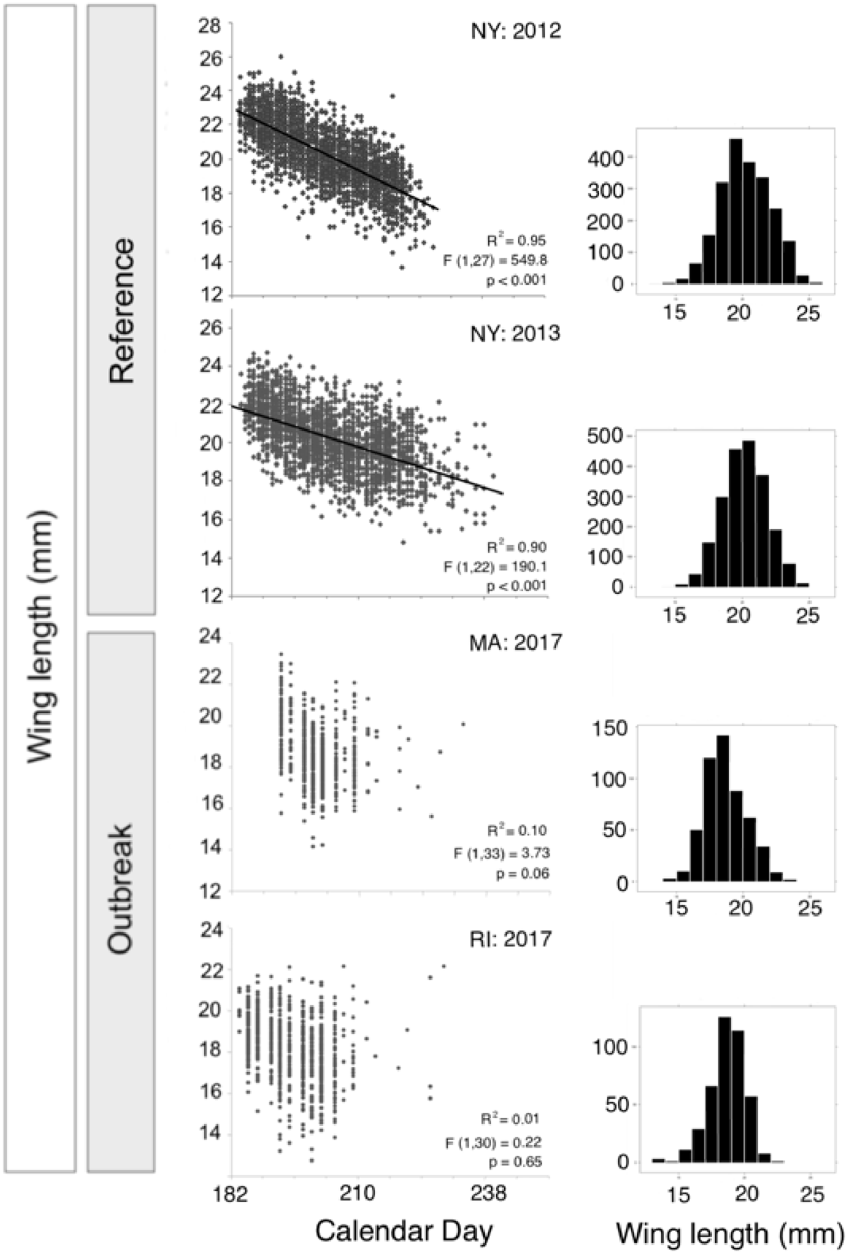


Figure 2.5. Male wing length on ordinal day illustrates the seasonal trend in moth size in outbreaking populations and low-density reference populations. F-values, p-values, and R-square values are reported for each regression. Data from moths trapped in Kirkville, NY, in 2012 and 2013 reference traps, and in high density populations in MA and RI in 2017. Histograms show wing length distribution for males caught throughout the trapping season.

Chapter 3: Size-dependent flight capacity and propensity of male European gypsy moth *Lymantria dispar*

Abstract

For capital-breeding insects, all resources available for reproduction and adult dispersal are accumulated during larval feeding and thus body size at adult eclosion represents the total energetic capacity of the individual. For female capital breeders, body size is strongly correlated with lifetime fecundity as measured by the number of eggs produced, and for males, body size is often representative of flight capacity metrics. Flight potential for male Lepidoptera has important implications for mate-finding, especially in species where females lack wings or are flightless. At low population densities, male flight capacity may become critically important in mating success. Failure to mate can be an important Allee effect, identified as a driver of the success or failure of invasive species at range edges and in species of conservation concern.

The gypsy moth, *Lymantria dispar* L., a capital breeder, was introduced to North America 151 years ago and now ranges across much of eastern North America. In the European strain introduced to North America, females are flightless and all mate finding is done by males. I quantified male European gypsy moth flight capacity and propensity to fly in relation to morphological and physiological characteristics using computer linked fixed-arm flight mills. Body size in males was varied using standard artificial diets where protein content was altered while holding other dietary components constant. Wing length, a proxy for body size, relative thorax mass, and forewing aspect were all important predictors of two components of flight capacity, total flight distance and maximum speed. These results have implications for gypsy moth population ecology and evolutionary ecology, including mate-finding ability, invasion dynamics, and potential for adaptation along the invasion front. Size-linked flight capacity and behavior in this species may have general application for other capital breeding Lepidoptera.

Keywords: Flight mill, *Lymantria dispar*, life-history, invasion dynamics, selective pressure

Introduction

Intraspecific variation in resource allocation affects the quantity and fitness of offspring an individual produces in any given environment (Boggs & Freeman, 2005; Boggs, 2009). Quality and quantity of nutrients, as well as the timing of intake, is critical for successful growth and reproduction. For holometabolous insects, including Lepidoptera, energy and nutrients acquired in the larval stage are later redistributed to meet metabolic needs, maintenance, storage, reproduction, and formation of adult body structures during the pupal stage (Boggs, 2009). While income breeding species feed as adults and use accumulated resources to supplement reproduction, capital breeders depend entirely on larval resources for adult functions such as reproduction, dispersal, and mate finding (Tammaru & Haukioja, 1996; Davis *et al*., 2016). Thus, in capital breeders, body size is indicative of the total energy and material reserve available for adult dispersal and reproduction. Capital breeding species present simplified models for studying resource allocation and life history trade-offs.

Estimating fitness for female capital-breeding insects is done relatively easily by counting the number of eggs a female produces (Hough & Pimentel, 1978; Tammaru *et al*., 1996a; Tisdale & Sappington, 2001, Parry *et al*., 2001). On the other hand, determining correlates of male fitness by quantifying the number of eggs fertilized following a controlled mating event or by measuring male flight capacity is more difficult, and done less frequently (e.g., Boggs & Gilbert, 1979; Svärd and Wiklund, 1989; Tammaru *et al*., 1996b; Tobin *et al*., 2014). Male flight may be particularly important for capital-breeding species where males do most or all of the mate finding (Roff, 1991; Berec *et al*., 2018; Javoiš *et al*., 2019).

The ability to successfully locate mates is critical for successful establishment in small isolated populations or those at low density near range edges (Contarini *et al*., 2009; Yamanaka & Liebhold, 2009; Rhainds, 2010). Dispersal away from low-density populations can exacerbate mate finding Allee effects by reducing male density, and increase the risk for local extinction. For example, gypsy moth (*Lymantria dispar* L.) infestations ahead of the invasion front often fail to establish, likely due to the failure of males to successfully locate females in low density populations (Contarini *et al*., 2009). In low density populations of Glanville fritillary *Melitaea cinxia* (Linneaus), individuals of both sexes are more likely to disperse, further reducing population density, and so at lower densities, mating success decreases (Kuusaari *et al*., 1998).

Gypsy moth was introduced to North America in 1869 and now ranges from northern Minnesota to the Outer Banks of North Carolina (Grayson and Johnson, 2018). Adult gypsy moths are sexually dimorphic with adults of the European strain in North America have flight-capable males, and winged but flightless females; therefore, resource allocation is widely divergent between the sexes (Doane & McManus, 1981). Energy expended by adult female gypsy moth is allocated primarily to production and emission of pheromone and manufacturing and provisioning of eggs. The relationship between female pupal mass and fecundity is well established with number of eggs produced strongly correlated with body mass (e.g., Hough & Pimentel, 1978, Faske *et al*., 2019), yet no studies to my knowledge have assessed the relationship between body size and flight capacity and behavior of male gypsy moths. Insect body size is often correlated with metrics of flight capacity (e.g., Davis *et* al., 2012; Evenden *et al*., 2014; Jones *et al.*, 2016), and flight-related morphology such as wing load and forewing aspect ratio are linked to greater flight capability for female Asian gypsy moth (Shi *et al*., 2015), and other Lepidoptera (Le Roy *et al*., 2019). Male gypsy moth flight capacity may be similarly affected by body size and flight-related morphology.

Fixed-arm flight mills provide a valuable avenue for investigating effects of physiological, morphological, and environmental variation on flight capability and dispersal under controlled conditions (Minter *et al*., 2018; Naranjo, 2019). Although tethered flight mills have been used to determine effects of morphological and physiological characteristics on females of the Asian gypsy moth, a strain with strong female flight ability (Yang et al. 2017), to my knowledge, none have done so for male gypsy moths. This study assessed the relationship between resource acquisition and allocation and male flight capacity for gypsy moth, a defoliating invasive capital breeder. Specifically, I used flight mills to assess the direction and influence of several morphological and physiological characteristics on male gypsy moth flight performance.

Materials and Methods

Flight mill design

Flight mills were assembled using the design described by Jones *et al*. (2010), which consisted of a rotating arm to which a moth was attached during each assay, a magnetic sensor that detected each full rotation, and a microcontroller that read up to eight sensors and sent rotation and timing information to a computer for storage and analysis (Fig. 3.1). The base of each flight mill was a 12.7 mm diameter vertical post which was attached to a painted 12.7 mm thick plywood base with a screw at the bottom. A small hole was drilled in the top to house a length of non-magnetic wire (0.85 mm diameter copper), which served as an axle for a 6.35 mm diameter x 25.4 mm vertical hub that carried the horizontal flight arm. Posts were cut to two lengths (139.7 mm and 63.5 mm) so that two flight mills could be attached to each plywood base with flight arms partially overlapping, but not colliding. To reduce friction, the hub and post were made of acetal plastic and the hub was suspended approximately 17.5 mm above the post by a pair of ring magnets with opposing poles (K&J Magnetics, Pipersville, PA). The flight arm was constructed of 0.85 mm diameter spring steel wire, which passed through a small hole in the hub, and was superglued in place. Arms extended 9.23 cm on either side of the hub. The harness was attached to one end by pushing the flight mill arm and the harness through a small piece of aquarium tubing, and a piece of soft polymer clay (approximately equal to the weight of the moth and harness) was attached to the opposite end as a counterbalance.

Each full rotation of the hub was detected using a hall effect sensor (Diodes Incorporated Hall Unipolar Switch Part No: AH3362Q-P-B) that was aligned with a small magnet on the hub. As the hub rotated, the output signal produced by the hall effect sensor was equal to the input voltage (3.3 volts in the present study) for approximately half of the revolution, and was zero volts for the other half. Up to eight, three-wire cables were attached to the microcontroller board at once (Appendix 1), which used an Arduino compatible Sparkfun pro micro 3.3 V 8 NHz microcontroller to check all sensor states at 10 Hz rate and output a message containing time since reset, flight mill number, and rotation count. The message was converted to a USB signal for logging by a terminal program (TeraTerm; Appendix 2) on a personal computer running Microsoft Windows8. Messages were stored on the microcontroller board by a Sparkfun OpenLog datalogger for backup in case of power failure or disrupted computer connection. Power was supplied to the board through the USB connection, but was also backed up by a set of three AA batteries. In the present study, sixteen flight mills were attached to two microcontroller boards feeding data to two instances of TeraTerm on the same netbook.

Insect rearing

In natural habitats, mass of male gypsy moths varies greatly as a function of diet quality, population density, and temperature experienced as a larva (Carter *et al*., 1991; Lindroth *et al*., 1997; Lazarevic *et al*., 2004). To investigate effects of male size on flight capacity methodology was developed to produce a range of sizes while minimizing effects of other confounding factors on flight metrics. To produce a size range of males for experimentation, we used an agar-based artificial gypsy moth diet (Leonard & Doane, 1966) and manipulated protein content while holding all other nutrients constant (Table 3.1). Males used in the flight mill experiment were reared on four formulations. When protein content was reduced, the equivalent amount by weight of food grade cellulose powder was added to keep the weight ratio of other ingredients equal across all diet mixes. Fresh diet was poured in 473 mL plastic-lined paper soup containers and given to larvae *ad libitum*. Larvae were reared in growth chamber at 25˚C and a photoperiod of 15:9 (L:D). Upon pupation, individuals were weighed to the nearest 0.0001 g and returned to the growth chamber in individual 60 mL plastic containers until adult emergence.

Flight assay

Pupae were checked at least twice a day for adult emergence. Moths that emerged and were not flown right away were held at 4˚C until ready to be flown. All flight assays were initiated within 24 hr of emergence. Prior to harness placement, moths were cold anesthetized at -10˚C for up to 5 min, then weighed to the nearest 0.0001 g. Inverted y-shaped wire harnesses (Fig. 3.2) were glued dorsally to the descaled first abdominal segment using cyanoacrylate gel super glue. Moths were reweighed with the harness, and attached to the distal end of the flight mill arm using thin plastic tubing. Flight assays began at the start of the photophase (light period) and ran for 24 hr with photoperiod of 15:9 (L:D). Photophase and scotophase temperatures were 25˚C and 15˚C, respectively. During flight assays, total distance and duration of each individual flight was recorded, along with morphological (pupal mass, moth mass, wing length, width, and area, thorax width, height and shape, relative thorax mass, relative abdominal mass, and forewing aspect) and physiological (mass lost during flight, relative mass lost during flight) characteristics. Moths that stopped flying were encouraged to fly every 15 min using gentle tactile stimulation (e.g., touch to the legs, posterior edge of the wings, antennae) for the first eight hours of each flight assay.

Following the 24 hr flight assay, moths were removed from flight mills, frozen, and reweighed to quantify mass lost during flight. The right forewing of each male was removed, scanned, and the digital image of each wing analyzed using ImageJ software to determine wing length, wing width, and wing area. Thorax mass and abdominal mass were measured by carefully separating each tagma using a scalpel and weighing them individually on a digital scale to the nearest 0.0001 g. Wing load was calculated as initial moth mass divided by two times the wing area (g/cm$_2$). Forewing aspect ratio was calculated as four times the squared length of the forewing divided by forewing area (mm/cm$_2$). Relative thorax mass was calculated as mass of the thorax divided by the pre-flight moth mass, and relative abdominal mass was calculated as the mass of the abdomen divided by the pre-flight moth mass. Thorax width and height were measured to the nearest 0.01 mm using a digital caliper. Thorax shape was calculated as the width of the thorax divided by the height. Mass lost during flight was calculated as initial mass of the moth minus he mass of the moth measured immediately after the flight assay, and ratio of mass lost was calculated as mass lost during flight divided by initial moth mass.

Flight propensity was measured as the number of flights consisting of more than 100 full rotations initiated upon tactile stimulation divided by the total number of touches for each individual moth. One-hundred rotations was equal to approximately 58 m of linear flight distance.

Variable selection

Total distance flown and maximum speed were used as dependent variables. Based on biological significance, wing length was chosen to represent body size, and proportionate mass lost during flight (RatioMD), forewing aspect ratio (FWa), and proportionate thorax mass (RTM) were also included in the model and each represented a different physiological or morphological component. Variables selected for the full models were not strongly correlated ($p < 0.4$). Flight mill identity was included in both models as a random effect to capture any variation in measured flight capacity metrics caused by slight physical differences in flight mill design. Since insects do not need to produce lift during tethered flight, wing load was not included in flight capacity analyses.

Statistical analysis

All data analyses were performed in R (version 3.6.1, R Core Development Team 2019). Significance level for all analyses was $\alpha = 0.05$. Flight propensity of small and large moths was compared using ordinary least squares regression with proportion of sustained flights initiated as the dependent variable and wing length and its squared coefficients as the explanatory variables.

Effects of morphological and physiological characteristics on metrics of male flight capacity were analyzed using mixed effects linear regression analysis. Fixed effects and pairwise interaction effects were tested using stepwise model selection (package *lmerTest*, function *step*). For each dependent variable, full and reduced models were compared using AIC and ANOVA hypothesis testing. Model normality and homoscedasticity were checked using residual plots. Total distance flown was square root transformed to improve normality.

Effect of wing length on flight propensity was tested using a separate linear regression and non-linear relationship was tested by adding squared and cubed coefficients, which were not significant in the final models. Model normality and homoscedasticity were checked using residual plots and were fund to be normally distributed. The proportion of variation in the response variable explained by predictors in the final models was estimated using the function *r.squaredGLMM* (package *MuMIn*; Barton & Barton, 2019).

Results

A total of 96 male gypsy moth were flown. Mean pupal mass of flown moths was 0.41 g ± 0.013 (mean ± SE) and ranged from 0.15–0.73 g. Mean wing length was 2.05 cm ± 0.021 and ranged from 1.64–2.53 cm.

Flight capacity

Wing length was the strongest predictor for both flight capacity metrics (total distance and maximum speed) in the mixed regression models (Table 3.3, 3.4). Total distance flown ($F_{1, 99} = 98.47$, $p < 0.0001$; Fig. 3.4) and maximum speed ($F_{1, 93} = 80.17$, $p < 0.0001$; Fig. 3.5) were significantly impacted by wing length. Large moths in the upper quartile for wing length (n = 27, $\bar{X}_{WL} = 2.33$ cm [2.22–2.53 cm]) flew on average 31.6 km [11.6–68.2 km], or 282% more than small moths in the lower quartile (n = 24, $\bar{X}_{WL} = 1.80$ cm [1.64–1.89 cm]) which flew on average 11.2 km [0.12–44.9 km]. Forewing aspect ratio had a significant negative effect on total distance ($F_{1, 91} = 30.32$, $p< 0.0001$) and maximum speed *(*$F_{1, 93} = 17.70$, $p < 0.0001$). Reduced forewing aspect was associated with greater flight speed and greater total distance flown. Relative thorax mass had a significant positive effect on total flight distance ($F_{1, 91} = 28.17$, $p < 0.001$).

Flight propensity

All but five moths recorded at least one response flight consisting of more than 100 full rotations; wing lengths of those that did not were 17.0, 17.22, 17.62, 19.13, and 20.34 mm. Mean wing length of moths flown was 20.55 mm (±0.21). Wing length had a positive exponential effect on proportion of sustained flights initiated ($F_{2,97} = 17.78$, $p < 0.001$; Fig. 3.6).

Discussion

Insect body size is often correlated with greater flight distance and speed (e.g., Dingle *et al*., 1980; Shirai, 1991; Bruzzone *et* al., 2009; Evenden *et al*., 2014; Fahrner *et al*., 2014; Jones *et al*., 2016). This study is the first to assess impacts of body size and flight morphometrics on measures of flight capacity for male gypsy moth. Wing length, a proxy for body size, was the most important predictor for both metrics of flight capacity; large moths were more likely than small moths to initiate sustained flight, and once initiated, larger moths tended toward longer faster sustained flights than smaller moths. The greatest distance flown in the present study was > 68 km, and was accomplished by one of the largest moths (24.6 mm wing length). However, many midsize moths (19–21 mm wing length) in our study also flew total distances > 30 km.

Moths with wing lengths between 19 and 21 mm are common in both endemic and high-density populations (Tobin *et* al., 2016; Jahant-Miller *et al*., *in review*). Due to altered physical properties of tethered flight relative to free flight, such as a forced flight path, lack of natural flight stimuli, and the fact tethered insects do not need to produce lift, extrapolation of tethered flight distances to flight potential in natural settings must be done with caution (Minter *et al*., 2018).

Other significant predictors for metrics of flight capacity included forewing aspect ratio for speed and distance, relative thorax mass for distance, and relative mass lost during flight for distance. Aspect ratio describes the shape of a wing with higher values indicating a longer narrower wing and lower values a shorter broader wing. For aircraft and some flying animals, including insects, aspect ratio is positively correlated with flight efficiency during gliding flight (since longer narrower wings produce less drag), and negatively correlated with flight speed (Savile, 1957; Dudley & Srygley, 1994; Davis *et al*., 2012). Insect energy storage for flight and flight musculature are thoracic components and so flight metrics such as distance and speed are often correlated with increased relative thorax mass (Dudley & Srygley, 1994; Marden, 2000; David *et al*., 2015). Relative mass lost during flight had a negative relationship with total distance flown, which may indicate larger moths were using proportionately less of their stored resources than smaller moths and so likely have the capacity to continue flying longer than the 24 hr assay period.

Most female gypsy moths mate only once, thus, the first male to reach a female has a reproductive advantage (Doane & McManus, 1981). This suggests being the fastest flier with the greatest endurance would be ideal; however, large body size can only be attained at the cost of longer larval development. All else being equal (e.g., foliar quality, temperature, etc.), genetically larger individuals take more time to develop than smaller conspecifics (Hough & Pimentel, 1978; Teder *et al*., 2014). Adaptation towards greater flight capacity could ultimately put larger males at a mating disadvantage, unless environmental conditions require mate-finding flights that cover greater distances, such as in low density populations. However, while body size is the greatest factor impacting flight capacity, this research suggests changes to flight morphology can also increase gypsy moth flight ability and could do so without requiring increases to development time.

Density-dependent shifts in mating or mate-finding morphology in other arthropods have been found. In the amphipod *Gammarus roeselii* Gervais, individuals exhibit differential allocation to mating morphology in response to population density (Lipkowski *et al*., 2019). In high density *G. roeselii* populations where mating competition is relatively high, individuals exhibit larger antennae to increase mate selectivity and larger grasping legs to reduce the likelihood of having a mate stolen. In a wing-dimorphic plant hopper *Prokelisia dolus* Wilson, individuals in low-density populations have fully developed wings and have greater mate-finding ability compared to wingless morphs at low female densities, but at the cost of producing fewer offspring (Langellotto & Denno, 2001).

If greater male gypsy moth body size, or altered flight-related morphology, increases dispersal and mate-finding success, selection may favor those traits, especially at the invasion front where populations are often at low densities. Morphological and physiological adaptation facilitating dispersal has been documented for several plant and animal phyla at invasion fronts, as well as areas in which species frequently undergo extinction and colonization events (Phillips *et al*., 2006; Darling *et al*., 2007; Forsman *et al*., 2010; Hill *et* al., 2011; Schäfer *et al*., 2018). In cane toads, *Rhinella marina* (Linneaus), for example, dispersal ability increases with leg length, and more recently established populations (e.g., those at the invasion front) in Australia have longer legs than populations behind the invasion front (Phillips *et al*., 2006). Similar intraspecific variation in dispersal related morphology and physiology has been reported for a butterfly, *Melitaea cinxia* (Linneaus), and invasive starlings, *Sturnus vulgaris* Linneaus, in Australia (Haag *et al*., 2005; Phair *et al*., 2018).

Population dynamics and gene structure at range limits comprise a paradox: adaptation facilitates invasion into new habitat, but low density populations tend to have low allelic diversity, making it less likely for a random beneficial mutation to occur (Bridle & Vines, 2007; Pujol & Pannell, 2008; Uller & Leimu, 2011). While high levels of gene flow can counteract beneficial mutations, a moderate amount of gene flow via dispersal towards the range margin from endemic populations can increase genetic diversity, ultimately facilitating adaptation and further invasion (Kirkpatrick & Barton, 1997; Alleaume-Benharira *et al*., 2006). Controlled studies on effects of gene flow in marginal populations are uncommon; however, a number of studies on evolution of plant species at range margins provide evidence for improved fitness at the range margin following introduction of genetic material from other populations, particularly when environmental conditions at the range margin and gene source were similar (Sexton *et al*., 2011; Bontrager & Angert, 2018).

In low density populations, emigration can exacerbate inverse density-dependent Allee effects such as mate-finding success and cooperative feeding behaviors, and can ultimately lead to range retraction (Taylor & Hastings, 2005; Tobin *et al*., 2009). However, male dispersal from within the range interior to the range margin could improve mating success by alleviating Allee effects caused by mate-finding failure occurring in low density gypsy moth populations, and could increase likelihood of establishment (Sharov *et al*., 1995; Liebhold & Bascompte, 2003; Contarini *et al*., 2009; Tobin *et al*., 2009). Increased dispersal ability of male gypsy moth from just behind the invasion front to nascent satellite populations ahead of it has the potential to increase establishment success and the rate of range expansion.

In light of ongoing human-mediated environmental impacts, including climate change and habitat fragmentation, mate-finding capacity, dispersal ability, and establishment success are all likely to become increasingly important for many insects as range shifts occur and populations become more fragmented (Hunter, 2002; Kingsolver *et al*., 2011). Implications for effects of flight ability on mate-finding success in low-density populations may be relevant to other capital-breeding Lepidoptera, especially populations experiencing Allee effects, and in which females are non-dispersing prior to mating. A body-size effect on mate finding success has been shown in some other capital-breeding Lepidoptera. For example, male autumnal moth *Epirrita autumnata* Borkhausen mating in low density field populations were larger than mean population size, indicating there may be a size-dependent advantage, although it is unclear whether it is from mate-finding or mating competition (Tammaru *et al*. 1996b). Evenden *et al*. (2015a) identified a mate-finding Allee effect in a capital-breeding spring defoliator, *Malacosoma disstria* Hubner; caged females were mated more frequently in high-density populations than low. Although large males originating from endemic populations were more likely to initiate mate-searching flight behavior than small males from outbreak populations, there was no apparent mate-finding advantage for larger body size (Evenden *et al*., 2015a; Evenden *et al*., 2015b). Mated males of the saturniid *Rothschildia lebeau* Guerin-Meneville tended to be larger on average than non-mated males in a field study that used caged females to attract endemic males (Agosta, 2010).

There are no studies, to our knowledge, assessing whether adaptive shifts facilitating dispersal ability are occurring in gypsy moth populations at the invasion front. There may be selective pressure for males to achieve larger body size at or near the invasion front in order to facilitate mate-finding and increase immigration, but predicting how body size responds in chronically low-density populations, such as at the invasion front, may be difficult or impossible. Selective pressures favoring small male body size, such as short growing seasons and cool climates, may limit the extent to which body size can change, and adaptive shifts favoring resource allocation to flight morphology in dispersing males may be favored. In addition, a significant component of gypsy moth range expansion is driven by human-vectored movement of life stages (Sharov & Liebhold, 1998; Bigsby *et al*., 2011; Tobin & Blackburn, 2014). Movement of propagules from established areas to range front populations may swamp any adaptive change.

Adaptive shifts in male gypsy moth flight ability have important implications for both understanding historical gypsy moth range expansion, as well as predicting future invasion success. Future studies should take advantage of common garden studies, which disentangle genetic and environmental effects on body size, or sample morphological traits along an establishment gradient, as per Phillips *et al*. (2006), to determine whether shifts in gypsy moth body size or flight morphology are occurring.

Acknowledgements

Many thanks to Melody Keena (USFS), Ann Hajek and Tonya Bittner (Cornell University) for their advice on improving larval rearing procedures. I thank Toomas Tammaru (University of Tartu) for graciously reviewing an earlier version of the manuscript. Thanks to Kiyoto Tanemura (Michigan State University) for help with writing R code, and to Dr Lianjun Zhang (SUNY-ESF) for input on statistical analyses. My lab technician Marina Wittman provided valuable assistance weighing and mixing diet ingredients. Funding was provided by Macrosystems Biology (Grant 1702701 to Kristine Grayson, Sal Agosta, and Dylan Parry) and Gypsy Moth Slow-the-Spread Foundation, Inc (Grant 19-01-13 to DP).

Table 3.1 Formulations for gypsy moth lab diet with varying quantities of protein. High protein diet is equivalent to the standard gypsy moth lab diet first described in Doane & McManus (1981). Protein content was modified by removing protein components (wheat germ and casein) and adding equal volumes of cellulose to maintain consistent proportions of constituent ingredients by volume. Ingredient quantities deviating from the standard recipe are in bold. Manufacturer information for each diet ingredient is included in the last column.

Ingredients	**High (standard)**	**Medium-high**	**Medium-low**	**Low**	**Ingredient source**
Wheat germ	120 g	120 g	**100 g**	**60 g**	Frontier Agricultural Sciences, Newark, DE, USA (Item #G1659)
Casein	25 g	**10 g**	**0 g**	**0 g**	Frontier Agricultural Sciences, Newark, DE, USA (Item #1100)
Wesson salt mix (no ferric phosphate)	8 g	8 g	8 g	8 g	LabDiet, St Louis, MO, USA (Item #5S92)
Sorbic acid	2 g	2 g	2 g	2 g	Frontier Agricultural Sciences, Newark, DE, USA (Item #6967)
Methyl paraben	1 g	1 g	1 g	1 g	Frontier Agricultural Sciences, Newark, DE, USA (Item #7685)
USDA Vitamin Premix	10 g	10 g	10 g	10 g	Frontier Agricultural Sciences, Newark, DE, USA (Item #6265)
Ferric citrate	0.1 g	0.1 g	0.1 g	0.1 g	Sigma-Aldrich Corp., St Louis, MO, USA (Item #F3388-250G)
Cellulose	0 g	**10 g**	**40 g**	**80 g**	Southland Products Inc., Lake Village, AR, USA (special order)
Agar	15 g	15 g	15 g	15 g	Frontier Agricultural Sciences, Newark, DE Item #7060
Water	800 ml	800 ml	800 ml	800 ml	

Table 3.2 Summary statistics (mean ± SE) for larval development on each diet formulation.

Metric	**High (standard)**	**Medium-high**	**Medium-low**	**Low**
Pupal mass (g)	0.4362 ± 0.014	0.4002 ± 0.048	0.3409 ± 0.027	0.1823 ± 0.014
Number flown	74	11	17	2
Relative survival	High	High	High	Low

Table 3.3 Summary statistics for dependent (total distance, maximum speed attained) and wing length.

Flight capacity metric	**Mean ± SD**	**Median**	**Minimum**	**Maximum**
Total distance (km)	19.96 ± 16.74	15.98	1.24	68.22
Maximum speed (m/s)	1.21 ± 0.37	1.21	0.32	1.93
Wing length (mm)	2.06 ± 0.21	2.03	1.64	2.53

Table 3.4 Intercept and slope coefficients for total distance (m) mixed linear regression. Total distance was square root transformed to improve residual normality.

Explanatory Variable	**Estimate**	**SE**	**Lower CI (5%)**	**Upper CI (95%)**	**t (df = 91)**	**P**	**Est. model R^2**
Intercept	75.77	110.41	-137.95	46.41	0.69	0.49	0.59
Wing length	204.78	20.63	161.58	239.59	9.92	<0.001	
Relative mass lost	-14.16	24.58	-120.40	-6.67	-0.58	0.57	
Forewing aspect ratio	-50.94	9.25	-67.40	-32.53	-5.51	<0.001	
Relative thorax mass	507.00	95.52	289.33	651.88	5.31	<0.001	

Table 3.5 Intercept and slope coefficients for maximum speed (m/s) mixed linear regression.

Explanatory Variable	**Estimate**	**SE**	**Lower CI (95%)**	**Upper CI (95%)**	**t (df = 91)**	**P**	**Est. model R_2**
Intercept	1.53	0.68	0.20	2.86	2.25	0.03	0.48
Wing length	1.21	0.13	0.94	1.47	8.95	<0.001	
Forewing aspect ratio	-0.26	0.06	-0.37	-0.14	-4.21	<0.001	

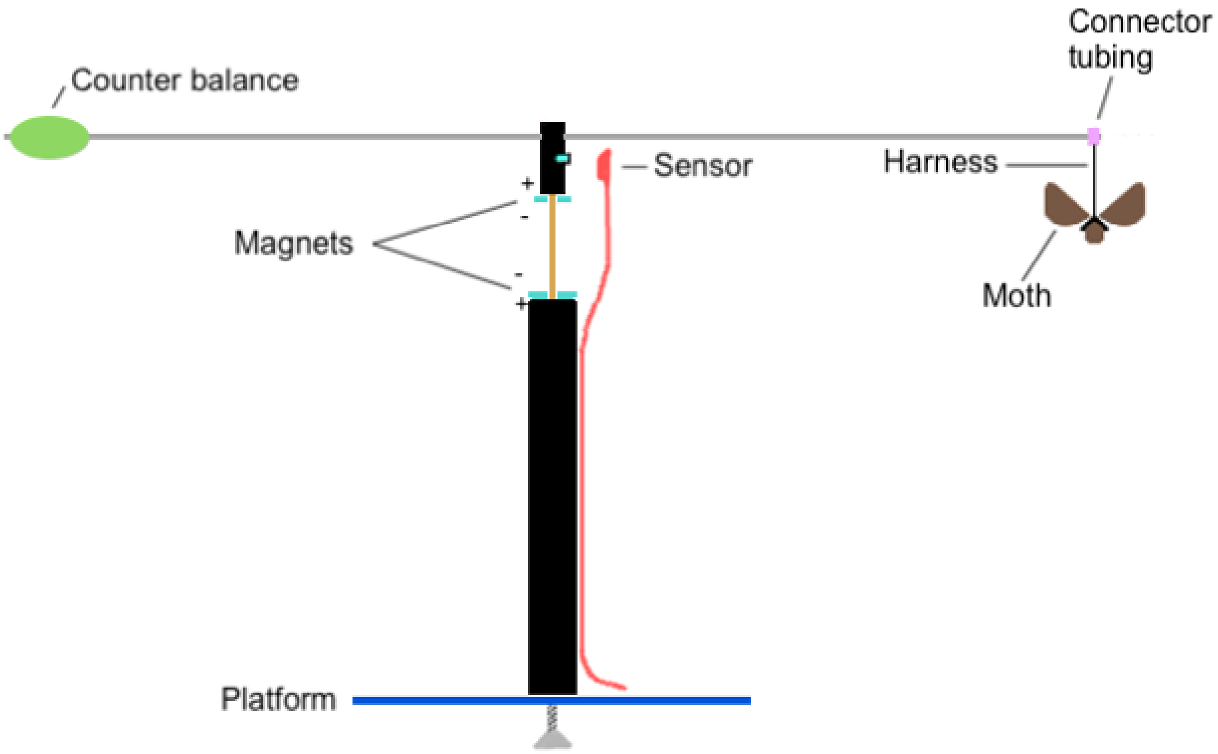


Figure 3.1 Schematic of fixed-arm flight mill with tethered moth. Rotating arm is held above mill using opposite-facing magnets to reduce rotational friction. A magnetic sensor detected magnetic impulse at each full rotation and computer program linked to flight mill recorded each rotation with a timestamp.

Figure 3.2 Male gypsy moth with inverted-y shaped harness glued to descaled first abdominal segment. Image was taken following 24 hr flight assay.

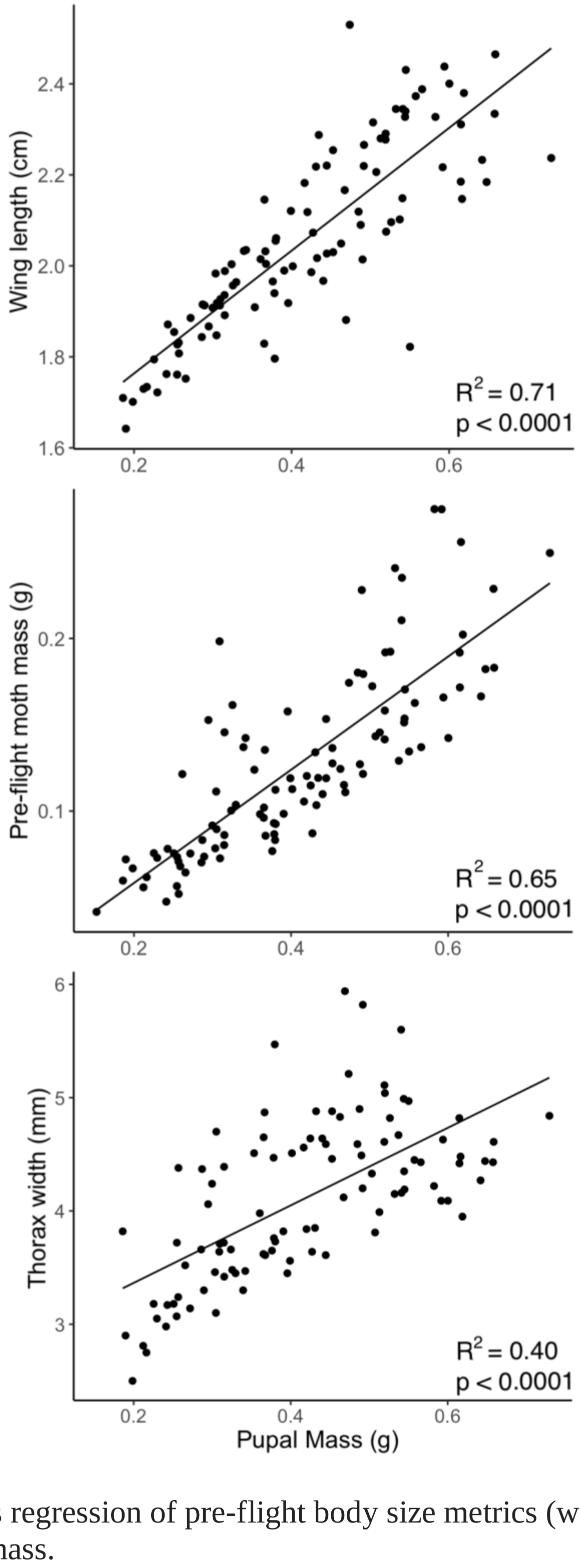


Figure 3.3 Least squares regression of pre-flight body size metrics (wing length, moth mass, and thorax width) on pupal mass.

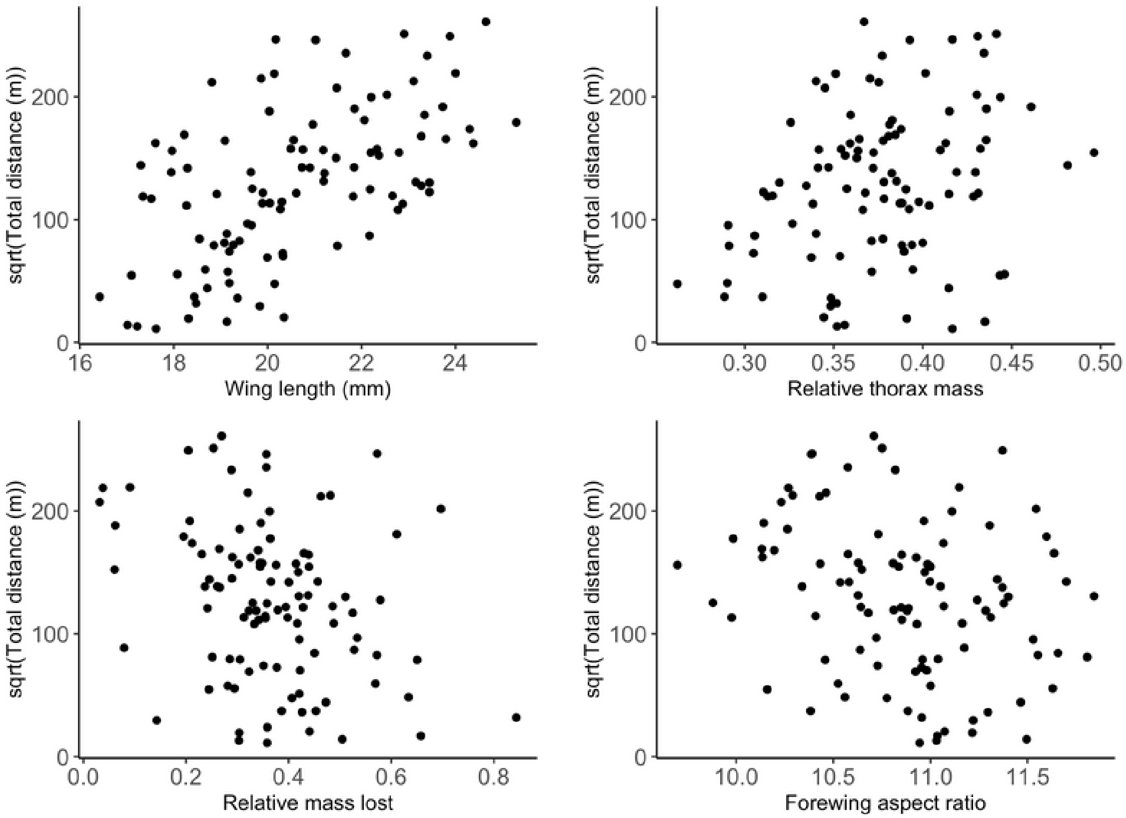


Figure 3.4 Scatter plots for the effect of wing length, forewing aspect ratio, relative thorax mass, and relative mass lost during flight on distance flown.

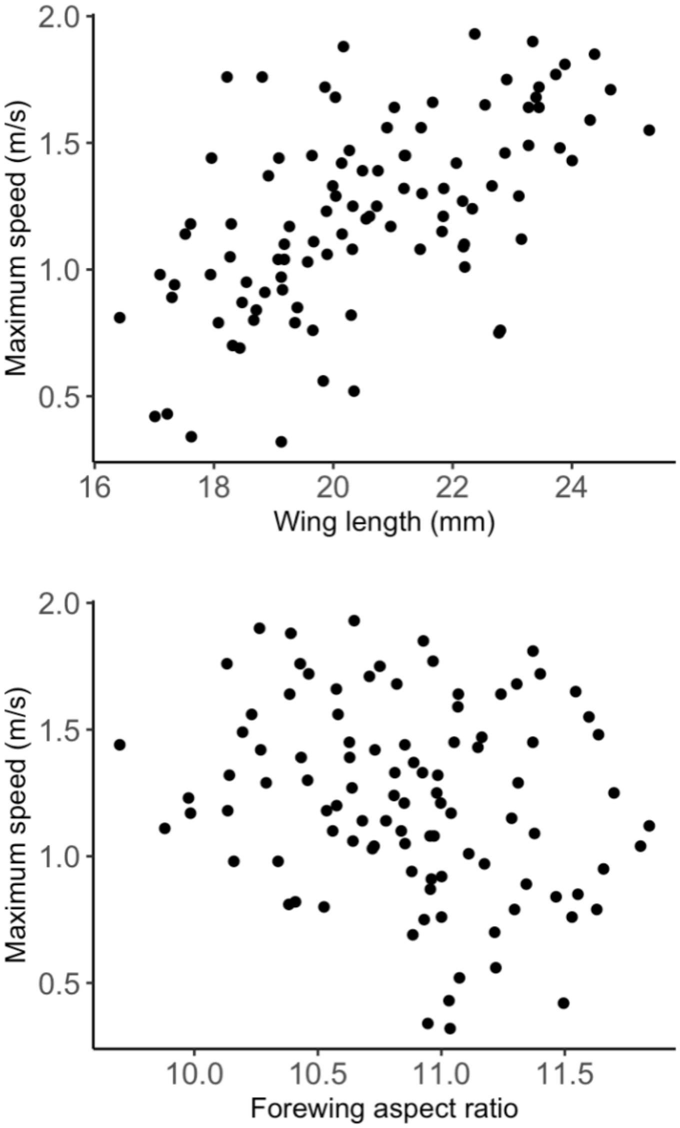


Figure 3.5 Scatter plots for significant factors impacting maximum speed attained by male gypsy moth. Raw data are plotted to visualize effects of wing length and forewing aspect ratio on maximum speed.

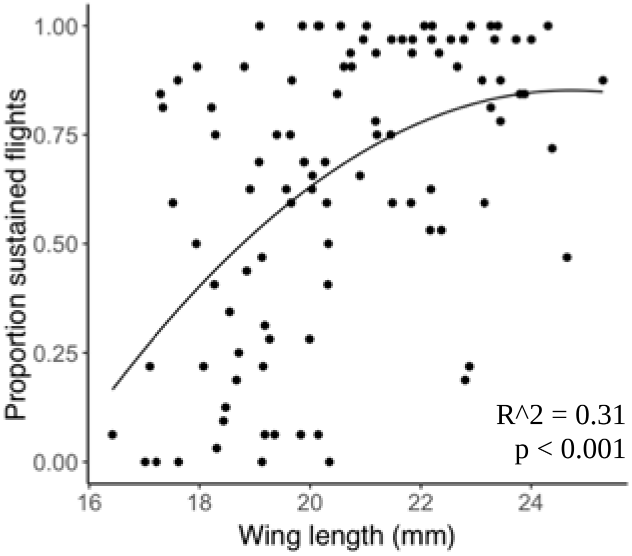


Figure 3.6 Least squares regression depicting relationship between mean proportion of sustained flights initiated and wing length.

Chapter 4: Climate-linked adaptive shift in hatch phenology of an invasive defoliating insect

Abstract

Synchronous, climatically-linked ecological processes are expected to experience unpredictable disruptions across taxa as human-mediated climate change continues, with consequences for trophic interactions, population dynamics, and realized range limits. However, adaptation to novel environmental conditions can improve fitness and mitigate negative consequences of climate change. Invasive species with ranges spanning large climatic gradients offer important opportunities to study selective pressures and rapid adaptation in response to novel climates.

Since its introduction near Boston, Massachusetts, in the late 1800’s, gypsy moth (*Lymantria dispar* L.) has increased its invasive North American range to span 12˚ latitude and wide range of climates. Phenological mismatch between the timing of gypsy moth egg hatch and leaf emergence of host trees is thought to occur in parts of its contemporary range. These asynchronies, if large, could impart fitness costs sufficiently large to drive adaptive changes in hatch timing.

Results from reciprocal transplant, common garden experiments in this study revealed divergent hatch timing in populations of gypsy moth spanning the north-south gradient. At both northern and southern sites, population of northern origin required fewer warm degree days to initiate hatch than mid-latitude populations with southern sourced populations requiring more. At two sites where leaf emergence was recorded simultaneously with egg hatch observations, all populations emerged well after leaf emergence and northern populations well before leaf emergence, in the northern and southern common gardens, respectively. The magnitude of the asynchrony, particularly at southern latitudes is likely to impart strong selection for a delay in hatching to better match tree phenology. These results may have important implications for future gypsy moth invasion dynamics and potential range limits.

Introduction

Anthropogenic climate change is causing a wide range of adverse environmental effects (IPCC, 2018). Altered thermal regimes have important consequences for many ecological phenomena linked by climatic cues, and the quantity and amplitude of disruptions across diverse taxa will increase as climates continue to warm (Parmesan & Yohe, 2003; Zhang *et al*., 2007; Forrest, 2016; Visser & Geinapp, 2019). Populations impacted by climate change are anticipated to respond in one of three ways: range shifts to track suitable climates, adapting in place, or local extinction (Parmesan & Yohe, 2003; Atkins & Travis, 2010). The ability for a population to adapt in place is dependent on sufficient genetic diversity for natural selection to occur, as well as having enough time for local adaptation to occur before populations dwindle below a sustainable threshold (Courchamp *et* al., 2008; Hoffmann & Sgro, 2011).

The role of selection and adaptation in range expansion of invasive species has been increasingly recognized (Lee, 2002; Allendorf & Lundquist, 2003; Prentis *et al*., 2008; Moran & Alexander, 2014). Latitudinal clines in fitness-related traits have been found for numerous invasive plant species (e.g., Weber & Schmid, 1998; Kollmann & Bañuelos, 2004; Monague *et al,* 2008; Woods *et al*., 2012; Novy *et al*., 2013). For example, successful reproduction at northern latitudes depends on the ability to develop within the constraints of a shorter cooler growing season (Colautti & Barrett, 2013; Way & Montgomery, 2015). As a result, populations of several invasive annual plants exhibit latitudinal tradeoffs between fecundity and developmental rate, with northern populations reaching reproductive maturity more quickly, but at a smaller size compared to southern populations (Flint, 1971; Ducousso *et al*., 1996; Kollman & Bañuelos, 2004; Colautti & Barrett, 2013). Latitudinal clines have also been identified in insect invaders (Garnas, 2018; O’Neill *et al*., 2017, Gilchrist *et al*., 2001) with several of the identified adaptive clines correlated with temperature. Seiter *et al*. (2013), for example, found invasive populations of *Pieris rapae* (Linneaus) on two continents had independently developed similar latitudinal clines in development time in < 300 years. Northern populations of the invasive hemlock wooly adelgid *Aldeges tsugae* Annand have increased cold tolerance compared to southern populations (Elkinton *et al*., 2017; Lombardo & Elkinton, 2017).

High reproductive rates and fast generation time contribute to the ability of insects to adapt quickly in response to novel or changing environments (e.g. Kettlewell, 1958, Singer *et al*., 1993; Gilchirst *et al*., 2008; Hoffman & Sgro, 2011). These characteristics make insects valuable models for understanding potential for adaptation in the face of large-scale human-mediated environmental disturbance across relatively short timescales (Seiter *et al*., 2013; Lombardo & Elkinton, 2017). Perhaps the most well-documented insect invasion on Earth has been the arrival and spread of gypsy moth (*Lymantria dispar* L.) in North America. Since its introduction in Medford, Massachusetts in 1869, it now ranges across 12˚ of latitude spanning a significant thermal gradient (Grayson & Johnson, 2018). Gypsy moth undergo a cold-dependent obligate diapause, followed by postdiapause which is terminated after sufficient warming (Gray, 2004). The latitudinal variation in gypsy moth hatch timing roughly matches that of bud burst in host trees (i.e., spring occurs progressively later in the calendar year as latitude increases), however, phenological asynchrony between gypsy moth hatch and leaf emergence has been observed. Foster *et al*. (2013) used satellite-derived estimates of leaf emergence in Appalachian forests and found gypsy moth hatch occurred 7–21 days after leaf emergence. Climatic conditions at the northern and southern range margins are likely to exacerbate fitness costs of hatch asynchrony with leaf emergence of host trees (Raupp *et al*., 1988; Hunter & Elkinton, 2000; Thompson *et al*., 2017; Keena & Shi, 2019).

Thermal clines are apparent for gypsy moth in both its native and invasive ranges (e.g., Keena, 2015; Thompson *et al*., 2015; Faske *et al*., 2019). In North America, gypsy moth populations in the southeastern coastal plains of Virginia and North Carolina exhibit increased heat tolerance in the egg and larval stage compared to those in an inland montane region, likely a result of fitness costs of increasing occurrence of supraoptimal temperatures in the coastal plain (Thompson *et al*., 2015; Faske *et al*., 2019). Observations of adaptive phenotypic traits have been corroborated by molecular analyses, which indicate physiological traits of invasive North American gypsy moth populations are diverging at least partially in response to climatic cues (Faske, 2017; Friedline *et al*., 2019), despite substantial founder effects evident in genetic analysis (Wu *et al*., 2015). In common garden experiments assessing thermal requirements for hatch for populations of Asian and European gypsy moth, populations encompassing a latitudinal-elevational gradient differed in warming requirements to initiate hatch, suggesting adaptation to local climates (Keena, 2015). No study that we are aware of has assessed the timing of gypsy moth hatch relative to leaf emergence across the entire North American latitudinal range.

My study assessed the timing of hatch for a suite of gypsy moth populations sourced to localities along the full latitudinal range of the species in eastern North America. The timing of egg hatch was recorded at ambient temperatures in three common garden sites and under a simulated climate representing a locality south of the current established range. At two sites (northern and southern) the timing of leaf emergence of a primary host tree species was recorded at the same time as egg hatch observations. I hypothesized that if local adaption has occurred 1) northern populations will require fewer warming degree days to initiate hatch than southern populations, 2) that northern populations will hatch too early and southern too late with respect to the phenology of local host trees, and 3) the simulated climate for the south-of-current range limit will exacerbate differences between northern and southern sourced populations in the onset of hatch.

Materials and Methods

Experimental design

Reciprocal transplant common garden studies are important for understanding whether variation in phenotypic traits among populations is a result of phenotypic plasticity or genetic variation (e.g., Colautti & Barret, 2013; Banahene *et al.*, 2018; Oduor *et al*, 2016). To determine whether variation in the timing of gypsy moth hatch across the latitudinal range in eastern North America is an adaptive or plastic response, I used a fully reciprocal design with four common gardens. Three experimental field sites and one growth chamber simulation were used to represent current and potential range of gypsy moth. The three experimental sites were located in Tully, New York (NY) (cool northern), Mountain Lake Biological Station (MLBS) near Blacksburg, Virginia (cool southern), and Richmond, VA (RVA) (warm southern), were selected to encompass climatic conditions experienced by gypsy moth populations in the current invasive range (Table 4.1).

The simulation was implemented using a Percival Growth Chamber (Model I-22VL with Intellus Connect, Percival Scientific, Inc.) to replicate the temperature regime for a geographical location south of the current southern range limit of gypsy moth. McClellanville, SC, was selected for the simulation because it is located ~ 160 km south of a recent (2015–2016; http://yt.ento.vt.edu/da/) gypsy moth satellite population that established near the southern tip of North Carolina; by doing so I was able to produce a realistic climate profile for gypsy moth populations if expansion continues southward. United States Climate Normals (1991–2010, www.ncdc.noaa.gov) temperature data were downloaded from three weather stations (GHCND: USC00386893, USW00003728, USC00385628) near McClellanville. Mean daily minimum and maximum temperatures were calculated and used to program the growth chamber, which alternated between daily minima and maxima in a ramping fashion. The lowest temperature that the growth chamber was capable of maintaining was 5˚C; thus, when mean minimum temperatures were < 5˚C, the growth chamber was programmed to 5˚C. Mean daily minimum temperatures for McClellanville, SC, fell below 5˚C for 71 days, by no more than 2˚C. Photoperiod of 16:8 (L:D) and 60% RH were used throughout the experiment.

Three individual northern red oak, *Quercus rubra* L., trees in RVA and three in NY with accessible low-hanging branches were photographed every 3–5 days in NY, and every two days in RVA, to assess bud phenology for the 2018 hatch phenology experiment. Digital photographs were used to produce a bud and leaf development phenological scale for northern red oak (Fig. 4.1) based on a phenological ranking for bud and leaf development developed for trembling aspen *Populus tremuloides* Michx (Parry *et al*., 1997). 'Early' was defined as the period where buds were softened and scales separated, but leaf expansion not initiated (Fig 4.1d). 'Late' was defined as the point when leaves were fully expanded, but not yet tough (Fig 4.1h).

Egg hatch phenology: Insects and overwintering

Eight populations sourced to localities along the full north-south latitudinal range were acquired in 2017 and overwintering at each common garden site. Eggs were previously collected from wild populations (Table 4.2) and reared through at least one generation under common conditions prior to this study in order to minimize maternal effects. Following captive rearing on red oak foliage and oviposition in the summer of 2017, at least 10 egg masses were mixed by population (n = 8) and divided into mesh pouches with ~ 150 eggs each. Replicate pouches (n =

2) from each population were sent to each of the four overwintering sites in December 2017. Pouches were kept in lidded plastic bins with holes for ventilation and each bin was equipped with a digital thermometer to record local temperatures. Eggs at MLBS and RVA were kept in permanent screen-sided shelters at ambient temperatures. Eggs in NY were located at Heiberg Memorial Forest in a vented opaque plastic box on the north facing side of a large tree with no direct sunlight. Prior to hatch, eggs were transferred from overwintering pouches to small Petri dishes fitted with fine mesh lids to simplify access for enumerating hatch. Eggs at each site were checked regularly until first hatch was recorded, after which eggs were checked daily or every other day, and newly hatched larvae counted and removed. Eggs were monitored for at least six days after the last recorded hatch.

After winter chilling requirements have been met, gypsy moth eggs begin accumulating warm degree days (DD) and the standard strain (Otis Laboratory) population often used as a bench mark hatch at ~282 DD (Johnson *et al.*, 1983). Daily minimum and maximum temperatures recorded at each site were used to calculate additive warming DD accumulation above a threshold of 3˚C (Johnson *et al.*, 1983). Due to large differences in winter conditions among sites, DD accumulation did not begin on the same day at each site (Gray *et al.*, 2001). Therefore, in order to compare warming requirements for each combination of population and site, DD accumulation was standardized at each site by sacrificing one population (MA1) to serve as a benchmark. This population was selected due to its mid-latitude location, as well as the redundancy of MA populations which were collected over a relatively small area of southern New England. I aligned 50% hatch in the MA1 population with 282 DD and calculated the relative difference in DD accumulation for the remaining populations. The same method was used to determine relative differences in DD accumulation for eggs of each population at each site at 5% hatch and 95% hatch. MA1 was not included in the statistical analyses comparing relative differences in DD accumulation needed for 5%, 50%, and 95% hatch for each population at each site.

In order to assess the effects of population source on the timing of egg hatch using a continuous metric, each population source location was described using mean annual temperature (Table 5.1). Mean annual temperature for each US population source coordinates were calculated using 30-year climate normals (1981 – 2010) from PRISM (PRISM Climate Group, 2012). Historical temperature data for Quebec City, Quebec, Canada (QC93) and Ville-Marie, Quebec, Canada (QC32) coordinates were acquired from Canadian Climate Normals (1981–2020; Government of Canada, 2019). Overwintering sites were also described using mean temperature for dates between 1 Jan 2018 – 1 June 2018 when eggs were completing diapause and accumulating DD. Mean temperatures were calculated from data recorded by iButton Thermochron (Maxim Integrated, San Jose, CA) temperature loggers deployed at each field site and inside the growth chamber used in the simulation.

Statistical analyses

All analyses were completed in R (version 3.5.2, R Core Development Team 2018). Significance level for all analyses was $\alpha = 0.05$. All variables were checked for normality using Shapiro-Wilk’s test, and were found to be normally distributed. In order to assess differences in hatch timing for populations within and among sites, three separate linear models were used with relative difference in DD accumulation by each population at 5%, 50%, and 95% as the response variable. Explanatory variables were population source climate, site climate, and the interaction. Duration of hatch in days for each population at each site was compared using linear modelling with population source climate, site climate, and the interaction as independent variables, and duration of hatch in days as the response variable.

Results

There was a strong dichotomy in the relationship between egg hatch and bud break of red oak at the northernmost and southernmost sites in this study. At the southern site (RVA), hatch in all gypsy moth populations preceded bud break significantly and was nearly complete before any foliage appeared (Fig. 4.2). In sharp contrast, egg hatch initiated well after bud break for all populations at the cool climate site in NY. In fact, hatch at the northern site (especially in the NC populations) continued even after leaves were already fully, or nearly fully, expanded (Fig 4.2).

Site climate, population source climate, and site climate× pop. source climate had significant effects on DD accumulation required for 5%, 50%, and 95% hatch, respectively (Table 4.3; Fig 4.3). Populations followed similar patterns of divergence from the DD accumulation standard at each site. The southern populations (e.g., NC) generally required more DD accumulation to reach 50% hatch than the mid-latitude populations (e.g., MA, NY), with the northern populations (e.g., QC) requiring fewer. As latitude decreased, southern populations required increasingly greater DD accumulation in order to reach 50% hatch.

Duration of egg hatch was also affected by site climate ($F_{1, 45} = 73.49$, $p < 0.001$) and population ($F_{1, 45} = 8.64$, $p = 0.005$) when measured in calendar days. There was a significant interaction between site and population ($F_{1, 45} = 4.81$, $p = 0.03$). Duration of hatch across all populations within sites became increasingly compressed in duration as site latitude increased (Fig 4.2) and as expected, as latitude increased, hatch occurred later in the calendar year. Total duration of hatch in days across all populations for each site was 24, 28, 29, 75 for NY, MLBS, RVA, and SC, respectively (listed from north to south; Table 4.4). Duration of hatch in days for each population tended to be shorter for southern populations than for northern, regardless of site. The pattern of hatch at each site was generally consistent with the two QC populations hatching first, followed by the mid-latitude populations MA and NY, and finally the two NC populations.

Discussion

Variation in direction and magnitude of insect and plant response to warming climate may result in the decoupling of phenologically-linked events, including the hatch of spring-feeding folivores and leaf emergence in their tree hosts (Visser & Holleman, 2001; Bale *et al.*, 2002; Foster *et* al., 2013; Forrest, 2016), resulting in reduced fitness and survival, and altered population dynamics (Raupp *et al.*, 1988; Hunter, 1992; Hunter & Elkinton, 1999; Jones and Despland, 2006; Forrest 2016). The present study demonstrates the ability of insects to respond rapidly to novel or changing environmental conditions across a climatic gradient. Specifically, shifts in hatch timing may illustrate an adaptive response to selective pressures that result from fitness costs of hatching out of synchrony with leaf emergence.

Hatching early or late relative to leaf emergence has important consequences for fitness and population dynamics of spring-feeding Lepidoptera (Raupp, 1988; Hunter, 1992; Hunter & Elkinton, 1999; Hunter & Elkinton, 2000; van Asch & Visser 2007). The directional shift in hatch timing at either end of the latitudinal range in this study improved synchrony between hatch and local red oak leaf emergence. At the cool northern site (NY), northern red oak leaf emergence occurred well before hatch, which commenced when leaves were partially to fully expanded. While northern-sourced populations exhibited the least degree of asynchrony, all populations were still hatching when leaves were beginning to mature. Conversely, at the warm southern site (RVA), the beginning of hatch for northern populations preceded leaf emergence, and leaves were still expanding when the southern populations hatched. For gypsy moth, hatching too early can result in starvation and death, with the duration of larval survival without food decreasing as temperature increases (Keena & Shi, 2019, Chapter 5). Conversely, hatching late increases risk of mortality and reduces pupal mass and larval development rate (Hunter & Elkinton, 2000; van Asch and Visser 2007, Chapter 5). The fitness consequences of asynchronous hatch relative to leaf emergence likely produce strong selective pressures on gypsy moth hatch timing capable of driving the adaptive shifts apparent in this study.

Duration of hatch in the present study increased from north to south, with total hatch duration in the SC simulation, the warmest overwintering condition, taking more than two times longer than the coolest overwintering site in NY. Gypsy moth diapause is temperature-dependent, and is terminated when inhibitory enzymes are depleted (Gray *et al*., 2001). Diapause is completed more quickly at lower temperatures (< 5˚C), though there is not a hard threshold and eggs overwintering above 5˚C may still complete diapause but require more time to initiate hatch (Tauber *et al*., 1990). As latitude decreases, mean winter temperatures become increasingly warmer (Table 4.1), and eventually become too warm to deplete diapause enzyme prior to onset of warmer spring temperatures (Gray *et al*., 2001). Conversely, colder mean winter temperatures at northern sites allow for rapid and complete depletion of inhibitory enzymes, and contribute to shorter hatch duration at northern latitudes compared to in the south. The pattern of hatch at MLBS in Virginia at nearly the same latitude as the coastal plain site (RVA) was more similar to the cool northern NY site, likely reflecting cooler spring temperatures at this high elevation location.

Similar patterns of divergence in hatch timing across populations of spring feeding Lepidoptera have been reported. For example, Fält-Nardmann *et al*. (2016) reported the amount of degree day accumulation required for hatch initiation increased from north to south for populations of three spring-feeding moth species sampled along a latitudinal climatic gradient in Fennoscandia, autumnal moth *Epirrita autumnata* Borkhausen, winter moth *Operophtera brumata* (Linneaus), and *Erannis defoliaria* (Clerck), and suggested fitness consequences of phenological mismatch as a mechanism for differential latitudinal hatch phenology. This argument is buttressed by the range expansion of another defoliator (*Agriopis aurantauria* Hubner) in the system as climate has warmed, apparently because of an increase in synchrony with their host trees phenology (Jepsen *et al*., 2011). Uelmen *et al.* (2016) found significant differences for hatch timing in forest tent caterpillar, *Malacosoma disstria* Hubner, at standardized temperatures for source populations collected over a 600 km latitudinal gradient. In hemlock looper *Lambdina fiscellaria* Guenée overwintered in a common garden, the number of days required to initiate hatch decreased with latitude of the source population (Berthiaume, 2007). In a study assessing hatch requirements for *Lymantria* species, four biotypes of *L. dispar* were invasive North American populations, and under standard warming treatments emerged in the following sequence: Massachusetts, West Virginia, Connecticut, and lastly North Carolina (Keena, 2015).

High fecundity and rapid generational turnover enable organisms such as insects and annual plant species to adapt to shifting environmental conditions with relative rapidity (Carroll *et al*., 2007) and can facilitate invasions as populations expand into habitat with novel environmental conditions (Lee, 2002; Colautti & Lau, 2015; Odour *et al*., 2016). Adaptive shifts have been documented for various traits in invasive insect species, both to facilitate dispersal at the invasion front, and in response to novel environmental conditions such as altered thermal regimes (e.g., Hill *et al*., 2011; Thompson *et al*., 2017; Renault *et al*., 2018). For example, following the introduction of the fall webworm *Hyphantria cunea* (Drury) to Japan, populations in the southern part of its invasive range adapted to a longer growing season by developing shorter photoperiodic response and greater temperature sensitivity, resulting in increased voltinism (Gomi, 2007). Gypsy moth populations originating from the southeastern coastal plain, have higher tolerance for temperatures above the thermal optimum compared to populations from the same latitude in the Appalachian Mountains, as well as a population from central New York (Thompson *et al*., 2017; Banahene *et al*., 2018). These changes are likely due to selective pressures from fitness costs of supraoptimal temperatures occurring more frequently in the coastal plain, although this currently seems insufficient to prevent a range retraction (Tobin *et al*., 2014a).

This study is the first to report adaptive shifts in gypsy moth hatch timing across the latitudinal gradient of the invasive range. The gypsy moth invasion represents a unique and valuable model for studying insect evolution because they now inhabit diverse forest types and temperature regimes ranging from continental to sub-tropical. Despite being present in North America for only 150 years and undergoing a profound founder effect (Wu *et al*., 2015), the evidence for rapid adaptation of gypsy moth to novel habitats is mounting. As climate changes, impacts of insects within their ecosystems is expected to change in a variety of ways including increased amplitude and frequency of insect outbreaks, which have the potential to negatively affect biodiversity and ecosystem services (Tobin *et al*., 2014b). Understanding the potential for adaption in novel environments, and the timescale on which it occurs, has important implications for management efforts and predictions for further range expansion in this important invasive defoliator.

Acknowledgments

I thank the numerous undergraduate research technicians from Grayson lab at University of Richmond who helped count newly hatched larvae, and especially Lily Thompson whose input and coordination were critical to the success of this project. Thank you to Jaime BeVille for allowing us to use the facilities at Mountain Lake Biological Station of University of Virginia, to Joel Slade (Michigan State University), and Sarah Wanamaker and Abby Kimmit (Indiana State University), for covering egg hatch checks at MLBS. Many thanks to Theresa and Jeff Given for opening their home to a traveling Ph.D. student. I thank the Parry lab undergraduate lab technicians including Chapin Czarnecki and Emma Livingston, who provided assistance with various components of this project. I am very grateful for the substantive comments from Colin Beier, Melisa Fierke and David Althoff which significantly improved this manuscript. This research was funded by the Edna Baily Sussman Foundation, National Science Foundation Macrosystems Biology (Grant 1702701 to Kristine Grayson, Sal Agosta, and Dylan Parry), Gypsy Moth Slow-the-Spread Foundation, Inc (Grant Number 19-01-13) to DP) and the USDA Forest Service, Northern Research Station (Grant 11-JV11242303-053 to DP).

Table 4.1 Location and elevation for the three outdoor sites used in the 2017–2018 hatch phenology experiment, and four-week average minimum and maximum temperatures for all four overwintering scenarios.

			27 Nov (˚C)		25 Dec (˚C)		22 Jan (˚C)		19 Feb (˚C)		19 Mar (˚C)	
Site	**Location**	**Elevation**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
Tully, NY (NY)	42.769512, -76.083395	573 m	-2.70	2.39	-13.46	-7.04	-8.14	-0.89	-4.40	1.29	-5.31	2.50
Mountain Lake Biological Station (MLBS)	37.375654, -80.522140	1,160 m	-2.15	5.37	-10.49	-2.29	-2.90	4.95	-0.45	7.76	-0.02	9.20
Richmond, VA (RVA)	37.573909, -77.539437	47 m	2.44	11.92	-4.80	5.34	2.58	12.58	4.18	13.91	6.31	18.03
McClellanville, SC (SC)*	33.156623, -79.604567	7.2 m	4.78	15.88	4.23	13.34	4.14	14.33	6.35	17.27	9.50	21.17

*Simulated in growth chamber in Richmond, VA. Values across row are means for three weather stations (GHCND: USC00386893, USW00003728, USC00385628).

Table 4.2 Source locations for the eight wild populations used in the hatch phenology reciprocal transplant design. Two replicates (~150 eggs/replicate) of each population overwintered in each of four settings: outdoor sites in NY, Richmond, VA, and Mountain Lake Biological Station in VA, and a growth chamber simulation of McClellanville, SC. Population source climate represents the annual mean temperature calculated using 30-year (1981–2010) climate normals.

Population	Locality	Elevation (ASL)	Köppen Climate Classification	Pop. source Climate (˚C)
Currituck, NC (NC 1)	36.449125˚N, 76.024672˚W	2 m	Humid sub-tropical	15.9
Hatteras, NC (NC 2)	35.250371°N, 75.581311°W	1 m	Humid sub-tropical	17.3
Dedham, MA. (MA 3)	42.25115˚N, 71.20961˚W	37 m	Warm continental	9.4
Waltham, MA MA 1	42.40502°N, 71.28713°W	49 m	Warm continental	9.5
Randolph, MA MA 2	42.20739°N, 71.08091°W	56 m	Warm continental	9.9
Kirkville, NY (NY)	43.080624˚N, 75.981274˚W	131 m	Temperate continental	8.9
Quebec City, QC (QC93)	46.9089631˚N, 70.8061075˚W	98 m	Temperate continental	3.6
Ville-Marie, QC (QC32)	47.2509807˚N, 79.4060515˚W	200 m	Temperate continental	3.1

Table 4.3 ANOVA table for linear models with degree day (DD) accumulation required for 5%, 50%, and 95% hatch as the response variable, and population source climate and overwintering site climate as independent variables. Three separate linear models were used for each level of hatch.

	5% hatch			50% hatch			95% hatch		
Variable	**F**	**df**	**p**	**F**	**df**	**p**	**F**	**df**	**p**
Pop. source climate	90.28	1, 53	< 0.001	72.60	1, 52	< 0.001	76.08	1, 52	< 0.001
Site climate	10.26	1, 53	0.002	7.95	1, 52	0.006	36.00	1, 52	< 0.001
Site climate×pop. source climate	26.86	1, 53	< 0.001	20.20	1, 52	< 0.001	16.55	1, 52	< 0.001

Table 4.4 Mean ± SE duration of hatch in days for each population by site in the hatch phenology reciprocal transplant experiment. Total duration is the number of days between first hatch and last hatch at each site for all populations.

	Sites			
Population	**SC Simulation**	**RVA**	**MLBS**	**NY**
NC1	22.0 ± 1.0	14.0 ± 0.0	14.0 ± 0.0	10.0 ± 2.0
NC2	21.0 ± 1.5	12.0 ± 0.0	7.5 ± 0.5	16.5 ± 1.5
MA1	23.0 ± 1.0	23.5 ± 0.5	9.5 ± 0.5	14.0 ± 2.0
MA2	29.0 ± 2.0	21.5 ± 0.5	11.0 ± 3.0	18.0 ± 1.0
MA3	34.0 ± 7.0	23.5 ± 2.5	10.0 ± 1.0	11.0 ± 1.0
NY	24.5 ± 1.5	22.5 ± 0.5	13.5 ± 0.5	15.0 ± 1.0
QC93	47.0 ± 7.0	17.5 ± 0.5	10.5 ± 6.5	16.0 ± 0.0
QC32	28.5 ± 4.5	16.5 ± 0.5	12.5 ± 2.5	15.0 ± 1.0
Total	75	29	28	24

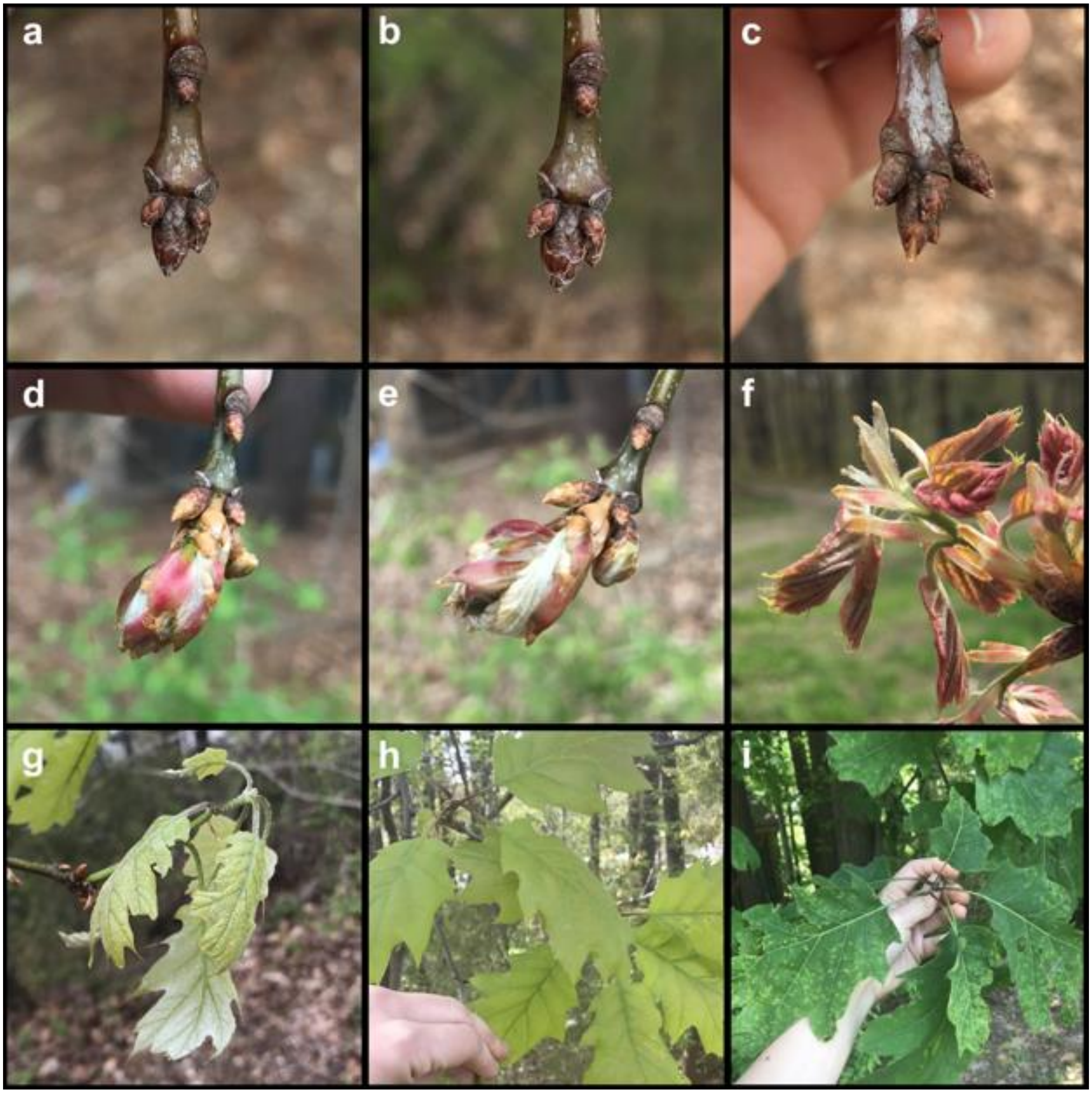


Figure 4.1 Red oak bud and leaf condition classes used to quantify the spring phenological development of leaves: a) firm winter bud, b) bud swelling, but still hard, c) bud softening and becoming green at scale edges, d) scales separating, leaf tips visible, e) leaf tips elongating and emerging from bud, f) leaves and petioles unrolling, g) scales shed, leaves and petioles elongating, h) leaves and petioles expanding, i) leaves fully or nearly fully expanded, light green, j) leaves fully expanded, dark green.

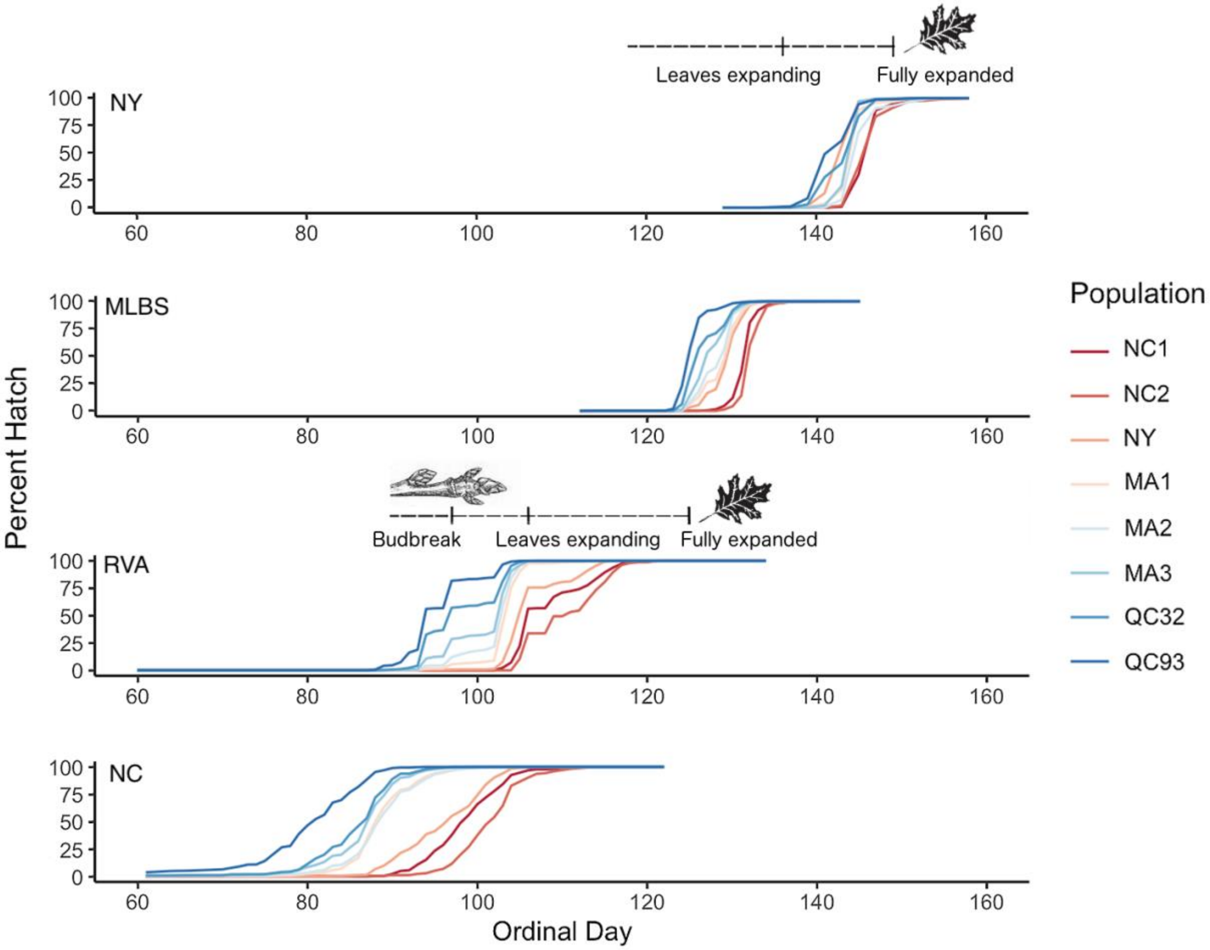


Figure 4.2 Cumulative percent hatch for each population (n = 2 replicates/combination) plotted against ordinal day at the three field sites and for the growth chamber climate simulation. Bud and leaf development for red oak, a primary host tree, was recorded at the NY (cool northern) and RVA (warm southern). Populations are color coded from north to south, with higher latitudes in blue and lower latitudes in red.

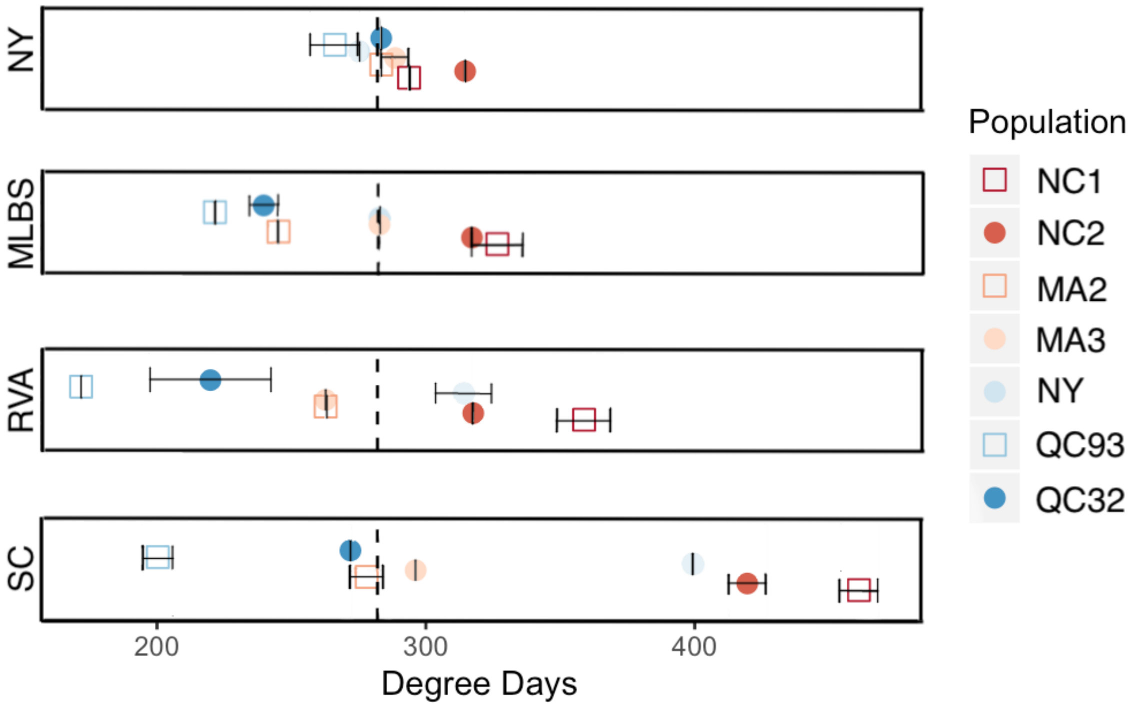


Figure 4.3 Degree days required for 50% hatch for each population at each site. Vertical dashed lines indicate 282 DD, which represents hatch timing benchmark based on the Otis Laboratory standard strain. Points indicate DD following MA1(a mid-latitude population) alignment to 282 DD. Error bars indicate standard error (n = 2 replicates/combination). Populations are color coded from north to south, with higher latitudes in blue and lower latitudes in red.

Chapter 5: Fitness consequences of phenological asynchrony with host trees for a widespread invasive defoliating insect

Abstract

Spring-feeding Lepidoptera are sensitive to the timing of leaf emergence of host trees, and hatching asynchronously can increase the risk of starvation and death, and reduce developmental rate and fecundity. Unlike native species that have evolved in concert with host tree species, invasive insect herbivores are likely to experience strong selective pressures to improve phenological synchrony with leaf emergence of novel hosts as their ranges expand. Gypsy moth (*Lymantria dispar* (L.)) was introduced to North America in 1869 and has spread to encompass diverse thermal regimes and habitats. Recent evidence suggests that the timing of egg hatch in gypsy moth populations at the northern and southern limits of the invasive range has changed, likely as a result of selection pressures to better synchronize with the phenology of leaf emergence.

In this Chapter, I quantified the relative magnitude of fitness costs for gypsy moth hatching outside the optimal phenological window. An experiment designed to simulate hatch prior to leaf emergence (too early) found that thermal regimes similar to those encountered in the southeastern portion of the invasive range greatly reduced the duration of neonate survival. To assess the consequences of later hatching (too late), the timing of hatch was manipulated so that gypsy moth neonates initiated feeding on either newly emerged leaves or leaves nearly two weeks more phenologically advanced on three common host trees in northeastern forests. For two moderate quality hosts (American beech and red maple), later hatch was associated with a reduction in mass at maturation and a decrease in survival. For red oak, a favored species for gypsy moth, late hatching decreased pupal mass but had no effect on survival. These results suggest that hatching too early is likely to be strongly selected against while selection against late hatch is weaker. In marginal habitats such as the current northern range edge, selection against late hatch is likely stronger than elsewhere. Understanding the environmental drivers of adaptive shifts has important implications for predicting future spread and informing management decisions.

Key words: *Lymantria dispar*, invasive species, selection pressures, phenology, climate

Introduction

Lepidoptera that depend on high quality, but ephemeral vernal foliage are especially vulnerable to shifts in the relative timing of leaf emergence (Parry *et al*., 1998; van Asch & Visser, 2007). Such changes are driven by idiosyncratic responses to the same set of critical cues such as temperature and photoperiod (Dewar & Watt, 1992; Forkner *et al*., 2008; Körner & Basler, 2010). Specifically, insects often rely primarily on temperature cues to break diapause, while deciduous host trees in temperate zones respond to warm temperatures only after winter chilling and photoperiod requirements have been met (Bale, 1987; Basler & Körner, 2014; Flynn & Wolkovich, 2018). As leaves mature, physical and biochemical properties change rapidly, resulting in increased leaf toughness, altered secondary chemical compound profiles, and reduced water and nitrogen levels (Feeny, 1970; Raupp *et al*., 1988). These changes ultimately lower nutritional value for folivores, and result in reduced insect fitness (Feeny, 1970; Raupp *et al*., 1988; Hunter & Elkinton, 2000; Fuentealba *et al*., 2017). Larvae hatching early relative to leaf emergence risk starvation and exposure to low temperatures (Fuentealba *et al*., 2017; Gray & Keena, 2019; Keena & Shi, 2019). Late-hatching larvae may have reduced developmental rates, pupal mass, fecundity, and survival when feeding on rapidly maturing foliage (Feeny, 1970; Ayres and McLean 1987; Raupp, 1988; Jones & Despland, 2006; van Asch and Visser 2007), and an increased risk of attack from predators and parasites (Parry *et al*., 1998; Hunter & Elkinton, 2000).

Understanding the relationship between leaf emergence and the onset of insect hatch has become increasingly important as one predicted consequence of climate change is the amplification of phenological asynchronies (Bale & Hayward, 2010; Forrest, 2016). Asynchrony with tree phenology generates strong selective pressures leading to adaptive shifts in the timing larval emergence to improve synchrony with leaf emergence (Davis *et al*., 2005; Franks *et al*., 2007; van Asch *et al*., 2013). Several studies have shown that hatch timing for spring-feeding native Lepidoptera varies across their geographic ranges with higher latitude populations generally requiring fewer degree-days to initiate hatch (Berthiaume, 2007; Fält-Nardmann *et al*., 2016; Uelmen *et al*., 2016. This change is assumed to be an adaptive response to patterns of local leaf emergence in favored tree host species (Bertiaume, 2007; Fält-Nardmann *et al*., 2016).

In native species, the relationship between spring leaf emergence and the timing of egg hatch has been shaped over long periods of evolutionary time. In contrast, invasive species, often feeding on novel hosts and encountering climatic regimes different from those with which they evolved, may be under considerable selection to increase synchrony. The gypsy moth (*Lymantria dispar* (L.)), exemplifies this relationship. This species was deliberately imported and accidentally released 150 years ago at a single location where it went through a pronounced genetic bottleneck (Forbush & Fernald, 1896; Wu *et al*., 2015). Subsequently, as it has spread across eastern North America, it has encountered increasingly divergent thermal regimes and environmental conditions (Grayson & Johnson, 2017). Despite reduced genetic diversity, common garden studies have identified regional variation among populations in traits thought to be adaptive to local climates (Thompson *et al*., 2017; Banahene *et al*., 2018; Faske *et al*., 2019). I showed (Chapter 4) that eggs from northern populations require fewer degree days to initiate hatch than mid-latitude populations, and southern populations require more. I hypothesized that these changes were driven by selective pressure to better align hatch with local patterns of leaf emergence in host trees, but did not quantify the magnitude of the fitness costs associated with asynchrony either early or late.

This study aims to quantify the relative magnitudes of the fitness costs of asynchronous gypsy moth hatch relative to leaf emergence. Specifically, I sought to quantify fitness costs of hatching late relative to leaf emergence by rearing larvae on early- and later-phenology foliage of three common host tree species for gypsy moth in eastern US deciduous forests, and measuring percent survival and pupal mass. A second component of this Chapter addresses the consequences of hatching prior to budbreak. I quantified the ability of neonates to survive starvation when held at a suite of temperatures representative of spring conditions across the invasive range. I expected that 1) larvae initiating feeding in synchrony with leaf emergence will perform better than those hatching later and 2) when fed maturing foliage, larvae reared on oak will perform better than on foliage of less suitable hosts. Reflecting hatch prior to leaf emergence, 3) survival duration for larvae in the absence of food will decrease as temperatures increase, with relatively short survival at warm temperatures more typical of spring near the southern range limits and 4) populations from regions where starvation is more likely will exhibit improved survival in the absence of food.

Materials and Methods

Phenological asynchrony feeding experiment

A two-way factorial feeding phenology experiment was conducted to quantify the fitness costs of hatching late relative to leaf emergence. In 2014, larvae were reared on early- and late-phenology foliage of three common eastern US deciduous host trees (beech *Fagus grandifolia* Ehrh., red maple *Acer rubrum* L., northern red oak *Quercus rubra*) in order to quantify fitness costs of hatching late relative to budburst. A single site was used in central NY (43.080624, -75.981274). Larvae were locally collected at the site (NY population: Table 5.1) and had been reared through at least one generation in captivity prior to use in the experiment.

Cohorts of eggs of the NY population were removed from cold storage and hatched at two time intervals, early and late (11 days apart) relative to local bud burst. Larvae (n = 30/bag) were reared in spun polyester mesh bags (n = 3/combination) on whole branches of either beech, red maple, or northern red oak. Three individual trees of each host species were used for the experiment, and each tree had one early-phenology bag and one later-phenology bag. Bags were moved to new branches as often as needed to ensure constant availability of foliage. Bags were checked every 2-3 days for larval survival. Pupae were removed every other day, weighed on an electronic balance, then placed individually in capped plastic cups and held at ambient temperature outdoors until adult emergence. Date of emergence and sex of adults was recorded.

Starvation survival duration

In order to assess the fitness consequences of hatching early relative to leaf emergence, I used a two-way factorial growth chamber experiment in which larvae (n = 45/combination) from eight wild populations encompassing the latitudinal breadth of the invasive range of gypsy moth were held without food under five temperature treatments, and the duration of survival was measured in days. Five experimental maximum temperatures were selected to represent the range of field temperatures larvae hatching during the 2018 egg phenology experiment would have experienced if they had hatched up to 14 days earlier than the date of first hatch at each site, based on temperature ranges recorded at each site (NY [6.9–22.1˚C], MLBS [8.4–24.9˚C], RVA [2.0–22.4˚C]). Gypsy moth larvae can survive 3–4 weeks at temperatures < 10˚C, as they become metabolically inactive (Keena & Shi, 2019). Therefore, temperatures < 10˚C were not used. Percival environmental chambers were set to run daily maximum (11˚C, 14˚C, 17˚C, 20˚C, 23˚C) temperatures for 15 hrs while the lights were on (photophase), and the minimum (10˚C) temperature for 9 hrs while lights were off (scotophase), corresponding to a 15:9 (L:D) photoperiod, as I felt that this was a more realistic spring temperature regime than a single constant day and night.

Populations used in the 2020 larval survival duration experiment are listed in Table 5.1. Each population had been reared through at least one generation in captivity under common conditions to minimize any maternal effects associated with the source collections. Within each population, egg masses (n = 29–60, depending on population) laid in 2019 were broken apart to release individual eggs and hand mixed to maximize genetic variation and then overwintered outdoors in Kirkville, NY, until mid-January 2020, when they were brought indoors to initiate hatch. Eggs were held at room temperature and checked four times daily for hatch. Newly hatched larvae were placed individually into labeled 1.5 mL microcentrifuge tubes and held at 4˚C until the beginning of scotophase (10˚C), when they were placed in the appropriate temperature treatment. Larvae (n = 45 per combination) were checked twice daily, 12 hr apart, for mortality by gently prodding with a fine-hair paintbrush. Larvae that did not move were considered dead. Survival duration was recorded in increments of 0.5 days, with integers indicating larvae were dead at the PM check. Larvae were removed from growth chambers for no more than 20 minutes at each mortality check. Digital thermometers were kept in each growth chamber for the duration of the experiment to measure temperature variation from set levels. Relative humidity was maintained between 50–70%. Photophase temperatures did not vary by > 1˚C for any temperature treatment except the 11˚C growth chamber, which averaged 9.4˚C.

In order to assess the effects of population source on survival using a continuous metric, each population source location was described using mean annual temperature (Table 5.1). Mean annual temperature for each US population source coordinates were calculated using 30-year climate normals (1981 – 2010) from PRISM (PRISM Climate Group, 2012). Historical temperature data for Quebec City, Quebec, Canada (QC93) coordinates were acquired from Canadian Climate Normals (1981–2020; Government of Canada, 2019).

Statistical analyses

All statistical analyses were completed in R (version 3.5.2, R Core Development Team 2018). Significance level for all analyses was $\alpha = 0.05$. For the 2014 feeding experiment separate ANOVAs were used to assess the effects of larval host tree species, phenology, and host species×phenology on pupal mass and larval survivorship. Due to developmental differences between males and females in later instars in this sexually dimorphic species, each sex was analyzed separately for pupal mass. However, data were pooled for larval survival. For the feeding experiment, if ANOVAs indicated significance, then pairwise Tukey HSD tests were used to determine which treatments differed from each other.

Effects of temperature, source climate, and temperature×source climate on the duration of larval survival when held without food was tested using linear mixed effects model (package *lmerTest*; Kuznetsova *et al*., 2017) with survival in days as the response variable. Date of hatch in ordinal days was included as a random effect to account for pseudoreplication. Degrees of freedom were calculated using Satterthwaite approximation due to unequal sample sizes. The proportion of variation in the response variable explained by predictors in the final models was estimated using the function *r.squaredGLMM* (package *MuMIn*; Barton & Barton, 2019).

Results

In the 2014 feeding phenology experiment, tree host had a significant effect on development time in days for males ($F_{2,12} = 91.86$, $p < 0.0001$) and females ($F_{2,12} = 139.35$, $p < 0.0001$). For both sexes, development was shortest on oak, intermediate on maple, and longest on beech. Host also had a significant effect on pupal mass for both males ($F_{2,12} = 14.65$, $p = 0.0006$) and females ($F_{2,12} = 58.67$, $p < 0.0001$; Fig. 5.1). As expected, larvae reared on oak produced larger pupae than the other two host species, regardless of sex and phenology. Beech generally produced larger individuals than maple, except in the case of females reared on early maple foliage, which were larger than females reared on early and late beech foliage. Later-phenology foliage significantly reduced female ($F_{2,12} = 30.10$, $p < 0.0001$), and male pupal masses ($F_{2,12} = 34.37$, $p < 0.0001$) and female ($F_{2,12} = 41.04$, $p < 0.0001$) but not male development time. There were significant host ($F_{2,12} = 56.97$, $p < 0.0001$) and phenology ($F_{1,12} = 5.67$, $p = 0.0347$) effects on survival. Larvae fed on oak foliage showed significantly higher survival rates than those fed beech or maple regardless of phenology (Fig. 5.2).

In the starvation experiment, temperature had a significant effect on survival in days ($F_{1, 1704}= 401.80$, $p < 0.0001$; Table 5.2). Survival duration decreased with increasing temperature (Fig. 5.3). Neither source climate nor the interaction term had significant effects on survival duration. Mean ± SE survival duration in days for larvae pooled across populations was 14.29 ± 0.31, 9.90 ± 0.17, 7.61 ± 2.21, 5.39 ± 0.08, and 4.86 ± 0.08 for 9.4˚C, 14˚C, 17˚C, 20˚C, and 23˚C, respectively (Table 5.3).

Discussion

The feeding experiment, which simulated later hatch relative to leaf emergence, corroborated results of previous phenology-based feeding experiments for gypsy moth, including those of Raupp *et al*. (1988) and Hunter and Elkinton (2000), in which early-phenology foliage generally supported superior development compared to later-phenology foliage, regardless of host species. In the present study, larvae feeding on northern red oak had higher survival, greater pupal mass, and faster development than those feeding on beech or red maple, regardless of phenology. This is similar to results reported by Hunter and Lechowicz (1992) which indicated that oak remains a more suitable host later into the growing season compared to other common eastern deciduous trees. Larvae tended to perform better on early-season maple and beech foliage than on late-season foliage of the same host; however, larvae feeding on early and late oak foliage performed equally well. Consequently, the pressure to shift hatch timing towards local bud burst may be reduced in habitats with a high proportion of oak. This may have important implications for gypsy moth as it approaches the northern limits of the genus *Quercus* in the upper Midwest and adjoining Canada. Indeed, the northern limit of gypsy moth in eastern North America roughly coincides with that of red oak and has moved little in two decades (Régnière *et al*., 2015).

Hatching prior to leaf emergence increases the likelihood of starvation for spring feeding Lepidoptera (Fuentealba *et al*., 2017; Gray & Keena, 2019; Keena & Shi, 2019). Although our simulations did not account for temperature fluctuations that would occur in nature, they do provide a valid estimate for effects of temperature on survival. Under spring conditions typical of much of the current gypsy moth range, larvae that hatch before budburst are unlikely to survive for longer than two weeks without food. As expected, survival decreased precipitously with increasing temperatures, a likely scenario in the southeastern portion of the current range. Results of this study align with those of a recent study assessing larval survival for *Lymantria* populations across Eurasia and North America under temperatures ranging from 1–30˚C (Keena

& Shi, 2019), which found increasing thermal regimes above 5˚C reduced survival time for all populations.

Starvation has large effects on fitness, thus natural selection should favor genotypes that initiate hatch in synchrony with leaf emergence of favored host trees. However, leaf emergence timing varies locally among tree genotypes both within and among species, and interannually so that a favored genotype in a given year may not be optimal in other years (Lechowicz, 1984; Rousi & Pusenius, 2005). Indeed, variation in leaf emergence may act to maintain variability in hatch within and among egg masses, which functions as a bet-hedging strategy to ensure at least some offspring hatch synchronously with leaf emergence in any given year. Such inherent variation may have contributed to the success of gypsy moth as it spread, even if local adaptation has shifted the mean hatch responses to temperature. Although starvation from early hatch appears to be more likely near the southern limits of gypsy moth, we found no evidence that these populations were more physiologically adept at enduring starvation.

The fitness consequence of hatching early relative to leaf emergence (i.e., starvation and death) is greater than the fitness consequences of hatching late (i.e., reduced fecundity and slower development). Gypsy moth is a generalist herbivore capable of feeding and developing on many genera of deciduous hardwoods (Liebhold *et al*., 1995). In the absence of oak foliage for early hatching individuals, larvae may use other highly suitable host genera including *Betula* and *Populus* that tend to leaf out earlier than oaks (Lechowicz, 1984), which would reduce selective pressures on hatch timing. Nonetheless, adaptive change in gypsy moth hatch timing is evident (Chapter 4). It is unclear whether this is being driven largely by delayed hatch in southern populations, advanced hatch in northern populations, or some combination of both. These results suggest that the fitness costs of asynchronous hatch with oak leaf emergence are large enough that they override the phenological flexibility provided by the broad array of hosts available to this generalist herbivore, as well as barriers to adaptation including founder effects and apparently high levels of gene flow from frequent movement of various life-stages through human-mediated transport (Bigsby *et* al., 2011; Wu *et al*., 2015). The apparent importance of oak spring phenology in influencing hatch timing of southern gypsy moth populations may be partially attributable to oak canopy dominance in mid-Atlantic deciduous forests (Braun, 1951; Küchler, 1965).

Spring-feeding Lepidoptera are dependent on young foliage for optimal development (van Asch & Visser, 2007), and as a result, are highly sensitive to phenological mismatch between initiation of larval feeding and leaf emergence of preferred hosts (van Asch & Visser, 2007). Fluctuations in the degree of synchrony between emergence of larvae and their preferred host foliage is thought to be an important determinant of population growth rates and outbreak occurrence for spring-feeding lepidopterans (van Asch & Visser, 2007; Jepsen *et al*., 2009). The fitness consequences of asynchrony between larval hatch and leaf emergence of preferred hosts has been suggested as a likely mechanism driving selective pressures for hatch timing in several other spring-feeding Lepidoptera including *Opheroptera brummata* (*L.*)*, Eppirita Aumnuata* Borkhausen, and *Agriopis aurantauria* Hubner (Jepsen *et* al., 2011; Fält-Nardmann *et al*., 2016), all of which display latitudinal clines in hatch timing similar to what I found for gypsy moth (Chapter 4).

Climate change is expected to cause widespread disruptions to climate-linked phenomena across diverse taxa (Parmesan & Yohe, 2003; Zhang *et al*., 2007; Thackeray *et a*l., 2016; Forrest, 2016; Cohen *et al*., 2018; Visser & Geinapp, 2019), including phenological synchrony between leaf emergence and hatch timing for gypsy moth. The ability to adapt to local conditions has important implications for gypsy moth establishment success and population growth rates. This study provides empirical support for the influence of phenological asynchrony between gypsy moth hatch timing and oak leaf emergence as a mechanism for driving local adaptation in hatch timing. Understanding the underlying environmental drivers of physiological adaptation in this invasive defoliator has important implications for predicting future range limits and informing management decisions about where to allocate resources and monitoring.

Acknowledgements

Thank you to Emily Booth, Shianne Lindsay, and volunteer Kayla Elkhay for providing assistance with checking for larval mortality in the growth chamber experiment. This research was funded by National Science Foundation Macrosystems Biology (Grant 1702701 to Kristine Grayson, Sal Agosta, and Dylan Parry), Gypsy Moth Slow-the-Spread Foundation, Inc (Grant Number 19-01-13) to DP) and the USDA Forest Service, Northern Research Station (Grant 11-JV11242303-053 to DP).

Table 5.1 Source locations for the eight wild populations used in the feeding phenology and starvation experiments. Source climate represents the annual mean temperature calculated using 30-year (1981–2010) climate normals. The last column indicates which experiment(s) each population was used in for the current study. Populations are listed latitudinally, from south to north.

Population	Locality	Source climate (˚C)	Experiment
Hatteras, NC (NC 2)	35.250371°N, 75.581311°W	17.3	Larval starvation
Currituck, NC (NC 1)	36.449125˚N, 76.024672˚W	15.9	Larval starvation
Wolf Creek, VA (WOLF)	37.28434082°N, 80.90811339°W	11.7	Larval starvation
MA 1	42.40502°N, 71.28713°W	9.5	Larval starvation
Kirkville, NY (NY)	43.080624˚N, 75.981274˚W	8.9	Larval starvation, Feeding phen.
Bayfield, WI (BAY)	46.812634°N, 90.820414°W	4.5	Larval starvation
Quebec City, QC (QC93)	46.9089631˚N, 70.8061075˚W	3.6	Larval starvation
Ely, MN (ELY)	47.8897227˚N, 91.846133˚W	3.3	Larval starvation

Table 5.2 ANOVA table for mixed linear effects models with survival in days as the response variable, temperature and source climate of each population as fixed effects, and hatch day in ordinal days as a random effect.

Variable	**MS**	**df**	**F**	**p**	**Estimated model R_2**
Temperature	4289.5	1, 1704	401.80	< 0.0001	0.50
Source pop. climate	9.6	1, 1705	0.90	0.34	
Hatch day				0.003	

Table 5.3 Survival in days for each population×temperature combination.

Population	Temp. (˚C)	Survival (days) ± SE	Range (˚C)	n
NC1	9.4	14.26 ± 0.62	5.0 – 22.5	43
NC2	9.4	12.35 ± 0.88	0.5 – 22	39
WOLF	9.4	16.36 ± 1.01	3.0 – 27.0	39
MA1	9.4	14.77 ± 0.76	5.5 – 24	42
NY	9.4	15.03 ± 0.79	0.5 – 23	45
QC93	9.4	13.80 ± 0.89	1.0 – 24.5	41
BAY	9.4	13.40 ± 1.13	1 – 25	39
ELY	9.4	14.24 ± 0.81	2.5 – 24.5	40
NC1	14	8.90 ± 0.52	0.5 – 13.5	45
NC2	14	9.02 ± 0.55	2.0 - 15	47
WOLF	14	11.55 ± 0.49	2.0 – 17.5	43
MA1	14	9.72 ± 0.52	2.5 – 16.5	45
NY	14	9.79 ± 0.40	2.5 – 14.5	43
QC93	14	9.48 ± 0.40	1.0 – 13.0	44
BAY	14	10.88 ± 0.35	3.0 – 16.5	45
ELY	14	9.99 ± 0.48	1.5 – 15.5	39
NC1	17	6.92 ± 0.32	0.5 – 10.5	45
NC2	17	7.17 ± 0.30	2.0 – 10.0	44
WOLF	17	9.15 ± 0.31	2.0 – 13.0	46
MA1	17	7.26 ± 0.42	0.5 – 12.0	44
NY	17	7.33 ± 0.36	1.5 – 10.5	43
QC93	17	8.10 ± 0.22	4.5 – 11.0	42
BAY	17	7.24 ± 0.30	2.5 – 12.0	46
ELY	17	7.66 ± 0.31	1.5 – 12.0	41
NC1	20	4.93 ± 0.22	0.5 – 9.5	38
NC2	20	5.20 ± 0.21	1.5 – 7	44
WOLF	20	6.30 ± 0.24	1.0 – 9.5	45
MA1	20	4.88 ± 0.29	0.5 – 9.0	39
NY	20	5.10 ± 0.18	3.0 – 7.0	42
QC93	20	5.43 ± 0.15	2.0 – 7.0	45
BAY	20	5.55 ± 0.26	1.5 – 11.0	44
ELY	20	5.56 ± 0.22	1.5 – 8.5	45
NC1	23	5.14 ± 0.14	2.5 – 7.5	43
NC2	23	4.41 ± 0.24	0.5 – 7.0	38
WOLF	23	5.14 ± 0.25	1.0 – 10.5	45
MA1	23	5.02 ± 0.21	0.5 – 7.0	43

NY	23	4.22 ± 0.26	0.5 – 6.5	44
QC93	23	4.89 ± 0.15	2.5 – 7.0	45
BAY	23	5.02 ± 0.26	0.5 – 9.0	42
ELY	23	4.97 ± 0.22	1.0 – 8.5	43

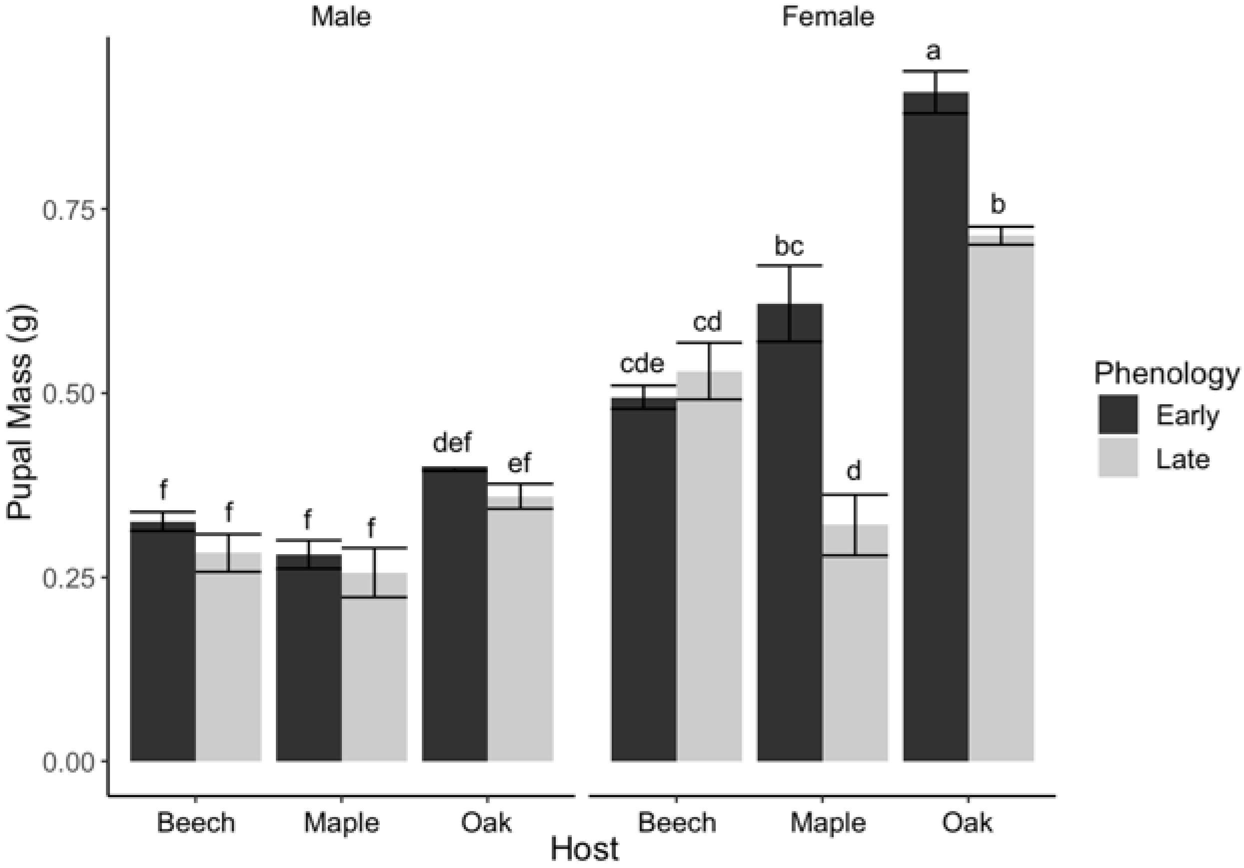


Figure 5.1 Pupal mass and total development time for male and female gypsy moth reared on early- and later-phenology foliage from three host species. Bars and error bars represent mean ± SE. Letters above bars indicate significant differences among combinations for each sex.

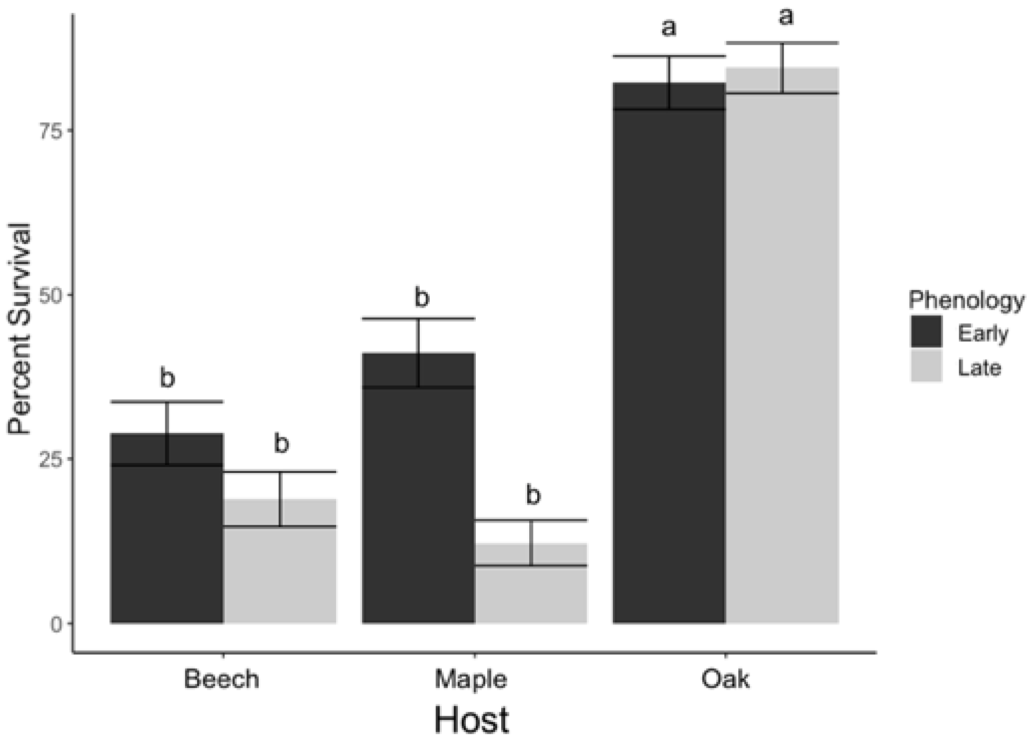


Figure 5.2 Survival for all moths for each combination of host species and phenology in 2014. Bars and error bars represent mean ± SE. Letters over bars indicate significant differences among hosts.

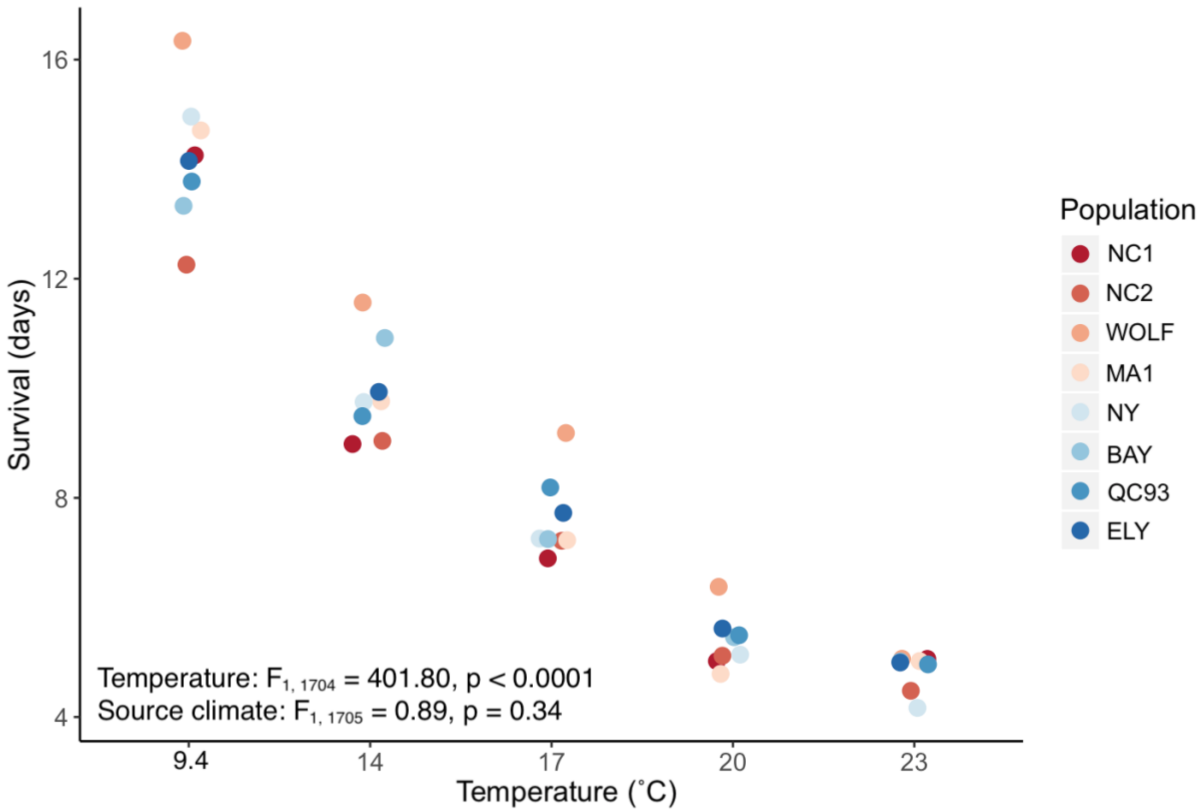


Figure 5.3 Mean survival time in days for each population/temperature combination. Populations are color coded from north to south with higher latitudes in blue and lower latitudes in red.

Chapter 6: Conclusion and Project Synthesis

Conclusions

Deviations from optimal biotic (e.g., host availability, phenology, etc) and abiotic (e.g., climate) environmental characteristics for a particular organism can result in reduced fitness, negative population growth, and range retraction or stasis (van Asch & Visser, 2007; White, 2008; Tobin *et al*., 2014b; Banahene *et al*., 2018). Thus, range limits are determined intrinsically by physiological limitations such as thermal tolerances (Sinclair *et al*., 2012; Tobin *et al*., 2014; Lancaster, 2016) and extrinsically by available habitat and resources, such as host plants (Morin *et al*., 2005; Régnièr *et al*., 2008). For invasive species, ranges are difficult to predict as species encounter environmental conditions that differ from those they evolved with in their native habitats. Furthermore, founder effects can be pronounced, thus limiting the genetic capacity to respond to novel environments (Dlugosh & Parker, 2008; Hill *et al*., 2011). Nonetheless, range expansion into novel environments can be quite rapid, facilitated by selection on beneficial mutations leading to increased fitness and population growth (Lee, 2002; Sinclair *et al*., 2012; Moran & Alexander, 2014).

Since its introduction to North America a century and a half ago, the gypsy moth invasion front has expanded to encompass an area stretching from Quebec to North Carolina, and west to northern Minnesota, inhabiting diverse climatic and biotic gradients (Grayson & Johnson, 2018). Despite the overwhelming success of gypsy moth, a pronounced range retraction over the past 20 years from its southern maximum, apparently due to an increase in supraoptimal temperatures (Tobin et al. 2014), has cued interest in climatic limitations at its range margins, and potential adaptive shifts in physiology and morphology (e.g., Thompson *et al*., 2017; Friedline *et al*., 2018).

My dissertation presented novel research addressing fitness of gypsy moth from three different perspectives. In chapter 2, I demonstrated that phenology, composition of forests, and population density all left signatures on gypsy moth males through their effect on wing morphology. As females are flightless, flight capacity of males is critical, especially at very low population densities along the leading edge of the invasion front. Such observations could refine predictions about population establishment beyond simple present/absence data currently gleaned from pheromone traps. Chapter 3 focuses on male flight capacity as a function of male size. For this component of my dissertation, I have pioneered the use of digital fixed arm flight mills for male gypsy moth and for the first time, recorded a series of flight metrics likely related to male success. This chapter further suggests that male gypsy moth fitness may have important implications for mate-finding success and establishment, especially in low density populations. Finally, in Chapter 4, I demonstrated that overwintering and spring climate has profound effects on gypsy moth hatching and that selection on populations along a latitudinal gradient has driven adaptive shifts in hatch timing at the population level. I provide a mechanistic basis for this adaptive change in hatch timing through two empirical experiments. In the following paragraphs, I will summarize the major findings of these three data chapters.

My analysis of the utility of pheromone-trapped male wing morphometrics in predicting habitat quality relies on easily measurable proxies for body size which reflect fitness consequences resulting from interspecific and phenological variation in host quality, and population density. Consistent with patterns previously reported for gypsy moth and other spring defoliators, moths from endemic populations exhibit a consistent seasonal decline in body size, which likely reflects the phenologically driven reduction in host quality as leaves mature (Raupp *et al.*, 1988; DuMerle & Cornic, 1991; Rhainds, 2015; Tobin *et al.*, 2016). Contrary to our expectations for seasonal pattern in wing length in high-density populations, which predicted an early increase in wing length to a mid-season peak, followed by reduction in wing length reflective of seasonally reduced host quality, there was an overall seasonal reduction in wing length consistent with the pattern seen in endemic populations. Though it is important to note that late-instar mortality due to a fungal epizootic could have partially obscured the full impact of population density on male wing length, these data suggest that the fitness costs of phenological reduction in host quality outweigh even the costs of density-related intraspecific competition. Additionally, males originating from endemic populations are significantly larger on average than conspecifics originating from high density populations, though there is overlap in wing lengths between the two groups.

Gypsy moth larvae reared on oak foliage develop more quickly, have greater survival, and increased pupal mass compared to larvae reared on other hosts commonly encountered in North American forests (Raupp *et al.*, 1988; Hunter & Lechowicz, 1992). Therefore, a primary hypothesis in the assessment of wing length as an informative tool for gypsy moth management efforts was that males originating from high-quality oak dominated/co-dominated forests would be larger than those originating from non-oak forests. While the results for our analyses that assessed the effect of binary forest type (oak dominated/co-dominated vs oak absent) supported this hypothesis, the effect of forest type as a continuous variable, measured either as oak basal area or our novel estimate of the impact of oak and non-oak hosts on habitat quality, FQI, did not have a significant effect on wing length.

As capital-breeders, gypsy moth accumulate all resources available for both reproduction and mate-finding during larval feeding. In female gypsy moth, fecundity is strongly correlated with pupal mass (Hough & Pimentel, 1978, Faske *et al.*, 2019); however, the effect of male gypsy moth body size on any aspect of fitness has not previously been estimated. I expected that flight capacity would increase with body size. Using fixed-arm flight assays, I assessed the relationship between male gypsy moth body size, as well as several morphological and physiological characteristics, and flight capacity and propensity, to link variation in body size resulting from environmental variation to measurements of a proxy for male fitness, flight capacity and propensity. Using linear mixed effect models, I found that wing length, a proxy for body size, is the most important predictor for total distance flown and maximum speed. Total distance was also significantly positively impacted by relative thorax mass. Total distance flown and maximum speed were both significantly negatively impacted by forewing aspect ratio. By estimating flight propensity as the proportion of tactile stimuli that resulted in sustained flights, I was also able to show that flight propensity is positively exponentially impacted by male wing length. Larger males could have a mate-finding advantage over smaller males in low density populations, which has important implications for adaptation at range margins, as well as establishment success in a range-expanding insect.

In populations that have adequate genetic diversity, the fitness consequences of maladaptation to local environmental conditions eventually result in adaptive shifts. In my reciprocal transplant experiment for egg hatch phenology, I demonstrated the occurrence of a latitudinal adaptive shift in hatch timing, in which northern populations require relatively fewer degree days to initiate hatch than mid-latitude populations, and southern populations require relatively more. The pattern of hatch across populations was consistent across the four sites; northern populations consistently hatched first, followed by mid-latitude populations, then southern. By monitoring oak budburst phenology at the northern and southern site, I showed that the respective shifts at either range extreme effectively reduce asynchrony between hatch and oak bud burst. Using empirical data from a starvation study and a feeding study in which larvae were intentionally hatched after budburst, then fed mature foliage, I suggested that the fitness costs incurred by hatching early or late relative to budburst could have provided sufficient selective pressure to result in the observed shifts in hatch requirements.

Broader Impacts, Limitations, and Future Directions

Male morphometrics as a management tool

Though several previous studies have associated variation in male gypsy moth wing length with population density (Bellinger *et al*., 1990; Carter *et al*., 1991; Tobin *et al*., 2016), the data presented in Chapter 2 were the first to link variation in wing length to differences in habitat quality. Wing morphometrics have been linked to habitat quality for other defoliating Lepidoptera, including tree cover (Dumerle & Cornic, 1991; Rhainds, 2015), and may indicate that wing length, or other easily-measured correlates of body size, could be used more generally as a tool for estimating habitat quality for other capital-breeding Lepidoptera. Future studies that assess the effect of forest composition, or other parameters of habitat quality, on wing length can determine whether morphometrics of trapped capital-breeding Lepidoptera can be used more broadly to inform management and conservation efforts.

A limitation on interpreting the non-significant relationship between oak basal area and male wing length is the mosaic forests where I conducted the studies. In central NY, oak dominated forests are uncommon and most are likely fragments that survived the extensive clearing and conversion to agriculture that dominated post-colonial New York. Oak forests in this region are embedded in much larger areas of maple dominated forests and male moths originating from these lower quality stands likely dilute the pool of larger males originating from oak. Despite the lack of significance, there were discernable differences between mean wing length for traps in oak and non-oak forests, where wing length from traps in oak forests were consistently larger than non-oak forests in early flying males. In a mark and recapture study, Elkinton and Carde (1980) found that the majority (95%) of dispersing males were caught at distances < 800 m when traps were placed every one or four kilometers apart. However, there is evidence that male moths are capable of dispersing much farther (Tobin & Blackburn, 2014; Tobin *et al.*, 2016). Future studies that use pheromone-baited traps to estimate habitat based differences among populations of gypsy moth should ensure that habitat patches are at least 800 m in diameter, if not larger.

The gypsy moth invasion front is characterized by stratified dispersal (Sharov & Liebhold, 1998), a combination of short distance diffusion via larval ballooning and long distance human-mediated dispersal (Doane & McManus, 1981; Bigsby *et al.*, 2011; Tobin & Blackburn, 2014). Isolated populations that establish ahead of the invasion front eventually merge with the expanding front, effectively increasing the invasion rate (Tobin & Blackburn, 2014). However, low-density populations are highly susceptible to Allee effects and stochastic events, and many fail to establish (Keitt *et al.*, 2001; Tobin *et al.*, 2009). Therefore, identifying and eradicating isolated populations that are most likely to successfully establish is a critical component of the Slow-the-Spread (STS) management efforts (Tobin & Blackburn, 2007; Tobin & Liebhold, 2011). Currently, the STS decision algorithm uses enumeration trap data to estimate population density and select areas for management efforts (Tobin & Sharov, 2007). Increased fitness at the population level corresponds to greater fecundity, and increased population growth rate (Hough & Pimentel, 1978; Bellinger *et al.*, 1990), and, importantly, variation in population density lags behind variation in fitness and fecundity. Therefore, monitoring a proxy for fitness, e.g., pheromone-trapped male wing length, could help inform predictions for successful establishment if mean wing length exceeds the expected value given estimates of population density and/or habitat parameters. Additionally, emigration of males to low-density populations can help overcome Allee effects and increase the likelihood of establishment (Tobin *et al*., 2009; Tobin & Blackburn, 2014). Monitoring seasonal patterns in wing length for low-density isolated populations could identify areas where long-distance dispersal is occurring (e.g., Tobin *et al*., 2016), and inform management decisions. Currently, however, moths caught in STS traps are collected only once per season and would not capture seasonal patterns, and visiting traps more frequently is costly (Sharov *et al*., 2002).

Male flight capacity

Though the relationship between female Lepidoptera body size and fecundity is directly related to fitness, is easy to estimate, and has long been known for gypsy moth (Hough & Pimentel, 1978; Faske *et al*., 2017), male fitness is more difficult to estimate, and is done less often. Male flight capacity, which can contribute to mate-finding ability, population growth, and establishment success (Contarini *et al*., 2009; Tobin *et al*., 2009), is a proxy for fitness. The relationship between insect body size and flight capacity varies interspecifically (Shirai, 1995; Elliott & Evenden, 2009; Evenden *et al*., 2014; Sappington & Burks, 2014; Lopez *et al*., 2017); increase in body size may or may not benefit male flight depending on the specific insect. Previous studies on gypsy moth flight capacity had assessed correlative relationships between morphology and flight ability for females of different *Lymantria* spp. including Asian gypsy moth (Shi & Keena, 2015; Yang *et al*., 2017), but my study on male European gypsy moth flight was the first of its kind. Using fixed-arm flight mills (Minter *et al*., 2018; Naranjo, 2019), I demonstrated that body size is the most important factor contributing to male gypsy moth flight capacity, and that various morphological characteristics (e.g., forewing aspect ratio and relative thorax mass) also significantly affect the distance and speed at which male moths fly.

Though the flight mill experiment demonstrated a significant relationship between body size and male gypsy moth flight capacity and propensity, and can be used to form hypotheses related to evolutionary ecology and population ecology, it cannot be used to estimate flight distances for wild moths. Gypsy moth flight path is naturally zig-zagging as males attempt to locate pheromone plumes of virgin females (Doane & McManus, 1981), whereas flight mills force a straight flight path. Despite my effort to reduce friction to a minimum using opposite-facing magnets and low-friction plastics in our design, tethered insects still must push the weight of the arm to fly forward. This extra energy expenditure may however be counterbalanced by the lift produced by the arm. In short, it is not possible to extrapolate the results reported here to estimate absolute natural flight distances. Estimates of flight capacity using wind tunnels or marked-release studies are more appropriate for predicting natural dispersal distances. Nonetheless, the metrics obtained are likely to correlate with at least some metrics of natural flight capacity.

In my study, relative thorax mass had a significant effect on the total distance flown, where moths with a greater proportion of body mass allocated to the thorax had increased flight ability. In insects, flight musculature is found in the thorax (Marden, 2000). Small amounts of energy for flight can be stored directly in the flight muscle, while most of it is stored as lipids in the fat body (Beenakkers *et al*., 1984), which is located within both the thorax and abdomen. This study was not designed to disentangle the specific underlying physiological mechanisms contributing to increased flight capacity; measuring relative thorax mass cannot rule out either allocation to flight musculature or lipid stores as a mechanism allowing for greater flight ability. Additionally, other underlying physiological mechanisms such as nutrient transport, increased enzyme activity, or respiration rate can affect flight capacity (Beenakkers *et al*., 1984; Haag *et al*., 2005; Lebeau *et al*., 2016). Future studies could implement more fine scale physiological methods to measure lipid content, muscle mass, respiratory rate, or enzyme activity for male gypsy moth to further inform the underlying mechanisms contributing to greater size-dependent variation in flight capacity.

Size-dependent variation in flight capacity for male gypsy moth could have important implications for selective pressures that may be acting on male body size, especially in low density populations where flight ability could affect mate-finding ability and establishment success (Tobin *et al*., 2009). In low density populations at the range front, Allee effects become critical for establishment and persistence of populations. Failure to mate is an important driver of failure in these populations (Tobin *et al*. 2009). Other organisms have been documented to have adaptive variation in dispersal morphology, physiology, and behavior both to increase dispersal ability at range margins, and to increase mate-finding ability (Langolotto & Denno, 2001; Haag *et al*., 2005; Phillips *et al*., 2006; Phair *et al*., 2018). Future studies could take advantage of common garden studies, which disentangle genetic and environmental effects on body size, or sample morphological traits along an establishment gradient, as Phillips *et al*. (2006) did for the range-expanding invasive cane toad in Australia, to determine whether gypsy moth body size or flight morphology has evolved in low-density populations at or behind the invasion front.

Two aspects implemented during my flight mill study improved upon existing methodology and could be used in future experiments. First, my novel flight mill design relied on readily available and fairly cheap off the shelf materials and software, which make it accessible to scientists who wish to use the technology at low cost. Details for the coding and schematics used in the electrical components of the flight mill design will be included in my online appendices upon publication. Second, the novel use of agar-based gypsy moth diet with varying quantities of protein, but constant concentrations of other dietary components, allowed us to test the effect of body size alone on flight capacity while controlling for other factors. This method could be used by future experimenters that aim to examine the effect of body size on fitness parameters without being influenced by foliage quality.

Adaptive shifts in hatch timing along a latitudinal cline

Much of the recent focus on climate-linked adaptation in gypsy moth has been on populations at the southern range limit, where a pronounced range retraction is occurring, likely due to the fitness costs of an increase in supraoptimal temperatures (Tobin *et* al., 2014; Banahene *et al*., 2018). Climate-linked shifts in phenotypic traits such as thermal tolerances during the larval and pupal stages in southern populations have been identified (e.g., Thompson *et al*., 2017; Banahene *et al*., 2018; Faske. 2018). However, few studies have assessed the direction and magnitude of adaptive shifts occurring across the latitudinal range of gypsy moth (but see Friedline *et al*., 2019), even though thermal tolerances are a critical component of range limits in this species (e.g., Gray, 2004; Régnière *et al*., 2008). Thus adaptation to local climate at range margins has important implications for continued expansion or contraction of gypsy moth range under both contemporary and future climates.

The results of my egg hatch phenology study indicate that rapid adaptation to local climatic conditions is occurring in gypsy moth populations across its latitudinal range. Specifically, an apparent adaptive shift in hatch phenology at both the northern and southern range extremes has occurred. Changes in the phenology of egg hatch are likely adaptive as the direction of change (faster in the north, slower in the south) serve to reduce the degree of asynchrony with bud break of host trees. I used a series of experiments to estimate the potential fitness costs of early and late asynchrony relative to budburst. A true estimate of fitness costs however, would require a carefully designed reciprocal transplant common garden experiment addressing the magnitude of asynchrony across several years and its effects on gypsy moth survival and reproductive output.

The pattern of variation in hatch timing across populations in my study may offer some insight into the underlying physiological mechanism resulting in the observed phenotypic shift in hatch phenology, which is likely the result of altered sensitivity to temperature during diapause. Gypsy moth diapause is divided into three stages, all of which are temperature-dependent, and are distinguishable by their disparate metabolic rates (Gray, 2004): pre-diapause, diapause, and post-diapause. Eggs used in my study were held under common conditions during pre-diapause (late-summer to early-fall) prior to allocation among the sites; therefore, the variation in hatch timing seen in the spring was not a result of pre-diapause conditions. Diapause is cold-dependent and transition to post-diapause occurs when diapause enzyme is depleted, following sufficient exposure to temperatures less than 5˚C (Tauber *et al.*, 1990; Gray *et al.*, 2001). Hatch occurs after the accumulation of sufficient warming degree days (Johnson *et al.*, 1983). Though the order of hatch across populations was consistent among the four sites, the study was conducted in a single year, and it is not clear if interannual variation in spring weather would alter the pattern significantly. Extensive work with the QC and NC egg masses across a range of natural and laboratory temperature envelopes indicates that the timing of egg hatch is a genetically fixed trait and research with a single strain of gypsy moth has shown that hatch timing can be selectively changed in relatively few generations through selection (Keena & Odell, 1994). The

South Carolina simulation used 20-year averages to approximate a southern spring, and fluctuated between day and nighttime temperatures using a smooth transition. Although the range of temperatures was realistic, the diurnal fluctuations and constrained lower limits depart from natural conditions, thus these results should be interpreted with caution.

In common garden experiments with Lepidoptera, diapause response to temperature is highly variable with northern cold-adapted populations in some species taking longer to terminate diapause than southern populations (Fu *et al*., 2015; Posledovich *et al*., 2015), and in other species less time is needed to terminate diapause in northern populations than southern populations (Masaki, 1965; Dambroski & Feder, 2007). If gypsy moth populations were adapting to local climate by altering diapause physiology, northern populations would be expected either to start with more diapause enzyme, or to deplete enzyme more slowly in response to cold temperature in order to compensate for the greater length of winter relative to lower latitudes. As a result, northern populations would take longer than southern populations to complete diapause in common garden experiments. In my study, however, northern populations consistently hatched earliest, followed by mid-latitude, then southern; therefore, it is likely that the observed pattern in hatch phenology is primarily a result of altered degree day requirements during post-diapause. Measuring diapause and post-diapause respiration rates for the populations used in my experiment could illuminate the underlying adaptive mechanism responsible in producing the observed adaptive shift in hatch timing (e.g., Gray *et al*., 2001; Linstad *et al*., 2019). Additionally, genetic analyses could reveal the genetic nature of the observed shifts (e.g., Faske, 2017; Friedline *et al*., 2019).

The data presented in the preceding chapters provide novel evidence of environmental characteristics driving patterns of adaptation, and has important implications for establishment and range expansion in an invasive forest pest. In context with the huge database of existing gypsy moth literature, which is the product of over a century of research, my contribution can help advise management efforts to limit the rate of gypsy moth invasion, inform new estimates of gypsy moth range limits, and offer insight for future research on adaptation in gypsy moth, dispersal ability, mate-finding, and establishment success. Additionally, the massive data base and fine scale resolution of occupied range and rate of expansion suggest that gypsy moth could be used as a model organism to understand environmental obstacles for establishment or range expansion during invasions. Many of the biotic and abiotic factors that lower fitness and limit establishment success for gypsy moth impact other insects, as well, and could either result in local extinction or stasis, or drive adaptive shifts that ultimately facilitate invasion and accelerate the rate of range expansion. These implications will be of particular importance as climate change and biological invasions continue to impact ecological processes and global biodiversity.

Literature Cited

Aalberg Haugen, I. M., & Gotthard, K. (2015). Diapause induction and relaxed selection on alternative developmental pathways in a butterfly. *Journal of Animal Ecology*, *84*(2), 464-472.

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1445), 739–745.

Agosta, S. J. (2010). Male body size and mating success and their relation to larval host plant history in the moth Rothschildia lebeau in Costa Rican dry forest. *Biotropica*, *42*(2), 201–207.

Alalouni, U., Schädler, M., & Brandl, R. (2013). Natural enemies and environmental factors affecting the population dynamics of the gypsy moth. *Journal of Applied Entomology*, *137*(10), 721–738.

Alleaume-Benharira, M., Pen, I. R., & Ronce, O. (2006). Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *Journal of evolutionary biology*, *19*(1), 203–215.

Allee, W. C. (1927). Animal aggregations. *The Quarterly Review of Biology*, *2*(3), 367–398.

Allee, W. C. (1938). The social life of animals. William Heinemann, London.

Allendorf, F. W., & Lundquist, L. L. (2003). Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, *17*(1), 24–30.

Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1743), 3843–3852.

Arnqvist, G., & Nilsson, T. (2000). The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*, *60*(2), 145–164.

Aukema, J.E., Leung, B., Kovacs, K., Chivers, C., Britton, K.O., Englin, J., Frankel, S.J., Haight, R.G., Holmes, T.P., Liebhold, A.M. and McCullough, D.G. (2011). Economic impacts of non–native forest insects in the continental United States. *PLoS one*, *6*(9), e24587.

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, *47*(1), 817–844.

Ayres, M.P. and MacLean, S.F., 1987. Development of birch leaves and the growth energetics of *Epirrita autumnata* (Geometridae). *Ecology*, *68*(3), pp.558–568.

Balanyá, J., Oller, J. M., Huey, R. B., Gilchrist, G. W., & Serra, L. (2006). Global genetic change tracks global climate warming in *Drosophila subobscura*. *Science*, *313*(5794), 1773–1775.

Bale, J. S. (1987). Insect cold hardiness: freezing and supercooling—an ecophysiological perspective. *Journal of Insect Physiology*, *33*(12), 899–908.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J. and Good, J.E. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global change biology*, *8*(1), 1–16.

Banahene, N., Salem, S.K., Faske, T.M., Byrne, H.M., Glackin, M., Agosta, S.J., Eckert, A.J., Grayson, K.L. and Thompson, L.M. (2018). Thermal sensitivity of gypsy moth (Lepidoptera: Erebidae) during larval and pupal development. *Environmental Entomology*, *47*(6), 1623–1631.

Barbehenn, R. V., Haugberg, N., Kochmanski, J., Menachem, B., & Miller, C. (2014). Physiological factors affecting the rapid decrease in protein assimilation efficiency by a caterpillar on newly-mature tree leaves. *Physiol Entomol*, *39*(1), 69–79.

Barbosa, P., Greenblatt, J., Withers, W., Cranshaw, W., & Harrington, E. A. (1979). Host-plant preferences and their induction in larvae of the gypsy moth, *Lymantria dispar*. *Entomologia Experimentalis et Applicata*, *26*(2), 180–188.

Barney, J. N., & Whitlow, T. H. (2008). A unifying framework for biological invasions: the state factor model. *Biological Invasions*, *10*(3), 259–272.

Barton, K., & Barton, M. K. (2019). Package 'MuMIn'. *R package version*, *1*(6).

Basler, D. and C. Körner. 2014. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiology*, 34: 377–388.

Bateson, W., & Mendel, G. (1913). *Mendel's principles of heredity*. University press.

Beenakkers, A. T., Van der Horst, D. J., & Van Marrewijk, W. J. A. (1984). Insect flight muscle metabolism. *Insect Biochemistry*, *14*(3), 243–260.

Bellinger, R. G., Ravlin, F. W., & McManus, M. L. (1990) Predicting egg mass density and fecundity in field populations of the gypsy moth (Lepidoptera: Lymantriidae) using wing length of male moths. *Environmental Entomology*, **19**, 1024–1028.

Berec, L., Kramer, A. M., Bernhauerová, V., & Drake, J. M. (2018). Density-dependent selection on mate search and evolution of Allee effects. *J Anim Ecol*, *87*(1), 24–35.

Berthiaume, R., 2007. Écologie évolutive des populations d'arpenteuse de la pruche. PhD Dissertation. University of Laval. 187 pp.

Bierl, B. A., Beroza, M., & Collier, C. W. (1970) Potent sex attractant of the gypsy moth: its isolation, identification, and synthesis. *Science*, **170**, 87–89.

Bigsby, K. M., Tobin, P. C., & Sills, E. O. (2011). Anthropogenic drivers of gypsy moth spread. *Biological Invasions*, *13*(9), 2077.

Bissoondath, C. J., & Wiklund, C. (1995). Protein content of spermatophores in relation to monandry/polyandry in butterflies. *Behavioral Ecology and Sociobiology*, *37*(6), 365–371.

Boag, P. T., & Grant, P. R. (1981). Intense natural selection in a population of Darwin's finches (Geospizinae) in the Galapagos. *Science*, *214*(4516), 82–85.

Boettner, G. H., Elkinton, J. S., & Boettner, C. J. (2000). Effects of a biological control introduction on three nontarget native species of saturniid moths. *Conservation Biology*, *14*(6), 1798–1806.

Bogdanowicz, S. M., Wallner, W. E., Bell, J., Odell, T. M., & Harrison, R. G. (1993). Asian gypsy moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. *Annals of the Entomological Society of America*, *86*(6), 710–715.

Boggs, C. L., & Gilbert, L. E. (1979). Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science*, *206*(4414), 83–84.

Bogich, T. L., Liebhold, A. M., & Shea, K. (2008). To sample or eradicate? A cost minimization model for monitoring and managing an invasive species. *Journal of Applied Ecology*, *45*(4), 1134–1142.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, *144*(3), 353–361.

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Funct Ecol*, *23*(1), 27–37.

Bontrager, M., & Angert, A. L. (2019). Gene flow improves fitness at a range edge under climate change. *Evol Lett*, *3*(1), 55–68.

Bradshaw, W. E., & Holzapfel, C. M. (2001). Genetic shift in photoperiodic response correlated with global warming. *Proceedings of the National Academy of Sciences*, *98*(25), 14509–14511.

Bradshaw, W. E., & Holzapfel, C. M. (2006). Evolutionary response to rapid climate change. *Science*, *312*(5779), 1477–1478.

Bradshaw, C.J., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.M., Simard, F. and Courchamp, F. (2016). Massive yet grossly underestimated global costs of invasive insects. *Nature Communications*, *7*, 12986.

Braun, E. L. (1950). Deciduous forests of eastern North America Blakiston Co. *Philadelphia, PA*.

Brewer, J. W., Capinera, J. L., Deshon, R. E., & Walmsley, M. L. (1985). Influence of foliar nitrogen levels on survival, development, and reproduction of western spruce budworm, *Choristoneura occidentals* (Lepidoptera: Tortricidae). *The Canadian Entomologist*, *117*(1), 23–32.

Brown, J. J., & Chippendale, G. M. (1974). Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *Journal of Insect Physiology*, *20*(7), 1117–1130.

Bruzzone, O. A., Villacide, J. M., Bernstein, C., & Corley, J. C. (2009). Flight variability in the woodwasp *Sirex noctilio* (Hymenoptera: Siricidae): an analysis of flight data using wavelets. *J Exp Biol*, *212*(5), 731–737.

Buckman, K. A., & Campbell, J. F. (2013). How varying pest and trap densities affect *Tribolium castaneum* capture in pheromone traps. *Entomologia Experimentalis et Applicata*, *146*(3), 404–412.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociol Methods Res*, *33*(2), 261–304.

Butlin, R. K., & Day, T. H. (1985). Adult size, longevity and fecundity in the seaweed fly, Coelopa frigida. *Heredity*, *54*(1), 107–110.

Butt, C., Quiring, D., Hébert, C., Delisle, J., Berthiaume, R., Bauce, E., & Royer, L. (2010) Influence of balsam fir (*Abies balsamea*) budburst phenology on hemlock looper (*Lambdina fiscellaria*). *Entomologia Experimentalis et Applicata*, **134**, 220–226.

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, *290*(5491), 521–523.

Campbell, R. W. (1975). *The gypsy moth and its natural enemies* (No. 381). US Department of Agriculture, Forest Service.

Campbell, R. W., & Sloan, R. J. (1977). Forest stand responses to defoliation by the gypsy moth. *Forest Science*, *23*(suppl_2), a0001–z0001.

Capinera, J. L., & Barbosa, P. (1976). Dispersal of first-instar gypsy moth larvae in relation to population quality. *Oecologia*, *26*(1), 53–60.

Carisey, N., & Bauce, E. (1997) Impact of balsam fir foliage age on sixth-instar spruce budworm growth, development, and food utilization. *Canadian Journal of Forest Research*, **27**, 257–264.

Carroll, S. P., Hendry, A. P., Reznick, D. N., & Fox, C. W. (2007). Evolution on ecological time-scales. *Functional Ecology*, *21*(3), 387–393.

Carter, M. R., Ravlin, F. W., & McManus, M. L. (1991) Changes in gypsy moth (Lepidoptera: Lymantriidae) fecundity and male wing length resulting from defoliation. *Environmental Entomology*, **20**, 1042–1047.

Casagrande, R. A., Logan, P. A., & Wallner, W. E. (1987). Phenological model for gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae and pupae. *Environmental entomology*, *16*(2), 556–562.

Cates, R. G. (1980). Feeding patterns of monophagous, oligophagous, and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. *Oecologia*, *46*(1), 22–31.

Catford, J. A., Jansson, R., & Nilsson, C. (2009). Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and distributions*, *15*(1), 22–40.

Charlton, R. E., & Cardé, R. T. (1990). Behavioral interactions in the courtship of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Annals of the entomological Society of America*, *83*(1), 89–96.

diapause induction and termination of the cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Journal of insect physiology*, *59*(9), 855–862.

Clark, J. S., Salk, C., Melillo, J., & Mohan, J. (2014). Tree phenology responses to winter chilling, spring warming, at north and south range limits. *Functional ecology*, *28*(6), 1344–1355.

Cohen, J. M., Lajeunesse, M. J., & Rohr, J. R. (2018). A global synthesis of animal phenological responses to climate change. *Nature Climate Change*, *8*(3), 224.

Colautti, R. I., & Barrett, S. C. (2013). Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science*, *342*(6156), 364–366.

Colautti, R. I., Grigorovich, I. A., & MacIsaac, H. J. (2006). Propagule pressure: a null model for biological invasions. *Biological Invasions*, *8*(5), 1023–1037.

Colautti, R. I., & Lau, J. A. (2015). Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology*, *24*(9), 1999–2017.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis?. *Ecology Letters*, *7*(8), 721–733.

Contarini, M., Onufrieva, K. S., Thorpe, K. W., Raffa, K. F., & Tobin, P. C. (2009). Mate-finding failure as an important cause of Allee effects along the leading edge of an invading insect population. *Entomol Exp Appl*, *133*(3), 307–314.

Cornell, H. V., & Hawkins, B. A. (1995). Survival patterns and mortality sources of herbivorous insects: some demographic trends. *The American Naturalist*, *145*(4), 563–593.

Courchamp, F., Berec, L., & Gascoigne, J. (2008). *Allee effects in ecology and conservation*. Oxford University Press.

Dambroski, H. R., & Feder, J. L. (2007). Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. *Journal of evolutionary biology*, *20*(6), 2101–2112.

Darling, E., Samis, K. E., & Eckert, C. G. (2008). Increased seed dispersal potential towards geographic range limits in a Pacific coast dune plant. *New Phytol*, *178*(2), 424–435.

David, G., Giffard, B., Van Halder, I., Piou, D., & Jactel, H. (2015). Energy allocation during the maturation of adults in a long-lived insect: implications for dispersal and reproduction. *Bull Entomol Res*, *105*(5), 629–636.

Davis, D. E. (2006). Historical significance of American chestnut to Appalachian culture and ecology. *Restoration of American Chestnut, The North Carolina Arboretum*.

Davis, A. K., Chi, J., Bradley, C., & Altizer, S. (2012). The redder the better: wing color predicts flight performance in monarch butterflies. *PLoS One*, *7*(7), e41323.

Davis, R. B., Javoiš, J., Kaasik, A., Õunap, E., & Tammaru, T. (2016). An ordination of life histories using morphological proxies: capital vs. income breeding in insects. *Ecology*, *97*(8), 2112–2124.

Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005a). Evolutionary responses to changing climate. *Ecology*, *86*(7), 1704–1714.

Davis, H. G., Taylor, C. M., Civille, J. C., & Strong, D. R. (2004). An Allee effect at the front of a plant invasion: ˆin a Pacific estuary. *Journal of Ecology*, *92*(2), 321–327.

Davidson, C. B., Gottschalk, K. W., & Johnson, J. E. (1999). Tree mortality following defoliation by the European gypsy moth (*Lymantria dispar* L.) in the United States: a review. *Forest Science*, *45*(1), 74–84.

De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O. and Gruwez, R. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, *101*(3), 784–795.

DeLucia, E. H., Nabity, P. D., Zavala, J. A., & Berenbaum, M. R. (2012). Climate change: resetting plant-insect interactions. *Plant physiology*, *160*(4), 1677–1685.

Denlinger, D. L. (2002). Regulation of diapause. *Annual review of entomology*, *47*(1), 93–122.

Denno, R. F., & Dingle, H. (Eds.). (1981). *Insect life history patterns: habitat and geographic variation* (p. 225). New York: Springer-Verlag.

Despres, L., David, J. P., & Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in ecology & evolution*, *22*(6), 298–307.

Dewar, R. C., & Watt, A. D. (1992). Predicted changes in the synchrony of larval emergence and budburst under climatic warming. *Oecologia*, *89*(4), 557–559.

Dingle, H., Blakley, N. R., & Miller, E. R. (1980). Variation in body size and flight performance in milkweed bugs (Oncopeltus). *Evolution*, 371–385.

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, *17*(1), 431–449.

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., & Gillette, H. D. (2015). The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular ecology*, *24*(9), 2095–2111.

Doane, C. C., & McManus, M. L. (Eds.). (1981). *The gypsy moth: research toward integrated pest management* (No. 1584). US Department of Agriculture.

Donnelly, A., Caffarra, A., & O'Neill, B. F. (2011). A review of climate-driven mismatches between interdependent phenophases in terrestrial and aquatic ecosystems. *International Journal of Biometeorology*, *55*(6), 805–817.

Drummond, B. A. (1984). Multiple mating and sperm competition in the Lepidoptera. *Sperm competition and the evolution of animal mating systems*, 291–370. Orlando, FL, USA: Academic Press Inc.

Ducousso, A., Guyon, J. P., & Kremer, A. (1996). Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (Quercus petraea (Matt) Liebl). In *Annales des sciences forestières* (Vol. 53, No. 2–3, pp. 775–782). EDP Sciences.

Dudley, R., & Srygley, R. (1994). Flight physiology of neotropical butterflies: allometry of airspeeds during natural free flight. *J Exp Biol*, *191*(1), 125–139.

Dudt, J. F., & Shure, D. J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, *75*(1), 86–98.

DuMerle, P. D., & Cornic, J. F. (1991) Monitoring the reproductive capacity of *Choristoneura murinana* (Lepidoptera: Tortricidae) populations by measuring the size of male moths caught in sex pheromone traps. *Acta oecologica*, **12**, 369–383.

Dwyer, G., & Elkinton, J. S. (1993). Using simple models to predict virus epizootics in gypsy moth populations. *Journal of Animal Ecology*, 1–11.

Dytham, C. (2009). Evolved dispersal strategies at range margins. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1661), 1407–1413.

Elkinton, J.S., Bittner, T.D., Pasquarella, V.J., Boettner, G.H., Liebhold, A.M., Gould, J.R., Faubert, H., Tewksbury, L., Broadley, H.J., Havill, N.P. and Hajek, A.E. (2019). Relating aerial deposition of *Entomophaga maimaiga* Conidia (Zoopagomycota: Entomophthorales) to mortality of gypsy moth (Lepidoptera: Erebidae) larvae and nearby defoliation. *Environmental Entomology*, *48*(5), 1214–1222.

Elkinton, J. S., & Cardé, R. T. (1981). Distribution, dispersal, and apparent survival of male gypsy moths as determined by capture in pheromone-baited traps. *Environmental Entomology*, *9*(6), 729–737.

Elkinton, J. S., Healy, W. M., Buonaccorsi, J. P., Boettner, G. H., Hazzard, A. M., & Smith, H. R. (1996). Interactions among gypsy moths, white-footed mice, and acorns. *Ecology*, *77*(8), 2332–2342.

Elkinton, J. S., Lombardo, J. A., Roehrig, A. D., McAvoy, T. J., Mayfield, A., & Whitmore, M. (2017). Induction of cold hardiness in an invasive herbivore: the case of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environmental entomology*, *46*(1), 118–124.

Elliott, C. G., & Evenden, M. L. (2009). Factors influencing flight potential of *Choristoneura conflictana*. *Physiological Entomology*, *34*(1), 71–78.

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics*, *24*(1), 217–242.

Elton, C. S. (1958). *The ecology of invasions by animals and plants*. University of Chicago Press.

Endler, J. A. (1977). *Geographic variation, speciation, and clines* (No. 10). Princeton University Press.

Evenden, M. L., Mori, B. A., Sjostrom, D., & Roland, J. (2015b). Forest tent caterpillar, *Malacosoma disstria* (Lepidoptera: Lasiocampidae), mate-finding behavior is greatest at intermediate population densities: implications for interpretation of moth capture in pheromone-baited traps. *Frontiers in Ecology and Evolution*, *3*, 78.

Evenden, M. L., Whitehouse, C. M., & Jones, B. C. (2015a). Resource allocation to flight in an outbreaking forest defoliator *Malacosoma disstria*. *Environmental Entomology*, *44*(3), 835–845.

Evenden, M. L., Whitehouse, C. M., & Sykes, J. (2014). Factors influencing flight capacity of the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). *Environmental entomology*, *43*(1), 187–196.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A., & David, P. (2006). A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution*, *21*(3), 130–135.

Fahrner, S. J., Lelito, J. P., Blaedow, K., Heimpel, G. E., & Aukema, B. H. (2014). Factors affecting the flight capacity of Tetrastichus planipennisi (Hymenoptera: Eulophidae), a classical biological control agent of Agrilus planipennis (Coleoptera: Buprestidae). *Environmental entomology*, *43*(6), 1603–1612.

Fält-Nardmann, J., Klemola, T., Roth, M., Ruohomäki, K., & Saikkonen, K. (2016). Northern geometrid forest pests (Lepidoptera: Geometridae) hatch at lower temperatures than their southern conspecifics: Implications of climate change. *European Journal of Entomology*, *113*, 337.

Faske, T. M. (2017). Effect of temperature and genetic structure on adaptive evolution at a dynamic range edge in the North American gypsy moth (*Lymantria dispar* L.). (Master's thesis). Retrieved from Graduate School at VCU Scholars Compass.

Faske, T.M., Thompson, L.M., Banahene, N., Levorse, A., Herrera, M.Q., Sherman, K., Timko, S.E., Yang, B., Gray, D.R., Parry, D. and Tobin, P.C. (2019). Can gypsy moth stand the heat? A reciprocal transplant experiment with an invasive forest pest across its southern range margin. *Biological Invasions*, *21*(4), 1365-1378.

Fajvan, M. A., & Wood, J. M. (1996). Stand structure and development after gypsy moth defoliation in the Appalachian Plateau. *Forest ecology and management*, *89*(1–3), 79–88.
Felt, E. P. (1924). The Gypsy Moth Problem in New York State. *Journal of Economic Entomology*, *17*(1), 64–67.

Feeny, P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology*, **51**, 565–581.

Fernald, C. H. (1888). *Division of entomology* (Vol. 1). Hatch Experiment Station of the Massachusetts Agricultural College.

Fischer, K., & Fiedler, K. (2001). Egg weight variation in the butterfly *Lycaena hippothoe*: more small or fewer large eggs?. *Population ecology*, *43*(1), 105–109.

Flint, H. L. (1972). Cold hardiness of twigs of *Quercus rubra* L. as a function of geographic origin. *Ecology*, *53*(6), 1163–1170.

Flynn, D. F. B., & Wolkovich, E. M. (2018). Temperature and photoperiod drive spring phenology across all species in a temperate forest community. *New Phytologist*, *219*(4), 1353–1362.

Forbush, E. & Fernald, C. (1896) *The Gypsy Moth*. State Board of Agriculture.

Forkner, R. E., Marquis, R. J., Lill, J. T., & CORFF, J. L. (2008). Timing is everything? Phenological synchrony and population variability in leaf‐chewing herbivores of Quercus. *Ecological Entomology, 33*(2), 276–285.

Forrest, J. R. (2016). Complex responses of insect phenology to climate change. *Current Opinion in Insect Science*, *17*, 49–54.

Forsman, A., Merilä, J., & Ebenhard, T. (2010). Phenotypic evolution of dispersal-enhancing traits in insular voles. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1703), 225–232.

Foster, J. R., Townsend, P. A., & Mladenoff, D. J. (2013). Mapping asynchrony between gypsy moth egg-hatch and forest leaf-out: Putting the phenological window hypothesis in a spatial context. *Forest Ecology and Management*, *287*, 67-76.

Frankham, R. (1995). Conservation genetics. *Annual Review of Genetics*, *29*(1), 305–327.

Frankham, R. (2005). Resolving the genetic paradox in invasive species. *Heredity*, *94*(4), 385–385.

Franks, S. J., Sim, S., & Weis, A. E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, *104*(4), 1278–1282.

Friedline, C.J., Faske, T.M., Lind, B.M., Hobson, E.M., Parry, D., Dyer, R.J., Johnson, D.M., Thompson, L.M., Grayson, K.L. and Eckert, A.J., 2019. Evolutionary genomics of gypsy moth populations sampled along a latitudinal gradient. *Molecular cology*, *28*(9), pp.2206–2223.

Fu, S., Chen, C., Xiao, L., He, H., & Xue, F. (2015). Inheritance of diapause in crosses between the northernmost and the southernmost strains of the Asian corn borer *Ostrinia furnacalis*. *PloS one*, *10*(2).

Fuentealba, A., Pureswaran, D., Bauce, É., & Despland, E. (2017). How does synchrony with host plant affect the performance of an outbreaking insect defoliator?. *Oecologia*, *184*(4), 847–857.

Fuenzalida, T. I., Hernández-Moreno, Á., & Piper, F. I. (2019). Secondary leaves of an outbreak-adapted tree species are both more resource acquisitive and more herbivore resistant than primary leaves. *Tree Physiology*, *39*(9), 1499-1511.

Fuester, R. W., Drea, J. J., Gruber, F., & Herard, F. (1981). Explorations in Europe and Iran by the ARS European Parasite Laboratory: 1972–77. *The gypsy moth: research toward integrated pest management. USDA Tech. Bull*, *1584*, 324–340.

García-Ramos, G., & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, *51*(1), 21–28.

Garnas, J.R., 2018. Rapid evolution of insects to global environmental change: conceptual issues and empirical gaps. *Current opinion in insect science*, *29*, pp.93–101.

Garrett, K. A., & Bowden, R. L. (2002). An Allee effect reduces the invasive potential of *Tilletia indica*. *Phytopathology*, *92*(11), 1152–1159.

Gibbs, M., Lace, L. A., Jones, M. J., & Moore, A. J. (2004). Intraspecific competition in the speckled wood butterfly *Pararge aegeria*: effect of rearing density and gender on larval life history. *J Insect Sci*, *4*(1), 16.

Giebultowicz, J. M., Raina, A. K., Uebel, E. C., & Ridgway, R. L. (1991). Two-step regulation of sex-pheromone decline in mated gypsy moth females. *Archives of Insect Biochemistry and Physiology*, 16(2), 95–105.

Giertych, M. J., Bąkowski, M., Karolewski, P., Zytkowiak, R., & Grzebyta, J. (2005). Influence of mineral fertilization on food quality of oak leaves and utilization efficiency of food components by the gypsy moth. *Entomologia experimentalis et applicata*, *117*(1), 59–69.

Gilchrist, G. W., Huey, R. B., Balanyà, J., Pascual, M., & Serra, L. (2004). A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution*, *58*(4), 768–780.

Gilchrist, G. W., Jeffers, L. M., West, B., Folk, D. G., Suess, J., & Huey, R. B. (2008). Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura*. *Evolutionary Applications*, *1*(3), 513–523.

Gilchrist, A. S., & Meats, A. W. (2010). The genetic structure of populations of an invading pest fruit fly, *Bactrocera tryoni*, at the species climatic range limit. *Heredity*, *105*(2), 165.

Gomi, T., & Takeda, M. (1996). Changes in life-history traits in the fall webworm within half a century of introduction to Japan. *Functional Ecology*, 384–389.

González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R. A., & Villalobos, F. (2020). Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biological Reviews*.

Gottschalk, K. W. (1990). Gypsy moth effects on mast production. In *In: McGee, Charles E., ed. Proceedings of the workshop: Southern Appalachian mast management; 1989 August 14–16; Knoxville, TN. Knoxville, TN: University of Tennessee: 42–50.* (pp. 42–50).

Gray, D. R. (2004). The gypsy moth life stage model: landscape-wide estimates of gypsy moth establishment using a multi-generational phenology model. *Ecological Modelling*, *176*(1-2), 155–171.

Gray, D. R. (2010). Hitchhikers on trade routes: a phenology model estimates the probabilities of gypsy moth introduction and establishment. *Ecological Applications*, *20*(8), 2300–2309.

Gray, D. R., & Keena, M. A. (2019). A phenology model for asian gypsy moth egg hatch. *Environmental Entomology*, *48*(4), 903–910.

Gray, D. R., Logan, J. A., Ravlin, F. W., & Carlson, J. A. (1991). Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature–time requirements of prediapause development. *Environmental Entomology*, *20*(6), 1645–1652.

Gray, D. R., Ravlin, F. W., & Braine, J. A. (2001). Diapause in the gypsy moth: a model of inhibition and development. *Journal of Insect Physiology*, *47*(2), 173–184.

Grayson, K. L., & Johnson, D. M. (2018). Novel insights on population and range edge dynamics using an unparalleled spatiotemporal record of species invasion. *Journal of Animal Ecology*, *87*(3), 581–593.

Guerra, P. A. (2011). Evaluating the life-history trade-off between dispersal capability and reproduction in wing dimorphic insects: a meta-analysis. *Biological Reviews*, *86*(4), 813–835.

Guerra, P. A., & Pollack, G. S. (2008). Flight behaviour attenuates the trade-off between flight capability and reproduction in a wing polymorphic cricket. *Biology letters*, *5*(2), 229–231.

Gurevitch, J., Fox, G. A., Wardle, G. M., & Taub, D. (2011). Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology letters*, *14*(4), 407–418.

Haag, C. R., Saastamoinen, M., Marden, J. H., & Hanski, I. (2005). A candidate locus for variation in dispersal rate in a butterfly metapopulation. *Proc R Soc Lond Ser B-Biol Sci*, *272*(1580), 2449–2456.

Hairston, N. G., Smith, F. E., & Slobodkin, L. B. (1960). Community structure, population control, and competition. *The american naturalist*, *94*(879), 421–425.

Hajek, A. E. (1997). Fungal and viral epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations in central New York. *Biological Control*, *10*(1), 58–68.

Hajek, A. E., & Tobin, P. C. (2009). North American eradications of Asian and European gypsy moth. In *Use of microbes for control and eradication of invasive arthropods* (pp. 71–89). Springer, Dordrecht.

Hajek, A. E., Tobin, P. C., & Haynes, K. J. (2015). Replacement of a dominant viral pathogen by a fungal pathogen does not alter the collapse of a regional forest insect outbreak. *Oecologia*, *177*(3), 785–797.

Hallegraeff, G. M., & Bolch, C. J. (1991). Transport of toxic dinoflagellate cysts via ships' ballast water. *Marine pollution bulletin*, *22*(1), 27–30.

Han, E. N., & Gatehouse, A. G. (1993). Flight capacity: genetic determination and physiological constraints in a migratory moth Mythimna separata. *Physiological entomology*, *18*(2), 183–188.

Hansen, M. M., Olivieri, I., Waller, D. M., Nielsen, E. E., & GeM Working Group. (2012). Monitoring adaptive genetic responses to environmental change. *Molecular ecology*, *21*(6), 1311–1329.

Hanula, J. L., Ulyshen, M. D., & Horn, S. (2011). Effect of trap type, trap position, time of year, and beetle density on captures of the redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). *Journal of Economic Entomology*, *104*(2), 501–508.

Harrington, R., Woiwod, I., & Sparks, T. (1999). Climate change and trophic interactions. *Trends in Ecology & Evolution*, *14*(4), 146–150.

Harvey, G. T. (1983). A geographical cline in egg weights in *Choristoneura fumiferana* (Lepidoptera: Tortricidae) and its significance in population dynamics. *The Canadian Entomologist*, *115*(9), 1103–1108.

Haukioja, E., Ossipov, V., & Lempa, K. (2002) Interactive effects of leaf maturation and phenolics on consumption and growth of a geometrid moth. *Entomologia Experimentalis et Applicata*, **104**, 125–136.

Heger, T., & Jeschke, J. M. (2014). The enemy release hypothesis as a hierarchy of hypotheses. *Oikos*, *123*(6), 741–750.

Hemming, J. D., & Lindroth, R. L. (1999). Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. *Journal of Chemical Ecology*, *25*(7), 1687–1714.

Hendry, A. P., Farrugia, T. J., & Kinnison, M. T. (2008). Human influences on rates of phenotypic change in wild animal populations. *Molecular ecology*, *17*(1), 20–29.

Hill, J. K., Griffiths, H. M., & Thomas, C. D. (2011). Climate change and evolutionary adaptations at species' range margins. *Annual review of entomology*, *56*, 143–159.

Hlásny, T., Trombik, J., Holuša, J., Lukášová, K., Grendár, M., Turčáni, M., Zúbrik, M., Tabaković-Tošić, M., Hirka, A., Buksha, I. and Modlinger, R. (2016). Multi-decade patterns of gypsy moth fluctuations in the Carpathian Mountains and options for outbreak forecasting. *Journal of pest science*, *89*(2), 413–425.

Hlásny, T., & Turčáni, M. (2009). Insect pests as climate change driven disturbances in forest ecosystems. In *Bioclimatology and natural hazards* (pp. 165–177). Springer, Dordrecht.

Hodek, I., & Hodková, M. (1988). Multiple role of temperature during insect diapause: a review. *Entomologia experimentalis et applicata*, *49*(1-2), 153–165.

Hoffmann, A. A., & Sgro, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485.

Hough, J.A. & Pimentel, D. (1978) Influence of host foliage on development, fecundity, and survival of the gypsy moth. *Environmental Entomology*, **7**, 97–102.

Huey, R. B., & Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *The Quarterly Review of Biology*, *51*(3), 363–384.

Hughes, C. L., Hill, J. K., & Dytham, C. (2003). Evolutionary trade-offs between reproduction and dispersal in populations at expanding range boundaries. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(suppl_2), S147–S150.

Hulme, P. E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of applied ecology*, *46*(1), 10–18.

Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J. and Pyšek, P. (2008). Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *Journal of Applied Ecology*, *45*(2), 403–414.

Hunter, A. F. (1991). Traits that distinguish outbreaking and nonoutbreaking Macrolepidoptera feeding on northern hardwood trees. *Oikos*, 275–282.

Hunter, M. D. (1992). A variable insect–plant interaction: the relationship between tree budburst phenology and population levels of insect herbivores among trees. *Ecological Entomology*, *17*(1), 91–95.

Hunter, A. F. (1993). Gypsy moth population sizes and the window of opportunity in spring. *Oikos*, 531–538.

Hunter, M. D. (2002). Landscape structure, habitat fragmentation, and the ecology of insects. *Agric For Entomol*, *4*(3), 159–166.

Hunter, A. F., & Elkinton, J. S. (2000). Effects of synchrony with host plant on populations of a spring-feeding lepidopteran. *Ecology*, *81*(5), 1248–1261.

Hunter, A. F., & Lechowicz, M. J. (1992) Foliage quality changes during canopy development of some northern hardwood trees. *Oecologia*, **89**, 316–323.

Hoffmann, A. A., & Willi, Y. (2008). Detecting genetic responses to environmental change. *Nature Reviews Genetics*, *9*(6), 421–432.

Houston, D. R., & Valentine, H. T. (1977). Comparing and predicting forest stand susceptibility to gypsy moth. *Canadian Journal of Forest Research*, *7*(3), 447–461.

Inderjit, Cadotte, M. W., & Colautti, R. I. (2005). The ecology of biological invasions: past, present and future. In *Invasive plants: Ecological and agricultural aspects* (pp. 19–43). Birkhäuser Basel.

Inglezakis, V. J., Poulopoulos, S. G., Arkhangelsky, E., Zorpas, A. A., & Menegaki, A. N. (2016). Aquatic Environment. In *Environment and Development* (pp. 137–212). Elsevier.
Janzen, D. H. (1973). Host plants as islands. II. Competition in evolutionary and contemporary time. *The American Naturalist*, *107*(958), 786–790.

IPCC, 2018: Summary for Policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.)]. World Meteorological Organization, Geneva, Switzerland, 32 pp.

Jaramillo, J., Chabi-Olaye, A., Kamonjo, C., Jaramillo, A., Vega, F. E., Poehling, H. M., & Borgemeister, C. (2009). Thermal tolerance of the coffee berry borer *Hypothenemus hampei*: predictions of climate change impact on a tropical insect pest. *PloS one*, *4*(8), e6487.

Javoiš, J., Davis, R. B., & Tammaru, T. (2019). A comparative morphometric study of sensory capacity in geometrid moths. *J Evol Biol*, *32*(4), 380-389.

Jedlicka, J., Vandermeer, J., Aviles-Vazquez, K., Barros, O., & Perfecto, I. (2004). Gypsy moth defoliation of oak trees and a positive response of red maple and black cherry: an example of indirect interaction. *The American midland naturalist*, *152*(2), 231-237.

Jepsen, J. U., Kapari, L., Hagen, S. B., Schott, T., Vindstad, O. P. L., Nilssen, A. C., & Ims, R. A. (2011). Rapid northwards expansion of a forest insect pest attributed to spring phenology matching with sub-Arctic birch. *Global Change Biology*, *17*(6), 2071–2083.

Jervis, M. A., Boggs, C. L., & Ferns, P. N. (2005). Egg maturation strategy and its associated trade-offs: a synthesis focusing on Lepidoptera. *Ecological Entomology*, *30*(4), 359–375.

Jeschke, J. M., Gómez Aparicio, L., Haider, S., Heger, T., Lortie, C. J., Pyšek, P., & Strayer, D. L. (2012). Support for major hypotheses in invasion biology is uneven and declining. *NeoBiota*, 14, 1–20.

Jing, X. H., & Kang, L. (2003). Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust Locusta migratoria L. *Ecological Entomology*, *28*(2), 151–158.

Johns, R. C., Tobita, H., Hara, H., & Ozaki, K. (2015). Adaptive advantages of dietary mixing different-aged foliage within conifers for a generalist defoliator. *Ecological research*, *30*(5), 793–802.

Johnson, C. G. (1969). Migration and dispersal of insects by flight. *Migration and dispersal of insects by flight.*

Johnson, P.C., Mason, D.P., Radke, S.L., & Tracewski, K.T. (1983) Gypsy moth, *Lymantria dispar*, (L.) (Lepidoptera: Lymantriidae) egg eclosion: degree-day accumulation. *Environmental Entomology*, **12**, 929-932.

Johnson, D., M. Liebhold, A., & N. Bjørnstad, O. (2006a). Geographical variation in the periodicity of gypsy moth outbreaks. *Ecography*, *29*(3), 367–374.

Johnson, D. M., Liebhold, A. M., Tobin, P. C., & Bjørnstad, O. N. (2006b). Allee effects and pulsed invasion by the gypsy moth. *Nature*, *444*(7117), 361.

Jones, B. C., & Despland, E. (2006) Effects of synchronization with host plant phenology occur early in the larval development of a spring folivore. *Canadian journal of zoology*, **84**, 628–633.

Jones, H. B., Lim, K. S., Bell, J. R., Hill, J. K., & Chapman, J. W. (2016). Quantifying interspecific variation in dispersal ability of noctuid moths using an advanced tethered flight technique. *Ecology and evolution*, *6*(1), 181–190.

Kanarek, A. R., & Webb, C. T. (2010). Allee effects, adaptive evolution, and invasion success. *Evolutionary Applications*, *3*(2), 122–135.

Karban, R., & Baldwin, I. T. (2007). *Induced responses to herbivory*. University of Chicago Press.

Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, *20*(1), 331–348.

Keena, M. A. (1996). Comparison of the hatch of *Lymantria dispar* (Lepidoptera: Lymantriidae) eggs from Russia and the United States after exposure to different temperatures and durations of low temperature. *Annals of the Entomological Society of America*, *89*(4), 564–572.

Keena, M. A. (2015). Inheritance and world variation in thermal requirements for egg hatch in *Lymantria dispar* (Lepidoptera: Erebidae). *Environmental entomology*, *45*(1), 1–10.

Keena, M. A., & Shi, J. (2019). Effects of temperature on first instar *Lymantria* (Lepidoptera: Erebidae) survival and development with and without food. *Environmental Entomology*, *48*(3), 655–666.

Keever, C. (1953). Present composition of some stands of the former oak-chestnut forest in the southern Blue Ridge Mountains. *Ecology, 34*(1), 44–54.

Keitt, T. H., Lewis, M. A., & Holt, R. D. (2001). Allee effects, invasion pinning, and species' borders. *The American Naturalist, 157*(2), 203–216.

Kenis, M., Rabitsch, W., Auger-Rozenberg, M. A., & Roques, A. (2007). How can alien species inventories and interception data help us prevent insect invasions?. *Bulletin of entomological research*, *97*(5), 489–502.

Kettlewell, H. B. D. (1955). Selection experiments on industrial melanism in the Lepidoptera. *Heredity*, *9*(3), 323.

Kingsolver, J. G., Arthur Woods, H., Buckley, L. B., Potter, K. A., MacLean, H. J., & Higgins, J. K. (2011). Complex life cycles and the responses of insects to climate change. *Integrative and Comparative Biology*, *51*(5), 719–732.

Kirkpatrick, M., & Barton, N. H. (1997). Evolution of a species' range. *Am Nat*, *150*(1), 1–23.

Klemola, N., Klemola, T., Rantala, M. J., & Ruuhola, T. (2007). Natural host-plant quality affects immune defence of an insect herbivore. *Entomologia Experimentalis et Applicata*, *123*(2), 167–176.

Klomp, H. (1964). Intraspecific competition and the regulation of insect numbers. *Annual review of entomology*, *9*(1), 17–40.

Knapp, R., & Casey, T. M. (1986). Thermal ecology, behavior, and growth of gypsy moth and eastern tent caterpillars. *Ecology*, *67*(3), 598–608.

Kollmann, J., & Bañuelos, M. J. (2004). Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions*, *10*(5-6), 377–385.

Koricheva, J., Larsson, S., & Haukioja, E. (1998b). Insect performance on experimentally stressed woody plants: a meta-analysis. *Annual review of entomology*, *43*(1), 195–216.

Koricheva, J., Larsson, S., Haukioja, E., & Keinänen, M. (1998a). Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, 212–226.

Körner, C., & Basler, D. (2010). Phenology under global warming. *Science, 327*(5972), 1461–1462.

Küchler, A. W. (1965). Potential natural vegetation of the conterminous United States. *Soil Science, 99*(5), 356.

Kuussaari, M., Saccheri, I., Camara, M., & Hanski, I. (1998). Allee effect and population dynamics in the Glanville fritillary butterfly. *Oikos*, 384–392.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, *82*(13).

Lameris, T.K., van der Jeugd, H.P., Eichhorn, G., Dokter, A.M., Bouten, W., Boom, M.P., Litvin, K.E., Ens, B.J. and Nolet, B.A. (2018). Arctic geese tune migration to a warming climate but still suffer from a phenological mismatch. *Current Biology*, *28*(15), 2467–2473.

Lancaster, L. T. (2016). Widespread range expansions shape latitudinal variation in insect thermal limits. *Nature climate change*, *6*(6), 618–621.

Lance, D., & Barbosa, P. (1981). Host tree influences on the dispersal of first instar gypsy moths, *Lymantria dispar* (L.). *Ecological Entomology*, *6*(4), 411–416.

Lance, D. R., Elkinton, J. S., & Schwalbe, C. P. (1986) Components of density-related stress as potential determinants of population quality in the gypsy moth (Lepidoptera: Lymantriidae). *Environmental entomology*, **15**, 914–918.

Langellotto, G. A., Denno, R. F., & Ott, J. R. (2000). A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. *Ecology*, *81*(3), 865–875.

Larsen, W. A., & McCleary, S. J. (1972). The use of partial residual plots in regression analysis. *Technometrics*, *14*(3), 781–790.

Lawrence, R. K., Mattson, W. J., & Haack, R. A. (1997). White spruce and the spruce budworm: defining the phenological window of susceptibility. *The Canadian Entomologist*, *129*(2), 291–318.

Lazarevic, J., Peric-Mataruga, V., Vlahovic, M., Mrdakovic, M., & Cvetanovic, D. (2004) Effects of rearing density on larval growth and activity of digestive enzymes in *Lymantria dispar* L.(Lepidoptera: Lymantriidae). *Folia Biologica-Krakow*, **52**, 105–112.

Lebeau, J., Wesselingh, R. A., & Van Dyck, H. (2016). Nectar resource limitation affects butterfly flight performance and metabolism differently in intensive and extensive agricultural landscapes. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1830), 20160455.

Lechowicz, M. J. (1984). Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. *The American Naturalist*, *124*(6), 821–842.

Lechowicz, M. J., & Mauffette, Y. (1986). Host preferences of the gypsy moth in eastern North American versus European forests. *Revue d'Entomologie du Quebec*, *31*(1), 43–51.

Lee, R. E. (1991). Principles of insect low temperature tolerance. In *Insects at low temperature* (pp. 17-46). Springer, Boston, MA.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in ecology & evolution*, *17*(8), 386–391.

Leonard, D. E., & Doane, C. C. (1966) An artificial diet for the gypsy moth, Porthetria dispar (Lepidoptera: Lymantriidae). *Annals of the Entomological Society of America*, **59**, 462–464.

Le Roy, C., Debat, V., & Llaurens, V. (2019). Adaptive evolution of butterfly wing shape: from morphology to behaviour. *Biol Rev*, *94*(4), 1261–1281.

Leung, B., Drake, J. M., & Lodge, D. M. (2004). Predicting invasions: propagule pressure and the gravity of Allee effects. *Ecology*, *85*(6), 1651–1660.

Levine, J. M., & D'Antonio, C. M. (1999). Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, 15–26.

Liebhold, A., & Bascompte, J. (2003). The Allee effect, stochastic dynamics and the eradication of alien species. *Ecol Lett*, *6*(2), 133–140.

Liebhold, A. M., Elmes, G. A., Halverson, J. A., & Quimby, J. (1994) Landscape characterization of forest susceptibility to gypsy moth defoliation. *Forest Science*, **40**, 18–29.

Liebhold, A.M., Gottschalk, K.W., Muzicka, R.M., Montgomery, M.E., Young, R., O’Day, K., & Kelley, B. (1995) *Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests*. USDA Forest Service.

Liebhold, A.M., Simons, E.E., Sior, A. and Unger, J.D. (1993). Forecasting defoliation caused by the gypsy moth from field measurements. *Environmental entomology*, *22*(1), pp.26–32.

Lindestad, O., von Schmalensee, L., Lehmann, P., & Gotthard, K. (2019). Variation in butterfly diapause duration in relation to voltinism suggests adaptation to autumn warmth, not winter cold. *Functional Ecology*.

Lindroth, R. L., Klein, K. A., Hemming, J. D., & Feuker, A. M. (1997). Variation in temperature and dietary nitrogen affect performance of the gypsy moth (Lymantria dispar L.). *Physiol Entomol*, *22*(1), 55–64.

Liu, H., & Stiling, P. (2006). Testing the enemy release hypothesis: a review and meta–analysis. *Biological invasions*, *8*(7), 1535–1545.

Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). *Invasion ecology*. John Wiley & Sons.

Loerch, C. R. (1984). Spermatophore persistence and mating determination in the gypsy moth (Lymantriidae). *J. Lepidopterists' Soc.*, *38*, 57–59.

Logan, J. A., Casagrande, R. A., & Liebhold, A. M. (1991). Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. *Environmental Entomology*, *20*(6), 1516–1525.

Lombardo, J. A., & Elkinton, J. S. (2017). Environmental adaptation in an asexual invasive insect. *Ecology and evolution*, *7*(14), 5123–5130.

Lopez, V. M., Hoddle, M. S., Francese, J. A., Lance, D. R., & Ray, A. M. (2017). Assessing flight potential of the invasive Asian longhorned beetle (Coleoptera: Cerambycidae) with computerized flight mills. *Journal of economic entomology*, *110*(3), 1070–1077.

Mack, M. C., & D'Antonio, C. M. (1998). Impacts of biological invasions on disturbance regimes. *Trends in Ecology & Evolution*, *13*(5), 195–198.

Marden, J. H. (2000). Variability in the size, composition, and function of insect flight muscles. *Annual Review of Physiology*, *62*(1), 157–178.

Maron, J. L., Vilà, M., Bommarco, R., Elmendorf, S., & Beardsley, P. (2004). Rapid evolution of an invasive plant. *Ecological Monographs*, *74*(2), 261–280.

Masaki, S. (1965). Geographic variation in the intrinsic incubation period: a physiological cline in the Emma field cricket (Orthoptera: Gryllidae: Teleogryllus).

Masaki, S. (1999). Seasonal adaptations of insects as revealed by latitudinal diapause clines. *Entomological Science*, *2*(4), 539–550.

Matsuki, M., Kay, M., Serin, J., Floyd, R., & Scott, J. K. (2001). Potential risk of accidental introduction of Asian gypsy moth (*Lymantria dispar*) to Australasia: effects of climatic conditions and suitability of native plants. *Agricultural and Forest Entomology*, *3*(4), 305–320.

McAnelly, M. L., & Rankin, M. A. (1986). Migration in the grasshopper Melanoplus sanguinipes (Fab.). I. The capacity for flight in non–swarming populations. *The Biological Bulletin*, *170*(3), 368–377.

McGeoch, M.A., Butchart, S.H., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J. and Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity and Distributions*, *16*(1), 95–108.

Melbourne, B.A., Cornell, H.V., Davies, K.F., Dugaw, C.J., Elmendorf, S., Freestone, A.L., Hall, R.J., Harrison, S., Hastings, A., Holland, M. and Holyoak, M. (2007). Invasion in a heterogeneous world: resistance, coexistence or hostile takeover?. *Ecology letters*, *10*(1), 77–94.
Menéndez, R. (2007). How are insects responding to global warming?. *Tijdschrift voor Entomologie*, *150*(2), 355.

Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-Kübler, K., Bissolli, P., Braslavská, O.G., Briede, A. and Chmielewski, F.M., 2006. European phenological response to climate change matches the warming pattern. *Global change biology*, *12*(10), pp.1969–1976.

Minter, M., Pearson, A., Lim, K. S., Wilson, K., Chapman, J. W., & Jones, C. M. (2018). The tethered flight technique as a tool for studying life-history strategies associated with migration in insects. *Ecological entomology*, *43*(4), 397–411.

Moles, A. T., Gruber, M. A., & Bonser, S. P. (2008). A new framework for predicting invasive plant species. *Journal of Ecology*, *96*(1), 13–17.

Mollot, G., Pantel, J. H., & Romanuk, T. N. (2017). The effects of invasive species on the decline in species richness: a global meta–analysis. In *Advances in Ecological Research* (Vol. 56, pp. 61–83). Academic Press.

Moran, E. V., & Alexander, J. M. (2014). Evolutionary responses to global change: lessons from invasive species. *Ecology Letters*, *17*(5), 637–649.

Morbey, Y. E., & Ydenberg, R. C. (2001). Protandrous arrival timing to breeding areas: a review. *Ecol Lett*, *4*(6), 663–673.

Morin, R. S., Liebhold, A. M., Luzader, E. R., Lister, A. J., Gottschalk, K. W., & Twardus, D. B. (2005). Mapping host–species abundance of three major exotic forest pests. *Res. Pap. NE–726. Newtown Square, PA: US Department of Agriculture, Forest Service, Northeastern Research Station. 11 p.*, *726*.

Montague, J. L., Barrett, S. C. H., & Eckert, C. G. (2008). Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (Lythrum salicaria, Lythraceae). *Journal of evolutionary biology*, *21*(1), 234–245.

Naranjo, S. E. (2019). Assessing insect flight behavior in the laboratory: a primer on flight mill methodology and what can be learned. *Annals of the Entomological Society of America*, *112*(3), 182–199.

Novy, A., Flory, S. L., & Hartman, J. M. (2013). Evidence for rapid evolution of phenology in an invasive grass. *Journal of Evolutionary Biology*, *26*(2), 443–450.

Nykänen, H., & Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos*, *104*(2), 247–268.

Odell, T. M. (1978). Periodicity of eclosion and pre-mating behavior of gypsy moth. *Annals of the Entomological Society of America*, *71*(5), 748-751.

Oduor, A. M., Leimu, R., & van Kleunen, M. (2016). Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. *Journal of Ecology*, *104*(4), 957–968.

O’neill, E.M., Hearn, E.J., Cogbill, J.M. and Kajita, Y., 2017. Rapid evolution of a divergent ecogeographic cline in introduced lady beetles. *Evolutionary Ecology*, *31*(5), pp.695–705.

Orr, H. A. (2002). The population genetics of adaptation: the adaptation of DNA sequences. *Evolution*, *56*(7), 1317–1330.

Ostfeld, R. S., Jones, C. G., & Wolff, J. O. (1996). Of mice and mast. *BioScience*, *46*(5), 323–330.

Paaijmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global change biology*, *19*(8), 2373–2380.

Paillet, F. L. (2002). Chestnut: history and ecology of a transformed species. *Journal of Biogeography*, *29*(10-11), 1517–1530.

Parker, W. E., & Gatehouse, A. G. (1985). The effect of larval rearing conditions on flight performance in females of the African armyworm, Spodoptera exempta (Walker)(Lepidoptera: Noctuidae). *Bull Entomol Res*, *75*(1), 35–48.

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*(6918), 37.

Parry D (2009) Beyond Pandora’s box: quantitatively evaluating non–target effects of parasitoids in classical biological control. Biol Invasions 11:47–58

Parry, D., & Goyer, R. A. (2004). Variation in the suitability of host tree species for geographically discrete populations of forest tent caterpillar. *Environmental Entomology*, *33*(5), 1477–1487.

Parry, D., Goyer, R.A. and Lenhard, G.J., (2001). Macrogeographic clines in fecundity, reproductive allocation, and offspring size of the forest tent caterpillar Malacosoma disstria. *Ecological Entomology, 26*(3): 281–291.

Parry, D., Spence, J.R., and W.J.A. Volney. (1998). Budbreak phenology and natural enemies mediate survival of first–instar forest tent caterpillar (Lepidoptera: Lasiocampidae). *Environmental Entomology*, 27(6): 1368–1374.

Pasquarella, V. J., Elkinton, J. S., & Bradley, B. A. (2018). Extensive gypsy moth defoliation in Southern New England characterized using Landsat satellite observations. *Biological Invasions*, *20*(11), 3047–3053.

Peterson, A. T., Williams, R., & Chen, G. (2007). Modeled global invasive potential of Asian gypsy moths, *Lymantria dispar*. *Entomologia Experimentalis et Applicata*, *125*(1), 39–44.

Phair, D. J., Le Roux, J., Berthouly-Salazar, C., Visser, V., van Vuuren, B. J., Cardilini, A., & Hui, C. (2018). Context-dependent spatial sorting of dispersal-related traits in the invasive starlings (Sturnus vulgaris) of South Africa and Australia. *bioRxiv*, 342451.

Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*(7078), 803.

Pimentel, D. (2014). *Biological invasions: economic and environmental costs of alien plant, animal, and microbe species*. CRC press.

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience*, *50*(1), 53-66.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'connell, C., ... & Tsomondo, T. (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment*, *84*(1), 1–20.

Pitt, J. P. W., Régnière, J., & Worner, S. (2007). Risk assessment of the gypsy moth, *Lymantria dispar* (L), in New Zealand based on phenology modelling. *International journal of biometeorology*, *51*(4), 295–305.

Plard, F., Gaillard, J. M., Coulson, T., Hewison, A. M., Delorme, D., Warnant, C., & Bonenfant, C. (2014). Mismatch between birth date and vegetation phenology slows the demography of roe deer. *PLoS biology*, *12*(4).

Pletsers, A., Caffarra, A., Kelleher, C. T., & Donnelly, A. (2015). Chilling temperature and photoperiod influence the timing of bud burst in juvenile *Betula pubescens* Ehrh. and *Populus tremulaL*. trees. *Annals of forest science*, *72*(7), 941–953.

Pogue, M., & Schaefer, P. W. (2007). Review of selected species of Lymantria hubner (1819)(Lepidoptera: Noctuidae: Lymantriinae) from subtropical and temperate regions of Asia, including the descriptions of three new species, some potentially invasive to North America.

Ponomarev, V. I., Klobukov, G. I., Ilyinykh, A. V., & Dubovskiy, I. M. (2019). Adaptation Features of Diapause Duration of the Gypsy Moth Lymantria dispar (L.) from Populations of Different Latitudinal Origination. *Contemporary Problems of Ecology*, *12*(1), 1–9.

Posledovich, D., Toftegaard, T., Wiklund, C., Ehrlén, J., & Gotthard, K. (2015). Latitudinal variation in diapause duration and post-winter development in two pierid butterflies in relation to phenological specialization. *Oecologia*, *177*(1), 181–190.

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in plant science*, *13*(6), 288–294.

Prosholf, F., & Bernon, G. L. (1994). Multiple mating in laboratory-reared gypsy moths (Lepidoptera: Lymantriidae). *Journal of economic entomology*, *87*(3), 661–666.

Pujol, B., & Pannell, J. R. (2008). Reduced responses to selection after species range expansion. *Science*, *321*(5885), 96–96.

Ratte, H. T. (1984). Temperature and insect development. In *Environmental physiology and biochemistry of insects* (pp. 33–66). Springer, Berlin, Heidelberg.

Raupp, M.J., Werren, J.H., and C.S. Sadof. 1988. Effects of short term phenological changes in leaf suitability on survivorship, growth, and development of gypsy moth (Lepidoptera: Lymantriidae) larvae. *Environmental Entomology*, 17(2): 316–319.

Redman, A. M., & Scriber, J. M. (2000). Competition between the gypsy moth, *Lymantria dispar*, and the northern tiger swallowtail, Papilio canadensis: interactions mediated by host plant chemistry, pathogens, and parasitoids. *Oecologia*, *125*(2), 218–228.

Régnière, J., Nealis, V., & Porter, K. (2008). Climate suitability and management of the gypsy moth invasion into Canada. In *Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems* (pp. 135–148). Springer, Dordrecht.

Reilly, J. R., & Hajek, A. E. (2008) Density-dependent resistance of the gypsy moth *Lymantria dispar* to its nucleopolyhedrovirus, and the consequences for population dynamics. *Oecologia*, **154**, 691-701.

Renner, S. S., & Zohner, C. M. (2018). Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. *Annual Review of Ecology, Evolution, and Systematics*, *49*, 165–182.

Rettelbach, A., Kopp, M., Dieckmann, U., & Hermisson, J. (2013). Three modes of adaptive speciation in spatially structured populations. *The American Naturalist*, *182*(6), E215–E234.

Rhainds, M. (2015). Size-dependent realized fecundity in two lepidopteran capital breeders. *Environmental entomology*, *44*(4), 1193–1200.

Ricciardi, A. (2007). Are modern biological invasions an unprecedented form of global change?. *Conservation Biology*, *21*(2), 329–336.

Richerson, J. V., Cameron, E. A., & Brown, E. A. (1976). Sexual activity of the gypsy moth. *American Midland Naturalist*, 299–312.

Robinet, C., Liebhold, A., & Gray, D. (2007). Variation in developmental time affects mating success and Allee effects. *Oikos*, *116*(7), 1227–1237.

Roden, D. B., & Mattson, W. J. (2008). Rapid induced resistance and host species effects on gypsy moth, *Lymantria dispar* (L.): Implications for outbreaks on three tree species in the boreal forest. *Forest ecology and management*, *255*(5–6), 1868–1873.

Roman, J., & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in ecology & evolution*, *22*(9), 454–464.

Rooney, T. P., & Waller, D. M. (2003). Direct and indirect effects of white-tailed deer in forest ecosystems. *Forest ecology and management*, *181*(1–2), 165–176.

Roth, S. K., Lindroth, R. L., & Montgomery, M. E. (1994). Effects of foliar phenolics and ascorbic acid on performance of the gypsy moth (*Lymantria dispar*). *Biochemical systematics and ecology*, *22*(4), 341–351.

Ruohomäki, K. (1992). Wing size variation in Epirrita autumnata (Lep., Geometridae) in relation to larval density. *Oikos*, 260–266.

Rutowski, R. L., Newton, M., & Schaeffer, J. (1983). Interspecific variation in the size of the nutrient investment made by male butterflies during copulation. *Evolution*, *37*(4), 708–713.

Sappington, T. W., & Burks, C. S. (2014). Patterns of flight behavior and capacity of unmated navel orangeworm (Lepidoptera: Pyralidae) adults related to age, gender, and wing size. *Environmental entomology*, *43*(3), 696–705.

Schäfer, M.A., Berger, D., Rohner, P.T., Kjaersgaard, A., Bauerfeind, S.S., Guillaume, F., Fox, C.W. and Blanckenhorn, W.U. (2018). Geographic clines in wing morphology relate to colonization history in New World but not Old World populations of yellow dung flies. *Evolution*, *72*(8), 1629–1644.

Schäfer, P. W. & Strothkamp, K.G. (2014). Mass flights of *Lymantria dispar japonica* and *Lymantria mathura* (Erebidae: Lymantriinae) to commercial lighting, with notes on female viability and fecundity. 1. *The Journal of the Lepidopterists' Society*, *68*(2), 124-129.

Schroeder, L. A. (1986) Changes in tree leaf quality and growth performance of lepidopteran larvae. *Ecology*, **67**, 1628–1636.

Schultz, J. C., & Lechowicz, M. J. (1986). Hostplant, larval age, and feeding behavior influence midgut pH in the gypsy moth (Lymantria dispar). *Oecologia*, *71*(1), 133–137.

Schwalbe, C.P. 1981. Disparlure baited traps for survey and detection, pp. 542–548.,*in* C.C. Doane and M.L. McManus (eds.). The Gypsy Moth: Research toward Integrated Pest Management.*U.S. Department of Agriculture Technical Bulletin 1584*.

Seiter, S., Ohsaki, N., & Kingsolver, J. (2013). Parallel invasions produce heterogenous patterns of life history adaptation: rapid divergence in an invasive insect. *Journal of evolutionary biology*, *26*(12), 2721–2728.

Sexton, J. P., Strauss, S. Y., & Rice, K. J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proc Natl Acad Sci U S A*, *108*(28), 11704–11709.

Sharov, A. A., Leonard, D., Liebhold, A. M., Roberts, E. A., & Dickerson, W. (2002). "Slow the spread": a national program to contain the gypsy moth. *Journal of Forestry*, *100*(5), 30–36.

Sharov, A. A., & Liebhold, A. M. (1998). Bioeconomics of managing the spread of exotic pest species with barrier zones. *Ecological applications*, *8*(3), 833–845.

Sharov, A. A., Liebhold, A. M., & Ravlin, F. W. (1995). Prediction of gypsy moth (Lepidoptera: Lymantriidae) mating success from pheromone trap counts. *Environmental Entomology*, *24*(5), 1239–1244.

Sharov, A. A., Pijanowski, B. C., Liebhold, A. M., & Gage, S. H. (1999). What affects the rate of gypsy moth (Lepidoptera: Lymantriidae) spread: winter temperature or forest susceptibility?. *Agricultural and Forest Entomology*, *1*(1), 37–45.

Shaw, J. T., Ruesink, W. G., Briggs, S. P., & Luckmann, W. H. (1984). Monitoring populations of corn rootworm beetles (Coleoptera: Chrysomelidae) with a trap baited with cucurbitacins. *Journal of economic entomology*, *77*(6), 1495–1499.

Shea, K., & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution*, *17*(4), 170–176.

Shi, J., Chen, F., & Keena, M. A. (2015). Differences in wing morphometrics of *Lymantria dispar* (Lepidoptera: Erebidae) between populations that vary in female flight capability. *Annals of the Entomological Society of America*, *108*(4), 528–535.

Shields, K. S., Hirth, R. T., Young, R. F., & Keena, M. A. (1997). Comparison of thoracic musculature of Asian and North American gypsy moths. In *Proceedings, US Department of Agriculture Interagency Gypsy Moth Research Forum, 1997: January 14–17, 1997, Loews Annapolis Hotel, Annapolis, Maryland* (Vol. 240). US Department of Agriculture, Forest Service, Northeastern Forest Experiment Station.

Shimazu, M., & Soper, R. S. (1986) Pathogenicity and sporulation of *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek (Entomophthorales: Entomophthoraceae) on larvae of the gypsy moth, *Lymantria dispar* L.(Lepidoptera: Lymantriidae). *Applied Entomology and Zoology*, **21**, 589–596.

Shirai, Y. (1995). Longevity, flight ability and reproductive performance of the diamondback moth, *Plutella xylostella* (L.)(Lepidoptera: Yponomeutidae), related to adult body size. *Population Ecology*, *37*(2), 269–277.

Showalter, C. R., & Whitmore, R. C. (2002). The effect of gypsy moth defoliation on cavity-nesting bird communities. *Forest Science*, *48*(2), 273-281.

Shure, D. J., Mooreside, P. D., & Ogle, S. M. (1998) Rainfall effects on plant-herbivore processes in an upland oak forest. *Ecology*, **79**, 604–617.

Sills, E. O. (2007). Assessment of the economic feasibility of the gypsy moth Slow the Spread project. *Raleigh (NC): North Carolina State University*.

Simberloff, D. (2006). Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both?. *Ecology Letters*, *9*(8), 912–919.

Simberloff, D. (2009). The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 81–102.

Simberloff, D. (2011). Charles Elton: neither founder nor siren, but prophet. *Fifty years of invasion ecology: The legacy of Charles Elton*, 11–24.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M. and Pyšek, P., (2013). Impacts of biological invasions: what's what and the way forward. *Trends in ecology & evolution*, *28*(1), 58–66.

Simberloff, D., and Von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown?. *Biological invasions*, *1*(1), 21–32.

Simmons, A. D., & Thomas, C. D. (2004). Changes in dispersal during species' range expansions. *The American Naturalist*, *164*(3), 378–395.

Sinclair, B. J., Williams, C. M., & Terblanche, J. S. (2012) Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, **85**, 594–606.

Singer, M. C., & Parmesan, C. (2010). Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy?. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3161–3176.

Singer, M. C., Thomas, C. D., & Parmesan, C. (1993). Rapid human-induced evolution of insect–host associations. *Nature*, *366*(6456), 681–683.

Skellam, J. G. (1951). Random dispersal in theoretical populations. *Biometrika*, *38*(1/2), 196–218.

Slow-the-Spread Foundation, Inc (2019) **http://www.gmsts.org/**

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional ecology*, *3*(3), 259-268.

Stephenson, S. L. (1986). Changes in a former chestnut–dominated forest after a half century of succession. *American Midland Naturalist*, 173–179.

Stephens, P. A., & Sutherland, W. J. (1999). Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in ecology & evolution, 14*(10), 401–405.

Stephens, P. A., Sutherland, W. J., & Freckleton, R. P. (1999). What is the Allee effect?. *Oikos*, 185–190.

Stockhoff, B. A. (1991) Starvation resistance of gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae): tradeoffs among growth, body size, and survival. *Oecologia*, **88**, 422–429.

Stoyenoff, J. L., Witter, J. A., Montgomery, M. E., & Chilcote, C. A. (1994) Effects of host switching on gypsy moth (*Lymantria dispar* (L.)) under field conditions. *Oecologia*, **97**, 143–157.

Streifel, M. A., Tobin, P. C., Kees, A. M., & Aukema, B. H. (2019). Range expansion of *Lymantria dispar* dispar (L.)(Lepidoptera: Erebidae) along its north-western margin in North America despite low predicted climatic suitability. *Journal of biogeography*, *46*(1), 58–69.

Svärd, L., & Wiklund, C. (1989). Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. *Behavioral Ecology and Sociobiology*, *24*(6), 395–402.

Tammaru, T., Esperk, T., & Castellanos, I. (2002). No evidence for costs of being large in females of *Orgyia* spp.(Lepidoptera, Lymantriidae): larger is always better. *Oecologia*, *133*(3), 430–438.

Tammaru, T., & Haukioja, E. (1996). Capital breeders and income breeders among Lepidoptera: consequences to population dynamics. *Oikos*, 561–564.

Tammaru, T., Kaitaniemi, P., & Ruohomäki, K. (1996). Realized fecundity in *Epirrita autumnata* (Lepidotera: Geometridae): Relation to body size and consequences to population dynamics. *Oikos*, *77*(3), 407–416.

Tammaru, T., Ruohomäki, K., & Saikkonen, K. (1996b). Components of male fitness in relation to body size in *Epirrita autumnata* (Lepidoptera, Geometridae). *Ecol Entomol*, *21*(2), 185-192.

Tardif, R., and J. P. Secrest. "Devices for cleaning and counting eggs of the gypsy moth." *J Econ Entomol* 63.2 (1970): 678–9.

Tauber, M.J., Tauber C.A., Ruberson, J.R., Tauber, A.J. and L.P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. *Annals of the Entomological Society of America*, 83(3): 494–503.

Taylor, C. M., & Hastings, A. (2005). Allee effects in biological invasions. *Ecology Letters*, *8*(8), 895–908.

Teder, T., Vellau, H., & Tammaru, T. (2014). Age and size at maturity: A quantitative review of diet-induced reaction norms in insects. *Evolution*, *68*(11), 3217–3228.

Thackeray, S. J., Henrys, P. A., Hemming, D., Bell, J. R., Botham, M. S., Burthe, S., ... & Mackay, E. B. (2016). Phenological sensitivity to climate across taxa and trophic levels. *Nature*, *535*(7611), 241.

Thompson, L. M., Faske, T. M., Banahene, N., Grim, D., Agosta, S. J., Parry, D., ... & Grayson, K. L. (2017). Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species. *Physiological entomology*, *42*(2), 181–190.

Thorpe, K. W., Ridgway, R. L., & Leonhardt, B. A. (1993). Relationship Between Gypsy Moth (Lepidoptera: Lymantriidae) Pheromone Trap Catch and Population Density: Comparison of Traps Baited with 1 and 500< g (+)-Disparlure Lures. *Journal of economic entomology*, *86*(1), 86–92.

Thurber, D. K., McClain, W. R., & Whitmore, R. C. (1994). Indirect effects of gypsy moth defoliation on nest predation. *The Journal of wildlife management*, 493–500.

Tigreros, N., Davidowitz, G., Trumbo, S.T., Bondy, E.C., Hunter, M.S., Stanley, D., Kim, Y., Kolosov, D., O'Donnell, M.J., Hayes, A.M. and Lavine, M.D., 2019. Flight-fecundity tradeoffs in wing-monomorphic insects. *Advances in Insect Physiology*, *56*, 1.

Tisdale, R. A., & Sappington, T. W. (2001). Realized and potential fecundity, egg fertility, and longevity of laboratory-reared female beet armyworm (Lepidoptera: Noctuidae) under different adult diet regimes. *Annals of the Entomological Society of America*, *94*(3), 415-419.

Tobin, P. C. (2008). *Cost analysis and biological ramifications for implementing the gypsy moth Slow the Spread Program* (Vol. 37). US Department of Agriculture, Forest Service, Northern Research Station.

Tobin, P. C., Berec, L., & Liebhold, A. M. (2011). Exploiting Allee effects for managing biological invasions. *Ecology letters*, *14*(6), 615–624.

Tobin, P. C., & Blackburn, L. M. eds. (2007). Slow the spread: a national program to manage the gypsy moth. *Gen. Tech. Rep. NRS-6. Newtown Square, PA: US Department of Agriculture, Forest Service, Northern Research Station. 109 p.*, *6*.

Tobin, P. C., Bolyard, J. L., Onufrieva, K. S., & Hickman, A. D. (2014a). The effect of male and female age on *Lymantria dispar* (Lepidoptera: Lymantriidae) fecundity. *Journal of economic entomology*, *107*(3), 1076–1083.

Tobin, P. C., Cremers, K. T., Hunt, L., & Parry, D. (2016) All quiet on the western front? Using phenological inference to detect the presence of a latent gypsy moth invasion in Northern Minnesota. *Biological Invasions*, **18**, 3561–3573.

Tobin, P. C., Gray, D. R., & Liebhold, A. M. (2014b). Supraoptimal temperatures influence the range dynamics of a non-native insect. *Diversity and Distributions*, *20*(7), 813–823.

Tobin, P. C., Parry, D., & Aukema, B. H. (2014b). The influence of climate change on insect invasions in temperate forest ecosystems. In *Challenges and Opportunities for the World's Forests in the 21st Century* (pp. 267–293). Springer, Dordrecht.

Tobin, P. C., Robinet, C., Johnson, D. M., Whitmire, S. L., Bjørnstad, O. N., & Liebhold, A. M. (2009). The role of Allee effects in gypsy moth, *Lymantria dispar* (L.), invasions. *Population Ecology*, *51*(3), 373–384.

Tobin, P. C., Whitmire, S. L., Johnson, D. M., Bjørnstad, O. N., & Liebhold, A. M. (2007). Invasion speed is affected by geographical variation in the strength of Allee effects. *Ecology Letters*, *10*(1), 36–43.

Turchin, P., & Odendaal, F. J. (1996). Measuring the effective sampling area of a pheromone trap for monitoring population density of southern pine beetle (Coleoptera: Scolytidae). *Environmental Entomology*, *25*(3), 582–588.

Uelmen Jr, J. A., Lindroth, R. L., Tobin, P. C., Reich, P. B., Schwartzberg, E. G., & Raffa, K. F. (2016). Effects of winter temperatures, spring degree-day accumulation, and insect population source on phenological synchrony between forest tent caterpillar and host trees. *Forest Ecology and Management*, *362*, 241–250.

Uller, T., & Leimu, R. (2011). Founder events predict changes in genetic diversity during human-mediated range expansions. *Global Change Biology*, *17*(11), 3478–3485.

[USDA] U.S. Department of Agriculture. 2001. *Gypsy moth program manual*.

Valentine, H. T., Wallner, W. E., & Wargo, P. M. (1983). Nutritional changes in host foliage during and after defoliation, and their relation to the weight of gypsy moth pupae. *Oecologia*, *57*(3), 298–302.

van Asch, M., Salis, L., Holleman, L. J., Van Lith, B., & Visser, M. E. (2013). Evolutionary response of the egg hatching date of a herbivorous insect under climate change. *Nature Climate Change*, *3*(3), 244.

van Asch, M. and M.E. Visser. (2007). Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annual Review of Entomology*, 52: 37–55.

Van Zandt, P. A., & Agrawal, A. A. (2004). Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology*, *85*(9), 2616–2629.

Veit, R. R., & Lewis, M. A. (1996). Dispersal, population growth, and the Allee effect: dynamics of the house finch invasion of eastern North America. *The American Naturalist*, *148*(2), 255–274.

Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2561–2569.

Visser, M. E., & Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nature ecology & evolution*, *3*(6), 879–885.

Vitasse, Y., François, C., Delpierre, N., Dufrêne, E., Kremer, A., Chuine, I., & Delzon, S. (2011). Assessing the effects of climate change on the phenology of European temperate trees. *Agricultural and Forest Meteorology, 151*(7), 969–980.

Vitousek, P. M. (1986). Biological invasions and ecosystem properties: can species make a difference?. In *Ecology of biological invasions of North America and Hawaii* (pp. 163–176). Springer, New York, NY.

Walker, M., Blackwood, J. C., Brown, V., & Childs, L. M. (2019). Modelling Allee effects in a transgenic mosquito population during range expansion. *Journal of biological dynamics*, *13*(sup1), 2–22.

Walsh, P. J. (1993). Asian gypsy moth: the risk to New Zealand. *New Zealand Forestry*, *38*(2), 41–43.

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior*, *7*(10), 1306–1320.

Way, D. A., & Montgomery, R. A. (2015). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant, Cell & Environment*, *38*(9), 1725–1736.

Weber, E., & Schmid, B. (1998). Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. *American journal of Botany*, *85*(8), 1110–1121.

Weseloh, R. M. (1998). Modeling the influence of forest characteristics and ant (Formicidae: Hymenoptera) predation on dispersal and survival of neonate gypsy moths (Lymantriidae: Lepidoptera). *Environmental entomology*, *27*(2), 288–296.

Weseloh, R. M., Andreadis, T. G., & Onstad, D. W. (1993). Modeling the influence of rainfall and temperature on the phenology of infection of gypsy moth, *Lymantria dispar*, larvae by the fungus Entomophaga maimaiga. *Biological Control*, *3*(4), 311–318.

Wesołowski, T., & Rowiński, P. (2006) Timing of bud burst and tree-leaf development in a multispecies temperate forest. *Forest Ecology and Management*, **237**, 387–393.

White, E. M., Wilson, J. C., & Clarke, A. R. (2006). Biotic indirect effects: a neglected concept in invasion biology. *Diversity and distributions*, *12*(4), 443–455.

Wickman, P. O. (1992). Sexual selection and butterfly design—a comparative study. *Evolution*, *46*(5), 1525–1536.

Wiklund, C., & Fagerström, T. (1977). Why do males emerge before females?. *Oecologia*, *31*(2), 153–158.

Wint, W. (1983). The role of alternative host-plant species in the life of a polyphagous moth, *Operophtera brumata* (Lepidoptera: Geometridae). *The Journal of Animal Ecology*, 439–450.

Woods, E. C., Hastings, A. P., Turley, N. E., Heard, S. B., & Agrawal, A. A. (2012). Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs*, *82*(2), 149–168.

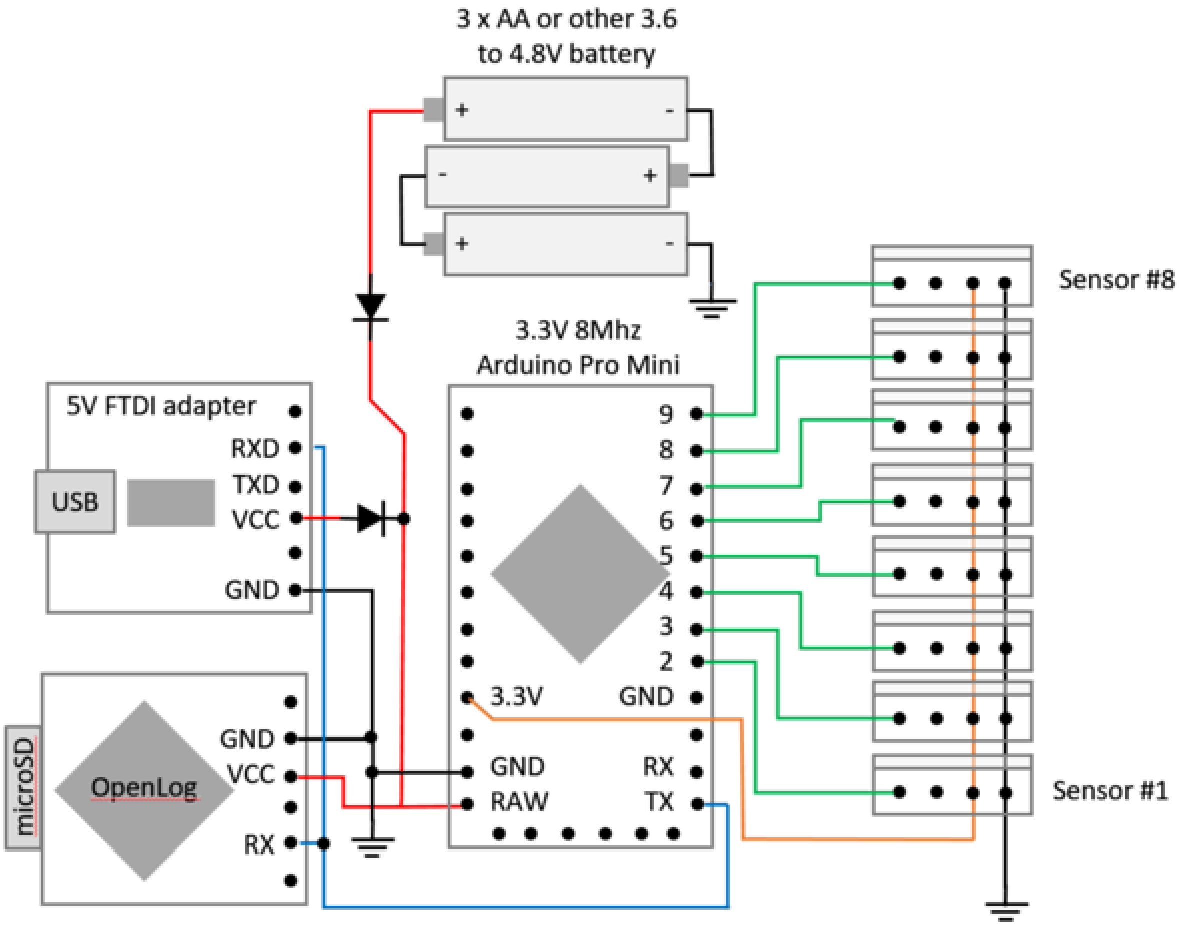
Wu, Y., Molongoski, J.J., Winograd, D.F., Bogdanowicz, S.M., Louyakis, A.S., Lance, D.R., Mastro, V.C. and Harrison, R.G. (2015). Genetic structure, admixture and invasion success in a Holarctic defoliator, the gypsy moth (*Lymantria dispar*, Lepidoptera: Erebidae). *Molecular Ecology*, *24*(6), 1275–1291

Wu, Y., Bogdanowicz, S.M., Andres, J.A., Vieira, K.A., Wang, B., Cossé, A. and Pfister, S.E. (2020).Tracking invasions of a destructive defoliator, the gypsy moth (Erebidae: Lymantria dispar): population structure, origin of intercepted specimens, and Asian introgression into North America. *Evolutionary Applications*. doi:10.1111/eva.12962.

Yamanaka, T., & Liebhold, A. M. (2009). Mate-location failure, the Allee effect, and the establishment of invading populations. *Popul Ecol*, *51*(3), 337–340.

Zhang, X., Tarpley, D., & Sullivan, J. T. (2007). Diverse responses of vegetation phenology to a warming climate. *Geophysical Research Letters*, *34*(19).

Appendix 1. Microcontroller schematic.



Appendix 2. Flight mill program. Arduino programming environment expects .ino file type.

```
// 8 channel flight mill counter
// 5/16/2017
// Use 8MHz 3.3V Sparkfun Pro-Mini or equivalent
//
// Wired for 8 hall effect switches on pins D2-D9
// input pins are pulled high internally, so hall
// effect switches should pull inputs to ground when
// activated by a magnet.
//
// record count data on a PC terminal emulator and/or
// attached OpenLog microSD card serial datalogger.
//
// Counter can be powered by USB convertor, but if PC
// sleeps the USB port may turn off and counts may be lost.
//


int LedPin =  13;

void setup()   {
  Serial.begin(9600);
  pinMode(2, INPUT_PULLUP);
  pinMode(3, INPUT_PULLUP);
  pinMode(4, INPUT_PULLUP);
  pinMode(5, INPUT_PULLUP);
  pinMode(6, INPUT_PULLUP);
  pinMode(7, INPUT_PULLUP);
  pinMode(8, INPUT_PULLUP);
  pinMode(9, INPUT_PULLUP);
  pinMode(LedPin, OUTPUT);

 Serial.println("v2 flight mill #n. 8 inputs"); // Customize message to differentiate files from
multiple counter units
 Serial.println("Time  and Counters reset!");
 Serial.println("Reports dummy channel 0 after every 5 sec with no new counts");
 Serial.println("Time(seconds*10), counter#, revolutions");    //time is reported in tenths of
seconds
}

long counts[10] = {0,0,0,0,0,0,0,0,0,0} ;
boolean last_state[10] {1,1,1,1,1,1,1,1,1,1};
boolean hall_state[10] {1,1,1,1,1,1,1,1,1,1};

long report_interval = 5000;        //output to terminal at least once every 5 sec
```

```
long last_report_time = millis();
long interval = 100;                //check pins every 100 mS
long last_time = millis();
long now;
int led_state;
int hall_num;
boolean output_counts = 1;

void loop()
{
 now = millis();

 while (now - last_time < interval)
 {
   now = millis();
 }

  last_time = now;

   if (led_state == LOW)        // heartbeat LED at half polling interval
      led_state = HIGH;
    else
      led_state = LOW;
   digitalWrite(LedPin, led_state);

  output_counts = 0;

  for ( int i = 2; i<10; i++ ) {     // poll pins 2 thru 9 and increment count on high going transitions
     hall_state[i] = digitalRead(i);
     if (hall_state[i] ==0 &&  last_state[i]==1) {
       counts[i]++;

       //v2 output each sensor seperately
       Serial.print(now/100 , DEC);
       Serial.print( ", ");
       Serial.print(i-1 , DEC);
       Serial.print( ", ");
       Serial.println( counts[i], DEC);

       output_counts = 1;
       last_state[i] = 0;
     } else if (hall_state[i] ==1 &&  last_state[i]==0){
       last_state[i] = 1;
     }
  }
```

```
  if (output_counts == 1){              //print line periodically if no counts detected to show
connection is alive.
    last_report_time = now;
    output_counts = 0;
  }

  if  (now - last_report_time >= report_interval){  //output dummy line if no new counts for
reportinterval/10 seconds
     Serial.print(now/100 , DEC);
     Serial.println(", 0, 0");
     last_report_time = now;
  }
}
```

Chelsea J. Jahant-Miller
(734) 972-6292 | cjjahant@syr.edu

Education

Doctor of Philosophy, Entomology, SUNY-ESF, Syracuse, NY Aug. 2015 - May 2020

Dissertation: Environmental drivers of morphological and physiological adaptation in an invasive defoliator, *Lymantria dispar*

Bachelor of Arts, Biology, Kalamazoo College, Kalamazoo, MI Sept. 2010 - May 2014

Awards and Grants

Tuition Scholarship Award, SUNY-ESF	2015-Present
OIGS Travel Grant, SUNY-ESF; $500, $350, $400	2016, 2017, & 2019
Edna Baily Sussman Internship, Sussman Foundation; $7,350 + $1,000 special merit award	2018
Simeone Fellowship Award, Dept. of Environmental and Forest Biology, SUNY-ESF; $8,425	2015

Publications

Jahant-Miller, C., Tobin, P.C., and Parry, D. Spatial and temporal changes in male gypsy moth wing morphology reflect host tree phenology and habitat quality. Submitted to *Agricultural and Forest Entomology*.

Barbehenn, R. V., Knister, J., Marsik, F., **Jahant-Miller, C.**, and Nham, W. (2015). Nutrients are assimilated efficiently by Lymantria dispar caterpillars from the mature leaves of trees in the Salicaceae. *Physiological Entomology*, *40*(1), 72-81.

Barbehenn, R. V., Haugberg, N., Kochmanski, J., Menachem, B., and **Miller, C.** (2014). Physiological factors affecting the rapid decrease in protein assimilation efficiency by a caterpillar on newly-mature tree leaves. *Physiological Entomology*, *39*(1), 69-79.

Conference Presentations

Jahant-Miller, C., and Parry, D. 2019. Flight behavior of male European Gypsy moth, *Lymantria dispar dispar*. Entomological Society of America. St. Louis, MO. Nov 17-20. (Oral)

Wu, J., **Jahant-Miller, C.**, Grayson, K., and Parry, D. 2019. Latitudinal clines in fecundity and resource allocation for an invasive forest defoliator. SUNY-ESF Spotlight on Student Research. Syracuse, NY. Apr 16. (Poster)

Jahant-Miller, C., Tobin, P., and Parry, D. 2017. Evaluating morphological characteristics of pheromone-trapped male gypsy moth (*Lymantria dispar* L.) as an index of habitat quality. Entomological Society of America. Denver, CO. Nov 5-8. (Oral)

Parry, D., **Jahant-Miller, C.**, and Tobin, P.C. 2016. More than a number: Biotic drivers of temporal and spatial change in the wing length of trap captured male gypsy moths. Annual Gypsy Moth Review. Columbus, OH. Oct 24-27. (Oral)

Jahant-Miller, C., Tobin, P., and Parry, D. 2016. Evaluating morphological characteristics of pheromone-trapped male gypsy moth (*Lymantria dispar* L.) as an index of habitat quality. International Congress of Entomology XXV. Orlando, FL. Sept 25-30. (Poster)

Research Experience

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2015-Present*
Doctoral thesis research conducted with Dr. Dylan Parry.
Investigated several physiological and evolutionary aspects of an invasive defoliating moth, *Lymantria dispar*.
Evaluated the combined effects of population density and habitat quality as determinants of male fitness using wing morphometrics of trapped male moths.
Elucidated the relationship between larval fitness and adult reproductive potential and fitness for male European gypsy moth using fixed arm flight mills.
Compared gypsy moth populations spanning the latitudinal range of the North American invasive range to detect a phenology-driven evolutionary shift in diapause and hatch physiology and revised a prediction for range expansion.

Department of Molecular, Cellular, and Developmental Biology, *University of Michigan, Ann Arbor, MI, 2011 & 2013*
Senior thesis research conducted under Dr. Ray Barbehenn.
Ran experiments to assess suitability for several host species of a major invasive forest insect, gypsy moth, by measuring protein and macronutrient assimilation efficiency.

University Teaching Experience

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2018-2019 (fall)*
Teaching assistant for Entomology. Led two weekly labs, wrote weekly quizzes and two practical exams, and designed lessons and activities to teach order and family level identification and ecology of insects to upper-level undergraduates. Directed undergraduate and graduate lab TAs.

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2019 (spring)*
Teaching assistant for Comparative Vertebrate Anatomy. Led two weekly labs, and wrote weekly quizzes and five practical exams. Prepared and implemented lab lectures, and managed undergraduate TAs to lead upper-level undergraduate students through vertebrate dissections designed to highlight evolutionary consistencies and modifications in anatomy across vertebrate taxa.

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2018 (fall)*
Guest lecturer for Plant-Insect Interactions. Designed and led two lessons to guide upper-level undergraduate students through the intricacies of plant-insect interactions in response to climate change. Lessons took place over two class periods, and consisted of a lecture followed by a seminar-style discussion.

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2018 (fall)*
Teaching assistant for Epidemiology. Supported faculty professor with grading, and offered critical lecture feedback. Designed and led a lecture on statistical methods in epidemiology.

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2016-2017 (spring)*
Teaching assistant for Diversity of Life II. Led two weekly labs and wrote weekly lab quizzes to highlight the global and local diversity and ecology of invertebrate and vertebrate divisions. Directed undergraduate lab TAs in order to provide seamless laboratory experience.

Department of Environmental and Forest Biology, *SUNY-ESF, Syracuse, NY, 2015-2016 (fall)*
Teaching assistant for General Biology. Led two weekly recitations and facilitated classroom discussions and activities to give students an introduction into topics in the diverse realm of biological sciences.

Professional and Academic Activities

Entomological Society of America	2016 - Present
Journal Reviewer Physiological Entomology	2017 - Present

Volunteer Outreach Educator, Baltimore Woods Nature Center, Marcellus, NY
Our Homes, Their Habitats, Volunteer January 2020
Insects, Earth Day Celebration 2020, Volunteer Outreach Educator April 2020
Invasive Species of Central NY, Volunteer Outreach Educator May 2020

Graduate Peer Mentor 2019 - Present

Undergraduate Research Mentor
Jeanne Wu, B.S., Spring 2019 Spring 2019
Adaptive shifts in resource allocation along latitudinal clines for an invasive forest pest, *Lymantria dispar*.
Matthew Carras, B.S., Spring 2020 Spring 2020
Estimating dispersal ability of *Philornis downsi* using fixed-arm flight mills.

Toastmaster International Member 2015 - 2017

Skills

- Fixed-arm flight mills
- Insect rearing
- Insect identification
- Statistical analysis
- Data collection and organization
- R statistical software
- Experimental design
- Insect trapping and monitoring
- Reciprocal transplant studies
- Oral and written communication
- Outreach and teaching
