# ECOLOGICAL FACTORS EXPLAINING GENETIC DIFFERENTIATION IN APHIDOMORPHA ASSOCIATED WITH PECAN AND WATER HICKORY TREES

A Dissertation

by

KYLE EDWARD HARRISON

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

| | |
|---|---|
| Chair of Committee, | Raul F. Medina |
| Committee Members, | Thomas J. DeWitt |
| | Cecilia Tamborindeguy |
| | Aaron M. Tarone |
| Head of Department, | David W. Ragsdale |

May 2017

Major Subject: Entomology

# ABSTRACT

Host-associated differentiation (HAD) is a form of ecologically mediated host-race formation between parasite populations. Since HAD can ultimately lead to speciation, it has been proposed as a way to account for the vast species diversity observed in parasitic arthropods. However, the importance of HAD to species diversity is unclear because the factors explaining the occurrence of HAD are only partially understood. Still, there are several examples of parasite-host case study systems for which there is a known cause of reproductive isolation between host-associated parasite populations. Thus, several biological and ecological factors (e.g., immigrant inviability or allochrony) have been proposed as explanatory factors for HAD occurrence. The body of research presented here represents the first quantitative assessment of the generalized relationship between HAD occurrence and the incidence of the proposed explanatory factors. This research was supported by field experiments that assessed the co-occurrence of HAD and particularly important explanatory factors. These experiments were conducted in a community of Aphidomorpha species living on pecan and water hickory trees. I found that HAD can be explained in general based on the incidence of specific explanatory factors (i.e. immigrant inviability, gall-making, short generation times, volatile preference, morphological differentiation, and host-shifting opportunities). These factors were used to create a hierarchy of conditional probabilities that can successfully separate the presence of HAD from its absence. The field experiments corroborated that the occurrence of HAD is correlated with immigrant inviability as well as allochrony.

# DEDICATION

This dissertation is dedicated to the people who loved and supported me throughout my doctorate. Thank you, Tammy Buffington, for being a devoted mother and mentor. Thank you, Steve Buffington, for always being there for me. Thank you, Lindsay Orlando, for sheltering me from hunger. Thank you, Chris Owens, for being my best friend for life. And thank you, Jordan Goebel, for your emotional support and your unwavering belief in me.

# ACKNOWLEDGEMENTS

This dissertation would not have been possible without Dr. Raul F. Medina's mentorship and limitless patience. I also thank Drs. Cecilia Tamborindeguy, Thomas J. DeWitt, and Aaron M. Tarone for allowing me to lean on their expertise throughout my doctorate program. I also extend special thanks to all of my lab mates, past and present. In particular, thanks go to Dr. Aaron Dickey for paving the way for this research by producing preliminary results within the pecan and water hickory Aphidomorpha community. Also, thank you, Drs. Josephine Antwi and Suhas Vyavhare, for your friendships and incredibly helpful advice. For helping me practice for both my preliminary examinations and defense talk, I want to thank Dr. Jason Carbaugh, Jocelyn Holt, Mackenzie Tietjen, and Crystal Wright. Lastly, thanks go to the Texas EcoLab program for providing invaluable funding opportunities.

# CONTRIBUTORS AND FUNDING SOURCES

## Contributors

This work was reviewed by a dissertation committee consisting of Associate Professor Raul F. Medina (Committee Chair) and Associate Professors Cecilia Tamborindeguy and Aaron M. Tarone of the Department of Entomology and Associate Professor Thomas J. DeWitt of Wildlife & Fisheries.

All work for the dissertation was completed by Kyle Harrison, under the advisement of Raul F. Medina. The genetic characterizations of pecan and water hickory Aphidomorpha supplied in Chapter I were analyzed in a collaboration between Kyle Harrison, Raul F. Medina of the Department of Entomology and Aaron M. Dickey and Larry J. Grauke of the USDA ARS and were published in *Entomologia Experimentalis et Applicata*, 2017.

## Funding Sources

This work was made possible in part by the Texas Ecolab Program. Partial financial support for this research was also provided by USDA Hatch funding (TEX09185, RFM). The contents of this dissertation are solely the responsibility of the authors and do not necessarily represent the official views of the Braun & Gresham, PLLC or the USDA.

# NOMENCLATURE

| | |
|---|---|
| B/CS | Bryan/College Station |
| HAD | Host-associated differentiation |
| TAMU | Texas A&M University |
| USDA ARS | United States Department of Agriculture: Agricultural Research Service |
| PCA | Principal Component Analysis |
| CCA | Canonical Correspondence Analysis |
| MANOVA | Multiple Analysis of Variance |

## TABLE OF CONTENTS

# LIST OF FIGURES

FIGURE Page

# LIST OF TABLES

# CHAPTER I

# INTRODUCTION TO RESEARCH AND STUDY SYSTEM[1]

This dissertation focuses on an ecologically-mediated form of host-race formation in which sympatric parasite populations become genetically distinct due to their host associations (Bush 1969, Feder, Chilcote and Bush 1988). This host-associated differentiation (HAD) represents an early stage in the continuum of ecological speciation and has the potential to account for the vast species diversity observed in parasitic arthropods, especially insect herbivores (Schluter 2001, Abrahamson, Blair, Eubanks and Morehead 2003, Stireman, Nason and Heard 2005) Theoretically, HAD is a phenomenon created and maintained by differential selection pressures experienced by host-associated parasite populations and leading to a subsequent reduction in gene flow between parasite populations (Nosil 2008, Feder, Egan and Nosil 2012). For example, if parasites experience strong, host-specific selection pressures linked to a trait important to parasite reproduction (e.g., traits determining host choice in parasite species that mate on their host), then reproductive isolation can develop between host-associated parasite populations alleles (Dres and Mallet 2002, Rundle and Nosil 2005, Geiselhardt, Otte, Hilker and Turlings 2012). Once reproductively isolated, parasite populations may accumulate genome-wide genetic differences due to genetic drift (especially if the

---

[1] Figure 1 of this chapter is the peer reviewed version of the following article: Host-associated differentiation in a pecan and water hickory Aphidomorpha community, which has been published in final form at the Journal of Entomologia Experimentalis et Applicata. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving. Figure 1 is reprinted with permission from Medina, Dickey, Harrison, and Miller, 2017.

number of individuals occurring on a novel host is initially small) and/or disruptive selection acting on adaptive alleles (Loxdale 2010, Michel, Sim, Powell, Taylor, Nosil and Feder 2010), this phenomenon can be detected by comparing genome-wide genetic markers between putatively host-associated populations (Hardison 2003, Stein et al. 2003). Since HAD leads to the accumulation of distinct genetic differences between reproductively isolated populations, HAD may constitute the beginning stages of ecological speciation (Schluter 2001, Wiens 2004, Rundle and Nosil 2005, Matsubayashi, Ohshima and Nosil 2010).

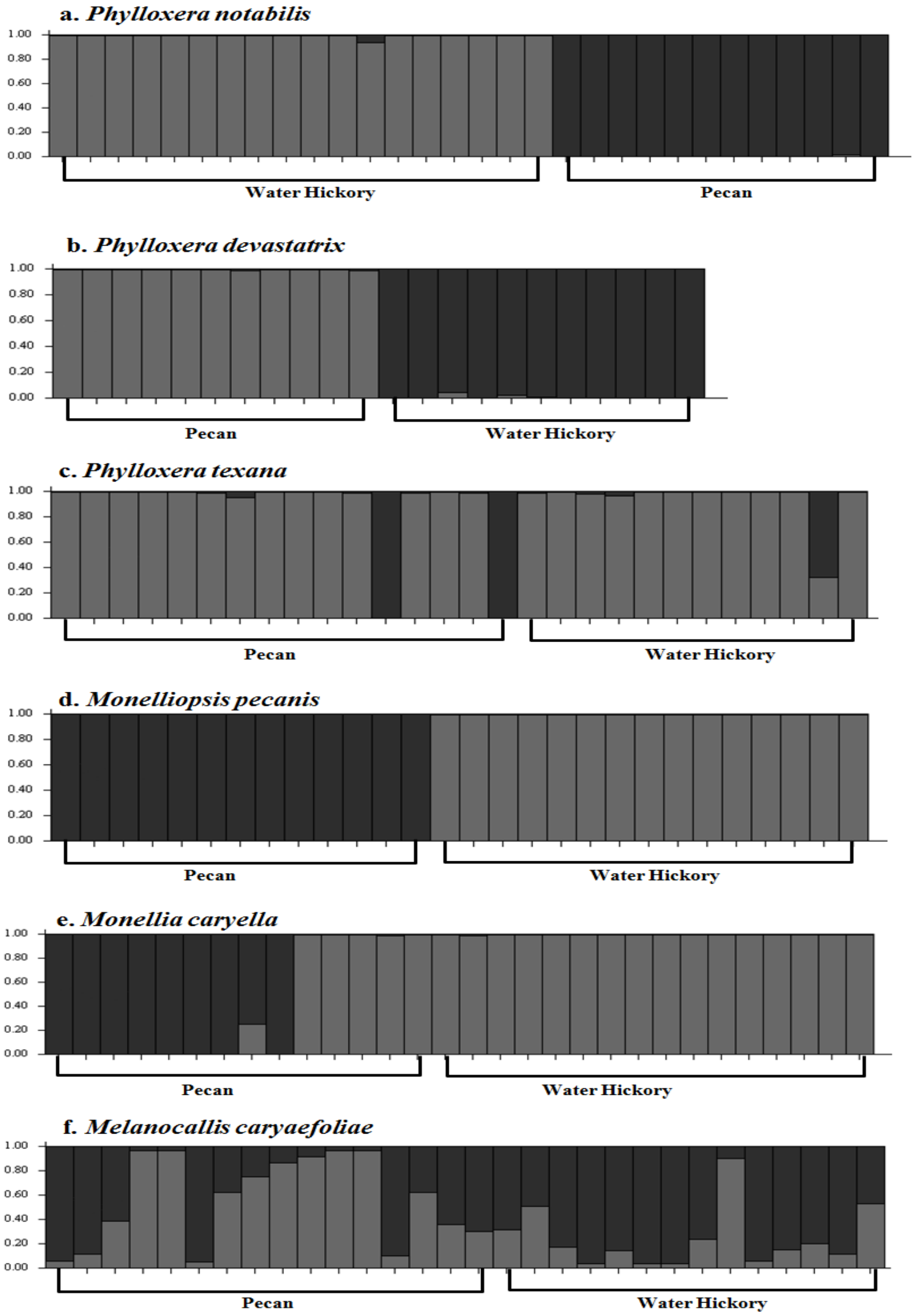
For example, multiple instances of host-mediated genetic differentiation or even speciation have been described among cactiphilic members of the Drosophila genus (Diptera: Drosophilidae) (Heed 1971, Fellows and Heed 1972). A complex of closely-related Drosophila species present in the Sonoran Desert (i.e., *D. arizonensis* Patterson & Wheller and *D. aldrichi* Patterson & Crow) experience host-mediated selection for choosing their natal cactus species over other available cactus species for oviposition (Ruiz and Heed 1988). This pattern of host-specificity in host/habitat choice by the Drosophila reflects the fact that these flies also experience a reduction in fitness when living on a novel cactus host. In addition to these fly species, *D. sechellia* Tsacas & Baechli and *D. simulans* Sturtevant also experience host-mediated selection but have instead adapted to plant secondary metabolites (R'kha, Capy and David 1991). *D. sechellia* has evolved to live exclusively on *Morinda citrifolia* L., a fruit-bearing citrus tree that is toxic to most insect species including the generalist D. simulans. This differential response to host-plant chemistry is sufficient for generating reproductive isolation between these species despite being genetically similar and living in sympatry (Kliman, Andolfatto, Coyne, Depaulis, Kreitman, Berry et al. 2000). For examples beyond Drosophila, Aphidomorpha appear to be useful for testing the relationship between factors potentially explaining the occurrence of HAD and the evolution of HAD (Via 1999, Via, Bouck and Skillman 2000, Peccoud, Ollivier, Plantegenest and Simon 2009). For example, several other aphid species have been shown to involve HAD: a figure in Dickey and Medina (2010) identifies 18 aphid species that have tested positive for HAD including the model pea aphid, *Acyrthosiphon pisum* Harris. However, these aphid species are all herbivores of herbaceous plants, meaning it is unclear whether arboreal aphids are prone to HAD. Like with most parasite-host systems involving HAD, the pecan and water hickory host-plants are perennial (Guldemond and Mackenzie 1994, Dres and Mallet 2002, Abrahamson, Blair, Eubanks and Morehead 2003, Blair, Abrahamson, Jackman and Tyrrell 2005, Leppänen, Malm, Värri and Nyman 2014). Unlike these systems, though, multiple generations of a single herbivore population can be sustained by an individual tree, meaning the host-specific selection pressures experienced by these parasites are in response to a single tree genotype within a species; this has the potential of increasing the stability and strength of selection for host-specific adaptations by decreasing the effect that environmental variability can have on HAD evolution (Edmunds and Alstad 1981, Mopper 2005, Dickey and Medina 2010, Craig, Itami, Ohgushi, Ando and Utsumi 2011). Aphids are also interesting for the study of HAD because they possess biological traits that have been hypothesized to increase the probability that HAD would evolve. Specifically, asexual organisms (such as aphids) may develop host-associated lineages faster than sexually reproducing organisms because recombination can break apart the accumulation of host-specific alleles under host-mediated divergent selection (Mitter, Futuyma, Schneider and Hare 1979, Lynch and Gabriel 1983, King 1993, Simon, Carre, Boutin, Prunier-Leterme, Sabater-Munoz, Latorre et al. 2003, Razmjou, Vorburger, Moharramipour, Mirhoseini and Fathipour 2010, Feder, Egan and Nosil 2012, Medina 2012).

Despite the existence of multiple parasite-host case study systems involving HAD, it is still unclear which set of biological and ecological factors best explain its occurrence (R'kha, Capy and David 1991, Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Feder, Hunt and Bush 1993, Komatsu and Akimoto 1995, Abrahamson and Weis 1997, Via 1999, Via, Bouck and Skillman 2000, Dres and Mallet 2002, Rundle and Nosil 2005, Stireman, Nason and Heard 2005, Sword, Joern and Senior 2005, Funk, Nosil and Etges 2006, Lozier, Roderick and Mills 2007, Dorchin, Scott, Clarkin, Luongo, Jordan and Abrahamson 2009, Peccoud, Ollivier, Plantegenest and Simon 2009, Schluter and Conte 2009, Matsubayashi, Ohshima and Nosil 2010, Barman, Parajulee, Sansone, Suh and Medina 2012, Dickey and Medina 2012, Hartfield and Keightley 2012, Medina, Szendrei, Harrison, Isaacs, Averill, Malo et al. 2013, Althoff, Fox and Frieden 2014). For example, Dr. Aaron Dickey, Dr. Raul F. Medina, and I characterized the host-associated genetic structuring in six species of arboreal Aphidomorpha (Medina, Dickey, Harrison, and Miller, 2017, *in press*). Initially, we hypothesized that feeding-mode would be the primary factor explaining the occurrence of HAD in pecan and water hickory Aphidomorpha. The Aphidomorpha community we studied consisted of three endophagous Phylloxeridae and three exophagous Aphididae (Dickey 2010). We endorsed this hypothesis because it was proposed that endophagous feeders may experience stronger differential selection than exophagous feeders such as prolonged and intense exposure to plant chemical defenses (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Cornell, Hawkins and Hochberg 1998, Stiling and Rossi 1998, Schick and Dahlsten 2003, Abbot and Withgott 2004). Additionally, galling parasites are further constrained since they require specific adaptations that allow them to manipulate host-plant metabolism to induce gall-formation; this paradigm is supported in the goldenrod system wherein two thirds of the endophagous feeders studied experienced HAD but no HAD was evident in either of the two exophagous feeders studied (Waring, Abrahamson and Howard 1990, Eubanks, Blair and Abrahamson 2003, Stireman, Nason and Heard 2005, Stireman, Nason, Heard and Seehawer 2006, Stireman, Janson, Carr, Devlin and Abbot 2008). In spite of our well-supported hypothesis, the genome-wide AFLP markers used to characterize population genetic structuring discovered that the pecan and water hickory Aphidomorpha community consists of two endophagous, gall-making phylloxeran species with complete HAD (*Phylloxera notabilis* Pergande and *P. devastatrix* Pergande), one exophagous aphid species with complete HAD (Monelliopsis pecanis Bissell), one phylloxeran without HAD (*P. texana* Stoetzel), one aphid without HAD (*Melanocallis caryaefoliae* Davis), and an aphid with a special case of ‘partial HAD’ in which a pecan-specialist population evolved alongside a host-generalist population (*Monellia caryella* Fitch) (Figure 1). Thus, the pattern of HAD occurrence in the pecan and water hickory Aphidomorpha community could not be explained by feeding-mode alone (Medina, Dickey, Harrison, and Miller, *in press*). Therefore, for the purposes of this dissertation, I proposed exploring other biological and ecological factors that could explain the occurrence of HAD in this community.

## STUDY SYSTEM DESCRIPTION

*Host-plants* – The study system in which I conducted my research involved two sympatric trees in the genus Carya: pecan, *Carya illinoinensis* Wangenh, and water hickory, *Carya aquatica* Michx (both Fagales: Juglandaceae). Since the Aphidomorpha involved in this study share the same host-plant species pair, this is an ideal system for making inferences about ecological traits potentially relevant to HAD without the confounding factors imposed by dealing with different host-plant species pairs. Pecan and water hickory, are large, deciduous trees which occur in sympatry in the study area. The Carya genus is native to North America and it is estimated to be at least 34 million years old (Manchester 1987). Modern pecan and water hickory trees are commonly found in areas of low water shed in the hardwood forests of eastern and southern North America (Fralish 2002). Even though pecan and water hickory trees are often difficult to distinguish visually, there are many differences between the plants which may promote or maintain HAD in insects. Specifically, water hickory trees break bud approximately three weeks after pecan trees (Grauke, Kalinsky and Strout 1984), making these trees phenologically distinct. Plants with different phenologies can create temporal selection

Figure 1. Population Genetic Structuring of Pecan and Water Hickory Aphidomorpha Based on AFLP's, Reprinted with Permission from Medina, Dickey, Harrison and Miller (2017). Each column represents an individual insect. The proportion of color in each column represents the probability that an individual is a member of one genetically distinct population (light grey) or another (dark grey). This figure is the peer reviewed version of the following article: Host-associated differentiation in a pecan and water hickory Aphidomorpha community, which has been published in final form at the Journal of Entomologia Experimentalis et Applicata. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.



pressures that influence the timing of adult insect diapause and eclosion, potentially isolating host-associated populations across time (i.e., allochronic isolation). Allochronic isolation has also been invoked to explain genetic differences in parasite-host systems involving HAD, including Rhagoletis pomonella Walsh (Diptera: Tephritidae) living on apple and hawthorn trees (Komatsu and Akimoto 1995, Feder and Filchak 1999). Phenological differences between pecan and water hickory trees are similar in magnitude to those of apple and hawthorn (Grauke, Pratt and Morris 1987). Therefore, Aphidomorpha experiencing host-associated selection pressures on these trees may have the potential to evolve allochronic isolation (further explored in Chapter IV). For the reasons stated above and because all Aphidomorpha considered in this system share the same host-plant species, I believe the pecan and water hickory systems provides an ideal system for testing the relationships between potentially explanatory factors (e.g., immigrant inviability and allochrony) and HAD.

*Parasites* – Likes their host-plants, the Aphidomorpha in this study system possess other traits hypothesized to increase the likelihood of HAD occurrence. Specifically, asexually reproductive insects (such as parthenogenetic aphids) may develop host-associated lineages faster than sexually reproducing insects because host-specific genetic changes are not lost to recombination during asexual generations. Thus, adaptive alleles rapidly increase in frequency and/or become fixed within populations, amplifying or accelerating host-specific responses to selection (discussed above). I studied three species of phylloxera (Hemiptera: Phylloxeridae) that all attack pecan and water hickory trees in central Texas: *Phylloxera notabilis, P. devastatrix*, and *P. texana*.

Unfortunately, little is known about these phylloxera species compared to their aphid counterparts. However, all three phylloxeran species are gall-forming, cyclically thelytokous parthenogens (Stoetzel 1985b). After overwintering, eggs hatch in the spring and summer and asexual females emerge. These females induce gall formation, living and taking food from inside the galls. These phylloxera also reproduce inside galls, laying eggs that develop into either sexual or asexual adults. Asexually reproducing phylloxera are all apterous while sexually reproducing adults are all winged. The sexual adults will eventually emerge from the gall and migrate. After mating, females either lay eggs on leaves or on the bark of tree branches. Eggs laid on trees will hatch during the same season, producing an asexual female that will induce gall-formation again (a generation of galls). Eggs laid on bark will overwinter and hatch during the next season (Stoetzel 1985a).

*P. notabilis* is commonly known as the pecan leaf phylloxera. Pecan leaf phylloxera galls form on pecan and water hickory leaves from mid-May to late July. Pecan leaf phylloxera produce five generations of galls annually.

*P. devastatrix* is commonly known as pecan stem phylloxera. Unlike the other phylloxera in this system, pecan stem phylloxera only produces a single generation of galls each year during May. Otherwise, their biology is quite similar to that of pecan leaf phylloxera.

*P. texana* have no common name. They are very similar to the pecan leaf phylloxera in appearance as well as life history and have only recently been described as a separate species (Stoetzel 1981). However, this phylloxeran exhibits a peculiar behavior in that it engages in host-alternation between asexual generations, a characteristic that the other pecan and water hickory phylloxera lack (Stoetzel 1985b).

I also study three aphid species (Hemiptera: Aphididae) that attack pecan and water hickory trees in central Texas: *Monelliopsis pecanis*, *Monellia caryella*, and *Melanocallis caryaefoliae*. All three aphid species feed on the underside of leaves and are also cyclically thelytokous parthenogens, producing dozens of asexual generations annually throughout the spring and summer months. Putatively, these asexual generations are followed by a single sexual generation in the fall.

*M. pecanis* is commonly known as the yellow pecan aphid and persists on pecan and water hickory trees from late-April to mid-August. Yellow pecan aphid populations experience between 22 and 32 overlapping generations of ovoviviparous thelytokous parthenogens annually, each adult producing 5 to 83 individuals in a life-time (Tedders 1978). The adults of the asexual generations are polyphenetic; they can be winged or apterous, meaning migration from one host tree to another is possible throughout the season. The yellow pecan aphid produces a single generation of winged, sexual adults in August (Tedders 1978). The asexual adults mate and produce eggs which will overwinter and hatch the following season.

*M. caryella* is commonly known as the black-margined aphid. Like the yellow pecan aphid, black-margined aphids persist on pecan and water hickory trees from late-April to mid-August. Black-margined aphid populations experience 16 to 32 overlapping generations of ovoviviparous thelytokous parthenogens annually, each adult producing 80 to 215 offspring in a life-time (Tedders 1978). All black-margined aphid adults are winged and, thus, have the potential to migrate all season. Like yellow pecan aphid, the black-margined aphid produces a single sexual generation during August. The sexual adults mate and produce eggs which will overwinter and hatch the following season.

*M. caryaefoliae* is commonly known as the black pecan aphid and persists on pecan and water hickory trees from late-April to September. Unlike the yellow pecan aphid and the black-margined aphid, though, the black pecan aphid has a relatively low population density until late-July to early-August (Tedders 1978). This is due to the first few generations reproducing less than later generations. All asexual black pecan aphid adults are winged and, thus, have the potential to migrate all season. The black pecan aphid produces a single generation of apterous sexual females and winged sexual males in September (Tedders 1978). The sexual adults mate and produce eggs which will overwinter and hatch the following season.

The goal of this dissertation was to explain the evolution of HAD in pecan and water hickory Aphidomorpha and HAD in general. For example, Figure 14.1 of Medina (2012) describes the theoretical relationship between the likelihood of HAD evolving in a given parasite-host system and two factors believed to mediate the evolution of HAD: recombination rate and level of concealment. Specifically, the less recombination a population undergoes (such as with thelytokous Aphidomorpha), the more likely that host-specific adaptations will accumulate and vice versa. Also, organisms that are completely concealed and live mostly inside their host-plant (e.g., fruit-feeders and gall-makers). The ideas presented by the verbal model are supported by parasite-host case study systems; for example, genetic recombination between populations experiencing disruptive, host-associated selection pressures can break up the independent accumulation of host-adapted alleles within host-associated populations (Rundle and Nosil 2005, Michel, Sim, Powell, Taylor, Nosil and Feder 2010, Feder, Egan and Nosil 2012, Flaxman, Feder and Nosil 2013). However, models such as the one presented in Medina 2012 have yet to be verified quantitatively; for more examples of these verbal models, see (Feder, Chilcote and Bush 1988, Via 1999, Via, Bouck and Skillman 2000, Nosil, Vines and Funk 2005, Stireman, Nason and Heard 2005, Funk, Nosil and Etges 2006, Nosil, Harmon and Seehausen 2009). Therefore, Chapter II of this dissertation resolves this issue by making a quantitative assessment of the explanatory power for several key factors previously proposed to explain the occurrence of HAD. I show that a few, key factors (e.g., gall-making, immigrant inviability, short parasite generation times, and volatile preference) are significantly related to the presence of HAD and can explain its occurrence based on conditional probabilities. Since the results of the Chapter II research show that immigrant inviability is an especially important factor explaining HAD occurrence, Chapter III is dedicated to testing for immigrant inviability in a pecan and water hickory aphid species (yellow pecan aphid). This aphid experiences a special form of HAD, called ‘partial HAD’ in which a host-specialist population lives in sympatry with a host-generalist population. This ‘partial HAD’ provides an exciting opportunity for testing the evolution of host-plant specialization by comparing a specialist population to a sympatric generalist population within the same species complex. Reciprocal transplant experiments showed that pecan-specialist aphids experience a reduction in fitness when transferred to water hickory while generalist aphids experience no such reduction. These results support the significance of immigrant inviability in explaining HAD occurrence. Finally, Chapter IV explores a factor that could not be properly assessed in the quantitative literature due to a lack of representative studies present in the current literature: allochrony. Despite the absence of supporting literature, allochronic isolation is thought to be important since it explains the occurrence of HAD in the model organism, *Rhagoletis pomonella* (Feder, Hunt and Bush 1993, Feder and Filchak 1999). Therefore, I tested for evidence of allochronic isolation in three pecan and water hickory aphid species: *M. pecanis*, *M. caryella*, and *M. caryaefoliae*; this was done by surveying the average aphid density per tree throughout the pecan and water hickory growing season. For all three species, the occurrence of

HAD correlates with the occurrence of allochrony, adding support to the importance of allochrony as a potential factor explaining HAD occurrence.

# CHAPTER II

# EXPLAINING THE OCCURRENCE OF HOST-ASSOCIATED DIFFERENTIATION: A QUANTITATIVE LITERATURE REVIEW

## SYNOPSIS

The evolution of reproductive isolation between parasite populations associated with different host species, or host-associated differentiation (HAD), has been proposed as a significant source of parasitic arthropod diversity. Previous research has described verbal models attempting to attribute the occurrence of HAD to specific organismal factors believed to generate or maintain reproductive isolation between host-associated parasite populations. However, the relevance of postulated factors thought to explain HAD is uncertain. Although, postulated factors have been shown to be important for the evolution of HAD in specific case studies, their generalizability have yet to be quantitatively assessed. In this research, I performed a quantitative literature review that evaluated the correlation between putative explanatory factors and the general occurrence of HAD. First, a MANOVA and canonical correspondence analysis (CCA) were used to characterize the simple co-occurrence between putative explanatory factors and HAD occurrence. Although the MANOVA showed that some factors and combinations of factors are significantly correlated with HAD occurrence (e.g., gall-making, immigrant inviability, short generation times, and volatile preference), the CCA showed that these factors, taken altogether, were not able to successfully distinguish HAD presence from absence. Thus, I used an infinite forest algorithm to test whether

conditional probabilities based on the incidence of significant explanatory factors could successfully separate the presence of HAD from the absence of HAD. The results of the infinite forest algorithm allowed us to develop a probabilistic decision tree to successfully explain the occurrence of HAD based on significant explanatory factors. The decision tree gives researchers a tool for making general predictions about the likelihood that a given parasite-host system would involve HAD and supports the hypothesis that HAD can arise through several distinct mechanisms involving combinations of reproductive isolating factors.

## INTRODUCTION

Parasitic Arthropoda are one of the most biodiverse groups of multicellular life. Of the estimated 1 million described arthropod species, over 60% are involved in some form of parasitism with plant or animal hosts (Price 1980, Poulin 1997, Dobson, Lafferty, Kuris, Hechinger and Jetz 2008). Parasites represent the majority of species in some of the most specious insect clades (e.g., Coleoptera, Hemiptera, Hymenoptera), especially among phytophagous insects (Mitter, Farrell and Wiegmann 1988, Wiegmann, Mitter and Farrell 1993, Farrell 1998). In fact, estimates of total species number for several obligate parasitic arthropod clades (e.g., Braconidae, Ichneumonidae, and Acariformes) are a magnitude greater than the current number of described species, meaning the actual prevalence of parasitism among arthropods is expected to be greater than 60% (Slowinski and Guyer 1989, Dolphin and Quicke 2001, Smith, Rodriguez, Whitfield, Deans, Janzen, Hallwachs et al. 2008). Forbes et al. succinctly describe the relationship between parasitism and species diversity when they theorized “a major cause for biodiversity may be biodiversity itself” (Forbes, Powell, Stelinski, Smith and Feder 2009). Ecological speciation has been proposed as a mechanism contributing to parasitic arthropod diversity (Futuyma and Moreno 1988, Schluter 2001, Via 2001, Rundle and Nosil 2005, Mallet, Meyer, Nosil and Feder 2009, Matsubayashi, Ohshima and Nosil 2010). Under ecological speciation, divergent selection imposed by parasite hosts can drive the evolution of reproductive isolation among parasites associated with different hosts (Price 1980, Funk 1998). If disruptive selection on different host species acts on parasite traits linked to their reproduction, then strong reproductive isolation can evolve relatively quickly between parasite populations on these different hosts (Bush 1969, Groman and Pellmyr 2000b, Althoff, Groman, Segraves and Pellmyr 2001). The host-mediated evolution of reproductive isolation between parasite populations associated with different host species can cause them to independently accumulate host-specific adaptations through selection and genetic drift (Bush 1975a, Jaenike 1990, Berlocher and Feder 2002, Abrahamson, Dobley, Houseknecht and Pecone 2005, Flaxman, Feder and Nosil 2013). Given enough time, this process of host-associated differentiation (HAD) can lead to the evolution of pre- and post-mating reproductive incompatibilities between parasite populations. Therefore, HAD constitutes an early stage of ecological speciation by which host-races evolve prior to co-cladogenesis (Bush 1994, Pritchard, Stephens and Donnelly 2000, Via and Hawthorne 2002, Linn, Dambroski, Feder, Berlocher, Nojima and Roelofs 2004, Futuyma 2008, Peccoud, Ollivier, Plantegenest and Simon 2009, Michel, Sim, Powell, Taylor, Nosil and Feder 2010, Powell, Hood, Murphy, Heilveil, Berlocher, Nosil et al. 2013). For this reason, HAD has been proposed as a way of explaining the origins of parasitic arthropod diversity (Funk 1998, Stireman, Nason and Heard 2005, Funk 2010).

It is still unclear, though, how common HAD is in nature and which parasitic arthropod systems are prone to its development (Stireman, Nason and Heard 2005). Multiple factors have been proposed to account for the occurrence of HAD (Bush 1974, Bush 1975b, Price 1977, Futuyma and Moreno 1988, Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Feder and Filchak 1999, Via 1999, Via, Bouck and Skillman 2000, Abrahamson, Eubanks, Blair and Whipple 2001, Craig, Horner and Itami 2001, Funk, Filchak and Feder 2002, Nosil, Crespi and Sandoval 2002, Nosil, Vines and Funk 2005, Funk, Nosil and Etges 2006, Stireman, Nason, Heard and Seehawer 2006, Dorchin, Scott, Clarkin, Luongo, Jordan and Abrahamson 2009, Forister, Dyer, Singer, Stireman and Lill 2012, Medina 2012). These factors are thought to promote the evolution of reproductive isolation between host-associated parasite populations. The most commonly discussed factors include:

*Host fidelity* – Host-associated parasitic arthropods can experience host fidelity or the tendency of insects to remain on or return to a host used as a nymph or larva (Bush 1969, Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Wood, Tilmon, Shantz, Harris and Pesek 1999, Craig, Horner and Itami 2001, Stelinski and Liburd 2005). When parasite populations use different host species, the evolution of preference for a natal host can result in divergent selection in response to differential cues (e.g., plant volatile preference) presented by different host species (Abrahamson, McCrea and Anderson 1989, McCall, Turlings, Lewis and Tumlinson 1993, Frey, Feder, Palma and Bush 1998, Pureswaran, Gries and Borden 2004). When host preference evolves in parasites that rely on their hosts to find a mate, then parasites populations may experience pre-mating reproductive isolation (Futuyma and Moreno 1988, Jaenike 1990, Egan and Funk 2006). For example, host fidelity has been demonstrated in pea aphid populations (*Acyrthosiphon pisum* Harris) experiencing HAD. Pea aphids from clover prefer to feed on clover compared to alfalfa and aphids from alfalfa prefer to feed on alfalfa or clover (Via 1999, Via, Bouck and Skillman 2000). Similarly, the apple maggot (*Rhagoletis pomonella* Walsh) consists of two host-associated populations, one preferring to oviposit and mate on Hawthorne trees and another population preferring apple trees (Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Dambroski, Linn, Berlocher, Forbes, Roelofs and Feder 2005). Importantly, the host fidelity of apple maggots is also reflected in their preference for host-specific volatiles that assist them in recognizing suitable habitats (Frey, Feder, Palma and Bush 1998).

*Allochronic isolation* – In order for parasites to survive, they must be able to appear in the same space and time as their hosts or when their host produces the resources on which they depend (e.g., fruits and seeds). This dependency imposes selection on parasites to match their hosts' phenology. If host-associated parasite populations adapt to different host species with divergent phenologies, then parasites will experience disruptive selection in traits relevant to seasonal timing. This can generate temporal or ‘allochronic’ reproductive isolation (Alexander and Bigelow 1960, Groman and Pellmyr 2000b, Raijmann and Menken 2000, Cooley, Simon, Marshall, Slon and Ehrhardt 2001, Abbot and Withgott 2004, Fudickar, Greives, Atwell, Stricker, Ketterson, Williams et al. 2016). For example, differences in fruit availability between apple and hawthorn trees impose differential selection pressures on adult emergence and eclosion times in the apple maggot, generating HAD (Bush 1969, Feder, Chilcote and Bush 1988, Feder and Filchak 1999, Raijmann and Menken 2000).

*Selection against migrants* – Pre-mating isolation can evolve when migrants moving from a natal host species to a novel host species experience a reduction in fitness, reducing contact between host-associated populations of a parasite species (Funk 1998, Via, Bouck and Skillman 2000, Nosil, Vines and Funk 2005, Nosil 2007, Dickey and Medina 2011a). Furthermore, if hybrids of migrants retain the maladapted traits of their parents, then post-mating isolation can also evolve between parasite populations (Wade and Johnson 1994, Wu and Palopoli 1994, Naisbit, Jiggins and Mallet 2001, Schluter 2001, Rundle 2002). In order for immigrant inviability to evolve, parasites must acquire pre-adaptations that allow them to survive on a novel host species, creating a barrier to reproduction by physically separating parasites with host-specific adaptations from parasites without similar adaptations. Thus, selection against migrants could result from the expression of one or several traits acting in concert (e.g., habitat preference and allochrony) to increase the specialization of parasite populations to the hosts they parasitize (Matsubayashi, Ohshima and Nosil 2010).

*Feeding mode* – Endophagous feeders are thought to experience stronger disruptive selection pressures compared to exophagous feeders (Cornell, Hawkins and Hochberg 1998, Stiling and Rossi 1998, Stireman, Nason and Heard 2005). For example, endophagous feeders encounter prolonged and intense exposure to plant chemical defenses compared to exophagous feeders (Waring, Abrahamson and Howard 1990, Abbot 2001, Abrahamson, Eubanks, Blair and Whipple 2001). This is especially true for gall-making phytophagous insects, which are presumed to experience particularly strong host-associated selection pressures due to the hypothetical gene-by-gene mechanism thought to be behind the hijacking of plant physiology in order to induce gall formation (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Brown, Abrahamson, Packer and Way 1995, Abrahamson, Eubanks, Blair and Whipple 2001, Stireman, Janson, Carr, Devlin and Abbot 2008, Dickey and Medina 2012). The selection pressures inherent in gall-making biology may promote the evolution of pre- and post-mating reproductive isolation since hybrids of parasites adapted to a natal host-plant species would likely fail to successfully induce gall formation in novel host-plants (Craig, Itami, Horner and Abrahamson 1994, Itami, Craig and Horner 1998).

*Reproductive mode* – Parthenogenetic parasites (e.g., aphids, thrips, and certain mites) may evolve reproductive isolation faster than sexually reproducing insects since host-specific adaptations would not be lost to recombination (or would be lost at a much lower rate in the case of cyclical parthenogens). Thus, adaptive alleles would rapidly increase in frequency and/or become fixed within populations, amplifying or accelerating host-specific genetic differentiation (Hartl 1972, Lynch and Gabriel 1983, Lynch 1984, King 1993, Neiman and Linksvayer 2006, Loxdale 2008, Dickey and Medina 2010). Parthenogens usually possess short generation times which could also result in the relatively rapid accumulation of host-specific adaptations (Martin and Palumbi 1993, Li, Ellsworth, Krushkal, Chang and Hewett-Emmett 1996). Furthermore, parasites with short generation times would experience several more rounds of host-mediated selection pressures per growing season compared to parasites with long generation times. The effect of low recombination rate and short generation times on the speed of host-specific adaptation is apparent in aphids since HAD appears to be particularly common in these parasites; for examples, see Dickey and Medina (2010).

*Sexual selection* – Divergent mating cues and habitat preference can reduce the probability of individual parasites successfully initiating mating between host-associated parasites, generating sexual reproductive isolation. For example, host-mediated mate choice has been documented between populations of goldenrod gall flies (*Eurosta solidaginis* Fitch) associated with different goldenrod species (Craig, Itami, Abrahamson and Horner 1993, Craig, Horner and Itami 1997). Also, sexual isolation has been observed between populations of walking sticks (*Timema cristinae* Vickery) associated with two different host-plant species (*Ceanothus spinosus* and *Adenostoma* *fasciculatum*) (Nosil, Crespi and Sandoval 2002). In these cases of sexual isolation, morphological differences arose between host-associated populations, meaning the relationship between sexual isolation and morphological differentiation is intrinsically linked. Sexual isolation might arise because of distinct morphological traits linked to host-plant use, however the actual factors contributing to sexual isolation between populations adapted to different hosts are usually unknown (Jiggins, Hurst and Majerus 2000, Dobson, Fox and Jiggins 2002, Fordyce and Nice 2003, Nosil 2007, Egan, Nosil and Funk 2008, Fordyce 2010). Therefore, I chose to examine morphological differentiation as a potential indicator of sexual selection. Such assertion, though, would need to be verified in the future due to the fact morphological differentiation can arise through other host-associated factors such as the ability to feed or oviposit on a particular host (Pappers, Velde, Ouborg and Groenendael 2002).

*Host shifting opportunities* – Parasites may use a novel host species if it is closely related to their natal host species and shares characteristics important to successful parasitism (Berlocher and Feder 2002, Dres and Mallet 2002). The closer a novel host species' characteristics are to the natal host species, the higher the probability that a parasite will mistakenly accept a novel host and the higher the probability that the parasite can survive on the novel host. For example, when parasite species are provided an opportunity to expand their range (e.g., invasive insect pests in a new continent), then host shifts may take place (Murphy 2004, Schwarz, Matta, Shakir-Botteri and McPheron 2005, Agosta 2006, Craig, Itami, Ohgushi, Ando and Utsumi 2011). Since the interaction between hosts and parasites is likely to select for host-specific adaptations in parasites (see ‘*Habitat fidelity*’ and ‘*Selection against migrants*’ above), then systems involving invasive or otherwise non-native hosts or parasites may be prone to the development of HAD.

Although many of these factors have been shown to cause reproductive isolation and are thought of as the most likely causes of HAD in the specific case studies in which they have been described (e.g., allochrony in apple maggots; habitat fidelity in pea aphids; and immigrant inviability in *Timema* walking sticks, etc.), it is unclear which (if any) of these factors can explain the occurrence of HAD in general. Highlighting this consideration is the existence of several examples in which HAD is absent in parasite-host systems even though they present the factors thought to explain HAD (Jaenike and Selander 1980, Clements, Sorenson, Wiegmann, Neese and Roe 2000, Baer, Tripp, Bjorksten and Antolin 2004, Gómez- Díaz, González- Solís, Peinado and Page 2007, Althoff 2008, Lozier, Roderick and Mills 2009, Dickey and Medina 2010, 2011b, Kohnen, Wissemann and Brandl 2011, Simonato, Battisti, Zovi and Medina 2012). For example, host-specific volatile preferences and allochrony failed to generate HAD in the cranberry fruitworm (*Acrobasis vaccinii* Riley) on blueberry and cranberry (Medina, Szendrei, Harrison, Isaacs, Averill, Malo et al. 2013). In contrast, these same factors were sufficient for generating HAD in the blueberry gall midge (*Dasineura oxycoccana* Johnson) in the same blueberry and cranberry system (Cook, Ozeroff, Fitzpatrick and Roitberg 2011). Similarly, pecan and water hickory trees share six Aphidomorpha species, all expected to exhibit some degree of HAD due to their shared parthenogenetic mode of reproduction yet only three species exhibit HAD (Medina, Dickey, Harrison, and Miller, *in press*). Thus, there exists a need to determine the specific biological and ecological factors that best explain the occurrence of HAD in general.

The ability to predict HAD occurrence has implications for evolutionary biology, agriculture, and disease ecology. First, as discussed above, HAD may account for the high level of diversity observed in parasitic arthropods. Second, host-associated populations of pestiferous insects can differ in host-associated adaptations relevant to pest control (Hufbauer and Roderick 2005, Medina 2012). Lastly, HAD has important implications for disease ecology specifically with respect to vector competence (Burban, Fishpool, Fauquet, Fargette and Thouvenel 1992, McCoy, Léger and Dietrich 2015, Esteve‐Gassent, Castro‐Arellano, Feria‐Arroyo, Patino, Li, Medina et al. 2016). In order to predict which parasite-host systems are prone to the evolution of HAD in general, I performed a quantitative literature review that compared the occurrence of HAD against the incidence of factors previously proposed to explain HAD occurrence in specific case studies. I hypothesized that the occurrence of HAD is non-random and, thus, significantly associated with the occurrence of specific explanatory factors.

## METHODS

*Data sources and searches*. I created a data matrix comprised of parasite-host case-study systems tested for HAD and the factors hypothesized to cause the reproductive isolation required for HAD evolution to take place. A single investigator (Harrison, K.) searched for SYNOPSISs that described case studies of parasite-host systems tested for genetic population structuring in Google Scholar, Web of Science, and JSTOR internet search engines. Key words and phrases employed in the search are provided in Table 1. Case studies were included in the database if 1) genetic markers (e.g., microsatellites, AFLP's, COI) were employed that characterized genetic structuring among multiple sympatric parasite populations on different hosts and if 2) the experimental design specifically characterized genetic structuring by host species associations. The literature cited in the case-studies was also searched for additional parasite-host systems. Case-studies included in the database were placed into one of two categories: Case-studies that unambiguously demonstrated genetic population structuring and host-species (HAD present) and case-studies that unambiguously demonstrated that population genetic structuring was not correlated with host species (HAD absent). Assignment of case studies to an HAD category was codified with a 1 or 0, creating an independent variable data matrix comprised of 97 case studies (64 'HAD present' and 33 'HAD absent' cases).

In addition to assigning case studies to HAD category, the main case studies and the scientific literature cited in the main case-studies themselves were searched for ecological and biological factors hypothesized to explain HAD observed in each parasite-host case study system. For each case study considered, key words representing proposed explanatory factors for HAD (see Table 1) were searched for all species

Table 1. Search Terms Used to Discover HAD Case Study Systems. Words in parentheses were included in search terms after searching for words outside of parentheses. The words “host” and “parasite” were also replaced with the specific names of each case study organism.

| *HAD case study search terms* | *HAD explanatory factor search terms* |
|---|---|
| HAD | allochrony |
| assortative mating | annual |
| differentiation | community ecology |
| ecological specialization | concealment |
| ecological speciation | (cyclical) parthenogenesis |
| genetic structuring | (differential/distinct) host (plant) phenology |
| habitat choice | endophagy |
| host (plant)-association (selection) | exophagy |
| host discrimination | gall (making) |
| host fidelity | generalist |
| host race | generation (time) |
| host-related | host (plant)/parasite invasive/non-native |
| host specific | host (plant)/parasite native |
| incipient speciation | hybrid unfitness |
| population structuring | immigrant inviability |
| reproductive incompatibility | longevity |

Table 1 continued.

| *HAD case study search terms* | *HAD explanatory factor search terms* |
|---|---|
| reproductive isolation | morphology/morphological (differentiation) |
| *Rhagoletis* | multivoltine |
| specialization | pest management |
| sympatric (speciation) | oviposition preference |
|  | perennial |
|  | recombination (rate) |
|  | sexual reproduction |
|  | specialist |
|  | trophic (level) |
|  | univoltine |
|  | volatile (preference) |

involved (i.e., all parasites and host species) in the case study and in the references each study cited. A total of 129 journal articles that were the main resources for descriptions of the case study systems were used to construct a data matrix of potential explanatory factors based on the following criteria: 1) The authors tested for the presence or absence of an explanatory factor that could cause reproductive isolation between parasite populations or 2) Provide citations of authors who had tested these factors in previous research. A list of the main case study system descriptions are provided in The Appendix. Explanatory factors were codified with a 1 (present) or 0 (absent), creating a data matrix comprised of 2247 cells. Matrix cells represented descriptions of explanatory factors across all case-study systems. Because several potentially explanatory factors have yet to be tested in some parasite-host case study systems, only 1882 out of 2247 (83.8%) matrix cells were available for my analysis. To avoid the exclusion of entire case studies or potential explanatory factors, I employed multiple imputation to simulate missing cell values (Hastie, Tibshirani, Sherlock, Eisen, Brown and Botstein 1999, Horton and Lipsitz 2001). The singular value decomposition method (SVD) of multiple imputation was performed in JMP®, Version 12 (SAS Institute Inc., Cary, NC, 1989-2016); the SVD method works well with large datasets (Schmitt, Mandel and Guedj 2015).

*Statistical analyses.* All statistical analyses were performed using the JMP®, Version 12. First, principal components (PCs) were calculated in a discriminant function analysis (DFA) to select a subset consisting only of the highest-order variables (i.e., the specific explanatory factors associated with PC’s that captured >95% of the total variance) (Jolliffe 2002). The significance of the selected subset of variables compared to unselected variables was evaluated using an ANOVA with $\alpha = 0.05$. The DFA used here is similar to the technique employed by de Ruiter et al. (2013) (de Ruiter, DeWitt, Carlson, Brophy, Schroeder, Ackermann et al. 2013), which reduces a large dataset with many, competing variable effects into a smaller, more informative dataset. This reduced dataset consisted of nine, informative explanatory factors (the significant factors shown in Table 2). A canonical correspondence analysis (CCA) was used to visualize the whole-model effect of distinguishing ‘HAD present’ cases from ‘HAD absent’ cases (Ter Braak 1986). A multivariate analysis of variance (MANOVA) was also performed to test the significance of individual effects among explanatory factors in explaining HAD occurrence as well as the interaction effects between factors. Effect strength scores were extracted from MANOVA and provided a heuristic estimate of the relative contribution of each independent variable (i.e., the presence or absence of explanatory factors) to the total variance separating HAD presence from HAD absence. This allowed us to observe whether HAD occurrence was positively or negatively correlated with a given explanatory factor. To characterize the interaction effects between dependent variables, an infinite random forest algorithm in JMP®, Version 12 (SAS Institute Inc., Cary, NC, 1989-2016) was used to create a LogWorth-supported decision tree that segregates ‘HAD present’ cases from ‘HAD absent’ cases based on the presence or absence of specific explanatory factors

Table 2. MANOVA Results Comparing HAD Occurrence and the Incidence of Biological and Ecological Factors. MANOVA table depicting whole model effects, individual variable effects, degrees of freedom for the numerator (numDF) and denominator (denDF), and significant interaction effects. Bolded values indicate significance for α=0.05.

| *Source* | *F-value* | *numDF* | *denDF* | *Effect Strength* | *Prob>F* |
|---|---|---|---|---|---|
| Whole Model | 1.1481 | 47 | 53 | **-** | **0.0354** |
| Asexual reproduction | 0.0234 | 1 | 91 | -0.155 | 0.1479 |
| Differential host phenology | 0.0008 | 1 | 91 | +0.025 | 0.7867 |
| Endophagy | 0.0003 | 1 | 91 | -0.018 | 0.8639 |
| Gall-making | 0.0582 | 1 | 91 | +0.342 | **0.0236** |
| Immigrant inviability | 0.0572 | 1 | 91 | +0.236 | **0.0248** |
| Morphological differentiation | 0.0179 | 1 | 91 | +0.120 | 0.2057 |
| Parasite or host non-native | 0.0095 | 1 | 91 | +0.088 | 0.3557 |
| Short generation times | 0.0458 | 1 | 91 | +0.213 | **0.0441** |
| Volatile preference | 0.0731 | 1 | 91 | +0.273 | **0.0115** |
| Immigrant inviability*Short generation times | 0.2034 | 1 | 53 | **-** | **0.0018** |
| Immigrant inviability*Morphological differentiation | 0.1075 | 1 | 53 | **-** | **0.0205** |
| Parasite or host non-native*Volatile preference | 0.0799 | 1 | 53 | **-** | **0.0444** |

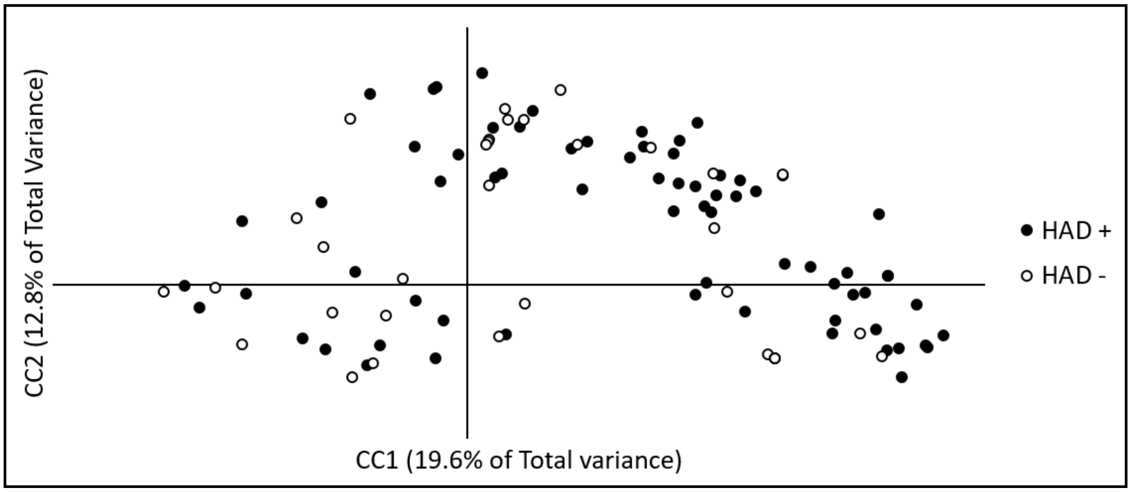
(Prasad, Iverson and Liaw 2006, Cutler, Edwards, Beard, Cutler, Hess, Gibson et al. 2007, Criminisi, Shotton and Konukoglu 2012). To determine the most appropriate number of end-points for the decision tree, the k-means clustering method was used to determine the number of distinct groups among HAD case studies based on the explanatory factors (Welling and Kurihara 2006).

Finally, to demonstrate the power of the proposed decision tree model, I conducted a validation test by adding parasite-host case study examples to the analysis and directly observing whether the model was able to correctly sort these specific case study examples into their respective ‘HAD present’ and ‘HAD absent’ categories. This was done by adding multiple parasite-host case study systems that were not originally included in the generation of the model and recalculating the conditional probabilities separating HAD presence from absence. The 11 parasite-host case study systems used in this validation test involved cases in which HAD was unambiguously present or absent. The parasite-host case study systems used for the validation study are highlighted in gray at the bottom of The Appendix.

## RESULTS

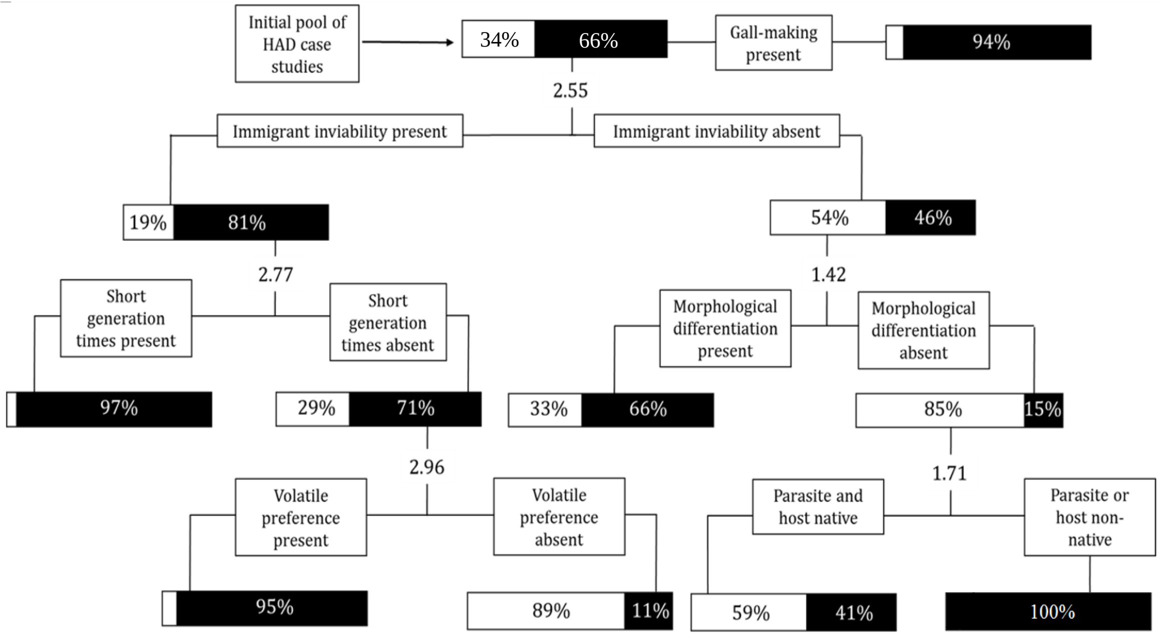
My analyses show that there is not a single, generalizable factor that can distinguish the presence of HAD from its absence, however an algorithm based on conditional probabilities can successfully separate HAD presence from HAD absence. My discriminant function analysis revealed that nine of the explanatory factors together could account for 95.1% of the total model variance ($P<0.001$). These were: parasite asexual reproduction, differential host-plant phenology, parasite endophagy, parasite gall-making, immigrant inviability in the parasite, parasite morphological differentiation, host-shifting opportunities, short generation times in parasites, and parasite volatile preference. My canonical correspondence analysis (CCA) was only able to capture 19.6% of the total variance on the first canonical axis while the second canonical axis captured 12.8% (Figure 2). In addition, the CCA did not reveal distinct clusters that unambiguously separate the presence of HAD from its absence. In contrast, results from the MANOVA comparing HAD presence and HAD absence showed a significant whole-model effect ($F_{47, 53}=1.1481$; $P<0.0354$). The MANOVA also showed that parasite gall-making ($F_{1, 91}=0.0581$; $P<0.0236$), parasite immigrant inviability ($F_{1, 91}=0.0572$; $P<0.0248$), parasite short generation time ($F_{1, 91}=0.0458$; $P<0.0441$), and parasite volatile preference ($F_{1, 91}=0.0731$; $P<0.0115$) were all significantly correlated with the presence of HAD (Table 2). In addition to these significant individual factors, the MANOVA indicated that three combinations of explanatory factors are significantly correlated with HAD occurrence: immigrant inviability-by-short generation times ($F_{1, 53}=0.2034$; $P<0.0018$), immigrant inviability-by-morphological differentiation ($F_{1, 53}=0.1075$; $P<0.0205$), and the host-shifting opportunities-by-volatile preference ($F_{1, 53}=0.0799$; $P<0.0444$). The MANOVA also showed that significant factors had relatively large effect strengths compared to non-significant factors and were all positively correlated with HAD presence: parasite gall-making (Effect Strength or ES +0.342), parasite

Figure 2. CCA of HAD Occurrence Based on the Incidence of Biological and Ecological Factors. CCA depicting the general pattern between potentially explanatory factors, HAD presence (+, black), and HAD absence (-, white). Canonical correspondence 1 (CC1) accounts for 19.6% of the total variance while CC2 accounts for 12.8% of the variance.



immigrant inviability (ES +0.236), parasite short generation times (ES +0.213), and parasite volatile preference (ES +0.273) (Table 2). Although these factors were significantly associated to HAD occurrence, the CCA showed that these factors, when considered altogether, cannot effectively distinguish HAD presence from absence. Consistent with the MANOVA findings, the infinite random forest algorithm generated conditional probabilities based on the nine significant explanatory factors. These conditional probabilities confidently separate case studies likely to involve HAD from case studies unlikely to involve HAD ($R^2$=0.371, P<0.001). Based on the conditional probabilities I calculated, I developed a hierarchical decision tree that shows the conditions most likely to involve HAD as well as the conditions least likely to involve HAD (Figure 3). Logworth support for branch splits were significant at $\alpha$=0.05 for all branches (1.42 to 2.96 where LogWorth= –log10 [P-value]). The decision tree also includes gall-making as a condition even though it lacks LogWorth support because all but one parasite-host case study system involved gall-making when HAD was present, meaning no informative decisions could be made beyond this factor. The importance of gall-making to HAD is also supported by the MANOVA as discussed above. Finally, results of my validation test support the results of the infinite forest analysis but only for the nine parasite-host case study systems where HAD was present (indicated with an asterisk in The Appendix). The two ‘HAD absent’ case study examples in the validation study were incorrectly sorted to the ‘HAD present’ category.

Figure 3. Hierarchy of Conditional Probabilities Separating HAD Presence from Absence Based on the Incidence of Biological and Ecological Factors. Dichotomous key depicting the conditional probabilities distinguishing HAD presence from its absence. Each level of the hierarchical dichotomy represents a condition in one of the explanatory factors (i.e., the presence or absence of immigrant inviability, morphological differentiation, native parasites and hosts, short generation times, and volatile preference). The values superimposed on branch splits in conditions represent the logworth statistic of a given split in the dichotomy. The black and white bars represent the proportion of case studies expected to have HAD present (black) or absent (white). The black-and-white bar at the top of the hierarchy represents the original dataset consisting of 33 case studies without HAD and 64 case studies with HAD; this initial proportion is modified based on the conditions met while moving down the decision-making hierarchy.



## DISCUSSION

The occurrence of HAD is non-random and is significantly associated with the occurrence of specific explanatory factors, supporting my initial hypothesis. My analyses revealed that there is no simple, generalizable pattern that can distinguish the presence of HAD from its absence, however an algorithm based on conditional probabilities is able to effectively explain HAD occurrence (Figure 3). The CCA showed the lack of a generalizable pattern among explanatory factors that unambiguously distinguishes the presence of HAD from its absence (Figure 2). In contrast, the MANOVA showed that four, specific explanatory factors were significantly correlated with the presence of HAD, contributing to a significant whole-model effect correlated with HAD occurrence ($P<0.0021$). These four factors were: 1) volatile preference, 2) immigrant inviability, 3) short generation times, and 4) gall-making (Table 2). In addition to these factors, three combinations of explanatory factors are significantly correlated with the HAD occurrence: 1) immigrant inviability-by-short generation times, 2) immigrant inviability-by-morphological differentiation, and 3) host-shifting opportunity-by-volatile preference (Table 2). Consistent with the MANOVA findings, the infinite tree model showed that conditional probabilities (based on the incidence of the above significant explanatory factors) can confidently separate case studies likely to involve HAD from case studies unlikely to involve HAD ($R^2=0.371$; $P<0.0001$). I employed these conditional probabilities in a hierarchical decision tree wherein the proportion of case-studies expected to involve HAD changes with the co-occurrence or absence of specific explanatory factors (Figure 3). Importantly, all of the significant explanatory factors as well as all of the significant interactions between factors were represented in the decision tree and the LogWorth support values for all branches were significant. The sole exception to this pattern, gall-making, is a special case: all but two of the sixteen gall-makers used to create the hierarchy of conditional probabilities exhibited HAD (the exceptions being *Procecidochares atra* Loew and *Epiblema scudderiana* Hodges, see The Appendix), meaning informative comparisons could not be made between case studies with HAD and case studies without HAD. However, this evidence suggests that gall-making parasites are highly likely to exhibit HAD and were, thus, incorporated as a branch on the decision tree despite the lack of LogWorth support. This idea is supported by the fact that the validation study successfully placed gall-makers into the ‘HAD present’ category for two out of three cases considering gall-makers. However, in the validation study a gall-maker without HAD (*Phylloxera texana* Stoetzel) was incorrectly sorted into the ‘HAD present’ category, suggesting that my model cannot accurately predict which conditions produce gall-making parasite populations without HAD. This limitation of my model reflects the bias introduced by the lack of ‘HAD absent’ case studies published. Lastly, the decision tree can be employed to create verbal models to explain the occurrence of HAD. For example, case studies involving immigrant inviability are more likely to involve HAD compared to case studies without immigrant inviability. Furthermore, case studies involving both immigrant inviability and short parasite generation times have an even higher probability of involving HAD than cases involving immigrant inviability alone.

Four specific findings are consistent with previously described models explaining the occurrence of HAD: 1) Gall-making can independently account for the occurrence of HAD since most gall-making parasites appear to experience HAD, supporting previously described models emphasizing the strength of host-associated selection pressures on gall-makers (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Craig, Itami, Horner and Abrahamson 1994, Brown, Abrahamson, Packer and Way 1995, Itami, Craig and Horner 1998, Abrahamson, Eubanks, Blair and Whipple 2001, Stireman, Janson, Carr, Devlin and Abbot 2008, Dickey and Medina 2012). It appears that gall-making is a special case among endophagous feeders since other forms of endophagy (i.e., fruit-feeding or endoparasitism) were not significantly associated to the occurrence of HAD and did not contribute to the conditional probabilities in the decision tree. 2) My findings support the importance of selection against migrants in creating a strong reproductive isolating barrier between populations with host-specific adaptations (Funk 1998, Via, Bouck and Skillman 2000, Nosil, Vines and Funk 2005, Nosil 2007, Dickey and Medina 2011a). Immigrant inviability had a comparatively large impact in explaining the occurrence of HAD since it is significantly correlated with the presence of HAD, significantly interacts with two other explanatory factors (i.e., short generation time and morphological differentiation), and it is the first factor (beyond gall-making) contributing to the conditional probabilities employed in the decision tree. Furthermore, the interaction effect between immigrant inviability and generation time was one of the best-supported branches in the decision tree. Even in the absence of short generation times, the presence of immigrant inviability had significant LogWorth branch support when combined with volatile preference to explain the presence of HAD (Figure 3). These interactions have important consequences for the ecological interpretation of the mechanistic relationship between these factors and the evolution of HAD. For example, immigrant inviability and short generation time may have a compounding effect in creating host-associated, reproductively isolated populations (Dickey and Medina 2011a). Specifically, parasites that experience rapid generation turnover (e.g., aphids) can quickly accumulate host-associated adaptations and evolve reproductive isolation (Groman and Pellmyr 2000a, Schwarz, Matta, Shakir-Botteri and McPheron 2005, Loxdale 2009, 2010). As another example, immigrant inviability may compound with morphological differentiation by imposing two related reproductive isolating barriers. Specifically, migrants with host-specific adaptations would limit interactions between populations, creating an opportunity for assortative mating to impose disruptive selection on morphological traits used in mate selection (Jiggins, Hurst and Majerus 2000, Dobson, Fox and Jiggins 2002, Fordyce and Nice 2003, Nosil 2007, Egan, Nosil and Funk 2008, Fordyce 2010). 3) The importance of differential responses to host-plant volatiles supports the importance of habitat isolation by way of disruptive selection for host fidelity (Futuyma and Moreno 1988, Jaenike 1990, Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Dambroski, Linn, Berlocher, Forbes, Roelofs and Feder 2005, Egan and Funk 2006). It is interesting that volatile differences independent of other host-specific cues are able to explain the occurrence of HAD, though not surprising since this effect has been shown to impose a strong reproductive isolating barrier in certain systems (Finch 1978, Finch and Collier 2000, Bruce, Wadhams and Woodcock 2005). 4)

I support one component of the ‘reproductive mode’ model explaining HAD occurrence: short generation time. I expected short generation time would be significantly related to HAD occurrence, however I also expected asexual reproduction to be related to HAD occurrence and it was not. It appears that in this analysis HAD cannot be explained by low recombination rates that allow the independent accumulation of alleles in host-associated populations but, instead, HAD is explained by rapid responses to selection pressures under some other mode of reproductive isolation, such as immigrant inviability (Gandon and Michalakis 2002, Dickey and Medina 2011a, 2012). Indeed, this hypothesis is supported by the significant interaction between immigrant inviability (which imposes strong, host-associated disruptive selection) and short generation times (which increases the speed of an evolutionary response to selection). Lastly, it is difficult to determine the exact nature of the significant interaction between volatile preference and host-shifting opportunities, yet this effect still makes a significant contribution to the conditional probabilities employed in the decision tree. This could mean that parasite-host systems where either the parasite or host are introduced to a novel habitat are likely to evolve HAD in instances where a pre-adaptation to a host volatile allows them to successfully radiate into a novel niche or allow them to maintain HAD due to the use of plant volatile as a host-plant recognition clue (Murphy 2004, Schwarz, Matta, Shakir-Botteri and McPheron 2005, Agosta 2006, Craig, Itami, Ohgushi, Ando and Utsumi 2011).

Although my findings support previously described verbal models explaining HAD occurrence, the analyses I developed have limitations. Specifically, information required to test the relative importance of certain models in explaining HAD occurrence could not be appropriately integrated into the analyses. For example, case studies supporting the importance of allochrony for the evolution of HAD have shown that allochronic barriers to reproduction can be quite strong (Feder, Hunt and Bush 1993, Feder and Filchak 1999, Mopper 2005, Svensson, Althoff and Pellmyr 2005, Ueno, Furukawa and Tsuchida 2006, Santos, Rousselet, Magnoux, Paiva, Branco and Kerdelhué 2007, Schöfl, Heckel and Groot 2009). However, relatively few case study examples could be found that tested for allochrony explicitly, meaning this factor was excluded from my analyses after the discriminant function analysis (DFA) showed allochrony to be comparatively uninformative. Although the DFA included differential host-plant phenology (a proxy for allochrony) as an informative factor, the MANOVA and infinite tree model both showed this to be an insignificant factor in explaining the occurrence of HAD. It is possible, though, that host-plant phenology is a poor proxy for allochrony, being necessary but insufficient for mediating the evolution of allochrony. Future research should consider testing for allochrony in more host-parasite systems. Lastly, gall-making may have an undiscovered, significant interaction effect with allochrony. Specifically, allochrony has been shown to create reproductive isolation between host-associated populations in a few aphid species experiencing HAD (Akimoto 1990, Komatsu and Akimoto 1995, Abbot 2001). For example, the galling aphid, *Kaltenbachiella japonica* Matsumura, consists of eight genetically distinct, host-associated populations which occur at different times in a growing season, corresponding to the timing of budburst in their natal host-plant species. In order to appropriately test the relative importance of allochrony for explaining HAD occurrence, future research needs to accumulate more examples of allochrony specifically tested in systems where HAD can been characterized.

Another factor potentially explaining HAD occurrence (i.e., differential infection of microbes in host-associated parasites) could not be tested due to the lack of case study examples. The exploration of this factor is relatively novel, so the small number of case-studies is not surprising. Still, evidence exists showing microbial symbionts of insect herbivores can play a role in HAD by allowing their host to use different host-plant species (Leonardo and Muiru 2003, Simon, Carre, Boutin, Prunier-Leterme, Sabater-Munoz, Latorre et al. 2003, Tsuchida, Koga and Fukatsu 2004, Ferrari, Scarborough and Godfray 2007). The pattern between host-plant use and bacteria composition can be tested in more organisms, opening up opportunities for exploring the relationship between this explanatory factor and HAD in general. For example, two bacteria (i.e., *Pantoea agglomerans* Gavini and *Serratia marcescens* Bizio) are differentially found in pecan leaf phylloxera, *Phylloxera notabilis* Pergande showing HAD in pecan and water hickory (Medina, Nachappa and Tamborindeguy 2011). This relationship between microbial community species composition and HAD has also been found in a few other systems (Hosokawa, Kikuchi, Shimada and Fukatsu 2007). As with allochrony, more case studies are needed that test the relationship between differential microbe infection and HAD. Lastly, my analyses are unintentionally biased against case study examples where HAD is absent. This fact is reflected in the hierarchical decision tree (Figure 3) where consistently lower LogWorth support was observed on branches where HAD is likely to be absent. This feature of my research highlights the importance of reporting ‘negative’ results in the scientific literature. Case study examples where a phenomenon is expected to be observed but fails to show up are absolutely necessary for quantitative literature comparisons (e.g., this study and meta-analyses in general) that attempt to explain generalizable patterns. Researchers going forward should place more emphasis on reporting negative results in their study systems since these examples are useful for my understanding of generalized patterns and I encourage editors of relevant journals to consider publication of such results in the future for this reason.

In conclusion, my research has shown that the occurrence of HAD is non-random and associated with gall-making, immigrant inviability, short generation time and volatile preference. My hierarchical decision tree model demonstrating the probabilistic relationship between postulated explanatory factors and HAD occurrence provides quantitative evidence suggesting the existence of a set of conditions that could predict the occurrence of HAD in a given system. Most likely, HAD will be common in systems where parasites produce galls on host-plants, fail to find or migrate to novel host-plants due to host-specific adaptations, or experience rapid generation turnover (e.g., aphids, mites, and thrips). Furthermore, certain combinations of factors (e.g., immigrant inviability and short generation times) can compound, meaning systems involving both of these factors are even more likely to experience HAD. These factors, integrated into conditional probabilities, explain which parasite-host systems are likely to exhibit HAD and which systems are not, providing a tool for testing the general occurrence of HAD. I stress the need for the continued study of additional explanatory factors that could not be appropriately tested in my research (i.e., allochrony and differential microbe infection), as well as advocate for the publication of negative results in HAD studies. Future research into the evolution of HAD and parasite diversity in general will refine the findings of this study and may provide novel factors that may contribute to HAD in currently unexplored species.

# CHAPTER III

# ASSESSING THE ROLE OF IMMIGRANT INVIABILITY IN A CASE OF PARTIAL HOST-ASSOCIATED DIFFERENTIATION (HAD) INVOLVING THE BLACK-MARGINED APHID, *MONELLIA CARYELLA*

## SYNOPSIS

Host-associated differentiation (HAD) is a form of ecologically mediated speciation by which two or more parasite populations (e.g., insect herbivores) of the same species become genetically distinct due to their associations with different host species (e.g., plants). HAD has been proposed as a way to partially account for the vast species diversity observed in herbivorous insects. However, the factors explaining the occurrence of HAD are not fully understood. For HAD to occur, parasite populations on different hosts need to experience reproductive isolation, which causes the accumulation of host-associated alleles resulting in genetically distinct populations on different hosts. An important mechanism of reproductive isolation involves selection against migrants moving from a native to a novel host (i.e., immigrant inviability). Immigrant inviability has been found in several organisms showing HAD. Immigrant inviability may appear to be important for the evolution of HAD, however it is difficult to separate the effect of immigrant inviability from the effects of other species-specific factors that may influence the evolution of HAD (e.g., the short generations and low recombination experienced by aphids). The present study assessed immigrant inviability in a special case of partial HAD in the black-margined aphid, *Monellia caryella* Fitch (Hemiptera:

Aphididae), as it occurs on two host-plant species: pecan, *Carya illinoiensis* Wangenh and water hickory *Carya aquatica* Michx. Under partial HAD, the black-margined aphid consists of two genetically distinct populations: one specialist population occurring exclusively on pecan and a generalist population occurring on both pecan and water hickory. If immigrant inviability is responsible for HAD, I hypothesized that the specialist population will experience it while the generalist population will not. Immigrant inviability was assessed by comparing fitness parameters (i.e., fecundity and longevity) during reciprocal transplant experiments where aphids were transferred from pecan to water hickory and vice versa; during these experiments, the population identities of tested aphids were confirmed through single-nucleotide polymorphism (SNP) characterizations. Immigrant inviability was only detected in the pecan-specific population supporting my hypothesis.

## INTRODUCTION

Host-associated differentiation (HAD) is a form of ecologically mediated speciation by which two or more populations of the same parasite species (e.g., insect herbivores) become genetically distinct due to each parasite population associating with a different host species (e.g., host-plants) (Abrahamson, Blair, Eubanks and Morehead 2003, Stireman, Nason and Heard 2005). Putatively, HAD is a phenomenon created and maintained by differential selection pressures experienced by host-associated parasite populations and depends on the reduction in gene flow between host-associated parasite populations. If parasites experience strong, host-specific selection pressures linked to parasite reproduction (e.g., traits involved in mate and/or habitat choice), then reproductive isolation between host-associated parasite populations can develop relatively quickly (Feder 1998, Groman and Pellmyr 2000a, Loxdale 2009). Once reproductively isolated, parasite populations accumulate genome-wide genetic differences due to genetic drift and/or disruptive selection acting on adaptive alleles (Michel, Sim, Powell, Taylor, Nosil and Feder 2010), this accumulation of genetic differences between parasite populations associated with different hosts (i.e., the genetic signature of HAD) can be detected using genome-wide molecular markers (Hardison 2003, Helyar, Hemmer- Hansen, Bekkevold, Taylor, Ogden, Limborg et al. 2011). Under the circumstances described above, even sympatric parasite populations can assume independent evolutionary trajectories so long as reproductive isolation is maintained (Flaxman, Feder and Nosil 2013). Given enough time, HAD may result in total reproductive incompatibility between parasite populations on different hosts. Thus, HAD may constitute the beginning stages of ecological speciation (Dres and Mallet 2002, Wiens 2004, Stireman, Nason and Heard 2005). Since most insects happen to be plant or animal parasites (Price 1980), HAD has been proposed as a way of partially explaining the vast species diversity observed in insects (Funk, Filchak and Feder 2002).

It is still unclear which combination of factors involved in parasite and host biology are most likely to explain the occurrence of HAD (Stireman, Nason and Heard 2005, Medina 2012). However, one mode of reproductive isolation potentially important to the evolution of HAD involves immigrant inviability or selection against maladapted migrants moving from a natal to a novel host. Immigrant inviability is commonly present in cases of HAD (Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Via, Bouck and Skillman 2000, Simon, Carre, Boutin, Prunier-Leterme, Sabater-Munoz, Latorre et al. 2003, Nosil, Vines and Funk 2005, Ferrari, Godfray, Faulconbridge, Prior and Via 2006, Nosil 2007, Ferrari, Via and Godfray 2008, Dickey and Medina 2011a, Matsubayashi, Kahono and Katakura 2011, Dickey and Medina 2012). In fact, immigrant inviability may be the first reproductive isolating barrier to develop between populations of parasites occurring on different host species (Nosil, Vines and Funk 2005). This is because HAD likely requires a rapid accumulation of divergent adaptive alleles that would otherwise be disrupted by even low levels of gene flow. Therefore, a reduction in fitness for migrants and hybrids of migrants would cause a nearly complete reduction in gene flow between host-associated populations and promote habitat-specific adaptation (Funk 1998, Via, Bouck and Skillman 2000). Evidence for rapid adaptive change under immigrant inviability has been shown in parasite populations as a response to differential selection pressures imposed by different host species (Bush 1994, Via 2001, Lowry, Modliszewski, Wright, Wu and Willis 2008, Funk 2010, Matsubayashi, Ohshima and Nosil 2010). For example, differential adaptive responses of insect herbivores to certain host-plant characteristics (e.g., plant traits that trigger insect host- or mate-seeking behaviors; plant chemical defenses; and plant nutrient content) can act as reproductive isolating barriers between host-associated insect herbivore populations and result in observable genetic differentiation in as few as 200 years (Bush 1969, Futuyma and Moreno 1988, Feder and Filchak 1999).

Immigrant inviability is not the only factor responsible for the evolution of HAD. Parthenogenesis (Sunnucks, De Barro, Lushai, Maclean and Hales 1997, Dixon 1998, Dickey and Medina 2010, 2011a), allochrony (Itami, Craig and Horner 1998, Feder and Filchak 1999, Cocroft, Rodríguez and Hunt 2008), and endophagy (Dreger-Jauffret and Shorthouse 1992, Stireman, Nason and Heard 2005) have also been postulated as factors that could explain the occurrence of HAD. Specifically, HAD can evolve in parthenogens more rapidly than in sexually reproducing organisms. This is due to the low level of gene flow between parthenogenetic lineages causing the independent accumulation of adaptive alleles; this effect is exacerbated by the fact that most aphid species experience upwards of 30 generations in a single growing season (Hartl 1972, Lynch and Gabriel 1983, Lynch 1984). In cases where two cyclically parthenogenetic lineages occur on different host-plants, each can evolve responses to differential selection pressures before mating can desegregate host-associated genetic structuring (King 1993, King and Murtaugh 1997, Loxdale 2008). In other words, the rapid evolution of host associations in aphids is mechanistically caused by the low levels of recombination inherent in cyclical parthenogens in conjunction with the short generation times experienced by aphids. . This is supported by the fact that aphids in general exhibit very strong host specificity with up to 99% of species considered host-plant specialists (Eastop 1973); in fact, Dickey and Medina (2010) identified 18 aphid species that consist of host races or host-associated genotypes. The low levels of recombination and short generation times can also interact with immigrant inviability to produce HAD: if a population of cyclical parthenogens accumulates alleles maladaptive on alternative hosts, then the potential for HAD to evolve is reinforced (Rundle and Nosil 2005). However, it is difficult to test this rationale because delineating the effect of immigrant inviability alone from the effect of other traits such as parthenogenesis (or of any other species specific trait) is complicated.

I hypothesize immigrant inviability occurs in host-specialist populations but not in host-generalist populations (Nosil, Harmon and Seehausen 2009, Forister, Dyer, Singer, Stireman and Lill 2012, Powell, Hood, Murphy, Heilveil, Berlocher, Nosil et al. 2013). This hypothesis was tested in the black-margined aphid (*Monellia caryella* Fitch), a winged arboreal aphid that feeds on pecan and water hickory leaves. The black-margined aphid was chosen for this study because it presents a special form of HAD I call ‘partial HAD’. Under partial HAD, the black-margined aphid’s genetic population structuring does not perfectly correspond to its host-associations (Raijmann and Menken 2000, Johnson, Adams and Clayton 2002, Vialatte, Dedryver, Simon, Galman and Plantegenest 2005, Ishiguro, Yoshida and Tsuchida 2006, Medina, Reyna and Bernal 2012, Antwi, Sword and Medina 2015). The black-margined aphid consists of two genetically distinct populations: one occurring exclusively on pecan trees (*Carya illinoiensis* Wangenh) and a second population occurring on pecan and water hickory trees (*Carya aquatica* Michx) (Medina, Dickey, and Harrison, in press). In other words, the black-margined aphid consists of a pecan-specialist population and a pecan and water hickory generalist population. This system was chosen for this study because it is ideal for testing the singular effect of immigrant inviability on the evolution of host-associated populations compared to other factors believed to important to the evolution of HAD. First, the specialist and generalist populations used for this study belong to the same species and the host-plant species share the same environment, allowing for direct comparisons between generalist and specialist performance during reciprocal transplant experiments (Mitter, Farrell and Wiegmann 1988, Zeh, Zeh and Smith 1989). More importantly, if parthenogenesis is the primary factor for determining the evolution of HAD, then any immigrant inviability (or the lack thereof) detected in this system would be independent of host associations. This is because the specialist and generalist black-margined aphids experience very similar parthenogenetic life histories (i.e., they share the same short generation times and low recombination rates); the only exception to this would involve mutations on sex-determining chromosomes (Lynch and Gabriel 1983, Lynch 1985, Howard and Lively 1994). However, if the evolution of immigrant inviability is the determinant factor or the evolution for HAD, then immigrant inviability would be present in the host-specialist population only.

## METHODS

*Aphid collection and reciprocal rearing.* The black-margined aphids persist from late-April to mid-August and annually produce 16 to 32 overlapping generations of thelytokous parthenogens (Tedders 1978). Back-margined aphids also produce a single sexual generation towards the end of their growing season that mate and lay overwintering eggs. Aphid life history parameters were assessed in a manner similar to Dickey and Medina

(2011a). Wild adults were collected from five southeastern Texas sites from June 9th to June 20th (pecan) and from July 1st to July 25th (water hickory), 2012. Pecan and water hickory trees occur in sympatry at my study area and share lowland plains near rivers and streams. One adult black-margined aphid was collected per tree per site (Table 3). Thus, it is reasonable to assume that each aphid sampled represents a different genetic lineage. All black-margined aphids were placed individually in a Newspring DELItainer® (Pactiv Corp., Lake Forest, IL) 8 cm high, 10 cm diameter, 473 mL plastic container and maintained in a rearing room heated and lit by a 1,000 W metal halide bulb at a LD 16h: 8h cycle. Temperatures fluctuated between 26 ˚C and 35 ˚C (average 30 ˚C). Aphids collected in the field were then reared until they deposited a nymph; the nymph's mother was then stored in -80 ˚C as a voucher specimen. The first generation ($F_1$) of nymphs was reared to adulthood on ~1 g of excised leaf material (leaflet) from their natal host tree (i.e., the same tree species from which they were collected). Once these aphids matured to adulthood, they were assigned to one of four treatments (i.e., on a leaflet from the natal or a novel host-plant, based on the host they were collected) and placed individually in randomly arranged plastic containers. Leaf material was replaced daily to reduce fungus growth and to ensure a continuous source of food for the aphids.

Table 3. Sample Information for Black-Margined Aphids Collected from Pecan and Water Hickory Trees.

| ***Location*** | ***Host-plant species*** | | |
|---|---|---|---|
| | *Pecan* | *Water Hickory* | *Total by Site* |
| Bryan, Texas | - | 3 | 3 |
| Highway 50 Roadside | 5 | - | 5 |
| Lake Somerville | 6 | 5 | 11 |
| Lick Creek Park | - | 12 | 12 |
| Pecan Breeding and Genetics | 15 | 6 | 21 |
| Texas A&M Campus | 4 | - | 4 |
| *Total by Host-plant* | 30 | 26 | 56 |

*Immigrant inviability data analyses.* Each day aphids were monitored for daily survival and number of nymphs produced. For each aphid, total longevity in days and total lifetime fecundity were calculated at the end of their lives; longevity was defined as the number of days from aphid birth to death whereas fecundity was defined as the total number of offspring produced by an aphid throughout its lifetime. To measure immigrant inviability, firstborn nymphs of the previously described $F_1$ adults were reared under the same conditions described above; likewise, longevity and fecundity of the $F_2$ generation were summed daily. Differences in longevity and fecundity among migrant treatments (i.e., migrants to natal or novel plant species) and differences among generations were assessed with Monte-Carlo tests for significance scripted in the PopTools package (Hood 2010) in Microsoft Excel™. This test was chosen because lifetime fecundity and longevity data for $F_1$ and $F_2$ generations were orthogonal and because a test for significance using a Wilcoxin signed-rank test would have been biased due to a high proportion of tied ranks (0's) in the data set. The relative fecundities ($f$) of pecan and water hickory immigrants ($f_P$ and $f_W$) were calculated as:

$$f_x = \frac{f_i}{f_r}$$

Where 'x' is relative fecundity for either pecan ($f_P$) or water hickory ($f_W$). Resident fecundity ($f_r$) is defined as the average fecundity of aphids transferred from a natal host-plant to natal host-plant; immigrant fecundity ($f_i$) is defined as the average fecundity of aphids transferred from a natal host-plant to novel host-plant material. The contribution of immigrant inviability was simply calculated as 1 minus relative fecundity (Nosil, Vines and Funk 2005).

*Aphid genotyping.* In order to precisely correlate the pattern of immigrant inviability observed in this study with the previously described genetic structuring of pecan-specialist and generalist populations of black-margined aphid (Dickey, Harrison, and Medina, in preparation), single nucleotide polymorphisms (SNPs) were developed using the RAD-tag sequencing protocol on the Illumina HiSeq platform (Baxter, Davey, Johnston, Shelton, Heckel, Jiggins et al. 2011, Davey and Blaxter 2011, Caporaso, Lauber, Walters, Berg-Lyons, Huntley, Fierer et al. 2012). Specifically, SNPs were developed from $F_2$aphids reared during the reciprocal transplant experiments; 5 aphids were chosen to represent each treatment group in the reciprocal transplant experiments, totaling 20 aphids submitted for sequencing. DNA was extracted from aphids using a Qiagen DNEasy Tissue Kit (Qiagen Inc., Valencia, CA) following the Qiagen recommended protocol (Qiagen 2002). Excess RNA was removed using the RNAseA plasmid cleanup kit following the Qiagen recommended protocol. The concentration (ng/µl) and purity (ratio of sample absorbance at 260 and 280 nm) of DNA was measured using a NanoDrop® spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Samples were only submitted if they contained a minimum of 50 ng of genomic DNA. Unfortunately, this reduced the number of aphid lineages that could be genotyped from the reciprocal transplant experiment because several of these failed to produce enough genomic DNA. RAD-tag marker development and Illumina HiSeq sequencing was performed by the Texas A&M University AgriLife Genomics and Bioinformatics Service (College Station, TX); this service provided raw sequence reads for comparative genomics. Comparative genomic analyses were performed in the Galaxy software (Giardine, Riemer, Hardison, Burhans, Elnitski, Shah et al. 2005, Blankenberg, Kuster, Coraor, Ananda, Lazarus, Mangan et al. 2010, Goecks, Nekrutenko and Taylor 2010). Reads were filtered for a minimum 5X coverage using FastQC (Andrews 2010) and then trimmed for quality using FastQ Trimmer by column (Blankenberg, Gordon, Von Kuster, Coraor, Taylor, Nekrutenko et al. 2010). The Galaxy software was also used to visualize genetic population structuring using a principal component analysis (PCA). Black-margined aphid sequences were aligned against the pea aphid (*Acyrthosiphon pisum* Harris) reference genome (Richards 2010) for comparative mapping and SNP discovery using the Burrows-Wheeler Aligner for Illumina (Li and Durbin 2009). These data were also used to generate a pileup and construct consensus sequences; the pileup was subsequently filtered to exclude base calls below a 99.9% accuracy threshold. Finally, nucleotide variants at each locus were quantified and compared in Galaxy to identify statistically significant SNPs; these SNPs were used to characterize the genetic structuring of host-associated black-margined aphids.

## RESULTS

*Immigrant Inviability Data Analysis.* The results of the of the reciprocal transplant experiment support the existence of immigrant inviability in the pecan-specialist population of the black-margined aphid. The effect of migration on overall black-margined aphid longevity and fecundity was dependent on host associations, with $F_2$ pecan-specialist aphids possessing lower fecundity and longevity on water hickory leaf material (Bootstrap statistic=100; P>0.001 and Bootstrap=98; P>0.001, respectively); Table 4 shows the comprehensive statistics for longevity and fecundity in the Monte-Carlo tests of significance. The reduction in both longevity and fecundity experienced by pecan-specialist aphids on water hickory relative to pecan indicates immigrant inviability. However, these reductions were not observed in $F_2$ host-generalist aphids. In addition, the effect of migration was significant in the $F_2$ generation but not in the $F_1$ generation for all black-margined aphids. Histograms of the fitness variables across the four migration treatments (i.e., pecan to water hickory, pecan to pecan, water hickory to water hickory, and water hickory to pecan) per generation are given in Figure 4. The relative fecundity of immigrants, the relative survival of immigrant's offspring, and the contribution to reproductive isolation of immigrant and offspring inviability are given in Table 5. *Aphid genotyping.* For an analysis of admixture and genetic clustering presented here, relatively simple methods of assembly are adequate, meaning the contiguity of the assembly need not be great and are not reported here. Trimmed reads (99% of raw reads) were aligned to the reference genome, and 89% of the trimmed reads per sample could be confidently and uniquely mapped to single positions in the genome; these were used for SNP characterization. Approximately 123,000 SNPs were identified per aphid with coverage ≥4.8X, allowing us to characterize the genetic clustering among sampled black-margined aphids. It was discovered that suspected pecan-specialist black-margined aphids were genetically distinct from host-generalist aphids (Figure 5).

Table 4. Monte-Carlo Test for Significance in Adult Longevity and Fecundity between Transfer Groups of Reciprocal Transplant Experiments

| *Transfer group 1* | *Transfer group 2* | *Measurement* | *Bootstrap Statistic* | *P-value* |
|---|---|---|---|---|
| Pecan-to-pecan | Pecan-to-water hickory | Longevity | 100 | <0.001 |
| | | Fecundity | 98 | <0.001 |
| Water hickory-to-water hickory | Water hickory-to-pecan | Longevity | 16 | 0.36 |
| | | Fecundity | 9 | 0.57 |

Table 5. Calculations for the Relative Fecundities and Degree of Reproductive Isolation between Pecan-Specialist and Generalist Black-Margined Aphids. Calculations for the relative fecundities of $F_2$ pecan-specialist and generalist black-margined aphids and the contribution to reproductive isolation due to immigrant inviability. The calculations of the relative fecundities depict the number of individuals produced in aphids transferred from their natal host over the number produced on a novel host (and vice versa).

| Collected from: | Transferred to: | Relative fecundity of immigrants | Contribution to reproductive isolation due to immigrant inviability |
|---|---|---|---|
| Pecan | Pecan | - | - |
| Pecan | Water hickory | 0.000/0.560 = 0.000 | 100% - 0.0% = 100.0% |
| Water hickory | Pecan | 1.060/0.920 = 1.152 | 100% - 115.2% = -15.2% |
| Water hickory | Water hickory | 0.920/1.060 = 0.868 | 100% - 86.8% = 13.2% |
| Collected from: | Transferred to: | Relative fecundity of immigrants | Contribution to reproductive isolation due to immigrant inviability |
| Pecan | Pecan | - | - |
| Pecan | Water hickory | 0.000/0.560 = 0.000 | 100% - 0.0% = 100.0% |
| Water hickory | Pecan | 1.060/0.920 = 1.152 | 100% - 115.2% = -15.2% |
| Water hickory | Water hickory | 0.920/1.060 = 0.868 | 100% - 86.8% = 13.2% |

Figure 4. Adult Longevity and Fecundity during Reciprocal Transplant Experiments Involving Host-Associated Black-Margined Aphids. Comparison of average $F_2$ black-margined aphid adult fecundity (a) and average adult longevity (b) during reciprocal transplant experiments. Aphids collected from pecan were transferred to pecan (P2P) or water hickory (P2W) and tested for significant differences; aphids collected from were hickory were transferred to pecan (W2P) or water hickory (W2W) and tested for significant differences. Significant differences are indicated by an asterisk (*).

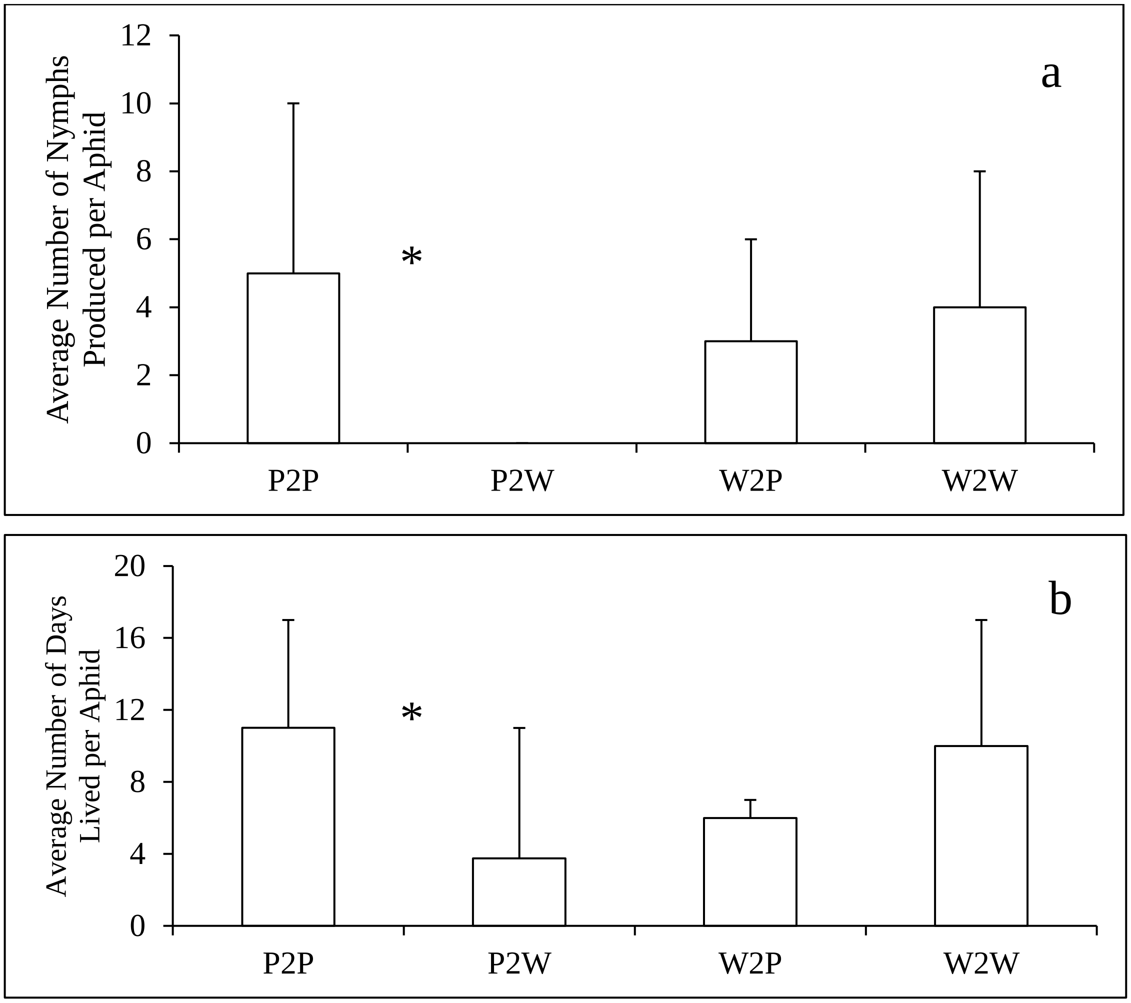
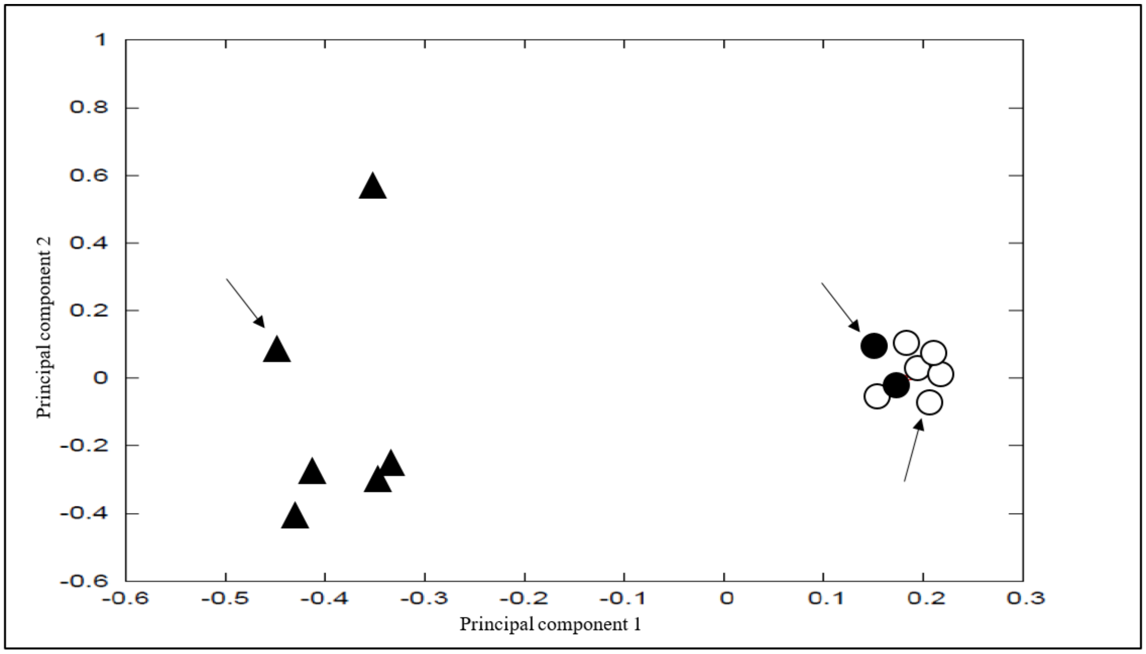


Figure 5. CCA of SNPs Characterizing Generalist and Specialist Black-Margined Aphid Populations. Principal component analysis (PCA) depicting the genetic differentiation between individual black-margined aphids from the reciprocal transplant experiment. Color indicates the whether an aphid was collected on pecan (black) or water hickory (white). Shape indicates whether an individual was suspected to belong to the pecan-specialist (triangle) or generalist (circle) genetic population based on the results of the reciprocal transplant experiment. Principal component 1 accounts for 93.8% of the total variance. The arrows point to individual aphids previously identified as belonging to the pecan-specialist population and the generalist population and were originally part of a different research data set (Medina et al., in review).



It was also discovered that black-margined aphids previously demonstrated to be part of the pecan-specialist population clustered together with aphids that could only survive on pecan during the reciprocal transplant experiment. Similarly, previously identified generalist black-margined aphids clustered with aphids that could survive and reproduce on either host-plant species. However, since only a single suspected generalist could be genotyped from the reciprocal transplant experiment, the conclusion that the individuals tested belong to the previously identified populations of pecan-specialist and generalist black-margined aphids is only partially supported.

## DISCUSSION

My reciprocal transplant experiments showed that pecan-collected black-margined aphids experienced a significant reduction in fitness parameters when transferred from pecan to water hickory (i.e., specialist aphids) while other pecan-collected aphids and all of the water hickory-collected aphids did not experience a fitness reduction when transferred from a natal host to a novel host (i.e., generalist aphids). The reason why this was observed only in the $F_2$ generation is most likely attributable to the strong maternal effects commonly experienced by aphids and because this effect can quickly be overcome by host-specific selection pressures (Via 1991). The characterization of population-wide SNPs among pecan- and water hickory-collected aphids showed that the specialist and generalist aphids observed in the reciprocal transplant experiments belong to the same specialist and generalist populations originally characterized by way of AFLP markers (Figure 1 of Medina, Dickey, and Harrison 2016). Furthermore, the survival rates and relative fecundities calculated from the reciprocal transplant experiments provided additional insight into the strength of immigrant inviability between the specialist and generalist populations of black margined aphids. By converting rates of into percentages and subtracting the survival rate of $F_2$ from the total (100), I derive the percent contribution to total reproductive isolation due to immigrant inviability (Nosil, Vines and Funk 2005, Dickey and Medina 2011a). First, the relative fecundity calculated for the pecan-specialist population showed that immigrant inviability could cause complete reproductive isolation by the second generation due to the failure of $F_2$ specialist aphids to accept water hickory as a host (100 - 0.0% survival rate of $F_2$ immigrants = 100.0% reproductive isolation due to immigrant inviability; these values and the ones used below are derived from the results shown in Table 5). In contrast, generalist aphids moving from pecan to water hickory do not experience complete reproductive isolation by the second generation because second generation offspring of generalist migrants have a relatively high fitness on water hickory (100 - 86.8% survival rate of $F_2$ immigrants = 13.2% reproductive isolation due to immigrant inviability). By extrapolating the survival rate of immigrants across generations, it can be shown that generalist aphids moving from pecan to water hickory should be able to produce viable offspring throughout an entire growing season, or 30 generations ($0.868^{30}$ = 1.4% relative survival rate) albeit at a very low rate. Though this survival rate is quite low, it still does not constitute complete reproductive isolation, especially considering 100’s to 1000’s of black-margined aphid clones can be produced and sustained by a single pecan or water hickory tree throughout the growing season (Harris 1985). Lastly, generalist aphids moving from water hickory to pecan do not experience immigrant inviability at all and, in fact, experience a small improvement in fitness on pecan (100 - 115.2% relative survival rate = -15.2% contribution to reproduction isolation).

The above calculations indicate that the reproductive isolating barrier created by immigrant inviability is sufficient to account for the reproductive isolation between pecan-specialist and black-margined aphids. These results parallel those of previous studies which have either been able to quantify the strength of reproductive isolation caused by immigrant inviability or have theorized that immigrant inviability plays an integral role in the development or maintenance of ecological specialization in general and HAD in particular (Nosil, Vines and Funk 2005, Nosil 2007, Lowry, Modliszewski, Wright, Wu and Willis 2008, Schluter and Conte 2009). In contrast, other studies have also suggested that the short generation times and low recombination rates of cyclically parthenogenetic aphids can explain the occurrence of host-specialist populations in aphids and may explain the diversification of host-races and cryptic species within aphids in general (Via 1999, Via, Bouck and Skillman 2000, Hawthorne and Via 2001, Dickey and Medina 2010, Peccoud, Simon, von Dohlen, Coeur d'acier, Plantegenest, Vanlerberghe-Masutti et al. 2010, Medina 2012). Therefore, I could not overlook the influence of short generation times or low recombination rate on the evolution of the pecan-specialist population in black-margined aphids. However, my results suggest that neither of these factors by themselves can explain the occurrence of the pecan-specialist population in the black-margined aphid. This is because the generalist and specialist populations of black-margined aphid experience the same number of generations per season and the same recombination rate, yet the generalist population is maintained in sympatry with the specialist population. It seems that immigrant inviability is the likely main source of reproductive isolation in the pecan-specialist genetic lineage whereas generation time and recombination rate play a negligible or not role at all. Perhaps, then, the reason why HAD and other forms of host specialization are observed so often in aphids is due short generation times allowing for the rapid development of reproductive isolation (e.g., immigrant inviability) between populations. Therefore, immigrant inviability could be a determinant factor explaining the occurrence of HAD while short generation times and low recombination may increase the probability that host-specialist populations develop in some organisms.

Unfortunately, the genetic identities of the individuals tested in the reciprocal transplant experiments could only partially be verified. This is because only a single suspected generalist black-margined aphid that was also collected from pecan could be included in the genetic verification test. This error was most likely committed due black-margined aphids emerging and achieving peek population density twice in a season: once in the early season (when all of the pecan-collected individuals were obtained) and again in the middle of the season (when all of the water hickory-collected individuals were obtained) (see the Methods section). It is now known that pecan-specialist black-margined aphids experience allochronic isolation (Chapter IV), meaning aphids captured early in the season may entirely belong to the pecan-specialist genetic population; although, the allochronically isolated population of black-margined aphids on pecan have yet to be genetically characterized. This phenomenon may also explain why no pecan-collected individuals could produce offspring by the $F_2$ generation in the reciprocal transplant experiment: It is possible that only pecan-specialist aphids were collected on pecan trees.

To establish a general pattern regarding the relationships between immigrant inviability, short generations, and low recombination, more parasites under partial HAD need to be tested for immigrant inviability. For example, the sexually obligate orchard ermine moth (*Yponomeuta padella* L.) consists of a genetically distinct, specialist population associated with Hawthorne trees (*Crataegus* spp. Tourn) in sympatry with a generalist population that can occur on several host-plants including mountain-ash (*Sorbus aucuparia* L.) and Juneberry trees (*Amelanchier lamarckii* L.) (Raijmann and Menken 2000). If reciprocal transplant experiments were performed on these populations moving moths collected on mountain-ash to Juneberry and vice versa, then comparisons in the strength of reproductive isolation by immigrant inviability could be made between a cyclical parthenogen (the black-margined aphid) and a sexually obligate organism (the orchard ermine moth). If I can determine that the strength of immigrant inviability is greater in the cyclical parthenogens, then this would support the theory that short generations and low recombination rates may increase the probability that reproductive isolation evolves.

The effects of immigrant inviability on the evolution of HAD in the black-margined aphid is also comparable to other insect species within the pecan and water hickory system. Multiple insect herbivore species within this system have also tested positive for host-plant specialization (Medina, Harrison, and Dickey, in preparation, Dickey and Medina 2010, 2012). Like the specialist population of black-margined aphids, pecan-water hickory-associated populations of yellow pecan aphid (*Monelliopsis pecanis* Bissell) and pecan leaf phylloxera (*Phylloxera notabilis* Pergande) also experienced immigrant inviability during reciprocal transplant experiments (Dickey and Medina 2011a, 2012). Unlike the black-margined aphid, the yellow pecan aphid and pecan leaf phylloxera experience full HAD and the immigrant inviability they experience is reciprocal for both the pecan- and water hickory-associated populations. As demonstrated in this study, it is possible that the immigrant inviability experienced by the yellow pecan aphid and pecan stem phylloxera are the cause of their host-associated reproductive isolaiton and that the short generations and low recombination experienced by all these aphid species only contributed to increasing the rate at which immigrant inviability evolved.

The evolution of the pecan specialist population in the black-margined aphid could be important to agricultural science because it fits into a theoretical narrative regarding evolutionary ecology and agriculture: It is theorized that human agricultural practices have provided a recent niche that selects for host-plant specialization in herbivorous insect pests (Jaenike 1990, Via 1990). Apparently, large-scale growing operations involving monocultures of crop plants can provide highly abundant and/or enemy-free spaces that allow populations of insect herbivores to thrive (Strong, McCoy and Rey 1977, Strong, Lawton and Southwood 1984, Ballabeni, Wlodarczyk and Rahier 2001, Murphy 2004, Heisswolf, Obermaier and Poethke 2005). This is because host switching onto monoculture would incur no cost for an insect herbivore pre-adapted to the crop host-plant while simultaneously incurring a search cost for switching back to wild host-plants. In this scenario, the rapid evolution of crop host-plant specialization is expected (Tan, Liu, Lin and Hsu 2014) and immigrant inviability could account for the strong, reproductive isolating barrier required for this specialization to take place.

In conclusion, I provide evidence that pecan-specialist black-margined aphids likely experience immigrant inviability while generalist black-margined aphids do not. However, future validations must be conducted that better test the genetic identities of black-margined aphid individuals. Still, this research is unprecedented with respect to the study of HAD because it provides us with the first characterization of the strength of immigrant inviability in a case of partial HAD. The discovery of immigrant inviability in a case of partial HAD improves my understanding of the evolution of reproductive isolation during sympatric speciation: a maladapted population of parasites can be removed from a host-plant species in as few as two asexual generations. This observation supports the hypothesis that ecological speciation is a common phenomenon in insect herbivores and that reproductive isolation can easily evolve in sympatric populations (Bush 1975b, Bush 1994, Schluter 2001, Via 2001). In order to make further assessments regarding the general relationship between immigrant inviability and the evolution of HAD, future research will need to involve more organisms experiencing partial HAD across a much broader diversity.

# CHAPTER IV

# ALLOCHRONY AND HOST-ASSOCIATED DIFFERENTIATION IN APHIDS

## SYNOPSIS

Sympatric populations can become reproductively isolated by being active at different times (i.e., allochrony). Allochrony has been proposed as a mechanistic explanation for the evolution of population genetic structuring among parasite populations associated with different hosts species (i.e., host-associated differentiation or HAD). Specifically, populations of insect herbivores can experience disruptive selection in response to phenological differences among their host-plant species. For example, host-associated populations of insect herbivores (e.g., aphids) will emerge and achieve peak population densities at different times in order to co-occur with the resources provided by their respective host-plant species. When differential responses to host-plant phenology result in allochronic isolation between parasite populations, then HAD can evolve. However, it is unclear whether the allochrony created by differences in host-plant phenology are sufficient to produce HAD in aphids. I hypothesize that if allochronic isolation via differences in host-plant phenology is sufficient for producing HAD, then I should observe allochronically isolated populations in exophagous aphids exhibiting HAD. I tested this hypothesis in three exophagous aphids occurring on pecan and water hickory. One aphid species shows HAD, one shows partial HAD and one lacks HAD. To assess the presence of allochrony among aphid populations living on different tree species, population density was collected for each aphid species on pecan and water hickory

throughout several weeks. I present evidence suggesting the occurrence of HAD and allochrony are correlated in aphids.

## INTRODUCTION

Sympatric populations can become reproductively isolated due to allochrony (Alexander and Bigelow 1960, Bush 1975a, Cooley, Simon, Marshall, Slon and Ehrhardt 2001, Ueno, Furukawa and Tsuchida 2006, Devaux and Lande 2008, Sota, Kagata, Ando, Utsumi and Osono 2014, Fudickar, Greives, Atwell, Stricker, Ketterson, Williams et al. 2016). Specifically, sympatric populations of insect herbivores can experience disruptive selection on time-dependent traits when host-plants produce ephemeral (e.g., flowers, fruits, seeds) resources at different times, causing reproductive isolation between populations active at different times or ‘allochronic isolation’ (Konno, Honda and Matsumoto 1981, Pashley, Hammond and Hardy 1992, Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Eubanks, Blair and Abrahamson 2003, Mopper 2005, Santos, Rousselet, Magnoux, Paiva, Branco and Kerdelhué 2007, Stireman, Janson, Carr, Devlin and Abbot 2008). For example, when host-plant species produce fruits, seeds, or nuts at different times, populations of short-lived insect herbivores can experience disruptive selection on traits that determine the timing of eclosion such that host-adapted insect herbivores emerge at distinct times and become allochronically isolated (Abrahamson, McCrea and Anderson 1989, Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Schluter 2001, Santos, Rousselet, Magnoux, Paiva, Branco and Kerdelhué 2007, Matsubayashi, Ohshima and Nosil 2010). Given several hundred generations of allochronic isolation, host-associated insect populations can accumulate genome-wide genetic differences, leading to host-associated differentiation (HAD) (Funk, Filchak and Feder 2002, Stireman, Nason and Heard 2005).

Allochrony has been invoked as an explanation for the evolution of HAD in the apple maggot (*Rhagoletis pomonella* Walsh), as it occurs on apple and hawthorn trees (Bush 1969, Feder, Chilcote and Bush 1988, Berlocher and Feder 2002, Powell, Forbes, Hood and Feder 2014). A mean difference of four weeks between the fruit availabilities of apple and hawthorn is sufficient for creating strong reproductive isolation between host-associated apple maggot populations. This difference in tree phenology is also sufficient to select for fly populations with divergent pupal emergence times (Feder and Filchak 1999) which translate into fly host-associated population densities peaking at different times (Feder, Hunt and Bush 1993). Similarly, phenological differences between two yucca species are thought to explain reproductive isolation between host-associated five-spotted bogus yucca moth (*Prodoxus quinquepunctellus* Chambers) populations (Groman and Pellmyr 2000a, Althoff, Fox and Frieden 2014). The relationship among host-plant phenology, allochrony, and HAD has been demonstrated in several other insect-plant systems as well (Akimoto 1990, Komatsu and Akimoto 1995, Raijmann and Menken 2000, Abbot and Withgott 2004, Bethenod, Thomas, Rousset, Frérot, Pélozuelo, Genestier et al. 2005, Stelinski and Liburd 2005). However, allochrony and HAD do not co-occur in all systems (Jaenike and Selander 1980, McLellan, Nordin and Haynes 1991, Baer, Tripp, Bjorksten and Antolin 2004, Antolin, Bjorksten and Vaughn 2006, Martinelli, Clark, Zucchi, Silva-Filho, Foster and Omoto 2007). For example, blueberry- and cranberry-associated populations of the cranberry fruitworm (*Acrobasis vaccinii* Riley) emerge and achieve peak population densities at significantly different times in parallel with the availability of their respective host-plant fruits, yet they do not exhibit HAD (Medina, Szendrei, Harrison, Isaacs, Averill, Malo et al. 2013). Interestingly, a gall-making wasp (*Dasineura oxycoccana* Johnson) exhibits HAD on the same host-plant pair (Cook, Ozeroff, Fitzpatrick and Roitberg 2011). Thus, the relationship between host-plant phenology, HAD, and allochrony appears to be complicated and may be affected by other factors.

Besides allochronic isolation, several other factors are believed to promote the evolution of reproductive isolation between insect herbivores under HAD (Bush 1975b, Price 1977, Futuyma and Moreno 1988, Via 1999, Via, Bouck and Skillman 2000, Abrahamson, Eubanks, Blair and Whipple 2001, Craig, Horner and Itami 2001, Funk, Filchak and Feder 2002, Nosil, Crespi and Sandoval 2002, Nosil, Vines and Funk 2005, Funk, Nosil and Etges 2006, Stireman, Nason, Heard and Seehawer 2006, Dorchin, Scott, Clarkin, Luongo, Jordan and Abrahamson 2009, Forister, Dyer, Singer, Stireman and Lill 2012, Medina 2012). Notably, gall-making insect herbivores are thought to be particularly prone to the evolution of HAD due to the hypothesized gene-by-gene mechanism required for the manipulation of host-plant phytohormones during insect-induced gall formation (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Craig, Itami, Horner and Abrahamson 1994, Brown, Abrahamson, Packer and

Way 1995, Abrahamson, Eubanks, Blair and Whipple 2001, Stireman, Janson, Carr, Devlin and Abbot 2008, Dickey and Medina 2012). However, the evolution of HAD in gall-making insects may also be tied to host-plant phenology (Mopper 2005). Specifically, gall-makers synchronize their biology with host-plant phenology (Yukawa 2000). Indeed, the existence of phenological differences between host-plant species has been shown to create allochronic isolation between host-associated populations in multiple gall-making aphid species that experience HAD (Akimoto 1990, Komatsu and Akimoto 1995, Abbot 2001). For example, the American gall-forming aphid, *Pemphigus obesinymphae* Riley, consists of two host-associated populations, each occurring on a phenologically distinct species of cottonwood tree (Abbot and Withgott 2004). It is unclear whether phenological differences alone or gall-making combined with phenological differences produces HAD in these aphids. Similarly, the role of allochrony in generating HAD in exophagous aphids is unclear. Although several exophagous aphid species have been shown to exhibit HAD on phenologically distinct host-plant species (Guldemond 1990, Vanlerberghe-Masutti and Chavigny 1998, Via 1999, Via, Bouck and Skillman 2000, Lozier, Roderick and Mills 2007, Miller, Favret, Carmichael and Voegtlin 2009, Peccoud, Ollivier, Plantegenest and Simon 2009, Dickey and Medina 2010, Mezghani-Khemakhem, Bouktila, Kharrat, Makni and Makni 2012), the occurrence of allochronic isolation in these aphids remains uncharacterized (Harrison et al., in review). The apparent dearth of tests for allochrony in exophagous aphids with HAD may be due to the fact that exophagous aphids are thought to rely on persistent (e.g., leaves and stems) rather than more ephemeral (e.g., fruits and seeds) host-plant resources, presumably freeing aphids from selection for emerging and living within a restricted window of time. However, the physiology of relatively persistent plant organs, and therefore their suitability to exophagous aphids, is still dependent on plant phenology (Senn, Hanhimäki and Haukioja 1992, Mopper 2005). For example, several leaf-chewing insects preferentially feed on younger, more nitrogen-rich leaves and their population densities reflect these phenological differences (Hunter 1992, Mopper and Simberloff 1995). It is still unclear how phloem feeders respond to phenological differences, though it is known that these insects respond to water stress which is, itself, dependent on plant phenology (Huberty and Denno 2004). Therefore, allochronic isolation may occur in exophagous phloem feeding aphids and possibly contribute to the evolution of HAD.

I hypothesized that HAD and allochrony may be correlated in exophagous aphids. This hypothesis was tested using three aphid species sharing the same pair of phenologically distinct hickory species: pecan (*Carya illinoinensis* Wangenh) and water hickory (*Carya aquatica* Michx). Water hickory trees break bud and produce leaves two weeks earlier than pecan; water hickory also begin nut water weight accumulation earlier than pecan (Grauke, Kalinsky and Strout 1984, Thompson and Grauke 1991). Pecan and water hickory aphids provide an excellent opportunity for assessing the relationship between HAD and allochrony in exophagous aphids because they represent a continuum of HAD living on a single host-plant pair. Thus, these aphids are faced with the same host-plant selection pressures and phenologies. These aphids include one species experiencing

HAD (yellow pecan aphid, *Monelliopsis pecanis* Bissell), one species which lacks HAD (black pecan aphid, *Melanocallis caryaefoliae* Davis), and one species which experiences a host-specialized population occurring in sympatry with a host-generalist population, or partial HAD (black-margined aphid, *Monellia caryella* Fitch) (Medina et al. 2017, in press). To assess the presence of allochrony across the HAD continuum, population density data was collected for each aphid species on pecan and water hickory trees during the 2013 growing season. My hypothesis predicts that genetically distinct, host-associated aphid populations will achieve peak population densities at significantly different times while undifferentiated, generalist populations will not.

## METHODS

To assess the presence of allochrony in pecan- and water hickory-associated populations of yellow pecan aphid, black-margined aphid, and black pecan aphid, the average population density of each aphid species was surveyed throughout the 2013 growing season and compared between tree species. Sympatric pecan and water hickory trees were surveyed for aphids during the aphid growing season from April 5th to October 15th, 2013 (Harris 1983). Aphids were sampled from five locations in Brazos, Burleson, and Lee counties, TX. GPS coordinates were recorded for all trees surveyed, allowing us to track the aphid population density on individual trees throughout the sampling period (Table 6). Aphid density was assessed in the same 30 pecan and 24 water hickory trees multiple times a week. During each survey date, aphids were counted on low-hanging

Table 6. Pecan and Water Hickory Locations and Collection Information. Number of sampled trees and their GPS coordinates for pecan and water hickory surveyed throughout Brazos, Burleson, and Lee counties, TX. Numbers under the “Pecan” “Water Hickory” and “Total by Site” columns indicate the number of trees sampled.

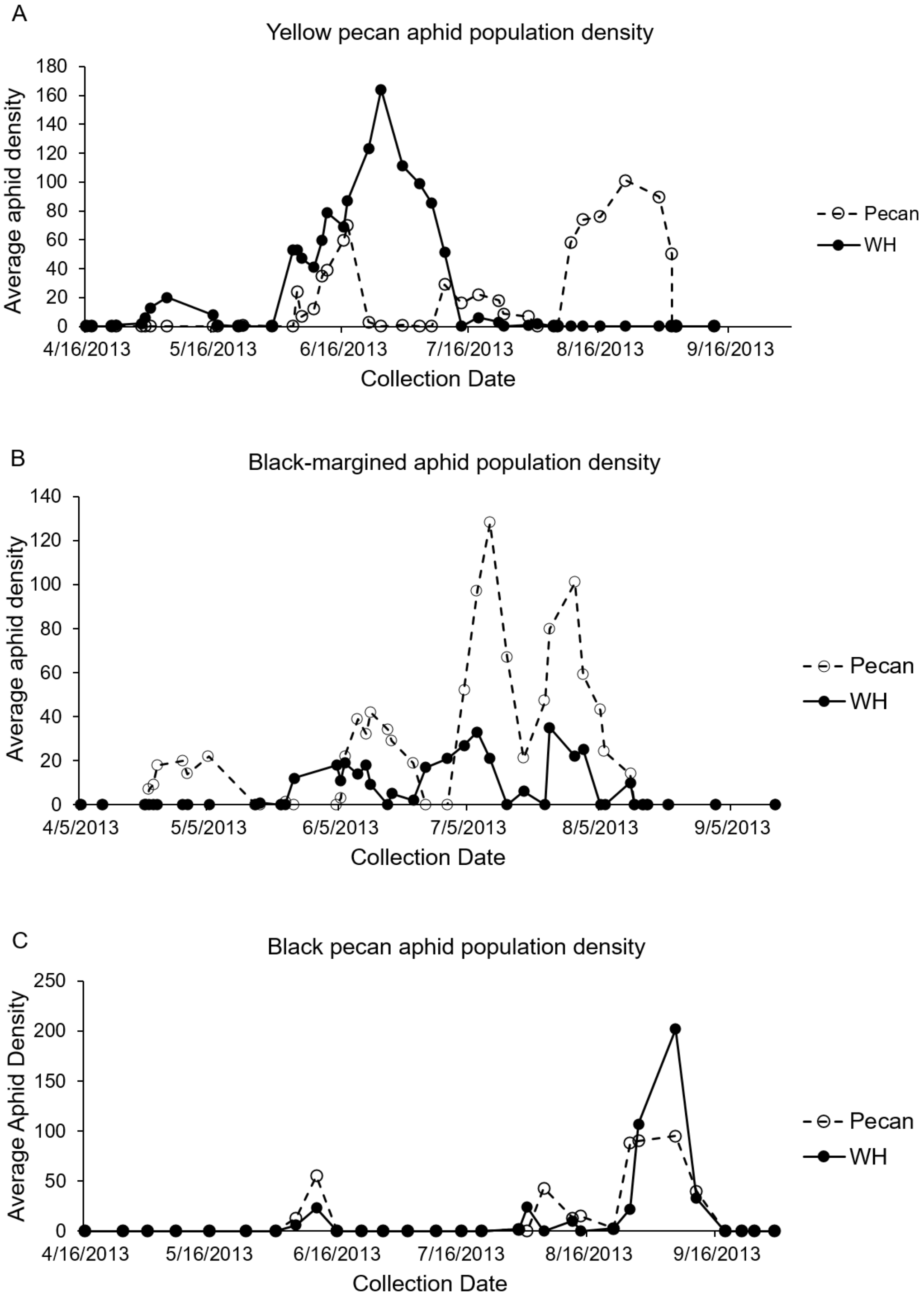
| Location | | Number of trees | | |
|---|---|---|---|---|
| Collection Site | GPS Coordinates | Pecan | Water Hickory | Total by Site |
| Bryan, Texas | 30°40'59.9"N, 96°22'7.4"W | - | 3 | 3 |
| San Salvador Pecan Orchard | 30°36'19.3"N, 96°31'53.2"W | 5 | - | 5 |
| Lake Somerville | 30°21'04.4"N 96°35'31.5"W | 6 | 5 | 11 |
| Lick Creek Park | 30°33'39.4"N, 96°12’52.1"W | - | 3 | 12 |
| | 30°33'37.1"N, 96°13'20.1"W | - | 3 | |
| | 30°33'33.0"N, 96°13'23.2"W | - | 3 | |
| | 30°33'24.9"N, 96°13'29.2"W | - | 3 | |
| Pecan Breeding and Genetics | 30°31'23.2"N, 96°25'27.1"W | - | 6 | 21 |
| | 30°31'15.3"N, 96°25'38.1"W | 5 | - | |
| | 30°31'11.8"N, 96°25'45.4"W | 5 | - | |
| | 30°31'07.3"N, 96°25'40.9"W | 5 | - | |
| Texas A&M Campus | 30°36'51.8"N, 96°20'59.0"W | 2 | - | 4 |
| | 30°36'53.2"N, 96°21'03.3"W | 2 | - | |
| Total by Host-plant | | 30 | 26 | 56 |

tree branches for 60 seconds; this count was repeated three times per tree to obtain an average aphid count per tree. Based on these counts, the average aphid density per aphid species per tree was calculated for each survey date. These aphid density data created distributions of average population densities per tree species across the sampling period. Distributions of aphid density data across time were compared between pecan and water hickory trees using a matched-pairs analysis in JMP® (Version 13. SAS Institute Inc., Cary, NC, 1989-2007). The Matched Pairs platform compares means between correlated variables (i.e., average aphid density across time) and assesses their differences; the null-hypothesis assumes that average aphid densities for a given week are different for each tree species. Aphid density distributions were checked for homoscedasticity using regression diagnostics and normality using a Kolmogorov-Smirnov test performed in JMP®

## RESULTS

HAD co-occurs with allochrony in pecan and water hickory Aphididae. For the yellow pecan aphid, the matched-pairs analysis for the average aphid density across time showed that peak population densities on pecan and water hickory do not significantly overlap (t=0.703; P>0.2666). This indicated that allochrony occurs between host-associated populations in the yellow pecan aphid (Figure 6). In my study area, yellow pecan aphid persisted on pecan and water hickory trees from late-April to mid-August in 2013. However, water hickory-associated yellow pecan aphids died out by mid-July which

Figure 6. Population Density of Three Pecan and Water Hickory Aphididae during 2013. Average number of aphids (A. yellow pecan aphids; B. black-margined aphids, and C. black pecan aphids) occurring on pecan (dashed line) and water hickory (solid line) from April 2nd to September 27th of 2013 (30 weeks).



allochronically isolated them from the pecan-associated yellow pecan aphid population that appeared in August.

For the black-margined aphid, the matched-pairs analysis for the average aphid density across time also showed that peak population densities on pecan and water hickory do not significantly overlap ($t=0.257$; $P>0.8627$). Like for the yellow pecan aphid, this indicated that allochrony occurs between host-associated populations in the black-margined aphid (Figure 6B). First, a population of black-margined aphid achieved peak density on pecan trees only from late-April to mid-May. This population occurred significantly earlier than the majority of black-margined aphid which fluctuated in population density on both pecan and water hickory trees from early June to mid-August.

Finally, the matched-pairs analysis for the black pecan aphid show that peak population densities on pecan and water hickory significantly overlap ($t=3.385$ $P<0.0001$; Figure 6C). That is, populations of black pecan aphids living on pecan and water hickory are not allochronically isolated. Black-margined aphids appear on both pecan and water hickory in early June and both experienced a second, larger peak population from the end of August to mid-September.

## DISCUSSION

Genetically distinct, host-specialized aphid populations in the pecan and water hickory system achieved peak population densities at significantly different times while undifferentiated, generalist aphids did not. Specifically, I observed that water hickory-associated populations of yellow pecan aphid achieved peak population density significantly earlier than pecan-associated aphids (Figure 6A), suggesting allochronic isolation may be present in an aphid species with complete HAD. Also, a pecan-associated population of black-margined aphid achieved peak population density significantly earlier than a generalist population associated with both pecan and water hickory (Figure 6B), suggesting that the pecan-specialist population in an aphid species with partial HAD may be allochronically isolated from the generalist populations; however, future research needs to corroborate the genetic identify of early-emerging black-margined aphid. Lastly, host-associated populations of black pecan aphid achieved peak population density simultaneously (Figure 6C), suggesting a lack of allochronic isolation in an aphid species without HAD.

My research provides the first examples of allochrony in exophagous aphids. Additionally, these aphids represent a continuum of HAD occurrence in aphids that share the same host-plant pair, meaning the specific conditions promoting the evolution of HAD and/or allochrony can be studied within the same parasite-host case study system. Furthermore, this research can be conducted in an aphids absent the gall-making trait. As stated in the introduction, previous examples of allochrony in aphids all involved gall-making (Akimoto 1990, Komatsu and Akimoto 1995, Abbot 2001), a condition believed to impose strong, host-associated selection pressures on parasite populations and drive the evolution of host-specific adaptations (e.g., allochrony) and

HAD (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Abrahamson and Weis 1997, Stireman, Nason and Heard 2005). In the pecan and water hickory system, though, the presence of allochrony is shown to be related to HAD occurrence in leaf-feeding aphids, implying that host-specific adaptations and HAD can evolve in aphids independent of the strong selection pressures imposed on gall-makers. I hypothesize that allochrony itself may drive the evolution of HAD in pecan and water hickory aphids (Abbot and Withgott 2004, Santos, Rousselet, Magnoux, Paiva, Branco and Kerdelhué 2007, Fudickar, Greives, Atwell, Stricker, Ketterson, Williams et al. 2016). This is supported by the fact that pecan- and water hickory-associated black pecan aphid lacked both allochrony and HAD.

However, this hypothesis also calls into question the presumed relationship between host-plant phenology and the evolution of allochrony because the black pecan aphid failed to evolve allochrony on a pair of phenologically distinct host-plants in which two other exophagous aphids evolved both allochrony and HAD. That is, if the phenological differences between pecan and water hickory trees are sufficient for creating allochronic isolation between host-associated populations, then why did the black pecan aphid fail to evolve allochrony or HAD? To assess the role of host-plant phenology in generating allochrony in pecan and water hickory aphids, I compared my parasite-host case study system to other case study systems involving allochrony and HAD. For example, the difference in bud break between pecan and water hickory is similar in magnitude to the phenological differences between apple and hawthorn hosting the apple maggot, *Rhagoletis pomonella* Walsh. Water hickory trees bud break and begin nut development two weeks earlier than pecan, just like hawthorn trees bud break and begin fruit development two weeks earlier than apple (Grauke, Kalinsky and Strout 1984, Grauke, Pratt and Morris 1987). Accordingly, hawthorn-associated apple maggot emerge from puparia and achieve peak population densities approximately two weeks before apple-associated apple maggot, adaptations evolved in response to differences in the availability of host-plant resources (Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Feder and Filchak 1999). The synchronization of parasite population density with host-plant phenology observed in the apple maggot is paralleled in multiple systems including the goldenrod gallfly (*Eurosta solidaginis* Fitch) which evolved HAD based on differences in bud availability between North American goldenrod species (Abrahamson, Brown, Roth, Sumerford, Horner, Hess et al. 1993, Stireman, Janson, Carr, Devlin and Abbot 2008), *Pemphigus* gall-making aphids on multiple oak species (Abbot and Withgott 2004), and *Prodoxus quinquepunctellus* Chambers on different yucca species (Groman and Pellmyr 2000b). However, the parasite-host case study systems presented above all involved ephemeral fruits or galls that can only be made within a restricted window of time (Felt 1940, Shorthouse and Rohfritsch 1992, Yukawa 2000). In contrast, the host-plant organ used by pecan and water hickory aphids (i.e., leaves) is accessible for several months, suggesting the selection pressure on time-dependent traits in exophagous aphids is looser than the selection pressure experienced by endophagous parasites. Indeed, this appears to be reflected in the asynchrony between host-plant resource phenology and aphid population density in the pecan and water hickory system: First, both yellow pecan aphid and black-margined aphid appear in the field weeks after pecan and water hickory leaves have matured. Next, the water hickory-associated population of yellow pecan aphid achieves peak population density approximately five weeks before the pecan-associated population rather than the two weeks that I would expect based on the two-week difference in phenology between pecan and water hickory trees. Last, the pecan-associated population of the black-margined aphid emerges before any water hickory-associated aphids, the opposite of what I would expect because water hickory phenology precedes pecan. The presented evidence suggests that, unlike other parasite-host case study systems, the evolution of allochrony in the pecan and water hickory system is not directly mediated by host-plant phenology and resource availability but instead by host-plant conditions that also vary in time.

Instead of the presence or absence of a specific host-plant resource, I hypothesize pecan and water hickory aphids may have evolved allochrony in response to differences in host-plant conditions that are indirectly influenced by host-plant phenology. For example, leaf quality conditions (e.g., nitrogen and allelochemical content, or water availability) vary across time and insect herbivores preferentially feed on leaves with high nitrogen and water content (Senn, Hanhimäki and Haukioja 1992, Mopper and Simberloff 1995, Mopper 2005). Aphids are able to rapidly evolve host-specific adaptations in response to the specific conditions presented by host-plant resources (Price, Fernandes and Waring 1987, Akimoto 1990, Via 1991, Guldemond and

Mackenzie 1994, Vanlerberghe-Masutti and Chavigny 1998, Abbot 2001, Tsuchida, Koga and Fukatsu 2004, Peccoud, Ollivier, Plantegenest and Simon 2009, Mezghani-Khemakhem, Bouktila, Kharrat, Makni and Makni 2012). This is particularly evident in the genetic structuring observed in the galling aphid, *Kaltenbachiella japonica* Matsumura, which have been shown to evolve HAD in response to individual host-plant bud break (Komatsu and Akimoto 1995). If aphid populations are able to evolve adaptations in response to individual variation in host-plant conditions (e.g., bud break phenology), then aphids may also evolve in response to individual variation in host-plant leaf quality across time. Future research should track the variation in several leaf quality characteristics in pecan and water hickory trees (e.g., water, nitrogen, and tannin concentration-per-leaf) across the growing season and compare these to the population densities of yellow pecan aphid, black-margined aphid, and black pecan aphid. I predict that the genetically distinct, pecan and water hickory aphid population densities synchronize with leaf quality conditions that promote aphid feeding (e.g., a high ratio of water/nitrogen content vs. allelochemicals in leaves).

Alternative to variation in phenology or host-plant leaf quality, I hypothesize that the evolution of allochrony in pecan and water hickory aphids may be explained by other factors correlated with time, such as horticultural practices. Large-scale growing operations involve monocultures of host-plants treated with insecticides, providing large, enemy-free spaces where insect herbivores thrive (Strong, McCoy and Rey 1977, Strong, Lawton and Southwood 1984, Ballabeni, Wlodarczyk and Rahier 2001, Murphy

2004, Heisswolf, Obermaier and Poethke 2005). Specifically, enemy-free spaces may promote the evolution of allochrony in exophagous aphids living on pecan because highly-damaging, early-season pecan-pests (e.g., the pecan nut casebearer, *Acrobasis nuxvorella* Neunzig, and pecan stem phylloxera, *Phylloxera devastatrix* Pergande) initiate the application of fast-acting, broad spectrum insecticides to counteract damage to pecan buds (Harris 1983, Tedders 1983). Certain insecticides previously used in my study sites (e.g., WARRIOR® or Lorsban™ 4E) likely killed-off early populations of important aphid predators (e.g., *Chrysoperla rufilabris* Burmeister and *Hippodamia convergens* Guérin-Méneville) (Mizell and Schiffhauer 1990), which are known to greatly reduce pecan aphid populations (Liao, Harris, Gilstrap and Mansour 1985). Without these biological control agents, early emergence would be highly selective in yellow pecan aphid living on pecan and pecan-specialist black-margined aphids, allowing allochronically-distinct populations to form. This scenario also supports the absence of allochrony in the black pecan aphid since their relatively late emergences in June and August prevent them from taking advantage of enemy-free spaces provided by the pest management practices described above.

Lastly, I hypothesize that allochrony may not be a cause of HAD in the pecan and water hickory system but a consequence. There are ecological and biological factors apart from gall-making and allochrony have been shown to be causes of re For example, immigrant inviability, or the reduction in fitness of migrants and hybrids of migrants, In fact, immigrant inviability exists in the yellow pecan aphid (Dickey and Medina 2011a) as well as the pecan-specialist population of black-margined aphid (Harrison & Medina, *in Preparation*). It is possible that host-specialist populations of yellow pecan aphid and black-margined aphid become reproductively isolated through the spontaneous acquisition of immigrant inviability and later acquired traits that optimized the timing of emergence on their respective host-plants, resulting in allochrony between host-associated populations. The necessity of immigrant inviability to host-specific adaptation may explain the absence of allochrony as well as HAD in the black pecan aphid. I suspect that black pecan aphid never acquired immigrant inviability between pecan or water hickory trees and, thus, never evolved a reproductive isolating barrier between host-associated populations. However, this hypothesis is contingent upon the fact that these aphids are indeed cyclically parthenogenetic and produce the sexual morphs that can disrupt the accumulation host-specific adaptation; these sexual morphs have yet to be discovered in my study system. Future research should test for the presence of immigrant inviability and sexual reproduction in the black pecan aphid to further test the relationship between immigrant inviability, allochrony, and HAD. If HAD develops in response to host-specific adaptations, then I would predict that the black pecan aphid migrants would experience no reduction in fitness during reciprocal transplant experiments moving pecan-collected individuals to water hickory and vice versa.

In conclusion, the discovery of allochrony in the yellow pecan aphid and the black-margined aphid has important implications for the continued effort to fully characterize HAD evolution. Specifically, I provided evidence suggesting allochrony and HAD are related in the pecan and water hickory parasite-host case study system. Also, I have provided examples of parasites evolving HAD and allochrony without a clear link to host-plant phenology. Furthermore, I have also shown, for the first time, that aphids without the gall-making trait evolve HAD under allochrony. The community of pecan and water hickory aphids examined in this research not only provide us with these novel insights, but they also provide a platform for future research that can test the mechanistic relationship between allochrony and HAD. Future research should include tests comparing host-plant leaf quality across time with aphid population densities, tests for the relationship between allochrony and enemy-free spaces, and tests for immigrant inviability in the black pecan aphid.

# CHAPTER V

# CONCLUSIONS AND FUTURE DIRECTIONS

Results from this dissertation have improved my understanding of the factors explaining the occurrence of HAD, both in general and in the pecan and water hickory Aphidomorpha system. First, the results of the quantitative literature (Chapter II) review demonstrated that the occurrence of HAD is non-random and likely common in systems where parasites produce galls on host-plants, fail to find or migrate to novel host-plants due to host-specific adaptations, or experience rapid generation turnover. In general, the combination of ecological factors that can best explain the occurrence of HAD seems to be more complex than previously proposed. Factors shown to be important in specific study systems are not necessarily generalizable to other study systems. Instead, a combination of factors (e.g., gall-making, immigrant inviability, short generation times in parasites, host-specific volatile preference, morphological differentiation, and whether the host or parasite is an invasive species) best explain the occurrence of HAD (Figure 3). Future research should employ the conditional probabilities I calculated to improve my estimates for the number of parasitic arthropod species likely to experience HAD. Based on my description of HAD occurrence in general, I predict that gall-making arthropods and arthropod species with short generation times will be highly diverse and consist of many cryptic species and host-race complexes. The hierarchy of conditional probabilities based on HAD explanatory factors provides a tool for estimating the parasite systems likely to involve HAD, providing a novel avenue for assessing the prevalence of HAD and its importance for the origins of parasite diversity (Colwell and Coddington 1994, Lewinsohn, Novotny and Basset 2005). This ‘decision tree’ tool can be used by ecologists and applied biologists to develop and to test novel verbal models to describe the interaction between explanatory factors that may account for HAD occurrence, models that can then be tested in natural systems. Furthermore, the tool is also helpful to agriculturalists or conservation biologists that want to know the likelihood of HAD occurrence on their crops or on natural reserves without having to conduct expensive and time-consuming genetic tests. Such valuable information can be used to develop more effective integrated pest management (IPM) strategies (Medina 2012). However, there are still limits to this model and avenues to improvement through future research. Specifically, the study systems used to design the decision tree were biased for case studies positive for HAD. This is most likely due to the current bias against publishing studies in which tested hypotheses were not supported by experimental results (Matosin, Frank, Engel, Lum and Newell 2014). These kinds of ‘negative’ results are an important contribution to the scientific literature because they provide a critical evaluation of the current paradigms in the HAD research (Rosenthal 1979, Knight 2003, da Silva 2015). For example, the pecan and water hickory Aphidomorpha system provides an example demonstrating that endophagous feeding is an insufficient predictor of HAD occurrence (Medina et al., in review).

I demonstrated that the pecan-specialist population of black-margined aphid (*Monellia caryella*) experiences immigrant inviability while generalist black-margined aphids do not (Chapter III). This supports the hypothesis that immigrant inviability may be an important mechanisms to explain HAD in some systems as described in Chapter II (Nosil, Vines and Funk 2005, Rundle and Nosil 2005, Feder, Egan and Nosil 2012) . This research is the first demonstration of immigrant inviability in a case of ‘partial HAD’. The discovery of immigrant inviability in this case study has an exciting implication for my understanding of ecological speciation: immigrant inviability can completely remove maladapted aphids from a host-plant species in as few as two asexual generations (calculations in Chapter III). This observation supports the hypothesis that ecological speciation may be a common phenomenon in insect herbivores and that genetic differences can accumulate between sympatric populations without invoking extraordinary circumstances (Dieckmann and Doebeli 1999, Schluter 2001, Via 2001, Futuyma 2008). Furthermore, the pattern of immigrant inviability in pecan and water hickory Aphidomorpha is consistent with the pattern of allochrony observed in Chapter IV. Both immigrant inviability and allochrony were observed in the yellow pecan aphid and pecan-specialist population of black-margined aphid. To corroborate this pattern, though, future research should test whether the black pecan aphid experiences immigrant inviability during reciprocal transplant experiments. I predict that they will not experience immigrant inviability.

Finally, I have provided evidence supporting the hypothesis that allochrony and HAD are correlated, demonstrating a lack of allochrony in an aphid species without HAD, allochrony in a specialist population of an aphid species, and allochrony in an aphid species with complete HAD. Despite previous research demonstrating allochrony as an important factor explaining HAD evolution (Feder, Hunt and Bush 1993, Komatsu and Akimoto 1995, Feder and Filchak 1999, Morrow, Scott, Congdon, Yeates, Frommer and Sved 2000, Abbot 2001, Abbot and Withgott 2004, Mopper 2005), this hypothesis could not be supported in the Chapter II research and is contradicted by other case study systems. For example, the cranberry fruitworm (*Acrobasis vaccinii* L.) is an endophagous, fruit-feeding moth that experiences allochronic isolation by its host yet has failed to evolve HAD (Medina, Szendrei, Harrison, Isaacs, Averill, Malo et al. 2013). Again, negative results like this provide an opportunity for critical analysis of current scientific thinking (like with the allochrony model in HAD). Consistent with the importance of negative results in research discussed above, the pecan and water hickory Aphidomorpha community provides curious examples of aphids evolving HAD under allochrony without a clear link to host-plant phenology. As mentioned above, previous research showed that host-associated populations of gall-making aphids were intimately linked with their host-plant's phenology (Komatsu and Akimoto 1995). This is likely due to the strong selection pressures operating on the gene-to-gene interaction between host-plant and gall-maker (Weis and Abrahamson 1986, Schick and Dahlsten 2003). Similarly, the relationship between HAD and host-plant phenology is clear in frugivorous insects, such as the apple maggot, which must emerge when a host-plant produces a resource with limited availability (i.e., fruits) (Jaenike 1981, Feder, Opp, Wlazlo, Reynolds, Go and Spisak 1994, Dekker, Ibba, Siju, Stensmyr and Hansson 2006). The exophagous pecan and water hickory aphids, though, are not so intimately linked with their host-plant's resources, yet still experience allochrony. As described in the 'Discussion' section of Chapter IV, other hypotheses may explain the origins of allochrony in each pecan and water hickory aphid species. For example, if the application of pesticides on pecan trees early in the growing season causes aphid predator populations to diminish, then the timing of emergence and propagation of the pecan-specialist black-margined aphid would correlate with the timing of these 'enemy-free-spaces' (Thompson 1988, Gratton and Welter 1999, Heard, Stireman III, Nason, Cox, Kolacz and Brown 2006). Testing this hypothesis is important because of its consequences for pesticide application decisions in pecan integrated pest management (IPM) programs (Harris 1983, Liao, Harris, Gilstrap, Dean, Agnew, Michels et al. 1984).

The community of pecan and water hickory aphids examined in this research not only provide us novel insights but also an ideal platform for future research that can test the mechanistic relationship between allochrony, gall-making, immigrant inviability, volatile preference, and HAD. While several tests have been described above and in Chapters 2 through 4, still others are applicable. For example, the gall-makers in the pecan and water hickory Aphidomorpha could not be characterized for allochrony or immigrant inviability due to my failure to sample generalist *M. caryella* from pecan trees during my dissertation research. Since immigrant inviability has already been shown to exist between host-associated populations of the pecan leaf phylloxera, so it is highly likely that immigrant inviability would also be observed in the pecan stem phylloxera. The existence of allochrony in these two phylloxera would also be expected since it would parallel the pattern between HAD and allochrony in this and other case studies (Komatsu and Akimoto 1995). *Phylloxera texana*, by comparison, would likely not experience either allochrony or immigrant inviability due to its lack of HAD. Lastly, volatile preference is entirely untested in pecan and water hickory Aphidomorpha; this is despite its apparent importance described in Chapter II and in other parasite-host case study systems (Wickremasinghe and Emden 1992, Ngi-Song, Overholt, Njagi, Dicke, Ayertey and Lwande 1996, Linn, Dambroski, Feder, Berlocher, Nojima and Roelofs 2004, Dambroski, Linn, Berlocher, Forbes, Roelofs and Feder 2005, Linn, Dambroski, Nojima, Feder, Berlocher and Roelofs 2005). Thus it would be interesting to compare the incidence of volatile preference among the members of this community.

In conclusion, I have shown that the evolution of HAD is non-random and mediated by a few key factors which can be integrated into a hierarchy of conditional probabilities to predict which parasite-host systems are most prone to HAD occurrence. I also show that HAD is likely common in specific systems, particularly in those involving gall-making and immigrant inviability. I also show that the study of HAD needs to be improved with the inclusion of more studies focused on the relationship between allochrony and HAD. With these contributions to the scientific community, I are now closer to explaining the vast species diversity observed in phytophagous insects and other parasitic arthropods.

# REFERENCES

Abbot, D. K. 2001. Evolutionary genetics of gall-forming aphids: population and behavioral processes. University of Arizona, Tucson, AZ, pp 82-132.

Abbot, P., and Withgott, J. H. 2004. Phylogenetic and molecular evidence for allochronic speciation in gall-forming aphids (*Pemphigus*). Evolution **58**:539-553.

Abrahamson, W. G., Blair, C. P., Eubanks, M. D., and Morehead, S. A. 2003. Sequential radiation of unrelated organisms: the gall fly *Eurosta solidaginis* and the tumbling flower beetle *Mordellistena convicta*. Journal of Evolutionary Biology **16**:781-789.

Abrahamson, W. G., Brown, J. M., Roth, S. K., Sumerford, D. V., Horner, J. D., Hess, M. D., How, S. T., Craig, T. P., Packer, R. A., and Itami, J. K. 1993. Gallmaker speciation: an assessment of the roles of host-plant characters, phenology, gallmaker competition, and natural enemies. North Central Forest Experimental Station, Forest Service, USDA, St. Paul, MN, pp 208-222.

Abrahamson, W. G., Dobley, K. B., Houseknecht, H. R., and Pecone, C. A. 2005. Ecological divergence among five co-occurring species of old-field goldenrods. Plant Ecology **177**:43-56.

Abrahamson, W. G., Eubanks, M. D., Blair, C. P., and Whipple, A. V. 2001. Gall flies, inquilines, and goldenrods: A model for host-race formation and sympatric speciation. American Zoologist **41**:928-938.

Abrahamson, W. G., McCrea, K. D., and Anderson, S. S. 1989. Host preference and recognition by the goldenrod ball gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). American Midland Naturalist **121**:322-330.

Abrahamson, W. G., and Weis, A. E. 1997. Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies. Princeton University Press, Princeton, NJ, pp 194-253.

Agosta, S. J. 2006. On ecological fitting, plant–insect associations, herbivore host shifts, and host plant selection. Oikos **114**:556-565.

Akimoto, S. 1990. Local adaptation and host race formation of a gall-forming aphid in relation to environmental heterogeneity. Oecologia **83**:162-170.

Alexander, R. D., and Bigelow, R. S. 1960. Allochronic speciation in field crickets, and a new species, *Acheta veletis*. Evolution **14**:334-346.

Althoff, D. M. 2008. A test of host-associated differentiation across the 'parasite continuum' in the tri-trophic interaction among yuccas, bogus yucca moths, and parasitoids. Molecular Ecology **17**:3917-3927.

Althoff, D. M., Fox, K. A., and Frieden, T. 2014. The role of ecological availability and host plant characteristics in determining host use by the bogus yucca moth *Prodoxus decipiens*. Ecological Entomology **39**:620-626.

Althoff, D. M., Groman, J. D., Segraves, K. A., and Pellmyr, O. 2001. Phylogeographic structure in the bogus yucca moth *Prodoxus quinquepunctellus* (Prodoxidae): Comparisons with coexisting pollinator yucca moths. Molecular Phylogenetics and Evolution **21**:117-127.

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data. Babraham Bioinformatics, Babraham, Cambridgeshire, UK, pp 175-176.

Antolin, M. F., Bjorksten, T. A., and Vaughn, T. T. 2006. Host- related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). Ecological Entomology **31**:242-254.

Antwi, J. B., Sword, G. A., and Medina, R. F. 2015. Host- associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. Ecology and Evolution **5**:2533-2543.

Baer, C., Tripp, D., Bjorksten, T., and Antolin, M. 2004. Phylogeography of a parasitoid wasp (*Diaeretiella rapae*): no evidence of host- associated lineages. Molecular Ecology **13**:1859-1869.

Ballabeni, P., Wlodarczyk, M., and Rahier, M. 2001. Does enemy- free space for eggs contribute to a leaf beetle’s oviposition preference for a nutritionally inferior host plant? Functional Ecology **15**:318-324.

Barman, A. K., Parajulee, M. N., Sansone, C. G., Suh, C. P., and Medina, R. F. 2012. Geographic pattern of host- associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus*. Entomologia Experimentalis et Applicata **143**:31-41.

Baxter, S. W., Davey, J. W., Johnston, J. S., Shelton, A. M., Heckel, D. G., Jiggins, C. D., and Blaxter, M. L. 2011. Linkage mapping and comparative genomics using next-generation RAD sequencing of a non-model organism. PloS One **6**:e19315.

Berlocher, S. H., and Feder, J. L. 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? Annual Review of Entomology **47**:773-815.

Bethenod, M., Thomas, Y., Rousset, F., Frérot, B., Pélozuelo, L., Genestier, G., and Bourguet, D. 2005. Genetic isolation between two sympatric host plant races of the European corn borer, *Ostrinia nubilalis* Hübner. II: assortative mating and host-plant preferences for oviposition. Heredity **94**:264-270.

Blair, C. P., Abrahamson, W. G., Jackman, J. A., and Tyrrell, L. 2005. Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle. Evolution **59**:304-316.

Blankenberg, D., Gordon, A., Von Kuster, G., Coraor, N., Taylor, J., Nekrutenko, A., and Team, G. 2010. Manipulation of FASTQ data with Galaxy. Bioinformatics **26**:1783-1785.

Blankenberg, D., Kuster, G. V., Coraor, N., Ananda, G., Lazarus, R., Mangan, M., Nekrutenko, A., and Taylor, J. 2010. Galaxy: a web- based genome analysis tool for experimentalists. Current Protocols in Molecular Biology **0 19**:Unit-19.1021.doi:1010.1002/0471142727.mb0471141910s0471142789.

Brown, J. M., Abrahamson, W. G., Packer, R. A., and Way, P. A. 1995. The role of natural enemy escape in a gallmaker host-plant shift. Oecologia **104**:52-60.

Bruce, T. J., Wadhams, L. J., and Woodcock, C. M. 2005. Insect host location: a volatile situation. Trends in Plant Science **10**:269-274.

Burban, C., Fishpool, L., Fauquet, C., Fargette, D., and Thouvenel, J. C. 1992. Host-associated biotypes within West African populations of the whitefly *Bemisia tabaci* (Genn.), (Homoptera, Aleyrodidae). Journal of Applied Entomology **113**:416-423.

Bush, G. 1974. The mechanism of sympatric host race formation in the true fruit flies (Tephritidae). *in* M. White, editor. Genetic mechanisms of speciation in insects. Springer, Australia & New Zealand Book Company, pp 3-23.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies in genus *Rhagoletis* (Diptera: Tephritidae). Evolution **23**:237-251.

Bush, G. L. 1975a. Modes of animal speciation. Annual Review of Ecology and Systematics **6**:339-364.

Bush, G. L. 1975b. Sympatric speciation in phytophagous parasitic insects. Evolutionary strategies of parasitic insects and mites. Springer, pp 187-206.

Bush, G. L. 1994. Sympatric speciation in animals - new wine in old bottles. Trends in Ecology & Evolution **9**:285-288.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., and Bauer, M. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal **6**:1621-1624.

Clements, K. M., Sorenson, C. E., Wiegmann, B. M., Neese, P. A., and Roe, R. M. 2000. Genetic, biochemical, and behavioral uniformity among populations of *Myzus nicotianae* and *Myzus persicae*. Entomologia Experimentalis et Applicata **95**:269-281.

Cocroft, R. B., Rodríguez, R. L., and Hunt, R. E. 2008. Host shifts, the evolution of communication, and speciation in the *Enchenopa binotata* species complex of treehoppers. *in* K. Tilmon, editor. Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley, CA, pp 88-100.

Colwell, R. K., and Coddington, J. A. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London B: Biological Sciences **345**:101-118.

Cook, M. A., Ozeroff, S. N., Fitzpatrick, S. M., and Roitberg, B. D. 2011. Host-associated differentiation in reproductive behaviour of cecidomyiid midges on cranberry and blueberry. Entomologia Experimentalis Et Applicata **141**:8-14.

Cooley, J. R., Simon, C., Marshall, D. C., Slon, K., and Ehrhardt, C. 2001. Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (Hemiptera: *Magicicada* spp.): genetic, morphological, and behavioural evidence. Molecular Ecology **10**:661-671.

Cornell, H. V., Hawkins, B. A., and Hochberg, M. E. 1998. Towards an empirically-based theory of herbivore demography. Ecological Entomology **23**:340-349.

Craig, T. P., Horner, J. D., and Itami, J. K. 1997. Hybridization studies on the host races of *Eurosta solidaginis*: implications for sympatric speciation. Evolution **51**:1552-1560.

Craig, T. P., Horner, J. D., and Itami, J. K. 2001. Genetics, experience, and host-plant preference in *Eurosta solidaginis*: implications for host shifts and speciation. Evolution **55**:773-782.

Craig, T. P., Itami, J. K., Abrahamson, W. G., and Horner, J. D. 1993. Behavior evidence for host-race formation in *Eurosta solidaginis*. Evolution **47**:1696-1710.

Craig, T. P., Itami, J. K., Horner, J. D., and Abrahamson, W. G. 1994. Host shifts and speciation in gall-forming insects. *in* P. W. Price, W. J. Mattson, and Y. N. Baranchikov, editors. Ecology and evolution of gall-forming insects. U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, MN, pp 194-207.

Craig, T. P., Itami, J. K., Ohgushi, T., Ando, Y., and Utsumi, S. 2011. Bridges and barriers to host shifts resulting from host plant genotypic variation. Journal of Plant Interactions **6**:141-145.

Criminisi, A., Shotton, J., and Konukoglu, E. 2012. Decision forests: A unified framework for classification, regression, density estimation, manifold learning and semi-supervised learning. Foundations and Trends® in Computer Graphics and Vision **7**:81-227.

Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T., Gibson, J., and Lawler, J. J. 2007. Random forests for classification in ecology. Ecology **88**:2783-2792.

da Silva, J. A. T. 2015. Negative results: negative perceptions limit their potential for increasing reproducibility. Journal of Negative Results in Biomedicine **14**:12.

Dambroski, H. R., Linn, C., Berlocher, S. H., Forbes, A. A., Roelofs, W., and Feder, J. L. 2005. The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host shifts. Evolution **59**:1953-1964.

Davey, J. W., and Blaxter, M. L. 2011. RADSeq: next-generation population genetics. Briefings in Functional Genomics **10**:108-108.

de Ruiter, D. J., DeWitt, T. J., Carlson, K. B., Brophy, J. K., Schroeder, L., Ackermann, R. R., Churchill, S. E., and Berger, L. R. 2013. Mandibular remains support taxonomic validity of *Australopithecus sediba*. Science **340**:1232997.

Dekker, T., Ibba, I., Siju, K., Stensmyr, M. C., and Hansson, B. S. 2006. Olfactory shifts parallel superspecialism for toxic fruit in *Drosophila melanogaster* sibling, *D. sechellia*. Current Biology **16**:101-109.

Devaux, C., and Lande, R. 2008. Incipient allochronic speciation due to non-selective assortative mating by flowering time, mutation and genetic drift. Proceedings of the Royal Society of London B: Biological Sciences **275**:2723-2732.

Dickey, A. M. 2010. Host-associated differentiation in an insect community. Texas A&M University, College Station, TX, pp 5-134.

Dickey, A. M., and Medina, R. F. 2010. Testing host-associated differentiation in a quasi-endophage and a parthenogen on native trees. Journal of Evolutionary Biology **23**:945-956.

Dickey, A. M., and Medina, R. F. 2011a. Immigrant inviability in yellow pecan aphid. Ecological Entomology **36**:526-531.

Dickey, A. M., and Medina, R. F. 2011b. Lack of sequential radiation in a parasitoid of a host-associated aphid. Entomologia Experimentalis Et Applicata **139**:154-160.

Dickey, A. M., and Medina, R. F. 2012. Host-associated genetic differentiation in pecan leaf phylloxera. Entomologia Experimentalis Et Applicata **143**:127-137.

Dieckmann, U., and Doebeli, M. 1999. On the origin of species by sympatric speciation. Nature **400**:354-357.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. Springer Science & Business Media, London, UK, pp 27-52.

Dobson, A., Lafferty, K. D., Kuris, A. M., Hechinger, R. F., and Jetz, W. 2008. Homage to Linnaeus: How many parasites? How many hosts? Proceedings of the National Academy of Sciences **105**:11482-11489.

Dobson, S. L., Fox, C. W., and Jiggins, F. M. 2002. The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems. Proceedings of the Royal Society of London B: Biological Sciences **269**:437-445.

Dolphin, K., and Quicke, D. L. 2001. Estimating the global species richness of an incompletely described taxon: an example using parasitoid wasps (Hymenoptera: Braconidae). Biological Journal of the Linnean Society **73**:279-286.

Dorchin, N., Scott, E. R., Clarkin, C. E., Luongo, M. P., Jordan, S., and Abrahamson, W. G. 2009. Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges. Journal of Evolutionary Biology **22**:729-739.

Dreger-Jauffret, F., and Shorthouse, J. 1992. Diversity of gall-inducing insects and their galls. Biology of Insect-Induced Galls. Oxford University Press, Oxford, UK, pp 8-33.

Dres, M., and Mallet, J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philosophical Transactions of the Royal Society B-Biological Sciences **357**:471-492.

Eastop, V. F. 1973. Deductions from the present day host plants of aphids and related insects in insect/plant relationships. *in* H. F. Van Emden, editor. Blackwell Scientific Publications, London, UK, pp 157-178.

Edmunds, G. F., and Alstad, D. N. 1981. Responses of black pineleaf scales to host plant variability. *in* R. F. Denno and H. Dingle, editors. Insect Life History Patterns. Springer-Verlag, New York, pp 29-38.

Egan, S. P., and Funk, D. J. 2006. Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. Proceedings of the Royal Society of London B: Biological Sciences **273**:843-848.

Egan, S. P., Nosil, P., and Funk, D. J. 2008. Selection and genomic differentiation during ecological speciation: Isolating the contributions of host association via a comparative genome scan of *Neochlamisus bebbianae* leaf beetles. Evolution **62**:1162-1181.

Esteve- Gassent, M. D., Castro- Arellano, I., Feria- Arroyo, T. P., Patino, R., Li, A. Y., Medina, R. F., León, A. A. P., and Rodríguez- Vivas, R. I. 2016. Translating ecology, physiology, biochemistry, and population genetics research to meet the challenge to tick and tick-borne diseases in North America. Archives of Insect Biochemistry and Physiology **92**:38-64.

Eubanks, M. D., Blair, C. P., and Abrahamson, W. G. 2003. One host shift leads to another? Evidence of host-race formation in a predaceous gall-boring beetle. Evolution **57**:168-172.

Farrell, B. D. 1998. "Inordinate fondness" explained: why are there so many beetles? Science **281**:555-559.

Feder, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*. Endless forms: species and speciation. Oxford Univ. Press, New York:130-144.

Feder, J. L., Chilcote, C. A., and Bush, G. L. 1988. Genetic differentiation between sympatric host races of the apple maggot fly, *Rhagoletis pomonella*. Nature **336**:61-64.

Feder, J. L., Egan, S. P., and Nosil, P. 2012. The genomics of speciation-with-gene-flow. Trends in Genetics **28**:342-350.

Feder, J. L., and Filchak, K. E. 1999. It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects. Entomologia Experimentalis Et Applicata **91**:211-225.

Feder, J. L., Hunt, T. A., and Bush, G. L. 1993. The effects of climate, host-plant phenology, and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. Entomologia Experimentalis Et Applicata **69**:117-135.

Feder, J. L., Opp, S. B., Wlazlo, B., Reynolds, K., Go, W., and Spisak, S. 1994. Host Fidelity Is an Effective Premating Barrier between Sympatric Races of the Apple Maggot Fly. Proceedings of the National Academy of Sciences of the United States of America **91**:7990-7994.

Fellows, D. P., and Heed, W. B. 1972. Factors affecting host plant selection in desert-adapted cactiphilic *Drosophila*. Ecology **53**:850-858.

Felt, E. P. 1940. Plant galls and gall makers. Comstock Publishing Company, Ithaca, NY, pp 240-340.

Ferrari, J., Godfray, H. C. J., Faulconbridge, A. S., Prior, K., and Via, S. 2006. Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. Evolution **60**:1574-1584.

Ferrari, J., Scarborough, C. L., and Godfray, H. C. J. 2007. Genetic variation in the effect of a facultative symbiont on host-plant use by pea aphids. Oecologia **153**:323-329.

Ferrari, J., Via, S., and Godfray, H. C. J. 2008. Population differentiation and genetic variation in performance on eight hosts in the pea aphid complex. Evolution **62**:2508-2524.

Finch, S. 1978. Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (*Delia brassicae*). Entomologia Experimentalis et Applicata **24**:350-359.

Finch, S., and Collier, R. 2000. Host-  plant selection by insects – a theory based on ‘appropriate/inappropriate landings’ by pest insects of cruciferous plants. Entomologia Experimentalis et Applicata **96**:91-102.

Flaxman, S. M., Feder, J. L., and Nosil, P. 2013. Genetic hitchhiking and the buildup of genetic divergence during speciation with gene flow. Evolution **67**:1-13.

Forbes, A. A., Powell, T. H., Stelinski, L. L., Smith, J. J., and Feder, J. L. 2009. Sequential sympatric speciation across trophic levels. Science **323**:776-779.

Fordyce, J. A. 2010. Host shifts and evolutionary radiations of butterflies. Proceedings of the Royal Society of London B: Biological Sciences **277**:3735-3743.

Fordyce, J. A., and Nice, C. C. 2003. Variation in butterfly egg adhesion: adaptation to local host plant senescence characteristics? Ecology Letters **6**:23-27.

Forister, M. L., Dyer, L. A., Singer, M. S., Stireman, J. O., III, and Lill, J. T. 2012. Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. Ecology **93**:981-991.

Fralish, J. S. F., S. B. 2002. Taxonomy and ecology of woody plants in North American forests (excluding Mexico and subtropical Florida). John Wiley & Sons, New York, NY, pp 210-221.

Frey, J. E., Feder, J. L., Palma, J., and Bush, G. L. 1998. Differences in the electroantennal responses of apple- and hawthorn-infesting races of *Rhagoletis pomonella* to host fruit volatile compounds. Chemoecology **8**:175-186.

Fudickar, A. M., Greives, T. J., Atwell, J. W., Stricker, C. A., Ketterson, E. D., Williams, T. D., and Michalakis, Y. 2016. Reproductive allochrony in seasonally sympatric populations maintained by differential response to photoperiod: implications for population divergence and response to climate change. The American Naturalist **187**:436-446.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. Evolution **52**:1744-1759.

Funk, D. J. 2010. Does strong selection promote host specialisation and ecological speciation in insect herbivores? Evidence from *Neochlamisus* leaf beetles. Ecological Entomology **35**:41-53.

Funk, D. J., Filchak, K. E., and Feder, J. L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica **116**:251-267.

Funk, D. J., Nosil, P., and Etges, W. J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. Proceedings of the National Academy of Sciences of the United States of America **103**:3209-3213.

Futuyma, D. 2008. Sympatric speciation: norm or exception. *in* K. Tilmon, editor. Specialization, speciation, and radiation: The evolutionary biology of herbivorous insects. Univesity of California Press, Berkeley, CA, pp 136-147.

Futuyma, D. J., and Moreno, G. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics **19**:207-233.

Gandon, S., and Michalakis, Y. 2002. Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, mutation, population size and generation time. Journal of Evolutionary Biology **15**:451-462.

Geiselhardt, S., Otte, T., Hilker, M., and Turlings, T. 2012. Looking for a similar partner: host plants shape mating preferences of herbivorous insects by altering their contact pheromones. Ecology Letters **15**:971-977.

Giardine, B., Riemer, C., Hardison, R. C., Burhans, R., Elnitski, L., Shah, P., Zhang, Y., Blankenberg, D., Albert, I., and Taylor, J. 2005. Galaxy: a platform for interactive large-scale genome analysis. Genome research **15**:1451-1455.

Goecks, J., Nekrutenko, A., and Taylor, J. 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol **11**:R86.

Gómez- Díaz, E., González- Solís, J., Peinado, M., and Page, R. D. 2007. Lack of host- dependent genetic structure in ectoparasites of Calonectris shearwaters. Molecular ecology **16**:5204-5215.

Gratton, C., and Welter, S. C. 1999. Does "enemy-free space" exist? Experimental host shiftsof an herbivorous fly. Ecology **80**:773-785.

Grauke, L. J., Kalinsky, R. G., and Strout, G. W. 1984. Identification, site adaptation and phenology of Louisiana hickories. Hortscience **19**:542-542.

Grauke, L. J., Pratt, J. W., and Morris, H. 1987. Phenology and site adaptation of Louisiana hickories. Louisiana Agricultural Experiment Station, Baton Rouge, pp 181-185.

Groman, J., and Pellmyr, O. 2000a. Rapid evolution and specialization following host colonization in a yucca moth. Journal of Evolutionary Biology **13**:223-236.

Groman, J. D., and Pellmyr, O. 2000b. Rapid evolution and specialization following host colonization in a yucca moth. Journal of Evolutionary Biology **13**:223-236.

Guldemond, J. A. 1990. Evolutionary genetics of the aphid *Cryptomyzus,* with a preliminary analysis of the inheritance of host plant preference, reproductive performance and host- alteration. Entomologia Experimentalis et Applicata **57**:65-76.

Guldemond, J. A., and Mackenzie, A. 1994. Sympatric speciation in aphids. I. Host race formation by escape from gene flow. *in* S. R. Leather, A. D. Watt, N. J. Mills , and K. F. A. Walters, editors. Individuals, populations and patterns in ecology. Intercept Ltd., Aukland, New Zealand, pp 367-378.

Hardison, R. C. 2003. Comparative genomics. Plos Biology **1**:156-160.

Harris, M. 1985. Population growth of the black-margined aphid on pecan in the field. Agriculture, Ecosystems & Environment **12**:253-261.

Harris, M. K. 1983. Integrated pest management of pecans. Annual Review of Entomology **28**:291-318.

Hartfield, M., and Keightley, P. D. 2012. Current hypotheses for the evolution of sex and recombination. Integrative zoology **7**:192-209.

Hartl, D. L. 1972. Fundamental theorem of natural selection for sex linkage or arrhenotoky. American Naturalist **106**:516-524.

Hastie, T., Tibshirani, R., Sherlock, G., Eisen, M., Brown, P., and Botstein, D. 1999. Imputing missing data for gene expression arrays. Stanford University Statistics Department Technical report, Stanford, CA, pp 1-9.

Hawthorne, D. J., and Via, S. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature **412**:904-907.

Heard, S. B., Stireman III, J. O., Nason, J. D., Cox, G. H., Kolacz, C. R., and Brown, J. M. 2006. On the elusiveness of enemy-free space: spatial, temporal, and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods. Oecologia **150**:421-434.

Heed, W. B. 1971. Host plant specificity and speciation in Hawaiian *Drosophila*. Taxon **20**:115-121.

Heisswolf, A., Obermaier, E., and Poethke, H. J. 2005. Selection of large host plants for oviposition by a monophagous leaf beetle: nutritional quality or enemy- free space? Ecological Entomology **30**:299-306.

Helyar, S. J., Hemmer- Hansen, J., Bekkevold, D., Taylor, M., Ogden, R., Limborg, M., Cariani, A., Maes, G., Diopere, E., and Carvalho, G. 2011. Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. Molecular Ecology Resources **11**:123-136.

Hood, G. M. 2010. PopTools version 3.2.5. Available on the internet, http://www.poptools.org, pp 1.

Horton, N. J., and Lipsitz, S. R. 2001. Multiple imputation in practice: comparison of software packages for regression models with missing variables. The American Statistician **55**:244-254.

Hosokawa, T., Kikuchi, Y., Shimada, M., and Fukatsu, T. 2007. Obligate symbiont involved in pest status of host insect. Proceedings of the Royal Society B-Biological Sciences **274**:1979-1984.

Howard, R. S., and Lively, C. M. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature **367**:554-557.

Huberty, A. F., and Denno, R. F. 2004. Plant water stress and its consequences for herbivorous insects: a new synthesis. Ecology **85**:1383-1398.

Hufbauer, R. A., and Roderick, G. K. 2005. Microevolution in biological control: mechanisms, patterns, and processes. Biological control **35**:227-239.

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**:91-95.

Ishiguro, N., Yoshida, K., and Tsuchida, K. 2006. Genetic differences between rice and water-oat feeders in the rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae). Applied Entomological and Zoology **41**:585-593.

Itami, J. K., Craig, T. P., and Horner, J. D. 1998. Factors affecting gene flow between the host races of *Eurosta solidaginis*. *in* S. Mopper and S. Y. Strauss, editors. Genetic structure and local adaptation in natural insect populations. Springer, pp 375-407.

Jaenike, J. 1981. Criteria for ascertaining the existence of host races. The American Naturalist **117**:830-834.

Jaenike, J. 1990. Host specialization in phytophagous insects. Annual Review of Ecology and Systematics **21**:243-273.

Jaenike, J., and Selander, R. K. 1980. On the question of host races in the fall webworm, *Hyphantria cunea*. Entomologia Experimentalis et Applicata **27**:31-37.

Jiggins, F. M., Hurst, G. D., and Majerus, M. E. 2000. Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. Proceedings of the Royal Society of London B: Biological Sciences **267**:69-73.

Johnson, K. P., Adams, R., and Clayton, D. H. 2002. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. Biological Journal of the Linnean Society **77**:233-247.

Jolliffe, I. 2002. Principal components as a small number of interpretable variables: some examples. Principal Component Analysis:63-77.

King, C. E. 1993. Random genetic drift during cyclical ameiotic parthenogenesis. Hydrobiologia **255**:205-212.

King, C. E., and Murtaugh, P. 1997. Effects of asexual reproduction on the neighborhood area of cyclical parthenogens. Live Food in Aquaculture. Springer, pp 55-62.

Kliman, R. M., Andolfatto, P., Coyne, J. A., Depaulis, F., Kreitman, M., Berry, A. J., McCarter, J., Wakeley, J., and Hey, J. 2000. The population genetics of the origin and divergence of the *Drosophila simulans* complex species. Genetics **156**:1913-1931.

Knight, J. 2003. Negative results: null and void. Nature **422**:554-555.

Kohnen, A., Wissemann, V., and Brandl, R. 2011. No host-associated differentiation in the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae) on three dog rose species. Biological Journal of the Linnean Society **102**:369-377.

Komatsu, T., and Akimoto, S. 1995. Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica*. Ecological Entomology **20**:33-42.

Konno, Y., Honda, H., and Matsumoto, Y. 1981. Mechanisms of reproductive isolation between the fruit-feeding and the *Pinaceae*-feeding types of the yellow peach moth, *Dichocrocis punctiferalis* Guenee (Lepidoptera: Pyralidae). Japanese Journal of Applied Entomology and Zoology (Japan) **25**:245-247.

Leonardo, T. E., and Muiru, G. T. 2003. Facultative symbionts are associated with host plant specialization in pea aphid populations. Proceedings of the Royal Society of London B: Biological Sciences **270**:S209-S212.

Leppänen, S. A., Malm, T., Värri, K., and Nyman, T. 2014. A comparative analysis of genetic differentiation across six shared willow host species in leaf- and bud-galling sawflies. PloS One **9**:e116286.

Lewinsohn, T. M., Novotny, V., and Basset, Y. 2005. Insects on plants: diversity of herbivore assemblages revisited. Annual Review of Ecological Evolutionary Systematics **36**:597-620.

Li, H., and Durbin, R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics **25**:1754-1760.

Li, W.-H., Ellsworth, D. L., Krushkal, J., Chang, B. H.-J., and Hewett-Emmett, D. 1996. Rates of nucleotide substitution in primates and rodents and the generation–time effect hypothesis. Molecular Phylogenetics and Evolution **5**:182-187.

Liao, H.-T., Harris, M., Gilstrap, F., and Mansour, F. 1985. Impact of natural enemies on the blackmargined pecan aphid, *Monellia caryella* (Homoptera: Aphidae). Environmental Entomology **14**:122-126.

Liao, H., Harris, M., Gilstrap, F., Dean, D., Agnew, C., Michels, G., and Mansour, F. 1984. Natural enemies and other factors affecting seasonal abundance of the blackmargined aphid on pecan. The Southwestern entomologist (USA).

Linn, C. E., Dambroski, H., Nojima, S., Feder, J. L., Berlocher, S. H., and Roelofs, W. L. 2005. Variability in response specificity of apple, hawthorn, and flowering dogwood- infesting Rhagoletis flies to host fruit volatile blends: implications for sympatric host shifts. Entomologia experimentalis et applicata **116**:55-64.

Linn, C. E., Dambroski, H. R., Feder, J. L., Berlocher, S. H., Nojima, S., and Roelofs, W. L. 2004. Postzygotic isolating factor in sympatric speciation in Rhagoletis flies: reduced response of hybrids to parental host-fruit odors. Proceedings of the National Academy of Sciences of the United States of America **101**:17753-17758.

Lowry, D. B., Modliszewski, J. L., Wright, K. M., Wu, C. A., and Willis, J. H. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants. Philosophical Transactions of the Royal Society of London B: Biological Sciences **363**:3009-3021.

Loxdale, H. D. 2008. The nature and reality of the aphid clone: genetic variation, adaptation and evolution. Agricultural and Forest Entomology **10**:81-90.

Loxdale, H. D. 2009. What's in a clone: the rapid evolution of aphid asexual lineages in relation to geography, host plant adaptation and resistance to pesticides. *in* I. Schön, K. Martens, and P. van Dijk, editors. Lost Sex. Springer, pp 535-557.

Loxdale, H. D. 2010. Rapid genetic changes in natural insect populations. Ecological Entomology **35**:155-164.

Lozier, J., Roderick, G., and Mills, N. 2009. Molecular markers reveal strong geographic, but not host associated, genetic differentiation in *Aphidius transcaspicus*, a parasitoid of the aphid genus *Hyalopterus*. Bulletin of entomological research **99**:83-96.

Lozier, J. D., Roderick, G. K., and Mills, N. J. 2007. Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host plant associated species in the aphid genus *Hyalopterus* (Hemiptera: Aphididae). Evolution **61**:1353-1367.

Lynch, M. 1984. The limits to life-history evolution in *Daphnia*. Evolution **38**:465-482.

Lynch, M. 1985. Spontaneous mutations for life-history characters in an obligate parthenogen. Evolution **39**:804-818.

Lynch, M., and Gabriel, W. 1983. Phenotypic evolution and parthenogenesis. American Naturalist **122**:745-764.

Mallet, J., Meyer, A., Nosil, P., and Feder, J. 2009. Space, sympatry and speciation. Journal of Evolutionary Biology **22**:2332-2341.

Manchester, S. R. 1987. The fossil history of the Juglandaceae. **41**:1-137.

Martin, A. P., and Palumbi, S. R. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proceedings of the National Academy of Sciences **90**:4087-4091.

Martinelli, S., Clark, P., Zucchi, M., Silva-Filho, M., Foster, J. E., and Omoto, C. 2007. Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. Bulletin of Entomological Research **97**:225-231.

Matosin, N., Frank, E., Engel, M., Lum, J. S., and Newell, K. A. 2014. Negativity towards negative results: a discussion of the disconnect between scientific worth and scientific culture. Disease Models and Mechanisms **7**:171-173.

Matsubayashi, K., Kahono, S., and Katakura, H. 2011. Divergent host plant specialization as the critical driving force in speciation between populations of a phytophagous ladybird beetle. Journal of Evolutionary Biology **24**:1421-1432.

Matsubayashi, K. W., Ohshima, I., and Nosil, P. 2010. Ecological speciation in phytophagous insects. Entomologia Experimentalis Et Applicata **134**:1-27.

McCall, P. J., Turlings, T. C., Lewis, W. J., and Tumlinson, J. H. 1993. Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera). Journal of Insect Behavior **6**:625-639.

McCoy, K. D., Léger, E., and Dietrich, M. 2015. Host specialization in ticks and transmission of tick-borne diseases: a review. Frontiers in Cellular and Infection Microbiology **3**:57.

McLellan, K. A., Nordin, G. L., and Haynes, K. F. 1991. Chemical communication and reproductive isolation in two types of the fall webworm (Lepidoptera: Arctiidae). Annals of the Entomological Society of America **84**:118-123.

Medina, R. F. 2012. Implications of host-associated differentiation in the control of pest species. *in* P. L. Barbosa, D.K; Agrawal, A. A., editor. Insect Outbreaks Revisited. Wiley-Blackwell Publishing, pp 291-310.

Medina, R. F., Dickey, A. M., Harrison, K., and Miller, G. L. 2017. Host-associated differentiation in a pecan and water hickory Aphidomorpha community Entomologia Experimentalis et Applicata ***in press***.

Medina, R. F., Nachappa, P., and Tamborindeguy, C. 2011. Differences in bacterial diversity of host-associated populations of *Phylloxera notabilis* Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory. Journal of Evolutionary Biology **24**:761-771.

Medina, R. F., Reyna, S. M., and Bernal, J. S. 2012. Population genetic structure of a specialist leafhopper on Zea: likely anthropogenic and ecological determinants of gene flow. Entomologia Experimentalis Et Applicata **142**:223-235.

Medina, R. F., Szendrei, Z., Harrison, K., Isaacs, R., Averill, A., Malo, E. A., and Rodrigues-Saona, C. 2013. Exploring host-associated differentiation in North

American native cranberry fruitworm, *Acrobasis vaccinii*, from blueberries and cranberries. Entomologia Experimentalis Et Applicata **150**:136-148.

Mezghani-Khemakhem, M., Bouktila, D., Kharrat, I., Makni, M., and Makni, H. 2012. Genetic variability of green citrus aphid populations from Tunisia, assessed by RAPD markers and mitochondrial DNA sequences. Entomological Science **15**:171-179.

Michel, A. P., Sim, S., Powell, T. H., Taylor, M. S., Nosil, P., and Feder, J. L. 2010. Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences **107**:9724-9729.

Miller, G. L., Favret, C., Carmichael, A., and Voegtlin, D. J. 2009. Is there a cryptic species within *Aulacorthum solani* (Hemiptera: Aphididae)? Journal of Economic Entomology **102**:398-400.

Mitter, C., Farrell, B., and Wiegmann, B. 1988. The phylogenetic study of adaptive zones - has phytophagy promoted insect diversification? American Naturalist **132**:107-128.

Mitter, C., Futuyma, D. J., Schneider, J. C., and Hare, J. D. 1979. Genetic variation and host plant relations in a parthenogenetic moth. Evolution **33**:777-790.

Mizell, R. F., and Schiffhauer, D. E. 1990. Effects of pesticides on pecan aphid predators *Chrysoperla rufilabris* (Neuroptera: Chrysopidae), *Hippodamia convergens*, *Cycloneda sanguinea* (L.), *Olla v-nigrum* (Coleoptera: Coccinellidae), and *Aphelinus perpallidus* (Hymenoptera: Encyrtidae). Journal of Economic Entomology **83**:1806-1812.

Mopper, S. 2005. Phenology: how time creates spatial structure in endophagous insect populations. Annales Zoologici Fennici **42**:327-333.

Mopper, S., and Simberloff, D. 1995. Differential herbivory in an oak population: the role of plant phenology and insect performance. Ecology **76**:1233-1241.

Morrow, J., Scott, L., Congdon, B., Yeates, D., Frommer, M., and Sved, J. 2000. Close genetic similarity between two sympatric species of tephritid fruit fly reproductively isolated by mating time. Evolution **54**:899-910.

Murphy, S. M. 2004. Enemy-free space maintains swallowtail butterfly host shift. Proceedings of the National Academy of Sciences **101**:18048-18052.

Naisbit, R. E., Jiggins, C. D., and Mallet, J. 2001. Disruptive sexual selection against hybrids contributes to speciation between *Heliconius cydno* and *Heliconius melpomene*. Proceedings of the Royal Society of London B: Biological Sciences **268**:1849-1854.

Neiman, M., and Linksvayer, T. A. 2006. The conversion of variance and the evolutionary potential of restricted recombination. Heredity **96**:111-121.

Ngi-Song, A. J., Overholt, W. A., Njagi, P. G., Dicke, M., Ayertey, J. N., and Lwande, W. 1996. Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron)(Hymenoptera: Braconidae), larval parasitoids of stemborers on graminae. Journal of Chemical Ecology **22**:307-323.

Nosil, P. 2007. Divergent Host Plant Adaptation and Reproductive Isolation between Ecotypes of *Timema cristinae* Walking Sticks. The American Naturalist **169**:151-162.

Nosil, P. 2008. Speciation with gene flow could be common. Molecular Ecology **17**:2103-2106.

Nosil, P., Crespi, B. J., and Sandoval, C. P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. Nature **417**:440-443.

Nosil, P., Harmon, L. J., and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. Trends in Ecology & Evolution **24**:145-156.

Nosil, P., Vines, T. H., and Funk, D. J. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. Evolution **59**:705-719.

Pappers, S. M., Velde, G. v. d., Ouborg, N., and Groenendael, J. M. v. 2002. Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphaeae*. Evolution **56**:1610-1621.

Pashley, D. P., Hammond, A. M., and Hardy, T. N. 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). Annals of the Entomological Society of America **85**:400-405.

Peccoud, J., Ollivier, A., Plantegenest, M., and Simon, J. C. 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. Proceedings of the National Academy of Sciences of the United States of America **106**:7495-7500.

Peccoud, J., Simon, J. C., von Dohlen, C., Coeur d'acier, A., Plantegenest, M., Vanlerberghe-Masutti, F., and Jousselin, E. 2010. Evolutionary history of aphid-plant associations and their role in aphid diversification. C R Biol **333**:474-487.

Poulin, R. 1997. Species richness of parasite assemblages: evolution and patterns. Annual Review of Ecology and Systematics **28**:341-358.

Powell, T. H. Q., Forbes, A. A., Hood, G. R., and Feder, J. L. 2014. Ecological adaptation and reproductive isolation in sympatry: genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*. Molecular Ecology **23**:688-704.

Powell, T. H. Q., Hood, G. R., Murphy, M. O., Heilveil, J. S., Berlocher, S. H., Nosil, P., and Feder, J. L. 2013. Genetic divergence along the speciation continuum: The transition from host race to species in *Rhagoletis* (Diptera: Tephritidae). Evolution **67**:2561-2576.

Prasad, A. M., Iverson, L. R., and Liaw, A. 2006. Newer classification and regression tree techniques: bagging and random forests for ecological prediction. Ecosystems **9**:181-199.

Price, P. W. 1977. General concepts on the evolutionary biology of parasites. Evolution **31**:405-420.

Price, P. W. 1980. Evolutionary biology of parasites. Princeton University Press, Princeton, NJ, pp 5-237.

Price, P. W., Fernandes, G. W., and Waring, G. L. 1987. Adaptive nature of insect galls. Environmental Entomology **16**:15-24.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Pureswaran, D. S., Gries, R., and Borden, J. H. 2004. Antennal responses of four species of tree-killing bark beetles (Coleoptera: Scolytidae) to volatiles collected from beetles and their host and nonhost conifers. Chemoecology **14**:59-66.

Qiagen. 2002. Dneasy® Tissue Handbook. Qiagen, Valencia, California, pp 1-25.

R'kha, S., Capy, P., and David, J. R. 1991. Host-plant specialization in the *Drosophila melanogaster* species complex: a physiological, behavioral, and genetical analysis. Proceedings of the National Academy of Sciences **88**:1835-1839.

Raijmann, L. E. L., and Menken, S. B. J. 2000. Temporal variation in the genetic structure of host-associated populations of the small ermine moth *Yponomeuta padellus* (Lepidoptera: Yponomeutidae). Biological Journal of the Linnean Society **70**:555-570.

Razmjou, J., Vorburger, C., Moharramipour, S., Mirhoseini, S. Z., and Fathipour, Y. 2010. Host-associated differentiation and evidence for sexual reproduction in Iranian populations of the cotton aphid, *Aphis gossypii*. Entomologia Experimentalis Et Applicata **134**:191-199.

Richards, S. 2010. Genome sequence of the pea aphid *Acyrthosiphon pisum*. Plos Biology **8**.

Rosenthal, R. 1979. The file drawer problem and tolerance for null results. Psychological Bulletin **86**:638.

Ruiz, A., and Heed, W. B. 1988. Host-plant specificity in the cactiphilic *Drosophila mulleri* species complex. The Journal of Animal Ecology **57**:237-249.

Rundle, H. D. 2002. A test of ecologically dependent postmating isolation between sympatric sticklebacks. Evolution **56**:322-329.

Rundle, H. D., and Nosil, P. 2005. Ecological speciation. Ecology Letters **8**:336-352.

Santos, H., Rousselet, J. m., Magnoux, E., Paiva, M.-R., Branco, M., and Kerdelhué, C. 2007. Genetic isolation through time: allochronic differentiation of a phenologically atypical population of the pine processionary moth. Proceedings of the Royal Society of London B: Biological Sciences **274**:935-941.

Schick, K. N., and Dahlsten, D. L. 2003. Gallmaking and insects. Encyclopedia of Insects, Academic Press, Amsterdam, pp 464-466.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology & Evolution **16**:372-380.

Schluter, D., and Conte, G. L. 2009. Genetics and ecological speciation. Proceedings of the National Academy of Sciences **106**:9955-9962.

Schmitt, P., Mandel, J., and Guedj, M. 2015. A comparison of six methods for missing data imputation. Journal of Biometrics & Biostatistics **6**:1.

Schöfl, G., Heckel, D., and Groot, A. 2009. Time- shifted reproductive behaviours among fall armyworm (Noctuidae: *Spodoptera frugiperda*) host strains: evidence for differing modes of inheritance. Journal of Evolutionary Biology **22**:1447-1459.

Schwarz, D., Matta, B. M., Shakir-Botteri, N. L., and McPheron, B. A. 2005. Host shift to an invasive plant triggers rapid animal hybrid speciation. Nature **436**:546-549.

Senn, J., Hanhimäki, S., and Haukioja, E. 1992. Among-tree variation in leaf phenology and morphology and its correlation with insect performance in the mountain birch. Oikos **63**:215-222.

Shorthouse, J. D., and Rohfritsch, O. 1992. Biology of insect-induced galls. Oxford University Press, London, UK, pp 25-185.

Simon, J. C., Carre, S., Boutin, M., Prunier-Leterme, N., Sabater-Munoz, B., Latorre, A., and Bournoville, R. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proceedings of the Royal Society B-Biological Sciences **270**:1703-1712.

Simonato, M., Battisti, A., Zovi, D., and Medina, R. F. 2012. Testing for host-associated differentiation in two egg parasitoids of a forest herbivore. Entomologia Experimentalis Et Applicata **145**:124-133.

Slowinski, J. B., and Guyer, C. 1989. Testing the stochasticity of patterns of organismal diversity: an improved null model. American Naturalist **134**:907-921.

Smith, M. A., Rodriguez, J. J., Whitfield, J. B., Deans, A. R., Janzen, D. H., Hallwachs, W., and Hebert, P. D. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings of the National Academy of Sciences **105**:12359-12364.

Sota, T., Kagata, H., Ando, Y., Utsumi, S., and Osono, T. 2014. Accelerated diversification by spatial and temporal isolation associated with life-history evolution in insects. *in* T. Sota, H. Kagata, Y. Ando, S. Utsumi, and T. Osono, editors. Species Diversity and Community Structure. Springer, pp 45-61.

Stelinski, L. L., and Liburd, O. E. 2005. Behavioral evidence for host fidelity among populations of the parasitic wasp, *Diachasma alloeum* Muesebeck. Naturwissenschaften **92**:65-68.

Stiling, P., and Rossi, A. M. 1998. Deme formation in a dispersive gall-forming midge. *in* S. Mopper and S. Y. Strauss, editors. Genetic Structure and Local Adaptation in Natural Insect Populations. Chapman & Hall, New York, NY, pp 22-36.

Stireman, J. O., III, Janson, E. M., Carr, T. G., Devlin, H., and Abbot, P. 2008. Evolutionary radiation of *Asteromyia carbonifera* (Diptera: Cecidomyiidae) gall morphotypes on the goldenrod *Solidago altissima* (Asteraceae). Biological Journal of the Linnean Society **95**:840-858.

Stireman, J. O., Nason, J. D., and Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: General phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution **59**:2573-2587.

Stireman, J. O., Nason, J. D., Heard, S. B., and Seehawer, J. M. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. Proceedings of the Royal Society B-Biological Sciences **273**:523-530.

Stoetzel, M. B. 1981. 2 new species of phylloxera (Phylloxeridae, Homoptera) on pecan. Journal of the Georgia Entomological Society **16**:127-144.

Stoetzel, M. B. 1985a. Host alternation: a newly discovered attribute of the Phylloxeridae (Homoptera, Aphidoidea). Proceedings of the Entomological Society of Washington **87**:265-268.

Stoetzel, M. B. 1985b. Life histories of the four species of Phylloxera on pecan (Homoptera: Phylloxeridae). Special publication - University of Georgia, Agriculture Experiment Stations **38**.

Strong, D. R., Lawton, J. H., and Southwood, S. R. 1984. Insects on plants: community patterns and mechanisms. Blackwell Scientific Publicatons, pp 37-187.

Strong, D. R., McCoy, E. D., and Rey, J. R. 1977. Time and the number of herbivore species: the pests of sugarcane. Ecology **58**:167-175.

Sunnucks, P., De Barro, P., Lushai, G., Maclean, N., and Hales, D. 1997. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. Molecular Ecology **6**:1059-1073.

Svensson, G. P., Althoff, D. M., and Pellmyr, O. 2005. Replicated host-race formation in bogus yucca moths: genetic and ecological divergence of *Prodoxus quinquepunctellus* on yucca hosts. Evolutionary Ecology Research **7**:1139-1151.

Sword, G., Joern, A., and Senior, L. 2005. Host plant- associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera: Acrididae). Molecular Ecology **14**:2197-2205.

Tan, W. H., Liu, T. H., Lin, Y. K., and Hsu, Y. F. 2014. Restoration of an endangered plant, *Hygrophila pogonocalyx,* leads to an adaptive host shift of the chocolate pansy (*Junonia iphita iphita*). Zoology **117**:237-244.

Tedders, W. 1983. Insect management in deciduous orchard ecosystems: habitat manipulation. Environmental Management **7**:29-34.

Tedders, W. L. 1978. Important biological and morphological characteristics of the foliar-feeding aphids of pecan. U.S. Department of Agriculture, Science, and Education Administration, Washington, District of Columbia, pp 1-29.

Ter Braak, C. J. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology **67**:1167-1179.

Thompson, J. N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologia Experimentalis et Applicata **47**:3-14.

Thompson, T. E., and Grauke, L. J. 1991. Pecans and other hickories (*Carya*). Acta Horticlogica **290**:839-906.

Tsuchida, T., Koga, R., and Fukatsu, T. 2004. Host plant specialization governed by facultative symbiont. Science **303**:1989-1989.

Ueno, H., Furukawa, S., and Tsuchida, K. 2006. Difference in the time of mating activity between host- associated populations of the rice stem borer, *Chilo suppressalis* Walker. Entomological Science **9**:255-259.

Vanlerberghe-Masutti, F., and Chavigny, P. 1998. Host- based genetic differentiation in the aphid *Aphis gossypii* Glover, evidenced from RAPD fingerprints. Molecular Ecology **7**:905-914.

Via, S. 1990. Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. Annual Review of Entomology **35**:421-446.

Via, S. 1991. Specialized host plant performance of pea aphid is not altered by experience. Ecology **72**:1420-1427.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. Evolution **53**:1446-1457.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. Trends in Ecology & Evolution **16**:381-390.

Via, S., Bouck, A. C., and Skillman, S. 2000. Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. Evolution **54**:1626-1637.

Via, S., and Hawthorne, D. J. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. The American Naturalist **159**:S76-S88.

Vialatte, A., Dedryver, C.-A., Simon, J.-C., Galman, M., and Plantegenest, M. 2005. Limited genetic exchanges between populations of an insect pest living on uncultivated and related cultivated host plants. Proceedings of the Royal Society of London B: Biological Sciences **272**:1075-1082.

Wade, M. J., and Johnson, N. A. 1994. Reproductive isolation between two species of flour beetles, *Tribolium castaneum* and *T. freemani*: variation within and among geographical populations of *T. castaneum*. Heredity **72**:155-162.

Waring, G. L., Abrahamson, W. G., and Howard, D. J. 1990. Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). Evolution **44**:1648-1655.

Weis, A. E., and Abrahamson, W. G. 1986. Evolution of host-plant manipulation by gall makers: ecological and genetic factors in the *Solidago-Eurosta* system. American Naturalist **127**:681-695.

Welling, M., and Kurihara, K. 2006. Bayesian K-means as a “maximization-expectation” algorithm. *in* Proceedings of the 2006 SIAM International Conference on Data Mining. SIAM, pp 474-478.

Wickremasinghe, M., and Emden, H. v. 1992. Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. Physiological Entomology **17**:297-304.

Wiegmann, B. M., Mitter, C., and Farrell, B. 1993. Diversification of carnivorous parasitic insects: extraordinary radiation or specialized dead end? American Naturalist **142**:737-754.

Wiens, J. J. 2004. Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. Evolution **58**:193-197.

Wood, T., Tilmon, K., Shantz, A., Harris, C., and Pesek, J. 1999. The role of host-plant fidelity in initiating insect race formation. Evolutionary Ecology Research **1**:317-332.

Wu, C.-l., and Palopoli, M. F. 1994. Genetics of postmating reproductive isolation in animals. Annual Review of Genetics **28**:283-308.

Yukawa, J. 2000. Synchronization of gallers with host plant phenology. Population Ecology **42**:105-113.

Zeh, D. W., Zeh, J. A., and Smith, R. L. 1989. Ovipositors, amnions and eggshell architecture in the diversification of terrestrial arthropods. Quarterly Review of Biology **64**:147-168.

# APPENDIX

Each row (numbered on each page, 1-108) lists the individual parasite species (Column 1), host species (Column 2), HAD occurrence (Column 3), and biological/ecological factors (Columns 4-21) that characterize the case study systems analyzed in the quantitative literature review; supporting literature for each case study system is provided in Columns 23-25 at the end of the Appendix. Each row represents a single parasite-host case study system. Case studies highlighted in grey were used for the validation study only and were not included in the development of my model. Underlined Column headers depict the factors that were significantly related to HAD occurrence in the MANOVA. Specifically, Column 3 describes the presence or absence of genetic structuring between host-associated populations of each parasite species. Columns 4 and 5 provide the Order and Family of each parasite species while Columns 6 and 7 provide the Order(s) and Family/Families of every host species involved in parasite-host system. Column 8 describes the trophic position of each parasite species. Column 9 describes the host resource used by each parasite species. Column 10 describes whether each parasite species is an endophagous or exophagous feeder. Column 11 describes the mode of reproduction used by each parasite species. Column 12 describes the approximate average number of generations experienced by each parasite species annually. Column 13 describes whether each parasite species uses their host as a habitat for discovering mates. Column describes whether morphological differences can be observed between host-associated parasite populations. Column 15 describes the timing of availability of the host resources used by each parasite. Column

16 describes whether host-associated populations of each parasite species will preferentially choose the volatiles specific to one host species over another during Y-tube choice experiments. Similarly, Column 17 describes whether host-associated parasites will preferentially choose to oviposit on one host species over another during choice-arena experiments and Column 18 describes whether host-associated parasites experience a reduction in fitness parameters while living on non-natal hosts during reciprocal transplant experiments. Column 19 describes whether host-associated parasite populations occur at significantly different times. Column 20 describes whether the parasite, host, or both occur in a geographic range outside of their natal range, creating opportunities for host-shifting events. Column 21 describes whether each system is maintained or cultivated by humans. Column 22 describes whether the species composition of microbiota infecting each parasite species is different depending on host association.

|  | 1. Parasite species | 2. Host species |
|---|---|---|
| 1 | *Acrobasis vaccinii* Riley | *Vaccinium corymbosum* L. and *Vaccinium macrocarpon* Aiton |
| 2 | *Aphelinus albipodus* Hayat & Fatima | *Aphis glycines* Matsumara and *Rhopalosiphum padi* L. |
| 3 | *Aphelinus gossypii* Timberlake | *Aphis gossypii* Timberlake, *Aphis spiraecolae* Patch, and *Toxoptera aurantii* Boyer de Fonscolombe |
| 4 | *Aphelinus perpallidus* Gahan | *Monelliopsis pecanis* Bissell |
| 5 | *Aphidius transcaspicus* Telenga | *Hyalopterus pruni* Geoffroy and *Aphis fabae* Scopoli |
| 6 | *Austromenopon echinatum* Edwards | *Calonectris diomedea diomedea* Scopoli, *Calonectris diomedea borealis* Cory, and *Calonectris diomedea edwardsii* Oustalet |
| 7 | *Bactrocera cucurbitae* Coquillett | *Cucumis sativus* L., *Cucurbita pepo* L., *Carica papaya* L., and *Solanum lycopersicum* L. |
| 8 | *Baryscapus servadeii* Domenichini | *Thaumetopoea pityocampa* Denis & Schiffermüller |
| 9 | *Columbicola adamsi* Clayton & Price | *Patagiones speciosa* Gmelin and *Patagiones plumbea* Vieillot |
| 10 | *Columbicola gracilicapitis* Carriker | *Leptotila jamaicensis* L. and *Leptotila verreauxi fulviventris* Lawrence |
| 11 | *Columbicola theresae* Ansari | *Streptopelia decaocto* Frivaldszky, *Streptopelia vinacea* Gmelin, and *Streptopelia senegalensis* L. |
| 12 | *Copidosoma gelechiae* Howard | *Gnorimoschema gallaesolidaginis* Hodges |
| 13 | *Cydia pomonella* L. | *Malus pumila* Miller, *Prunus persica* Batsch, and *Pyrus* spp. L. |
| 14 | *Dendroctonus ponderosae* Hopkins | *Pinus contorta* Douglas and *Pinus flexilis* James |
| 15 | *Diaeretiella rapae* M'Intosh | *Brevicoryne brassicae* L. and *Diuraphis noxia* Kurdjumov |
| 16 | *Diaphorina citri* Kuwayama | *Citrus* spp. L. and *Murraya paniculata* Jack |
| 17 | *Drosophila pachea* Patterson & Wheeler | *Lophocereus schottii* Hunt |
| 18 | *Epiblema scudderiana* Clemens | *Solidago altissima* L. and *Solidago gigantea* Aiton |
| 19 | *Eusandalum barteli* Gourlay | *Prodoxus phylloryctus* Wagner & Powell |
| 20 | *Gretchena bolliana* Slingerland | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 21 | *Halipeurus abnormis* Thompson | *Calonectris diomedea diomedea* Scopoli, *Calonectris diomedea borealis* Cory, and *Calonectris diomedea edwardsii* Oustalet |
| 22 | *Hyphantria cunea* Drury | *Prunus serotina* Ehrh and *Juglans nigra* L. |
| 23 | *Myzus nicotianae* Blackmann | *Brassica campestris* L., *Capsella bursa-pastoris* L., *Capsicum annuum* L., *Celosia argentea* L., *Hibiscus rosa-sinensis* L., *Nicotiana tabacum* L., *Physalis angulata* L., *Solanum dulcamara* L., and *Solanum tuberosum* L. |
| 24 | *Ooencyrtus pityocampae* Mercet | *Thaumetopoea pityocampa* Denis & Schiffermüller |
| 25 | *Procecidochares atra* Loew | *Solidago altissima* L. *and Solidago gigantea* Aiton |
| 26 | *Saemundssonia peusi* Eichler | *Calonectris diomedea diomedea* Scopoli, *Calonectris diomedea borealis* Cory, and *Calonectris diomedea edwardsii* Oustalet |
| 27 | *Thaumetopoea pityocampa* Denis & Schiffermüller | *Pinus nigra* Arnold and *Pinus sylvestris* L. |
| 28 | *Trirhabda convergens* LeConte | *Solidago altissima* L. *and Solidago gigantea* Aiton |
| 29 | *Trirhabda virgata* LeConte | *Solidago altissima* L. *and Solidago gigantea* Aiton |
| 30 | *Tuta absoluta* Meyrick | *Solanum lycopersicum* L. and *Solanum tuberosum* L. |
| 31 | *Yponomeuta cagnagellus* Hübner | *Crataegus monogyna* Jacq. and *Prunus spinosa* L. |
| 32 | *Yponomeuta padella* L. | *Crataegus monogyna* Jacq. and *Prunus spinosa* L. |
| 33 | *Xenopsylla gratiosa* Jordan | *Calonectris diomedea diomedea* Scopoli, *Calonectris diomedea borealis* Cory, and *Calonectris diomedea edwardsii* Oustalet |
| 34 | *Aceria parapopuli* Keifer | *Populus angustifolia* James and *Populus fremontii* Watson |
| 35 | *Acrocercops transecta* Meyrick | *Juglans ailantifolia* Carr. and *Lyonia ovalifolia* Drude |

|  | 1. Parasite species | 2. Host species |
|---|---|---|
| 36 | *Acyrthosiphon pisum* Harris | *Cytisus scoparius* L., *Lathyrus pratensis* L., *Lotus corniculatus* L., *Lotus pedunculatus* Cav., *Medicago lupulina* L., *Medicago sativa* L., *Melilotus albus* Medik., *Melilotus officinalis* L., *Ononis repens* L., *Onosis spinosa* L., *Pisum sativum* L., *Trifolium campestre* Schreb., *Trifolium dubium* Sibth., *Trifolium pratense* L., *Trifolium repens* L., *Vicia cracca* L., *Vicia faba* L., and *Vicia sativa* L. |
| 37 | *Aphelinus asychis* Walker | *Aphis glycines* Mastumara, *Aphis gossypii* Glover, *Diuraphis noxia* Kurdjumov, *Myzus persicae* Sulzer, and *Rhopalosiphun padi* L. |
| 38 | *Aphelinus varipes* Foerster | *Aphis glycines* Mastumara, *Aphis gossypii* Glover, *Diuraphis noxia* Kurdjumov, *Myzus persicae* Sulzer, and *Rhopalosiphun padi* L. |
| 39 | *Aphidius ervi* Haliday | *Acyrthosiphon pisum* Harris and *Aulacorthum solani* Kaltenbach |
| 40 | *Aphis fabae* Scopoli | *Arctium lappa* L., *Euonymus europaeus* L., *Impatiens glandulifera* Royle, *Tropaeolum majus* L., *Vicia faba* L., and *Vibernum opulus* L. |
| 41 | *Aphis gossypii* Glover | *Capsicum annuum* L., *Chrysanthemum* L., *Citrus* L., *Cucumus* spp. L., *Cucumus sativa* L., *Cucurbita pepo* L., *Hibiscus* spp. L., *Solanum tubersum* L. |
| 42 | *Aphis spiraecola* Patch | *Citrus* x *paradisi* Macfad, *Citrus jambhiri* Lush, *Citrus* x *sinensis* Osbeck, *Spiraea* spp. L., *Polyscias crispatum* Hort., *Polyscias scutellaria* Burm., and *Viburnum suspensum* Lindl. |
| 43 | *Aulacorthum solani* Kaltenbach | *Apium graveolens* L., *Capsicum annuum* L., *Cucumis sativus* L., *Daucus carota subsp. sativus* Schübl. & Martens, *Nicotiana tabacum* L., *Solanum lycopersicum* L., and *Tulipa gesneriana* L., |
| 44 | *Asteromyia carbonifera* Sacken | *Solidago altissima* L. *and Solidago gigantea* Aiton |
| 45 | *Baryscapus fumipennis* Girault | *Asteromyia carbonifera* Sacken |
| 46 | *Brevicoryne brassicae* L. | *Brassica oleraceae* L. and *Brassica rapa* L. |
| 47 | *Callosobruchus maculatus* Fabricius | *Cajanus cajan* Millsp., *Cajanus scarabaeoides* Thouars, *Dunbaria bella* Prain, *Dunbara podocarpa, Lablab purpureus* Sweet, *Phaseolus vulgaris* L., *Vigna angularis* Ohwi & H. Ohashi, *Vigna radiata* Wilczek, *Vigna subterranea* Verdc., *Vigna umbellata* Ohwi & H. Ohashi, and *Vigna unguiculata* Walp. |
| 48 | *Chilo suppressalis* Walker | *Oryza sativa* L. and *Zizania latifolia* Turcz. ex Stapf |
| 49 | *Columbicola passerinae* Wilson | *Columbina inca* Lesson and *Columbina passerina* L. |
| 50 | *Columbicula macrourae* Wilson | *Geotrygon montana* L., *Leptotila jamaicensis* L., *Leptotila verreauxi fuviventris* Lawrence, *Leptotila verreauxi angelica* Bangs & T. E. Penard, *Leptotila plumbeiceps* Sclater & Salvin, *Zenaida asiatica* L., *Zenaida galapagoensis* Gould, and *Zenaida macroura* L. |
| 51 | *Cotesia melitaearum* Wilkinson | *Melitaea cinxia* L. and *Melitaea athalia* Rottemburg |
| 52 | *Cryptomyzus galeopsidis* Kaltenbach | *Ribes nigrum* L. and *Ribes rubrum* L. |
| 53 | *Dasineura oxycoccana* Johnson | *Vaccinium corymbosum* L. *and Vaccinium macrocarpon* Aiton |
| 54 | *Diachasma alloeum* Muesbeck | *Rhagoletis pomonella* Walsh |
| 55 | *Drosophila mettleri* Heed | *Carnegiea gigantea* Britton & Rose and *Pachycereus pringlei* Britton & Rose |
| 56 | *Drosophila mojavensis* Patterson | *Stenocereus gummosus* Engelmann and *Stenocereus thurberi* Buxbaum |
| 57 | *Drosophila nigrospiracula* Patterson & Wheeler | *Carnegiea gigantea* Britton & Rose *and Pachycereus pringlei* Britton & Rose |
| 58 | *Enchenopa binotata* Say | *Celastrus* L., *Cercis* L., *Juglans* L., *Ptelea* L., and *Viburnum* L. |
| 59 | *Epilachna niponica* Lewis | *Caulophyllum robustum* Maxim. and *Cirsium* spp. Mill. |
| 60 | *Eurosta solidaginis* Fitch | *Solidago altissima* L. *and Solidago gigantea* Aiton |
| 61 | *Euura mucronata* Hartig | *Salix glauca* L., *Salix hastata* L., *Salix lanata* L., *Salix lapponum* L., and *Salix myrsinifolia* Salisb. |
| 62 | *Gnorimoschema gallaesolidaginis* Riley | *Solidago altissima* L. *and Solidago gigantea* Aiton |

| | 1. Parasite species | 2. Host species |
|---|---|---|
| 63 | *Hesperotettix viridis* Thomas | *Gutierrezia sarothrae* Pursh and *Solidago mollis* Bartlett |
| 64 | *Hyalesthes obsoletus* Signoret | *Convolvulus arvensis* L., *Lavandula angustifolia* Mill., *Solanum tuberosum* L., *Urtica dioica* L., and *Vitex agnus-castus* L. |
| 65 | *Hyalopterus pruni* Geoffrey | *Prunus dulcis* Mill, *Prunus armeniaca* L., *Prunus spinosa* L., *Prunus persica* Batsch, *Prunus australis* Yü & Lu |
| 66 | *Ixodes ricinus* L. | *Anthus pratensis* L., *Apodemus sylvaticus* L., *Apodemus agrarius* Pallas, *Apodemus flavicollis* Melchior, *Capreolus capreolus* L., *Erithacus rubecula* L., *Lacerta viridis* Laurenti, *Luscinia megarhynchos* Brehm, *Phylloscopus collybita* Vieillot, *Parus major* L., *Nannus troglodytes* L., *Myodes glareolus* Schreber, *Sus crofa* L., *Sylvia atricapilla* L., *Sylvia communis* Latham, *Turdus philomelos* Brehm, and *Turdus merula* L. |
| 67 | *Ixodes uriae* White | *Uria lomvia* L. and *Rissa tridactyla* L. |
| 68 | *Jadera haematoloma* Herrich-Schäffer | *Cardiospermum corindum* L. and *Koelreuteria elegans* Laxm. |
| 69 | *Kaltenbachiella japonica* Matsumara | *Ulmus davidiana* Planch. |
| 70 | *Lochmaea capreae* L. | *Betula* L. and *Salix caprea* L. |
| 71 | *Maconellicoccus hirsutus* Green | *Annona muricata* L., *Acacia pennatula* Schltdl. & Cham., and *Mimosa pudica* L. |
| 72 | *Melitaea cinxia* L. | *Plantago lanceolata* L. and *Veronica spicata* L. |
| 73 | *Mordellistena convicta* LeConte | *Euthamia graminifolia L., Solidago altisima L., Solidago gigantea Aiton, and Solidago juncea Aiton, Symphyotrichum lateriflorum* Á. & D. Löve, and *Symphyotrichum pilosum* Wild. |
| 74 | *Myzus persicae* Sulzer | *Brassica campestris* L., *Capsella bursa-pastoris* L., *Capsicum annuum* L., *Celosia argentea* L., *Hibiscus rosa-sinensis* L., *Nicotiana tabacum* L., *Physalis angulata* L., *Solanum dulcamara* L., and *Solanum tuberosum* L. |
| 75 | *Nilaparvata lugens* Stål | *Leersia hexandra* Sw. and *Oryza sativa* L. |
| 76 | *Ostrinia nubilalis* Hübner | *Artemisia vulgaris* L. and *Zea mays* subsp. *mays* L. |
| 77 | *Pemphigus bursarius* L. | *Lapsana communis* L. and *Lactuca sativa* L. |
| 78 | *Phytomyza glabricola* Kulp | *Ilex coriacea* L. and *Ilex glabra* L. |
| 79 | *Platycampus luridiventris* Fallén | *Alnus glutinosa* L. and *Alnus incana* L. |
| 80 | *Platygaster variabilis* Fouts | *Rhopalomyia solidaginis* Fitch |
| 81 | *Pontania viminalis* L. | *Salix borealis* Fries, *Salix glauca* L., *Salix hastata* L., *Salix lanata* L., *Salix lapponum* L., *Salix myrsinifolia* Salisb., *Salix myrsinites* L., and *Salix reticulata* L. |
| 82 | *Prodoxus coloradensis* Riley | *Yucca aloifolia* L., *Yucca constricta* Buckley, and *Yucca glauca* Nutt. |
| 83 | *Prodoxus decipiens* Riley | *Yucca baccata* Torr., *Yucca filamentosa* L., *Yucca schidigera* Roezl, and *Yucca treculeana* Carrière |
| 84 | *Pseudatomoscelis seriatus* Reuter | *Ambrosia psilostachya* DC, *Croton argyranthemum* Michx., *Croton lindheimerianus* Scheele, *Croton monanthogynus* Michx., *Gaura parviflora* Dougl. ex Lehm., *Glandularia bipinnatifida* Nutt., *Gossypium hirsutum* L., *Hymenopappus scabiosaeus* L'Hér, *Malvella lepidota, Marrubium vulgare, Monarda punctata, Oenothera speciosa,* and *Solanum elaegnifolium* Cav. |
| 85 | *Rhagoletis pomonella* Walsh | *Crataegus rhipidophylla* Gand. and *Malus pumila* Miller |
| 86 | *Rhinusa antirrhini* Paykull | *Linaria genistifolia* L., *Linaria rubioides* Pančić, and *Linaria vulgaris* Mill. |
| 87 | *Rhopalomyia solidaginis* Fitch | *Solidago altissima* L. and *Solidago gigantea* Aiton |
| 88 | *Schistocerca lineata* Scudder | *Ptelea trifoliata* L. and *Rubus trivialis* Michx. |
| 89 | *Schizaphis graminum* Rondani | *Sorghum halepense* L., *Sorghum bicolor* L., *Hordeum vulgare* L., *and Triticum spp.* L. |
| 90 | *Sitobion avenae* Fabricius | *Dactylis glomerata* L. and *Triticum aestivum* L. |

|  | 1. Parasite species | 2. Host species |
|---|---|---|
| 91 | *Spodoptera frugiperda* Smith | *Gossypium hirsutum* L. and *Zea mays* subsp. *mays* L. |
| 92 | *Tephritis conura* Loew | *Cirsium heterophyllum* L. and *Cirsium oleraceum* L. |
| 93 | *Tetraneura yezoensis* Matsumura | *Ulmus davidiana* Planch. and *Ulmus laciniata* Trautv. |
| 94 | *Tetranychus kanzawai* Kishida | *Camellia sinensis* L., *Hydrangea macropylla* Thunb., *Pueraria lobata* Kudzu, and *Pyrus pyrifolia* Burm. |
| 95 | *Thrips tabaci* Linderman | *Allium ampeloprasum* L. and *Nicotiana tabacum* L. |
| 96 | *Uroleucon nigrotuberculatum* Olive | *Solidago altissima* L. |
| 97 | *Zeiraphera diniana* Hübner | *Pinus contorta* Douglas and *Larix decidua* Mill. |
| 98 | *Melanaphis sacchari* Zehntner | *Sorghum halepense* L., *Sorghum bicolor* L., *Hordeum vulgare* L., and *Triticum* spp. L. |
| 99 | *Melanocallis caryaefoliae* Davis | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 100 | *Phylloxera texana* Stoetzel | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 101 | *Cimex lectularius* Latreille | *Eptesicus serotinus* Schreber, *Myotis emarginatus* Geoffroy, *Myotis myotis* Borkhausen, Pipistrellus auritus Thompson, and Rhinolophus spp. Lacépède, |
| 102 | *Conogethes punctiferalis* Guenée | *Castanea* Mill., *Elettaria cardamomum* Maton, *Malus pumila* Miller, *Prunus persica* L., *Pyrus* L., *Ricinus communis* L., and *Zea mays* subsp. *mays* L. |
| 103 | *Dalbulus maidis* Delong and Wolcott | *Zea mays* subs. *mays* L. and *Zea mays* subsp. p*arviglumis* Iltis & Doebley |
| 104 | *Euproctis chrysorrhoea* L. | *Arbutus unedo* L., *Crataegus monogyna* Jacq., *Prunus cerasifera* Ehrh., *Quercus faginea* Lam., *Quercus robur* L., and *Sorbus torminalis* L. |
| 105 | *Monellia caryella* Fitch | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 106 | *Monelliopsis pecanis* Bissell | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 107 | *Phylloxera devastatrix* Pergande | Carya aquatica Michx. and Carya illinoinensis Wangenh |
| 108 | *Phylloxera notabilis* Pergande | Carya aquatica Michx. and Carya illinoinensis Wangenh |

|  | 3. HAD occurrence | 4. Parasite Order | 5. Parasite Family | 6. Host Order(s) | 7. Host Family/Families | 8. Parasite trophic position |
|---|---|---|---|---|---|---|
| 1 | absent | Lepidoptera | Pyralidae | Ericales | Ericaceae | herbivore |
| 2 | absent | Hymenoptera | Aphelinidae | Hemiptera | Aphididae | parasitoid |
| 3 | absent | Hymenoptera | Aphelinidae | Hemiptera | Aphididae | parasitoid |
| 4 | absent | Hymenoptera | Aphelinidae | Hemiptera | Aphididae | parasitoid |
| 5 | absent | Hymenoptera | Braconidae | Hemiptera | Aphididae | parasitoid |
| 6 | absent | Phthiraptera | Menoponidae | Procellariiformes | Procellariidae | ectoparasite |
| 7 | absent | Diptera | Tephritidae | Cucurbitales | Cucurbitaceae | herbivore |
| 8 | absent | Hymenoptera | Eulophidae | Lepidoptera | Thaumetopoeidae | parasitoid |
| 9 | absent | Phthiraptera | Philopteridae | Columbiformes | Columbidae | ectoparasite |
| 10 | absent | Phthiraptera | Philopteridae | Columbiformes | Columbidae | ectoparasite |
| 11 | absent | Phthiraptera | Philopteridae | Columbiformes | Columbidae | ectoparasite |
| 12 | absent | Hymenoptera | Encyrtidae | Lepidoptera | Gelechiidae | parasitoid |
| 13 | absent | Lepidoptera | Tortricidae | Rosales | Rosaceae | herbivore |
| 14 | absent | Coleoptera | Scolytidae | Pinales | Pinaceae | herbivore |
| 15 | absent | Hymenoptera | Aphidiidae | Hemiptera | Aphididae | parasitoid |
| 16 | absent | Hemiptera | Liviidae | Hemiptera | Aphididae | herbivore |
| 17 | absent | Diptera | Drosophila | Caryophyllales | Cactaceae | fungivore |
| 18 | absent | Lepidoptera | Tortricidae | Asterales | Asteraceae | herbivore |
| 19 | absent | Hymenoptera | Eupelmidae | Lepidoptera | Prodoxidae | parasitoid |
| 20 | absent | Lepidoptera | Tortricidae | Fagales | Juglandaceae | herbivore |
| 21 | absent | Phthiraptera | Philopteridae | Procellariiformes | Procellariidae | ectoparasite |
| 22 | absent | Lepidoptera | Arctiidae | Fagales, Rosales | Juglandaceae, Rosaceae | herbivore |
| 23 | absent | Hemiptera | Aphididae | Brassicales, Caryophyllales, Solanales | Brassicaceae, Amaranthaceae, Solanaceae | herbivore |
| 24 | absent | Hymenoptera | Encyrtidae | Lepidoptera | Thaumetopoeidae | parasitoid |
| 25 | absent | Diptera | Tephritidae | Asterales | Asteraceae | herbivore |
| 26 | absent | Phthiraptera | Philopteridae | Procellariiformes | Procellariidae | ectoparasite |
| 27 | absent | Lepidoptera | Notodontidae | Pinales | Pinaceae | herbivore |
| 28 | absent | Coleoptera | Chrysomelidae | Asterales | Asteraceae | herbivore |
| 29 | absent | Coleoptera | Chrysomelidae | Asterales | Asteraceae | herbivore |
| 30 | absent | Lepidoptera | Gelechiidae | Solanales | Solanaceae | herbivore |
| 31 | absent | Lepidoptera | Yponomeutidae | Rosales | Rosaceae | herbivore |
| 32 | absent | Lepidoptera | Yponomeutidae | Rosales | Rosaceae | herbivore |
| 33 | absent | Siphonaptera | Pulicidae | Procellariiformes | Procellariidae | ectoparasite |
| 34 | present | Acari | Eriophydae | Malpighiales | Salicaceae | herbivore |
| 35 | present | Lepidoptera | Gracillariidae | Fagales, Ericales | Juglandaceae, Ericaceae | herbivore |

|  | 3. HAD occurrence | 4. Parasite Order | 5. Parasite Family | 6. Host Order(s) | 7. Host Family/Families | 8. Parasite trophic position |
|---|---|---|---|---|---|---|
| 36 | present | Hemiptera | Aphididae | Fabales | Fabaceae | herbivore |
| 37 | present | Hymenoptera | Aphelinidae | Hemiptera | Aphididae | parasitoid |
| 38 | present | Hymenoptera | Aphelinidae | Hemiptera | Aphididae | parasitoid |
| 39 | present | Hymenoptera | Braconidae | Hemiptera | Aphididae | parasitoid |
| 40 | present | Hemiptera | Aphididae | Asterales, Celastrales | Asteraceae, Celastraceae | herbivore |
| 41 | present | Hemiptera | Aphididae | Solanales, Asterales, Sapindales, Cucurbitales, Malvales | Solanaceae, Asteraceae, Rutaceae, Cucurbitaceae, Malvaceae | herbivore |
| 42 | present | Hemiptera | Aphididae | Sapindales, Apiales, Dipsacales | Rutaceae, Araliaceae, Adoxaceae | herbivore |
| 43 | present | Hemiptera | Aphididae | Apiales, Solanales, Cucurbitales, Liliales | Apiaceae, Solanaceae, Cucurbitaceae, Liliaceae | herbivore |
| 44 | present | Diptera | Cecidomyiideae | Asterales | Asteraceae | herbivore |
| 45 | present | Hymenoptera | Eulophidae | Diptera | Cecidomyiideae | herbivore |
| 46 | present | Hemiptera | Aphididae | Brassicales | Brassicaceae | herbivore |
| 47 | present | Coleoptera | Chrysomelidae | Fabales | Fabaceae | herbivore |
| 48 | present | Lepidoptera | Crambidae | Poales | Poaceae | herbivore |
| 49 | present | Phthiraptera | Philopteridae | Columbiformes | Columbidae | ectoparasite |
| 50 | present | Phthiraptera | Philopteridae | Columbiformes | Columbidae | ectoparasite |
| 51 | present | Hymenoptera | Braconidae | Lepidoptera | Nymphalidae | parasitoid |
| 52 | present | Hemiptera | Aphididae | Saxifragales | Nymphalidae | herbivore |
| 53 | present | Diptera | Cecidomyiideae | Ericales | Ericaceae | parasitoid |
| 54 | present | Hymenoptera | Braconidae | Diptera | Tephritidae | parasitoid |
| 55 | present | Diptera | Drosophila | Caryophyllales | Cactaceae | fungivore |
| 56 | present | Diptera | Drosophila | Caryophyllales | Cactaceae | fungivore |
| 57 | present | Diptera | Drosophila | Caryophyllales | Cactaceae | fungivore |
| 58 | present | Hemiptera | Membracidae | Celastrales, Fabales, Sapindales, Dipsacales | Celastraceae, Fabaceae, Rutaceae, Adoxaceae | herbivore |
| 59 | present | Coleoptera | Coccinellidae | Ranunculales | Berberidaceae | herbivore |
| 60 | present | Diptera | Tephritidae | Asterales | Asteraceae | herbivore |
| 61 | present | Hymenoptera | Tenthredinidae | Malpighiales | Salicaceae | herbivore |
| 62 | present | Lepidoptera | Gelechiidae | Asterales | Asteraceae | herbivore |

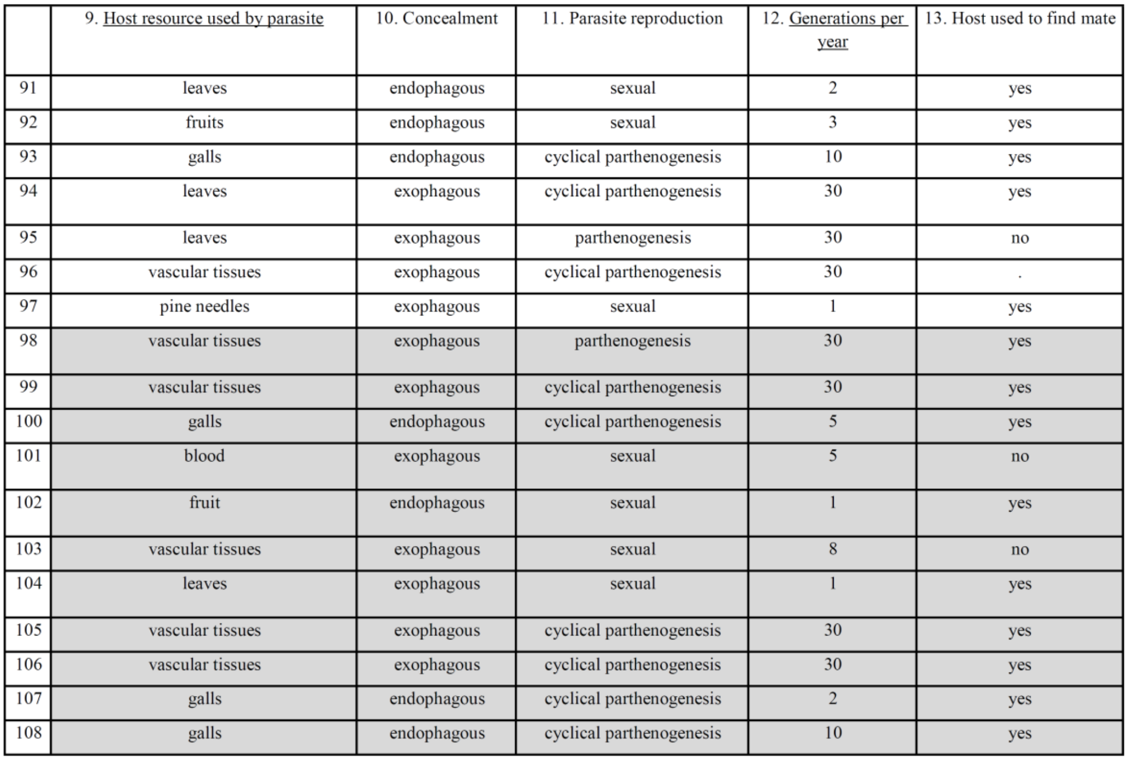
| | 3. HAD occurrence | 4. Parasite Order | 5. Parasite Family | 6. Host Order(s) | 7. Host Family/Families | 8. Parasite trophic position |
|---|---|---|---|---|---|---|
| 63 | present | Orthoptera | Acrididae | Asterales | Asteraceae | herbivore |
| 64 | present | Hemiptera | Cixiidae | Solanales, Lamiales, Rosales | Convolvulaceae, Lamiaceae, Urticaceae, Verbenaceae | herbivore |
| 65 | present | Hemiptera | Aphididae | Rosales | Rosaceae | herbivore |
| 66 | present | Acari | Ixodidae | Passeriformes, Rodentia, Artiodactyla | Motacillidae, Muridae, Cervidae, Muscicapidae, Phylloscopidae, Paridae, Cricetidae, Troglodytidae, Suidae, Sylviidae, Turdidae | ectoparasite |
| 67 | present | Acari | Ixodidae | Charadriiformes | Alcidae, Laridae | ectoparasite |
| 68 | present | Hemiptera | Rhopalidae | Sapindales | Sapindaceae | herbivore |
| 69 | present | Hemiptera | Aphididae | Rosales | Ulmaceae | herbivore |
| 70 | present | Coleoptera | Chrysomelidae | Fagales, Malpighiales | Betulaceae, Salicaceae | herbivore |
| 71 | present | Hemiptera | Pseudococcidae | Magnoliales, Fagales | Annonaceae, Fabaceae | herbivore |
| 72 | present | Lepidoptera | Nymphalidae | Lamiales | Plantaginaceae | herbivore |
| 73 | present | Coleoptera | Mordellidae | Asterales | Asteraceae | herbivore |
| 74 | present | Hemiptera | Aphididae | Brassicales, Solanales | Brassicaceae, Solanaceae | herbivore |
| 75 | present | Hemiptera | Delphacidae | Poales | Poaceae | herbivore |
| 76 | present | Lepidoptera | Crambidae | Asterales, Poales | Asteraceae, Poaceae | herbivore |
| 77 | present | Hemiptera | Aphididae | Asterales | Asteraceae | herbivore |
| 78 | present | Diptera | Agromyzidae | Aquifoliales | Aquifoliaceae | herbivore |
| 79 | present | Hymenoptera | Tenthredinidae | Fagales | Betulaceae | herbivore |
| 80 | present | Hymenoptera | Platygastridae | Diptera | Cecidomyiideae | parasitoid |
| 81 | present | Hymenoptera | Tenthredinidae | Malpighiales | Salicaceae | herbivore |
| 82 | present | Lepidoptera | Prodoxidae | Asparagales | Asparagaceae | herbivore |
| 83 | present | Lepidoptera | Prodoxidae | Asparagales | Asparagaceae | herbivore |
| 84 | present | Hemiptera | Miridae | Asterales, Malpighiales, Lamiales, Malvales, Myrtales, Solanales | Asteraceae, Euphorbiaceae, Verbenaceae, Malvaceae, Lamiaceae, Onagraceae, Solanaceae | herbivore |
| 85 | present | Diptera | Tephritidae | Rosales | Rosaceae | herbivore |
| 86 | present | Coleoptera | Curculionidae | Lamiales | Plantaginaceae | herbivore |
| 87 | present | Diptera | Cecidomyiideae | Asterales | Asteraceae | herbivore |
| 88 | present | Orthoptera | Acrididae | Sapindales, Rosales | Rutaceae, Rosaceae | herbivore |
| 89 | present | Hemiptera | Aphididae | Poales | Poaceae | herbivore |
| 90 | present | Hemiptera | Aphididae | Poales | Poaceae | herbivore |

|  | 3. HAD occurrence | 4. Parasite Order | 5. Parasite Family | 6. Host Order(s) | 7. Host Family/Families | 8. Parasite trophic position |
|---|---|---|---|---|---|---|
| 91 | present | Lepidoptera | Noctuidae | Malvales, Poales | Malvaceae, Poaceae | herbivore |
| 92 | present | Diptera | Tephritidae | Asterales | Asteraceae | herbivore |
| 93 | present | Hemiptera | Pemphigidae | Rosales | Ulmaceae | herbivore |
| 94 | present | Acari | Tetranychidae | Cornales, Ericales, Fabales, Rosales | Hydrangeaceae, Theaceae, Fabaceae, Rosaceae | herbivore |
| 95 | present | Thysanoptera | Thripidae | Asparagales, Solanales | Amaryllidaceae, Solanaceae | herbivore |
| 96 | present | Hemiptera | Aphididae | Asterales | Asteraceae | herbivore |
| 97 | present | Lepidoptera | Tortricidae | Pinales | Pinaceae | herbivore |
| 98 | absent | Hemiptera | Aphididae | Poales | Poaceae | herbivore |
| 99 | absent | Hemiptera | Aphididae | Fagales | Juglandaceae | herbivore |
| 100 | absent | Hemiptera | Phylloxeridae | Fagales | Juglandaceae | herbivore |
| 101 | present | Hemiptera | Cimicidae | Chiroptera, Primates | Vespertilionidae, Hominidae | ectoparasite |
| 102 | present | Lepidoptera | Crambidae | Fagales | Fagaceae | herbivore |
| 103 | present | Hemiptera | Cicadellidae | Poales | Poaceae | herbivore |
| 104 | present | Lepidoptera | Erebidae | Ericales, Rosales | Ericaceae, Rosaceae | herbivore |
| 105 | present | Hemiptera | Aphididae | Fagales | Juglandaceae | herbivore |
| 106 | present | Hemiptera | Aphididae | Fagales | Juglandaceae | herbivore |
| 107 | present | Hemiptera | Phylloxeridae | Fagales | Juglandaceae | herbivore |
| 108 | present | Hemiptera | Phylloxeridae | Fagales | Juglandaceae | herbivore |

|  | 9. Host resource used by parasite | 10. Concealment | 11. Parasite reproduction | 12. Generations per year | 13. Host used to find mate |
|---|---|---|---|---|---|
| 1 | fruits | endophagous | sexual | 1 | yes |
| 2 | aphids | endophagous | sexual | 20 | yes |
| 3 | aphids | endophagous | parthenogenesis | 20 | no |
| 4 | aphids | exophagous | sexual | 20 | no |
| 5 | aphids | endophagous | sexual | 20 | no |
| 6 | vascular tissues | exophagous | sexual | 10 | yes |
| 7 | fruits | endophagous | sexual | 8 | yes |
| 8 | caterpillars | endophagous | parthenogenesis | 1 | yes |
| 9 | detritivore | exophagous | sexual | 1 | yes |
| 10 | detritivore | exophagous | sexual | 1 | yes |
| 11 | detritivore | exophagous | sexual | 1 | yes |
| 12 | caterpillars | endophagous | sexual | 1 | yes |
| 13 | fruits | endophagous | sexual | 1 | yes |
| 14 | pine needles | endophagous | sexual | 1 | yes |
| 15 | aphids | endophagous | sexual | 5 | yes |
| 16 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 17 | yeast | exophagous | sexual | 10 | yes |
| 18 | galls | endophagous | sexual | 1 | yes |
| 19 | caterpillars | endophagous | sexual | 1 | yes |
| 20 | leaves | endophagous | sexual | 5 | yes |
| 21 | detritivore | exophagous | sexual | 10 | yes |
| 22 | leaves | exophagous | sexual | 1 | no |
| 23 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 24 | caterpillars | endophagous | parthenogenesis | 1 | yes |
| 25 | galls | endophagous | sexual | 4 | yes |
| 26 | vascular tissues | exophagous | sexual | 10 | yes |
| 27 | pine needles | exophagous | sexual | 1 | yes |
| 28 | leaves | exophagous | sexual | 1 | yes |
| 29 | leaves | exophagous | sexual | 1 | yes |
| 30 | leaf mines | endophagous | sexual | 8 | yes |
| 31 | fruit | endophagous | sexual | 1 | no |
| 32 | fruit | endophagous | sexual | 1 | no |
| 33 | vascular tissues | exophagous | sexual | 8 | no |
| 34 | galls | endophagous | cyclical parthenogenesis | 30 | yes |
| 35 | leaf mines | endophagous | sexual | 1 | yes |

|  | 9. Host resource used by parasite | 10. Concealment | 11. Parasite reproduction | 12. Generations per year | 13. Host used to find mate |
|---|---|---|---|---|---|
| 36 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 37 | aphids | endophagous | parthenogenesis | 20 | yes |
| 38 | aphids | endophagous | sexual | 20 | yes |
| 39 | aphids | endophagous | sexual | 20 | yes |
| 40 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 41 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 42 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 43 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 44 | galls | endophagous | sexual | 8 | yes |
| 45 | parasitoid larvae | endophagous | sexual | 1 | yes |
| 46 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 47 | leaves | endophagous | sexual | 1 | no |
| 48 | leaf mines | endophagous | sexual | 1 | yes |
| 49 | detritivore | exophagous | sexual | 1 | yes |
| 50 | detritivore | exophagous | sexual | 1 | yes |
| 51 | caterpillars | endophagous | parthenogenesis | 1 | no |
| 52 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 53 | galls | endophagous | sexual | 4 | yes |
| 54 | fly larvae | endophagous | sexual | 1 | yes |
| 55 | yeast | exophagous | sexual | 10 | yes |
| 56 | yeast | exophagous | sexual | 10 | yes |
| 57 | yeast |  | sexual | 10 | yes |
| 58 | vascular tissues | exophagous | sexual | 1 | yes |
| 59 | leaves | exophagous | sexual | 4 | yes |
| 60 | galls | endophagous | sexual | 8 | yes |
| 61 | galls | endophagous | sexual | 1 | yes |
| 62 | galls | endophagous | sexual | 1 | yes |

|  | 9. Host resource used by parasite | 10. Concealment | 11. Parasite reproduction | 12. Generations per year | 13. Host used to find mate |
|---|---|---|---|---|---|
| 63 | leaves | exophagous | sexual | 1 | no |
| 64 | vascular tissues | exophagous | sexual | 5 | yes |
| 65 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 66 | blood | exophagous | sexual | 1 | yes |
| 67 | blood | exophagous | sexual | 1 | yes |
| 68 | seeds | exophagous | sexual | 1 | yes |
| 69 | galls | endophagous | cyclical parthenogenesis | 10 | yes |
| 70 | leaves | exophagous | sexual | 1 | yes |
| 71 | leaves | exophagous | sexual | 30 | yes |
| 72 | leaves | exophagous | sexual | 1 | yes |
| 73 | galls | endophagous | sexual | 1 | yes |
| 74 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 75 | vascular tissues | exophagous | sexual | 5 | yes |
| 76 | stems | endophagous | sexual | 2 | yes |
| 77 | vascular tissues | exophagous | cyclical parthenogenesis | 10 | yes |
| 78 | leaf mines | endophagous | sexual | 5 | yes |
| 79 | leaves | endophagous | sexual | 1 | yes |
| 80 | larvae | endophagous | sexual | 1 | yes |
| 81 | larvae | endophagous | parthenogenesis | 1 | yes |
| 82 | leaf mines | endophagous | sexual | 1 | yes |
| 83 | leaf mines | endophagous | sexual | 1 | yes |
| 84 | vascular tissues | exophagous | sexual | 5 | yes |
| 85 | fruits | endophagous | sexual | 4 | yes |
| 86 | galls | endophagous | parthenogenesis | 1 | yes |
| 87 | galls | endophagous | sexual | 10 | yes |
| 88 | leaves | exophagous | sexual | 1 | no |
| 89 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 90 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |



| | 9. Host resource used by parasite | 10. Concealment | 11. Parasite reproduction | 12. Generations per year | 13. Host used to find mate |
|---|---|---|---|---|---|
| 91 | leaves | endophagous | sexual | 2 | yes |
| 92 | fruits | endophagous | sexual | 3 | yes |
| 93 | galls | endophagous | cyclical parthenogenesis | 10 | yes |
| 94 | leaves | exophagous | cyclical parthenogenesis | 30 | yes |
| 95 | leaves | exophagous | parthenogenesis | 30 | no |
| 96 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | . |
| 97 | pine needles | exophagous | sexual | 1 | yes |
| 98 | vascular tissues | exophagous | parthenogenesis | 30 | yes |
| 99 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 100 | galls | endophagous | cyclical parthenogenesis | 5 | yes |
| 101 | blood | exophagous | sexual | 5 | no |
| 102 | fruit | endophagous | sexual | 1 | yes |
| 103 | vascular tissues | exophagous | sexual | 8 | no |
| 104 | leaves | exophagous | sexual | 1 | yes |
| 105 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 106 | vascular tissues | exophagous | cyclical parthenogenesis | 30 | yes |
| 107 | galls | endophagous | cyclical parthenogenesis | 2 | yes |
| 108 | galls | endophagous | cyclical parthenogenesis | 10 | yes |

|  | 14. Host phenology | 15. Host volatile preference | 16. Oviposition preference | 17. Immigrant inviability | 18. Allochrony |
|---|---|---|---|---|---|
| 1 | host resources available at different times | yes | . | . | yes |
| 2 | host resources available at the same time | yes | yes | no | . |
| 3 | host resources available at the same time | . | yes | yes | . |
| 4 | host resources available at the same time | . | . | . | . |
| 5 | host resources available at different times | . | no | . | . |
| 6 | host resources available at the same time | . | . | . | . |
| 7 | host resources available at different times | yes | yes | yes | . |
| 8 | host resources available at different times | . | yes | . | . |
| 9 | host resources available at the same time | . | . | . | . |
| 10 | host resources available at the same time | . | . | . | . |
| 11 | host resources available at the same time | . | . | . | . |
| 12 | host resources available at the same time | . | . | . | . |
| 13 | host resources available at the same time | yes | yes | . | . |
| 14 | host resources available at different times | no | no | yes | . |
| 15 | host resources available at the same time | . | yes | yes | yes |
| 16 | host resources available at different times | no | no | no | . |
| 17 | host resources available at different times | yes | yes | yes | . |
| 18 | host resources available at the same time | no | no | . | . |
| 19 | host resources available at the same time | . | . | . | . |
| 20 | host resources available at different times | . | . | . | . |
| 21 | host resources available at the same time | . | . | . | yes |
| 22 | host resources available at the same time | yes | no | no | yes |
| 23 | host resources available at different times | yes | . | no | . |
| 24 | host resources available at different times | . | . | . | . |
| 25 | host resources available at different times | no | no | no | yes |
| 26 | host resources available at the same time | . | . | . | yes |
| 27 | host resources available at different times | . | . | yes | . |
| 28 | host resources available at different times | . | . | yes | . |
| 29 | host resources available at different times | . | . | . | . |
| 30 | host resources available at different times | yes | yes | yes | . |
| 31 | host resources available at different times | . | . | . | . |
| 32 | host resources available at different times | . | . | . | . |
| 33 | host resources available at different times | . | . | . | . |
| 34 | host resources available at different times | . | yes | yes | . |
| 35 | host resources available at the same time | yes | yes | yes | yes |

|  | 14. Host phenology | 15. Host volatile preference | 16. Oviposition preference | 17. Immigrant inviability | 18. Allochrony |
|---|---|---|---|---|---|
| 36 | host resources available at the same time | yes | yes | yes | . |
| 37 | host resources available at different times | . | yes | yes | . |
| 38 | host resources available at different times | yes | . | . | . |
| 39 | host resources available at different times | . | yes | yes | . |
| 40 | host resources available at different times | yes | yes | yes | . |
| 41 | host resources available at different times | . | . | yes | . |
| 42 | host resources available at different times | . | . | yes | . |
| 43 | host resources available at the same time | yes | . | yes | . |
| 44 | host resources available at the same time | yes | . | . | yes |
| 45 | host resources available at the same time | . | . | . | . |
| 46 | host resources available at the same time | . | no | no | . |
| 47 | host resources available at different times | . | yes | no | no |
| 48 | host resources available at different times | yes | yes | yes | . |
| 49 | host resources available at the same time | . | . | . | . |
| 50 | host resources available at the same time | . | . | . | . |
| 51 | host resources available at the same time | . | yes | yes | . |
| 52 | host resources available at the same time | yes | yes | yes | . |
| 53 | host resources available at different times | yes | yes | yes | yes |
| 54 | host resources available at different times | yes | yes | yes | yes |
| 55 | host resources available at different times | yes | yes | yes | . |
| 56 | host resources available at different times | yes | yes | yes | . |
| 57 | host resources available at different times | yes | yes | yes |  |
| 58 | host resources available at different times | yes | yes | yes | yes |
| 59 | host resources available at different times | yes | yes | yes | . |
| 60 | host resources available at the same time | yes | yes | yes | yes |
| 61 | host resources available at different times | . | . | . | . |
| 62 | host resources available at different times | . | . | . | yes |

|  | 14. Host phenology | 15. Host volatile preference | 16. Oviposition preference | 17. Immigrant inviability | 18. Allochrony |
|---|---|---|---|---|---|
| 63 | host resources available at different times | . | no | yes | . |
| 64 | host resources available at the same time | . | yes | . | . |
| 65 | host resources available at different times | . | . | . | . |
| 66 | host resources available at different times | . | . | no | yes |
| 67 | host resources available at different times | . | . | no | yes |
| 68 | host resources available at different times | . | . | yes | . |
| 69 | host resources available at the same time | . | yes | . | yes |
| 70 | host resources available at different times | . | yes | yes | . |
| 71 | host resources available at different times | . | . | . | . |
| 72 | host resources available at the same time | yes | . | yes | . |
| 73 | host resources available at the same time | yes | yes | yes | yes |
| 74 | host resources available at different times | yes | . | no | . |
| 75 | host resources available at different times | yes | yes | yes | . |
| 76 | host resources available at different times | yes | yes | yes | yes |
| 77 | host resources available at different times | . | . | yes | . |
| 78 | host resources available at different times | . | yes | yes | yes |
| 79 | host resources available at different times | . | . | . | . |
| 80 | host resources available at different times | . | . | . | yes |
| 81 | host resources available at the same time | . | . | . | . |
| 82 | host resources available at different times | . | yes | . | no |
| 83 | host resources available at the same time | . | yes | . | no |
| 84 | host resources available at different times | . | . | . | . |
| 85 | host resources available at different times | yes | yes | yes | yes |
| 86 | host resources available at different times | . | yes | . | no |
| 87 | host resources available at different times | . | . | yes | yes |
| 88 | host resources available at different times | . | no | yes | no |
| 89 | host resources available at different times | . | . | . | . |
| 90 | host resources available at different times | . | . | yes | . |

|  | 14. Host phenology | 15. Host volatile preference | 16. Oviposition preference | 17. Immigrant inviability | 18. Allochrony |
|---|---|---|---|---|---|
| 91 | host resources available at the same time | yes | . | yes | no |
| 92 | host resources available at the same time | . | . | . | yes |
| 93 | host resources available at the same time | . | . | . | yes |
| 94 | host resources available at different times | . | yes | yes | yes |
| 95 | host resources available at different times | . | . | . | . |
| 96 | host resources available at the same time | . | . | yes | . |
| 97 | host resources available at different times | yes | yes | yes | . |
| 98 | host resources available at different times | . | . | yes | . |
| 99 | host resources available at different times | . | . | yes | yes |
| 100 | host resources available at different times | . | . | . | . |
| 101 | host resources available at different times | . | . | . | . |
| 102 | host resources available at different times | . | . | yes | . |
| 103 | host resources available at different times | yes | yes | yes | . |
| 104 | host resources available at different times | . | . | . | . |
| 105 | host resources available at different times | . | . | yes | yes |
| 106 | host resources available at different times | . | . | yes | yes |
| 107 | host resources available at the same time | . | . | . | . |
| 108 | host resources available at different times | yes | yes | yes | no |

|  | 19. Morphological differentiation between populations | 20. Host shift opportunity | 21. System management | 22. Differential infection by microbes |
|---|---|---|---|---|
| 1 | no | parasite and hosts native | managed | . |
| 2 | no | parasite non-native, hosts native or non- | managed | . |
| 3 | no | parasite non-native, hosts native or non-native | managed | . |
| 4 | no | parasite and host native | managed | . |
| 5 | no | parasite and hosts native | managed | . |
| 6 | . | parasite and hosts native | wild | . |
| 7 | . | parasite and hosts non-native | managed | . |
| 8 | . | parasite native, host non-native | managed | . |
| 9 | . | parasite and hosts native | wild | . |
| 10 | . | parasite and hosts native | wild | . |
| 11 | . | parasite and hosts native | wild | . |
| 12 | no | parasite and hosts native | wild | . |
| 13 | . | parasite and hosts non-native | managed | . |
| 14 | yes | parasite and hosts native | wild | . |
| 15 | . | parasite and hosts non-native | managed | . |
| 16 | no | parasite non-native, hosts native | managed | yes |
| 17 | . | parasite and host native | wild | . |
| 18 | . | parasite and hosts native | wild | . |
| 19 | no | parasite non-native, host native | wild | no |
| 20 | . | parasite and hosts native | managed | . |
| 21 | . | parasite and hosts native | wild | . |
| 22 | no | parasite and hosts native | managed | . |
| 23 | no | parasite and hosts non-native | managed | . |
| 24 | . | parasite and host native | wild | . |
| 25 | . | parasite and hosts native | wild | . |
| 26 | . | parasite and hosts native | wild | . |
| 27 | . | parasite non-native, hosts native | managed | . |
| 28 | . | parasite and hosts native | wild | . |
| 29 | . | parasite and hosts native | wild | . |
| 30 | . | parasite and hosts native | managed | . |
| 31 | no | parasite non-native, hosts native or non- | wild | . |
| 32 | no | parasite non-native, hosts native or non- | wild | . |
| 33 | . | parasite and hosts native | wild | . |
| 34 | no | parasite and hosts native | wild | . |
| 35 | no | parasite native, hosts native or non-native | managed | . |

|  | 19. Morphological differentiation between populations | 20. Host shift opportunity | 21. System management | 22. Differential infection by microbes |
|---|---|---|---|---|
| 36 | yes | parasite non-native, hosts native or non-native | managed | yes |
| 37 | no | parasite and hosts non-native | managed | . |
| 38 | yes | parasite and hosts non-native | managed | . |
| 39 | no | parasite and hosts non-native | managed | . |
| 40 | no | parasite non-native, hosts native or non-native | managed | yes |
| 41 | yes | parasite and hosts non-native | managed | yes |
| 42 | . | parasite and hosts non-native | managed | yes |
| 43 | yes | parasite and hosts non-native | managed | yes |
| 44 | yes | parasite and hosts native | wild | . |
| 45 | . | parasite and host native | wild | . |
| 46 | yes | parasite and hosts non-native | managed | . |
| 47 | yes | parasite non-native, hosts native or non-native | managed | . |
| 48 | . | parasite native, hosts native or non-native | managed | yes |
| 49 | . | parasite and hosts native | wild | . |
| 50 | . | parasite and hosts native | wild | . |
| 51 | . | parasite and hosts native | wild | no |
| 52 | yes | parasite and hosts native | wild | . |
| 53 | . | parasite and hosts native | managed | . |
| 54 | no | parasite and host native | managed | . |
| 55 | . | parasite and hosts native | wild | . |
| 56 | . | parasite and hosts native | wild | . |
| 57 | . | parasite and hosts native | wild |  |
| 58 | no | parasite native, hosts native or non-native | wild | . |
| 59 | no | parasite native, one host native, one host non-native | wild | . |
| 60 | . | parasite and hosts native | wild | . |
| 61 | yes | parasite and hosts native | wild | . |
| 62 | . | parasite and hosts native | wild | . |

| | 19. Morphological differentiation between populations | 20. Host shift opportunity | 21. System management | 22. Differential infection by microbes |
|---|---|---|---|---|
| 63 | no | parasite native, one host native, one host non-native | wild | . |
| 64 | . | parasite native, hosts mixed | wild | yes |
| 65 | yes | parasite non-native, hosts native | managed | . |
| 66 | . | parasite and hosts native | wild | . |
| 67 | . | parasite and hosts native | wild | . |
| 68 | yes | parasite native, one host native, one host non-native | wild | . |
| 69 | yes | parasite non-native, host native | managed | . |
| 70 | . | parasite and hosts native | wild | . |
| 71 | . | parasite non-native, hosts native | managed | . |
| 72 | . | parasite and hosts native | wild | . |
| 73 | no | parasite and hosts native | managed | . |
| 74 | no | parasite and hosts non-native | managed | . |
| 75 | no | parasite and hosts non-native | managed | . |
| 76 | no | parasite non-native, hosts native | managed | . |
| 77 | . | parasite and hosts non-native | managed | yes |
| 78 | no | parasite and hosts native | wild | . |
| 79 | . | parasite and hosts native | wild | . |
| 80 | . | parasite and host native | managed | . |
| 81 | yes | parasite and hosts native | wild | . |
| 82 | . | parasite and hosts native | wild | . |
| 83 | . | parasite and hosts native | wild | . |
| 84 | no | parasite native, hosts native or non-native | managed | . |
| 85 | no | parasite native, one host native, one host non-native | managed | . |
| 86 | . | parasite and hosts native | wild | no |
| 87 | yes | parasite and hosts native | wild | . |
| 88 | no | parasite and hosts native | wild | . |
| 89 | . | parasite native, hosts mixed | managed | yes |
| 90 | . | parasite and hosts non-native | managed | no |

|  | 19. Morphological differentiation between populations | 20. Host shift opportunity | 21. System management | 22. Differential infection by microbes |
|---|---|---|---|---|
| 91 | no | parasite native, hosts mixed | managed | yes |
| 92 | yes | parasite and hosts native | managed | yes |
| 93 | yes | parasite non-native, hosts native | managed | . |
| 94 | . | parasite native, hosts native or non-native | managed | no |
| 95 | . | parasite and hosts non-native | managed | yes |
| 96 | . | parasite and host native | wild | yes |
| 97 | . | parasite and hosts native | wild | . |
| 98 | . | parasite non-native, hosts native or non-native | managed | . |
| 99 | yes | parasite and hosts native | managed | . |
| 100 | . | parasite and hosts native | managed | . |
| 101 | . | parasite and hosts native | managed | . |
| 102 | . | parasite and hosts native | managed | . |
| 103 | . | parasite and hosts native | managed | yes |
| 104 | . | parasite native, hosts native or non-native | wild | . |
| 105 | . | parasite and hosts native | managed | . |
| 106 | . | parasite and hosts native | managed | . |
| 107 | . | parasite and hosts native | managed | . |
| 108 | . | parasite and hosts native | managed | yes |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 1 | Medina, R. F., Szendrei, Z., Harrison, K., Isaacs, R., Averill, A., Malo, E. A., & Rodriguez-Saona, C. 2014. Exploring host-associated differentiation in the North American native cranberry fruitworm, *Acrobasis vaccinii* , from blueberries and cranberries. Entomologia experimentalis et applicata, **150**: 136-148. |  |  |
| 2 | Wu, Z., Hopper, K. R., O'Neil, R. J., Voegtlin, D. J., Prokrym, D. R., & Heimpel, G. E. 2004. Reproductive compatibility and genetic variation between two strains of *Aphelinus albipodus* (Hymenoptera: Aphelinidae), a parasitoid of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). Biological control, **31**: 311-319. |  |  |
| 3 | Weathersbee Iii, A. A., Shufran, K. A., Panchal, T. D., Dang, P. M., & Evans, G. A. 2004. Detection and differentiation of parasitoids (Hymenoptera: Aphidiidae and Aphelinidae) of the brown citrus aphid (Homoptera: Aphididae): species-specific polymerase chain reaction amplification of 18S rDNA. Annals of the entomological society of America. **97**: 286-292. | Yokomi, R. K., & Tang, Y. Q. 1995. Host preference and suitability of two aphelinid parasitoids (Hymenoptera: Aphelinidae) for aphids (Homoptera: Aphididae) on citrus. Journal of economic entomology, **88**: 840-845. |  |
| 4 | Dickey, A. M., & Medina, R. F. 2011. Lack of sequential radiation in a parasitoid of a host-associated aphid. Entomologia experimentalis et applicata, **139**: 154-160. |  |  |
| 5 | Lozier, J. D., Roderick, G. K., & Mills, N. J. 2009. Molecular markers reveal strong geographic, but not host associated, genetic differentiation in Aphidius transcaspicus, a parasitoid of the aphid genus Hyalopterus. Bulletin of entomological |  |  |
| 6 | Gómez-Díaz, E., González-solís, J., Peinado, M. A., & Page, R. D. 2007. Lack of host-dependent genetic structure in ectoparasites of Calonectris shearwaters. Molecular ecology, **16**: 5204-5215. |  |  |
| 7 | Prabhakar, C. S., Mehta, P. K., Sood, P., Singh, S. K., Sharma, P., & Sharma, P. N. 2012. Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Coquillett)(Diptera: Tephritidae) based on mitochondrial cytochrome oxidase (COI) gene sequences. Genetica, **14**: 83- | Piñero, J. C., Jácome, I., Vargas, R., & Prokopy, R. J. 2006. Response of female melon fly, Bactrocera cucurbitae, to host-associated visual and olfactory stimuli. Entomologia experimentalis et applicata, **121**: 261-269. |  |
| 8 | Simonato, M., Battisti, A., Zovi, D., & Medina, R. F. 2012. Testing for host-associated differentiation in two egg parasitoids of a forest herbivore. Entomologia experimentalis et applicata, **145**: 124-133. |  |  |
| 9 | Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? Systematic biology, **52**: 37-47. |  |  |
| 10 | Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? Systematic biology, **52**: 37-47. |  |  |

| | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 11 | Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? Systematic biology, **52**: 37-47. | | |
| 12 | Stireman, J. O., Nason, J. D., Heard, S. B., & Seehawer, J. M. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. Proceedings of the royal society of London B: Biological sciences, **273**: | | |
| 13 | Timm, A. E., Geertsema, H., & Warnich, L. 2006. Gene flow among *Cydia pomonella* (Lepidoptera: Tortricidae) geographic and host populations in South Africa. Journal of economic entomology, **99**: 341-348. | Witzgall, P., Ansebo, L., Yang, Z., Angeli, G., Sauphanor, B., & Bengtsson, M. 2005. Plant volatiles affect oviposition by codling moths. Chemoecology, **15**: 77-83. | |
| 14 | Langor, D. W., & Spence, J. R. 1991. Host effects on allozyme and morphological variation of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). The Canadian entomologist, **123**: 395-410. | | |
| 15 | Baer, C. F., Tripp, D. W., Bjorksten, T. A., & Antolin, M. F. 2004. Phylogeography of a parasitoid wasp (*Diaeretiella rapae* ): no evidence of host-associated lineages. Molecular ecology, **13**: 1859-1869. | Antolin, M. F., Bjorksten, T. A., & Vaughn, T. T. 2006. Host-related fitness trade-offs in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera: Aphidiidae). Ecological entomology, **31**: 242-254. | |
| 16 | Guidolin, A. S., Fresia, P., & Cônsoli, F. L. 2014. The genetic structure of an invasive pest, the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae). PloS One, **9**: e115749. | Wenninger, E. J., Stelinski, L. L., & Hall, D. G. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environmental entomology, **38**: 225-234. | |
| 17 | Heed, W. B. 1978. Ecology and genetics of Sonoran desert Drosophila. In Ecological genetics: The interface. Springer New York, pp. | Markow, T. A., Fogleman, J. C., & Heed, W. B. 1983. Reproductive isolation in Sonoran desert Drosophila. Evolution, **37**: 649-652. | |
| 18 | Stireman III, J. O., Nason, J. D., & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution, **59**: 2573-2587. | | |
| 19 | Althoff, D. M. 2008. A test of host-associated differentiation across the 'parasite continuum'in the tri-trophic interaction among yuccas, bogus yucca moths, and parasitoids. Molecular ecology, **17**: 3917-3927. | | |
| 20 | Dickey, A. M., & Medina, R. F. 2010. Testing host-associated differentiation in a quasi-endophage and a parthenogen on native trees. Journal of evolutionary biology, **23**: 945- | | |
| 21 | Gómez-Díaz, E., González-solís, J., Peinado, M. A., & Page, R. D. 2007. Lack of host-dependent genetic structure in ectoparasites of Calonectris shearwaters. Molecular ecology, **16**: 5204-5215. | | |
| 22 | Jaenike, J., & Selander, R. K. 1980. On the question of host races in the fall webworm, *Hyphantria cunea* . Entomologia experimentalis et applicata, **27**: 31-37. | | |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 23 | Clements, K. M., Sorenson, C. E., Wiegmann, B. M., Neese, P. A., & Roe, R. M. 2000. Genetic, biochemical, and behavioral uniformity among populations of *Myzus nicotianae* and *Myzus persicae* . Entomologia experimentalis et applicata, **95**: 269-281. |  |  |
| 24 | Simonato, M., Battisti, A., Zovi, D., & Medina, R. F. 2012. Testing for host-associated differentiation in two egg parasitoids of a forest herbivore. Entomologia experimentalis et applicata, **145**: 124-133. |  |  |
| 25 | Stireman III, J. O., Nason, J. D., & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution, **59**: 2573-2587. |  |  |
| 26 | Gómez-Díaz, E., González-solís, J., Peinado, M. A., & Page, R. D. 2007. Lack of host-dependent genetic structure in ectoparasites of Calonectris shearwaters. Molecular ecology, **16**: 5204-5215. |  |  |
| 27 | Salvato, P., Battisti, A., Concato, S., Masutti, L., Patarnello, T., & Zane, L. 2002. Genetic differentiation in the winter pine processionary moth (*Thaumetopoea pityocampa* —wilkinsoni complex), inferred by AFLP and mitochondrial DNA markers. Molecular ecology, **11**: 2435- | Paiva, M. R., Mateus, E., Santos, M. H., & Branco, M. R. 2011. Pine volatiles mediate host selection for oviposition by *Thaumetopoea pityocampa* (Lepidoptera: Notodontidae). Journal of applied entomology, **135**: 195-203. |  |
| 28 | Stireman III, J. O., Nason, J. D., & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution, **59**: 2573-2587. |  |  |
| 29 | Stireman III, J. O., Nason, J. D., & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution, **59**: 2573-2587. |  |  |
| 30 | Cifuentes, D., Chynoweth, R., & Bielza, P. 2011. Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. Pest management science, **67**: 1155-1162. | Pereyra, P. C., & Sánchez, N. E. 2006. Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). Neotropical entomology, **35**: 671-676. | Proffit, M., Birgersson, G., Bengtsson, M., Reis, R., Witzgall, P., & Lima, E. 2011. Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. Journal of chemical ecology, **37**: 565-574. |
| 31 | Bakker, A. C., Roessingh, P., & Menken, S. B. 2008. Sympatric speciation in Yponomeuta: no evidence for host plant fidelity. Entomologia experimentalis et applicata, **128**: 240-247. |  |  |
| 32 | Bakker, A. C., Roessingh, P., & Menken, S. B. 2008. Sympatric speciation in Yponomeuta: no evidence for host plant fidelity. Entomologia experimentalis et applicata, **128**: 240-247. |  |  |
| 33 | Gómez-Díaz, E., González-solís, J., Peinado, M. A., & Page, R. D. 2007. Lack of host-dependent genetic structure in ectoparasites of Calonectris shearwaters. Molecular ecology, **16**: 5204-5215. |  |  |
| 34 | Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., Frazier, M., et al. 2009. Colony collapse disorder: a descriptive study. PloS One, **4**: e6481. |  |  |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 35 | Ohshima, I. 2008. Host race formation in the leaf-mining moth Acrocercops transecta (Lepidoptera: Gracillariidae). Biological journal of the Linnean society, **93**: 135-145. | Ohshima, I. 2010. Host-associated pre-mating reproductive isolation between host races of *Acrocercops transecta* : mating site preferences and effect of host presence on mating. Ecological entomology, **35**: 253-257. |  |
| 36 | Peccoud, J., Ollivier, A., Plantegenest, M., & Simon, J. C. 2009. A continuum of genetic divergence from sympatric host races to species in the pea aphid complex. Proceedings of the national academy of sciences, **106**: 7495-7500. | Hawthorne, D. J., & Via, S. 2001. Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature, **412**: 904-907. | Ferrari, J., West, J. A., Via, S., & Godfray, H. C. J. 2012. Population genetic structure and secondary symbionts in host-associated populations of the pea aphid complex. Evolution, **66**: 375-390. |
| 37 | Kazmer, D. J., Maiden, K., Ramualde, N., Coutinot, D., & Hopper, K. R. 1996. Reproductive compatibility, mating behavior, and random amplified polymorphic DNA variability in some *Aphelinus asychis* (Hymenoptera: Aphelinidae) derived from the Old World. Annals of the entomological society of America, **89**: 212- |  |  |
| 38 | Chen, Y., Giles, K. L., & Greenstone, M. H. 2002. Molecular evidence for a species complex in the genus Aphelinus (Hymenoptera: Aphelinidae), with additional data on aphidiine phylogeny (Hymenoptera: Braconidae). Annals of the entomological society of America, **95**: 29-34. |  |  |
| 39 | Henry, L. M., May, N., Acheampong, S., Gillespie, D. R., & Roitberg, B. D. 2010. Host-adapted parasitoids in biological control: Does source matter?. Ecological applications, **20**: 242-250. | Henry, L. M. 2008. Assortative mating and the role of phenotypic plasticity in male competition: implications for gene flow among host-associated parasitoid populations. Biology letters, **4**: 508-511. |  |
| 40 | Raymond, B., Searle, J. B., & Douglas, A. E. 2001. On the processes shaping reproductive isolation in aphids of the *Aphis fabae* (Scop.) complex (Aphididae: Homoptera). Biological Journal of the Linnean society, **74**: 205-215. | Adams, D., & Douglas, A. E. 1997. How symbiotic bacteria influence plant utilisation by the polyphagous aphid, *Aphis fabae* . Oecologia, **110**: 528-532. | Nottingham, S. F., & Hardie, J. 1993. Flight behaviour of the black bean aphid, *Aphis fabae* , and the cabbage aphid, *Brevicoryne brassicae* , in host and non-host plant odour. Physiological Entomology, **18**: 389-394. |
| 41 | Vanlerberghe-Masutti, F., & Chavigny, P. 1998. Host-based genetic differentiation in the aphid *Aphis gossypii* Glover, evidenced from RAPD fingerprints. Molecular ecology, **7**: 905-914. | Carletto, J., Lombaert, E., Chavigny, P., Brévault, T., Lapchin, L., & Vanlerberghe-Masutti, F. 2009. Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. Molecular ecology, **18**: 2198-2212. | Carletto, J., Lombaert, E., Chavigny, P., Brévault, T., Lapchin, L., & Vanlerberghe-Masutti, F. 2009. Ecological specialization of the aphid *Aphis gossypii* Glover on cultivated host plants. Molecular ecology, **18**: 2198-2212. |
| 42 | Mezghani-Khemakhem, M., Bouktila, D., Kharrat, I., Makni, M., & Makni, H. 2012. Genetic variability of green citrus aphid populations from Tunisia, assessed by RAPD markers and mitochondrial DNA sequences. Entomological science, **15**: 171-179. | Komazaki, S. 1998. Difference of egg diapause in two host races of the spirea aphid, Aphis spiraecola. Entomologia experimentalis et applicata, **89**: 201-205. |  |
| 43 | Miller, G. L., Favret, C., Carmichael, A., & Voegtlin, D. J. 2009. Is there a cryptic species within *Aulacorthum solani* (Hemiptera: Aphididae)?. Journal of economic entomology, **102**: 398-400. | Damsteegt, V. D., & Voegtlin, D. J. 1990. Morphological and biological variation among populations of *Aulacorthum solani* (Homoptera: Aphididae), the vector of soybean dwarf virus. Annals of the entomological society of America, **83**: 949-955. |  |
| 44 | Stireman, J. O., Janson, E. M., Carr, T. G., Devlin, H., & Abbot, P. 2008. Evolutionary radiation of *Asteromyia carbonifera* (Diptera: Cecidomyiidae) gall morphotypes on the goldenrod *Solidago altissima* (Asteraceae). Biological journal of the Linnean |  |  |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 45 | Howell, J. L. 2016. Host location and host-associated divergence in parasitoids of the gall midge, *Asteromyia carbonifera.* PhD thesis, Wright State University, pp. 35-67. |  |  |
| 46 | Ruiz-Montoya, L., Nunez-Farfan, J., & Vargas, J. 2003. Host-associated genetic structure of Mexican populations of the cabbage aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae). Heredity, **91**: 415-421. | Ruiz-Montoya, L., Nùñez-Farfán, J., & Domínguez, C. A. 2005. Changes in morphological traits of the cabbage aphid (*Brevicoryne brassicae* ) associated with the use of different host plants. Ecological research, **20**: 591-598. | Nottingham, S. F., & Hardie, J. 1993. Flight behaviour of the black bean aphid, *Aphis fabae* , and the cabbage aphid, *Brevicoryne brassicae* , in host and non-host plant odour. Physiological Entomology, **18**: 389-394. |
| 47 | Tuda, M., Kagoshima, K., Toquenaga, Y., & Arnqvist, G. 2014. Global genetic differentiation in a cosmopolitan pest of stored beans: effects of geography, host-plant usage and anthropogenic factors. PLoS One, **9**: e106268. |  |  |
| 48 | Ishiguro, N., Yoshida, K., & Tsuchida, K. 2006. Genetic differences between rice and water-oat feeders in the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae). Applied entomology and zoology, **41**: 585-593. | Ishiguro, N., & Tsuchida, K. 2006. Polymorphic microsatellite loci for the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae). Applied entomology and zoology, **41**: 565-568. |  |
| 49 | Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? Systematic biology, **52**: 37-47. |  |  |
| 50 | Johnson, K. P., Adams, R. J., Page, R. D., & Clayton, D. H. 2003. When do parasites fail to speciate in response to host speciation? Systematic biology, **52**: 37-47. |  |  |
| 51 | Kankare, M., Van Nouhuys, S., & Hanski, I. 2005. Genetic divergence among host-specific cryptic species in *Cotesia melitaearum* aggregate (Hymenoptera: Braconidae), parasitoids of checkerspot butterflies. Annals of the entomological society of America, **98**: 382-394. |  |  |
| 52 | Guldemond, J. A. 1990. Evolutionary genetics of the aphid Cryptomyzus, with a preliminary analysis of the inheritance of host plant preference, reproductive performance and host-alteration. Entomologia experimentalis et | Guldemond, J. A. 1990. Choice of host plant as a factor in reproductive isolation of the aphid genus Cryptomyzus (Homoptera, Aphididae). Ecological entomology, **15**: 43-51. |  |
| 53 | Cook, M. A. 2011. *Dasineura oxycoccana* (Diptera: Cecidomyiidae) populations on cranberry and blueberry in British Columbia: same species, host races or sibling species? PhD thesis, Simon Fraser University, pp. 54-60. |  |  |
| 54 | Forbes, A. A., Powell, T. H., Stelinski, L. L., Smith, J. J., & Feder, J. L. 2009. Sequential sympatric speciation across trophic levels. Science, **323**: 776-779. | Stelinski, L. L., & Liburd, O. E. 2005. Behavioral evidence for host fidelity among populations of the parasitic wasp, *Diachasma alloeum* | Hamerlinck, G. 2015. Coevolution of Rhagoletis hosts and their parasitic wasps. PhD thesis, University of Iowa, pp.47-61. |
| 55 | Heed, W. B. 1978. Ecology and genetics of Sonoran desert Drosophila. In Ecological genetics: The interface. Springer New York, pp. | Markow, T. A., Fogleman, J. C., & Heed, W. B. 1983. Reproductive isolation in Sonoran desert Drosophila. Evolution, **37**: 649-652. | Castrezana, S., & Bono, J. M. 2012. Host plant adaptation in *Drosophila mettleri* populations. PloS One, **7**: e34008. |

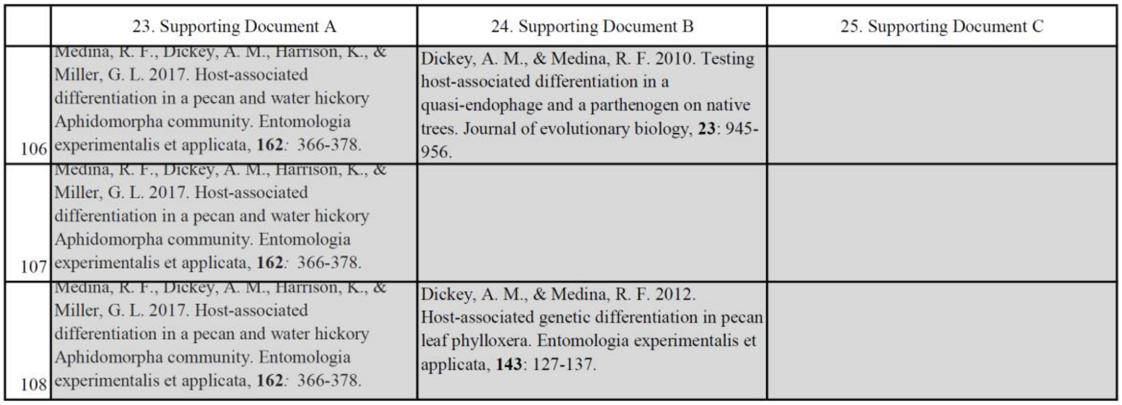
|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 56 | Heed, W. B. 1978. Ecology and genetics of Sonoran desert Drosophila. In Ecological genetics: The interface. Springer New York, pp. 109-126. | Reed, L. K., Nyboer, M., & Markow, T. A. 2007. Evolutionary relationships of Drosophila mojavensis geographic host races and their sister species *Drosophila arizonae* . Molecular ecology, **16**: 1007-1022. |  |
| 57 | Heed, W. B. 1978. Ecology and genetics of Sonoran desert Drosophila. In Ecological genetics: The interface. Springer New York, pp. |  |  |
| 58 | Wood, T. K., & Keese, M. C. 1990. Host-plant-induced assortative mating in Enchenopa treehoppers. Evolution, **109**: 619-628. | Wood, T. K., & Guttman, S. I. 1983. *Enchenopa binotata* complex: sympatric speciation? Science, **220**: 310-312. | Guttman, S. I., Wood, T. K., & Karlin, A. A. 1981. Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex (Homoptera: Membracidae). Evolution, **35**: 205-217. |
| 59 | Shirai, Y., & Morimoto, N. 1999. A host shift from wild blue cohosh to cultivated potato by the phytophagous ladybird beetle, *Epilachna yasutomii* (Coleoptera, Coccinellidae). Researches on population ecology, **41**: 161-167. | Katakura, H., Shioi, M., & Kira, Y. 1989. Reproductive isolation by host specificity in a pair of phytophagous ladybird beetles. Evolution, **3**: 1045-1053. | Koji, S., Nakamura, K., & Yamashita, M. 2004. Adaptive change and conservatism in host specificity in two local populations of the thistle-feeding ladybird beetle *Epilachna niponica* . Entomologia experimentalis et applicata, **112**: 145-153. |
| 60 | Waring, G. L., Abrahamson, W. G., & Howard, D. J. 1990. Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). Evolution, **44**: 1648-1655. | Craig, T. P., Itami, J. K., Abrahamson, W. G., & Horner, J. D. 1993. Behavioral evidence for host-race formation in *Eurosta solidaginis* . Evolution, **47**: 1696-1710. | Craig, T. P., Horner, J. D., & Itami, J. K. 2001. Genetics, experience, and host-plant preference in *Eurosta solidaginis* : implications for host shifts and speciation. Evolution, **55**: 773-782. |
| 61 | Leppänen, S. A., Malm, T., Värri, K., & Nyman, T. 2014. A comparative analysis of genetic differentiation across six shared willow host species in leaf-and bud-galling sawflies. PloS |  |  |
| 62 | Nason, J. D., Heard, S. B., & Williams, F. R. 2002. Host-associated genetic differentiation in the goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae). Evolution, **56**: 1475-1488. |  |  |
| 63 | Sword, G. A., Joern, A., & Senior, L. B. 2005. Host plant-associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera: Acrididae). Molecular ecology, **14**: 2197-2205. |  |  |
| 64 | Imo, M., Maixner, M., & Johannesen, J. 2013. Sympatric diversification vs. immigration: deciphering host-plant specialization in a polyphagous insect, the stolbur phytoplasma vector *Hyalesthes obsoletus* |  |  |
| 65 | Lozier, J. D., Roderick, G. K., & Mills, N. J. (2007). Genetic evidence from mitochondrial, nuclear, and endosymbiont markers for the evolution of host plant associated species in the aphid genus Hyalopterus (Hemiptera: Aphididae). Evolution, **61**: 1353-136. |  |  |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 66 | Kempf, F., De Meeûs, T., Vaumourin, E., Noel, V., Taragel'ová, V., Plantard, O., et al., & McCoy, K. D. 2011. Host races in *Ixodes ricinus* , the European vector of Lyme borreliosis. Infection, genetics and evolution, **11**: |  |  |
| 67 | McCoy, K. D., Boulinier, T., Tirard, C., & Michalakis, Y. 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae* . Journal of evolutionary biology, **14**: 395- |  |  |
| 68 | Carroll, S. P., Dingle, H., Famula, T. R., & Fox, C. W. 2001. Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma* . Genetica, **112**: 257-272. | Carroll, S. P., Dingle, H., & Klassen, S. P. 1997. Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. Evolution, **51**: 1182-1188. | Carroll, S. P., & Boyd, C. 1992. Host race radiation in the soapberry bug: natural history with the history. Evolution, **46**: 1052-1069. |
| 69 | Komatsu, T., & Akimoto, S. 1995. Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica* . Ecological entomology, **20**: 33-42. |  |  |
| 70 | Kreslavskiia, A. G., Mikheev, A. V., Solomatin, V. M., & Gritsenko, V. V. 1981. Genetic exchange and isolating mechanisms in sympatric races of *Lochmaea capreae* (Coleoptera, Chrysomelidae). Journal of evolutionary biology, **16**: 208-218. | Soudi, S., Reinhold, K., & Engqvist, L. 2015. Host-associated divergence in sympatric host races of the leaf beetle Lochmaea capreae: implications for local adaptation and reproductive isolation. Biological journal of the Linnean society, **116**: 169-182. |  |
| 71 | Rosas-García, N. M., Sarmiento-Benavides, S. L., Villegas-Mendoza, J. M., Hernández-Delgado, S., & Mayek-Pérez, N. 2010. Genetic differentiation among *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) populations living on different host plants. Environmental entomology, **39**: 1043- |  |  |
| 72 | Kuussaari, M., Singer, M., & Hanski, I. 2000. Local specialization and landscape-level influence on host use in an herbivorous insect. Ecology, **81**: 2177-2187. |  |  |
| 73 | Blair, C. P., Abrahamson, W. G., Jackman, J. A., & Tyrrell, L. 2005. Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle. Evolution, **59**: 304-316. |  |  |
| 74 | Margaritopoulos, J. T., Malarky, G., Tsitsipis, J. A., & Blackman, R. L. 2007. Microsatellite DNA and behavioural studies provide evidence of host-mediated speciation in Myzus persicae (Hemiptera: Aphididae*).* Biological journal of the Linnean society, **91**: 687-702. | Margaritopoulos, J. T., Shigehara, T., Takada, H., & Blackman, R. L. 2007. Host-related morphological variation within Myzus persicae group (Homoptera: Aphididae) from Japan. Applied entomology and zoology, **42**: 329-335. |  |
| 75 | Sezer, M., & Butlin, R. K. 1998. The genetic basis of oviposition preference differences between sympatric host races of the brown planthopper (*Nilaparvata lugens* ). Proceedings of the royal society of London B: biological sciences, **265**: 2399-2405. | Saxena, R. C., & Barrion, A. A. 1985. Biotypes of the brown planthopper *Nilaparvata lugens* (Stål) and strategies in deployment of host plant resistance. International journal of tropical insect science, **6**: 271-289. |  |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 76 | Thomas, Y., Bethenod, M. T., Pelozuelo, L., Frérot, B., & Bourguet, D. 2003. Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hübner. I. Sex pheromone, moth emergence timing, and parasitism. Evolution, **57**: 261-273. | Bethenod, M. T., Thomas, Y., Rousset, F., Frérot, B., Pélozuelo, L., Genestier, G., & Bourguet, D. 2005. Genetic isolation between two sympatric host plant races of the European corn borer, Ostrinia nubilalis Hübner. II: assortative mating and host-plant preferences for oviposition. Heredity, **94**: 264-270. |  |
| 77 | Miller, N. J., Birley, A. J., Overall, A. D. J., & Tatchell, G. M. 2003. Population genetic structure of the lettuce root aphid, *Pemphigus bursarius* L., in relation to geographic distance, gene flow and host plant usage. Heredity, **91**: 217- | Miller, N. J., Kift, N. B., & Tatchell, G. M. 2005. Host-associated populations in the lettuce root aphid, *Pemphigus bursarius* L. Heredity, **94**: 556-564. |  |
| 78 | Scheffer, S. J., & Hawthorne, D. J. 2007. Molecular evidence of host-associated genetic divergence in the holly leafminer *Phytomyza glabricola* (Diptera: Agromyzidae): apparent discordance among marker systems. Molecular | Hebert, J. B., Scheffer, S. J., & Hawthorne, D. J. 2013. Reproductive Isolation between Host Races of *Phytomyza glabricola* on *Ilex coriacea* and *I. glabra* . PloS One, **8**: e73976. |  |
| 79 | Herbst, J., & Heitland, W. 1994. Genetic differentiation among populations of the sawfly-species *Platycampus luridiventris* , associated with different Alder species (Hymenoptera: Tenthredinidae). Entomologia generalis, **19**: 39- |  |  |
| 80 | Stireman, J. O., Nason, J. D., Heard, S. B., & Seehawer, J. M. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. Proceedings of the royal society of London B: biological sciences, **273**: |  |  |
| 81 | Leppänen, S. A., Malm, T., Värri, K., & Nyman, T. 2014. A comparative analysis of genetic differentiation across six shared willow host species in leaf-and bud-galling sawflies. PloS |  |  |
| 82 | Drummond, C. S., Xue, H. J., Yoder, J. B., & Pellmyr, O. 2010. Host-associated divergence and incipient speciation in the yucca moth *Prodoxus coloradensis* (Lepidoptera: Prodoxidae) on three species of host plants. Heredity, **105**: 183-196. |  |  |
| 83 | Darwell, C. T., Fox, K. A., & Althoff, D. M. 2014. The roles of geography and founder effects in promoting host-associated differentiation in the generalist bogus yucca moth Prodoxus decipiens. Journal of evolutionary biology, **27**: 2706-2718. |  |  |
| 84 | Barman, A. K., Parajulee, M. N., Sansone, C. G., Suh, C. P., & Medina, R. F. 2012. Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatus* . Entomologia experimentalis et applicata, **143**: 31-41. | Barman, A. K., Parajulee, M. N., Sansone, C. G., & Medina, R. F. (2012). Host preference of cotton fleahopper, Pseudatomoscelis seriatus (Reuter) is not labile to geographic origin and prior experience. Environmental entomology, **41**: 125-132. | Antwi, J. B., Sword, G. A., & Medina, R. F. 2015. Host-associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. Ecology and evolution, **5**: 2533-2543. |
| 85 | Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus Rhagoletis (Diptera, Tephritidae). Evolution **23**: 237-251. | Feder, J. L., & Filchak, K. E. 1999. It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella* , and its implications for fitness trade-offs in phytophagous insects.<br>In Proceedings of the 10th International Symposium on Insect-Plant Relationships. Springer Netherlands, pp. 211-225. | Feder, J. L., Hunt, T. A., & Bush, L. 1993. The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella* . Entomologia experimentalis et applicata, **69**: 117-135. |

| | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 86 | Hernández-vera, G., Mitrović, M., Jović, J., Toševski, I., Caldara, R., Gassmann, A., & Emerson, B. C. 2010. Host-associated genetic differentiation in a seed parasitic weevil *Rhinusa antirrhini* (Coleptera: Curculionidae) revealed by mitochondrial and nuclear sequence data. Molecular ecology, **19**: 2286-2300. | | |
| 87 | Stireman III, J. O., Nason, J. D., & Heard, S. B. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution, **59**: 2573-2587. | | |
| 88 | Sword, G. A., & Dopman, E. B. 1999. Developmental specialization and geographic structure of host plant use in a polyphagous grasshopper, *Schistocerca emarginata* (= *lineata* )(Orthoptera: Acrididae). Oecologia, **120**: 437-445. | | |
| 89 | Anstead, J. A., Burd, J. D., & Shufran, K. A. 2002. Mitochondrial DNA sequence divergence among *Schizaphis graminum* (Hemiptera: Aphididae) clones from cultivated and non-cultivated hosts: haplotype and host associations. Bulletin of entomological | | |
| 90 | Sunnucks, P., De Barro, P. J., Lushai, G., Maclean, N., & Hales, D. 1997. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. Molecular ecology, **6**: 1059-1073. | Simon, J. C., Baumann, S., Sunnucks, P., Hebert, P. D. N., Pierre, J. S., Le Gallic, J. F., & Dedryver, C. A. 1999. Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. Molecular ecology, **8**: 531-545. | |
| 91 | Martinelli, S., Clark, P. L., Zucchi, M. I., Silva-Filho, M. C., Foster, J. E., & Omoto, C. 2007. Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton fields in Brazil. Bulletin of entomological research, **97**: | Pashley, D. P., Hammond, A. M., & Hardy, T. N. 1992. Reproductive isolating mechanisms in fall armyworm host strains (Lepidoptera: Noctuidae). Annals of the entomological society of America, **85**: 400-405. | |
| 92 | Diegisser, T., Seitz, A., & Johannesen, J. E. S. 2006. Phylogeographic patterns of host-race evolution in *Tephritis conura* (Diptera: Tephritidae). Molecular ecology, **15**: 681-694. | | |
| 93 | Akimoto, S. 1990. Local adaptation and host race formation of a gall-forming aphid in relation to environmental heterogeneity. Oecologia, **83**: 162-170. | | |
| 94 | Koh, G. & GoToH, T. 1996. Host plant preference and genetic compatibility of the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). Applied entomology and aoology, **31**: 417-425. | Takafuji, A., Santoso, S., & Hinomoto, N. 2001. Host-related differences in diapause characteristics of different geographical populations of the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), in Japan. Applied entomology and zoology, **36**: 177-184. | |

|  | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 95 | Brunner, P. C., Chatzivassiliou, E. K., Katis, N. I., & Frey, J. E. (2004). Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. Heredity, **93**: 364-370. | Westmore, G. C., Poke, F. S., Allen, G. R., & Wilson, C. R. 2013. Genetic and host-associated differentiation within Thrips tabaci Lindeman (Thysanoptera: Thripidae) and its links to Tomato spotted wilt virus-vector competence. Heredity, **111**: 210-215. |  |
| 96 | Williams, R. S., & Avakian, M. A. 2015. Colonization of Solidago altissima by the specialist aphid Uroleucon nigrotuberculatum: effects of genetic identity and leaf chemistry. Journal of chemical ecology, **41**: 129- |  |  |
| 97 | Emelianov, I., Mallet, J., & Baltensweiler, W. 1995. Genetic differentiation in *Zeiraphera diniana* (Lepidoptera: Tortricidae, the larch budmoth): polymorphism, host races or. Heredity, **75**: 16-424. | Emelianov, I., Simpson, F., Narang, P., & Mallet, J. 2003. Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana* . Journal of evolutionary biology, **16**: 208-218. |  |
| 98 | Nibouche, S., Fartek, B., Mississipi, S., Delatte, H., Reynaud, B., & Costet, L. 2014. Low genetic diversity in *Melanaphis sacchari* aphid populations at the worldwide scale. PloS One, **9**: | Nibouche, S., Mississipi, S., Fartek, B., Delatte, H., Reynaud, B., & Costet, L. 2015. Host plant specialization in the sugarcane aphid *Melanaphis sacchari* . PloS One, **10**: |  |
| 99 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. (2017). Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162***:* 366-378. |  |  |
| 100 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. (2017). Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162***:* 366-378. |  |  |
| 101 | Booth, W., Balvín, O., Vargo, E. L., Vilímová, J., & Schal, C. 2015. Host association drives genetic divergence in the bed bug, *Cimex lectularius* . Molecular ecology, **24**: 980-992. |  |  |
| 102 | Shashank, P. R., Chakravarthy, A. K., Raju, B. R., & Bhanu, K. R. M. 2014. DNA barcoding reveals the occurrence of cryptic species in host-associated population of *Conogethes punctiferalis* (Lepidoptera: Crambidae). Applied entomology and zoology, **49**: 283-295. | Li, D. Y., Ai, P. P., Du, Y. L., Sun, S. L., & Zhang, M. Z. 2015. Effects of different host plants on the development and reproduction of Yellow Peach Moth, *Conogethes punctiferalis* (Guenée, 1854)(Lepidoptera: Crambidae). Austral entomology, **54**: 149-153. |  |
| 103 | Medina, R. F., Reyna, S. M., & Bernal, J. S. 2012. Population genetic structure of a specialist leafhopper on Zea: likely anthropogenic and ecological determinants of gene flow. Entomologia experimentalis et | Davila-Flores, A. M. (2012). Host plant influences on performance and haplotype diversity of Dalbulus maidis, a specialist herbivore of Zea. PhD thesis, Texas A&M University, pp. 60-93. |  |
| 104 | Marques, J. F., Wang, H. L., Svensson, G. P., Frago, E., & Anderbrant, O. 2014. Genetic divergence and evidence for sympatric host-races in the highly polyphagous brown tail moth, *Euproctis chrysorrhoea* (Lepidoptera: Erebidae). Evolutionary ecology, **28**: 829-848. |  |  |
| 105 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. 2017. Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162***:* 366-378. |  |  |



| | 23. Supporting Document A | 24. Supporting Document B | 25. Supporting Document C |
|---|---|---|---|
| 106 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. 2017. Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162**: 366-378. | Dickey, A. M., & Medina, R. F. 2010. Testing host-associated differentiation in a quasi-endophage and a parthenogen on native trees. Journal of evolutionary biology, **23**: 945-956. | |
| 107 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. 2017. Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162**: 366-378. | | |
| 108 | Medina, R. F., Dickey, A. M., Harrison, K., & Miller, G. L. 2017. Host-associated differentiation in a pecan and water hickory Aphidomorpha community. Entomologia experimentalis et applicata, **162**: 366-378. | Dickey, A. M., & Medina, R. F. 2012. Host-associated genetic differentiation in pecan leaf phylloxera. Entomologia experimentalis et applicata, **143**: 127-137. | |