DISSERTATION

PATTERNS OF ASSOCIATION IN A CO-INTRODUCED INSECT HERBIVORE AND PARASITOID WASP

Submitted by

Cheryl Lindsay Bowker

Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2017

Doctoral Committee:

Advisor: Paul J. Ode

William C. Black IV
Louis Bjostad
Deane Bowers
Joseph C. von Fischer

ABSTRACT

PATTERNS OF ASSOCIATION IN A CO-INTRODUCED INSECT HERBIVORE AND PARASITOID WASP

Species within trophic networks that experience a range expansion can lead to populations of coevolved species re-associating in some areas, not associating in others, or creating novel associations with native species at one or more trophic level(s). Although most studies have focused predominantly on coevolved interactions between pairs of species, a growing number of studies investigate interactions within a broader community context across geographically widespread areas. By tracing the invasion routes of co-introduced species that share a close evolutionary history in their native range, I address the relative importance of long-term historical associations versus ecological fitting in community assembly. In the context of invasion ecology, ecological fitting refers to the process whereby exotic species form novel associations with native species that lack a shared coevolutionary relationship.

In Chapter 1 of my dissertation, I review the literature that has taken a population ecology approach to understanding community assembly of introduced coevolved species. Collectively, these studies suggest that multiple factors ranging from local adaptation and ecological fitting are important in shaping multi-species associations in the introduced range and can occur simultaneously in one system. For example, coevolution can be an important factor in the re-association of many exotic plant-herbivore species, but ecological fitting also readily occurs as evidenced by the prevalence of native species host switching to exotics, and vice-versa.

For the remainder of my dissertation, I used molecular methods to determine the invasion routes of the herbivorous parsnip webworm (*Depressaria pastinacella*; Lepidoptera: Depressariidae), and its primary parasitoid, *Copidosoma sosares* (Hymenoptera: Encyrtidae) to address the importance of shared population history and ecological fitting in shaping species interactions in introduced ranges. The

coevolved reciprocal interaction between wild parsnip, parsnip webworm, and *C. sosares* has been well documented. Throughout its native range in Europe and much of its introduced range in North America, the parsnip webworm attacks wild parsnip (*Pastinaca sativa;* Apiaceae). Wild parsnips produce furanocoumarins, allelochemicals that are broadly biocidal because they intercalate DNA and interfere with transcription. Selection for chemically-based resistance occurs in plant populations by increasing concentrations of three furanocoumarins: xanthotoxin, bergapten, and sphondin. Genetic variation also exists in webworm populations in the rate at which these furanocoumarins are metabolized indicating that plant chemistry can act as a selective force on insect physiology.

The wild parsnip was introduced to the United States (U.S.) as a food crop in the 17th century, where it escaped cultivation and spread throughout the U.S. By the 1860s, parsnip webworm was accidentally introduced to the U.S. and has re-associated with its coevolved wild parsnip plant, and formed novel associations with cow parsnip (*Heracleum maximum*; Apiaceae), a plant native to the U.S. *Copidosoma sosares* is also found in the U.S., but its arrival date is unclear. *Copidosoma sosares* attacks webworms on both wild parsnip and cow parsnip, but its distribution is patchy compared to parsnip webworms; with rare exception, *C. sosares* populations are restricted to the western U.S.

In Chapter 2, I used a mitochondrial molecular marker to determine: 1) the source population(s) of U.S. and New Zealand parsnip webworms, and 2) whether parsnip webworm populations in the U.S. or Europe are locally adapted to the host plant species they attack. I found that parsnip webworms experienced a genetic bottleneck during introduction to the U.S. and New Zealand. U.S. parsnip webworm populations were founded by a single (or a few) population(s) of parsnip webworms from the British Isles. British Isles populations of parsnip webworm are themselves genetically isolated from continental European parsnip webworm populations. The introduction pattern of webworms stands in contrast to that of its host plant, wild parsnip, in the introduced ranges of the U.S. and New Zealand. The acquisition of the non-coevolved cow parsnip by webworms in the U.S has led to genetic divergence from webworms feeding on their coevolved plant species. In contrast, European webworm populations are not restricted to the host plant species on which they feed.

In Chapter 3, I describe the development of novel microsatellite markers for tracing the routes of *C. sosares* within the introduced range of the U.S. Thirty-four candidate loci were identified, 12 of which were ultimately chosen for detailed screening. Seven of the 12 loci were polymorphic, but only 5 of the 7 were successfully amplified across samples and ranges. Of these 5 polymorphic loci, there were 3 - 9 alleles per locus. Inbreeding coefficients and the null allele frequency ranged from -0.04 to 0.74 and 0 to 0.73, respectively. After Bonferroni correction, only one locus (Csos 4) significantly deviated from Hardy-Weinberg Equilibrium (HWE, $P < 0.05$) across all populations sampled. When the data were partitioned by location (European and U.S. populations), loci Csos 2, Csos 3, and Csos 4 in European populations conformed to HWE ($P > 0.05$), whereas loci Csos 1, Csos 2, and Csos 3 in the U.S. conformed to HWE ($P>0.05$). No pairs of loci demonstrated linkage disequilibrium, neither across all populations, nor when the data was partitioned into European and U.S populations ($P > 0.05$). These results indicate the developed microsatellite markers for *C. sosares* are well suited for use as genetic markers for elucidating the population structure of *C. sosares*.

In Chapter 4, I used the microsatellite markers developed in Chapter 3 and an additional mitochondrial marker to test three hypotheses: 1) *C. sosares* populations in the U.S. came from the same European location as their webworm hosts in Europe (Host-Pursuit Hypothesis), 2) once in the U.S., *C. sosares* followed a similar host plant switch onto cow parsnip as parsnip webworms (Continued Host-Pursuit Hypothesis), and 3) *C. sosares* populations will have limited geneflow between sites that are geographically farther apart, and differ in elevation (isolation by distance and elevation). The molecular data indicate the Netherlands and several other European sites served as sources for U.S. *C. sosares* populations, following the predictions from the Host-Shift Hypothesis. European *C. sosares* populations from hogweed have directly established on U.S. cow parsnip, and U.S. *C. sosares* populations on wild parsnip have host plant switched to cow parsnip. However, *C. sosares* geneflow is not restricted to either host plant species in the U.S., contrary to our findings with U.S. webworms. Overall, the factors that can influence the establishment of an herbivore (i.e., host plant species, concentrations of plant toxins) in an introduced area, may not be important for its primary parasitoid.

ACKNOWLEDGEMENTS

I would like to extend a word of appreciation to my advisor, Paul Ode, and to the members of my committee, Lou Bjostad, Bill Black, Deane Bowers, and Joe von Fischer for guidance throughout my doctoral program. Thanks to Janet Dill for help in navigating the business side of graduate school and for always showing interest in how I was doing. Laura Sample McMeeking provided me with a wonderful job at the STEM Center that provided the foundation I needed to balance family life and research. My own research is built on the decades of work by May Berenbaum and her colleagues at the University of Illinois, that enabled me to tell a more complete story. May, and others, including Paul Ode, Tania Jogesh, Margaret Stanley, Charles Dean, and Bernarda Calla, helped collect the samples used in this project.

I was fortunate to have the help of many undergraduate students, who in turn, helped me become a better mentor. These included Emily Yannarella, Erika Peirce, Jasmine Cherry, Taniesha Bracken, Andrew Wintergerst, and Lauren Kleine. Also a note of gratitude to all the members of the Ode lab: Theresa Barosh, Ryan Paul, Britt Smith, and Dhaval Vyas, for their camaraderie over the years. Emotional support was provided by my family: my father, my sisters Karen and Linda, my brother James, and their partners and children. My in-laws, Bill and Linda Bowker, encouraged me to celebrate my achievements, both minor and major, which helped me make it through challenging times. To my daughter, Evie Bowker, who is the smartest and sweetest 3 year old a mother could ask for, and who loves referring to her mother as a “doctor scientist”. Finally, thanks to my husband, Daniel Bowker, my biggest champion. I may never be able to fully express my gratitude, but I will always remember the many sacrifices he made in his own life that allowed me to earn this Ph.D.

DEDICATION

This dissertation is dedicated to the memory of my mother, Gloria Ann Lindsay. She never doubted that I would complete my doctorate, and I'm glad that I got to prove her right.

TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGEMENTS v
DEDICATION vi
CHAPTER 1: The influence of shared population history on introduced multi-trophic species assembly 1
Introduction 1
Theoretical Predictions 5
Empirical Evidence 14
Conclusions and Future Directions 19
Figures 21
CHAPTER 2: The rapid establishment and spread of the introduced parsnip webworm (*Depressaria pastinacella*, Lepidoptera: Depressaridae) despite a genetic bottleneck 23
Introduction 23
Materials and Methods 30
Results 34
Discussion 37
Figures 44
Tables 52
CHAPTER 3: Development of novel microsatellite markers for the polyembryonic parasitoid wasp, *Copidosoma sosares* (Hymenoptera: Encyrtidae) 61
Introduction 61
Materials and Methods 62
Results and discussion 64
Tables 66
CHAPTER 4: The introduced parasitoid wasp, *Copidosoma sosares* (Hymenoptera: Encyrtidae), exploits multiple routes of invasion in pursuit of its herbivorous insect host 70
Introduction 70
Methods 77
Results 82
Discussion 86
Conclusion 89
Figures 92
Tables 100

REFERENCES 110

CHAPTER 1: THE INFLUENCE OF SHARED POPULATION HISTORY ON INTRODUCED MULTI-TROPHIC SPECIES ASSEMBLY

Introduction

How members of terrestrial communities assemble in geographically widespread environments has been a major question in population biology and community ecology (Morin 2011; Mittlebach 2012). Studies of introduced species that examine the underlying processes and mechanisms of community assembly have steadily increased since the early-20th century when such cases were first labelled as 'experiment[s] in nature' by Joseph Grinnell in 1919. Many subsequent studies have focused on a single invasive organism, often a plant or herbivorous insect, and how it interacts with the native community (Godfray et al. 1995; Schönrogge et al. 1996; Girardoz et al. 2006; Grabenweger et al. 2010). For example, Vitousek et al. (1987) showed that a single introduced plant species, *Myrica faya* (Myricaceae), can alter nutrient availability and disturbance regimes in Hawaii by significantly increasing the amount of nitrogen in volcanic sites and the overall biologically available nitrogen. The accumulation of native herbivores on introduced economically important crop cultivars has been well documented (Strong 1974, 1979; Wastie 1975; Strong et al. 1977, 1984; Rey et al. 1981; Pimentel 1986; Simberloff 1988; Wilson et al. 1990; Pearse et al. 2013). The myriad of studies documenting introduced herbivores switching to novel native host plants has underscored the importance and prevalence of *ecological fitting* in community assembly (Janzen 1985; Thomas et al. 2001; Shapiro 2002). In the context of invasion ecology, ecological fitting refers to the process whereby exotic species form novel associations with native species that lack a shared coevolutionary relationship.

Studies on exotic plants and insects have generally been restricted to pairwise interactions between plants and their herbivores (Torchin et al. 2003; Verhoeven et al. 2009). Recently, studies have included interactions between exotic plants, herbivores, and their natural enemies (e.g., predators and parasitoids) because of the significant role that higher trophic levels play in affecting the structure and function of terrestrial communities (Hairston et al. 1960; Price et al. 1980; Hunter and Price 1992; Harvey

et al. 2003; Hunter 2003; Gröbler and Lewis 2008; Wang et al. 2009; Fortuna et al. 2012; Mooney et al. 2012; Wang et al. 2013). This multitrophic perspective on co-introduced (from the same ancestral range) plant-herbivore-natural enemy systems will greatly increase our understanding of the relative importance of long-term historical association versus ecological fitting in shaping species associations in an introduced range.

Typically, invasive species do not move in isolation; rather, they can facilitate the introduction of other trophic levels that have a shared association in their area of origin. This accidental co-introduction is a common feature of community development involving invading hosts (Dunn 2009). One such example is the tri-trophic system consisting of wild parsnip *Pastinaca sativa* (Apiaceae), the parsnip webworm *Depressaria pastinacella* (Lepidoptera: Depressariidae), and its parasitoid *Copidosoma sosares* (Hymenoptera: Encyrtidae), which is the focus of Chapters 2 - 4 of this dissertation. All three species are native to Europe, where their coevolutionary relationship has been well documented (Berenbaum and Zangerl 1992; Berenbaum and Zangerl 1998; Berenbaum et al. 1993; Lampert et al. 2008; Zangerl and Berenbaum 2003; Zangerl et al. 2008). The parsnip plant was likely intentionally introduced to North America in the early 17th century (Nuttall 1818) as a food crop, while parsnip webworm was accidentally introduced in the mid-19th century (Bethune 1869; Riley 1889). The predominant parasitoid wasp of parsnip webworm, *C. sosares* is also found in the United States, although the date of its arrival is unknown (Carroll et al. 2007; Ode et al. 2004; Lampert et al. 2008). Across the United States, parsnip webworms have re-associated with wild parsnip, but have also formed novel associations with the native cow parsnip, *Heracleum maximum* (Apiaceae). In turn, *C. sosares* has re-associated with webworm populations feeding on both cow parsnip and wild parsnip, but its distribution is mainly restricted to the western United States.

Alternatively, co-introduced plant-herbivore systems can re-associate in areas where their coevolved parasitoid has failed to follow. In such cases, herbivores have escaped selective pressure from coevolved higher trophic levels (enemy-free space) but they may acquire novel associations with native parasitoids. The latter scenario has occurred within the oak-cynipid gall wasp system within Europe. The

Turkey oak (*Quercus cerris)* and the pedunculate oak (*Q. robur*) were historically restricted to the Iberian peninsula, northern Italy, and the Balkans (Huntley & Birks 1983). In these regions, both oak species are attacked by a cynipid gall wasp, *Andricus quercuscalicis,* which, in turn, was parasitized by a suite of generalist and specialist parasitoid species (Stone and Sunnucks 1993; Stone et al. 1995). Intentional plantings of both oak species have led to their range expansion into northern and western Europe (Jalas & Suominen 1987; Huntley & Birks 1983) and the co-introduction of the cynipid gall wasp, which has re-associated with its ancestral host plant species. However, extensive sampling of parasitoid communities attacking cynipid gall wasps in Britain have shown that no parasitoids from the ancestral range of the gall wasp have followed its host into the introduced range. Rather, several local parasitoids now attack the invasive cynipid gall wasp, forming novel herbivore-parasitoid associations (Stone et al. 1995).

In another example, the plant *Pyracantha coccinea* (Rosaceae), which has been planted across Europe in gardens from its ancestral range in the Caucasus, has facilitated the introduction of its ancestral herbivore, the leaf mining moth, *Phyloonorycter leucographella* (Gröbler and Lewis 2008). In the southern United Kingdom, the leaf mining moth has re-associated with its ancestral host plant but has also formed a novel association with a local plant (*Crataegus monogyna*: Rosaceae), allowing its distribution to expand northwards into Scotland (Emmet 1989; Bland 2002; Sefrova 2003). The recruitment of local parasitoids on the exotic leaf mining moth has occurred rapidly in the United Kingdom on both invasive and native plants (Urbaneja et al. 2000; Amalin et al. 2002), but no ancestral parasitoids of the leaf mining moth have been found in the United Kingdom.

These examples illustrate the range of possible associations that occur when two (or more) species with shared population histories move into a new area. Three questions are of interest: 1) how will exotic species respond to each other in the novel environment (by re-associating or not), 2) how will exotic species respond to native species (e.g., exotic herbivores host-switch onto native plants), and 3) how will native species respond to exotic species (e.g., native parasitoids host-switch onto exotic herbivores).

To better predict the likelihood of each of these associations, which are illustrated in Figure 1.1, we need to first understand the population histories of the invaders from their native range and compare them with their population structure in the introduced range (Estoup and Guillemaud 2010). This will reveal the relative importance of shared population histories, local adaptation, and/or ecological fitting in shaping plant-herbivore-parasitoid systems in novel ranges. With the advancement of inexpensive molecular marker development and new statistical analyses, there has been an increase in the number of invasive biology studies that determine the sources and routes of invasions, and the genetic make-up of the founding populations (Girardoz et al. 2006; Grobler and Lewis 2008; Lozier et al. 2009; Grabenweger et al. 2010; Nicholls et al. 2010; Morrison and Hay 2011; Auger-Rozenberg et al. 2012; Stone et al. 2012; Wang et al. 2013; Allen et al. 2015). Although the aim of these studies has ranged from studying community ecology, biodiversity, or trophic links in food webs, their findings can shed light on the relative importance of shared population histories, local adaptation, and/or ecological fitting in shaping trophic associations in novel ranges.

The aims of this paper are to: 1) present current theoretical predictions and hypotheses on introduced plant, herbivore, and parasitoid systems; 2) present empirical evidence on plant-herbivore-parasitoid introductions and how they fit theory; and 3) discuss the future implications of research into co-introduced multitrophic systems. There are numerous reviews on species interactions in the introduced range within the context of biological control programs (weed biological control, Hinz et al. 2014; arthropod biological control, González-Chang et al. 2016; Hajek et al. 2016), and therefore we excluded these systems in our empirical evidence section. However, the insights gained from biological control programs have aided in the development and refinement of hypotheses regarding introduced species assembly, and therefore, we cite this research in the theoretical predictions and future implications section. Rather, this review focuses on terrestrial plant-herbivore-parasitoid systems that have undergone range expansions, undirected by humans. Multitrophic systems chosen for the empirical evidence section additionally contained population history data from at least two of the trophic levels.

Theoretical Predictions

As illustrated in Figure 1.1, there are a range of possible interactions that can occur when a plant, an herbivore, and a parasitoid are introduced to a new area. We grouped these interactions by asking three questions; 1) what enables co-introduced species to re-associate (Fig 1.1 A, B, O), 2) what prevents co-introduced species from re-associating (Fig. 1.1 D, F, Q), and 3) what allows for novel associations in the introduced range (Fig. 1.1 C, H, I, M, P)? To answer each of these questions, we explored the hypotheses and predictions specific to each interaction in the following sections.

What enables co-introduced species to re-associate?

Many plant and insect interactions have been used as examples supporting the theory of *coevolution* (Ehrlich and Raven 1964; Fox and Morrow 1981; Jermy 1984; Bernays and Graham 1988; Janz et al. 2001). Between plant-herbivore interactions, the plant evolves defenses that repel or deter herbivore attack and the herbivore evolves counter adaptations to overcome plant defenses, creating an evolutionary arms race (Thompson 1988). Overtime, this arms race is predicted to lead to specialization in herbivore diet, leading to intimate associations between plants with phylogenetically conserved chemical defenses and certain herbivore lineages (Feeny 1976). In the family Pieridae, many species of butterfly larvae feed only on plants in one order, Brassicales, that produce the secondary defensive compounds glucosinolates (Gols et al. 2008; Hopkins et al. 2009). If the plant and herbivore species within these coevolved associations are introduced to a different area, in tandem, their re-association could be pre-determined (Fig. 1.1A).

The phylogenetically conserved chemical defenses plants evolve in response to coevolved herbivores, may also benefit the plant in the introduced range. Native herbivores, when faced with exotic plant defensive chemicals in which they have not evolved counter adaptations, may not be able to establish or feed on these exotic plants (Fig. 1.1 G). There are a variety of physiological mechanisms herbivores use against plant chemical defenses (Harvey et al. 2010) which might prove ineffective against novel phytotoxins. Collectively, this could explain why exotic plants are attacked less than related natives

in some systems (Cappuccino and Arnason 2006), or why native herbivores fail to host plant switch on exotic plants entirely (Fig. 1.1 G).

As with some plant-herbivore interactions, parasitoids can inflict significant fitness costs on their herbivorous hosts (Godfray and Shimada 1999). Parasitoids are insects that lay their eggs on or in the bodies of other insects, and upon hatching, the larvae consume the host, either immediately, or as the host continues to feed and develop (Godfray and Shimada 1999). Given their major impact on host fitness, host traits that help avoid or reduce parasitism should therefore be under strong selection (Abrams 2000). Research has documented the evolution of several host traits, including behavioral defenses to avoid parasitism (Gross 1993; Le Ralec et al. 2010), and physiological defenses to prevent parasitoid development after oviposition (i.e., encapsulation (Hochberg 1997), host immunity (Griffiths 1960; Li et al. 2002; Poirie and Coustau 2011)). Populations of hosts attacked by parasitoids therefore tend to exhibit substantial genetic variation for resistance to parasitoids in a variety of host taxa (Henter 1995; Henter and Via 1995; Kraaijeveld and Godfray 1999; Ferrari et al. 2001; von Burg et al. 2008; Vorburger et al .2009). For example, aphids have evolved a number of behavioral defenses such as kicking or dropping off the plant to avoid parasitism (Gross 1993; Le Ralec et al. 2010).

As with plant defenses leading to the evolution of herbivore counter adaptations, herbivorous host defensive traits impose selection for parasitoid counter adaptations (Thompson 1994). In general, these counter adaptations increase the virulence of parasitoids (i.e., ability of eggs and larvae to survive within the host body). For example, the aphid parasitoid, *Lysiphlebus fabarum* has evolved counter adaptations to aphid hosts harboring defensive endosymbionts (Rouchet and Vorburger 2014). The exact counter adaptation(s) in this system are unknown (Rouchet and Vorburger 2014). However, leading theories based on observational data suggest female parasitoid wasps may lay multiple eggs in a single aphid host to increase their success (Oliver et al. 2012), female parasitoids avoid aphids infected with toxin-producing endosymbionts (Lukasik et al. 2013), or there is an increase tolerance of the parasitoid egg or larva to the toxins produced by aphid endosymbionts (Oliver et al. 2009).

In another example, there is a positive correlation between *Drosophila* flies and parasitoids in the genera *Asobara* and *Leptopilina* between the virulence of the parasitoid and resistance of the host (Kraaijeveld and Godfray 1999). The primary mechanism of host resistance is through encapsulation of parasitoid eggs (Kraaijeveld and van Alphen 1994; Russo et al. 1996). In response, textural changes to the chorion of parasitoid eggs enables them to become embedded in host tissue, and avoid encapsulation by the host's blood cells (Kraaijeveld and van Alphen 1994). Collectively, this indicates selective interactions and reciprocal genetic changes (i.e., coevolution) between herbivorous hosts and their parasitoids readily occur (Price 1980; Thompson 1982). Therefore, the re-association between herbivorous hosts and their parasitoids in the introduced range could be a product of their long evolutionary history (Fig. 1.1 B, O).

As outlined above, coevolution has been documented between species in multitrophic interactions, but this strong reciprocal interaction can also vary geographically, among populations, referred to as *local adaptation* (Lively and Dybdahl 2000; Gandon 2002; Kawecki and Ebert 2004). When herbivores are locally adapted to their host plants, their performance is expected to be higher than that achieved by allopatric herbivore populations (Kawecki and Ebert 2004). When host plants are locally adapted to their herbivores, they are expected to experience lower amounts of damage, and higher levels of resistance, than when consumed by allopatric herbivore populations (Garrido et al. 2011). Further, locally adapted plant-herbivore interactions can experience geographic mosaics of coevolution (Thompson 1988, 1994, 1999). This local adaptation leads to "hot spots", where the plant-herbivore interaction is tightly coevolved, whereas "cold spots" occur when plant traits and herbivore traits are mismatched and not tightly coevolved (Thompson 1988, 1994, 1999; Agrawal et al. 2006). Studies on the wild parsnip, and its primary herbivore, the parsnip webworm, as discussed in the Introduction, has demonstrated that wild parsnip defensive phenotypes matches the detoxification abilities in some webworm populations, but mis-matches occurred in populations where the alternate host plant species, cow parsnip, was present (Zangerl and Berenbaum 2003).

Within herbivorous hosts and parasitoid systems, defensive traits and counter adaptations have also been shown to vary within and among populations, but evidence for local adaptation within these populations is lacking (Dupas et al. 2009; Kraaijeveld and Godfray 2009; Branca et al. 2011; Ndemah et al. 2012; Calatayud et al. 2011; van Nouhuys et al. 2012). However, the majority of this work is confined to host-parasitoid systems attacking three hosts; pea aphids (*Acyrthosiphon pisum*; Henter 1995; Henter and Via 1995; Hufbauer and Via 1999; Hufbauer 2001), *Drosophila* species (Kraaijeveld and van Alphen 1994; Pannebakker et al. 2008; Dupas et al. 2003; Dupas et al. 2009), and tropical stem boring moths (Calatayud et al. 2011; Catherine et al. 2010). Therefore, we cannot rule out the possibility of local adaptation in shaping herbivore and parasitoid associations in the introduced range. Overall, this could further explain why some coevolved species are able to re-associate in the introduced range; they are derived from populations that were locally adapted in the ancestral range (Fig. 1.1. A, B, O).

What prevents co-introduced species from re-associating?

The success of an exotic plant species has been attributed to the fact that they leave behind their coevolved natural enemies (e.g., herbivores and pathogens) (Maron and Vila 2001; Keane and Crawley 2002; Wolfe 2002; Mitchell and Power 2003; Reinhart et al. 2003; Stastny et al. 2005; Liu and Stiling 2006; Castells et al. 2013), as predicted by the *enemy-release hypothesis* (Elton 1958; Keane and Crawley 2002). However, the enemy-release hypothesis relies on the assumption that specialist enemies of the exotic species are absent from the introduced region, and host-switching by specialist local enemies to the exotic species will be rare (Keane and Crawley 2002). However, as we have noted in this review, there are several examples of co-introduced plants and herbivores, and their co-introduced parasitoids.

Therefore, the lack of association between co-introduced, coevolved species could be a consequence of three factors; 1) one of the species in the trophic level cannot establish in the environmentally different range, 2) the introduced populations are derived from populations in the ancestral range that were not locally adapted to one another, or 3) native species in the introduced range host switch to the exotic species, disrupting associations between the coevolved species. Each of these factors are discussed in detail in the following sections.

Environmental Mis-Matches (Fig. 1.1 F, D, N, Q): The climate in the introduced range is rarely the same as that in the native range, therefore, introduced species may not be able to establish in habitats with different environmental traits such as climate, soil type, or topography (Day and McAndrew 2003; Goolsby et al 2006; Fig. 1.1F, D, N, Q). Most of the insights into the effects of environment on introduced herbivores and parasitoids have come from biological control research (Van Lenteren et al. 2006). More specifically, the environmental conditions that influence the establishment of herbivore or parasitoid agents in the introduced range. Many weed biological control programs now choose potential herbivore agents based on climate-matching approaches to increase the establishment and success of intentionally introduced herbivores on exotic plants (Wapshere 1974, 1983, 1993; Dennill and Gordon 1990; Kleinjan and Scott 1996; Adair and Scott 1997; Goolsby et al. 2004; Lawson et al. 2010). For example, the tortoise beetle, *Gratiana spadicea* (Coleoptera: Chrysomelidae) was released as a biological control agent for the invasive weed, *Solanum sisymbriifolium* (Solanaceae) in parts of South Africa (Hill and Hulley 1995; Olckers et al. 1999), but its establishment has been variable (Bryne et al. 2002). Research revealed the beetle failed to establish in some areas because the winters dropped below the lower lethal humidity levels for the beetle eggs (Bryne et al. 2002).

There are also many examples of parasitoids not pursuing their herbivorous hosts into the introduced range (Schönrogge and Crawley 2000; Torchin et al. 2003; Yang et al. 2010), which could be due to suboptimal environmental conditions in the introduced range (Torchin et al. 2002; Fig. 1.1D, Q). In the debate over introducing specialized or generalist parasitoids to suppress exotic pest species (in classical biological control programs; Chang and Kareiva 1999; Symondson et al. 2002; Van Lenteren 2012), specialists may have decreased non-target effects (Stiling and Corneliseen 2005), but they have been shown to be more vulnerable to ecological conditions in their introduced range (Wang et al. 2009). In a review of parasitoids used as biological control agents, the main abiotic factor affecting the establishment of intentionally introduced parasitoid species was temperature (Van Lenteren et al. 2006). More specifically, parasitoids were unable to establish in areas with winters that reached temperatures

below their "developmental threshold" (Van Lenteren et al. 2006). The development threshold is the temperature below which there is no development of an arthropod (Hart et al. 2002).

Population Mis-Matches (Fig. 1.1 F, D, Q): Populations of plants can vary in their concentration and suite of defensive chemicals (Zangerl and Berenbaum 1997; Berenbaum and Zangerl 2006). As mentioned earlier, studies on wild parsnip, and its primary herbivore, the parsnip webworm, documented the occurrence of matched populations, in terms of wild parsnip chemical profiles and the detoxification abilities of webworms (Zangerl and Berenbaum 2003). However, studies have also documented mis-matches between wild parsnip and webworm populations (Zangerl and Berenbaum 2003; Carroll and Berenbaum 2006), in some cases, created by the presence of an alternate host plant species, cow parsnip (Zangerl and Berenbaum 2003). If introduced plants and herbivores came from source populations with mis-matched chemical profiles and detoxification abilities, the herbivore might be unable to re-associate with its host plant in the introduced range (Fig. 1.1 F).

The performance of parasitoid wasps is also affected by population-related differences in plant chemistry (Ode et al. 2004; Harvey 2005; Ode 2006; Gols and Harvey 2009). In studies on multitrophic systems within their ancestral ranges, parasitoids that are less well-adapted compared to their hosts on plants with increased defenses have been shown to perform worse (Harvey et al. 2003, 2005; Ode 2006; Gols et al. 2008). If the founding population of exotic plants and herbivores came from a population with matched plant chemical profiles and herbivore metabolism capability, they could re-associate (Fig. 1.1 A). However, if founding populations of parasitoids are derived from plant and herbivore populations in which they have no shared population history in the ancestral range, the parasitoid might fail to establish on hosts in the introduced range (Fig. 1.1 D, Q).

These scenarios further illustrate how local adaptation between plants, herbivores, and parasitoids in the ancestral range can determine their association, or lack thereof, in the introduced range.

Native Species Disrupt Coevolved Associations (Fig. 1.1 Q): Parasitoids may be able to exploit the same introduction pathways as their herbivorous host, but their establishment may be dependent on traits specific to the host plant species (Fig. 1.1 Q). In several systems, parasitoids rely on host plant

structure and morphology to locate, and subsequently accept, herbivorous hosts (Turlings and Wackers 2004; Wang et al. 2009). If exotic herbivores host plant switch to native plants in the introduced range, the introduced parasitoid may be unable to establish on herbivores attacking the novel host plant (Fig. 1.1Q). Chemical cues that are used to locate suitable plants may differ between exotic and native plant species with which they are usually associated (Harvey et al. 2010). Because of this, parasitoids may not initially respond to novel odors associated with exotic plants, at least during the initial creation of these interactions (Vet and Dicke 1992). In this situation, the herbivore escapes from their parasitoid natural enemies (e.g., enemy-release hypothesis; Berdegue et al. 1996; Jeffries and Lawton 1984; Futuyma and Moreno 1988), by host plant switching.

What allows novel associations in the introduced range?

The multitude of studies documenting host-switching between exotic and native species in the introduced range that lack any evolutionary history (herbivores host plant switching reviewed in Keane and Crawley 2002; parasitoids host switching reviewed in Roy et al. 2011) suggests species can have a discrete set of phenotypes that predisposes them to utilize novel species, a concept referred to as *ecological fitting* (Janzen 1985). Ecological fitting was originally formulated to describe plant-herbivore interactions that were not the result of any coevolutionary history (Agosta 2006; Agosta et al. 2010). Species interactions within an ecological fit framework are potentially more plastic, and not conserved over time (Verhoeven et al. 2009; Harvey et al. 2010). In the following sections, we discuss each possible set of novel species interactions that can occur in the introduced range.

Exotic Herbivores Host-Switch to Native Plants (Fig. 1.1 M): Introduced insect herbivores can interact with both novel plants (lower trophic level) and parasitoids (higher trophic level), creating a variety of novel species associations in their invaded range (Godfray et al. 1995; Schönrogge et al. 1996; Girardoz et al 2006; Grobler and Lewis 2008; Grabenweger et al 2010). Many intentionally introduced coevolved herbivores of exotic plants have been used in biological control efforts, but have instead formed novel associations with native plants (non-target effects; Louda et al. 1997; Pearson and Callaway 2003), suggesting native plants are an 'ecological fit' for some introduced herbivores.

Further, exotic plants and their herbivores can both be accidentally co-introduced, but the exotic herbivore starts attacking native plants (Fig. 1.1 M), sometimes at greater rates than their coevolved introduced plant species (Lambert et al. 2007; Lambert and Casagrande 2007; Park and Blossey 2008; Allen et al. 2015; Cronin et al. 2015). This has emerged has a common phenomenon across multiple species and guilds of introduced herbivores, creating an invasional meltdown (Simberloff and Von Holle 1999). During an invasional meltdown multiple invasive species facilitate one another's spread or exacerbate their impact on native species. Returning to our plant-herbivore example, introduced herbivores can indirectly facilitate the growth and spread of invasive plant species by preferentially feeding on their native competitors (Parker et al. 2006; Relva et al. 2010), although these studies have been limited to introduced generalists herbivores.

Native Parasitoids Host-Switch to Exotic Herbivores (Fig. 1.1 C, P): In addition to exotic herbivores forming novel associations with native plants, native parasitoids can host-switch to exotic herbivores (reviewed by Roy et al. 2011). The oak and exotic gall wasp system in Britain has been well studied and has revealed that a diverse and abundant native parasitoid species complex can host-switch to an exotic herbivore in a span of 30 years (Collins et al. 1983; Hails et al. 1990; Hails and Crawley 1992; Schönrogge et al. 1998). However, the process of native parasitoids host-switching to exotic herbivores is highly variable (Cornell and Hawkins 1993). In an analysis of 87 exotic herbivorous insects, between less than 10 years to more than 140 years elapsed before native parasitoid complexes were as rich or richer on the exotic herbivore than parasitoid complexes on the herbivore in its native range (Cornell and Hawkins 1993).

Native parasitoid species may also show a high level of plasticity in its ability to exploit an exotic host on a wide range of host plant species (Fig. 1.1 C). In another example, the U.S. native parasitoid, *Habrobracon gelechiae*) has established on the light brown apple moth (*Epiphyas postvittana*), an introduced herbivore in the U.S. (Brown et al. 2010), which feeds on a range of host plant species (Suckling and Brockerhoff 2010; Brockerhoff et al. 2011). The parasitoid could locate the host larvae on

all plants equally well, and the fitness correlates examined in the parasitoid did not differ based on the host plant species with which the light brown apple moth fed (Wang et al. 2013).

Native Herbivores Host-Switch to Exotic Plants (Fig. 1.1 H): There are many studies that document novel interactions between exotic plants that have become established in new habitats with native herbivores (Agrawal et al. 1999; Agrawal and Kotanen 2003; Louda et al. 2005; Siemann et al. 2006; Lankau and Strauss 2007). These novel interactions with exotic plants can have either positive or negative effects on the behavior and development of non-coevolved herbivores (Keeler and Chew 2008). Exotic plants may be toxic for non-adapted insect herbivores and prevent host switching onto exotic plants (Fig. 1.1 G), but ecological 'fits' can also occur by chance without the need for any evolutionary precedent (Harvey et al. 2010; Fig. 1.1 H).

The *biotic resistance hypothesis* (Elton 1958; Parker and Hay 2005) predicts that herbivores in the introduced range cause greater mortality to invasive plant species than their herbivores in the ancestral range (Agrawal and Kotanen 2003; Chun et al. 2010; Morrison and Hay 2011; Fan et al. 2013). This phenomenon is often attributed to the invasive plant lacking effective defenses to resist attack by herbivores with which they do not share an evolutionary history (Morrison and Hay 2011; Fig. 1.1H). Although the biotic resistance hypothesis assumes no ancestral herbivores are co-introduced with the plant, the biotic resistance hypothesis can explain why native herbivores are able to host switch onto exotic plants, regardless of the presence or absence of the coevolved, co-introduced exotic herbivore. In contrast to situations where exotic plants experience herbivore-free space because native herbivores have not evolved counter adaptations to exotic plant defenses, this mis-matching between plant defensive traits and native herbivore counter adaptations could benefit the native herbivore (Hokkanen and Pimental 1989; Colautti et al. 2004; Morrison and Hay 2011). As a result, native herbivores can successfully establish on exotic herbivores (Fig. 1.1 H). For example, in a field experiment of 30 plant species, in which 15 plant pairs were used (14 congeneric pairs and 1 confamilial pair) between a plant species native to North America and an exotic species of Eurasian origin, Agrawal and Kotanen (2003) found exotic plants suffer levels of leaf herbivory damage equal to or greater than levels suffered by their paired

native plant species. Additionally, the exotic plant species were no more resistant to herbivores than the native congeners (Agrawal and Kotanen 2003).

The hypotheses discussed thus far have mainly focused on the pair-wise interactions between plants and herbivores, or between herbivores and parasitoids. The original intent of these hypotheses was not to address interactions across three trophic levels. However, the tightly-linked interactions between some plant–herbivore–parasitoid systems are well-suited to investigate whether these hypotheses can be applied across multiple trophic levels. In addition, the extent to which each of the possible interactions outlined in Fig. 1.1 occurs in the field is unclear. In the following sections, we will take the empirical studies that have been conducted on introduced plant-herbivore-parasitoid systems with known source population data to assess the importance of each hypothesis in introduced species assembly.

Empirical Evidence

Cedars and seeding-feeding chalcids

If an introduced plant and herbivore do not re-associate in the new range, but rather the introduced herbivore host-switches to a native plant (Fig. 1.1 M), the parasitoid may not be able to follow its insect host (Fig. 1.1 Q). This is especially true if the parasitoid uses host plant cues to locate its host, such as the parasitoid of the seed feeding chalcid wasp in the following example. In Europe, the seed feeding chalcid, *Megastigmus schimitscheki*, has a native range throughout the eastern Mediterranean region, where it develops on two species of *Cedrus* trees. In Turkey, Syria, and Lebanon it develops on C. *libani*, and in Cyprus, on *C. brevifolia* (Fabre et al. 2004). In 1995, the chalcid was detected on cedar seeds in southern France, and has progressively invaded most of the planted *C. atlantica* stands (Fabre et al. 2004; Roques et al. 2008). *C. atlantica* invaded France when its seeds were imported from North Africa in the 1860s for reforestation of the Mediterranean zone (Auger-Rozenberg et al. 2012). Although it is obvious that the seed chalcid shifted host plants, it was unclear from which region and host plant species it originated. Genetic data revealed that French invasive seed chalcid populations originated from *C. brevifolia* populations in Cyprus, where *C. atlantica* does not occur (Auger-Rozenberg et al. 2012). However, as predicted by ecological fitting, natural crosses between *C. atlantica* and *C. brevifolia*

produces fertile hybrids (Fady et al. 2003), suggesting phylogenetic proximity. In France, *C. atlantica* represents a directly exploitable resource for the seed chalcid, even outcompeting the native seed-feeder (Boivin et al. 2008), although they did not coevolve. No specialist natural enemies of the seed chalcid known in the native area have been detected in the introduced range of southeastern France, suggesting the host plant switching of an introduced herbivore created an enemy-free space from coevolved parasitoids (Auger-Rozenberg et al. 2012).

Oaks, oak gall wasps, and their parasitoids

Oak gall wasps are prevalent throughout Europe, and many studies have explored the genetic history of gall wasps and their parasitoids to aid in control measures (Stone and Sunnucks 1993; Stone et al. 1995; Nicholls et al. 2010; Stone et al. 2012). As mentioned earlier in the introduction, oak gall wasps in the genus *Andricus* can have an obligate relationship with more than one oak species during their asexual and sexual generations. The cynipid oak gall wasp, *Andricus kollari*, has a native range in two distinct locations which geographically divides the oak species on which they develop. In Eastern Europe and Turkey, the gall wasp sexually develops on Turkey oak (*Q. cerris*), but the parthenogenetic generation develops on section Quercus oaks (*Q. robur, Q. petraea*, and *Q. pubescens*). In the Iberian Peninsula and northern Africa, the sexual generation develops on the cork oak (*Q. suber*), and its parthenogenetic generation develops on oaks in the section Quercus, but not on Turkey oak, which does not naturally occur in Iberia. The Turkey oak has been planted intentionally throughout Europe over the last 500 years and expanded its range northward and westward, now overlapping with northern Iberian populations of cork oak (Stone and Sunnucks 1993). Not surprisingly, the oak gall wasp has also been found on these planted Turkey oaks (Stone et al. 2001, 2002), and genetic data reveal all invading oak gall wasps reaching northern continental Europe are derived from populations native to Italy and the Balkans, with a long evolutionary history of exploiting Turkey oaks (Stone et al. 2001, Fig. 1.1 A). Even though Turkey oaks and cork oaks now overlap in the Iberian peninsula, oak gall wasps from the Iberian region have not host-switched onto Turkey oaks to expand their range northwards, even though this species can develop on Turkey oaks elsewhere.

Studies on oaks and the cynipid oak gall wasp (*A. kollari*) were later extended to examine the roles of coevolution, ecological fitting, and anthropogenic disturbance among the natural enemy of the invasive gall wasp, the parasitoid, *Megastigmus stigmatizans* (Nicholls et al. 2010). Genetic data from European *M. stigmatizans* populations collected from Turkey oaks revealed some populations had Balkan origins just as their gall wasp hosts, but others have an Iberian origin. This latter result was surprising, because Iberian gall wasps are confined to cork oak and do not occur on Turkey oaks. Although Iberian gall wasps can't host plant switch to Turkey oaks, its parasitoid, Iberian *M. stigmatizans*, can successfully colonize gall wasps that utilize different host plant species. Overall, this system indicates that a long evolutionary history can be important between one trophic link (oak and gall wasp), but not the other trophic link (gall wasp-parasitoid).

Chestnut trees, the horse chestnut leaf-mining moth, and its parasitoid

The horse chestnut leaf-mining moth, *Cameraria ohridella*, is a pest of horse chestnut trees and has experienced a rapid range expansion from its native area in the southern Balkans (Valade et al. 2009; Lees et al. 2011), to almost all of Europe within the last 25 years (Augustin et al. 2010). The generalist parasitoid, *Pediobius saulius*, is the dominant parasitoid species on *C. ohridella* in the native range of the Balkans (Grabenweger et al. 2010), while in the introduced ranges of central and western Europe, *P. saulius* is a common parasitoid of other leaf miners (Noyes 2002; Girardoz et al. 2007) and rarely attacks *C. ohridella* (Freise et al. 2002; Stojanovic and Markovic 2004; Grabenweger et al. 2005; Lupi 2005; Volter and Kenis 2006; Girardoz et al. 2007; Grabenweger et al. 2010). Genetic data revealed central and northern European populations of *P. saulius* do not come from the Balkans, but rather consist of *P. saulius* lineages that were already present in most of Europe prior to the time of *C. ohridella*'s invasion (Hernandez-Lopez et al. 2012). This suggests that when *C. ohridella* spread from the Balkans to Austria in 1989 and then to central and western Europe this was probably made without its associate Balkan *P. saulius* haplotypes (Fig. 1.1 Q). However, the researchers of this study have noted Austrian *P. saulius* parasitism rates on *C. ohridella* has gradually increased since 1996 (Fig. 1.1 O). This system suggests the strength of some herbivore-parasitoid associations vary by geographical location, with Balkan *C.*

ohridella and *P. saulius* populations locally adapted to each other. When Balkan *C. ohridella* expanded its range into Europe, it experienced escape from its natural enemy in two ways. First, the locally adapted Balkan parasitoid *P. saulius* did not pursue *C. ohridella*, and second, European *P. saulius* populations were unable to parasitize *C. ohridella*. Nevertheless, *C. ohridella* release from natural enemies appears to have been temporary. Whether the population origin of *P. saulius* that attacks *C. ohridella* in Europe is Balkans or European (that have formed associations with allopatric *C. ohridella* populations) is unknown at this time.

A parasitoid species that does attack *C. ohridella* throughout Europe is the generalist ectoparasitoid, *Pnigalio mediterraneus* (Hymenoptera: Eulophidae), even though it was previously restricted to southern Europe (Freise et al. 2002; Grabenweger & Lethmayer 1999; Fig 1J). In fact, prior to *C. ohridella's* invasion from the Balkans into the rest of Europe, the preferred host of *P. mediterraneus* was *B. oleae*, an invasive fly, originally from the Mediterranean. Therefore, *P. mediterraneus* has become the primary parasitoid of two invaders that colonized Europe thousands of years apart. Genetic data revealed the natural host of *P. mediterraneus* in the Mediterranean-Balkan region was *B. oleae*. Then, over the past 25 years, some haplotypes of *P. mediterraneus* switched to *C. ohridella* (Gebiola et al. 2013). When *C. ohridella* expanded its range from the southern Balkans, some of these haplotypes then tracked *C. ohridella* all over Europe. Hence, herbivore host-switching (a form of ecological fitting) occurred first by the parasitoid, followed by herbivore host pursuit into new ranges.

A single host species, the horse chestnut leaf miner, can be parasitized by multiple species of parasitoids. However, these various parasitoid species will respond differently when its host undergoes a range expansion. In the case of *P. saulius*, it could not follow its host into central and western Europe from its shared area of origin in the Balkans. However, *P. mediterraneus* could follow its host into central and western Europe after only forming an association with it in the Mediterranean/Balkan region in the past 25 years. It is unclear, in this system, how a parasitoid with a long evolutionary history with its host cannot pursue it across landscapes, but a parasitoid with a relatively short history can.

Responses of native parasitoids to exotic herbivores

Thus far, we have considered how parasitoids, or communities of parasitoids, adapt to the range expansion of their hosts. However, we also need to consider how native parasitoids respond to the arrival of a novel exotic host, whether it's the same host species (but from an allopatric population), or an entirely new species of host (no evidence of a historical evolutionary relationship from any region). After the invasion, the invader might interact with native species through local processes, such as competition, predation, or relevant to the focus of this review, host-switching (Simberloff 1999, Williamson 1996). Studying the impacts of invading plant-herbivore-parasitoid species on native communities can reveal the relative importance of both regional/historical and local/contemporary processes.

Do parasitoids locally adapt both behaviorally and physiologically to the arrival of a novel host by shifting hosts? Few studies have focused on whether and how parasitoids adapt to the arrival of a new potential host at the invasion front (Klug et al. 2008). Therefore, the mechanisms in such adaptive processes are poorly understood. For instance, what are the adaptations that allow a parasitoid to switch to a new host and what are the adaptations that take place after the host switch? As discussed previously with *P. saulius*, a parasitoid of the horse chestnut leaf miner, local central and western European *P. saulius* populations were not parasitizing the invasive Balkan horse chestnut leaf miner even though *P. saulius* is the primary parasitoid of horse chestnut leaf miners in the Balkans. In this system, local parasitoid populations did not host-switch to the invading host, even though the invading host was a species that has been attacked by the same parasitoid species in other regions. Different examples however, show that after a certain time, native parasitoids can exploit introduced host species (Roy et al. 2011). Cornell and Hawkins (1993), after investigating many host-parasitoid systems, conclude that the accumulation of native parasitoids on introduced herbivores reaches an asymptote within 100 years. Over the past 25 years, Britain has seen the introduction of eight herbivorous gall wasps (Hymenoptera: Cynipinae), but there is no evidence of pursuit by natural enemies from the continental Europe (Schönrogge et al. 2012). Instead, exotic gall wasps have recruited native British parasitoids in the short time since their arrival. After its arrival in Korea from its native China, *Dryocosmus kuriphilus*

(Hymenoptera, Cynipidae) recruited a parasitoid assemblage of 17 chalcid species over a period of only several decades (Ko, 1971; Yasumatsu & Kamijo, 1979; Kamijo, 1981, 1982; Murakami et al., 1985, 1994, 1995; Otake, 1989; Otake et al. 1982; Kim, 1998).

However, local parasitoids that host switch have also been demonstrated to play only a limited role in the regulation of the population dynamics of the invader (Stojanovic & Markovic, 2005; Vercher et al. 2005). Cornell & Hawkins (1993) compared the structure and diversity of 87 parasitoid complexes on introduced herbivores, and native herbivores. Parasitoid attack rates are generally lower on introduced hosts and contain more generalist parasitoids when compared to their native herbivorous hosts. It seems to achieve the high parasitism rates that a host experienced in its native range, a specialized parasitoid species needs to follow the invader, as evidenced by *Phyllonorycter platani* in England (Godfray et al. 1995).

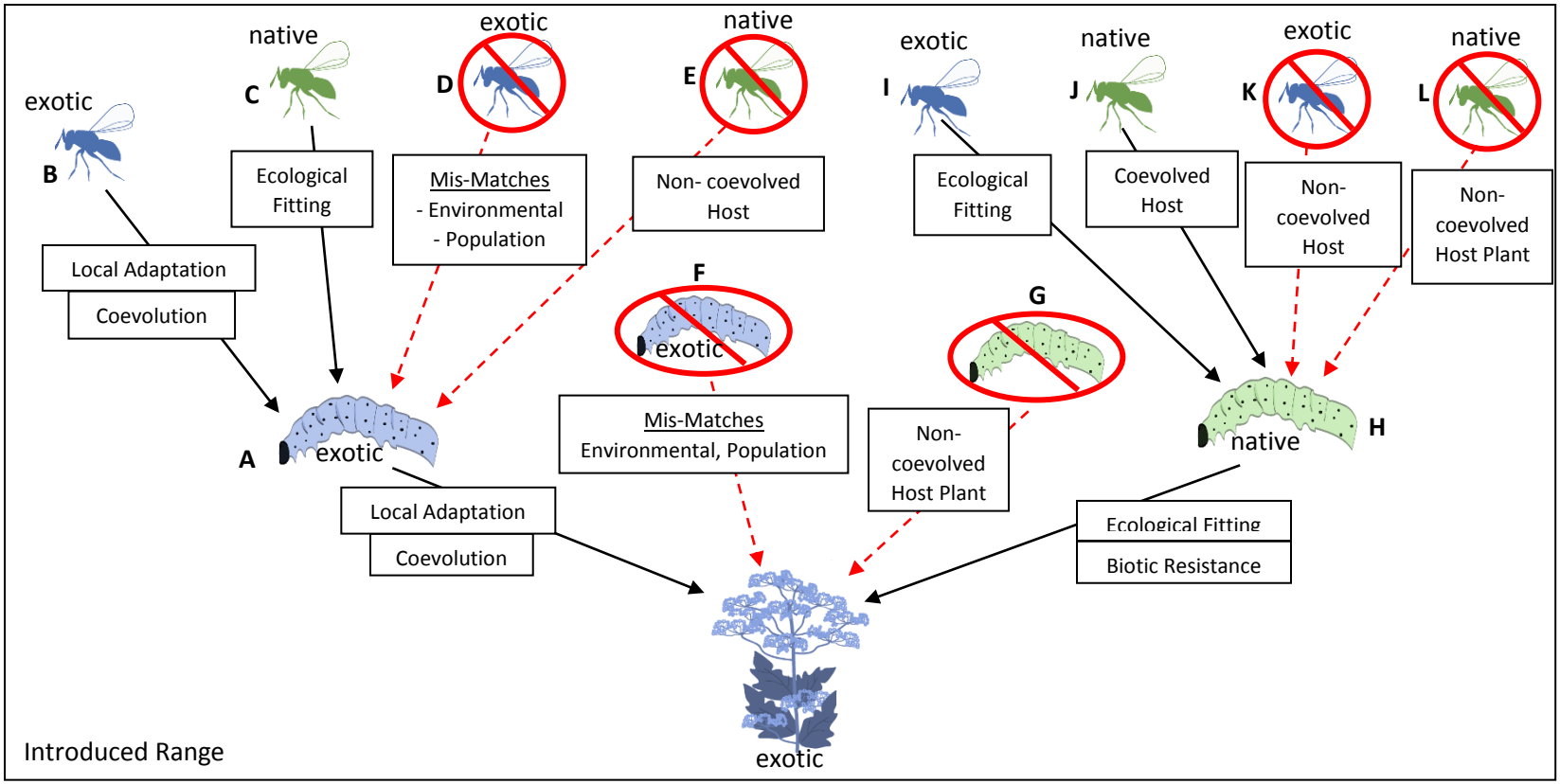
Conclusions and Future Directions

Understanding the genetic structure of a natural enemy in its native range may provide important information for biological control practitioners by aiding in the identification of populations that exhibit local adaptations to environmental conditions or organisms with which they interact. It has generally been assumed that natural enemy populations from a pest's region of origin, once identified, should be favored for biological control (Mackauer 1976; Hoelmer and Kirk 2005; Hufbauer and Roderick 2005). The foundation of this assumption is that such natural enemies are more likely to possess genetic variation that is most compatible with the climate or host genotypes in areas from which invasive pest populations are derived (Messenger and van den Bosch, 1971). However, other strategies, such as introductions from the most genetically diverse sources or from multiple ecologically and genetically distinct populations have also been proposed (Remington 1968; Mackauer 1976). Unfortunately, the utility of these various approaches is still largely untested in biocontrol programs because both the intraspecific lineages of the various geographic areas of the parasitoid species must be determined, and then common garden experiments with the various lineages has to be conducted to reveal whether local adaptations favors the establishment of certain lineages.

The research presented in this review has tackled both issues by distinguishing distinct intraspecific lineages of host-parasitoid systems that have re-ordered themselves in introduced areas. Overall, these studies suggest that multiple factors encompassing both coevolution and ecological fitting are important when an introduced parasitoid associates with a host, and can occur simultaneously in one system. For example, the data on the origins of *M. stigmatizans*, *P. saulius*, and *P. mediterraneus* associated with exotic herbivores suggest that the invading natural enemy populations were derived from numerous sources; allopatric, sympatric and/or a novel host species. Coevolution can be an important factor in an exotic plant-herbivore association (e.g., Balkan Turkey oaks and *Andricus kollari* gall wasps), but ecological fitting readily occurs when local parasitoid species are recruited on exotic herbivores, as documented in the previous section. However, their impact on exotic herbivore population levels may be lower. This highlights the diversity of mechanisms that must be considered when trying to predict the outcome of community-level modifications, including the intentional release of biocontrol agents.

Although there are numerous studies that have documented the co-introduction of plants and herbivores fewer studies have incorporated co-introduced natural enemies, and the resulting tri-trophic associations in the introduced range. However, such co-introduced multitrophic studies are needed to understand the importance of shared population histories on species interactions in the native range and the introduced range. With the advent of cheaper molecular markers for non-model organisms, discovering the population origins and spread of multiple exotic species is more feasible. Once population histories are known for each trophic level, experimental assays need to be conducted to corroborate the species associations of plants, herbivores and parasitoids seen in the field. Also, studies are needed to address whether parasitoid populations that have host-switched onto novel host species can persist.

Figures


exotic
B
native
C
Ecological Fitting
exotic
D
Mis-Matches
- Environmental
- Population
native
E
Non- coevolved Host
exotic
I
Ecological Fitting
native
J
Coevolved Host
exotic
K
Non-coevolved Host
native
L
Non-coevolved Host Plant
Local Adaptation
Coevolution
A
exotic
F
exotic
Mis-Matches
Environmental, Population
G
Non-coevolved Host Plant
native
H
Local Adaptation
Coevolution
Ecological Fitting
Biotic Resistance
exotic
Introduced Range

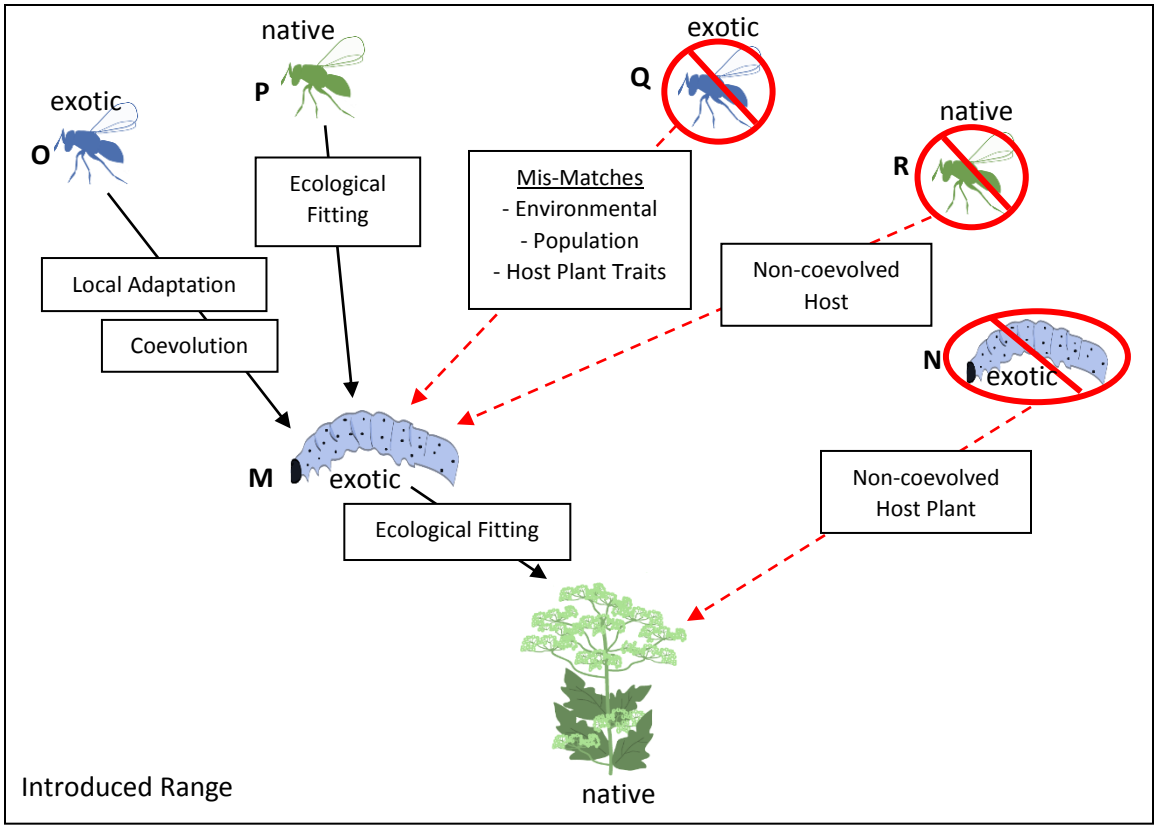


Figure 1.1: Trophic interactions that could occur between a native or exotic plant with exotic or native herbivores and parasitoids. Exotic refers to the introduced species and are colored blue. Native refers to the species already present in the introduced area and are colored green. Trophic associates that share the same color share the same population origin. Species with a red circle and line through them indicate no association with the lower trophic level The text boxes overlaying each arrow are the hypotheses or theories that may have shaped each interaction and are discussed in the text, with the letters next to each species used as reference.

CHAPTER 2: THE RAPID ESTABLISHMENT AND SPREAD OF THE INTRODUCED PARSNIP WEBWORM (DEPRESSARIA PASTINACELLA, LEPIDOPTERA: DEPRESSARIDAE) DESPITE A GENETIC BOTTLENECK

Introduction

How biological communities assemble across space and time has been a major question in ecology (Weiher and Keddy 1999). Given the dramatic increase in the number of invasive and introduced organisms around the world (Sala et al. 2000; Nentwig 2009; Simberloff et al. 2013), this question has never been more important. Although studies on invasion biology have historically focused on single exotic species of concern and how it impacts native communities (Elton 1958; Lodge 1993a, b; Simberloff 1996; Wilcove et al. 1998), numerous studies now document the occurrence of species within multitrophic interactions being introduced to novel ranges, simultaneously or gradually, over time (Gröbler and Lewis 2008; Nicholls et al. 2010; Stone et al. 2012; Wang et al. 2013). Tracing the invasion routes of co-introduced species that share a close evolutionary history in their native range can address many questions regarding community re-assembly. For example, what is the relative importance of long-term historical associations between species in the ancestral range to shaping interactions in the introduced range? Alternatively, are these coevolved, co-introduced species less likely to re-associate, particularly if alternate, ecologically similar local species are available?

Within terrestrial ecosystems, there are numerous examples of exotic plants and their natural enemies moving in tandem, particularly plants and their insect herbivores (O'Dowd *et al.* 2003; Grosholz 2005; Green *et al.* 2011). These co-introductions have been a result of both intentional human introduction of the plant (e.g., cultivation) and insect (e.g., biological control) or accidental, assisted by human trade or travel. Although much theoretical and empirical data have been collected on plant-herbivore community assembly, few studies have incorporated population history into models of multispecies interactions (Hober and Brooks 2008; Smith et al. 2011; Whiteman et al. 2007) because of the lack of detailed knowledge of the coevolutionary relationships between species in multitrophic

systems. One interaction whose reciprocal selective impacts have been well documented is that of the wild parsnip (*Pastinaca sativa* L.) (Apiaceae) and its primary herbivore, parsnip webworm (*Depressaria pastinacella*; Lepidoptera: Depressariidae) (Berenbaum and Zangerl 1992; Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003). Wild parsnip and parsnip webworm have both been introduced to North America and New Zealand, allowing us to investigate the relative importance of historical, shared population histories on plant-herbivore species assembly in two different habitats.

Parsnips are native to Eurasia and have been cultivated for more than five centuries (Cain et al. 2010). The current global distribution and invasive status of wild parsnip in temperate climates may have been linked originally to its cultivation as a food crop (Cain et al. 2010). The relationship between wild and cultivated parsnips is unclear, but recent evidence using molecular markers indicates they are genetically similar (Jogesh et al. 2015). Parsnips were introduced in eastern North America in the early 17th century (Sturtevant 1890), and have since spread throughout the United States, becoming widely naturalized. In addition to being introduced into North America, wild parsnip also occurs as an invasive species in southern South America, South Africa, China, Australia, and New Zealand (Jogesh et al. 2015). In New Zealand, the first written record of wild parsnip in North America dates to 1867 (Webb 1978).

The parsnip webworm has followed its host plant into these new ranges. Webworm larvae construct webs around part of the umbel and feed primarily on buds, flowers, and developing fruits (Berenbaum et al. 1993; Nitao and Berenbaum 1988), causing substantial reductions to host plant fitness. Wild parsnips produce furanocoumarins, allelochemicals that are broadly biocidal because they intercalate DNA and interfere with transcription (Berenbaum 1990). Selection for chemically-based resistance occurs in plant populations by increasing concentrations of three furanocoumarins: xanthotoxin, bergapten, and sphondin (Berenbaum et al. 1986; Zangerl and Berenbaum 1993). Genetic variation also exists in webworm populations in the rate at which these furanocoumarins are metabolized (Berenbaum and Zangerl 1992) indicating that plant chemistry can act as a selective force on insect physiology.

In the United States (U.S.), the arrival of parsnip webworm occurred in the mid-nineteenth century (Bethune 1869; Riley 1889), approximately 200 years after the arrival of parsnips. In New Zealand, parsnip webworms were first reported in 2004, more than 125 years after the arrival of parsnip plants (Zangerl et al. 2008). In both the U.S. and New Zealand, the parsnip webworm has re-associated with wild parsnips, but within the U.S., webworms have also formed novel associations with the native cow parsnip (*Heracleum maximum*, Apiaceae).

Cow parsnip is a monocarpic species common in shady, moist habitats and is found throughout North America (Hendrix 1984; Berenbaum and Zangerl 1991). Although, parsnip webworm attacks another species of *Heracleum* in Europe, the common hogweed *H. sphondylium*, which is chemically and phylogenetically closely related to wild parsnips (Batten et al. 1982, Downie et al. 2000), webworms have not previously interacted with cow parsnip prior to its arrival in the U.S.

The overall aim of this study was to investigate the structure of webworm populations within Europe, the U.S., and New Zealand. We tested two hypotheses. First, we wanted to test the hypothesis that parsnip webworms in the introduced ranges of the U.S. and New Zealand have a level of genetic diversity comparable to that found in parsnip webworm populations in the native range of Europe. Second, we hypothesize that parsnip webworm populations will have a high degree of population structure based on the furanocoumarin profiles of the host plant species on which they attack.

Specific to our first hypothesis, we asked, "How are webworms able to establish in ranges ecologically dissimilar from their native range?" In western Europe, webworms have been found at elevations slightly below sea level up to 100m and within 6° of latitude (Ode et al. 2004; Berenbaum and Zangerl 2006). In contrast, since their introduction to the U.S., webworms have established widely in North America, ranging in the north from Nova Scotia to British Columbia, in the south to Washington DC, and westward to Arizona (Riley 1889; Hodges 1974; Berenbaum and Zangerl 1991; McKenna and Berenbaum 2003). In some of these areas, webworms have been collected at elevations up to 2600m above sea level (Ode et al. 2004). In addition to these varying environmental conditions, webworms in the introduced ranges are also establishing on host plant species with varying phenologies.

In Europe, adult webworms oviposit on host plants, prior to plant bolting (Berenbaum and Zangerl 2006). Upon hatching, webworm larvae feed initially on meristems and eventually move to infest floral structures (Nitao and Berenbaum 1988; Berenbaum et al. 1993). Larvae ready to pupate leave the flowering parts and burrow into the hollow stem (Nitao and Berenbaum 1988; Berenbaum et al. 1993). Parsnip webworms are univoltine, and adults emerge in late summer, enter reproductive diapause in autumn, overwinter, and become active again in early spring (Nitao and Berenbaum 1988; Berenbaum et al. 1993). Although host plant species in the western U.S. follow a similar phenology to European host plant species, this is not the case in the midwestern U.S. nor in New Zealand.

In the midwestern U.S., host plant phenology is shifted to earlier in the season, with wild parsnips bolting in early May, flowering in June, and setting seed in mid-late July 1993). In mid-April, prior to host plant bolting, parsnip webworm adults oviposit on rosette leaves (Gorder and Mertins 1984; Nitao and Berenbaum 1988). Although webworms in the midwest feed on wild parsnip and cow parsnip, phenological differences do exist between them (Zangerl et al. 2002). The perennial cow parsnip tends to flower several weeks earlier even at the same sites where the biennial wild parsnip is found (Berenbaum, pers. obs.).

Aside from being in the Southern Hemisphere, which causes New Zealand parsnip rosettes to bolt in late November, flower throughout December and set seed in mid-late January; webworms oviposit in early December, *after* the plants have already bolted (Jogesh et al. 2014).

The ability of a species to respond to different ecological conditions in the introduced range has been attributed to increased levels of genetic variation, created by either multiple introductions or a single introduction event with a genetically diverse founding individuals (Ellstrand and Schierenbeck 2000; Kolar and Lodge 2001). Although numerous studies have reported reduced levels of genetic variation in introduced populations (Barett and Kohn 1991; Tsutsui et al. 2000; Havill et al. 2006; Puillandre et al. 2007; Schmid-Hempel et al. 2007; Dlugosch and Parker 2008), others have found genetic diversity in the introduced range was the same as or higher than that in the native range (Bossdorf et al. 2005; Genton et al. 2005; reviewed in aquatic invasions by Roman and Darling, 2007).

Previous population genetics studies on wild parsnip introductions also provides support to our hypothesis that introduced parsnip webworm populations have at least similar levels of genetic diversity to European populations. Wild parsnip populations in the introduced ranges of the U.S. and New Zealand had high genetic diversity in both regions, suggesting multiple introductions (Jogesh et al. 2015). These same routes of introduction could have been exploited by the parsnip webworm, potentially creating multiple introduction events. Multiple introduction events increase the levels of genetic diversity by bringing together unusually large amounts of variation and novel genetic combinations, and can be common in invasions (Ellstrand and Schierenbeck 2000; Bossdorf et al. 2005; Novak and Mack 2005).

Specific to our second hypothesis, we asked, "What is the amount of gene flow between webworm populations attacking different host plant species?" Plant furanocoumarin chemistry can act as a selective agent on webworm metabolism (Zangerl and Berenbaum 1993) and webworms can act as a selective agent on plant chemistry (Berenbaum et al. 1986). Phenotype matching between plant defense and webworm detoxification profiles have been documented in European (Berenbaum and Zangerl 2006) and midwestern U.S. populations (Zangerl and Berenbaum 2003), and the level of matching varies geographically within regions (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003). However, it is unclear the amount of geneflow occurring between these geographically widespread regions of webworm populations, as no studies to date have investigated the population genetics of webworms.

As outlined in Figure 2.1, Berenbaum and Zangerl (2006) characterized the level of phenotype matching in three plant-webworm associations in Europe. First, populations of European wild parsnip without webworms had lower levels of xanthotoxin and bergapten (Fig. 2.1A). Second, wild parsnip populations with webworms had overall higher concentrations of furanocoumarins, and these webworms had increased detoxification capability to match wild parsnip (Fig. 2.1B). Lastly, hogweed populations had overall lower concentrations of furanocoumarins and increased rates of infestation by webworms that had decreased detoxification capabilities compared to webworms on wild parsnip (Fig 2.1C). In these instances, webworms are found attacking hogweed in greater numbers than wild parsnip, even though they suffer greater rates of attack by its primary parasitoid, *Copidosoma sosares* (Hymenoptera:

Encyrtidae), most likely due to the overall lower furanocoumarin concentrations of hogweed (Ode et al. 2004). Does the degree of phenotype matching influence the level of gene flow between webworms attacking hogweed and webworms attacking wild parsnip within Europe? If so, we expect to see a high degree of genetic isolation based on the host plant species on which European webworms occur.

Phenotype matching between plant defenses and insect detoxification profiles also exist in the introduced range of the U.S. (Fig 2.1 D-K). In areas of the midwestern U.S. where webworms have only wild parsnip available, webworms with higher detoxification capacities matching the chemistry of wild parsnip had higher levels of survivorship in 60% of the populations examined (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Fig. 2.1E). In the remaining 40% of populations in which webworms were mismatched with wild parsnip populations, wild parsnips had significantly higher xanthotoxin, imperatorin, and sphondin concentrations (Zangerl and Berenbaum 2003), and webworms suffered reduced growth (Zangerl and Berenbaum 1993; Fig. 2.1F). Webworms appear to use cow parsnip preferentially as a host plant where it is available in central Illinois (Zangerl et al. 2002) even though it suffers higher rates of predation by birds while pupating in cow parsnip stems and the higher concentrations of imperatorin and sphondin in cow parsnip (Zangerl and Berenbaum 2003) results in reduced webworm growth (Berenbaum and Zangerl 1991; Fig. 2.1G).

In the western U.S., where cow parsnip is the more abundant host plant species, the majority of webworm populations examined were found to have high levels of metabolic detoxification capabilities (Carroll and Berenbaum 2006; Fig. 2.1H-K). This was the case irrespective of the level of furanocoumarin concentrations, which differed based on the presence of the European introduced primary parasitoid of webworms, *C. sosares* (Fig. 2.1I, K). Does the degree of phenotype matching influence the level of gene flow between webworms attacking wild parsnip and webworms attacking cow parsnip within the U.S.? If so, we expect to see a high degree of genetic isolation based on the host plant species on which U.S. webworms occur.

Specific to the U.S, we can also ask, "Are U.S. webworm populations derived from European webworm populations attacking wild parsnip (high levels of furanocoumarins) or hogweed (low levels of

furanocoumarins)?" The various detoxification capacities documented in U.S. webworm populations that feed on wild parsnip and cowparsnip populations (Fig. 2.1E-K) suggests multiple European webworm populations from both European host plant species were introduced to the U.S. However, within the U.S., not all webworm populations are phenotypically matched to the furanocoumarin concentrations of their host plant species (Fig. 2.1F, G, I, K), suggesting two possibilities. First, webworm gene flow could be restricted within the U.S. leading to isolated populations of webworms feeding on the only available host plant. Second, contrary to our first hypothesis, there could be little genetic variation within introduced webworm populations. Zangerl et al. (2002) suggested the persistent use of cow parsnip by webworms even though they suffer higher predation and higher levels of furanocoumarins could be due to a loss of host plant discrimination because of reduced genetic variation during introduction.

Within New Zealand, webworm populations are only found on wild parsnip populations. The lack of an alternate host plant species in New Zealand does not allow us to test whether webworm populations are structured by the furanocoumarin concentrations of their host plant species. However, by identifying the source populations of New Zealand webworms, we can address the potential detoxification capabilities of the introduced webworms. New Zealand wild parsnips differ substantially in chemical composition from those of European and U.S. parsnips (Zangerl et al. 2007). Almost all of New Zealand populations examined fell into a furanocoumarin phenotype cluster distinct from the phenotypes of Europe and the U.S., characterized by lower levels of imperatorin, bergapten, and isopimpinellin and slightly above-average levels of xanthotoxin (Zangerl et al. 2007). If multiple populations of European (or U.S.) webworms were introduced to New Zealand, there would exist the genetic variation in webworm detoxification capabilities to establish on New Zealand wild parsnips with these strikingly different furanocoumarin profiles.

A mitochondrial marker with population level variability was used to test these hypotheses. Mitochondrial DNA (mtDNA) is widely used in phylogeographic studies, due to its low or absent recombination, uniparental inheritance, conserved structure and relatively high evolutionary rate (Moritz et al. 1987; Harrison 1989; Avise 2000). The analysis of intraspecific mtDNA variation can reveal

information about the interconnectivity of populations and past demographic events such as genetic bottlenecks after introduction.

Materials and Methods

Samples

Larvae and pupae of parsnip webworms were collected from wild parsnip, cow parsnip, or common hogweed plants from 23 sites in the United States between 2004-2013, 19 sites in Europe between 2004-2012, and 9 sites in New Zealand in 2012. GPS coordinates, elevation, and host plant species were recorded for each sample (Table 2.1). Webworms were collected from a variety of public lands, roadways, and rights of way. For those sites within National Forest Service lands, collection permits from the U.S Department of Agriculture-Forest Service were obtained, none of the collected specimens are protected organisms. New Zealand parsnip webworm larvae were collected and immediately placed in 100% ethanol until further processing. Eastern U.S. webworms were collected as pupae, immediately frozen, and stored in a -80°C ultralow freezer until further processing. European and western U.S. parsnip webworm were collected as either larvae or pupae. To check for the presence of the egg-larval parasitoid, *C. sosares*, within field collected larvae, individuals were maintained in 30mL solo cups and fed seeds from their respective host plants until pupation (not parasitized). Upon pupation, webworms were moved to glass vials until adult emergence. Both field-collected pupae and those larval samples that pupated after collection were maintained in a 27ºC with a LD 16:8 h incubator until adult emergence. Adults were immediately frozen and stored in a -80°C ultralow freezer until further processing.

DNA extraction, amplification and sequencing

Genomic DNA was extracted using a Genomic DNA-Tissue MiniPrep Kit (Zymo Research) in accordance with the manufacturer's instructions for solid tissue. Webworm tissue was ground in a 1.5 mL centrifuge tube using a Kontes pestle (Kimble) and incubated for 2 hours at 56°C. Since pupae, larvae, and adults were collected, the type of tissue varied with life stage. Legs of adult webworms were

used, while the posterior three segments of larval and pupal samples were used. Isolated DNA was checked for quantity and purity using the BioTek Epoch Take3 plate reader.

Several mitochondrial genes were amplified to screen for intraspecific variable markers, including the cytochrome oxidase subunit 1 (COI), cytochrome *b*, and NADH dehydrogenase subunits *4* and *5* using primers described in Table 2.2. Ultimately, one mtDNA gene, *ND* 5, was chosen for phylogenetic analyses based on its higher variability within and between collections. Polymerase chain reaction (PCR) was performed on either an Eppendorf Mastercycler gradient or Thermo Scientific PCR machine. PCR reactions were performed in a 20 µL reaction volume containing 2.5 µL of 10X PCR Buffer (100mM Tris-HCl, 500mM KCl, 15 mM $MgCl_2$), 2.5 mM of each dNTP, 5 pmol of each primer, 0.625 U of TaKaRa Taq HotStart Version polymerase (Clontech), 2.5 µL of genomic DNA template, and up to 20 µL of sterile distilled water. The thermal profile consisted of an initial denaturation for 2 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 59°C, and 30 seconds at 65°C, with a subsequent final 5 minute extension at 70°C. Successfully amplified samples were sent to the University of Arizona Genetics Core for clean-up, quantification, and DNA sequencing on an ABI 3730 DNA Analyzer (Applied Biosystems). Each sample was sequenced in both directions using PCR primers. Sequence data were manually checked using CHROMASLITE and reverse sequences were used to resolve ambiguous base calls in the forward sequence. The sequencing region was checked for an open reading frame using EXPASY online translation tool using the invertebrate mitochondrial codon table. Sequences were aligned using CLUSTAL W (Thompson et al. 1994). No insertions, deletions, or stop codons were observed. The sequence dataset of the mitochondrial *ND* 5 was truncated to the same length (703bp) and translated to amino acid sequences to check for nuclear mitochondrial pseudogenes (numts). The haplotype sequence matrix for *ND* 5 was used for all subsequent phylogenetic analyses.

Phylogenetic Analysis

Standard molecular diversity indices were calculated using DNASP v5 (Librado and Rozas 2009; http://www.ub.edu/dnasp/). Diversity calculations included the number of haplotypes, the number of segregating (polymorphic) sites, the nucleotide diversity, the mutation rate within codon position, and

haplotype diversity of all variable nucleotide sites. The DNA polymorphism using the sliding window method was estimated for the determination of the number of segregating sites (S) across each portion of the mtDNA sequences, set with a window length=100 and a step size = 25 (Librado and Rozas, 2009).

A phylogenetic tree was constructed using the maximum-likelihood method in the program package *MEGA* version 7 (Kumar et al 2016). The tree was rooted using *Endrosis sarcitrella* (Lepidoptera: Oecophoridae) as an outgroup (GenBank Accession #KJ508037.1).

Population structure

Genetic diversity within populations was estimated by computing haplotype diversity (*H*) and nucleotide diversity (π). Haplotype diversity (also known as gene diversity) represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences. Partitioning of genetic variation within and among populations was calculated using analysis of molecular variance (AMOVA; Excoffier et al 1992), by computation of conventional *F*-statistics from haplotypes with 10000 permutations as implemented in the program ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). More specifically, genetic variation was calculated within Europe (native range), within the U.S (introduced range) and within New Zealand (introduced range) to test whether introduced U.S. and New Zealand webworm populations had similar levels of genetic variation to European populations (hypothesis 1). Additionally, because of the large geographical distance between parsnip webworms in the midwestern U.S. (Illinois and Wisconsin) and the western U.S. sites, we performed an AMOVA between eastern and western U.S. parsnip webworms to test whether these two regions are genetically distinct.

We constructed 3 models that reflect different genetic structures across the landscape: 1) linear geographical distance, 2) elevational differences, and 3) clustering of sites into groups based on host plant species (hypothesis 2). To test these models, we employed several grouping schemes. For the first model, we calculated linear distances between sites from GPS coordinates for the collected specimens. We clustered sites within 50 linear km of each other to assess isolation by distance. We constructed the second model based on the extreme elevational differences between European and western U.S. sites and

between western and midwestern U.S. sites. Elevation (m) data was recorded from each site during collection and we clustered sites within 50 linear km of each other *and* with less than 50 m elevation difference. The difference in elevation between clusters was used to assess isolation by elevation. Both models were assessed with the Mantel test (Mantel 1967) with 10,000 permutations, using Slatkin's linearized F_{ST} (Slatkin 1995). New Zealand samples were excluded from analyses testing genetic isolation due to the lack of genetic diversity occurring among the sites.

We constructed the third model to test our second hypothesis that webworms will assemble based on host plant species, because of the documented reciprocal co-evolution occurring between webworms and parsnip plants. Since some sites and regions contained webworms collected from more than one host plant species, we clustered webworms within each site and region based on the host plant species on which they were collected. We used this clustering method in model 3 to perform two AMOVA analyses within the U.S. and within Europe; 1) among European individuals collected from wild parsnip or hogweed, and 2) among U.S. individuals collected from cow parsnip or wild parsnip. New Zealand webworms were excluded from this analysis since they were only collected from one host plant species, wild parsnip. Additionally, we performed a series of eight AMOVA analyses between webworms from the U.S., New Zealand, and Europe collected from different host plant species to assess whether webworms assembled differently between the introduced ranges and the native range: 1) among European individuals collected from wild parsnip and U.S. individuals collected from wild parsnip, 2) among European individuals collected from wild parsnip and U.S. individuals collected from cow parsnip, 3) among European individuals collected from hogweed and U.S. individuals collected from cow parsnip, 4) among European individuals collected from hogweed and U.S individuals collected from wild parsnip, 5) among European individuals collected from hogweed and New Zealand individuals (all are from wild parsnip), 6) among European individuals collected from wild parsnip and New Zealand individuals, 7) among U.S individuals collected from wild parsnip and New Zealand individuals, and 8) among U.S. individuals collected from cow parsnip and New Zealand individuals.

To determine the potential source populations of U.S. and New Zealand webworm populations pairwise linearized F_{ST} values (Slatkin 1995) were estimated as implemented in ARLEQUIN v. 3.5. by haplotype frequencies. Very low and non-significant F_{ST} values between populations indicate a close relationship, whereas high and significant F_{ST} values between populations indicate no or very distant relationship.

Results

Phylogenetic analyses

A total of 722 webworm individuals, all sequenced at a 703bp length of the *ND* 5 gene were used in analyses. Overall there were 11 polymorphic (segregating) sites leading to the definition of 13 haplotypes (Fig 2.2, 2.4). There were 149.33 synonymous sites and 549.67 nonsynonymous sites, due to the high frequency of A-T nucleotide bases over the entire sequence (0.818). The majority of individuals fell within two haplotypes; Hap1 (0.165) and Hap2 (0.791). Haplotype 2 has a global distribution, representing webworms collected in Europe, the U.S., and New Zealand (Fig. 2.4, Table 2.3). Additionally, Hap2 represents webworms collected from all host plant species; wild parsnip, cow parsnip, and common hogweed. Five haplotypes were unique to Europe (Hap 3, 4, 6, 8, 9), and six haplotypes were unique to the U.S. (Hap 5, 7, 10, 11, 12, 13; Fig. 2.5). Haplotype 1 is mostly confined to European populations, except for 2 individual webworms collected from a site in Illinois (midwestern U.S.). All New Zealand samples were Hap2. Eight of the 13 haplotypes were singletons, in which a single individual contained that particular sequence. Excluding the singletons, Hap4 is the only haplotype confined to a single collection site (Netherlands M). Overall, across all collection sites, the haplotype diversity was 0.347, and the mutation rates of the different codon positions were 0.5162, 0.9588, and 1.5268 for 1st, 2nd, and 3rd positions, respectively. The high mutation rate at the second codon position is unusual, and may be attributed to the high frequency of A-T nucleotide bases at the second codon position (proportion AT = 0.718), which increases the number of transversions at this position.

Introduced webworm populations underwent a severe bottleneck

The molecular diversity indices within each region (Europe, the U.S., and New Zealand) revealed a high level of haplotype diversity in Europe ($\hat{\theta}_\pi$ = 0.67 ± 0.57), very little haplotype diversity in the U.S. ($\hat{\theta}_\pi$ = 0.04 ± 0.12), and no haplotype diversity in New Zealand ($\hat{\theta}_\pi$ = 0.00 ± 0.00; Table 2.3). This is further supported by the AMOVA analyses within and between European, U.S., and New Zealand populations. Variation among the four European collection sites differed significantly ($P < 0.001$), with the highest percentage of variation occurring within the European sites (65.83%, Table 2.4). On the other hand, there were no significant differences among the 10 sites in the U.S. ($P = 0.18$; Table 2.4), including no significant differences between western and midwestern U.S. sites ($P = 0.08$).

The low genetic diversity of U.S. webworm populations is additionally supported by the pairwise linearized F_{ST} values (Table 2.7). Sites within the U.S. did not have significantly high pairwise F_{ST} values, indicating that U.S. webworm populations are all genetically similar. Within Europe, Denmark and the Netherlands significantly differed between the rest of the European collections (Germany, United Kingdom, Ireland); whereas Germany, the United Kingdom, and Ireland were similar (Fig 2.4). Overall this indicates European webworm populations are highly structured, with the most distinct populations occurring between Denmark and the Netherlands (Fig 2.4B, Table 2.7).

Source Populations of U.S. and New Zealand Webworms

Between the U.S. and European sites, the highest pairwise linearized F_{ST} Slatkin's values were found between all U.S. sites and Denmark (F_{ST} values ranged from 4.77 to 13.68; $P < 0.001$ in all comparisons; Table 2.7) and the Netherlands (F_{ST} values ranged from 0.62 to 1.16; $P < 0.001$ in all comparisons). The lowest values were found between Ireland and all of the U.S. sites (F_{ST} values ranged from 0 to 0.06; $P > 0.05$ in all comparisons), between the United Kingdom and six U.S. sites (Montana, $F_{ST} = 0$; Black Hills, $F_{ST}= 0.03$; the Wasatch Mountains, $F_{ST} = 0.02$; Medicine Bow Mountains, $F_{ST} = 0.02$; Wisconsin, $F_{ST} = 0.02$; Illinois, $F_{ST} = 0.03$; $P > 0.05$ for all comparisons), and between Germany and Montana ($F_{ST} = 0.01$; $P > 0.05$), the Wasatch Mountains ($F_{ST} = 0.09$, $P > 0.05$), and the Medicine Bow Mountains ($F_{ST} = 0.09$; $P > 0.05$). The Slatkin's F_{ST} values for the New Zealand sites and European

sites were all significantly high (F_{ST} = 0.07 - 13.45; $P < 0.001$), except for Ireland (F_{ST} = 0; $P > 0.05$), while the values were all significantly low for New Zealand and the U.S. (F_{ST} = 0 – 0.23; $P > 0.05$). The similarity between Ireland, United Kingdom, and Germany webworm populations to U.S. webworm populations indicate the British Isles and Germany are likely source populations (Fig. 2.3). Additionally, the U.S., and not Europe, is a likely source for New Zealand populations based on the significantly low Slatkin's F_{ST} values between the U.S and New Zealand.

The low levels of genetic diversity in the introduced range of the U.S and New Zealand leads us to reject our hypothesis that U.S. webworms were multiply introduced from genetically diverse source populations or underwent a single introduction event with many founders from genetically diverse sources (hypothesis 1).

U.S. webworm populations are structured by host plant species

The AMOVA analyses based on host plant species within Europe revealed parsnip webworms collected from hogweed sites did not significantly differ from those webworms collected from wild parsnip sites (P = 0.292; Table 2.5), even though furanocoumarin levels have been shown to be significantly different (Berenbaum and Zangerl 2006). Within the U.S., webworms from wild parsnip sites slightly differed from those webworms collected from cow parsnip sites (P = 0.078; Table 2.5). Additionally, 3 unique haplotypes occurred only in webworms feeding on cow parsnip (Hap 5, 7, 11; Table 2.3), and 3 unique haplotypes occurred only in wild parsnip-feeding webworms (Hap 10, 12, 13; Table 2.3). Collectively, these results follow previous findings that wild parsnip and cow parsnip have different furanocoumarin profiles in the U.S. (Zangerl et al. 2002; Zangerl and Berenbaum 2003; Carroll and Berenbaum 2006).

To determine whether a webworm population feeding on a specific host plant species in Europe served as the founding webworm population in the introduced ranges, we did a series of AMOVAs between each possible host plant species interactions across the three ranges (Europe, New Zealand, and the U.S.). European webworms from wild parsnip sites and U.S. webworms from wild parsnips were different, albeit slightly (P = 0.058; Table 2.6). European webworms from hogweed and U.S webworms

from wild parsnip ($P = 0.168$) were not significantly different. This matches our earlier finding that British Isles webworms served as the founding population of U.S. webworms, because all British Isles webworms were collected from hogweed. Not surprisingly, U.S. webworms from cow parsnips are significantly different from all European webworms, regardless of host plant species (between European wild parsnip, $P < 0.05$; between European hogweed, $P < 0.05$). Combined with our earlier finding that webworms feeding on cow parsnip are genetically diverged from U.S. wild parsnip-feeding webworms; our results indicate European webworm populations feeding on hogweed were introduced to the U.S., established on U.S. wild parsnip, then host-switched to cow parsnip.

New Zealand webworm populations were only collected from wild parsnip, and these populations were not significantly different from any of the webworm populations in the U.S. nor Europe, regardless of host plant species ($P > 0.05$; Table 2.6). Due to the universal presence of Hap2 in New Zealand webworms and the lack of an alternate host plant species in New Zealand, our mtDNA marker, which reveals historical levels of geneflow, was not able to recover any significant signal in these recently introduced populations.

Isolation by distance (IBD)

Within the United States, the correlation analysis revealed the lack of a significant association between genetic distance and 1) geographic distance (Mantel test, $P = 0.28$; Fig. 2.5B), or 2) elevational differences (Mantel test, $P= 0.53$; Fig. 2.6B). Within Europe, geographic distance revealed a significant positive association (Mantel test, $P < 0.05$, Fig. 2.5A) while elevational difference did not (Mantel test, $P = 0.21$; Fig. 2.6A). Overall, within Europe, differing furanocoumarin profiles of the two different host plant species are not hindering geneflow between webworm populations, rather sites are isolated by distance alone.

Discussion

There is ample evidence documenting the co-introduction of plants and their herbivorous insects into novel habitats (Gröbler and Lewis 2008; Nicholls et al. 2010; Stone et al. 2012; Wang et al. 2013). Once introduced, herbivores may re-associate with their host plant species of shared origin, switch host

plants, or instead attack novel species that lack a shared history. Within the parsnip-webworm system, webworms have re-associated with their coevolved host plants (U.S., New Zealand), and have host plant switched to a novel species (onto cow parsnip in the U.S.). Using a molecular marker, we determined the British Isles served as the main source of U.S. webworm populations, but these introductions were either few in number, or consisted of large numbers of founders with very little genetic variation. This is further supported by the genetic similarity between U.S. webworms feeding on wild parsnip and European webworms feeding on hogweed ($P = 0.168$; Table 2.6), because all British Isles webworm samples were collected from hogweed populations (Table 2.1).

The loss of genetic variability of webworms in the introduced range of New Zealand is even more drastic, as indicated by the presence of only a single haplotype (Hap2). This could be due to a single introduction compounded by its very recent introduction to New Zealand less than 20 years ago. Although our results indicate U.S. webworm populations are more likely to serve as the source population for New Zealand than Europe, the global distribution of Hap2 does not allow us to say this with certainty. The high proportion of webworms in the U.S. that have Hap2 (0.982) compared to Europe (0.505) does increase the probability of a webworm with Hap2 coming from the U.S., but we lack any further molecular indices to test this conclusion. The low genetic variation of parsnip webworms in the introduced range could have resulted either from a single introduction, selection after introduction to the new environment, or fixation because of genetic drift. The loss of genetic variation in introduced populations relative to source populations has occurred in several different taxa ((Barett and Kohn 1991; Tsutsui et al. 2000; Havill et al. 2006; Puillandre et al. 2007; Schmid-Hempel et al. 2007; Dlugosch and Parker 2008).

Our molecular data also allowed us to test the amount of geneflow within European and within U.S. webworm populations. Phenotype matching between host plant furanocoumarin concentrations and webworm detoxification profiles has been documented in European (Berenbaum and Zangerl 2006) and midwestern U.S. populations (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003). Based on these studies, we hypothesized that webworm populations would be locally adapted to their respective

host plant species, with little to no gene flow occurring between wild parsnip and hogweed in Europe, and between wild parsnip and cow parsnip in the U.S.

Within Europe, our molecular data revealed that webworms from the Netherlands and Denmark are genetically isolated from each other, and from webworms in the British Isles and Germany, yet this genetic isolation is not correlated with host plant species, rather with geographic distance alone. The majority of genetic variation does not occur among European webworms feeding on wild parsnip and hogweed (4.068%, Table 2.5), rather among European webworm populations feeding on wild parsnip and among European webworm populations feeding on hogweed (36.887%, Table 2.5), and further still, within these individual populations (59.044%, Table 2.5). We formed our hypothesis based on studies examining furanocoumarin levels and webworm detoxification profiles in Europe, where wild parsnip populations, on average, have higher furanocoumarin concentrations and hogweed populations have overall lower furanocoumarin levels (Ode et al. 2004; Berenbaum and Zangerl 2006). As a result, webworms on wild parsnip have higher furanocoumarin-detoxification capacities, whereas webworms feeding on cow parsnip have reduced detoxification capacities (Ode et al. 2004; Berenbaum and Zangerl 2006). However, these studies were restricted to Netherlands populations, where webworms are most abundant (Ode et al. 2004). To date, no studies have examined the furanocoumarin content of host plants in other European host plant populations, nor the metabolic capacity of other European webworms. Yet, our molecular data indicates that, at least in the Netherlands, geneflow is not hindered between webworms with high metabolic capacities feeding on wild parsnip and webworms with low metabolic capacities feeding on cow parsnip.

As mentioned earlier, geographic distance alone is significantly correlated with the amount of gene flow between European webworm collection sites. There was a significant genetic dissimilarity between webworm populations on the British Isles and webworms in the Netherlands, Belgium, and Denmark, but a genetic similarity with German webworms (Fig. 2.4B; Table 2.7). This indicates a lack of mixing between webworms in the British Isles and continental Europe, with the English Channel acting as a likely barrier to gene flow. The initial introduction of webworms to the British Isles likely occurred

from Germany, based on the nonsignificant F_{ST} values between Germany and the British Isles (United Kingdom, $P = 0.182$; Ireland, $P = 0.119$; Table 2.7), and significant values between the British Isles populations and all other European sites ($P < 0.001$; Table 2.7). Although Germany is geographically farther from the British Isles than either Belgium or the Netherlands, the German webworms used in this study were collected from the North Sea coast of Germany, close to Jade Bay (site C) and Dollart Bay (site A) and the northern peninsula of Germany (site D), indicating a close geographic proximity increased the likelihood of German webworm populations reaching the British Isles.

Within the U.S., despite the low level of genetic diversity in webworms, we were still able to recover a slight signal that webworms feeding on cow parsnip are different than webworms feeding on wild parsnip ($P = 0.078$). This signal is most likely caused by the fact that three webworm haplotypes are unique to cow parsnip populations (Hap 5, 7, 11; Table 2.3), and three haplotypes unique only to wild parsnip populations (Hap 10, 12, 13; Table 2.3). This result follows previous findings on webworm metabolic capacities and the level of matching to wild parsnip and cow parsnip furanocoumarin profiles (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Carroll and Berenbaum 2006). The majority of our U.S. webworm samples collected on wild parsnip came from midwestern sites, whose furanocoumarin metabolism rates have been shown to vary across wild parsnip populations with varying levels of furanocoumarins (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Fig. 2.1E-F). For the majority (60%) of these populations examined webworms were phenotypically matched with wild parsnip chemotypes. The remaining 40% of webworm populations were phenotypically mis-matched due to the presence of the alternate host plant species, cow parsnip (Zangerl and Berenbaum 2003; Fig. 2.1G). In the western U.S., furanocoumarin metabolism rates did not vary among webworm populations (Carroll and Berenbaum 2006), instead western webworms displayed metabolism rates near the maximum rates reported for webworms in the midwest (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003). Additionally, there were significant mis-matches between metabolic capacities and host plant furanocoumarins, even though only one host plant species was present (Carroll and Berenbaum 2006). Our results indicate host plant-switching by webworms has led to genetic divergence from webworms that

feed on U.S. wild parsnip, and coupled with findings from previous studies (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Carroll and Berenbaum 2006), this genetic difference coincides with the fact that only webworms with high metabolic capacities are establishing on cow parsnip populations (Carroll and Berenbaum 2006). Not surprisingly, we also found European webworms feeding on both host plant species are genetically divergent from U.S webworms feeding on cow parsnip (European wild parsnip $P = 0.0147$; European hogweed, $P = 0.035$; Table 2.6)

Although theory suggests genetic bottlenecks lead to lost opportunities for adaptive evolutionary change and can decreases a species' success, there is evidence to the contrary (Brooks and Endler 2001a, b; Gilchrist et al. 2001; Koskenen et al. 2002a, b; Frankham 2005; Lindholm et al. 2005). For example, the European bumblebee, *Bombus terrestris*, is a highly invasive species in Tasmania, but genetic data revealed the initial founding population contained a very small number of individuals, potentially as few as two (Schmid-Hempel et al. 2007). The potato tuber moth, *Tecia solanivora*, has also successfully invaded South America and the Canary Islands in less than 20 years despite a genetic bottleneck (Puillandre et al. 2007). North American herbarium collections of wild parsnips spanning the 152 years pre- and post-introduction of webworms suggest introduced webworms had high adaptive potential despite a genetic bottleneck. Before and shortly after webworms were introduced to the U.S. (1850-1889) wild parsnips were lower in furanocoumarins than all plants collected post-introduction and lower than European plant samples collected before 1889 (Zangerl and Berenbaum 2005). Within 60 years after the introduction of webworms, wild parsnip furanocoumarin levels started to increase (Zangerl and Berenbaum 2005). Consistent with increased plant defenses, between 1930 and 1969 webworm infestation rates began to decline (Zangerl and Berenbaum 2005). After 1969, webworm infestation levels started to increase, indicating evolution of webworm resistance to increased levels of plant furanocoumarins (Zangerl and Berenbaum 2005).

Overlaying our results with data from herbarium specimens, the initial founding population of webworms into the U.S in the late 19th century came from the British Isles. These founding populations did not contain high levels of genetic variation. However, the wild parsnip plants available at the time of

their arrival had low levels of furanocoumarins, providing no resistance against webworm attack. Over time, the lack of genetic variation and potential lack of variation in metabolic capacities would have reasonably slowed the establishment of webworms on wild parsnip with increased furanocoumarin levels. However, in a matter of 40 years, webworms were able to adapt to wild parsnip increased defenses.

In addition to U.S. wild parsnip populations having low plant defenses during the initial introduction of webworms, the lack of webworm natural enemies could have aided in the successful establishment of webworms despite a genetic bottleneck. The parsnip webworm suffers high rates of parasitism by its primary parasitoid, *C. sosares*, in its native range, with attack rates often exceeding 80% (Ode et al. 2004). Although *C. sosares* has also followed parsnip webworm in the U.S, its distribution is patchier, resulting in areas of enemy-free space (Ode et al. 2004). In fact, populations of parsnip webworm in eastern and midwestern North America are largely free from any significant natural enemy pressure (Ode et al. 2004). The loss of natural enemies at the start of an introduction could have allowed parsnip webworms to quickly spread and establish in the U.S. and New Zealand, but additional studies are needed to assess the introduction history of *C. sosares* (see chapter 4).

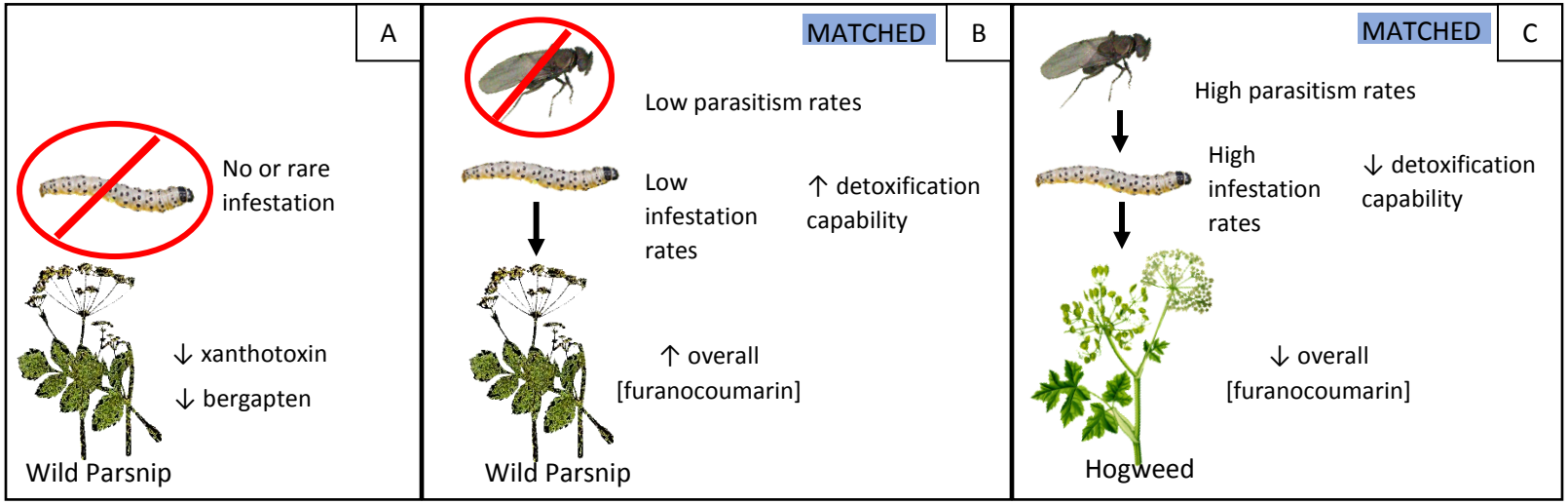
As mentioned earlier, Hap2 has a global distribution, occurring in European, U.S. and New Zealand webworms and indeed this haplotype is found on all three host plant species. Although Jogesh et al. (2015) found high levels of genetic diversity within wild parsnip populations across ranges, the majority of plants (79%) also shared a single haplotype (in the internal transcribed spacer (ITS) region). The prevalence of a single, global haplotype shared by the majority of populations across ranges is not unique to this plant-webworm system, but has also been documented in other species (Tsutsui et al. 2001; Downie 2002; Havill et al. 2006). The position of Hap2 in the phylogenetic tree indicates this haplotype is more recent than the other haplotypes. Both Europe and the U.S. contain webworms exhibiting 7 unique haplotypes, but the occurrence of singletons was higher in the U.S, with 6 of the 7 haplotypes only collected from one webworm individual. Although western and midwestern U.S. sites did not significantly differ between haplotype frequency, it is interesting to note that midwestern sites had a

higher number of unique haplotypes, only found in the midwest (Hap 10, 11, 12, 13), than all sites combined in the western U.S. (Hap 5, 7).

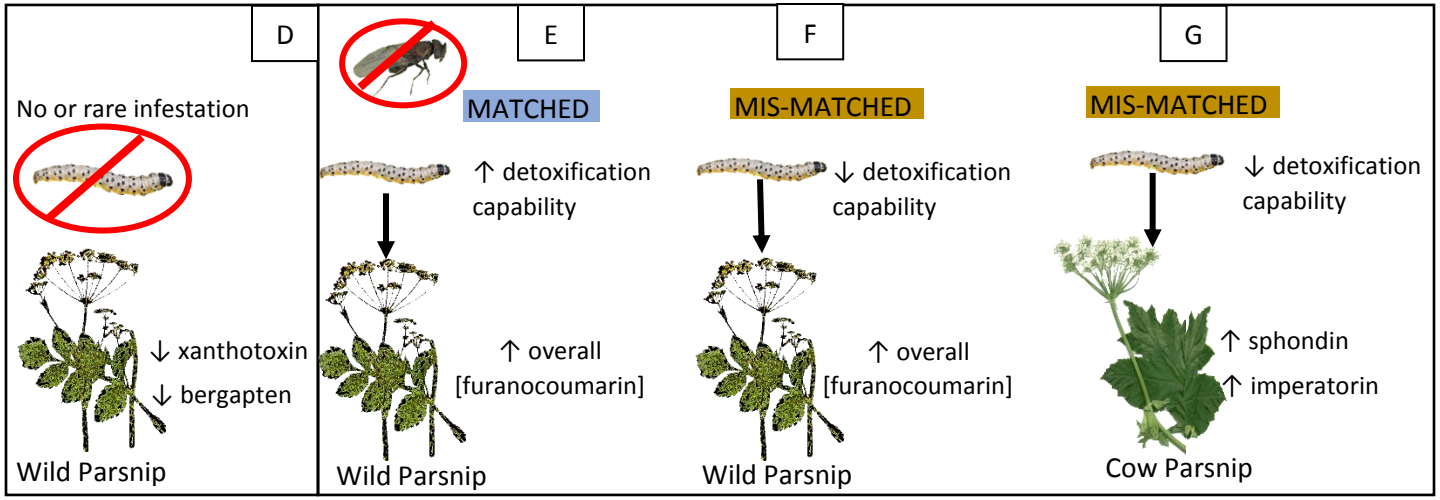
This study provides evidence that parsnip webworms harbor high genetic diversity in their native range of Europe, but a significantly lower level in the introduced ranges of the U.S. and New Zealand, indicating a genetic bottleneck. Yet, webworms were able to establish and rapidly spread throughout their introduced ranges despite low genetic diversity. This could be due to two possible reasons; 1) wild parsnip populations in the U.S. had low levels of plant defenses during the initial introduction of webworms, and 2) no natural enemies followed webworms into the U.S, initially, and none have been documented in New Zealand. One possible consequence of low genetic diversity within the U.S., is phenotype mis-matching between the novel cow parsnip and webworm detoxification profiles. The acquisition of the non-coevolved cow parsnip by webworms in the U.S has also led to genetic divergence from webworms feeding on their coevolved plant species. In contrast, European webworm populations are not restricted to the host plant species on which they feed, and webworm populations from both host plant species are genetically similar. Parsnip webworms were sampled extensively in the native and introduced ranges, suggesting that the reduction of genetic diversity was not a result of sampling bias and our molecular data are an accurate representation of genetic diversity. Future studies documenting the furanocoumarin content and webworm detoxification capabilities from additional sites in Europe, the U.S., and New Zealand will provide further support to our findings.

Figures

Europe (native range)



Midwestern United States (introduced range)



Western United States (introduced range)

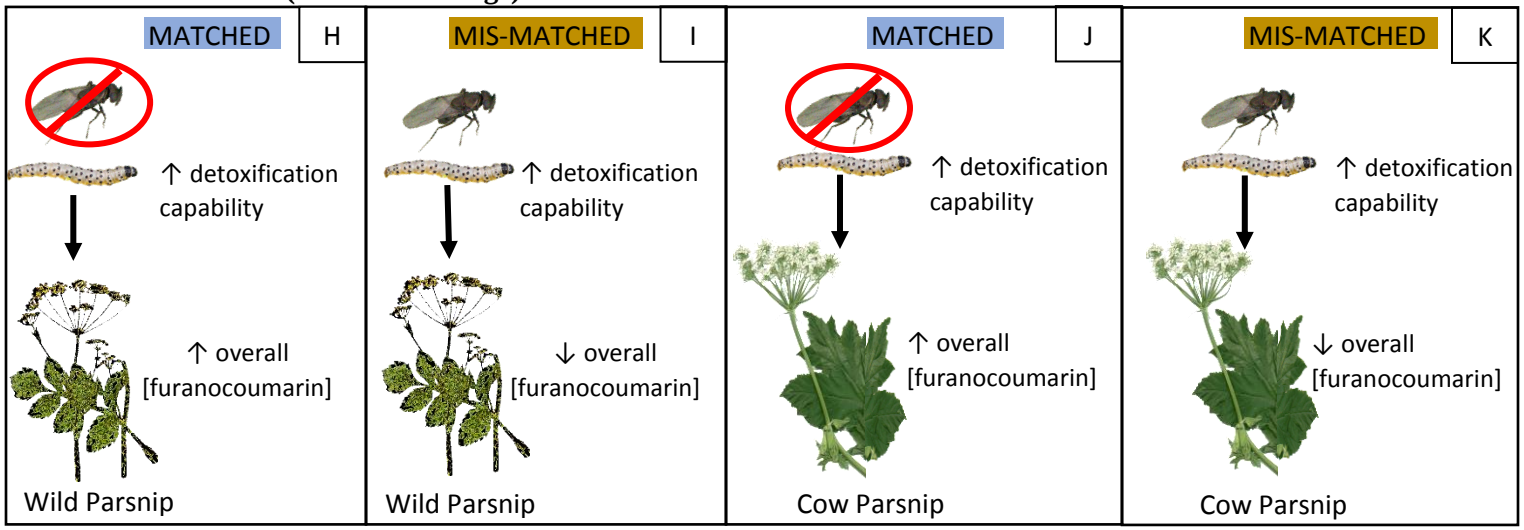


Figure 2.1: Illustration of host plant species and webworm interactions documented within Europe, the midwestern U.S., and the western U.S. Next to each host plant is the furanocoumarin content that has been reported for each interaction. Next to each webworm is the level of infestation, if reported, and their overall level of detoxification capability when compared to webworms feeding on the same host plant species. Above the larval webworm is the primary parasitoid of webworms, *Copidosoma sosares*. Next to *C. sosares* is the rates of parasitism, if known. Illustrations with a red cross through them indicate the species is not present within the interaction. Colored boxes labelled as "Matched" and "Mis-Matched" refer to whether plant furanocoumarin profiles and webworm detoxification capabilities are matched or mis-matched. Letters across the top are used as a reference within the text.

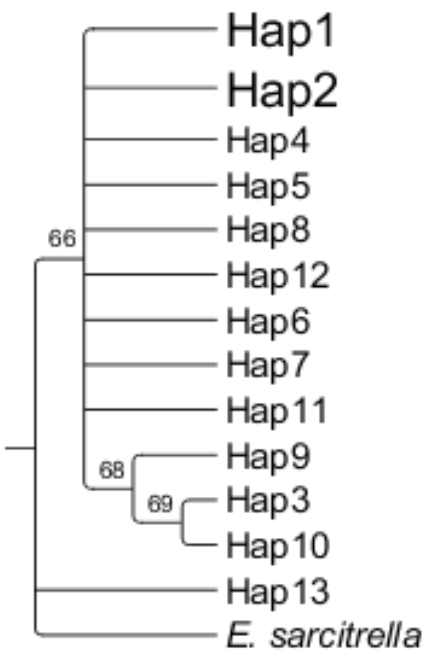


Figure 2.2: Phylogenetic tree of haplotype sequence data using maximum likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-938.3542) is shown. Bootstrap values > 50 are shown at the branches, values < 50 were collapsed. Haplotypes with the greatest proportion of webworm individuals have an increased font size. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4631)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 14 amino acid sequences and a total of 232 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

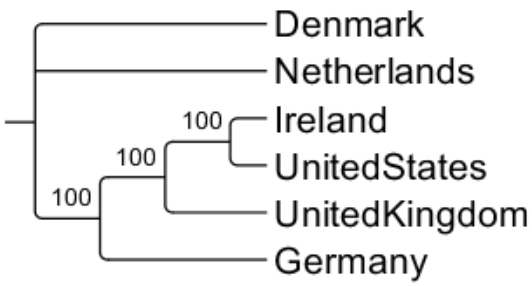
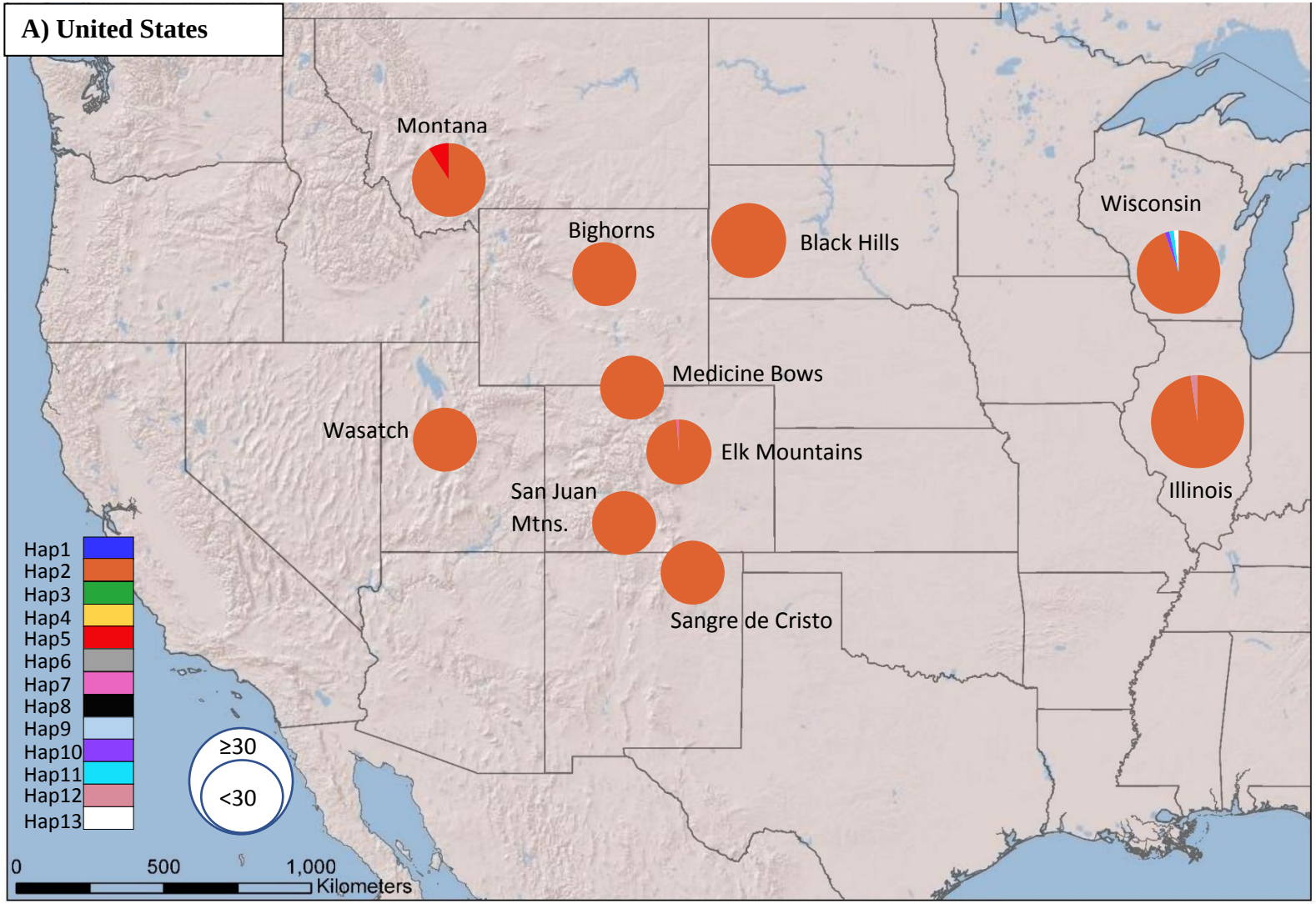


Figure 2.3: Neighbor-joining tree was constructed in POPTREE2 (Takezaki et al. 2010) using linearized Slatkin's pairwise F_{ST} values for European webworm populations and all U.S. populations. U.S. populations were collapsed together and New Zealand populations were excluded from analysis because of the lack of haplotype diversity. Bootstrap was run for 10000 replications, bootstrap values > 50 are listed at the branches, values < 50 are collapsed and not show


A) United States
Montana
Bighorns
Black Hills
Wisconsin
Medicine Bows
Wasatch
Elk Mountains
Illinois
San Juan
Mtns.
Sangre de Cristo
Hap1
Hap2
Hap3
Hap4
Hap5
Hap6
Hap7
Hap8
Hap9
Hap10
Hap11
Hap12
Hap13
≥30
<30
0
500
1,000
Kilometers

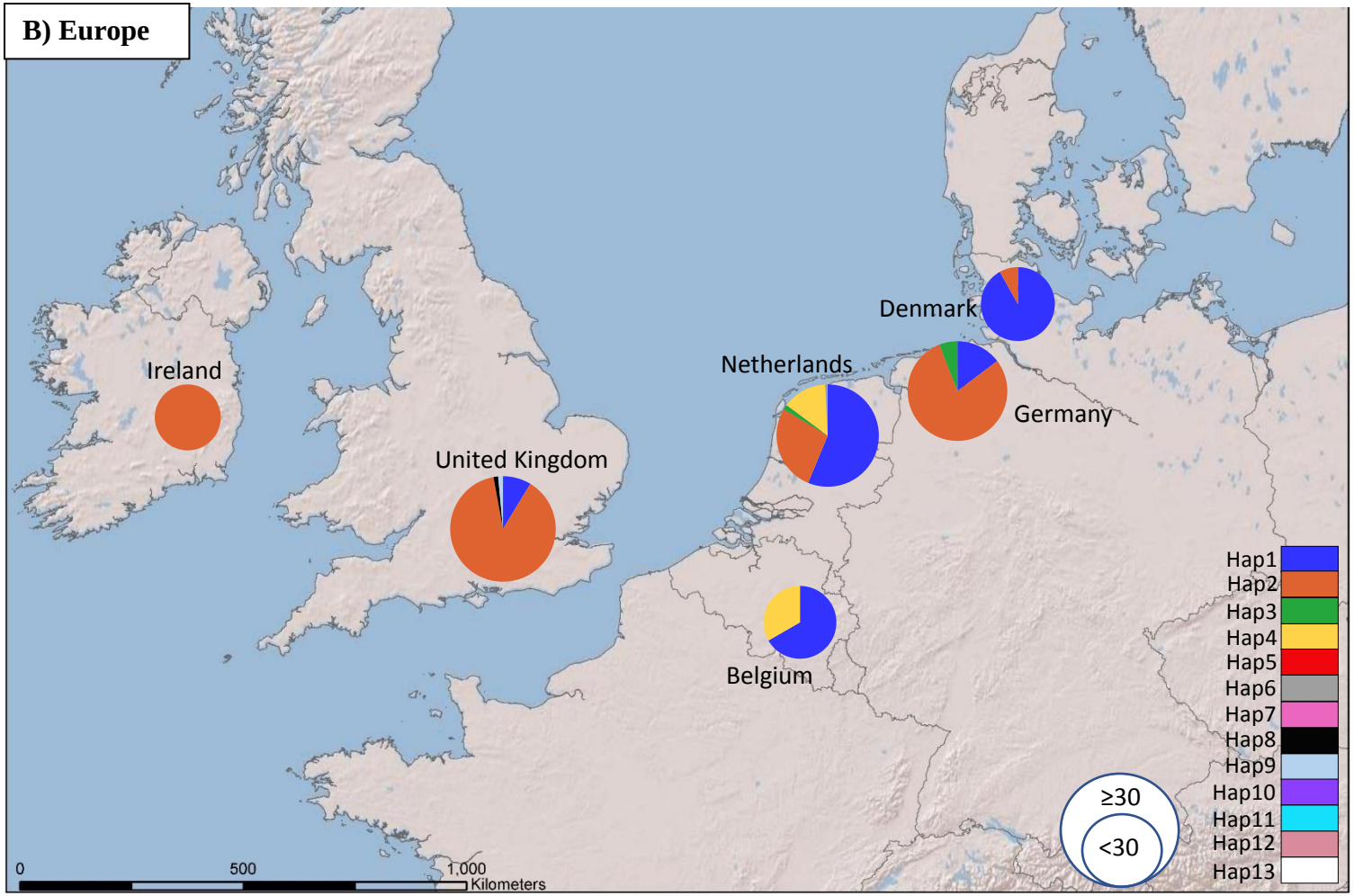


Figure 2.4: Proportion of parsnip webworm individuals with each *ND* 5 haplotype at each collection site (A: United States, B: Europe). The size of each pie chart represents the number of samples at each site (≥ 30, or < 30 samples)

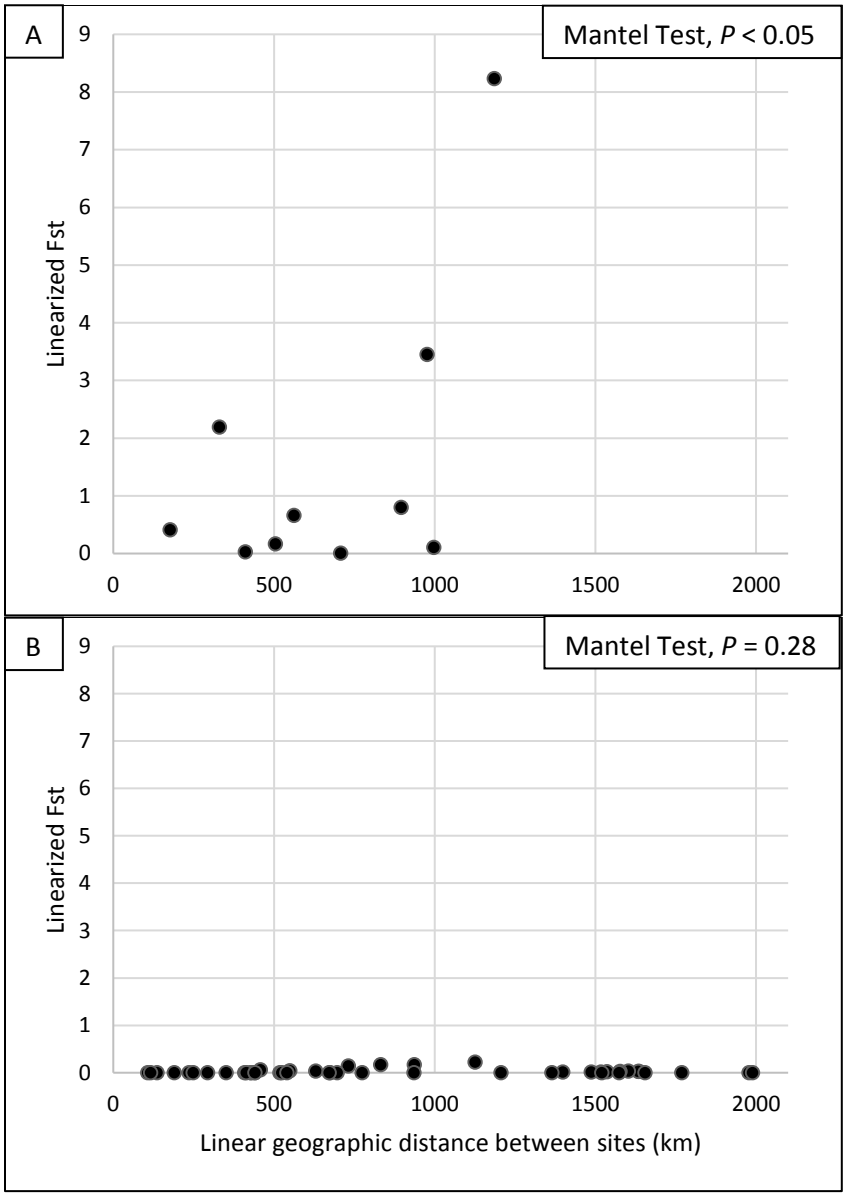


Figure 2.5: The correlation between genetic distance (linearized Slatkin's F_{ST}) and geographic distance (km) in the mtDNA region, *ND* 5, of parsnip webworm sites within Europe (A) and within the U.S. (B).

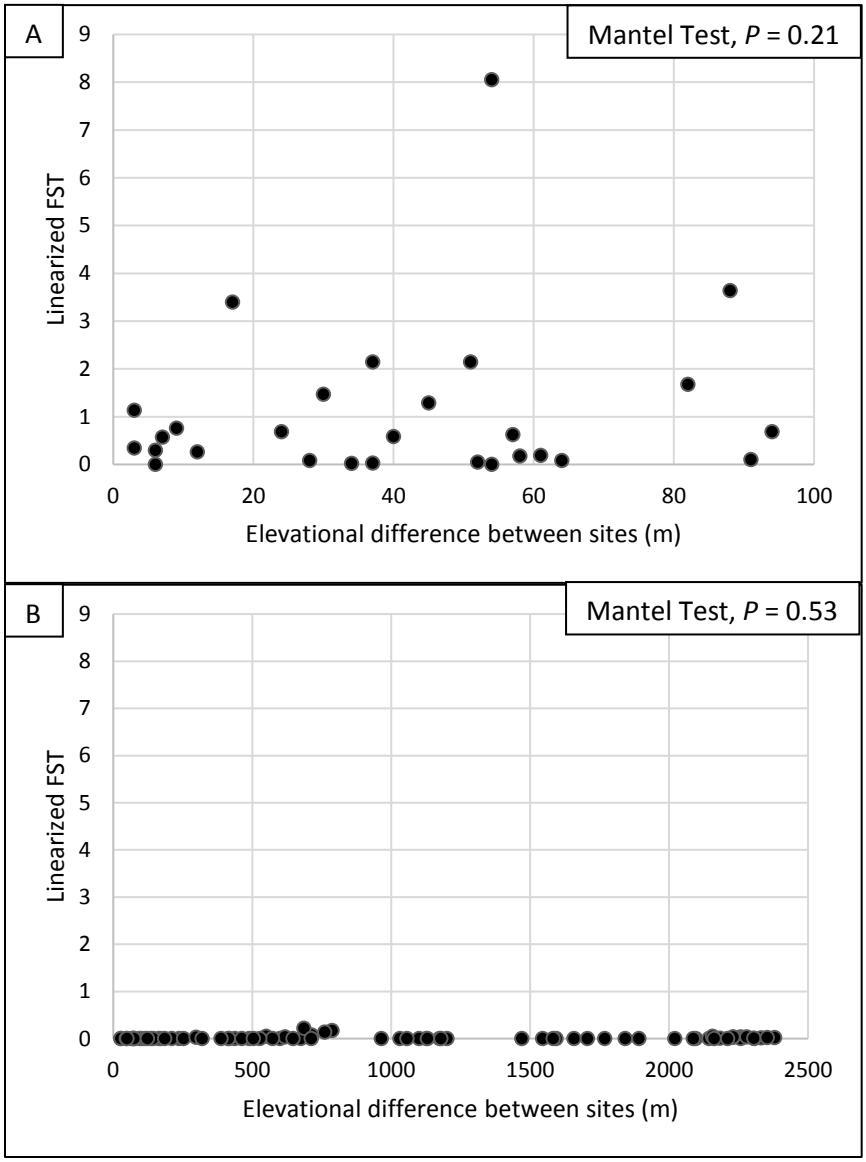


Figure 2.6: The correlation between genetic distance (linearized Slatkin's F_{ST}) and elevational differences (m) in the mtDNA region, *ND* 5, of parsnip webworm sites within Europe (A) and within the U.S. (B).

Tables

Table 2.1: Locality information (country/region, latitude, longitude), the host plant species parsnip webworms were collected from, and the number of individual parsnip webworms used in analysis (n).

Collection	Latitude	Longitude	Plant species	n
Europe (native range)				
Netherlands-E	N 52° 28' 6.84"	E 6° 6' 51.42"	*H. sphondylium*	8
Netherlands-G	N 51° 59' 11.60"	E 5° 40' 20.63"	*H. sphondylium*	7
Netherlands-H	N 51° 56' 49.38"	E 5° 45' 21.78"	*H. sphondylium*	53
Netherlands-I	N 52° 3' 42.78"	E 5° 11' 45.24"	*P. sativa*	11
Netherlands-K	N 52° 29' 31.2"	E 4° 54' 4.26"	*H. sphondylium*	13
Netherlands-M	N 53° 5' 30.84"	E 5° 22' 36.78"	*P. sativa*	27
Netherlands-N	N 50° 52' 52.44"	E 5° 54' 22.86"	*H. sphondylium*	3
Belgium-A	N 50° 57' 24.9"	E 5° 41' 38.22"	*H. sphondylium*	5
Denmark-B	N 55° 49' 4.38"	E 9° 47' 20.46"	*H. sphondylium*	13
Denmark-C	N 55° 53' 0.42"	E 9° 49' 33.84"	*P. sativa*	1
Denmark-D	N 56° 5' 47.04"	E 10° 3' 9"	*P. sativa*	18
Denmark-E	N 55° 18' 33.06"	E 9° 29' 56.58"	*H. sphondylium*	5
Germany-A	N 53° 11' 30.84"	E 7° 23' 26.34"	*H. sphondylium*	23
Germany-C	N 53° 22' 54.78"	E 8° 16' 15.06"	*P. sativa*	2
Germany-D	N 54° 11' 37.74"	E 9° 38' 39.06"	*H. sphondylium*	9
Ireland-1	N 52° 13' 19.14"	W 7° 21' 24.48"	*H. sphondylium*	15
Ireland-2	N 53° 24' 53.7"	W 6° 48' 28.68"	*H. sphondylium*	4
United Kingdom	N 50° 35' 29.46"	W 2° 2' 15.96"	*H. sphondylium*	69
United States (introduced range)				
San Juans-AE	N 36˚59'30"	W 106˚46'52.8"	*P. sativa*	6
San Juans-H	N 38˚11'0.84"	W 107˚03'13.98"	*H. maximum*	41
San Juans-T	N 36˚50'36.06"	W 106˚34'13.38"	*P. sativa*	12
San Juans-S	N 37˚13'58.08"	W 106˚46'29.46"	*H. maximum*	22
Montana-Z	N 44° 51' 55.56"	W 111° 33' 18.78"	*H. maximum*	8
Sangre de Cristo-J	N 35˚46'19.92"	W 105˚42'5.58"	*H. maximum*	51
Wasatch-K	N 39° 55' 48.84"	W 111° 38' 27.72"	*H. maximum*	16
Elk Mountains-F	N 39˚22'11.52"	W 106˚40'51.96"	*H. maximum*	3
Bighorns-C	N 44˚11'17.04"	W 105˚51'34.92"	*H. maximum*	11
Medicine Bow-AC	N 40˚24'25.62"	W 106˚48'23.94"	*P. sativa*	15
Black Hills-B	N 44˚04'35.7"	W 103˚38'20.76"	*H. maximum*	5
Black Hills-P	N 44˚25'17.1"	W 103˚52'54.6"	*H. maximum*	3
Illinois-W	N 40° 5' 0.708"	W 88° 13' 34.4958"	*P. sativa*	7
Illinois-PT	N 40° 7' 51.4956"	W 88° 13' 34.4958"	*P. sativa*	9
Illinois-C/P	N 40° 6' 24.4548"	W 88° 27' 46.8966"	*P. sativa*	8
Illinois-100E	N 40° 6' 25.0236"	W 88° 27' 33.3144"	*P. sativa*	13
Illinois-Rt10	N 40° 6' 22.9356"	W 88° 28' 21.5796"	*P. sativa*	9
Illinois-106	N 40° 6' 24.0336"	W 88° 27' 47.7396"	*P. sativa*	3
Wisconsin-Jayco	N 43° 32' 54.5712"	W 90° 48' 57.549"	*P. sativa*	8

Wisconsin-KHS	N 43° 29' 22.56"	W 90° 49' 45.915"	*P. sativa*	4
Wisconsin-Wayside	N 43° 28' 36.8868"	W 90° 49' 45.915"	*H. maximum*	12
Wisconsin	N 43° 31' 23.7894"	W 90° 42' 53.3982"	*H. maximum*	12
			P. sativa	12
Wisconsin-DC	N 43° 25' 13.6128"	W 90° 46' 11.1894"	*H. maximum*	7
New Zealand (introduced range)				
New Zealand-Crimp	S 45° 54' 0.1044"	E 170° 26' 24.0324"	*P. sativa*	2
New Zealand-Kane	S 45° 53' 59.622"	E 170° 26' 28.8198"	*P. sativa*	3
New Zealand-Lumber	S 45° 53' 59.0856"	E 170° 26' 33.6114"	*P. sativa*	7
New Zealand-Townley	S 45° 53' 32.6832"	E 170° 27' 28.605"	*P. sativa*	4
New Zealand-Ocean	S 45° 42' 3.441"	E 170° 36' 9.8562"	*P. sativa*	7
New Zealand-Cottage	S 45° 40' 44.1192"	E 170° 37' 29.424"	*P. sativa*	8
New Zealand-Rock	S 45° 39' 21.312"	E 170° 38' 28.5936"	*P. sativa*	8
New Zealand-Bush	S 45° 28' 16.359"	E 170° 46' 24.4452"	*P. sativa*	2
New Zealand-Moeraki	S 45° 21' 48.9774"	E 170° 50' 55.7406"	*P. sativa*	5

Table 2.2: Primers to amplify segments of *Depressaria pastinacella* mitochondrial DNA (mtDNA). T_a (°C), annealing temperature used during PCR.

Gene/ Region	Primer Name	Sequence (5'-3')	T_a (°C)	Reference
COI	LepF1	F: ATTCAACCAATCATAAAGATATTGG	60°	Hebert et al. 2004
	LepR1	R: TAAACTTCTGGATGTCCAAAAAATCA		
COI	K698	F: TACAATTTATCGCCTAAACTTCAGCC	55°	Simmons and Weller 2001
	PAT2K837	R: TCCATTACATATAATCTGCCATATTAG		
Cyt *b*	CYTBF_PS	F: CCGAAAAACTCACCCAATTT	60°	developed from Park et al 2014*
	CYTBR_PS	R: CCCGTTTGCATGGATAGTTC		
Cyt *b*	REVCB2H	F: TGAGGACAAATATCATTTGAGGT	59°	Simmons and Weller 2001
	REVCBJ	R: ACTGGTCGAGCTCCAATTCATGT		
ND4	LF02-S01-F1	F: TTATAATACCHCCAATWAC	54°	Kim et al 2014
	LF02-S01-R1	R: GGTTTAATTTTATTAAGAATTTG		
ND5	LF01-S09-F1	F: AWAHTTCTCTTCAACCYAWATC	46.7°	Kim et al 2014
	LF01-S09-R2	R: GCTTTATCWACTTTAAGWCA		

*Park et al (2014) sequenced the entire mitochondrial genome of *Promalactis suzukiella* (Lepidoptera: Oecophoridae), which is the closest phylogenetic species to parsnip webworm that had a complete mitochondrial genome sequenced, at the time of this study. Primers were developed flanking the cytochrome *b* gene in *Promalactis suzukiella*.

Table 2.3: Molecular diversity indices within populations of Europe, the United States, and New Zealand *ND* 5 haplotypes. Numbers of individuals (N), number of haplotypes (N_h), and haplotype diversity ($\hat{\theta}_\pi \pm s.d$)

Collection	N	N_h	Haplotypes	$\hat{\theta}_\pi \pm s.d$
Europe	287	7	Hap 1, 2, 3, 4, 6, 8, 9,	0.67 ± 0.57
Denmark	37	2	Hap 1, 2	0.15 ± 0.24
Netherlands	128	5	Hap 1, 2, 3, 4, 6	0.78 ± 0.63
Germany	34	3	Hap 1, 2, 3	0.45 ± 0.46
United Kingdom	69	4	Hap 1, 2, 8, 9	0.28 ± 0.36
Ireland	19	1	Hap 2	0.00 ± 0.00
United States	380	7	Hap 2, 5, 7, 10, 11, 12, 13	0.04 ± 0.12
San Juan Mtns. 1	40	1	Hap 2	0.00 ± 0.00
San Juan Mtns. 2	41	1	Hap 2	0.00 ± 0.00
Montana	11	2	Hap 2, 5	0.18 ± 0.29
Sangre de Cristo Mtns.	51	1	Hap 2	0.00 ± 0.00
Elk Mountains	88	2	Hap 2, 7	0.02 ± 0.09
Black Hills	19	1	Hap 2	0.00 ± 0.00
Wasatch Mtns.	16	1	Hap 2	0.00 ± 0.00
Medicine Bows	15	1	Hap 2	0.00 ± 0.00
Wisconsin	56	4	Hap 2, 10, 11, 13	0.14 ± 0.23
Illinois	43	3	Hap 1, 2, 12	0.09 ± 0.18
New Zealand	53	1	Hap 2	0.00 ± 0.00

Table 2.4: Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) for parsnip webworm collections, using *ND* 5 haplotype frequencies. Significance levels; **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European populations	4	26.83	0.13	34.17	0.000***
Within European populations	282	69.07	0.24	65.83	
Total	286	95.90	0.37		
Among U.S. populations	9	0.24	0.00	0.76	0.179
Within U.S. populations	370	7.73	0.02	99.24	
Total	379	7.97	0.02		
Among Europe and U.S.	1	30.25	0.08	33.05	0.0167*
Among populations within Europe and U.S.	13	27.07	0.05	19.39	0.000***
Within populations	652	76.81	0.12	47.56	0.000***
Total	666	134.12	0.25		
Among Europe and New Zealand	1	8.28	0.01	2.21	0.334
Among populations within Europe and New Zealand	4	26.83	0.13	37.37	0.000***
Within populations	334	69.07	0.21	60.42	0.000***
Total	339	104.18	0.34		
Among U.S. and New Zealand	1	0	0	-1.6	0.442
Among populations within U.S. and New Zealand	9	0.24	0	1.26	0.182
Within populations	422	7.73	0.02	100.35	0.170
Total	432	7.98	0.02		

Table 2.5: Analysis of molecular variance (AMOVA) (Excoffier et al 1992) between host plant species parsnip webworms were collected, within the native range (Europe) and the introduced range (U.S.), using *ND* 5 haplotype frequencies. Significance levels; **P* < 0.05, ***P* < 0.001, ****P* < 0.0001

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and hogweed	1	6.935	0.015	4.068	0.292
Among populations within European wild parsnip and hogweed	5	27.371	0.137	36.887	0.000***
Within populations	280	61.597	0.220	59.044	0.000***
Total	286	95.902	0.373		
Among U.S. wild parsnip and cow parsnip	1	0.047	0.000	0.764	0.078
Among populations within U.S. wild parsnip and cow parsnip	11	0.252	0.000	0.360	0.351
Within populations	368	7.674	0.021	98.876	0.239
Total	380	7.974	0.021		

Table 2.6: Analysis of molecular variance (AMOVA) (Excoffier et al 1992) between host plant species parsnip webworms were collected, between native range (Europe) host plants species and introduced range (U.S. and New Zealand) host plant species, using *ND* 5 haplotype frequencies. Significance levels; **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and U.S wild parsnip	1	17.355	0.204	48.38	0.058
Among populations within European wild parsnip and U.S wild parsnip	4	6.967	0.065	15.38	0.000***
Within populations	154	23.516	0.153	36.25	0.000***
Total	159	47.837	0.421		
Among European wild parsnip and U.S cow parsnip	1	22.681	0.223	69.83	0.0147*
Among populations within European wild parsnip and U.S cow parsnip	9	6.992	0.025	7.72	0.000***
Within populations	328	23.536	0.072	22.46	0.000***
Total	338	53.209	0.319		
Among European hogweed and U.S cow parsnip	1	17.961	0.060	29.59	0.035*
Among populations within European hogweed and U.S cow parsnip	12	20.656	0.050	24.54	0.000***
Within populations	494	45.755	0.092	45.87	0.000***
Total	507	84.372	0.202		
Among European hogweed and U.S wild parsnip	1	10.011	0.046	17.02	0.168
Among populations within European hogweed and U.S wild parsnip	7	20.631	0.083	30.60	0.000***
Within populations	320	45.735	0.143	52.39	0.000***
Total	328	76.377	0.273		
Among European hogweed and New Zealand wild parsnip	1	6.144	0.000	-3.01	0.492
Among populations within European hogweed and New Zealand wild parsnip	4	20.517	0.121	45.66	0.000***
Within populations	275	41.908	0.152	57.35	0.000***
Total	280		0.266		
Among European wild parsnip and New Zealand wild parsnip	1	12.993	0.039	8.43	0.679
Among populations within European wild parsnip and New Zealand parsnip	1	6.853	0.247	52.86	0.000***
Within populations	109	19.689	0.181	38.71	0.000***
Total	111	39.536	0.467		
Among U.S wild parsnip and New Zealand wild parsnip	1	0.020	0.000	-1.59	0.605
Among populations within U.S wild parsnip and New Zealand parsnip	3	0.114	0.000	1.99	0.439
Within populations	149	3.827	0.026	99.59	0.155

Total	153	3.961	0.026		
Among U.S cow parsnip and New Zealand wild parsnip	1	0.002	0.000	-1.84	0.398
Among populations within U.S cow parsnip and New Zealand parsnip	8	0.139	0.000	1.58	0.294
Within populations	323	3.847	0.012	100.26	0.220
Total	332	3.988	0.012		

Table 2.7: Estimates of parsnip webworm subpopulation differentiation from Slatkin's linearized pairwise F_{ST} values between and within collection sites in Europe, the United States, and New Zealand using *ND* 5 haplotypes. Significant comparisons indicate the pairs are significantly different and are marked with an asterisk(s) based on significance level; $P < 0.05$*, $P < 0.001$**

		Denmark	Netherlands	Germany	United Kingdom	Ireland	San Juan Mtns. 1	San Juan Mtns. 2	Montana	Sangre de Cristo Mtns.	Elk Mtns.	Black Hills	Wasatch Mtns.	Medicine Bow Mtns.	Wisconsin	Illinois
Europe	Netherlands	**0.17****														
	Germany	**2.19****	**0.41****													
	United Kingdom	**3.45****	**0.66****	0.08												
	Ireland	**8.23****	**0.80****	0.11	0.03											
United States	San Juan Mtns. 1	**11.46****	**0.94****	**0.18****	**0.06***	0										
	San Juan Mtns. 2	**11.61****	**0.94****	**0.18****	**0.06***	0	0									
	Montana	**4.77****	**0.62****	0.01	0	0.06	0.17	0.17								
	Sangre de Cristo Mtns.	**13.15****	**1.00****	**0.22****	**0.07***	0	0	0	0.22							
	Elk Mtns.	**13.68****	**1.16****	**0.26****	**0.08****	0	0	0	0.14	0						
	Black Hills	**8.23****	**0.80****	**0.11***	0.03	0	0	0	0.06	0	0					
	Wasatch Mtns.	**7.77****	**0.78****	0.09	0.02	0	0	0	0.04	0	0	0				
	Medicine Bow Mtns.	**7.61****	**0.77****	0.09	0.02	0	0	0	0.03	0	0	0	0			
	Wisconsin	**6.40****	**0.87****	**0.10****	0.02	0	0.01	0.01	0	0.02	0.01	0	0	0		
	Illinois	**6.70****	**0.84****	**0.10****	0.03	0	0.02	0.02	0	0.03	0.02	0	0	0	0	
	New Zealand	**13.45****	**1.013****	**0.22****	**0.07****	0	0	0	0.23	0	0	0	0	0	0.02	0.03

CHAPTER 3: DEVELOPMENT OF NOVEL MICROSATELLITE MARKERS FOR THE POLYEMBRYONIC PARASITOID WASP, COPIDOSOMA SOSARES (HYMENOPTERA: ENCYRTIDAE)

Introduction

Copidosoma sosares (Walker) (Hymenoptera: Encyrtidae) is a specialized polyembryonic parasitoid wasp whose sole known host is the parsnip webworm, *Depressaria pastinacella* (Duponchel) (Lepidoptera: Depressariidae), a specialist herbivore that feeds exclusively on the reproductive tissues of apiaceous plants within the genera *Pastinaca, Heracleum,* and *Angelica* (Apiaceae) (Hardy 1996; Ode et al. 2004). In its native range in Europe, *C. sosares* attacks *D. pastinacella* feeding on wild parsnip, *P. sativa* (L.) and the common hogweed, *H. sphondylium* (L.). In its introduced range within the United States, *C. sosares* attacks parsnip webworm populations that feed on both the introduced wild parsnip and the cow parsnip (*H. maximum* Bartram), a plant native to North America. Like all copidosomatine encyrtids, *C. sosares* is a polyembryonic egg-larval parasitoid. Female *C. sosares* adults oviposit in the egg stage of their webworm host and progeny complete development in the host's final (sixth) instar. Development time from egg to adult wasp is approximately 30 days. *C. sosares* produces all-male and all-female broods by laying single male or female eggs, and mixed-sex broods by laying one male and one female egg per host (Lampert et al. 2008). During host development, *C. sosares* egg(s) proliferate clonally, producing clutch sizes ranging from 100 to 300 genetically-identical offspring per host (Ode et al. 2004; Lampert et al. 2008). Like its webworm host, *C. sosares* is univoltine. Males and females immediately mate after emergence in late summer; females must overwinter as adults before maturing eggs the following late spring to early summer.

The population genetics of this species has yet to be examined, as no genetic markers have been developed to date. Polymorphic microsatellites are well suited to investigating a variety of phenomena in *C. sosares* including paternity analysis, analysis of mating populations, patterns of introduction and

spread across ranges, and population level associations with their host. Here we describe the development and characterization of nine variable microsatellite loci in *C. sosares*.

Materials and Methods

Samples

Copidosoma sosares broods were collected from the western United States (U.S.) during the summer of 2004 from parasitized parsnip webworms feeding on wild parsnip or cow parsnip. These individuals were used for microsatellite library construction. Additional *C. sosares* broods collected from 7 western United States and 4 European sites between 2004 and 2012 were used for microsatellite loci characterization across variable populations (Table 3.1). Within Europe, parasitized parsnip webworms are also found feeding on the common hogweed plant, *H. sphondylium* and were also collected for this study. In total, 296 individuals were used; 117 females and 81 males from the U.S., and 69 females and 29 males from Europe. Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) in accordance with the manufacturer's instructions for solid tissue. A single adult female from all-female and mixed-sex broods, and a single adult male from all-male broods were ground using a Kontes pestle (Kimble) in the extraction mixture and incubated for 3 hours at 56°C. Isolated DNA was checked for quantity and purity using the BioTek Epoch Take3 plate reader.

Isolation and characterization of microsatellite loci

Adult *C. sosares* individuals were sent to the Life Sciences Core Laboratories Center at Cornell University for microsatellite library construction. Library construction followed modified protocols from Hamilton et al. (1999). CODONCODE ALIGNER software (version 2.0.3) was used to trim vector and linker sequences and to determine which sequences formed contigs. Contigs were assembled from scratch and "slip ends" and "trim vector" options were enabled in the preprocess step. Sequences were aligned with built-in assembly algorithm. Sequences that met the minimum criteria for repeat length and amount of flanking sequence were exported as fasta files. From these sequences primers were designed, tested, and genotyped. The software PRIMER 3 (Rozen and Skaletsky 2000) was used to design primers between 18 and 35 bases long with annealing temperatures between 50°-70°C.

Polymerase chain reaction (PCR)

PCR reactions were performed on either an Eppendorf Mastercycler gradient thermal cycler (www.eppendorfna.com) or a Thermo Scientific Arktik thermal cycler (www.thermoscientific.com). A twelve-degree Celsius annealing temperature gradient was run for each primer pair. PCR was performed in a 25 µL reaction volume containing 2.5 µL of 10X PCR Buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM MgCl2), 2.5 mM of each dNTP, 5 pmol of each primer, 0.625 U of TaKaRa Taq HotStart Version polymerase (Clontech), 2.5 µL of genomic DNA template extracted from a single adult, and up to 25 µL of sterile distilled water. The thermal profile consisted of 2 min at 94°C followed by 40 cycles of 94°C for 30 s, the optimal annealing temperature for 30 s (Table 3.2), and for 65°C for 30 s with a final extension of 70°C for 5 min. Loci exhibiting discrete bands on an agarose gel had one primer re-synthesized with a fluorescent phosphoramidite dye (6-FAM, HEX). The PCR products and Genescan size standard ROX 500 were genotyped at the University of Arizona Genetics Core on a ABI 3730 DNA Analyzer (Applied Biosystems) and the resulting genotype data were analyzed using ABI GeneMapper software (version 4). Loci that were polymorphic and had reduced allelic stutter were deemed appropriate for use in *C. sosares* populations. The heterozygosities, expected (H_E) and observed (H_O), null allele frequencies (r), and inbreeding coefficient (F_{IS}) were analyzed for each population using GENEPOP (ver. 4.1.4; Rousset 2008). Additionally, because our dataset included populations from the ancestral range of *C. sosares* in Europe as well as introduced populations of *C. sosares* (U.S.), we investigated whether null allele frequencies differed at each locus from each of these two ranges. Since our microsatellite library was constructed from individuals collected in the U.S., null allele frequencies in Europe might be higher due to divergence of introduced populations compared to ancestral European populations. This information will be important for future studies seeking to investigate the population structure within Europe (see chapter 4). Because of the haplodiploid nature of inheritance, only diploid, female data were included in analyses of observed and expected heterozygosity, and haploid, male data were excluded.

Results and discussion

Microsatellite library construction identified 34 candidate loci, of which 12 were ultimately chosen for detailed screening based on amplification success and levels of polymorphism. Seven of the 12 loci were polymorphic with 3-9 alleles per locus (Table 3.2), but only 5 of the 7 were consistently amplified across all samples and regions. Inbreeding coefficients and the null allele frequency ranged from -0.04 to 0.74 and 0 to 0.73, respectively (Table 3.3). After Bonferroni correction, only one locus (Csos 4) significantly deviated from Hardy-Weinberg Equilibrium (HWE, $P < 0.05$) across all populations sampled. When the data was partitioned by location (European and U.S populations), loci Csos 2, Csos 3, and Csos 4 in European populations conformed to HWE ($P > 0.05$), whereas loci Csos 1, Csos 2, and Csos 3 in the U.S. conformed to HWE ($P > 0.05$). No pairs of loci demonstrated linkage disequilibrium neither across all populations nor when the data was partitioned into European and U.S populations ($P > 0.05$). Heterozygosity for locus Csos 3 was lower than expected and had the highest null allele frequency across all loci in European populations tested (avg. = 0.442), but not in U.S populations (avg. = 0.185) suggesting segregating null alleles are a common occurrence within locus Csos 3 in Europe. Locus Csos 5 had significantly reduced amplification success in all but the Netherlands samples, suggesting the flanking regions of the microsatellite repeat motif in the other ten populations no longer bind the designed primers. In males, null allele frequencies ranged from 0.134 - 0.350. The null allele frequencies were inferred by the number of "unamplified samples" as males are haploid. However, it is possible that the null allele frequencies were overestimated as absence of amplification could be attributed to other factors.

The main aim of this study was to develop molecular markers for *C. sosares* for use in studies of parasitoid biology and evolutionary ecology. The congener of *C. sosares*, *C. floridanum*, has been used in studies on kinship (Ode and Strand 1995; Giron et al. 2004; Giron and Strand 2004), competition (Strand et al. 1990; Harvey et al. 2000; Utsunomiya and Iwabuchi 2002; Uka et al. 2006; Bowker et al. 2015), developmental biology (Baehrecke and Strand 1990; Strand and Ode 1990; Baehrecke et al. 1992; Grbic et al. 1996; Corley et al. 2005; Donnell et al. 2006; Gordon and Strand 2009), and social biology (Grbic et

al. 1997; Giron et al. 2007; Segoli et al. 2009) owing to its unique polyembryonic development and evolution of a larval caste system inside its host caterpillar. *C. sosares* exhibits many of these same features, but has rarely been studied, mainly due to the difficulties associated with maintaining *C. sosares* lab cultures. As a result, most experimental work on *C. sosares* is conducted on field populations, making the development and use of molecular markers for *C. sosares* necessary. The reciprocal selection between parsnip webworms and its hostplant, wild parsnip, have been studied extensively (Berenbaum and Zangerl 1992; Berenbaum and Zangerl 1998; Berenbaum et al. 1993; Zangerl and Berenbaum 2003; Zangerl et al. 2008). Recently, this relationship has been extended to include *C. sosares* to better understand tritrophic dynamics (Ode et al. 2004; Lampert et al. 2008; Lampert et al. 2011). These results indicate microsatellite markers for *C. sosares* are well suited for use as genetic markers for elucidating the population structure of *C. sosares*.

Tables

Table 3.1: Collection sites of *C. sosares*, the number of broods collected from each site, the host plant species from which parasitized parsnip webworm hosts were collected, and the number of individuals from each site subsequently used in microsatellite characterization.

	Collection Sites	**Hostplant**	**n**		
United States			Female	Male	**Total**
Black Hills	N 44°14' 9.34" W 103°28' 57.79"	*H. maximum*	32	19	51
Bighorns	N 44˚11'17.04" W 105˚51'34.92"	*H. maximum*	32	17	49
Elk Mountains	N 39˚22'48.96" W 107˚04'42.24"	*H. maximum*	6	18	24
		P. sativa	4	4	8
San Juan Mtns.	N 38˚11'0.84" W 107˚03'13.98"	*H. maximum*	12	6	18
		P. sativa	8	3	11
Sangre de Cristo Mtns.	N 35˚46'19.92" W 105˚42'5.58"	*H. maximum*	9	7	16
Wasatch Range	N 39°55' 48.84" W 111°38'27.72"	*H. maximum*	6	5	11
Montana	N 46°42'19.872" W 114°32'14.53"	*H. maximum*	8	2	10
Europe					
Denmark	N 55° 49' 4.38" E 9° 47' 20.46"	*P. sativa*	1	2	3
		H. sphondylium	3	4	7
Belgium	N 50° 57' 24.9" E 5° 41' 38.22"	*H. sphondylium*	4	0	4
Germany	N 53° 22' 54.78" E 8° 16' 15.06"	*P. sativa*	17	5	22
Netherlands	N 52° 9' 22.62" E 4° 28' 48"	*P. sativa*	44	18	62

Table 3.2: Characteristics of 12 microsatellite loci, tested on samples of *C. sosares* from populations in Europe and the United States, including repeat motif, primer sequences, number of detected alleles (*A)*, size range (bp), annealing temperature (T_a) and significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$ for European populations (*) and U.S populations (**).

Locus	Repeat Motif	Primer Sequences (5'-3') (F = forward, R = reverse)	*A*	Fragment size (bp)	T_a (°C)
Csos 1*	$(CAGA)_{5-9}$	F:GGCAAACAGCTCACTCCGGCA R:TCCTTTTCCGTTTCGGGCCGT	5	463-479	58
Csos 2	$(AG)_{0-17}$	F:GCCGCGTTGCTGGCGTTTTT R:AGGAGAGCGCACGAGGGGAG	9	236-270	50
Csos 3	$(TG)_{2-4}$	F:CGTCGATCCCCGCAGTCACG R:TCGAGCTGGACCTCTCGGGC	3	299-303	50
Csos 4**	$(ACAG)_{5-10}$	F:TCCTCTGCAGAAGCGTGGGT R:GGCATCCCGAAAGCGCGTTACA	5	414-534	59.7
Csos 5	$(CAT)_{4-37}$	F:AGGTGGTGGTGGTGTATCATCT R:GTTTCGAGTAATCAGTGCGTTGGGAG	6	261-361	55
Csos 6	$(GTT)_{21}$	F:TTCAATATTCTCGCACTCACG R:AGACAAGAAAACGGGGGACT	1	235	50
Csos 7	$(GTT)_{6}$	F:GGACGGAGACCGAGGAGGGG R:CGGCTCCTCTAAGCGCGCAA	2	254-256	50
Csos 8	$(AC)_{14}$	F:ACCCACTCTTTGATTTTTGCTC R:GTTTGATTGTTTAATGGCGGTGGC	1	302	55
Csos 9	$(AC)_{10}$	F:AGAAGCAGCAACACCCATAG R:GTTTGGAAACACAAAGAAAGTCATCC	1	268	55
Csos 10	(GT)	F:GGCTGTCCCCCATTGT R:GTTTATCCACCCCGTCATTTCCA	4	312-320	55
Csos 11	$(GAT)_{5}$	F:CGCGTTTTATATTCACTCGTTCACAC R:GTTTCCGGTGCGTCGTTGTCAT	1	323	55
Csos 12	$(GAT)_{63}$	F:CAATATGTTAATGTCGGTCCAAA R:CAACCGCTTCTGCTTGTTTA	1	298	55

Table 3.3: Analysis of 5 polymorphic loci in *C. sosares* at 11 collection sites using GENEPOP (ver. 4.1.4; Rousset 2008) The number of female individuals used in the analysis for each loci (*n*), the number of alleles at each loci (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), and null allele frequencies (*r*).

Population	Loci	*n*	N_A	H_E	H_O	F_{IS}	*r*
Black Hills	Csos 1	32	3	18.56	9	0.52	0.26
	Csos 2	33	6	22.46	14	0.38	0.13
	Csos 3	32	2	13.12	6	0.55	-
	Csos 4	32	5	21.46	17	0.21	0.16
	Csos 5	2	2	1.00	1	0.000	-
Montana	Csos 1	8	3	3.93	3	0.25	0.10
	Csos 2	8	4	4.80	3	0.39	0.13
	Csos 3	8	2	2.60	5	-0.17	-
	Csos 4	6	2	1.71	2	-0.20	-
	Csos 5	NA	-	-	-	-	-
Bighorn Mountains	Csos 1	34	4	17.01	10	0.42	0.19
	Csos 2	34	4	17.35	18	-0.04	0.01
	Csos 3	31	3	15.61	12	0.23	0.12
	Csos 4	32	5	21.84	17	0.22	0.28
	Csos 5	3	2	1	1	0.00	-
Elk Mountains	Csos 1	15	1	-	-	-	-
	Csos 2	14	3	8.70	8	0.08	0.01
	Csos 3	14	2	5.93	4	0.33	-
	Csos 4	13	5	9.88	9	0.09	0.23
	Csos 5	NA	-	-	-	-	-
Sangre de Cristo Mountains	Csos 1	9	2	1.00	2	0.00	-
	Csos 2	8	2	3.67	1	0.74	-
	Csos 3	8	4	2.67	3	-0.14	0.00
	Csos 4	8	3	5.33	4	0.26	0.33
	Csos 5	2	2	1	1	0.00	-
Wasatch Range	Csos 1	4	1	0	0	-	-
	Csos 2	4	3	1.86	2	-0.09	0.00
	Csos 3	4	2	1.71	2	-0.20	-
	Csos 4	4	4	3.29	3	0.10	0.21
	Csos 5	1	1	0.00	0	-	-
San Juan Mountains	Csos 1	26	2	10.43	6	0.43	-
	Csos 2	25	4	15.00	5	0.67	0.26
	Csos 3	23	3	10.02	9	0.10	0.25
	Csos 4	25	5	17.82	16	0.10	0.25
	Csos 5	NA	-	-	-	-	-
Denmark	Csos 1	4	1	0.00	0	-	-
	Csos 2	3	3	2.20	2	0.11	0.00
	Csos 3	4	3	2.86	0	1.00	0.73
	Csos 4	4	1	-	-	-	-
	Csos 5	NA	-	-	-	-	
Belgium	Csos 1	4	2	1.71	2	-0.20	-
	Csos 2	4	3	2.71	2	0.29	0.05
	Csos 3	4	2	2.14	1	0.57	-
	Csos 4	4	1	0.00	0	-	-
	Csos 5	NA	-	-	-	-	-
Germany	Csos 1	16	5	12.68	8	0.38	0.22
	Csos 2	14	4	9.74	10	-0.03	0.00
	Csos 3	13	3	6.92	3	0.58	0.33
	Csos 4	16	5	11.81	11	0.07	0.01

	Csos 5	1	1	-	-	-	
Netherlands	Csos 1	43	5	33.32	14	0.58	0.34
	Csos 2	42	7	31.02	23	0.26	0.18
	Csos 3	40	3	23.41	20	0.15	0.26
	Csos 4	42	5	28.25	21	0.26	0.21
	Csos 5	23	4	9.98	8	0.20	0.02

CHAPTER 4: THE INTRODUCED PARASITOID WASP, *COPIDOSOMA SOSARES* (HYMENOPTERA: ENCYRTIDAE), EXPLOITS MULTIPLE ROUTES OF INVASION IN PURSUIT OF ITS HERBIVOROUS INSECT HOST

Introduction

Species within coevolved trophic networks that experience a range expansion can either re-associate with populations of the same species in the original coevolved trophic network (Schönrogge et al. 1996; Grobler and Lewis 2008), fail to re-associate with one or more species in the original trophic network (Allen et al. 2015), or they may create new interactions with novel species at one or more trophic level(s) (Godfray et al. 1995; Schönrogge et al. 1996; Thomas et al. 2001; Girardoz et al. 2006: Grobler and Lewis 2008; Grabenweger et al. 2010; Harvey and Gols 2011; Fortuna et al. 2012; Fortuna et al. 2013). For example, the effectiveness of some classical biological control programs has relied on the intentionally introduced coevolved enemy re-associating with the exotic pest species in the introduced range (Cullen et al. 1982; Cullen and Moore 1983; Giblin-Davis et al. 2001; Evans et al. 2005; Mills 1994, 2000, 2001). Conversely, the ineffectiveness of some biocontrol agents has been due to their lack of re-association with the exotic pest species in the introduced range (Mills 2000; Palmer and Witt 2006).

In addition, coevolved introduced multitrophic systems have re-ordered themselves without human assistance. The invasion of the horse chestnut leaf miner *Cameraria ohridella* (Lepidoptera: Gracillariidae) throughout the European continent serves as an example for the occurrence of new interactions with novel species at one or more trophic level(s). In all the invaded areas, *C. ohridella* is attacked by a large number of native European predators and parasitoids (Hellrigl 2001; Grabenweger et al. 2005). In some cases, a single multitrophic system can form a variety of interactions in the introduced range. For example, the leaf mining moth *Phyllonorycter leucographella* (Lepidoptera: Gracillariidae) has invaded the southern United Kingdom and has re-associated with its ancestral host plant *Pyracantha coccinea* (Rosaceae), which is widely cultivated in the UK (Emmet 1989; Sefrova 2003; Grobler and

Lewis 2008). Additionally, the moth has spread northwards into central Scotland (Bland 2002) and has formed a novel association with the UK native plant, *Crataegus monogyna* (Grobler and Lewis 2008).

Although these studies have contributed to the understanding of invasive species ecology and more broadly, community assembly, these and other empirical and theoretical studies on coevolved interactions have, historically, been concentrated on interactions between pairs of species (Cronin and Haynes 2004; Harvey et al. 2010; Harvey and Gols 2011; Stenberg 2012), most notably between plants and herbivores (Louda et al. 1997; Keane and Crawley 2002; Parker et al. 2006). Recently, there is a growing number of studies that investigate multispecies interactions within a broader community context across geographically widespread areas (Grobler and Lewis 2008; Nicholls et al. 2010; Harvey and Gols 2011). The species-rich communities of plants, insect herbivores, and their natural enemies have been the focus of this recent research on the assembly of geographically widespread species, due in part to their pervasiveness in terrestrial communities and their crucial role in ecosystem services such as pollination and biological control (Nicholls et al. 2010; Fortuna et al. 2012; Stone et al. 2012; Quacchia et al. 2013; Wang et al. 2013).

Studies documenting the associations between multispecies interactions within introduced ranges are difficult because detailed knowledge of the coevolutionary relationships between species and the population history of each species in the ancestral and introduced ranges is needed (Hoberg and Brooks 2008; Smith et al. 2011; Whiteman et al. 2007). One multitrophic system whose interactions have been well documented, and population history data exists for two of the species, is that of the wild parsnip (*Pastinaca sativa* L; Apiaceae), its primary herbivore, parsnip webworm (*Depressaria pastinacella*; Lepidoptera: Depressariidae) (Berenbaum and Zangerl 1992; Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003), and its parasitoid, *Copidosoma sosares* (Hymenoptera: Encyrtidae) (Ode et al. 2004; Lampert et al. 2008).

The interaction between wild parsnips and webworms has been discussed in detail in Chapter 2. Briefly, webworms are the primary herbivore of wild parsnips, in which the larvae construct webs around part of the wild parsnip umbel and feed primarily on buds, flowers, and developing fruits (Berenbaum et

al. 1993; Nitao and Berenbaum 1988), causing substantial reductions to host plant fitness. Wild parsnips produce furanocoumarins, allelochemicals that are broadly biocidal because they intercalate DNA and interfere with transcription (Berenbaum 1990). Selection for chemically-based resistance occurs in plant populations by increasing concentrations of three furanocoumarins: xanthotoxin, bergapten, and sphondin (Berenbaum et al. 1986; Zangerl and Berenbaum 1993). Genetic variation also exists in webworm populations in the rate at which these furanocoumarins are metabolized (Berenbaum and Zangerl 1992) indicating that plant chemistry can act as a selective force on insect physiology.

Like all copidosomatine encyrtids (Strand et al. 1991), *C. sosares* is a polyembryonic egg-larval parasitoid, in which females oviposit in the egg stage of their webworm host and progeny complete development in the host's final (sixth) instar (Hardy 1996; Ode et al. 2004). As the webworm continues development, *C. sosares* embryos clonally divide resulting in 100 to 400 genetically identical offspring (Ode et al. 2004). Owing to the development of *C. sosares* within the hemolymph of webworm larvae, immature *C. sosares* encounter unmetabolized furanocoumarins (McGovern et al. 2006). Additionally, *C. sosares* larvae lack the ability to metabolize these furanocoumarins (McGovern et al. 2006; Lampert et al. 2008). Field studies showed that two furanocoumarins; isopimpinellin and xanthotoxin, were negatively associated with *C. sosares* fitness correlates (Ode et al. 2004). The likelihood of *C. sosares* parasitism was negatively correlated with host plant isopimpinellin content, while xanthotoxin decreased within-brood survivorship and clutch size (Ode et al. 2004). Successful development of *C. sosares* embryos and larvae likely depends in part on the ability of the webworm to detoxify furanocoumarins as well as the furanocoumarin content of the host plant tissues consumed by the webworm (Ode et al 2004; McGovern et al. 2006; Lampert et al. 2008).

Introduction Histories of Wild Parsnip and Parsnip Webworm

Wild parsnip was first introduced to the United States (U.S.) as a food crop in the 17th century (Sturtevant 1890), where it escaped cultivation and spread throughout the U.S. Population genetics studies on wild parsnip in the U.S indicate wild parsnips have high levels of genetic diversity, comparable to their ancestral European populations (Jogesh et al. 2015). This indicates either one large population

containing genetically diverse wild parsnips were introduced or multiple introductions of wild parsnips from Europe have occurred.

By the 1860s, parsnip webworm had been accidentally introduced to the U.S. (Bethune 1869; Riley 1889) and has not only re-associated with its coevolved wild parsnip plant, but has also formed novel associations with cow parsnip (*Heracleum maximum*; Apiaceae), a plant native to the U.S. (Berenbaum and Zangerl 1991). Although parsnip webworm attacks other species of *Heracleum*, such as the common hogweed (*H. sphondylium*), which are chemically and phylogenetically closely related to wild parsnip (Batten et al. 1982; Downie et al. 2000) as hosts in Europe, its association with cow parsnip apparently dates back only to its arrival in the U.S. (Berenbaum and Zangerl 2006). Genetic data revealed that introduced U.S webworm populations are derived from a single (or few) webworm populations in the British Isles (Chapter 2). The webworm populations in the British Isles were themselves derived from parsnip webworm populations in Germany. Although it is unclear when parsnip webworms arrived in the British Isles, their populations have become genetically isolated from continental Europe based on significant correlations in pairwise F_{ST} values and geographic distance (Chapter 2).

Copidosoma sosares is also found in the U.S., but its arrival date is unclear. *C. sosares* attacks webworms on both wild parsnip and cow parsnip, but its distribution is patchy compared to parsnip webworms. Aside from a single site in the midwestern U.S. (Wisconsin), which was reported to have *C. sosares* in the summer of 2015 (Berenbaum, pers. obs.), *C. sosares* populations are restricted to the western U.S.

In this study, we formed our hypotheses from a set of factors which earlier work indicated might be important in structuring plant-webworm-*C. sosares* communities. These factors included webworm furanocoumarin-detoxification capabilities, host plant furanocoumarin concentrations, and the geographical locations of samples sites (i.e. distance and elevational differences between sites). We developed three hypotheses incorporating each factor. First, we hypothesized *C. sosares* populations in the U.S. came from the same European location as their webworm hosts in Europe (Host-Pursuit Hypothesis, Fig 4.1A). Second, once in the U.S., *C. sosares* followed a similar host plant switch onto cow

parsnip as parsnip webworms (Continued Host-Pursuit Hypothesis). Third, *C. sosares* populations will have limited geneflow between sites that are geographically farther apart, and differ in elevation (isolation by distance and elevation).

Specific to our first hypothesis, we asked, "From which European *C. sosares* populations are U.S populations derived?" As noted previously, all the webworm populations in the U.S. are derived from British Isles webworm populations (Chapter 2). Under the Host-Pursuit Hypothesis, species are predicted to disperse together from a shared origin, such that biotic associations that occurred in the ancestral range (and hence, potentially, coevolution) are maintained at the population level in the exotic range (Nicholls et al. 2010). A biotic association that is important between webworms and *C. sosares* is that of the furanocoumarin content of host plant tissue, and in turn, the furanocoumarin-metabolizing capacity of webworms. Webworms from different geographical regions vary in their metabolic capacities (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Berenbaum and Zangerl 2006; Carroll and Berenbaum 2006). Similarly, plant populations vary considerably in terms of the furanocoumarin profiles produced, both within a species (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003) and among species (Zangerl and Berenbaum 2003; Ode et al. 2004; Carroll and Berenbaum 2006).

If *C. sosares* populations are locally adapted to the detoxification profiles of webworms in the British Isles, then we would expect only *C. sosares* from the British Isles to have successfully established on U.S. webworms (Fig. 4.1A). The alternative to the Host-Pursuit Hypothesis is that of the Host-Shift Hypothesis (Nicholls et al. 2010), which predicts species may have dis-concordant dispersal pathways, and any re-association in the new range is independent of prior population history in the ancestral range. If we find U.S. *C. sosares* populations are derived from multiple European populations (Fig. 4.1A), this indicates webworms and *C. sosares* are not locally adapted and webworm detoxification capability is not structuring *C. sosares* populations in the introduced range. The Host-Shift Hypothesis does not preclude the predictions under the Host-Pursuit Hypothesis; *C. sosares* populations from the British Isles pursued parsnip webworm populations from the British Isles, but rather predicts additional European populations of *C. sosares* to be introduced, and associate with parsnip webworm populations in the U.S.

Specific to our second hypothesis we asked, "From which populations does *C. sosares* attacking webworms on cow parsnip in the U.S. come from?" Genetic analysis of U.S. webworm populations indicated webworm populations attacking cow parsnip did not come directly from European webworm populations (Chapter 2). Rather European webworm populations became established on U.S wild parsnip populations, then these webworm populations host plant switched onto cow parsnip (Chapter 2). The ability for parsnip webworm to form a novel association with cow parsnip has been discussed in Chapter 2. Briefly, in the midwest, the use of cow parsnip by webworms, even though they suffer higher predation and higher levels of furanocoumarins, could be due to a loss of host plant discrimination because of the reduced genetic variation during introduction. In the western U.S., webworm populations exhibited high levels of detoxification capabilities, regardless of the furanocoumarin content of the ingested host plant. Therefore, in the western U.S., those webworm populations on wild parsnip with high detoxification capabilities could host plant switch to cow parsnip. If *C. sosares* followed the same introduction pathways as webworms (hypothesis 1; Fig. 4.1A), including following webworms from wild parsnip onto cow parsnip, this would constitute a continued host pursuit by U.S. *C. sosares*, post-introduction (Fig. 4.1B). Collectively, this would indicate a sustained reciprocal association between webworms and *C. sosares* within the introduced range.

Conversely, U.S. *C. sosares* on cow parsnip may not be derived from U.S. *C. sosares* on wild parsnip, rather they could be directly derived from European *C. sosares*, on any host plant species (Fig. 4.1B). This could indicate one of two things. First, *C. sosares* can establish on webworm hosts they are not locally adapted to, and second, introduced *C. sosares* populations are able to readily switch host plant species. In an earlier study the furanocoumarin chemistry of western cow parsnip populations with *C. sosares* were found to have lower concentrations of all furanocoumarins except sphondin, when compared to cow parsnip populations attacked by webworms, but where *C. sosares* was absent (Carroll and Berenbaum 2006). In addition, western webworm populations did not differ significantly in their furanocoumarin-metabolizing capabilities (Carroll and Berenbaum 2006). Rather, western webworm populations had detoxification capabilities nearing the maximum of any levels found in midwestern

webworm populations (Berenbaum and Zangerl 1998; Zangerl and Berenbaum 2003; Carroll and Berenbaum 2006). The authors, Carroll and Berenbaum (2006), speculate that the lower furanocoumarin chemistry of cow parsnip in the western U.S. is more conducive to the survival of *C. sosares* than webworm detoxification capabilities and would indicate the host plant switch onto cow parsnip by *C. sosares*.

Our first and second hypotheses investigates the relative importance of webworms and the furanocoumarin content of host plant species in structuring *C. sosares* populations. Our third hypothesis investigates the influence of two geographical factors on the geneflow of *C. sosares* within the U.S: distance and elevation. As indicated in Fig. 4.4 and Table 4.1, *C. sosares* has been collected throughout the U.S., at elevations ranging from sea level to 2500 m (Carroll et al. 2007; pers. obs.) and distances spanning 145 km between the closest sites, with about 2410 km between the most distant edges of the *C. sosares* U.S. distribution. These vast distances, marked by geographical features such as mountain ranges and rivers, may create the most significant barrier impeding dispersal between *C. sosares* populations, and not features associated with their parsnip webworm hosts or host plant species.

Our approach is to use a combination of mitochondrial sequence and nuclear microsatellite data to identify genetic variation within and between *C. sosares* populations from introduced and ancestral ranges relevant to our hypotheses. First, we will determine the degree of genetic variation within European (native range) and U.S. (introduced range) *C. sosares* populations, and the source locations for U.S. *C. sosares* populations. If *C. sosares* underwent a genetic bottleneck in the U.S. and are most genetically similar to *C. sosares* collected from the British Isles, the Host-Pursuit Hypothesis is supported. On the other hand, a comparable level of genetic variation in the introduced range would indicate multiple introduction events, a result that would be further supported by finding high levels of genetic similarity between U.S. *C. sosares* populations and multiple populations in Europe, supporting the Host-Shift Hypothesis. Second, we will determine the source populations of *C. sosares* populations on cow parsnip. If *C. sosares* populations on cow parsnip are derived from *C. sosares* populations on U.S. wild parsnip, then *C. sosares* has continued to pursue its webworm hosts within the introduced range

(Continued Host-Pursuit Hypothesis). Lastly, we will determine the migration/immigration rates between *C. sosares* sites in the U.S., and whether distance and/or elevation is correlated with the genetic distance between sites.

Methods

Insect sampling and DNA extraction

Copidosoma sosares samples were obtained by collecting larvae of parsnip webworms in the native and introduced ranges between 2004 and 2013. In Europe, parsnip webworm larvae were collected at four sites feeding on wild parsnip and from 12 sites feeding on hogweed. In the U.S., parsnip webworm larvae were collected on five sites feeding on wild parsnip and from 14 sites feeding on cow parsnip. Parsnip webworm larvae that weren't readily identified as being parasitized (the parasitoids had not begun pupation) were brought back to the laboratory and reared in a 27ºC, LD 16:8 h incubator until either the webworms pupated (not parasitized) or became a mummy (parasitized). After emerging. each *C. sosares* brood was sexed and immediately frozen in an -80°C freezer. In total, 401 broods of *C. sosares* were collected; 123 from Europe and 278 from the U.S. (Table 4.1).

Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) in accordance with the manufacturer's instructions for solid tissue. A single, adult *C. sosares* wasp from each brood was ground using a Kontes pestle (Kimble) and incubated in the extraction mixture for 3 hours at 56°C. Isolated DNA was checked for quantity and purity using the BioTek Epoch Take3 plate reader.

Mitochondrial haplotypes

A variety of mitochondrial genes were amplified to screen for intraspecific variable markers, including the cytochrome oxidase subunit 1 (COI), tRNA-Leucine, cytochrome *b*, NADH dehydrogenase subunit 1, the large ribosomal subunit (16S), and the small ribosomal subunit (12S) using primers described in Table 4.2. Ultimately one mtDNA gene, cytochrome *b* (cyt *b*), was chosen for phylogenetic analyses based on its high amplification success and high variability within and between collections. Polymerase chain reactions (PCR) were performed on either an Eppendorf Mastercycler gradient thermal cycler (www.eppendorfna.com) or a Thermo Scientific Arktik thermal cycler

(www.thermoscientific.com). PCR reactions were performed in a 20 µL reaction volume containing 2.5 µL of 10X PCR Buffer (100mM Tris-HCl, 500mM KCl, 15 mM $MgCl_2$), 2.5 mM of each dNTP, 5 pmol of each primer, 0.625 U of TaKaRa Taq HotStart Version polymerase (Clontech), 2.5 µL of genomic DNA template, and up to 20 µL of sterile distilled water. The thermal profile consisted of an initial denaturation for 2 minutes at 94°C, followed by 40 cycles of 30 seconds at 94°C, 30 seconds at 48-55°C, and 30 seconds at 65°C, with a subsequent final 5-minute extension at 70°C. Successfully amplified samples were sent to the University of Arizona Genetics Core for clean-up, quantification, and DNA sequencing on an ABI 3730 DNA Analyzer (Applied Biosystems). Each sample was sequenced in both directions using PCR primers. Sequence data were manually checked using CHROMASLITE and reverse sequences were used to resolve ambiguous base calls in the forward sequence. The sequencing region was checked for an open reading frame using EXPASY online translation tool using the invertebrate mitochondrial codon table. Sequences were aligned using CLUSTAL W (Thompson et al. 1994). No insertions, deletions, or stop codons were observed. Sequences of the mitochondrial cyt *b* region were truncated to the same length (387bp) and translated to amino acid sequences to check for nuclear mitochondrial pseudogenes (numts). The haplotype sequence matrix for cyt *b* was used for all subsequent phylogenetic analyses.

Microsatellite genotyping

To increase the resolution of genetic structure and to corroborate the independent evolutionary status of mitochondrial lineages (Edwards 2009), we genotyped 354 (214 females, 140 males) of the 404 individuals for 4 nuclear microsatellite loci (Csos 1, 2, 3, and 4) following protocols described in chapter 3. PCR fragments were sized on an ABI 3730 DNA Analyzer (Applied Biosystems) using capillary electrophoresis and scored using the software GENEMAPPER v4.0 (Applied Biosystems).

Phylogenetic inference from mtDNA

Standard molecular diversity indices were calculated using DNASP v5 (Librado and Rozas 2009; http://www.ub.edu/dnasp/). Diversity calculations included the number of haplotypes, the number of segregating (polymorphic) sites, the nucleotide diversity, the mutation rates within codon position, and

haplotype diversity of all variable nucleotide sites. The DNA polymorphism using the sliding window method was estimated for the determination of the number of segregating sites (S) across each portion of the mtDNA sequences, set with a window length=100 and a step size = 25 (Librado and Rozas, 2009).

A phylogenetic tree was constructed using the maximum-likelihood method in the program package *MEGA* version 7 (Kumar et al. 2016). The tree was rooted using the congener *C. floridanum* obtained from a laboratory colony maintained at Colorado State University.

Analyses of mtDNA

Genetic diversity within populations was estimated by computing haplotype diversity (*H*) and nucleotide diversity (π). Haplotype diversity (also known as gene diversity) represents the probability that two randomly sampled alleles are different, while nucleotide diversity is defined as the average number of nucleotide differences per site in pairwise comparisons among DNA sequences. Partitioning of genetic variation within and among populations was calculated using analysis of molecular variance (AMOVA; Excoffier et al. 1992), by computation of conventional *F*-statistics from haplotypes with 10,000 permutations as implemented in the program ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). More specifically, genetic variation was calculated within Europe (native range), and within the U.S (introduced range) to test whether introduced U.S *C. sosares* populations had similar levels of genetic variation to European populations. To test whether *C. sosares* populations are structured around host plant species within their ranges (introduced or native), another series of AMOVA analyses were conducted: 1) among European individuals collected from wild parsnip or hogweed, and 2) among U.S. individuals collected from cow parsnip or wild parsnip.

We tested two hypotheses of genetic isolation: 1) by geographical distance, using linear geographic distances and 2) by elevation, using the difference in elevation between collection sites. Both hypotheses were assessed with the Mantel test (Mantel 1967) with 10,000 permutations, using Slatkin's linearized F_{ST} (Slatkin 1995).

To determine if U.S. *C. sosares* populations were derived from the British Isles (Host-Pursuit Hypothesis) or from multiple locations in Europe (Host-Shift Hypothesis) pairwise linearized F_{ST} (Slatkin

1995) were estimated as implemented in ARLEQUIN v. 3.5 by haplotype frequencies. Very low and non-significant F_{ST} values between populations indicate a close relationship, whereas high and significant F_{ST} values between populations indicate no or very distant relationship. To determine the source population(s) of U.S. *C. sosares* wasps attacking webworms on cow parsnip (hypothesis 2), we conducted a series of five AMOVA analyses: 1) between European wasps collected from wild parsnip and U.S. wasps collected from cow parsnip, 2) between European wasps collected from hogweed and U.S. wasps collected from cow parsnip, 3) between U.S. wasps collected from wild parsnip and U.S. wasps collected from cow parsnip, 4) between European individuals collected from hogweed and U.S individuals collected from wild parsnip, and 5) between European individuals collected from wild parsnip and U.S. individuals collected from wild parsnip. The first three analyses tested the Continued Host Pursuit Hypothesis directly, while the last two runs tested the possibility of alternative source populations.

Analyses of microsatellite data

Individuals were screened for 4 loci with 3 to 9 (mean = 5.5) alleles per locus. As wasps are haplodiploid, with haploid males and diploid females, only diploid (female) genotypes were initially used to assess concordance with the assumptions of further population genetic analyses. Departure from Hardy-Weinberg and linkage disequilibrium were tested using ARLEQUIN v. 3.5 within each of the sampling regions specified in Table 4.1. Significance levels were adjusted for multiple tests using a sequential Bonferroni correction and a table-wide alpha of 0.05. Significant departures were obtained for 11 of 60 tests of Hardy-Weinberg equilibrium and 9 of 856 tests of linkage equilibrium; however, these departures showed no consistency across loci or populations so all data were included in subsequent analyses.

Phylogeographic structure in the complete microsatellite data was assessed using STRUCTURE v. 2.3.4 (Pritchard et al. 2000) on European and U.S. wasps to identify the number (K) of discrete population clusters in the data, and then mapping the geographic extent of these cluster. Analyses incorporating K values of 1-18 were run for 100,000 generations with a burn-in of 1,000 generations, with convergence in estimated parameter values checked over 20 independent runs for each K. The K values having the

highest levels of convergence were re-run (K=1-9) for 750,000 generations with a burn-in of 500,000 generations, with convergence in estimated parameter values checked over 20 independent runs for each K. The ΔK method (Evanno et al. 2005) estimated the most likely value of K. An admixture model was used, allowing individuals to have ancestry from multiple populations, with population allele frequencies assumed to be correlated among populations. STRUCTURE also quantifies the likelihood that each wasp belongs to each of the K clusters and assigns wasps to a cluster based upon this information. The program DISTRUCT (Rosenberg 2004) was used to display membership likelihood coefficients. Cluster membership was represented as different colors, and individual wasps were depicted as vertical bars partitioned into segments that correspond to membership coefficients in each of the nine collection sites in the U.S. A neighbor-joining tree (Saitou and Nei 1987) was constructed using F_{ST} distance measures (Latter 1972) in the program POPTREE2 (Takezaki et al. 2010). Node support was assessed from 10,000 bootstrap replicates (Felsenstein 1985).

The migration rate and population size were estimated in MIGRATE 3.0.3 (Beerli 2009) using coalescent and maximum likelihood inference (Beerli 1998; Beerli and Felsenstein 1999, 2001). The single step model for microsatellite data was used, and missing data was excluded from the analyses. The Markov chain settings were as follows; 50,000 short chain trees sampled, 500 recorded; 500,000 long chain trees sampled, 5,000 recorded; burn-in per chain = 1,000,000. The effective migration rate $4N_em$ was calculated between a donor and recipient population as M (generated from an F_{ST} calculation) multiplied by the recipient population θ (generated from an F_{ST} calculation).

If $M = 1$, the rate that new alleles enter a population through immigration is equal to the rate new alleles arise by mutation. A $4N_em < 1$ suggests that immigration is insufficient to offset the effects of genetic drift so that populations diverge in allele frequencies. A $4N_em > 1$ suggests that there is sufficient immigration to offset the effects of genetic drift to maintain homogeneity among populations. We compared each pairwise $4N_em$ value for all collection sites to determine the direction and rate of gene flow between the collections.

Results

Phylogenetic analyses

A total of 401 *C. sosares* individuals, all sequenced at a 387 bp length of the cyt *b* gene were used in analyses. Overall there were 50 polymorphic (segregating) sites leading to the definition of 61 haplotypes (Figs. 4.2, 4.3, 4.4). There were 81 synonymous sites and 305 nonsynonymous sites, owing to the increased frequency of A-T nucleotide bases across the entire sequence (0.788). The majority of individuals fell within Hap1 (n=313) and there was at least one individual from each collection site with the Hap1 sequence. Therefore, Hap1 represents *C. sosares* individuals collected in the native range, the introduced range, and from all host plant species; wild parsnip, cow parsnip, and hogweed (Fig. 4.4). Eight haplotypes were shared between Europe and the U.S (Hap1, Hap8, Hap5, Hap11, Hap12, Hap13, Hap 26, Hap46), whereas 29 haplotypes were unique to the U.S and 24 haplotypes were unique to Europe (Fig. 4.4). Overall, across all collection sites, the haplotype diversity was 0.399, and the mutation rates of the different codon positions were 0.5226, 0.445, and 2.0323 for 1st, 2nd, and 3rd positions, respectively.

A total of 214 female individuals were analyzed across four microsatellite loci. The average number of alleles per locus was 3.75 across all sites. The average observed heterozygosity across all loci and sites was 0.348 ± 0.148. A total of 142 U.S. and 72 European female individuals were analyzed, and the ΔK method identified four genetic groups in the U.S and Europe.

Genetic diversity within introduced C. sosares populations

The molecular diversity indices within Europe and U.S revealed a high level of haplotype diversity in Europe ($\hat{\theta}_\pi$ = 1.29 ± 0.90), and a lower level in the United States ($\hat{\theta}_\pi$ = 0.65 ± 0.56). This is further supported by the cyt *b* AMOVA analyses within and between European and U.S. populations. There was a significant difference between European collections sites ($P < 0.05$), with the highest percent of variation occurring within each site (92.85%, Table 4.4A). On the other hand, there was no significant differences among sites in the U.S. ($P = 0.22$). The low genetic diversity of U.S. *C. sosares* populations is additionally supported by the cyt *b* pairwise linearized F_{ST} values. Within the U.S the only significantly

high pairwise Slatkin's values was that between the Elk Mountains and all other U.S *C. sosares* populations, whereas all other U.S. sites were similar (Table 4.7).

Source populations of U.S. C. sosares populations

The origins of U.S. *C. sosares* populations were assumed to be European, and our AMOVA (Table 4.4A) and F_{ST} values using the cyt *b* dataset confirms this assumption (Fig. 4.3). Overall, there was no significant difference between all European and all U.S. *C. sosares* sites (AMOVA $P = 0.12$), which is further corroborated by several pairwise associations between continental sites that were genetically similar. For example, pairwise F_{ST} values indicate three sites in the U.S. (Medicine Bow Mountains, Elk Mountains, Oregon) shared genetic similarity with all European sites, and one site (Black Hills) was similar to all European sites, except Denmark. Additionally, all U.S. sites were genetically similar to the Netherlands and the British Isles. The only European sites genetically different from the majority of U.S. sites were those of Denmark ($F_{ST} = 0.34 - 0.44$) and Germany ($F_{ST} = 0.13 - 0.47$; Table 4.7).

Contrary to the cyt *b* findings, which traces historic rates of geneflow, our microsatellite dataset indicates an overall significant genetic difference between all European and all U.S. *C. sosares* populations (AMOVA $P < 0.05$; Table 4.4B). This difference could be attributed to two things. First, microsatellite markers trace more current rates of geneflow compared to mitochondrial markers. Second, genetic drift after the initial introduction of *C. sosares* into the U.S. could have occurred, which might not be recovered from our mtDNA, but rather from our microsatellite data. Additionally, the high degree of genetic similarity between numerous pairwise F_{ST} values between U.S and European sites in our cyt *b* dataset was not fully recovered in our microsatellite dataset (Table 4.8). Rather, only four U.S. sites shared a genetic similarity with any European sites ($P > 0.05$): Wasatch Mountains and British Isles sites, Oregon and the Netherlands and Denmark, Montana and the Netherlands, and Wisconsin and Germany and Belgium. The neighbor-joining tree constructed from F_{ST} values of our microsatellite dataset positioned all U.S. sites except Wisconsin into the same clade, with Netherlands and British Isles samples posterior to this U.S. clade (Fig. 4.3B). Collectively, this indicates that the British Isles and Netherlands served as a likely source of *C. sosares* to the U.S. (as indicated by the strong cyt *b* haplotype signal), with

subsequent introductions from European countries to a few sites in the U.S. (as indicated by microsatellite pairwise F_{ST} values), fitting predictions under the Host-Shift Hypothesis.

U.S. C. sosares on cow parsnip are derived from European hogweed and U.S. wild parsnip populations

The AMOVA analyses within European and within U.S. populations based on host plant species were not significant for either cyt *b* ($P > 0.05$; Table 4.5A) or the microsatellite dataset ($P > 0.05$; Table 4.5B). Within Europe, hogweed sites did not significantly differ from those *C. sosares* collected from wild parsnip sites (cyt *b*, $P = 0.10$; microsatellite, $P = 0.39$; Table 4.5). Within the U.S, wild parsnip sites did not significantly differ from those *C. sosares* collected from cow parsnip sites (cyt *b*, $P = 0.71$; microsatellite, $P = 0.71$). In tests comparing host plant species associations between continents, European *C. sosares* from wild parsnip sites and U.S. *C. sosares* from wild parsnips were not significantly different (cyt *b*, $P = 0.06$; microsatellite, $P = 0.51$; Table 4.6). European *C. sosares* from hogweed sites and U.S. *C. sosares* from wild parsnips were not significantly different (cyt *b*, $P = 0.56$ microsatellite, $P = 0.65$; Table 4.6). However, European *C. sosares* from wild parsnip and U.S. *C. sosares* from cow parsnip were significantly different (cyt *b*, $P < 0.05$; microsatellite, $P < 0.0001$; Table 4.6). Between European *C. sosares* hogweed and U.S. *C. sosares* cow parsnip populations we obtained contradictory results from our two datasets. There was no significant difference according to our cyt *b* dataset ($P = 0.20$; Table 4.6A), but there was with our microsatellite dataset ($P < 0.0001$; Table 4.6B). This indicates European *C. sosares* collected from either host plant species (hogweed or wild parsnip) are genetically similar to U.S *C. sosares* collected from wild parsnip. However, this relationship does not hold true for U.S. cow parsnip. Rather European *C. sosares* collected from wild parsnip is significantly different from U.S. *C. sosares* collected from cow parsnip plants. Overall, this suggests *C. sosares* populations on wild parsnip in the U.S. are derived from European *C. sosares* populations on both host plant species (hogweed and wild parsnip). Some of these U.S. *C. sosares* populations on wild parsnip then switched to attacking webworms on cow parsnip plants, following the pattern of host plant switching by parsnip webworms (Chapter 2; Continued Host-Pursuit). However, European *C. sosares* populations on hogweed are

genetically similar, indicating these populations in Europe could directly establish on U.S. cow parsnip, which do not support our predictions under the Continued Host-Pursuit Hypothesis.

Migration patterns among U.S. C. sosares populations

Rates and directions of gene flow among U.S. collections were estimated for all microsatellite loci using the program MIGRATE and Table 4.9 lists the average $4N_em$ among U.S. collections. Our results indicate a high degree of bidirectional geneflow between the majority of sites in the U.S., a pattern supported by the genetic similarity between most of the U.S. sites found with our F_{ST} analysis. Montana and the Bighorn Mountains receive, on average, the most reproductive immigrant individuals per generation (Montana, $4N_em$ = 7.47; Bighorn Mountains, $4N_em$ = 6.63) and have the most reproductive migrant individuals going to other sites in the U.S. (Montana, $4N_em$ = 4.16; Bighorn Mountains, $4N_em$ = 3.53). Sangre de Cristo Mountains, on average, has the least number of immigrants ($4N_em$ = 1.83) and migrants ($4N_em$ = 1.75). There is bi-directional geneflow between Wisconsin and the Sangre de Cristo Mountains (migrant $4N_em$ = 3.03, immigrant $4N_em$ = 2.89) and Montana (migrant $4N_em$ = 9.89, immigrant $4N_em$ = 12.87). Additionally, Wisconsin receives 2.39 to 6.29 reproductive immigrant individuals from multiple other U.S. sites (Black Hills, Bighorn Mountains, Elk Mountains, Wasatch Mountains, San Juan Mountains), but very few migrants from Wisconsin go to these same sites ($4N_em$ = 0.23 – 1.35), indicating the prevalence of nonequilibrium geneflow at the Wisconsin site.

The Wisconsin site was also genetically dissimilar from the majority of U.S. sites (San Juan Mountains, Elk Mountains, Wasatch Mountains, Bighorn Mountains, and the Black Hills) according to our F_{ST} analysis at a highly significant level, $P < 0.001$. Sangre de Cristo Mountains and Montana were also genetically dissimilar from Wisconsin, contradicting our MIGRATE analysis. However, Montana was only slightly significantly different from Wisconsin (P = 0.03). Although the Oregon site is geographically far from other U.S. sites in our study, there is a high amount of geneflow between Oregon and the Bighorn Mountains (migrant $4N_em$ = 7.41, immigrant $4N_em$ = 10.57), the Wasatch Mountains (migrant $4N_em$ = 4.50, immigrant $4N_em$ = 3.36), the San Juan Mountains (migrant $4N_em$ = 5.85, immigrant $4N_em$ = 4.60), and Montana (migrant $4N_em$ = 4.05), which are the four sites closest to Oregon.

Collectively, this indicates that a high degree of bi-directional geneflow occurs between the majority of U.S. sites. The one exception is the Sangre de Cristo Mountains on the southern edge of our collecting range that experiences low levels of geneflow, from either migrants or immigrants to the site. Wisconsin, on the eastern edge of our collecting range, experiences high levels of geneflow, but the majority of reproductive individuals per generation are immigrants into Wisconsin, and only a couple of sites (Sangre de Cristo Mountains and Montana) receive migrants from Wisconsin.

Our correlation analysis revealed the lack of a significant association between genetic and geographic distance (Mantel test, U.S. P=0.12; Europe, P = 0.30; Fig. 4.3), and elevational differences (Mantel test, U.S., P = 0.27; Europe, P = 0.72; Fig. 4.4) within the U.S or Europe. Therefore, the genetic isolation of the Sangre de Cristo Mountains compared to the other U.S. sites is not caused by geographic distance nor elevational differences.

Discussion

Our molecular data show that *C. sosares* introductions and associations in the U.S. have followed the predictions from the Host-Shift Hypothesis. Although the British Isles was a source population for U.S. *C. sosares* populations, as for parsnip webworms (supporting the Host-Pursuit Hypothesis); the Netherlands and several other European sites served as sources for *C. sosares*. Our finding of multiple source populations was supported by pairwise F_{ST} comparisons with both the cyt *b* and microsatellite markers, and further by the clustering of all *C. sosares* samples into just four genetic clusters across the introduced and native ranges. A similar pattern was reported for other co-introduced herbivore-parasitoid systems, including the parasitoid *Megastigmus stigmatizans* on the invasive gall wasp in Europe (Nicolls et al. 2010), and the parasitoids *Pediobius saulius* and *Pnigalio mediterraneus* on the invasive chestnut leaf-mining moth (Valade et al. 2009; Lees et al. 2011; Hernandez-Lopez et al. 2012, Gebiola et al. 2013), in which the invading natural enemy populations were derived from numerous sources: allopatric, sympatric and/or a novel host species.

Although introduced *C. sosares* populations had lower genetic diversity compared to their native range in Europe, this was not a significant difference. This finding runs contrary to the parsnip webworm,

which had a single (or few) introductions to the U.S. from the British Isles, resulting in a genetic bottleneck. The accidental introduction of parsnip webworm and *C. sosares* into the U.S. was thought to be a direct result of the intentional introduction of wild parsnip. However, *C. sosares* could exploit different introduction routes than parsnip webworm, becoming established on U.S. webworms that lacked a shared population history with *C. sosares* in Europe.

The variability of *C. sosares* distribution in the U.S., and the lack of *C. sosares* in the eastern U.S., has been attributed to the variation in the ability of parsnip webworms to metabolize host plant toxins (Fig. 2.1; Berenbaum and Zangerl 1992; Ode et al. 2004 Lampert et al. 2008; Carroll and Berenbaum 2006; Carroll et al. 2007). Webworm populations in the midwestern U.S have been documented attacking cow parsnip and wild parsnip populations with high levels of furanocoumarins. Yet, these webworm populations are mis-matched to the host plant chemical profiles, in terms of exhibiting less efficient furanocoumarin-metabolizing capabilities (Fig 2.1F, G). In the western U.S., webworms on both wild parsnip and cow parsnip have high levels of detoxification capability, reaching the maximum levels seen in midwestern and European webworm populations (Carroll and Berenbaum 2006). The lack of *C. sosares* populations in some U.S. sites could be due to an inability of *C. sosares* to parasitize webworms with different metabolism capabilities. As a result, eastern U.S. webworm populations may be poorer hosts for *C. sosares* in terms of reduced host survivorship or body size if these webworms contain higher levels of furanocoumarins in their hemolymph where they would be encountered by developing parasitoids. However, the multiple European sites serving as introduction sources, the high levels of geneflow between sites with varying host plant species, and elevations, suggest *C. sosares* can establish on different host plant species and parasitize webworms with various metabolism capabilities.

Considering these findings, the spread of *C. sosares* into Wisconsin, which is the farthest east in the U.S. *C. sosares* has ever been documented, could be attributed to random chance alone. Geographically speaking, the most likely source populations could be from the Black Hills in western South Dakota, because it is geographically closer to Wisconsin than any other *C. sosares* site, albeit still 1,030 km away.

Although migrants from the Black Hills have arrived in Wisconsin, the genetic structure of Wisconsin *C. sosares* individuals indicates a more complex history. Our mtDNA haplotype and microsatellite data indicate *C. sosares* Wisconsin individuals are genetically more similar to Germany and Belgium then *C. sosares* individuals from other U.S sites based on F_{ST} values. However, our microsatellite MIGRATE analysis indicate geneflow coming into Wisconsin from multiple U.S. sites, including the Black Hills ($4N_em = 3.24$), the Bighorn Mountains ($4N_em = 4.66$), Elk Mountains ($4N_em = 2.39$), Sangre de Cristo Mountains ($4N_em = 2.89$), Wasatch Mountains ($4N_em = 6.29$), San Juan Mountains ($4N_em = 4.02$), and Montana ($4N_em = 12.87$). Of these sites however, the Black Hills and Elk Mountains share mtDNA haplotype similarity with Germany and Belgium ($P > 0.05$), while the Bighorn Mountains, Sangre de Cristo Mountains, San Juan Mountains share mtDNA haplotype similarity with Belgium ($P > 0.05$). Therefore, U.S *C. sosares* individuals derived from Belgium and/or Germany are migrating to Wisconsin, but the highest number of migrants into Wisconsin are those U.S. *C. sosares* with no genetic similarity between Belgium and Germany (Montana and Wasatch Mountains). As a result, Wisconsin could share a high genetic similarity with Germany and Belgium indirectly (from U.S. *C. sosares* individuals derived from Belgium and Germany migrating to Wisconsin), or directly from *C. sosares* migrants from Germany (supported by mtDNA haplotype and microsatellite genotype similarity) and Belgium (supported by microsatellite genotype similarity).

Additionally, we hypothesized *C. sosares* attacking webworms on cow parsnip in the U.S. followed the same pattern of host plant switching as their parsnip webworm hosts (Continued Host-Pursuit Hypothesis). We based this hypothesis on the idea that webworms and *C. sosares* are locally adapted to one another, with their interactions are shaped by the furanocoumarin content present in a host plant species tissue. Our results indicate U.S. *C. sosares* collected from wild parsnip are derived from European *C. sosares* collected on both wild parsnip and hogweed plants. Cow parsnip populations harboring *C. sosares* in the U.S. were different, genetically, then European *C. sosares* found on wild parsnip plants. Cow parsnip is native to the U.S. and does not occur in Europe, therefore all introduced parsnip webworms and *C. sosares* that host-switched to cow parsnip were derived from either European

wild parsnip and/or hogweed. Genetic analysis of parsnip webworms (Chapter 2) indicated European webworms from hogweed populations were introduced and established on U.S. wild parsnip plants. At some point, these introduced webworms then host plant switched to cow parsnip. There was a lack of genetic similarity between European webworms and U.S webworms on cow parsnip, indicating webworms on cow parsnip populations started to diverge genetically from webworms in their native range. Our genetic analysis of *C. sosares* on cow parsnip populations reveal a more complex pattern of host plant switching. European *C. sosares* from webworm populations attacking both wild parsnip and hogweed populations followed webworms onto U.S. wild parsnip plant populations. At some point, U.S. *C. sosares* started attacking webworms that had host plant switched onto cow parsnip. Yet, unlike webworms, *C. sosares* on cow parsnip is still genetically similar to *C. sosares* on wild parsnip. The association between European *C. sosares* on hogweed and U.S. *C. sosares* on cow parsnip is unclear due to the contradictory results from our two molecular markers. According to our cyt *b* analysis there remains a genetic similarity between *C. sosares* on cow parsnip and European *C. sosares* on hogweed plants. However, our microsatellite analysis indicates the opposite; there is no similarity between *C. sosares* on cow parsnip and European *C. sosares* on hogweed plants. In general, mitochondrial markers trace historical patterns of geneflow, whereas microsatellite markers trace more recent patterns of geneflow. Accordingly, our data indicates European *C. sosares* populations on hogweed directly established on U.S. cow parsnip plants, but this has not occurred recently. The ability of C. *sosares* hogweed populations to establish on cow parsnip plants fits ecologically, given hogweed shares phylogenetic proximity to U.S. cow parsnip.

Conclusion

Within the multitrophic system consisting of wild parsnip, parsnip webworm, and *C. sosares*, the introduction history and introduction pathways have been different for each trophic level. The wild parsnip has been introduced to the U.S. multiple times, most likely due to its cultivation as a food crop (Jogesh et al. 2015). This initial introduction of wild parsnip runs contrary to both parsnip webworm and *C. sosares* that have been accidentally introduced to the U.S. Accidental introduction by anthropogenic

causes (e.g., trade, travel) can create vary different introduction patterns between co-evolved species, resulting in seemingly contradictory patterns of introduction. For the parsnip webworm, its introduction into the U.S. has been rather restrictive, consisting of only British Isles populations of parsnip webworm (Chapter 2). On the other hand, *C. sosares* has exploited multiple routes of introduction, from multiple sources in Europe to re-associate with sympatric populations of parsnip webworms derived from the British Isles; also allopatric populations of *C. sosares* have associated with these same British Isles-derived parsnip webworms. Both parsnip webworm and *C. sosares* have established populations on the novel, native host plant species, cow parsnip. Although host plant species is driving webworm population structure in the U.S. (Chapter 2), this is not the case for *C. sosares*. Instead, European *C. sosares* populations from hogweed have directly established on U.S. cow parsnip. Additionally, U.S. *C. sosares* populations on wild parsnip have host plant switched to cow parsnip, but *C. sosares* geneflow is not restricted to either host plant species, contrary to our findings with U.S. webworms (Chapter 2).

Overall, the factors that can influence the establishment of an herbivore (i.e., host plant species, concentrations of plant toxins) in an introduced area, may not be significant for its primary parasitoid. Similarly, in a study between introduced oaks, cynipid oak gall wasps (*Andricus kollari*), and its parasitoid (*Megastigmus stigmatizans*), Nicholls et al (2010), found differences in the factors driving the establishment between oak gall wasps and *M. stigmatizans*. In their study, the authors set out to examine the roles of coevolution, ecological fitting, and anthropogenic disturbance in the establishment of *M. stigmatizans* in Europe (Nicholls et al. 2010), building upon earlier research on the cynipid oak gall wasp (Stone and Sunnucks 1993; Stone et al. 2001, 2002). Genetic data from European *M. stigmatizans* populations collected from Turkey oaks revealed some populations had Balkan origins just as their gall wasp hosts, but others had an Iberian origin. This latter result was surprising, because Iberian gall wasps are confined to cork oak and do not occur on Turkey oaks. Although Iberian gall wasps can't host plant switch to Turkey oaks, its parasitoid, Iberian *M. stigmatizans*, can successfully colonize gall wasps that utilize different host plant species. Overall, this system indicates that a long evolutionary history can be

important between one trophic link (oak and gall wasp), but not the other trophic link (gall wasp and parasitoid).

More studies on the co-introduction of multitrophic systems can reveal the relative importance of coevolutionary relationships in the ancestral range in forming associations in the introduced range. Ultimately, these studies can help predict the consequences of other biological invasions and inform biological control of these invasive organisms.

Figures

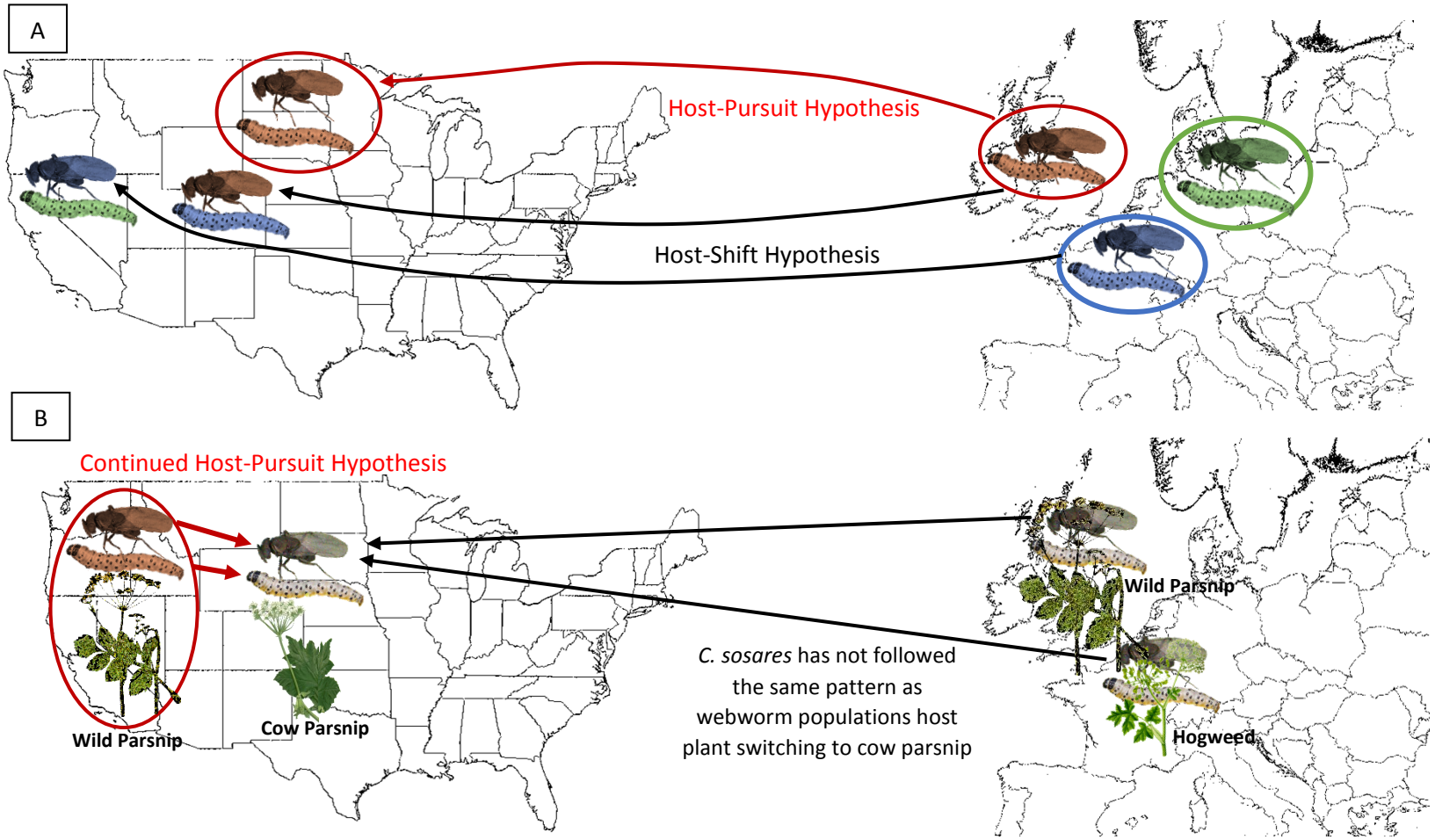


Figure 4.1: Illustration of the introduction history, and dispersal patterns of *C. sosares* populations as predicted under the Host-Pursuit, Host-Shift, and Continued Host-Pursuit Hypotheses in the U.S. (introduced range) and Europe (native range). A: Webworms and *C. sosares* that are locally adapted to each other are the same color. Interactions in the U.S. where the two species do not share population histories are not the same color.

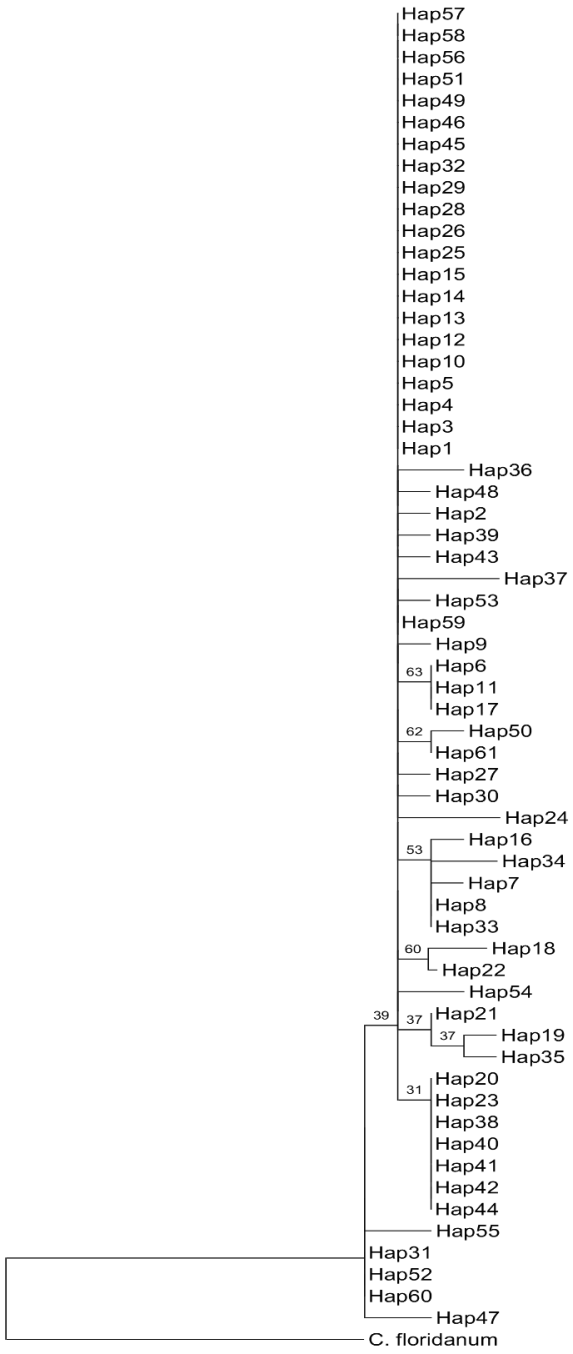


Figure 4.2: Phylogenetic tree of haplotype sequence data using maximum likelihood method based on the JTT matrix-based model (Jones et al. 1992). The tree with the highest log likelihood (-938.3542) is shown. Bootstrap values > 30 are shown at the branches, values < 30 were collapsed and not shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4631)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

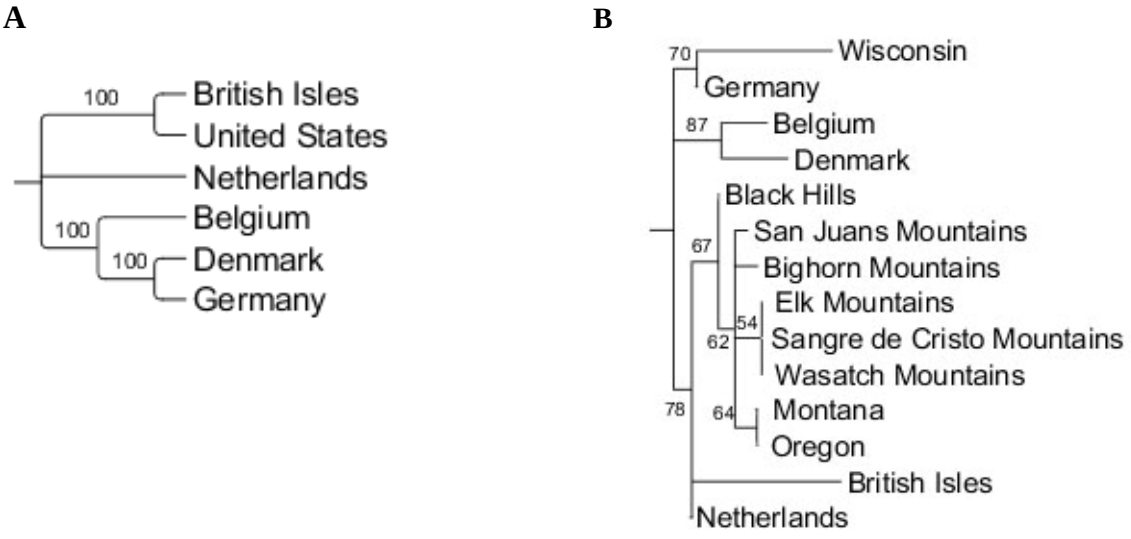
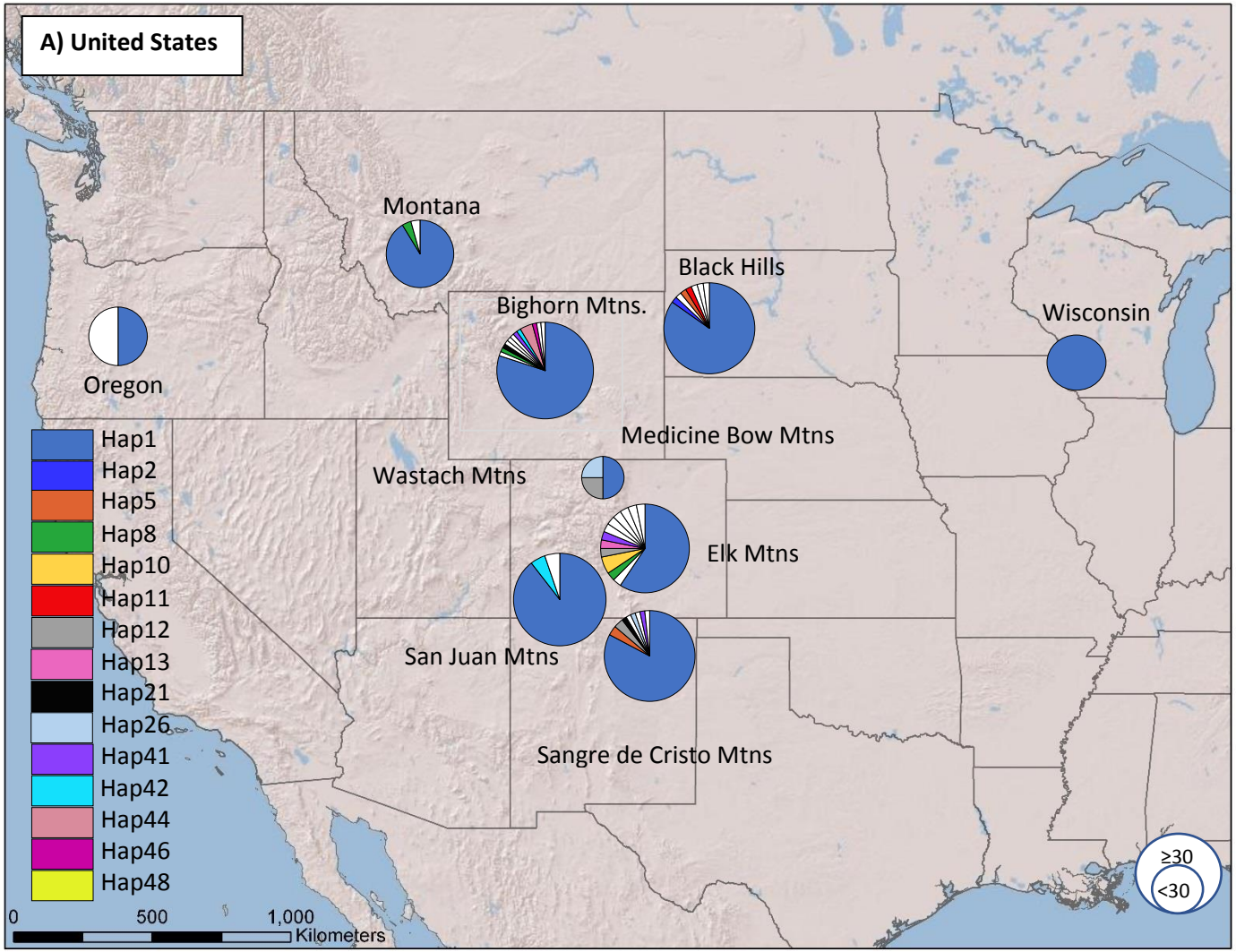


Figure 4.3: Neighbor-joining trees for European and U.S. *C. sosares* populations using mtDNA (A) and microsatellite (B) data. The U.S populations were collapsed together in the mtDNA dataset because of the lack of haplotype diversity. Construction of the neighbor-joining tree (Saitou and Nei 1987) and calculation of the distance matrix (F_{ST}, Latter 1972), was performed in PopTree2 (Takezaki et al. 2010), with 10,000 bootstrap (Felsenstein 1985) replications. Bootstrap values >50 are listed at the branches, values <50 are collapsed and not shown.


A) United States
Montana
Black Hills
Bighorn Mtns.
Wisconsin
Oregon
Medicine Bow Mtns
Wastach Mtns
Elk Mtns
San Juan Mtns
Sangre de Cristo Mtns
Hap1
Hap2
Hap5
Hap8
Hap10
Hap11
Hap12
Hap13
Hap21
Hap26
Hap41
Hap42
Hap44
Hap46
Hap48
0
500
1,000
Kilometers
≥30
<30

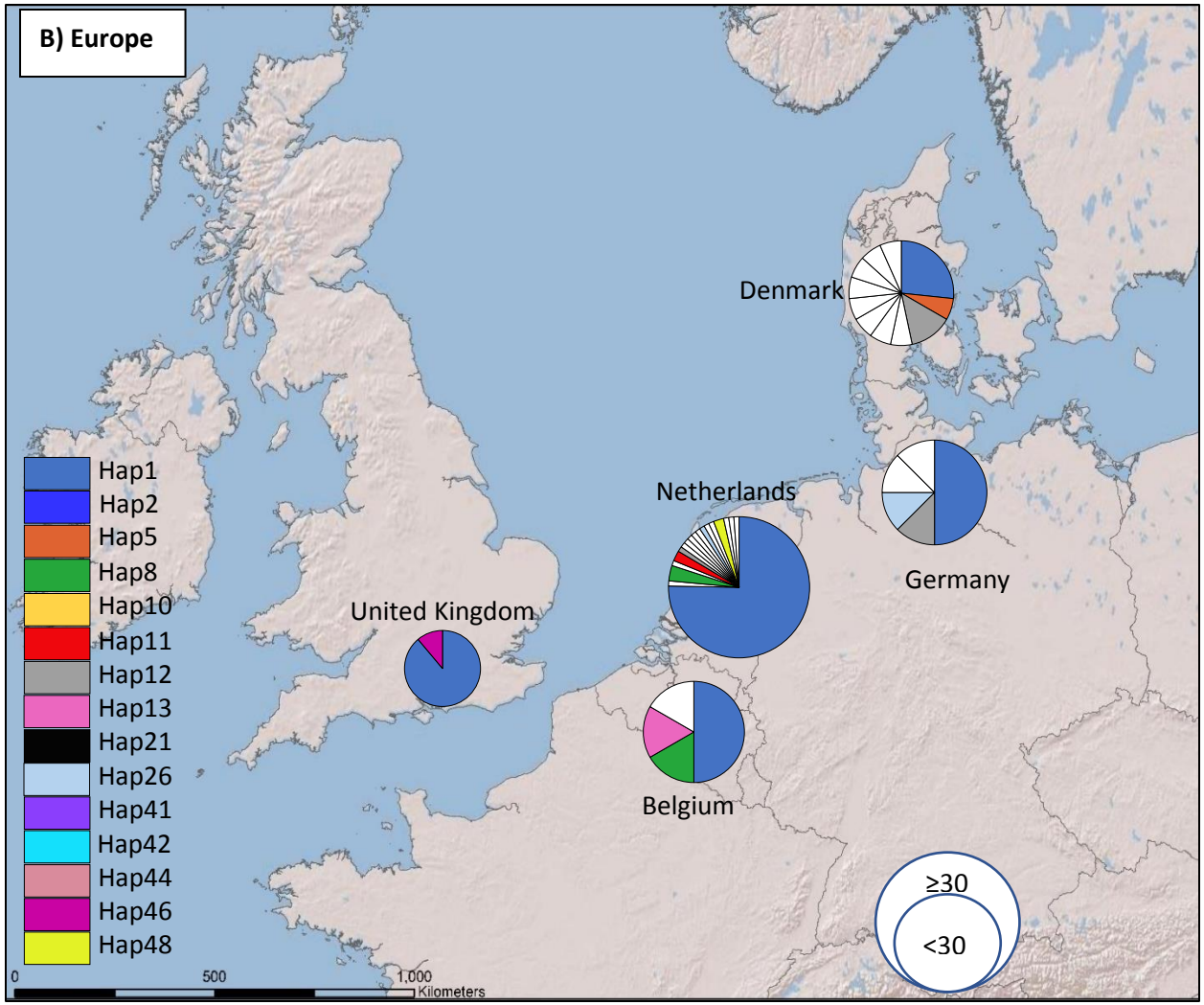


Figure 4.4: Proportion of individuals with each cyt *b* haplotype at each collection site (A: United States, B: Europe). The size of each pie chart represents the number of samples at each site (≥ 30, or < 30 samples). Haplotypes that were shared with more than one individual were assigned a color. Haplotypes that were singletons (only one individual with that haplotype) are white.

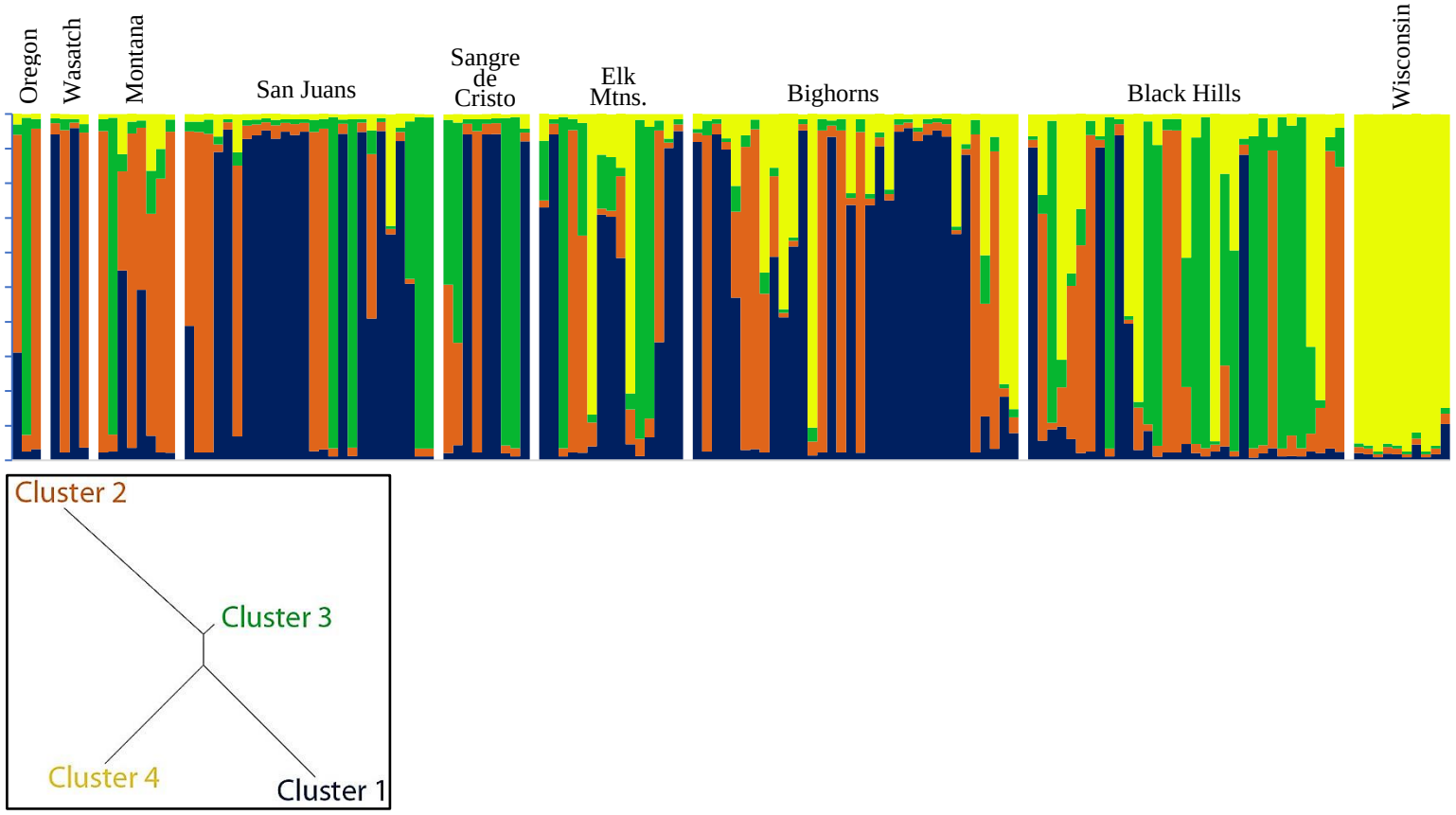


Figure 4.5: DISTRUCT plot and neighbor-joining tree created from STRUCTURE output. Cluster membership is represented as different colors, and individual wasps were depicted as vertical bars partitioned into segment that correspond to membership coefficients in each of the nine collection sites in the U.S. The number of genetic clusters (K=4) of wasps was determined based on their 4 microsatellite loci. The tree was computed by applying the neighbor joining algorithm (Saitou and Nei 1987) to the matrix of allele-frequency divergence among clusters (net nucleotide distance). The plot was produced using DRAWTREE as part of the PHYLIP phylogeny package (Felsenstein 2005).

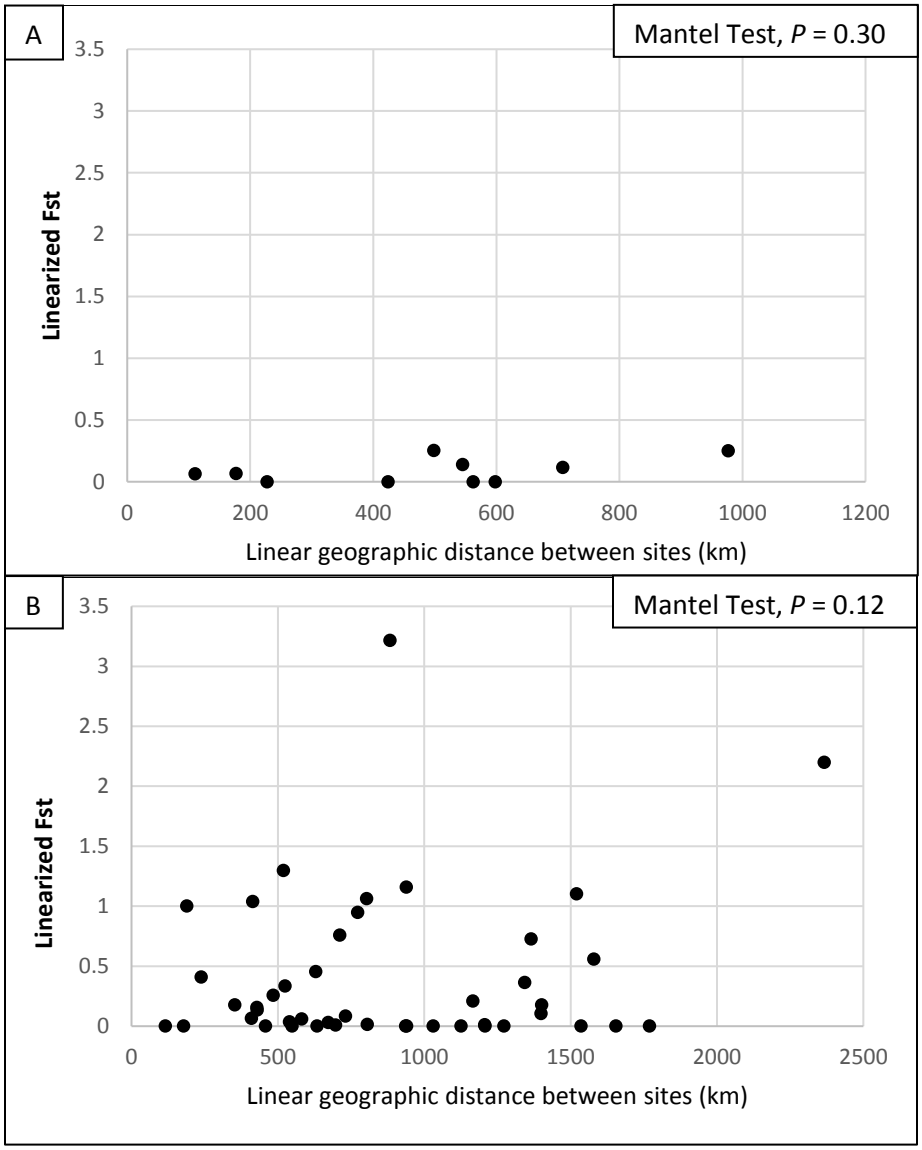


Figure 4.6: The correlation between genetic distance (linearized Slatkin's F_{ST}) and geographic distance (km) in the mtDNA region, cyt *b*, of *C. sosares* sites within Europe (A) and within the U.S. (B).

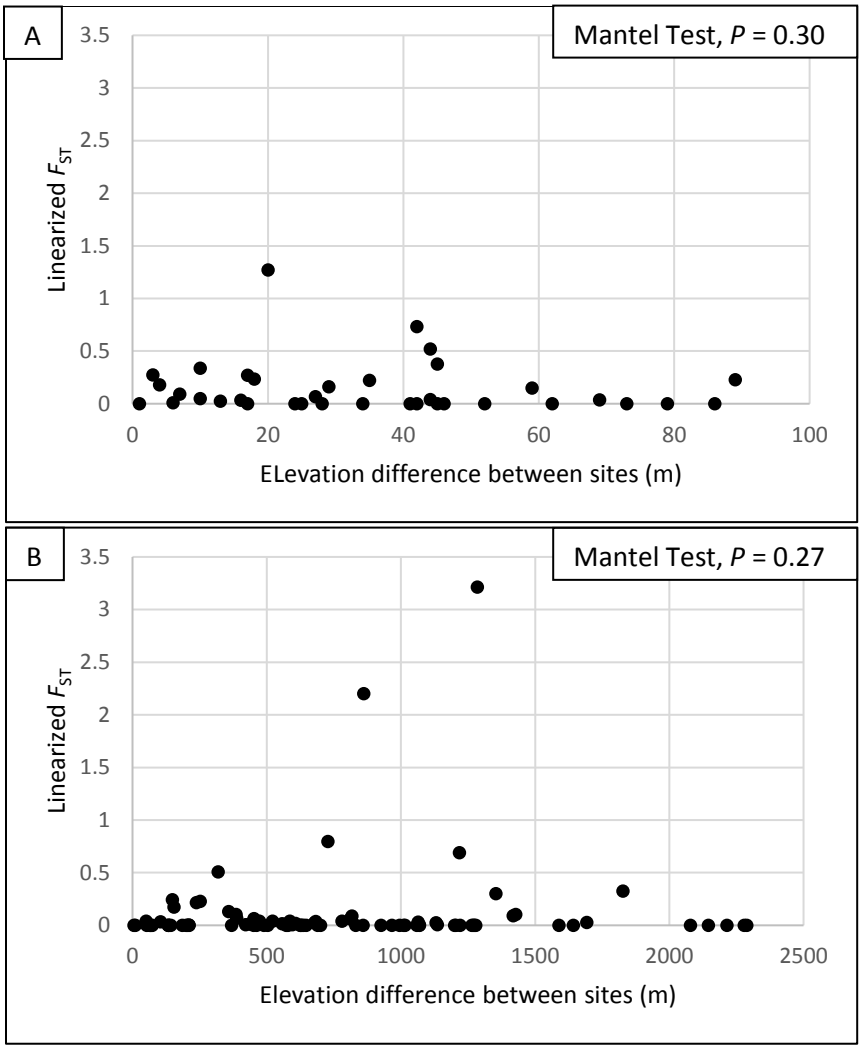


Figure 4.7: The correlation between genetic distance (linearized Slatkin's F_{ST}) and elevational differences (m) in the mtDNA region, cyt *b*, of *C. sosares* sites within Europe (A) and within the U.S. (B).

Tables

Table 4.1: Locality information (Latitude, Longitude) from collection sites of *C. sosares* in the native range (Europe) and the introduced range (US). The table includes information on the host plant species wasp hosts were collected and the number of individuals (n).

Collection	Latitude	Longitude	Plant species	n
Europe (native range)				
Netherlands-A	N 52° 9' 22.62"	E 4° 28' 48"	*H. sphondylium*	3
Netherlands-B	N 52° 8' 59.28"	E 4° 29' 1.92"	*H. sphondylium*	5
Netherlands-E	N 52° 28' 6.84"	E 6° 6' 51.42"	*H. sphondylium*	19
Netherlands-G	N 51° 59' 11.60"	E 5° 40' 20.63"	*H. sphondylium*	44
Netherlands-H	N 51° 56' 49.38"	E 5° 45' 21.78"	*H. sphondylium*	14
Belgium-A	N 50° 57' 24.9"	E 5° 41' 38.22"	*H. sphondylium*	3
Belgium-C	N 51° 7' 10.14"	E 4° 27' 1.44"	*P. sativa*	1
Belgium-D	N 51° 7' 11.76"	E 4° 28' 9.42"	*H. sphondylium*	2
Denmark-B	N 55° 49' 4.38"	E 9° 47' 20.46"	*H. sphondylium*	4
Denmark-C	N 55° 53' 0.42"	E 9° 49' 33.84"	*P. sativa*	1
Denmark-D	N 56° 5' 47.04"	E 10° 3' 9"	*P. sativa*	1
Denmark-E	N 55° 18' 33.06"	E 9° 29' 56.58"	*H. sphondylium*	9
Germany-A	N 53° 11' 30.84"	E 7° 23' 26.34"	*H. sphondylium*	7
Germany-C	N 53° 22' 54.78"	E 8° 16' 15.06"	*P. sativa*	1
Ireland-2	N 53° 24' 53.7"	W 6° 48' 28.68"	*H. sphondylium*	4
United Kingdom-1	N 50° 35' 29.46"	W 2° 2' 15.96"	*H. sphondylium*	5
United States (introduced range)				
San Juans-AE	N 36˚59'30"	W 106˚46'52.8"	*P. sativa*	6
San Juans-H	N 38˚11'0.84"	W 107˚03'13.98"	*H. maximum*	4
San Juans-T	N 36˚50'36.06"	W 106˚34'13.38"	*P. sativa* *H. maximum*	25 2
San Juans-S	N 37˚13'58.08"	W 106˚46'29.46"	*H. maximum*	21
Montana-4	N 46° 42' 14.22"	W 114° 32' 14.4"	*H. maximum*	7
Montana-5	N 44° 51' 55.56"	W 111° 33' 18.78"	*H. maximum*	12
Montana-Y	N 46° 42' 19.87"	W 114° 32' 14.53"	*H. maximum*	4
Sangre de Cristo-J	N 35˚46'19.92"	W 105˚42'5.58"	*H. maximum*	19
Wasatch-K	N 39° 55' 48.84"	W 111° 38' 27.72"	*H. maximum*	14
Elk Mountains-F	N 39˚22'11.52"	W 106˚40'51.96"	*H. maximum*	17
Elk Mountains-G	N 39˚22'48.96"	W 107˚04'42.24"	*P. sativa* *H. maximum*	8 7
Bighorns-C	N 44˚11'17.04"	W 105˚51'34.92"	*H. maximum*	70
Medicine Bow-AC	N 40˚24'25.62"	W 106˚48'23.94"	*P. sativa*	4
Black Hills-B	N 44˚04'35.7"	W 103˚38'20.76"	*H. maximum*	33
Black Hills-P	N 44˚25'17.1"	W 103˚52'54.6"	*H. maximum*	13
Oregon-X	N 44° 22' 57.36"	W 120° 31' 12.24"	*H. maximum*	2
Wisconsin	N 43° 32' 54.57"	W 90° 48' 57.54"	*P. sativa*	10

Table 4.2: Primers to amplify segments of *C. sosares* mitochondrial DNA (mtDNA). T_a (°C), annealing temperature used during PCR.

Gene/ Region	Primer Name	Sequence (5'-3')	T_a (°C)	Reference
cyt *b*	CB-J-10933	F: TATGTACTACCATGAGGACAAATATC	48	Crozier & Crozier 1992
	CB-N-11367	R: ATTACACCTCCTAATTTATTAGGAAT		
COI	C1-J-1751	F: GGATCACCTGATATAGCATTCCC	50	Simon et al. (1994)*
	C1-N-2191	R: CCAGGTAAAATTAAAATATAAACTTC		
16s rRNA	LR-J-13017	F: TTACGCTGTTATCCTAA	50	Kambhampati & Smith (1995)
	LR-N-13398	R; CACCTGTTTAACAAAAACAT		
12S	SR-J-14233	F: GAAATTGACGGGCGATTTGT	50	Kocher et al. 1989
	SR-N-14588	R: AAACTAGGATTAGATACCCTACTAT		

*primer sequence derived from *Apis mellifera*

Table 4.3: Molecular diversity indices within European and U.S. populations of mtDNA *C. sosares*. Numbers of individuals (N), number of haplotypes (N_h), and haplotype diversity ($\hat{\theta}_\pi \pm s.d$)

Collection	N	N_h	Haplotypes	$\hat{\theta}_\pi \pm s.d$
Europe	123	32		1.29 ± 0.90
Denmark	15	11	Hap 1, 5, 12, 23, 28, 29, 30, 32, 34, 58, 59	2.63 ± 1.67
Germany	8	5	Hap 1, 12, 26, 31, 33	2.02 ± 1.44
Netherlands	85	18	Hap 1, 7, 8, 9, 11, 12, 16, 17, 18, 20, 25, 26, 35, 47, 48, 57,60, 61	1.08 ± 0.79
Belgium	6	4	Hap 1, 8, 13, 27	1.34 ± 1.11
British Isles	9	2	Hap 1, 46	0.22 ± 0.33
United States	278	37		0.65 ± 0.56
Black Hills	46	8	Hap 1, 2, 3, 5, 11, 14, 15, 19	0.61 ± 0.54
San Juan Mtns.	58	9	Hap 1, 5, 12, 21, 24, 26, 39, 41, 45	0.45 ± 0.45
Medicine Bows	4	3	Hap 1, 12, 26	1.00 ± 0.99
Sangre de Cristo Mtns.	19	3	Hap 1, 42, 50	0.42 ± 0.45
Elk Mtns.	32	13	Hap 1, 6, 8, 10, 12, 13, 41, 43, 49, 51, 52, 53, 56	1.18 ± 0.87
Montana	23	3	Hap 1, 8, 38	0.35 ± 0.40
Bighorn Mtns.	70	13	Hap 1, 4, 8, 21, 36, 37, 40, 41, 42, 44, 46, 54, 55	0.94 ± 0.73
Wasatch Mtns.	14	1	Hap 1	0.00 ± 0.00
Oregon	2	2	Hap 1, 22	1.00 ± 1.42
Wisconsin	10	1	Hap 1	0.00 ± 0.00

Table 4.4: Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) for *C. sosares* collections using the A) cyt *b* and B) microsatellite datasets. Significance levels; *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$

A: Cyt *b*

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European populations	4	5.04	0.05	7.15	*P* =0.049*
Within European populations	118	73.41	0.62	92.85	
Total	122	78.45	0.67		
Among U.S. populations	11	4.10	0.00	0.72	*P* = 0.217
Within U.S. populations	266	85.59	0.32	99.28	
Total	277	89.69	0.32		
Among Europe and U.S	1	1.03	0.00	0.02	*P* = 0.117
Among populations within Europe and U.S	15	9.14	0.01	2.35	*P* = 0.065
Within populations	384	159.00	0.41	97.62	*P* = 0.040*
Total	400	169.17	0.42		

B: Microsatellite

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European populations	4	17.54	0.12	15.93	0.000***
Within individuals within European populations	67	74.30	0.26	25.21	0.000***
Within individuals	72	43.00	0.60	58.86	0.000***
Total	143	134.83	1.01		
Among U.S. populations	8	30.30	0.098	12.93	0.000***
Among individuals within U.S. populations	133	117.73	0.223	29.24	0.000***
Within individuals	142	62.50	0.440	57.83	0.000***
Total	283	210.53	0.761		
Among Europe and U.S	1	16.15	0.045	5.05	0.022*
Among populations within Europe and U.S	12	47.84	0.115	12.93	0.000***
Among individuals within populations	200	192.02	0.234	26.36	0.000***
Within individuals	214	105.50	0.493	55.65	0.000***
Total	427	361.51	0.886		

Table 4.5: Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) between host plant species *C. sosares* were collected, within the native range (Europe) and the introduced range (U.S.) using the A) cyt *b* and B) microsatellite datasets. Significance levels; **P* < 0.05, ***P* < 0.001, ****P* < 0.0001

A: Cyt *b*

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and hogweed	1	1.26	0.06	8.00	0.105
Among populations within European wild parsnip and hogweed	3	3.78	0.04	5.50	0.013*
Within populations	118	73.41	0.62	86.50	0.061
Total	122	78.45	0.72		
Among U.S wild parsnip and cow parsnip	1	0.30	0.00	0.00	0.707
Among populations within U.S wild parsnip and cow parsnip	9	3.06	0.00	0.47	0.192
Within populations	259	79.59	0.31	99.75	0.278
Total	269	82.94	0.31		

B: Microsatellite

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and hogweed	1	1.69	0.00	-.012	0.394
Among populations within European wild parsnip and hogweed	13	32.42	0.16	17.09	0.000***
Among individuals within wild parsnip and hogweed populations	57	57.72	0.21	21.43	0.000***
Within individuals	72	43.00	0.60	61.60	0.000***
Total		134.83	0.97		
Among U.S wild parsnip and cow parsnip	1	4.33	0.02	2.31	0.134
Among populations within U.S wild parsnip and cow parsnip	14	29.01	0.07	9.73	0.000***
Among individuals within wild parsnip and cow parsnip populations	126	114.71	0.24	31.25	0.000***
Within individuals	142	61.50	0.433	56.71	0.000***
Total	283	209.55	0.76		

Table 4.6: Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) between host plant species *C. sosares* were collected, between native range (Europe) host plants species and introduced range (U.S.) host plant species using the A) cyt *b* and B) microsatellite datasets. Significance levels; *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$.

A: Cyt *b*

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and U.S wild parsnip	1	1.34	0.12	30.14	0.061
Among populations within European wild parsnip and U.S wild parsnip	4	2.45	0.04	10.87	0.029*
Within populations	51	12.40	0.24	58.99	0.017*
Total	56	16.19	0.41		
Among European wild parsnip and U.S cow parsnip	1	1.44	0.14	27.43	0.022*
Among populations within European wild parsnip and U.S cow parsnip	8	3.87	0.01	1.15	0.248
Within populations	219	77.19	0.35	71.42	0.055
Total	228	82.49	0.49		
Among European hogweed and U.S cow parsnip	1	0.85	0.00	0.13	0.196
Among populations within European hogweed and U.S cow parsnip	9	5.15	0.01	1.16	0.054
Within populations	333	146.60	0.44	98.71	0.054
Total	343	152.59	0.45		
Among European hogweed and U.S wild parsnip	1	0.58	0.00	-1.37	0.559
Among populations within European hogweed and U.S wild parsnip	5	3.73	0.01	3.16	0.014*
Within populations	165	81.81	0.49	98.20	0.067
Total	171	86.13	0.50		

B: Microsatellite

Source of variation	d.f.	Sum of squares	Variance components	% of variation	*F* (prob)
Among European wild parsnip and U.S wild parsnip	1	2.18	-0.07	-7.09	0.509
Among populations within European wild parsnip and U.S wild parsnip	5	20.81	0.42	41.24	0.000***
Among individuals within European wild parsnip and U.S wild parsnip populations	24	23.64	0.32	32.15	0.000***
Within individuals	31	10.50	0.34	33.70	0.000***
Total	61	57.13	1.00		
Among European wild parsnip and U.S cow parsnip	1	5.04	0.26	27.38	0.000***

Among populations within European wild parsnip and U.S cow parsnip	13	19.89	0.05	5.01	0.000***
Among individuals within European wild parsnip and U.S cow parsnip populations	104	87.18	0.20	21.28	0.000***
Within individuals	119	52.00	0.44	46.34	0.000***
Total	237	164.11	0.94		
Among European hogweed and U.S cow parsnip	1	14.75	0.07	7.77	0.000***
Among populations within European hogweed and U.S cow parsnip	22	41.96	0.07	8.27	0.000***
Among individuals within European hogweed and U.S cow parsnip populations	159	149.65	0.21	24.67	0.000***
Within individuals	183	94.00	0.51	59.29	0.000***
Total	365	300.36	0.87		
Among European hogweed and U.S wild parsnip	1	3.11	-0.03	-3.37	0.648
Among populations within European hogweed and U.S wild parsnip	14	44.98	0.21	21.68	0.000***
Among individuals within European hogweed and U.S wild parsnip populations	79	81.86	0.24	24.86	0.000***
Within individuals	95	52.50	0.55	56.83	0.000***
Total	189	182.45	0.97		0.000***

Table 4.7: Estimates of *C. sosares* subpopulation differentiation from Slatkin's linearized pairwise F_{ST} values between and within collection sites in Europe and the U.S. using cyt *b* haplotype data. Significant comparisons indicate the pairs are significantly different and are marked with an asterisk(s) based on significance level; $P < 0.05$*, $P < 0.001$**

		San Juans	Sangre de Cristo	Elk Mtns.	Montana	Wasatch Mtn.	Oregon	Bighorn Mtns.	Wisconsin	Black Hills	Medicine Bow	British Isles	Netherlands	Denmark	Germany
United States	San Juans														
	Sangre de Cristo	0													
	Elk Mtns	**0.06***	**0.07***												
	Montana	0	0	**0.09***											
	Wasatch Mtns.	0.02	0.01	**0.14****	0										
	Oregon	0.29	0.56	0	0.76	3.21									
	Bighorn Mtns	0	0	**0.04****	0	0.03	0.21								
	Wisconsin	0	0	**0.11***	0	0	2.20	0.01							
	Black Hills	0	0	**0.07***	0	0.01	0.36	0	0						
	Medicine Bow	0.17	0.33	0	0.45	1.04	0	0.16	0.73	0.26					
Europe	British Isles	0	0	0.03	0	0.05	0.36	0	0.01	0	0.19				
	Netherlands	0	0.01	0.02	0.01	0.04	0.11	0	0.03	0	0.07	0			
	Denmark	**0.37****	**0.38****	0.07	**0.46****	**0.55****	0	**0.34****	**0.44****	**0.41****	0	**0.25****	**0.25****		
	Germany	**0.15***	**0.22****	0	**0.29***	**0.47***	0	**0.13***	0.34	0.19	0	0.11	0.07	0	
	Belgium	0.18	0.26	0	**0.32***	**0.63***	0	0.13	**0.45***	0.22	0	0.14	0.06	0	0

Table 4.8: Estimates of *C. sosares* subpopulation differentiation from Slatkin's linearized pairwise F_{ST} values between and within collection sites in Europe and the U.S using microsatellite data. Significant comparisons indicate the pairs are significantly different and are marked with an asterisk(s) based on significance level; $P < 0.05$*, $P < 0.001$**

		San Juans	Sangre de Cristo	Elk Mtns.	Montana	Wasatch Mtn.	Oregon	Bighorn Mtns.	Wisconsin	Black Hills	British Isles	Netherlands	Denmark	Germany
United States														
	San Juans													
	Sangre de Cristo	0.05												
	Elk Mtns	**0.11***	-0.00											
	Montana	-0.02	0.04	**0.11***										
	Wasatch Mtns.	0.05	0.04	0.06	0.03									
	Oregon	-0.07	-0.18	-0.05	-0.11	0.01								
	Bighorn Mtns	0.02	**0.09***	**0.14****	-0.02	0.04	-0.03							
	Wisconsin	**0.26****	**0.34***	**0.38****	**0.17***	**0.30****	0.16	**0.21****						
	Black Hills	0.038	0.07	**0.12****	0.00	0.12	-0.07	**0.06****	**0.19****					
Europe	British Isles	**0.50****	**0.75***	**0.71****	**0.50****	0.77	**0.72***	**0.45****	**0.46****	**0.41***				
	Netherlands	**0.08****	**0.15****	**0.19****	0.04	**0.16***	-0.00	**0.08****	**0.07***	**0.04***	**0.34****			
	Denmark	**0.46****	**0.69***	**0.67****	**0.42****	**0.70***	0.61	**0.41****	**0.25***	**0.31****	**0.78***	**0.22****		
	Germany	**0.18****	**0.33****	**0.36****	**0.14***	**0.30****	**0.18***	**0.17****	0.06	**0.14****	**0.39****	0.03	**0.32****	
	Belgium	**0.39****	**0.55****	**0.58****	**0.32****	**0.51***	**0.37***	**0.33****	0.03	**0.29****	**0.60***	**0.13***	**0.39***	**0.15***

Table 4.9 Pairwise $4N_em$ of immigrants and migrants among U.S. collections sites. The first value in the cell is the $4N_em$ of migrants, the second value in the cell is the $4N_em$ of immigrants between each pair of collection sites. For example, the first cell is read as; 4.88 reproductive migrants per generation from Bighorn Mountains into Black Hills, and 4.70 reproductive immigrants per generation into the Bighorn Mountains from Black Hills. A $4N_em < 1$ suggests that immigration is insufficient to offset the effects of genetic drift so that populations diverge in allele frequencies. A $4N_em > 1$ suggests that there is sufficient immigration to offset the effects of genetic drift to maintain homogeneity among populations.

	Black Hills	Bighorns	Elk Mountains	Sangre de Cristo Mtns.	Wasatch	San Juan Mountains	Montana	Oregon	Wisconsin
Black Hills	-								
Bighorns	4.88, 4.70	-							
Elk Mountains	1.34, 6.32	2.83, 9.40	-						
Sangre de Cristo	1.67, 0.36	1.39, 0.35	2.52, 0.82	-					
Wasatch Mountains	3.10, 3.11	1.33, 3.03	5.71, 0.93	1.62, 4.08	-				
San Juan Mountains	2.80, 6.92	5.12, 2.48	2.96, 0.42	2.01, 2.02	5.22, 2.48	-			
Montana	6.68, 8.42	4.83, 17.88	4.87, 8.30	0.30, 3.31	1.80, 7.00	0.82, 1.13	-		
Oregon	1.95, 1.26	7.41, 10.57	1.40, 2.39	1.45, 0.79	4.50, 3.36	5.85, 4.60	4.05, 0.88	-	
Wisconsin	0.46, 3.24	0.44, 4.66	1.35, 2.39	3.03, 2.89	0.23, 6.29	0.81, 4.02	9.89, 12.87	0.10, 1.94	-
Averages	**2.86, 4.29**	**3.53, 6.63**	**2.87, 3.87**	**1.75, 1.83**	**2.94, 3.79**	**3.20, 3.01**	**4.16, 7.47**	**3.34, 3.22**	**2.04, 4.79**

REFERENCES

Abrams, P. A. (2000). The evolution of predator-prey interactions: theory and evidence. *Annual Review of Ecology and Systematics*, *31*(1), 79-105.

Adair RJ & Scott JK. (1997). Distribution, life history and host specificity of *Chrysolina picturata* and *Chrysolina* sp. B (Coleoptera: Chrysomelidae), two biological control agents for *Chrysanthemoides monilifera* (Compositae). *Bulletin of Entomological Research* 87, 331–341.

Aebi, A., Schönrogge, K., Melika, G., Alma, A., Bosio, G., Quacchia, A., Picciau, L., Abe, Y., Moriya, S., Yara, K. & Seljak, G. (2006). Parasitoid recruitment to the globally invasive chestnut gall wasp *Dryocosmus kuriphilus*. *Galling arthropods and their associates*, 103-121.

Agrawal, A. A. (1999). Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecology*, 80(5), 1713-1723.

Agrawal, A. A., & Kotanen, P. M. (2003). Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, 6(8), 712-715.

Agrawal, A. A., Lau, J. A., & Hambäck, P. A. (2006). Community heterogeneity and the evolution of interactions between plants and insect herbivores. *The Quarterly Review of Biology*, 81(4), 349-376.

Agosta, S. J. (2006). On ecological fitting, plant–insect associations, herbivore host shifts, and host plant selection. *Oikos*, *114*(3), 556-565.

Agosta, S. J., & Klemens, J. A. (2008). Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecology Letters*, *11*(11), 1123-1134.

Agosta, S.J., Janz, N., & Brooks, D.R. (2010). How specialists can be generalists: resolving the "parasite paradox" and implications for emerging infectious disease. *Zoologia (Curitiba)*, *27*(2), 151-162.

Agrawal, A.A., & Kotanen, P.M. (2003). Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, 6(8), 712-715.

Agrawal, A.A., Lau, J.A., & Hambäck, P.A. (2006). Community heterogeneity and the evolution of interactions between plants and insect herbivores. *The Quarterly Review of Biology*, *81*(4), 349-376.

Allen, W.J., Young, R.E., Bhattarai, G.P., Croy, J.R., Lambert, A.M., Meyerson, L.A., & Cronin, J.T. (2015). Multitrophic enemy escape of invasive *Phragmites australis* and its introduced herbivores in North America. *Biological invasions*, 17(12), 3419-3432.

Amalin, D.M., Pena, J.E., Duncan, R.E., Browning, H.W. & McSorley, R. (2002) Natural mortality factors acting on citrus leafminer, *Phyllocnistis citrella*, in lime orchards in South Florida. *Biocontrol*, 47, 327–347.

Auger-Rozenberg, M.A., Boivin, T., Magnoux, E., Courtin, C., Roques, A., & Kerdelhué, C. (2012). Inferences on population history of a seed chalcid wasp: invasion success despite a severe founder effect from an unexpected source population. *Molecular Ecology*, 21(24), 6086-6103

Avise, J.C. (2000). *Phylogeography: the history and formation of species*. Cambridge: Harvard University Press. 447

Baehrecke, E.H., & Strand, M.R. (1990). Embryonic morphology and growth of the polyembryonic parasitoid *Copidosoma floridanum* (Ashmead)(Hymenoptera: Encyrtidae). *International Journal of Insect Morphology and Embryology*, *19*(3-4), 165-175.

Baehrecke, E.H., Grbić, M., & Strand, M.R. (1992). Serosa ontogeny in two embryonic morphs of *Copidosoma floridanum*: The influence of host hormones. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *262*(1), 30-39.

Barrett, S.C.H. & Kohn, J.R. (1991). Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pages 3-20 in D.A. Falk and K.E. Holsinger, editors. *Genetics and conservation of rare plants*. Oxford University Press, New York.

Bartomeus, I., Vilà, M., & Santamaría, L. (2008). Contrasting effects of invasive plants in plant–pollinator networks. *Oecologia*, 155(4), 761-770.

Batten, A.R., & Murray, D.F. (1982). A Literature Survey on the Wetland Vegetation of Alaska. *Alaska University Institute of Artic Biology and Museum.*

Beerli, P. (1998). Estimation of migration rates and population sizes in geographically structured populations. In: Advances in molecular ecology (Ed. G. Carvalho). NATO-ASI workshop series. IOS Press, Amsterdam. Pp. 39-53.

Beerli, P. (2009). How to use migrate or why are markov chain monte carlo programs difficult to use? In G. Bertorelle, M. W. Bruford, H. C. Hauffe, A. Rizzoli, and C. Vernesi, editors, Population Genetics for Animal Conservation, volume 17 of Conservation Biology, pages 42–79. Cambridge University Press, Cambridge UK.

Beerli, P. & Felsenstein, J. (1999). Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics*, 152(2):763–73,

Beerli, P. & Felsenstein, J. (2001). Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences* of the USA, 98(8):4563–4568.

Berdegue, M., Trumble, J.T., Hare, J. D., & Redak, R. A. (1996). Is it enemy-free space? The evidence for terrestrial insects and freshwater arthropods. *Ecological Entomology*, *21*(3), 203-217.

Berenbaum, M.R. (1990). Evolution of specialization in insect-umbellifer associations. *Annual Review of Entomology* 35, 319-343.

Berenbaum, M.R., & Zangerl, A.R. (1991). Acquisition of a native hostplant by an introduced oligophagous herbivore. *Oikos* 62, 153-159.

Berenbaum, M.R., & Zangerl, A.R. (1992). Genetics of physiological and behavioral resistance to host furanocoumarins in the parsnip webworm. *Evolution*, 1373-1384.

Berenbaum, M.R., & Zangerl, A.R. (1998). Chemical phenotype matching between a plant and its insect herbivore. *Proceedings of the National Academy of Sciences*, 95(23), 13743-13748.

Berenbaum, M. R., & Zangerl, A.R. (2006). Parsnip webworms and host plants at home and abroad: trophic complexity in a geographic mosaic. *Ecology*, *87*(12), 3070-3081.

Berenbaum, M.R., Zangerl, A.R. & Nitao, J.K (1986). Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution*, 40, 1215-1228.

Berenbaum, M.R., Green E.S., & Zangerl, A.R (1993). Web costs and web defense in the parsnip webworm (Lepidoptera: Oecophoridae). *Environmental Entomology* 22, 791-795.

Bernays, E., & Graham, M. (1988). On the evolution of host specificity in phytophagous arthropods. *Ecology*, *69*(4), 886-892.

Bethune, C.J.S. (1869). Larvae infesting the parsnip. *Canadian Entomology* 2: 1–4

Bland, K. (2002). *Phyllonorycter leucographella* (Zeller, 1850) (Lep.: Gracillariidae), newly arrived in Scotland. *Entomologist's Record and Journal of Variation*, 114, 164.

Boivin, S., Kerdelhué, C., Rozenberg, M.A., Cariou, M. L., & Roques, A. (2003). Characterization of microsatellite loci in a seed chalcid, *Megastigmus spermotrophus* (Hymenoptera: Torymidae). *Molecular Ecology Resources*, 3(3), 363-365.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144(1), 1-11.

Bowker, C.L., Ross, K.G., & Strand, M.R. (2014). The metabolic enzyme *phosphoglucose isomerase* (*Pgi*) affects the outcome of intra- specific competition in a polyembryonic wasp. *Ecological Entomology*, *39*(5), 648-655.

Branca, A., Le Ru, B.P., Vavre, F., Silvain, J.F., & Dupas, S. (2011). Intraspecific specialization of the generalist parasitoid *Cotesia sesamiae* revealed by polydnavirus polymorphism and associated with different *Wolbachia* infection. *Molecular Ecology*, 20, 959–971.

Brockerhoff, E.G., Suckling, D.M., Ecroyd, C.E., Wagstaff, S.J., Raabe, M.C., Dowell, R.V., & Wearing, C.H. (2011). Worldwide host plants of the highly polyphagous, invasive *Epiphyas postvittana* (Lepidoptera: Tortricidae). *Journal of economic entomology*, *104*(5), 1514-1524.

Brooks, R. & Endler J.A. (2001a). Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulate*). *Evolution*, 55, 1002-1015.

Brooks, R. & Endler, J.A. (2001b). Female guppies agree to differ; phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*, 55, 1644-1655.

Brown, J.W., Epstein, M.E., Gilligan, T.M., Passoa, S.C., & Powell, J.A. (2010). Biology, identification, and history of the light brown apple moth, *Epiphyas postvittana* (Walker)(Lepidoptera: Tortricidae: Archipini) in California: an example of the importance of local faunal surveys to document the establishment of exotic insects. *American Entomologist*, *56*(1), 34-43.

Byrne, M. J., Currin, S., & Hill, M. P. (2002). The influence of climate on the establishment and success of the biocontrol agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biological Control*, *24*(2), 128-134.

Cain, N., Darbyshire, S. J., Francis, A., Nurse, R. E., & Simard, M. J. (2010). The biology of Canadian weeds. 144. *Pastinaca sativa* L. *Canadian Journal of Plant Science*, 90(2), 217-240.

Calatayud, P.A., Gitau, C., Calatayud, S., Dupas, S., Le Ru, B., & Silvain, J.F. (2011) Variability in the reproductive biology and in resistance against *Cotesia sesamiae* among two *Busseola fusca* populations. *Journal of Applied Entomology*, 135, 423–429.

Cappuccino, N., & Arnason, J. T. (2006). Novel chemistry of invasive exotic plants. *Biology Letters*, *2*(2), 189-193.

Carroll, M. J., & Berenbaum, M. R. (2006). Lutein sequestration and furanocoumarin metabolism in parsnip webworms under different ultraviolet light regimes in the montane west. *Journal of chemical ecology*, 32(2), 277-305.

Carroll, M. J., Lampert, E. C., Berenbaum, M. R., Noyes, J. S., & Ode, P. J. (2007). New records of *Copidosoma sosares* (Walker) (Hymenoptera: Encyrtidae), a parasitoid of the parsnip webworm (*Depressaria pastinacella* (Duponchel))(Lepidoptera: Elachistidae), in Western North America. *Journal of the Kansas Entomological Society*, 80(4), 309-318.

Catherine, G.W., Schulthess, F., & Stephane, D. (2010) An association between host acceptance and virulence status of different populations of *Cotesia sesamiae*, a braconid larval parasitoid of lepidopteran cereal stemborers in Kenya. *Biological Control*, 54, 100–106.

Chun, Y. J., Van Kleunen, M., & Dawson, W. (2010). The role of enemy release, tolerance and resistance in plant invasions: linking damage to performance. *Ecology Letters*, 13(8), 937-946.

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

Corley, L. S., White, M. A., & Strand, M. R. (2005). Both endogenous and environmental factors affect embryo proliferation in the polyembryonic wasp *Copidosoma floridanum*. *Evolution & development*, *7*(2), 115-121.

Cronin, J. T., & Haynes, K. J. (2004). An invasive plant promotes unstable host–parasitoid patch dynamics. *Ecology*, 85(10), 2772-2782.

Cullen, J.M., Groves, R.H. & Alex, J.F. (1982). The influence of *Aceria chondrillae* on the growth and reproductive capacity of *Chondrilla juncea*. *Journal of Applied Ecology* 19, 529–537.

Cullen, J.M., & Moore, A.D. (1983). The influence of three populations of *Aceria chondrillae* on three forms of *Chondrilla juncea*. *Journal of Applied Ecology* 20, 235–243.

Day, M.D., & McAndrew, T.D., 2003. The biology and host range of *Falconia intermedia* (Hemiptera: Miridae), a potential biological control agent for *Lantana camara* (Verbenaceae) in Australia. *Biocontrol Science and Technology* 13, 13–22

Dennill, G.B. & Gordon, A.J. 1990. Climate-related differences in the efficacy of the Australian gall wasp (Hymenoptera: Pteromalidae) released for the control of *Acacia longifolia* in South Africa. *Environmental Entomology* 19, 130–136.

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

Donnell, D. M., & Strand, M. R. (2006). Caste-based differences in gene expression in the polyembryonic wasp *Copidosoma floridanum*. *Insect biochemistry and molecular biology*, *36*(2), 141-153.

Downie, S. R., Katz-Downie, D. S., & Watson, M. F. (2000). A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily Apioideae. *American Journal of Botany*, 87(2), 273-292.

Downie, D. A. (2002). Locating the sources of an invasive pest, grape phylloxera, using a mitochondrial DNA gene genealogy. *Molecular Ecology*, 11(10), 2013-2026.

Dunn, A. M. (2009). Parasites and biological invasions. *Advances in parasitology*, 68, 161-184.

Dupas, S., Carton, Y. and Poirie, M. (2003). Genetic dimension of the coevolution of virulence-resistance in *Drosophila*-parasitoid wasp relationships. *Heredity , 90*, 84–89.

Dupas, S., Dubuffet, A., Carton, Y., Poirie, M. (2009) Local, geographic and phylogenetic scales of coevolution in *Drosophila*-parasitoid interactions. In *Advances in Parasitology: Parasitoids of Drosophila*; Prevost, G., Ed.; Elsevier: London, UK; Volume 70, pp. 281–295.

Edwards S.V. (2009). Is a new and general theory of molecular systematics emerging? *Evolution*, 63:1-19.

Ehrlich, P.R., & Raven, P.H. (1964) Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.

Ellstrand, N. C., & Schierenbeck, K. A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences*, 97(13), 7043-7050.

Elton, C.S. (1958) *The Ecology of Invasions by Animals and Plants.* University of Chicago Press, Chicago, IL.

Emmet, A.M. (1989) *Phyllonorycter leucographella* (Zeller, 1850) (Lepidoptera: Gracillariidae) in Essex: a new species to Britain. *Entomologist's Record*, 101, 189–194.

Estoup, A., & Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular ecology*, 19(19), 4113-4130.

Evanno, G., Regnaut, S,,Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 2005; 14(8):2611–20.

Evans, K. J., Jones, M. K., & Roush, R.T. (2005). Susceptibility of invasive taxa of European blackberry to rust disease caused by the uredinial stage of *Phragmidium violaceum* under field conditions in Australia. *Plant Pathology* 54, 275–286.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131(2), 479-491.

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular ecology resources, 10(3), 564-567.
Gilchrist, G.W, Huey, R.B., & Serra, L. (2001) Rapid evolution of wing size clines in *Drosophila subobscura*. *Genetica*, 112, 273-286.

Fabre, J. P., Auger-Rozenberg, M. A., Chalon, A., Boivin, S., & Roques, A. (2004). Competition between exotic and native insects for seed resources in trees of a Mediterranean forest ecosystem. *Biological Invasions*, 6(1), 11-22.

Fady, B., Lefèvre, F., Reynaud, M., Vendramin, G. G., Dagher-Kharrat, M. B., Anzidei, M., ... & Bariteau, M. (2003). Gene flow among different taxonomic units: evidence from nuclear and cytoplasmic markers in *Cedrus* plantation forests. *Theoretical and Applied Genetics*, *107*(6), 1132-1138.

Fan, S., Yu, D., & Liu, C. (2013). The invasive plant *Alternanthera philoxeroides* was suppressed more intensively than its native congener by a native generalist: implications for the biotic resistance hypothesis. *PloS one*, 8(12), e83619.

Feeny, P. (1976). Plant apparency and chemical defense. In *Biochemical interaction between plants and insects* (pp. 1-40). Springer US.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 783-791.

Ferrari, J., Müller, C.B., Kraaijeveld, A.R. and Godfray, H.C.J. (2001) Clonal variation and covariation in aphid resistance to parasitoids and a pathogen. *Evolution*, 55, 1805–1814.

Fortuna T. M., Vet L.E.M., Harvey J.A. (2012) Effects of an invasive plant on the performance of two parasitoids with different host exploitation strategies. *Biological Control*, 62, 213–220.

Fortuna, T. M., Woelke, J. B., Hordijk, C. A., Jansen, J. J., van Dam, N. M., Vet, L. E., & Harvey, J. A. (2013). A tritrophic approach to the preference–performance hypothesis involving an exotic and a native plant. *Biological invasions*, 15(11), 2387-2401.

Fox, L. A., & Morrow, P. A. (1981). Specialization: species property or local phenomenon. *Science*, 211(4485), 887-893.

Futuyma, D. J., & Moreno, G. (1988). The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, *19*(1), 207-233.

Gandon, S. (2002) Local adaptation and the geometry of host-parasite coevolution. *Ecology Letters*, 5, 246–256

Genton, B. J., Shykoff, J. A., & Giraud, T. (2005). High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. *Molecular ecology*, *14*(14), 4275-4285.

Giblin-Davis, R. M., Makinson, J., Center, B. J., Davies, K. A., Purcell, M., Taylor, G. S., ... & Center, T. D. (2001). Fergusobia/Fergusonina-induced shoot bud gall development on *Melaleuca quinquenervia*. *Journal of Nematology*, 33(4), 239.

Girardoz, S., Kenis, M., & Quicke, D. L. (2006). Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridella*: host-parasitoid synchronization and influence of the environment. *Agricultural and Forest Entomology*, 8(1), 49-56.

Giron, D., & Strand, M. R. (2004). Host resistance and the evolution of kin recognition in polyembryonic wasps. *Proceedings of the Royal Society of London B: Biological Sciences*, *271*(Suppl 6), S395-S398.

Giron, D., Dunn, D. W., Hardy, I. C., & Strand, M. R. (2004). Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. *Nature*, *430*(7000), 676-679.

Giron, D., Harvey, J. A., Johnson, J. A., & Strand, M. R. (2007). Male soldier caste larvae are non-aggressive in the polyembryonic wasp *Copidosoma floridanum*. *Biology letters*, *3*(4), 431-434.

Godfray, H. C. J., Agassiz, D. J., Nash, D. R., & Lawton, J. H. (1995). The recruitment of parasitoid species to two invading herbivores. *Journal of Animal Ecology*, 393-402.

Godfray, H.C.J. & Shimada, M. 1999. Parasitoids as model organisms for ecologists. *Research in Population Ecology* 41:3-10.

Gols, R., Wagenaar, R., Bukovinszky, T., Dam, N. M. V., Dicke, M., Bullock, J. M., & Harvey, J. A. (2008). Genetic variation in defense chemistry in wild cabbages affects herbivores and their endoparasitoids. *Ecology*, 89(6), 1616-1626.

Goolsby JA, Zonneveld R & Bourne A. 2004. Prerelease assessment of impact on biomass production of an invasive weed, *Lygodium microphyllum* (Lygodiaceae: Pteridophyta), by a potential biological control agent, Floracarus perrepae (Acariformes: Eriophyidae). *Environmental Entomology* 33, 997–1002.

Goolsby, J. A., Van Klinken, R. D., & Palmer, W. A. (2006). Maximising the contribution of native-range studies towards the identification and prioritisation of weed biocontrol agents. *Austral Entomology*, *45*(4), 276-286.

González-Chang, M., Wratten, S. D., Lefort, M. C., & Boyer, S. (2016). Food webs and biological control: A review of molecular tools used to reveal trophic interactions in agricultural systems. *Food Webs*, *9*, 4-11.

Gordon, S. D., & Strand, M. R. (2009). The polyembryonic wasp *Copidosoma floridanum* produces two castes by differentially parceling the germ line to daughter embryos during embryo proliferation. *Development genes and evolution*, *219*(9-10), 445-454.

Grabenweger, G. & Lethmayer, C. (1999) Occurrence and phenology of parasitic Chalcidoidea on the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae). *Journal of Applied Entomology*, 123, 257–260.

Grabenweger, G., Kehrli, P., Schlick- Steiner, B., Steiner, F., Stolz, M., & Bacher, S. (2005). Predator complex of the horse chestnut leafminer *Cameraria ohridella*: identification and impact assessment. *Journal of Applied Entomology*, 129(7), 353-362.

Grabenweger G., Kehrli P., Zweim¨ullerI., Augustin S., Avtzis N., Bacher S., Freise J., Girardoz S., Guichard S., Heitland W., Lethmayer C., Stolz M., Tomov R., Volter L., Kenis M. (2010) Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe. *Biological Invasions*, 12, 2797–2813

Grbic, M., Nagy, L. M., & Strand, M. (1996). Pattern duplications in larvae of the polyembryonic wasp *Copidosoma floridanum*. *Development genes and evolution*, *206*(4), 281-287.

Grbic, M., Rivers, D., & Strand, M. R. (1997). Caste formation in the polyembryonic wasp *Copidosoma floridanum* (Hymenoptera: Encyrtidae): in vivo and in vitro analysis. *Journal of insect physiology*, *43*(6), 553-565

Green, P. T., O'Dowd, D. J., Abbott, K. L., Jeffery, M., Retallick, K., & Mac Nally, R. (2011). Invasional meltdown: invader–invader mutualism facilitates a secondary invasion. *Ecology*, 92(9), 1758-1768.

Griffiths, D.C. (1960) Immunity of aphids to insect parasites. *Nature*, 187, 346.

Grinnell, J. (1919). The English sparrow has arrived in death valley: an experiment in nature. *The American Naturalist*, 53(628), 468-472.

Grobler, C.B., & Lewis, O. T. (2008). Response of native parasitoids to a range-expanding host. *Ecological Entomology*, 33(4), 453-463.

Grosholz, E. D. (2005). Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences of the United States of America*, 102(4), 1088-1091.

Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology*, 38, 251–273.

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

Hajek, A.E., Hurley, B.P., Kenis, M., Garnas, J.R., Bush, S.J., Wingfield, M.J., van Lenteren, J.C., Matthew, J. and Cock, W. (2016) Exotic biological control agents: A solution or contribution to arthropod invasions? *Biological invasions*, 18(4), 953.

Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends Ecololgy and Evolution* 4: 6–11.

Hart AJ, Tullett AG, Bale JS, Walters KFA. 2002. Effects of temperature on the establishment potential in the UK of the non-native glasshouse biocontrol agent *Macrolophus caliginosus*. *Physiological Entomology* 27:112–23

Harvey, J. A., Corley, L. S., & Strand, M. R. (2000). Competition induces adaptive shifts in caste ratios of a polyembryonic wasp. *Nature*, *406*(6792), 183-186.

Harvey J.A., van Dam N.M., Gols R. (2003) Interactions over four trophic levels: food plant quality affects development of a hyperparasitoid as mediated through an herbivore and its primary parasitoid. *Journal of Animal Ecology*, 72, 520–531.

Harvey, J. A., Bukovinszky, T., & van der Putten, W. H. (2010). Interactions between invasive plants and insect herbivores: a plea for a multitrophic perspective. *Biological Conservation*, 143(10), 2251-2259.

Harvey, J.A., Biere, A., Fortuna, T., Vet, L.E., Engelkes, T., Morriën, E., Gols, R., Verhoeven, K., Vogel, H., Macel, M. & Heidel-Fischer, H.M., 2010. Ecological fits, mis-fits and lotteries involving insect herbivores on the invasive plant, *Bunias orientalis*. *Biological Invasions*, 12(9), 3045-3059.

Harvey, J. A., & Gols, R. (2011). Population-related variation in plant defense more strongly affects survival of an herbivore than its solitary parasitoid wasp. *Journal of chemical ecology*, 37(10), 1081.

Havill, N. P., Montgomery, M. E., Yu, G., Shiyake, S., & Caccone, A. (2006). Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America*, *99*(2), 195-203.

Hayes, K. R., & Barry, S. C. (2008). Are there any consistent predictors of invasion success? *Biological Invasions*, 10(4), 483-506.

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101(41), 14812-14817.

Hellrigl, K. (2001). Neue Erkenntnisse und Untersuchungen über die Rosskastanien-Miniermotte *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae). Gredleriana, 1(2001), 9-81.

Hendrix, S. D. (1984). Reactions of *Heracleum lanatum* to floral herbivory by *Depressaria pastinacella*. *Ecology*, 65(1), 191-197.

Henter, H.J. (1995) The potential for co-evolution in a host-parasitoid system. II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution* 49:439–45

Henter, H.J. and Via, S. (1995) The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* 49:427–38

Hill, M. P., & Hulley, P. E. (1995). Biology and host range of *Gratiana spadicea* (Klug, 1829) (Coleoptera: Chrysomelidae: Cassidinae), a potential biological control agent for the weed *Solanum sisymbriifolium* Lamarck (Solanaceae) in South Africa. *Biological Control*, *5*(3), 345-352.

Hinz, H. L., Schwarzländer, M., Gassmann, A., & Bourchier, R. S. (2014). Successes we may not have had: a retrospective analysis of selected weed biological control agents in the United States. *Invasive Plant Science and Management*, 7(4), 565-579.

Hoberg, E. P., & Brooks, D. R. (2008). A macroevolutionary mosaic: episodic host- switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography*, 35(9), 1533-1550.

Hochberg, M.E. (1997) Hide or fight? The competitive evolution of concealment and encapsulation in parasitoid-host associations. *Oikos* 80:342–52

Hodges, R.W. (1974). *Moths of America North of Mexico*. (E.W. Classey & R.B.D Publications Inc., London), Fasc. 6.2.

Hokkanen, H. M., & Pimentel, D. (1989). New associations in biological control: theory and practice. *The Canadian Entomologist*, 121(10), 829-840.

Hopkins, R. J., van Dam, N. M., & van Loon, J. J. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual review of entomology*, 54, 57-83.

Hufbauer, R.A.; Via, S. (1999) Evolution of an aphid-parasitoid interaction: Variation in resistance to parasitism among aphid populations specialized on different plants. *Evolution*, 53, 1435–1445.

Hufbauer, R.A. (2001) Pea aphid-parasitoid interactions: Have parasitoids adapted to differential resistance? *Ecology*, 82, 717–725.

Hunter, M. D. (2003). Effects of plant quality on the population ecology of parasitoids. *Agricultural and Forest Entomology*, 5(1), 1-8.

Hunter, M. D., & Price, P. W. (1992). Playing chutes and ladders: heterogeneity and the relative roles of bottom- up and top- down forces in natural communities. *Ecology*, 73(3), 724-732.

Huntley, B.H & Birks, J.B. (1983) *An Atlas of Past and Present Pollen Maps for Europe: 0-13,000 Years Ago*. Cambridge University Press. Cambridge

Jalas, J. & Suominen, J. (1987) *Atlas Florae Europeae. Distribution of Vascular Plants in Europe*, Vol. 2 Cambridge University Press, Cambridge.

Janz, N., Nyblom, K., & Nylin, S. (2001). Evolutionary dynamics of host-plant specialization: a case study of the tribe Nymphalini. *Evolution*, 55(4), 783-796.

Janzen, D. H. (1985). The natural history of mutualisms. The biology of mutualism: *Ecology and evolution*, 40-99.

Jeffries, M. J., & Lawton, J. H. (1984). Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, *23*(4), 269-286.

Jermy, T. (1984). Evolution of insect/host plant relationships. *The American Naturalist*, 124(5), 609-630.

Jogesh, T., Peery, R., Downie, S. R., & Berenbaum, M. R. (2015). Patterns of genetic diversity in the globally invasive species wild parsnip (*Pastinaca sativa*). *Invasive Plant Science and Management*, 8(4), 415-429.

Kambhampati, S., & Smith, P. T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect molecular biology*, 4(4), 233-236.

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17(4), 164-170.

Keeler, M. S., & Chew, F. S. (2008). Escaping an evolutionary trap: preference and performance of a native insect on an exotic invasive host. *Oecologia*, *156*(3), 559-568.

Kempel, A., Chrobock, T., Fischer, M., Rohr, R. P., & van Kleunen, M. (2013). Determinants of plant establishment success in a multispecies introduction experiment with native and alien species. *Proceedings of the National Academy of Sciences*, 110(31), 12727-12732.

Kleinjan, C.A. & Scott, J.K. (1996). Selection of *Cassida* spp. from southern Africa for the biological control of *Chrysanthemoides monilifera* in Australia. *Annals of Applied Biology* 128, 373-385.

Klug, T., Meyhöfer, R., Kreye, M., & Hommes, M. (2008). Native parasitoids and their potential to control the invasive leafminer, *Cameraria ohridella* DESCH. & DIM.(Lep.: Gracillariidae). *Bulletin of entomological research*, 98(04), 379-387.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., & Wilson, A.C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Science*, U.S.A 86: 6196-6200.

Kolar, C.S., & Lodge, D.M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16:199-204.

Koskinen, M.T., Haugen, T.O, Primmer, C.R. (2002a) Contemporary Fisherian life-history evolution in small salmonid populations. *Nature*, 419, 826-830

Koskinene, M.T, Nilsson, J., Veselov, A.J., Potutkin, A.G., Ranta, E. & Primmer, C.R (2002b). Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae, *Heredity*, 88, 391-401.

Kraaijeveld, A.R. and van Alphen, J.J.M. (1994) Geographical variation in resistance of the parasitoid *Asobara tabida*, against encapsulation by *Drosophila melanogaser* larvae- the mechanism explored. *Physiological Entomology*, 19, 9–14.

Kraaijeveld, A.R., Godfray, H.C.J. (1999) Geographic patterns in the evolution of resistance and virulence in *Drosophila* and its parasitoids. *American Naturalist* 153:S61–S74.

Kraaijeveld, A.R.; Godfray, H.C.J. (2009) Evolution of host resistance and parasitoid counter-resistance. In A*dvances in Parasitology*; Elsevier: London, UK; Volume 70, pp. 257–280.

Kumar, S., Stecher, G., & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.

Lampert, E. C., Zangerl, A. R., Berenbaum, M. R., & Ode, P. J. (2008). Tritrophic effects of xanthotoxin on the polyembryonic parasitoid *Copidosoma sosares* (Hymenoptera: Encyrtidae). *Journal of chemical ecology*, 34(6), 783-790.

Lampert, E. C., Zangerl, A. R., Berenbaum, M. R., & Ode, P. J. (2011). Generalist and specialist host–parasitoid associations respond differently to wild parsnip (*Pastinaca sativa*) defensive chemistry. *Ecological Entomology*, 36(1), 52-61.

Lankau, R. A., & Strauss, S. Y. (2007). Community complexity drives patterns of natural selection on a chemical defense of *Brassica nigra*. *The American Naturalist*, *171*(2), 150-161.

Latter, B. D. H. (1972). Selection in finite populations with multiple alleles. III. Genetic divergence with centripetal selection and mutation. *Genetics*, 70(3), 475-490.

Lawson, B. E., Day, M. D., Bowen, M., van Klinken, R. D., & Zalucki, M. P. (2010). The effect of data sources and quality on the predictive capacity of CLIMEX models: an assessment of *Teleonemia scrupulosa* and *Octotoma scabripennis* for the biocontrol of *Lantana camara* in Australia. *Biological Control*, *52*(1), 68-76.

Le Ralec, A., Anselme, C., Outreman, Y., Poirié, M., van Baaren, J., Le Lann, C. and van Alphen, J.J.M. (2010) Evolutionary ecology of the interactions between aphids and their parasitoids. *Comptes Rendus Biologies*, 333, 554–565.

Li, S., Falabella, P., Giannantonio, S., Fanti, P., Battaglia, D., Digilio, M.C., Volkl, W., Sloggett, J.J., Weisser, W. and Pennacchio, F. (2002) Pea aphid clonal resistance to the endophagous parasitoid *Aphidius ervi*. *Journal of Insect Physiology*, 48, 971–980.

Librado P., Rozas J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451-1452

Lindholm, A.K., Breden, F., Alexander, H.J., Chan, W.K, Thakurta, S.G., & Brooks, R (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Molecular Ecology*, 14, 3671-3682.

Lively, C.M., & Dybdahl, M.F. (2000) Parasite adaptation to locally common host genotypes. *Nature*, 405, 679–681.

Lodge, D. M. (1993a). Biological invasions: lessons for ecology. *Trends in Ecology & Evolution*, 8(4), 133-137.

Lodge, D.M. (1993b). Species invasions and deletions: community effects and responses to climate and habitat change. In *Biotic Interactions and Global Change*, ed. PM Karieva, JG Kingsolver, RB Huey, pp. 367-87. Sunderland, MA: Sinauer. 559 pp

Louda, S. M., Kendall, D., Connor, J., & Simberloff, D. (1997). Ecological effects of an insect introduced for the biological control of weeds. *Science*, 277(5329), 1088-1090.

Lozier, J. D., Roderick, G. K., & Mills, N. J. (2008). Evolutionarily significant units in natural enemies: Identifying regional populations of *Aphidius transcaspicus* (Hymenoptera: Braconidae) for use in biological control of mealy plum aphid. *Biological Control*, 46(3), 532-541.

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 research*, 99(01), 83-96.

Łukasik, P., van Asch, M., Guo, H., Ferrari, J. & Godfray, H.C.J. (2013) Unrelated facultative endosymbionts protect aphids against a fungal pathogen. *Ecology Letters* 16:214–218.

Lupi, D. (2005). A 3 year field survey of the natural enemies of the horse-chestnut leaf miner *Cameraria ohridella* in Lombardy, Italy. *BioControl*, 50(1), 113-126.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer research*, 27(2 Part 1), 209-220.

Maron, J. L., & Vila, M. (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, 95(3), 361-373.

McGovern, J. L., Zangerl, A. R., Ode, P. J., & Berenbaum, M. R. (2006). Furanocoumarins and their detoxification in a tri-trophic interaction. *Chemoecology*, 16(1), 45-50.

Mills, N. J. (1994). Parasitoid guilds: defining the structure of the parasitoid communities of endopterygote insect hosts. *Environmental Entomology*, 23(5), 1066-1083.

Mills, N. J. (2000). Biological control: the need for realistic models and experimental approaches to parasitoid introductions. *Parasitoid population biology*, 217-234.

Mills, N. J. (2001). Factors influencing top-down control of insect pest populations in biological control systems. *Basic and Applied Ecology*, 2(4), 323-332.

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature*, 421(6923), 625-627.

Mittelbach, G. G. (2012). *Community ecology*. Sinauer Associates.

Mooney K.A., Pratt R.T., Singer M.S. (2012) The tri-trophic interactions hypothesis: interactive effects of host plant quality, diet breadth and natural enemies on herbivores. *PLoS One*, 7, e34403.26.

Morin, P. J. (2009). *Community ecology*. John Wiley & Sons.

Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecological Systematics* 18: 269–292.

Morrison, W. E., & Hay, M. E. (2011). Herbivore preference for native vs. exotic plants: generalist herbivores from multiple continents prefer exotic plants that are evolutionarily naïve. *PLoS One*, 6(3), e17227.

Ndemah, R., Schulthess, F., Abang, A., Ghogomu, R.T., Ntonifor, N., Dupas, S., LeRu, B. (2012) Suitability of cereal stemborers in Cameroon to Kenyan populations of the braconid larval parasitoid *Cotesia sesamiae*. *Journal of Applied Entomology*, 136, 60–69.

Nentwig, W. (2009). *Handbook of alien species in Europe* (Vol. 3). Springer Verlag.

Nicholls, J. A., Fuentes-Utrilla, P., Hayward, A., Melika, G., Csóka, G., Nieves-Aldrey, J. L., ... & Stone, G. N. (2010). Community impacts of anthropogenic disturbance: natural enemies exploit multiple routes in pursuit of invading herbivore hosts. *BMC Evolutionary Biology*, 10(1), 322.

Nitao, J.K, & Berenbaum, M.R. (1988). Laboratory rearing of the parsnip webworm, *Depressaria pastinacella* (Lepidoptera: Oecophoridae). *Annals Entomological Society of America* 81, 485-487.

Novak, M. A., Kershner, J. L., & Mock, K. E. (2005). Molecular genetic investigation of Yellowstone cutthroat trout and finespotted Snake River cutthroat trout. *Wyoming Game and Fish Commission, a Report in Partial Fulfillment of Agreement*, 165(04).

Nuttall, T. (1818). The Genera of North American Plants: And a Catalogue of the Species, to the Year 1817 (Vol. 1).

Ode, P. J., & Strand, M. R. (1995). Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. J*ournal of Animal Ecology*, 213-224.

Ode, P.J., Berenbaum, M.R., Zangerl, A.R, & Hardy, I.C.W (2004). Host plant, host plant chemistry and the polyembryonic parasitoid *Copidosoma sosares*: indirect effects in a tritrophic interaction. Oikos, 104(2), 388-400

O'Dowd, D. J., Green, P. T., & Lake, P. S. (2003). Invasional 'meltdown'on an oceanic island. *Ecology Letters*, 6(9), 812-817.

Olckers, T., Hoffmann, J. H., Moran, V. C., Impson, F. A. C., & Hill, M. P. (1999). The initiation of biological control programmes against *Solanum elaeagnifolium* Cavanilles and *S. sisymbriifolium* Lamarck (Solanaceae) in South Africa. *African Entomology Memoir*, *1*, 1990-98.

Oliver, K.M., Degnan, P.H., Hunter, M.S. & Moran, N.A. (2009) Bacterio-phages encode factors required for protection in a symbiotic mutualism. *Science* 325:992–994

Oliver, K.M., Noge, K., Huang, E.M., Campos, J.M., Becerra, J.X., & Hunter, M.S. (2012). Parasitic wasp responses to symbiont-based defense in aphids. *BMC Biology*. 10:11

Palmer, W. A., & Witt, A. B. R. (2006). On the host range and biology of *Acizzia melanocephala* (Hemiptera: Psyllidae), an insect rejected for the biological control of *Acacia niloticai* subsp. *indica* (Mimosaceae) in Australia: short communication.

Pannebakker, B.A.; Garrido, N.R.T.; Zwaan, B.J.; van Alphen, J.J.M. (2008) Geographic variation in host-selection behaviour in the Drosophila parasitoid *Leptopilina clavipes*. *Entomologia Experimentalis et Applicata*, 127, 48–54.

Park, J. S., Kim, S. S., Kim, K. Y., & Kim, I. (2016). Complete mitochondrial genome of Suzuki's *Promolactis* moth *Promalactis suzukiella* (Lepidoptera: Oecophoridae). *Mitochondrial DNA Part A*, 27(3), 2093-2094.

Parker, J. D., & Hay, M. E. (2005). Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters*, 8(9), 959-967.

Parker, J. D., Burkepile, D. E., & Hay, M. E. (2006). Opposing effects of native and exotic herbivores on plant invasions. *Science*, 311(5766), 1459-1461.

Pimentel, D. (1986). Biological invasions of plants and animals in agriculture and forestry. In *Ecology of biological invasions of North America and Hawaii* (pp. 149-162). Springer New York.

Poirié, M. and Coustau, C. (2011) The evolutionary ecology of aphids' immunity. *ISJ-Invertebrate Survival Journal*, 8, 247–255.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., & Weis, A. E. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, 11(1), 41-65.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959.

Puillandre, N., Dupas, S., Dangles, O., Zeddam, J. L., Capdevielle-Dulac, C., Barbin, K., ... & Silvain, J. F. (2008). Genetic bottleneck in invasive species: the potato tuber moth adds to the list. *Biological Invasions*, 10(3), 319-333.

Quacchia, A., Ferracini, C., Nicholls, J. A., Piazza, E., Saladini, M. A., Tota, F., ... & Alma, A. (2013). Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north- western Italy. *Insect Conservation and Diversity*, 6(2), 114-123.

Rey, J. R., McCoy, E. D. and Strong, D. R. (198). Herbivore pests, habitat islands, and the species-area relation. *American Naturalist* 117: 611-622.

Riley, C. V. (1889). The parsnip webworm (*Depressaria heracliana* DeG.). *Insect Life*, 1, 94-98.

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

Roques, L., Auger-Rozenberg, M. A., & Roques, A. (2008). Modelling the impact of an invasive insect via reaction-diffusion. *Mathematical biosciences*, 216(1), 47-55.

Rosenberg, N.A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4(1): 137-138.

Rouchet, R., & Vorburger, C. (2014). Experimental evolution of parasitoid infectivity on symbiont-protected hosts leads to the emergence of genotype specificity. *Evolution*, *68*(6), 1607-1616.

Russo, J., Dupas, S., Frey, F., Carton, Y., & Brehelin, M. (1996). Insect immunity: early events in the encapsulation process of parasitoid (Leptopilina boulardi) eggs in resistant and susceptible strains of Drosophila. *Parasitology*, 112(1), 135-142.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., ... & Leemans, R. (2000). Global biodiversity scenarios for the year 2100. *Science*, 287(5459), 1770-1774.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4(4), 406-425.

Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P. C., Seeman, O. D., & Allen, G. R. (2007). Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. *Heredity*, 99(4), 414-422.

Schönrogge, K., Stone, G. N., & Crawley, M. J. (1996). Abundance patterns and species richness of the parasitoids and inquilines of the alien gall-former *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Oikos*, 507-518.

Sefrova, H. (2003) Invasions of Lithocolletinae species in Europe – causes, kinds, limits and ecological impact (Lepidoptera, Gracillariidae). *Ekologia-Bratislava*, 22, 132–142.

Segoli, M., Harari, A. R., Bouskila, A., & Keasar, T. (2009). Brood size in a polyembryonic parasitoid wasp is affected by relatedness among competing larvae. *Behavioral Ecology*, arp057.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, 139(1), 457-462.

Shapiro, A.M. (2002) The Californian urban butterfly fauna is dependent on alien plants. *Diversity and Distributions*, 8, 31–40.

Siemann, E., Rogers, W. E., & Dewalt, S. J. (2006). Rapid adaptation of insect herbivores to an invasive plant. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1602), 2763-2769.

Simberloff D. 1996. Impacts of introduced species in the United States. Consequences: *Nat. Implic. Environ. Change* 2:13–22.

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

Smith, D. S., Bailey, J. K., Shuster, S. M., & Whitham, T. G. (2011). A geographic mosaic of trophic interactions and selection: trees, aphids and birds. *Journal of evolutionary biology*, 24(2), 422-429.

Stenberg, J. A. (2012). Plant-mediated effects of different Salix species on the performance of the braconid parasitoid *Perilitus brevicollis*. *Biological Control*, 60(1), 54-58.

Stone, G. N., & Sunnucks, P. (1993). Genetic consequences of an invasion through a patchy environment—the cynipid gallwasp *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Molecular Ecology*, 2(4), 251-268.

Stone, G. N., Schönrogge, K., Crawley, M. J., & Fraser, S. (1995). Geographic and between-generation variation in the parasitoid communities associated with an invading gallwasp, *Andricus quercuscalicis* (Hymenoptera: Cynipidae). *Oecologia*, 104(2), 207-217.

Stone, G. N., Lohse, K., Nicholls, J. A., Fuentes-Utrilla, P., Sinclair, F., Schönrogge, K., ... & Tavakoli, M. (2012). Reconstructing community assembly in time and space reveals enemy escape in a Western Palearctic insect community. *Current Biology*, 22(6), 532-537.

Strand, M. R., & Ode, P. J. (1990). Chromosome number of the polyembryonic parasitoid *Copidosoma floridanum* (Hymenoptera: Encyrtidae). *Annals of the Entomological Society of America*, *83*(4), 834-837.

Strand, M. R., Johnson, J. A., & Culin, J. D. (1990). Intrinsic interspecific competition between the polyembryonic parasitoid *Copidosoma floridanum* and solitary endoparasitoid *Microplitis demolitor* in *Pseudoplusia includens*. *Entomologia Experimentalis et Applicata*, 55(3), 275-284.

Strong, D. R. (1974). Rapid asymptotic species accumulation in phytophagous insect communities: the pests of cacao. *Science*, 185(4156), 1064-1066.

Strong, D. R. 1(979). Biogeographic dynamics of insect-host plant communities. *Annual Review of Entomology* 24: 89-119

Strong, D. R., McCoy, E. D., & Rey, J. R. (1977). Time and the number of herbivore species: the pests of sugarcane. *Ecology*, 58(1), 167-175.

Strong, D. R., Lawton, J. H. and Southwood, R. (1984). *Insects on plants*: community patterns and mechanisms. Harvard University Press, Cambridge, USA

Sturtevant, E.L (1890) The history of garden vegetables. *American Naturalist* 24:30-49.

Suckling, D. M., & Brockerhoff, E. G. (2010). Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). *Annual review of entomology*, *55*, 285-306.

Takezaki, N., Nei, M., & Tamura, K. (2010). POPTREE2: Software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. *Molecular biology and evolution*, 27(4), 747-752.

Thomas, C.D., Bodsworth, E.J., Wilson, R.J., Simmons, A.D., Davies, Z.G., Musche, M. *et al*. (2001) Ecological and evolutionary processes at expanding range margins. *Nature*, 411, 577–581.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, 22(22), 4673-4680.

Thompson, J. N. (1988). Coevolution and alternative hypotheses on insect/plant interactions. *Ecology*, 69(4), 893-895.

Thompson, J. N. (1994). The coevolutionary process. University of Chicago Press, Chicago.

Thompson, J. N. (1999). Specific hypotheses on the geographic mosaic of coevolution. *The American Naturalist*, 153(S5), S1-S14.

Torchin, M. E., Lafferty, K. D., Dobson, A. P., McKenzie, V. J., & Kuris, A. M. (2003). Introduced species and their missing parasites. *Nature*, 421(6923), 628-630.

Tsutsui, N. D., Suarez, A. V., Holway, D. A., & Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences*, 97(11), 5948-5953.

Uka, D., Hiraoka, T., & Iwabuchi, K. (2006). Physiological suppression of the larval parasitoid *Glyptapanteles pallipe*s by the polyembryonic parasitoid *Copidosoma floridanum*. *Journal of insect physiology*, *52*(11), 1137-1142.

Urbaneja, A., Llácer, E., Tomás, O., Garrido, A. & Jacas, J. (2000) Indigenous natural enemies associated with *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in eastern Spain. *Biological Control*, 18, 199–207.

Utsunomiya, A., & Iwabuchi, K. (2002). Interspecific competition between the polyembryonic wasp *Copidosoma floridanum* and the gregarious endoparasitoid *Glyptapanteles pallipes*. *Entomologia experimentalis et applicata*, *104*(2- 3), 353-362.

Van Lenteren, J. C., Bale, J., Bigler, F., Hokkanen, H. M. T., & Loomans, A. J. M. (2006). Assessing risks of releasing exotic biological control agents of arthropod pests. *Annual Review of Entomology*, *51*, 609-634.

van Nouhuys, S., Niemikapee, S., & Hanski, I. (2012). Variation in a host–parasitoid interaction across independent populations. *Insects*, 3(4), 1236-1256.

Verhoeven, K. J., Biere, A., Harvey, J. A., & Van Der Putten, W. H. (2009). Plant invaders and their novel natural enemies: who is naive? *Ecology Letters*, 12(2), 107-117.

Vilà, M., Bartomeus, I., Dietzsch, A. C., Petanidou, T., Steffan-Dewenter, I., Stout, J. C., & Tscheulin, T. (2009). Invasive plant integration into native plant–pollinator networks across Europe. *Proceedings of the Royal Society of London B: Biological Sciences*, 276(1674), 3887-3893.

Vitousek, P. M., Walker, L. R., Whiteaker, L. D., Mueller-Dombois, D., & Matson, P. A. (1987). Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science*, 238(4828), 802-804.

von Burg, S., Ferrari, J., Müller, C.B. and Vorburger, C. (2008) Genetic variation and covariation of susceptibility to parasitoids in the aphid Myzus persicae–no evidence for trade-offs. *Proceedings of the Royal Society B-Biological Sciences*, 275, 1089–1094.

Vorburger, C., Sandrock, C., Gouskov, A., Castañeda, L.E. and Ferrari, J. (2009) Genotypic variation and the role of defensive endosymbionts in an all-parthenogenetic host–parasitoid interaction. *Evolution*, 63, 1439–1450.

Wang X.G., Nadel H., Johnson M.W., Daane K.M., Hoelmer K., Walton V.M., Pickett C.P., Sime K.R. (2009) Crop domestication relaxes both bottom-up and top-down effects on a specialist herbivore. *Basic and Applied Ecology*, 10, 216–227.

Wang, X. G., Hogg, B. N., Levy, K., & Daane, K. M. (2013). Predicting the outcomes of a tritrophic interaction between an indigenous parasitoid and an exotic herbivorous pest and its host plants. *Annals of applied biology*, 163(2), 288-297.

Wapshere A.J. (1974) A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* 77, 201–211

Wapshere A.J. (1983) Discovery and testing of a climatically adapted strain of *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae) for Australia. *Entomophaga* 28, 27–32.

Wapshere A.J. (1993) Climate and the prospects for biological control of weeds: the contrasting examples of agents for *Solanum elaeagnifolium* and *Heliotropium amplexicaule*. In: *Pest Control and Sustainable Agriculture* (eds SA Corey, DJ Dall & WM Milne), pp. 200–202. CSIRO, Melbourne, Australia.

Wastie, R. L. (1975). Diseases of rubber and their control. *PANS Pest Articles & News Summaries*, 21(3), 268-288.

Webb, C. J. (1978). Checklist of dicotyledons naturalised in New Zealand 1. Umbelliferae. *New Zealand Journal of Botany*, 16(3), 387-390.

Weiher, E. & Keddy, P. (1991). *Ecological assembly rules: perspective, advances, retreats*. Cambridge University Press, Cambridge.

Whiteman, N. K., Kimball, R. T., & Parker, P. G. (2007). Co- phylogeography and comparative population genetics of the threatened Galápagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Molecular Ecology*, 16(22), 4759-4773.

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying Threats to Imperiled Species in the United States Assessing the relative importance of habitat destruction, alien species, pollution, overexploitation, and disease. *BioScience*, 48(8), 607-615.

Wilson, C. G., Flanagan, G. J. and Gillet, J. D. 1990. The phytophagous insect fauna of the introduced shrub *Mimosa pigra* in northern Australia and its relevance to biological control. *Environmental Entomology* 19: 776-784.

Zappala, L., Bernardo, U., Biondi, A., Cocco, A., Deliperi, S., Delrio, G., ... & Siscaro, G. (2012). Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bulletin of Insectology*, 65(1), 51-61.

Zangerl, A. R., & Berenbaum, M. R. (1997). Cost of chemically defending seeds: furanocoumarins and Pastinaca sativa. *The American Naturalist*, *150*(4), 491-504.

Zangerl, A.R. Huang, T., McGovern, J.L. et al (2002). Paradoxical host shift by *Depressaria pastinacella* in North America: is enemy-free space involved? *Oikos* 98, 431-436.

Zangerl, A. R., & Berenbaum, M. R. (2003). Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. *Evolution*, 57(4), 806-815.

Zangerl, A., Stanley, M. & Berenbaum, M. (2008). Selection for chemical trait remixing in an invasive weed after reassociation with a coevolved specialist. *Proceedings of the National Academy of Science* USA 102: 15529–15532.