

DISSERTATION

COMPETITIVE INTERACTIONS OF TWO PARASITIC WASPS THAT REQUIRE THE
SAME HOST

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ABSTRACT

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Behavior is a dynamic trait that allows animals to cope with their environment. When individuals of different species compete for the same resources, certain behaviors become important for affecting the outcome of competition. These behaviors can undergo significant changes as a species exists in different ecological communities in which the level of competition is altered. Comparisons of spatially separated populations enhances our understanding of how interspecific interactions influence animal behavior.

Cotesia glomerata and *C. rubecula* are two parasitoids wasps with different relationships based on their geography. In their original habitats in Europe, the two species coexist with little direct competition because each wasp parasitizes a different species of caterpillar. Coexistence changed to competition when the two parasitoids were established in North America where the primary host of *C. glomerata* is absent leading *C. glomerata* to attack *Pieris rapae*, the sole host of *C. rubecula*. Competition favors *C. rubecula* leading to competitive exclusion of *C. glomerata* in most parts of the US and southern Canada, yet there are some populations of *C. glomerata* that live without *C. rubecula* and other populations that coexist with this congener. While we know that the host shift to *P. rapae* led to differences in parasitoid-host interactions between North American and European populations of *C. glomerata*, it is unknown whether competition with *C. rubecula* has affected the behavior of *C. glomerata* in North America. My goal was to examine the behavioral and ecological factors that might explain the outcomes

(competitive displacement or coexistence) of interspecific interactions between these two parasitoid wasps.

The introductory chapter introduces competitor-free space and geographic mosaics of selection, two concepts that are relevant to the *Cotesia* parasitoids at the center of my research. *Cotesia glomerata* had unchallenged access to *P. rapae* in North America for 80 years before *C. rubecula* arrived and began competing with *C. glomerata* for the same host. *Cotesia rubecula* failed to reach every *C. glomerata* population and while some *C. glomerata* populations were displaced by *C. rubecula*, others persist with this competitor. Thus, North American *C. glomerata* occur in a patchwork of competitive environments with some populations experiencing competitor-free space, others having been extirpated by *C. rubecula*, and a few where *C. glomerata* evades competitive displacement. Chapter 2 provides details into the natural history and biology of the *Pieris-Cotesia* system. I also present the timeline of the introductions of *C. glomerata* and *C. rubecula* to North America, and their subsequent spread throughout parts of the continent. These two parasitoids offer a rare opportunity to understand how animal behavior is affected by competitive interactions that vary across the range of a broadly distributed species.

In Chapter 3, I explore the differences in foraging behaviors between Colorado and Maryland *C. glomerata*, as well as how *C. glomerata* differed from *C. rubecula*. My focus is on evaluating differences between the species in their foraging efficiency as they foraged in patches of low to high densities of hosts. I also examine the time spent finding a host (i.e., search time) and how long the wasps took to attack a host (i.e., handling time). Wasps were observed foraging alone or with the heterospecific since some parasitoids can adjust their foraging behaviors in the presence of stronger competitors. In addition to *C. rubecula* from Maryland, I

used *C. rubecula* from Minnesota where *C. rubecula* has displaced *C. glomerata*. Regardless of their place of origin, both *Cotesia* species attacked more hosts as host density increased. Interspecific differences in foraging performance were minimal with some evidence suggesting that *C. glomerata* from Maryland attacked a fewer number of hosts in the presence of *C. rubecula*. Colorado *C. glomerata* had the slowest search and handling times, whereas Maryland *C. rubecula* had the fastest times. Interestingly, the Colorado *C. glomerata* were slightly slower at finding hosts than were Maryland *C. glomerata*, which were just as fast as Minnesota *C. rubecula*. Foraging efficiency influences the rate at which a parasitoid exploits its hosts, which is important for interspecific competition. Given that *C. rubecula* parasitized hosts are deadly for *C. glomerata*, the inability of *C. glomerata* to find and attack hosts first could be disadvantageous where it to suddenly share its habitat with *C. rubecula*.

In Chapter 4, I investigate whether *C. glomerata* females avoid hosts parasitized by *C. rubecula* since competition inside the host favors *C. rubecula*. I compare the foraging decisions of females from Colorado, where *C. rubecula* is absent, to conspecifics from Maryland where *C. glomerata* coexists with *C. rubecula*. In a patch of *P. rapae* caterpillars where some are unparasitized and others have been attacked by *C. rubecula*, selecting the latter should negatively affect *C. glomerata* population growth as mortality would increase in these hosts. I found that *C. glomerata* avoided *C. rubecula* parasitized caterpillars, and this avoidance was greater in *C. glomerata* from Maryland than in conspecifics from Colorado. *Cotesia glomerata* populations may show differences in foraging behaviors based on the length of time they have associated with *C. rubecula*. *Cotesia rubecula* is a mortality factor for *C. glomerata*, which may only experience selection for avoiding hosts previously parasitized by *C. rubecula* if sharing the

habitat with the congener. The failure to avoid *C. rubecula* parasitized hosts should contribute to the displacement of *C. glomerata* once *C. rubecula* enters the same habitat.

Lastly, I compare the diversity of hyperparasitoids attacking *C. glomerata* in Colorado and Maryland. The goal was to identify differences in the community of hyperparasitoids between *C. glomerata* populations, and to determine if this higher trophic level had differential impact on *C. glomerata* and *C. rubecula*. As a top-down pressure, hyperparasitoids have been implicated in the failure of *C. rubecula* to establish in parts of North America. At the per host, brood level, *Cotesia rubecula*, with its single larva, is much more susceptible to mortality from hyperparasitoids than is a typical brood of 20-30 *C. glomerata*. I suspected that the coexistence of *C. glomerata* and *C. rubecula* in Maryland could be partly explained by differences in mortality experienced by these two parasitoids from the shared community of natural enemies. Field observations showed that both *Cotesia* species were attacked by the same hyperparasitoids, but that *C. rubecula* experienced greater mortality from these enemies. In addition, a greater abundance and diversity of hyperparasitoids emerged from *C. glomerata* broods. My research suggests that through apparent competition, hyperparasitoids may reduce the competitive advantage experienced by *C. rubecula* over *C. glomerata*, allowing for coexistence in places like Maryland. As dispersing *C. rubecula* populations enter habitats previously occupied by *C. glomerata* (e.g., Colorado) and its hyperparasitoids, the success of the colonizing population may be contingent on top-down factors.

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DEDICATION

To my parents, whose countless sacrifices paved the way for my accomplishments.

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CHAPTER 1: Competitor-free spaces and mosaics of selection among parasitoids

1.1 INTRODUCTION

Species undergo significant changes as their populations spread to new environments and experience new selection pressures (Sax et al. 2007; Prentis et al 2008). An important driver of these changes is the formation of interspecific interactions (e.g., predation, competition, mutualism) between the community of resident species and the new species. These interactions create selection pressures that shape physical and behavioral traits (predation: Abrams and Matsuda 1997; competition: Brown and Wilson 1956; mutualism: Janzen 1966). As a species expands its range and encounters new heterospecifics, it may experience changes in traits that originally evolved in response to interactions established in the ancestral community. For example, when populations of prey escape predation and occupy enemy-free space (Jeffries and Lawton 1984; Berdegue et al. 1996), these populations can undergo changes in physical features (loss of physical defenses – Marchinko and Schluter 2007; Mezquida and Benkman 2005) and behavioral traits (lack of predator avoidance – Cousyn et al. 2001; Stoks et al 2003). Within a community of species with overlapping resource requirements, competition becomes a significant selection pressure. While much is known about how prey change following release from predators (Jeffries and Lawton 1984), less is known about how weaker competitors change following a reduction in the severity of competition. We also have an insufficient understanding of how, following dissociation, species react to reuniting with interspecific competitors with which an ecological and evolutionary history once existed. After a reunion, do communities reassemble in a manner prior to separation, or does separation cause enough change to create a novel outcome following the reassociation?

Competition was originally proposed to explain the composition of species within a community (Gause 1934; MacArthur 1958; Connell 1978; Tilman 1982). After much debate regarding its significance, competition has re-established its importance within evolutionary and community ecology (Wiens 1977; Schoener 1982; Ferson et al. 1986). Overlapping resource requirements can cause individuals to engage in competitive interspecific interactions, especially if these resources are essential and limited in supply. In exploitative competition, the winner of competitive interactions may indirectly outcompete its rivals by being more efficient at using a resource, thus reducing the availability of that resource for competitors (Begon et al. 1986). In contrast, interference competition occurs when an individual of one species directly impedes individuals of another species from accessing a resource, often using physical (e.g., aggression) or chemical (e.g., pheromone markers) mechanisms (Begon et al. 1986). When faced with competition, weaker competitors must use strategies that reduce the negative impact from stronger competitors, otherwise the population of weaker individuals risks extinction.

Competition can be more severe within a guild where species have similar resource demands (Root 1967; Pianka 1980; Simberloff and Dayan 1991). Guild members can evolve mechanisms to reduce the costs of competition. Studies on character displacement demonstrate how interspecific competition affects the traits and niche preferences of similar species across a diversity of taxa (mammals: Dayan et al. 1989; lizards: Losos 1992; fish: Schluter 1994; birds: Grant and Grant 2006; amphibians: Pfenning and Pfenning 2010). In each of these systems, competition caused the species to be more dissimilar in regions of sympatry than in allopatry. These studies confirm that competition is a selection pressure shaping the behavioral and physical parameters of a population to avoid losing during competitive encounters.

Resources are central to competition and since animals use foraging behaviors to acquire resources, these behaviors can determine the outcome of competitive interactions. When the outcome of interspecific competition is asymmetrical and consistently favors the stronger species, the foraging strategies of weaker competitors should be under strong directional selection. Animals use a diversity of behavioral strategies to avoid the costs of losing to a stronger competitor. Temporal and spatial shifts in foraging patterns, as well as changes in resource preferences, are mechanisms leading to coexistence (Roughgarden 1974; Chesson 1985; Chesson and Rosenzweig 1991; Chesson 2000). For a broadly distributed species, each population may experience a different severity of competition if the community of competing heterospecifics differ among populations. This patchwork of interactions can lead to diverse behaviors used by species to mediate competition. Assessing the geographic patterns in ecological relationships enhances our understanding of how competition shapes the behaviors used to acquire vital resources.

1.2 PARASITOIDS & COMPETITION

Competitive displacement is a phenomenon documented across numerous insect taxa (Reitz and Trumble 2002). Parasitoid insects are particularly susceptible to competition because they require hosts for reproduction, thus the host is directly linked to fitness. Six orders of insects contain parasitoid lifestyles, but wasps are the most numerous and well-studied group of parasitoids (Godfray 1994; Quicke 1997; Wajnberg et al. 2008). The life cycles of these wasps include a host-dependent immature (egg and larvae) stage and a free-living adult stage. The importance of interference and exploitative competition differs based on the parasitoid's age. Interference competition is most often observed among larval wasps that can have large

mandibles used to attack and kill conspecifics and heterospecifics developing on or in the same host individual (Harvey et al. 2013). In contrast, adult wasps usually engage in exploitative competition where the winner is often the most efficient at foraging for hosts (Boivin and Brodeur 2006). At each developmental stage, interspecific competition has shaped the behavior of parasitoid wasps.

Parasitoid wasps are useful models for investigating the relationship between behavior and competition across multiple populations for several reasons. First, interspecific competition for an individual host among larval parasitoids often results in mortality for the losing species. Hosts can be attacked by more than one species of parasitoid in what is known as multiparasitism (May and Hassell 1981; Harvey et al 2013). However, often only one species successfully develops and emerges from a multiparasitized host, leaving the weaker competitors to die in or on the host. Second, wasp behavior plays an essential role in mediating competition. Foraging efficiency, defined by search and handling time, is often greater for parasitoid wasps that outcompete and displace heterospecifics that attack the same host (Dowell and Horn 1977; Harcourt 1990; Chua et al. 1990; Pijls et al. 1995). Parasitoid wasps can distinguish multiparasitized hosts and avoid hosts previously attacked by a superior competitor (Vinson 1976; van Baaren et al. 1994;). Even immature parasitoid wasps (i.e., larvae) have behaviors used during competition as they often attack and kill competitors (Harvey et al. 2013). Lastly, many parasitoid species can be found in geographically separated populations that experience different communities of heterospecifics (Mitsui et al. 2007; Rull et al. 2009; Klapwijk and Lewis 2011; Wood et al. 2017). Some of these populations inhabit communities where numerous parasitoids attack the same host species (i.e., high levels of competition), whereas other populations are in communities with few parasitoid species with overlapping host

preferences (i.e., fewer interspecific competitors). These spatial patterns of competition could lead to populations experiencing different types of selection for behaviors important for interspecific competition (Fig. 1.1).

In this dissertation, I use parasitoid wasps as a model system to argue that variability in the strength of competition can drive changes in animal behavior. I start by reviewing studies that document competitor-free space, a subset of enemy-free space, among parasitoids. These examples are important to establish that the evolution of parasitoid behavior is affected by release from competitors. Thereafter, I summarize literature showing differences among populations of parasitoids with broad geographical distributions. These studies demonstrate that parasitoid populations can diverge in behavioral traits as they experience different degrees of selection pressures throughout their range. Together, these examples form the foundation for my thesis that differences in the competitive environment of the parasitoid wasp, *Cotesia glomerata* (Hymenoptera: Braconidae), have influenced the foraging behaviors that are important for competing with its stronger competitor, *C. rubecula*, in North America.

1.3 THEORETICAL BACKGROUND: How Parasitoids Gain Competitor-free Space

Jefferies and Lawton (1984) suggested that enemy-free spaces (EFS) reduce or eliminate a species' vulnerability to one or more types of natural enemies. In most EFS studies, predators have been the enemy exerting top-down pressure on prey. As they escape from predation, prey populations undergo evolutionary changes resulting from weakened selection on traits that evolved to deal with predation (Berdegue et al. 1996; Lahti et al. 2009). Prey populations in enemy-free spaces are often different from conspecific populations that continue to experience predation pressure. As an ecological concept, EFS has been used to explain niche partitioning

among prey species in several aquatic and terrestrial communities. In their review of EFS, Berdegue et al. (1996) refine EFS as a discrete moment in evolutionary time where prey experience higher fitness through mechanisms that reduce selection pressures imposed by natural enemies. Observable coping strategies range from changing the preferred habitat to modifications of the physiology, morphology and behavior of prey species. In addition to improving the definition of EFS, Berdegue et al. (1996) developed three conditions required for EFS to exist. First, there must be reduced fitness in the presence of an enemy in the preferred habitat. Second, when the enemy is present, fitness is higher in an alternate habitat than in the preferred habitat with the natural enemy. Third, when enemies are lacking, fitness in the alternate habitat is lower than in the preferred habitat. The attention to predators as enemies restricts our understanding of EFS to just trophic interactions. We need to expand our focus to other interspecific interactions, such as competition, to gain a more complete view of how EFS affects ecological and evolutionary relationships between species.

Like predation, competition can cause mortality and loss of fitness. The outcome of competition between species is asymmetrical if a weaker species consistently suffers most of the costs. When the consequences of competition favor one species, the weaker competitors must use strategies to escape competition. Competitor-free space (CFS) is a type of EFS whereby a weaker competitor experiences higher fitness away from the stronger competitor. As a subset of EFS, CFS needs more attention because it impacts the structure of ecological communities. Unlike predator-prey based EFS that spans trophic levels, CFS can help explain the dynamics between species within the same trophic level or guild (e.g., parasitoids).

While EFS is recognized as an important factor shaping the ecological niche of a diversity of herbivorous insects (Jefferies and Lawton, 1984; Feder 1995; Mulatu et al, 2004;

Murphy 2004; Heard et al, 2006), few studies examine how EFS impacts parasitoid insects. The most thorough review of EFS among parasitoids focused on how parasitoids seek EFS to avoid mortality from predators (e.g., birds) and hyperparasitoids (i.e., wasps that parasitize parasitoids) (Murphy et al., 2014), but not in the context of intraguild competitors. Parasitoid insects are beneficial models for studying the evolution of adaptations that create competitor-free spaces. Within parasitoid communities, there is often overlap in host preferences, significantly increasing competitive pressure for parasitoid species. As insect pests colonize new environments, biological control efforts move one or more of the pests' parasitoids from their original habitats to the newly inhabited area to control the unwanted insects. Previous introductions of parasitoids into new ecological communities often created competitive interactions with existing parasitoids (Ehler 1982; Keller 1984; Bennett 1993), thus allowing unique opportunities to study the impact of interspecific competition on community assemblages. The competitive interactions between introduced and existing parasitoid species have led to two important strategies by which parasitoids achieve CFS: switching to new hosts and evolving new traits to exploit existing hosts.

1.3.1 Competitor-free space from host switching

The host insect is essential for parasitoid reproduction and incompatible hosts lead to evolutionary dead-ends as the immature parasitoids fail to develop into successful adults. Parasitoids have evolved behavioral adaptations that enable them to find the appropriate habitat of the host, identify the correct host species, and develop in the host (Vinson 1998). Specialist parasitoids usually attack one or a few host species, whereas generalist parasitoids have a wider range of acceptable hosts (Godfray 1994). Competition between multiple species of parasitoids

for the same host can lead to displacement or extinction of weaker competitors (DeBach and Sundby 1963; Selhime et al. 1969; Harcourt 1990; Pijls et al. 1995). For weaker competitors to survive, they must adopt strategies to avoid competition with stronger competitors. One solution is to exploit a host option that offers refuge from competition. Host switching is an important strategy used by parasitoids to find competitor-free space.

Messing and Wang (2009) examined a system where host switching occurred following the arrival of a stronger competitor that preferred the hosts of an established parasitoid. *Diachasmimorpha tryoni* is a parasitoid wasp from Australia that was brought to Hawaii in 1913 to control introduced Mediterranean fruit flies. The introductions were successful and *D. tryoni* became established in Hawaii. In the 1950s, *Fopius arisanus*, another parasitoid wasp of fruit flies, was brought from Asia to Hawaii to control Oriental fruit flies attacking coffee and other fruit crops (Bess et al., 1961). However, *F. arisanus* attacks many species of host insects and it eventually began to attack Mediterranean fruit flies, the preferred host of *D. tryoni*. *Fopius arisanus* displaced *D. tryoni* to the point where *D. tryoni* was undetectable from Mediterranean fruit fly hosts (Clancy, 1950; Bess et al., 1961). *Diachasmimorpha tryoni* avoided extinction by switching to lantana gall flies (*Eutreta xanthochaeta*), which were never documented to be attacked by *D. tryoni* before the arrival of *F. arisanus* (Clancy, 1950). The switch to the lantana gall fly was remarkable because it required *D. tryoni* to forage on lantana, which is taxonomically unrelated to the plants on which Mediterranean fruit flies fed. Parasitoids are sensitive to specific plant volatiles emitted by herbivory, and these volatiles direct foraging parasitoids to their hosts (Vet and Dicke 1992; Hare 2011). Lantana gall flies are found on lantana plants, which occur in habitats different from the agricultural fruit fields that are home to

Mediterranean fruit fly hosts. In order to exploit competitor-free space, *D. tryoni* had to evolve a mechanism of finding the new habitat, identifying the novel host and developing in this host.

The behavioral plasticity exhibited by *D. tryoni* was likely selected as a result of competition with *F. arisanus* (Messing and Wang 2009). If *D. tryoni* was unable to adapt to the competitive pressure, it is likely to have been excluded and driven to extirpation in Hawaii. The lantana gall fly serves as a competitor-free space based on the three tests for enemy-free space (Berdegue et al. 1996). *Diachasmimorpha tryoni* experienced lower fecundity and slower development time when attacking the lantana gall fly, which *D. tryoni* avoids in preference for the Mediterranean fruit fly in experiments where *F. arisanus* is removed. Only when *F. arisanus* is present does *D. tryoni* achieve fitness benefits from attacking lantana gall fly. Despite the costs for *D. tryoni* on its new host species, data from 60 years of surveys on the Hawaiian Islands have documented *D. tryoni* continuing to parasitize lantana gall flies, likely due to the dominance of *F. arisanus* over *D. tryoni*.

1.3.2 Competitor-free space from novel traits for exploiting existing hosts

A complete switch to a novel host species is one method of avoiding competition, but some parasitoid species have evolved methods of partitioning different niches within the same host or host plant to create competitor-free spaces. For female parasitoids that lay eggs inside of their hosts, the size of the ovipositor can vary depending on the substrate being penetrated. The variation in ovipositor size makes this trait susceptible to selection pressures as the host defend themselves against attacks by evolving thicker cuticles (Gross 1993; Vogelweith et al. 2014) or finding refuge deeper within plant tissues (e.g., bark, fruit, seeds) (Gross 1993; Greeney et al. 2012). In response, the size of the ovipositor can evolve to increase successful injection of eggs

into the host. Three species of parasitoid wasps, in the genus *Megarhyssa*, have undergone sympatric speciation because of competition for hosts. Each species specializes in different depths at which the ovipositor can reach their wood-boring hymenopteran host *Tremex columba* (Heatwole and Davis 1965). Hosts at the shallowest depths are exploitable by each of the three species, leading to strong competition where only one wasp species is the strongest competitor. Wasps with longer ovipositors gain a fitness advantage by exploiting hosts unreachable by the other wasps, and these wasps may have experienced directional selection for longer ovipositors. Competitor-free space was created by attacking hosts at greater depths, away from the competitively superior species of *Megarhyssa* that primarily attacks hosts at shallower depths. Gibbons (1979) considered this an example of sympatric speciation resulting from competitive interactions, which acted as selection pressures leading to polymorphic ovipositors. Courtship behaviors and prezygotic barriers are thought to contribute to selection against hybrids between the three *Megarhyssa* species.

Directional selection on ovipositor length impacted the interspecific interactions between two sympatric parasitoid wasps that attack fruit flies. *Utetes anastrephae* and *Doryctobracon areolatus* share the same geographic range throughout the Neotropics, inhabit the same altitudinal ranges and are recovered from the same fruit and fruit fly hosts (Aluja et al. 2013). Despite these similarities, there are differences in the abundance of each parasitoid depending on the type of fruit. *Doryctobracon areolatus* is more commonly found in larger fruits, whereas *U. anastrephae* flourishes in smaller fruit (Sivinski 1991, Sivinski et al. 1997). A longer ovipositor in *D. areolatus* enables it to access fruit fly larvae that are deeper within the pulp. The shorter ovipositors of *U. anastrephae* restrict it to smaller fruits. When both species attack fruit flies in smaller sized fruits, *U. anastrephae* is the stronger competitor and *D. areolatus* larvae are often

killed within the host by the aggressive larvae of *U. anastrephae* (Aluja et al. 2013). The longer ovipositor length enables *D. areolatus* to have competitor-free space and access hosts beyond the reach of *U. anastrephae*. The introduction of *Diachasmimorpha longicaudata*, a parasitoid wasp with an ovipositor longer than that of *D. areolatus*, verified the importance of the longer ovipositor and its role in competitive interactions for *D. areolatus*. *Diachasmimorpha longicaudata* shares the same host range as *D. areolatus* and *U. anastrephae*, but it competitively displaced *D. areolatus* from the shared range (Paranhos et al. 2013). The longer ovipositor of *D. longicaudata* eliminated the competitor-free space occupied by *D. areolatus* and resulted in competition with *D. longicaudata*, an interaction that had severe fitness costs for *D. areolatus*.

There are at least two possible reasons why *D. areolatus* failed to evolve an ovipositor longer than that of *D. longicaudata*. First, there may be a lack of larger fruits in which the fruit fly host are found. If the largest fruit size has been reached, then competitor-free spaces are unavailable and longer ovipositors would fail to give a fitness advantage. Second, there are trade-offs in ovipositor length with costs to having long ovipositors. Askew (1965) found that Torymid wasps often broke their ovipositors in the galls that they attacked. The authors did not mention whether broken ovipositors are prevalent among *D. areolatus* and *D. longicaudata*, but longer ovipositors can be more brittle and susceptible to breaking than the shorter ovipositors. In addition to physical damage, accessing hosts at deeper depths increases the time that a female parasitoid is immobile and vulnerable to predators (Heatwole and Davis 1965). *Utetes anastrephae* ovipositor length may have remained short because *U. anastrephae* had another mechanism for outcompeting other species: aggressive larvae. The *U. anastrephae* larvae have formidable mandibles that they use to kill other larvae and to win bouts of interference competition (Aluja et al. 2013). The defenseless and weaker larvae of *D. areolatus* made it

vulnerable to displacement from *U. anastrephae*, but a longer ovipositor created competitor-free space, until *D. areolatus* encountered a species that eliminated this space.

1.3.3 Conclusion

These studies suggest that parasitoids undergo behavioral and physical changes because of interspecific competition. Host selection is the most important behavior for adult parasitoid insects since the wrong choice results in reduced reproductive fitness. When multiple parasitoids use the same host, the asymmetrical outcome of competition causes one or more species to incur severe fitness costs (e.g., competitive displacement). If the weaker competitors have behavioral plasticity in exploiting hosts, they can expand their range of suitable hosts and escape competition.

An operational definition of competitive-free space can be an area where a weaker competitor reduces or eliminates the threat to its fitness from stronger competitors. By using a resource ignored by or unsuitable for the stronger species, populations of the weaker competitor can experience a growth rate that allows them to coexist with the stronger competitor. Just as prey species undergo behavioral and morphological changes in predator-free spaces, weaker competitors also experience evolutionary changes resulting from competitor-free space. Competitor-free spaces allow for coexistence within a community and may contribute to greater biodiversity.

Variability in foraging strategies can occur because of a stronger competitor that acts as a selection pressure. The changes required to achieve competitor-free space can cause a population to be different from conspecifics that experience a weaker degree of pressure from

competition. For species with wide geographical distributions, each population may encounter its own unique set of competitors, leading to a patchwork of traits used to mediate competition.

1.4 THEORETICAL BACKGROUND: Differences in Foraging Behaviors Among Broadly Distributed Parasitoid Populations

The studies described in the previous sections dealt only with parasitoids competing within the same habitat and community. Ecological relationships are malleable, and as a species experiences changes in the community composition of its heterospecific competitors, we expect variation in the behaviors (e.g., aggression, foraging) that result from these interspecific interactions. When individuals disperse to new areas, behavioral changes may occur as species dissociate from heterospecifics that once had significant influence on their behavior (Coss 1999; Cousyn et al. 2001; Stoks et al. 2003; Blumstein 2004). In addition, behaviors may be modified to cope with novel species in the new habitat(s) (Holway and Suarez 1999; Sih et al. 2010). For wide-ranging species, a patchwork of traits can occur as each population experiences different selection pressures from interactions with members of their specific ecological communities (Fig. 1) (Brodie and Brodie 1991; Carroll and Boyd 1992; Wilkinson et al. 1996; Benkman 1990; Althoff & Thompson 1999).

While much is known about how coevolutionary relationships produce mosaics of selection for trophic interactions (predator-prey, parasitoid-hosts, plant-pollinator) (Thompson 1994), less is known about geographic patterns of selection among intraguild interactions. Competition is known to play a significant role in shaping communities and species, but interspecific competitive interactions have yet to be examined in the context of geographic mosaics of selection. Interspecific competition is important to parasitoid behavioral ecology,

particularly the foraging behaviors that are essential for finding hosts. When parasitoid species are found in geographically separated populations, each differing in its ecological community, there is potential for foraging behaviors to experience mosaics of selection from competitors.

Few studies have directly examined how parasitoid foraging behaviors can change across multiple populations experiencing different degrees of interspecific competition. Rather, most studies on geographic mosaics of selection for parasitoids have focused on parasitoid-host dynamics (Henter 1995; Kraaijeveld and Godfray 1999; Dixon et al. 2009; Jancek et al. 2013). These studies allow us to infer that if geographic variability in bottom-up (host-parasitoid) interactions causes behavioral changes, then it is conceivable that similar effects can occur from intraguild interactions (competition). The host is directly linked to reproductive success and the failure to adapt to hosts leads to high fitness costs. In addition to overcoming competitors, a parasitoid must adapt to changes in the host's habitat, as well as the host's defenses against attack. The following studies provide evidence that parasitoid foraging behaviors vary among populations.

1.4.1 Parasitoids track changes in host habitats

Local adaptation can occur in parasitoid-host relationships as host populations occupy different habitats. *Eurosta solidaginis*, a tephritid fly, forms galls on goldenrod (*Solidago altissima*) in the US. In Minnesota, two subspecies of goldenrod are partitioned by habitat: with *S. a. gilvocanescens* in the prairie and *S. a. altissima* in the forests. The galls sizes differ between the habitats with prairie galls being larger and rounder than the forest galls (Dixon et al. 2009). In congruence with its food plants, *E. solidaginis* has two host races, one that inhabits the prairies and the other preferring forests. *Eurosta solidaginis* is likely undergoing genetic

divergence as suggested by differences in the two fly populations in wing patterns and allozyme frequencies (Itami et al. 1998). The fly is a host for the parasitoid wasp *Eurytoma gigantea*, which attacks the fly larvae through the gall. Reciprocal transplant and common garden experiments show that differences in the plant cascade upward to produce differences in the insect host and its parasitoid (Dixon et al. 2009). The parasitoids in the prairie have to penetrate larger galls, so these wasps have longer ovipositors than conspecifics in the forests. The authors suggest the differences in the ovipositor length could be evidence of local adaptation in the wasp.

1.4.2 Differential ability to overcome host defenses

Hosts are rarely defenseless against parasitoid attacks. Following parasitism, the host's immune system can produce specialized cells (e.g., plasmatocytes) to encapsulate and kill the parasitoid egg. Encapsulation is an immune response that causes mortality for parasitoids, but a host's encapsulation ability can vary geographically. The parasitoid, *Asobara tabida*, can change its foraging behavior based on the survival probabilities in their *Drosophila* hosts (Kraaijeveld and van Der Wel 1994; Kraaijeveld et al. 1995). *Asobara tabida* has a wide geographic range in which populations encounter different host species. Its main hosts are *D. subobscura* and *D. melanogaster*, and each differs in its resistance to *A. tabida* with *D. subobscura* being more poorly defended. Survivorship in these hosts is dependent on the origin of *A. tabida*. Parasitoids from north-western and central Europe experience high mortality from encapsulation when they attack *D. melanogaster*, whereas wasps from southern Europe survive equally on both *Drosophila* hosts (Nappi 1981; Rizki and Rizki 1984; Kraaijeveld and van Der Wel 1994). Thus, the quality of the host varies with species and location. Five strains of *A. tabida* were tested for host preference behavior when foraging for *D. melanogaster* and *D. subobscura*.

Wasps with low survival probabilities in *D. melanogaster* preferred *D. subobscura*, whereas a lack of preference was found for wasp strains with intermediate or high survivorship in *D. melanogaster* (Kraaijeveld et al. 1995). Across its distribution, *A. tabida* populations have diverged in foraging behaviors resulting from differing degrees of selection pressure from their hosts. This parasitoid-host system suggests that parasitoids display covariance between the host's immune competency and the parasitoids foraging behavior. However, hosts are often attacked by numerous parasitoids and their immune responses are likely general defenses against parasitism. The geographic patterns observed in *A. tabida* could be a product of the parasitoid adapting to the resistance of its host without a reciprocal evolutionary response from the host (Kraaijeveld and Godfray 1999).

Patterns of local adaption to hosts can occur from factors other than behavioral preferences. Physiological compatibility with the host was shown to be the most important factor differentiating six strains of the parasitoid *Cotesia flavipes* across four geographic regions (North America, South American, African, and Asian). This old-world wasp attacks sugarcane stemborer caterpillars in the families Noctuidae and Pyralidae, which are widespread pests. The spotted stalk borer (*Chilo partellus*) is the ancestral host of *C. flavipes* (Potting et al. 1997), but a novel parasitoid-host association was created when *C. flavipes* was brought to North America for the control of *Diatraea saccharalis*, a new-world stemborer. Each of the six strains of *C. flavipes* shows signs of local adaptation to specific hosts. A comparison of the six strains in host preferences tests showed a lack of preference for hosts from the same region as the wasps, thus foraging behaviors fail to explain differences in the parasitoid-host associations (but see Shami and Mohyuddin 1992). Instead, parasitoid virulence (i.e., ability to successfully develop in the host), was the key factor leading to the parasitoid-host complex for *C. flavipes*. While all six

strains had higher mortality in *D. saccharalis*, the North American strain from Texas had the highest survival rate in this host. Reproductive success was closely linked to the longevity of contact between the parasitoid strain and its host. Of the six strains, the *C. flavipes* from Texas had the longest period of co-existence with *D. saccharalis*.

1.4.3 Conclusion

Parasitoid foraging behaviors can be under intense selection because they are the link between the parasitoid to the hosts, which directly affect reproductive success. These behaviors must change as their hosts colonize new habitats or as hosts evolve defenses against attacks. The case studies above show that parasitoids can react to changes in host distribution and host defenses. In addition to these bottom-up interactions, parasitoids must also cope with intraguild interactions, such as those with competing parasitoids. Heterospecific competitors exert selection pressures on the foraging behaviors important for competition between weaker and stronger species. Thus, in a community where multiple parasitoids have overlapping host preferences, selection should favor weaker competitors with foraging strategies that reduce mortality from competition. If competition is relaxed or removed, either by dispersal to new ecological communities or changes in existing communities, then these behavioral strategies may wane or remain in the population if they are not costly.

Within a community, interspecific interactions are some of the most important biotic factors influencing the behaviors of animals. Unique patterns of behaviors are produced as species expand their ranges and interact with new communities. Animal behavior is arguably the most dynamic and complex phenotype. Our appreciation and understanding of animal

biodiversity will become more complete with explorations into the ecological and evolutionary factors shaping animal behavior.

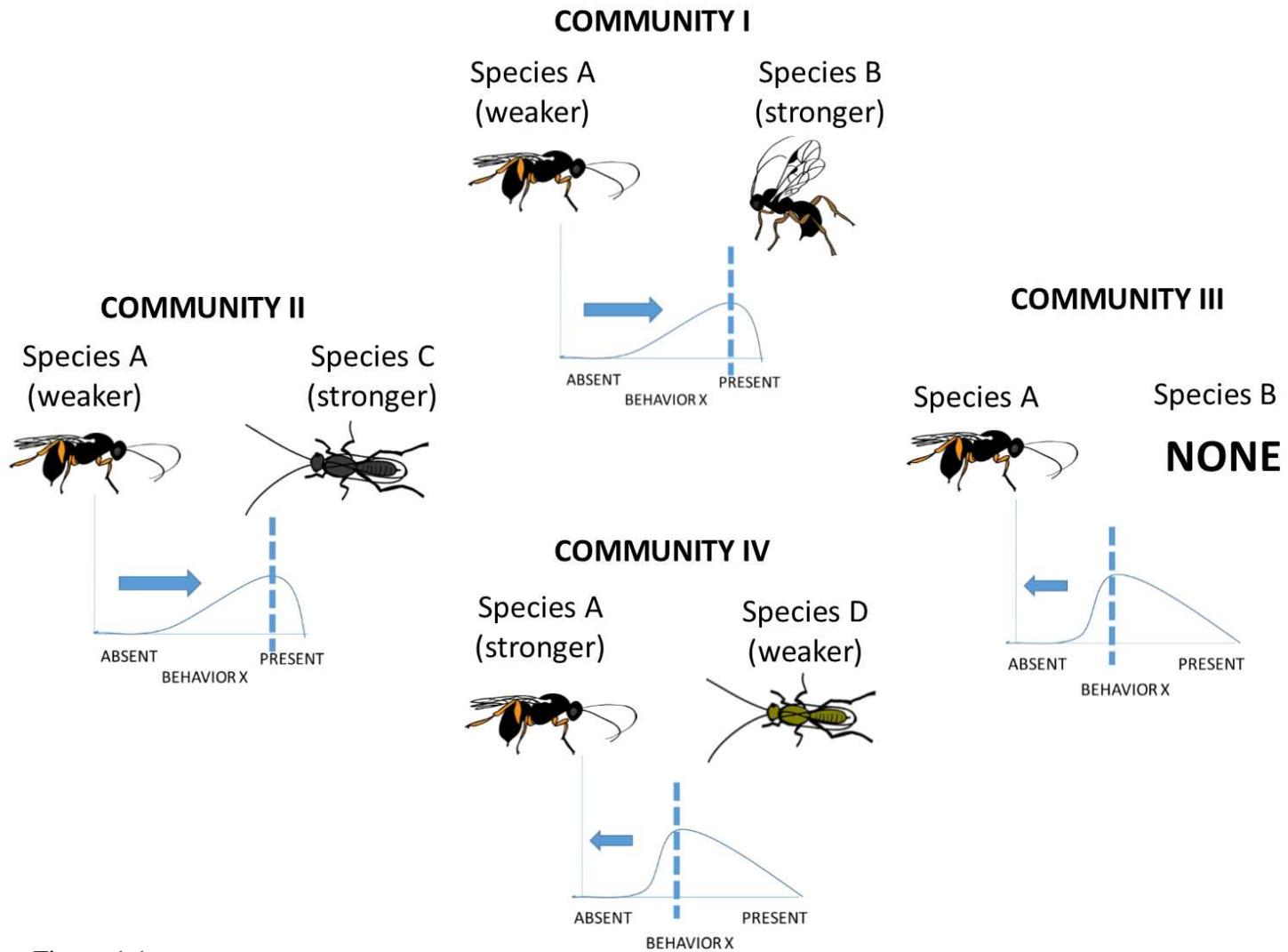


Figure 1.1.

Figure 1.1. A parasitoid (Species A) whose populations are distributed across different ecological communities. Community I is the ancestral community from which the other three populations of Species A originated. In communities I, II & III, Species A competes with a heterospecific over a shared host. This scenario assumes that only one host option is available and only one heterospecific competes with Species A. Behavior X is a behavior used by Species A to mitigate the effects of competition with the competing species. The presence of behavior X allows Species A to coexist with the heterospecific, whereas the absence of the behavior is detrimental to coexistence. Behavior X is costly (e.g., energy and time expenditure required), therefore this behavior is selected to be absent when it is unnecessary for Species A, such as in communities III and IV. Each community represents the different directions of selection for behavior X, which is affected by the competitive ability of Species A in a given community.

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CHAPTER 2: The *Cotesia-Pieris* system

2.1 THE COMMUNITY

My research was conducted in agroecosystems consisting of cultivated varieties of cabbage (*Brassica oleracea*). These plants serve as a common oviposition site and host plants for the imported cabbageworm (*Pieris rapae* [Lepidoptera: Pieridae]), a butterfly whose caterpillars are the primary host for *Cotesia glomerata* (Hymenoptera: Braconidae) and *C. rubecula* (Hymenoptera: Braconidae). In addition to *P. rapae*, cabbage cultivars are inhabited by numerous other insects that feed on the plants. Diamondback moth (*Plutella xylostella*) (Lepidoptera: Plutellidae), cabbage looper (*Trichoplusia ni*) (Lepidoptera: Noctuidae), green peach aphid (*Myzus persicae*) (Hemiptera: Aphididae) and cabbage aphid (*Brevicoryne brassicae*) (Hemiptera: Aphididae) are the most common heterospecific herbivores found in these agroecosystems. These herbivores are attacked by their own species of predators and parasitoids.

2.2 THE HERBIVORE HOST

The imported cabbageworm (a.k.a., small white) is a butterfly with a palearctic distribution. Originating in Europe (Scudder 1887; Richards 1940), *P. rapae* was first documented in North America in Quebec during the 1860s and is thought to have been brought from Europe in shipments of various cabbage cultivars (Richards 1940; Harcourt 1963). Since its arrival to the continent, *P. rapae* has expanded its range to become a ubiquitous butterfly in the US and Canada. Females lay a single yellow egg on the underside of plants in the family Brassicaceae. The larva feeds and develops on the natal plant. Each larva goes through five

instars (i.e., developmental stages). After the fifth instar, the larva develops into a chrysalis from which a new adult butterfly eventually emerges.

2.3 THE *COTESIA* PARASITOIDS

Cotesia glomerata is a gregarious endoparasitoid that can inject 10-50 eggs per attack into a host (Laing and Levin 1982). In their natal region of Europe, female wasps prefer to attack first and second instar caterpillars of *Pieris brassicae* (Lepidoptera: Pieridae) (Hamilton 1935; Brodeur and Geervliet 1992; Mattiacci and Dicke 1995). *Cotesia glomerata* will attack other pierid caterpillars (Sato 1976; Laing and Levin 1982), although *P. brassicae* is the main host species in Europe. Immature *C. glomerata* feed on the host's hemolymph and fat bodies as the host continues to grow. Once the host reaches its fifth instar, the larvae exit the host by chewing through the host's cuticle and then spin yellow silk around themselves. Each *C. glomerata* larva spins its own cocoon, but the collection of cocoons forms a cocoon mass comprised of the brood *C. glomerata* larvae. The host dies soon after the *C. glomerata* larvae finish emerging.

The morphology and appearance of *C. rubecula* resembles that of *C. glomerata*, but *C. rubecula* are usually larger (Wilkinson 1945). Like *C. glomerata*, *C. rubecula* prefers the first and second instar stages of *P. rapae* for oviposition (Brodeur and Geervliet 1992; Harvey et al 1999). There are important life history differences between these sympatric *Cotesia* species. Unlike *C. glomerata*, *C. rubecula* is a solitary parasitoid wasp that lays only one egg per attack. Field observations show that the host range of *C. rubecula* is restricted to *P. rapae* (Brodeur and Geervliet 1992; Brodeur et al. 1996; van Driesche et al. 2003); therefore *C. rubecula* is considered a specialist. The immature *C. rubecula* hatches with large sclerotized mandibles and

a caudal appendage, two features lacking in the young *C. glomerata*. Previous studies note that *C. rubecula* uses its mandibles to attack conspecific and heterospecific parasitoid larvae in the same host (Laing and Corrigan 1987). The caudal appendage is presumed to facilitate movement through the host's hemolymph. A key phenological difference between *C. glomerata* and *C. rubecula* is that the *C. rubecula* larva emerges from the host during its third or fourth instar. The solitary larva spins a white cocoon in which it pupates.

It remains unclear how these closely related wasps diverged and remain different in their host preferences in Europe. *Cotesia glomerata* will attack and successfully develop in *P. rapae* caterpillars, but wasps growing in the larger *P. brassicae* hosts produce larger adults, which correlates with fitness advantages (i.e., more eggs) among parasitoids (Godfray 1994; Visser 1994; King 1987). Perhaps this fitness gain was a strong enough selection pressure that led to *C. glomerata* preferences for *P. brassicae*. Another explanation for differential host preferences is the consequence of *C. glomerata* and *C. rubecula* attacking the same *P. rapae* caterpillar. Inside these hosts, larval competition favors *C. rubecula*, which usually outcompetes and kills *C. glomerata* larvae (Laing and Corrigan 1982). The costs of competition with *C. rubecula* may greatly influence *C. glomerata* to prefer *P. brassicae* in Europe (Geervliet et al. 2000). Thus, interspecific competition could be an important selection pressure causing *C. glomerata* to forage in a way that reduces their attacks on risky *P. rapae* hosts. In fact, researchers in the Netherlands reported low occurrence of *P. rapae* hosts containing both *C. glomerata* and *C. rubecula* (Geervliet et al. 2000).

2.4 THE HYPERPARASITIDS

Parasitoids are often attacked by their own parasitoids, which are called hyperparasitoids (Sullivan 1987; Sullivan and Völkl 1999). Hyperparasitoids are wasps that use parasitoid insects (e.g., wasps and flies) as hosts for the development of hyperparasitoid offspring. These wasps can attack the immature parasitoid while it is developing in or on its herbivore host, or the hyperparasitoids attack the parasitoid in its pupal stage (i.e., after it completes development in or on the herbivore host). A single parasitoid species can be attacked by a community of hyperparasitoids, many of which are generalists and attack numerous species of parasitoids. Hyperparasitoid attacks are a significant source of mortality for parasitoid insects (Frago 2016). *Cotesia glomerata* and *C. rubecula* are attacked by hyperparasitoid communities that are somewhat similar across their range in North America (Nealis 1983; McDonald and Kok 1991; Weis et al. 2016), but differ greatly from the hyperparasitoid communities in Europe (Poelman et al. 2012; Zhu et al. 2016).

2.5 HISTORY OF *COTESIA* AND *PIERIS* IN NORTH AMERICA

Soon after its arrival in North America during the 1960s, *P. rapae* became an agricultural pest in need of management. Biological control efforts were initiated in 1884 with the introduction of *C. glomerata* from Europe to areas near Washington, DC (Clausen 1978). *Cotesia glomerata* subsequently spread to other regions and became the predominant parasitoid of *P. rapae* in North America. In 1963, the first record of *C. rubecula* in North America was made of a population of unknown origins in Vancouver Island, British Columbia (Wilkinson 1966). Shortly after its discovery in Canada, four redistributions of *C. rubecula* were made with the attempt to establish this wasp in southern regions of North America.

In the 1960s, individuals from the Vancouver Island population was introduced to Missouri, New Jersey, South Carolina and Ontario with only the Ontario introduction achieving establishment (Puttler et al. 1970; Corrigan 1982). A second attempt to introduce *C. rubecula* into the US (Missouri and Virginia) was made in the 1980s using a strain from former Yugoslavia (McDonald and Kok 1991). These wasps also failed to reach long-term establishment. In 1988, a Chinese strain of *C. rubecula* was released in New England where it established and by 2002, had spread throughout the region (Van Driesche and Nunn 2002). The final documented introduction of *C. rubecula* was in Minnesota where Yugoslavian and Chinese strains were released in the early 1990s and found again in 2000 (Wold-Burkness et al 2005; Lee and Heimpel 2005). It is worthwhile to note that a *C. rubecula* population of uncertain origin was found in farmlands in Quebec in 1993 (Godin and Boivin 1998). The supposed barriers to *C. rubecula* spread and establishment have included a lack of cold tolerance (Corrigan 1982), failure to meet diapause requirements (Nealis 1985) and mortality from hyperparasitoid attacks (McDonald and Kok 1992; Gaines and Kok 1999).

North America represents a more challenging shift in the ecological community for *Cotesia glomerata* than for *C. rubecula*. The major difference compared with Europe is that *C. glomerata* has had to adapt to attacking *P. rapae* because *P. brassicae* is absent in North America. In response, *C. glomerata* adjusted its foraging behaviors to the distribution of *P. rapae*, which is drastically different from *P. brassicae* (le Masurier and Waage 1993; Vos et al 1998). *Pieris rapae* females often lay one or a few eggs per leaf, whereas *P. brassicae* lays clusters of 7-150 eggs per leaf. Vos (1998) describes the spatial patterns of these two pierid butterflies in the field as "...few plants with many *P. brassicae* and many plants with few *P. rapae*.". The change in the primary host has led to North American *C. glomerata* becoming

behaviorally different from European *C. glomerata* (le Masurier and Waage 1993; Vos et al. 1998). When searching for hosts, North American *C. glomerata* search more plants and spend less time searching per plant, whereas European *C. glomerata* do the opposite (Vos 1998). North American *C. glomerata* display a foraging strategy similar to *C. rubecula* (Wiskerke and Vet 1991; Vos 1998; Vos et al. 1998).

The second major change in the ecological community facing *C. glomerata* is the separation from and reunion with *C. rubecula*. *Cotesia glomerata* lived in North America for nearly a century without *C. rubecula*. The reliance of both *Cotesia* species on *P. rapae* has created severe competition, a selection pressure that was weak or absent in Europe because of different host preferences. North American *C. glomerata* adapted their foraging behaviors to forage for *P. rapae*, and these behaviors may have also changed to mediate competitive interactions with *C. rubecula*.

As early as 1982, researchers in North America began to note the decline of *C. glomerata* in regions where *C. rubecula* established (Biever 1992). Since then, the competitive displacement of *C. glomerata* by *C. rubecula* has been documented in several parts of North America (Herlihy et al. 2012), yet the mechanism of this displacement remains unknown. I investigated several questions about how *C. glomerata* foraging behaviors influence the competitive interactions with *C. rubecula*. First, how do North American *C. glomerata* respond to hosts previously parasitized by *C. rubecula*? These hosts are costly for *C. glomerata* since its larvae fail to emerge from a *C. rubecula* parasitized host (Laing and Corrigan 1987). Second, there remain North America populations of *C. glomerata* that have yet to encounter *C. rubecula* (e.g., Colorado) and others that coexist with *C. rubecula* (e.g., Maryland). For *C. glomerata* that are naïve to *C. rubecula*, do they lack behaviors that make them vulnerable to displacement, as

has happened to most of *C. glomerata* populations across North America? And what behavioral and ecological factors set apart those North American *C. glomerata* that coexist with *C. rubecula*?

Since host foraging behaviors are critical to parasitoid reproductive success, these behaviors are susceptible to natural selection. When *C. rubecula* is present and both *Cotesia* species attack *P. rapae*, we expect directional selection for *C. glomerata* to avoid *C. rubecula* parasitized hosts since these hosts represent high mortality risks for *C. glomerata* (Fig. 1.1 Community I or II). The assessment and avoidance of multiparasitized hosts can be time consuming and costly for *C. glomerata*; therefore, the absence of *C. rubecula* should reduce or preclude selection for avoidance of multiparasitism (Fig 1.1 Community III). *Cotesia glomerata* search time and handling time, both measures of foraging efficiency, may also be affected by the presence of *C. rubecula*. In addition to exploitative and interference competition, *C. glomerata* and *C. rubecula* may engage in apparent competition if they have shared enemies that have asymmetrical impacts on either parasitoid.

2.5 DISSERTATION OVERVIEW

I used laboratory and greenhouse experiments to examine the foraging behaviors of *C. glomerata* from two populations with different historical associations with *C. rubecula*. In addition, I conducted field surveys in agroecosystems to understand the top-down trophic interactions that affect both *Cotesia* species. The objectives of this project were to:

- Review literature describing the significance of interspecific competition among parasitoid insects with an emphasis on how these insects change when freed from such competition (Chapter 1.1-1.3.3).

- Review studies documenting that for broadly distributed parasitoid species, geographic mosaics of selection can cause populations to diverge in important traits (Chapter 1.4-1.4.3).
- Describe the natural history of *C. glomerata* and *C. rubecula*, and review their history in North America.
- Quantify foraging success and efficiency of *C. glomerata* (living with and without *C. rubecula*) and *C. rubecula* when given a range of hosts (low to high), and whether either species affects the other's foraging performance (Chapter 3).
- Compare the response to *C. rubecula*-parasitized hosts between *C. glomerata* that are naïve to competition with *C. rubecula* to conspecifics living with *C. rubecula* (Chapter 4)
- Compare the diversity of hyperparasitoids attacking *C. glomerata* where it lives without *C. rubecula* and in a region where both *C. glomerata* and *C. rubecula* coexist (Chapter 5).
- Summarizing discussion explaining the main findings, future directions and significance of the research (Chapter 6).

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CHAPTER 3: Foraging behavior mitigates the effects of interspecific competition between two parasitic wasps

3.1 SYNOPSIS

Overlapping resource requirements between individuals of different species frequently results in intense interspecific competition. The outcome of such competition is often mediated by foraging behaviors used to exploit resources. Here we explore whether differences in foraging efficiency plays a role in the outcome of competitive interactions between *Cotesia glomerata* and *C. rubecula*, two parasitic wasps that attack the cabbage white butterfly *Pieris rapae*. These wasps differ in their co-occurrence throughout North America with some areas having only *C. glomerata*, others with only *C. rubecula*, and still other regions containing both species. When these two species attack the same individual host, *C. rubecula* invariably wins. Therefore, we expected differences in foraging efficiency between *C. glomerata* populations that co-occur with *C. rubecula* and *C. glomerata* populations that are naïve to *C. rubecula*. We used two greenhouse experiments to examine the foraging behaviors of different populations of the two species based on their association with each other. Experiment I compared the ability of *C. glomerata* and *C. rubecula* populations to exploit a range of host densities per plant. Experiment II examined interspecific differences in search and handling time when attacking a single host. Both experiments were conducted with wasps foraging alone, or with the heterospecific. We found that *C. glomerata* was generally less efficient with longer search and handling times. However, among *C. glomerata* populations, foraging efficiency was greater for the *C. glomerata* individuals from populations that coexist with *C. rubecula*. *Cotesia glomerata* populations naïve to *C. rubecula* may suffer competitive displacement if adult foraging behaviors fail to

compensate for the competitive weakness of immature *C. glomerata*. Our results suggest that foraging behaviors influence the outcome of interspecific competition between North American *C. glomerata* and *C. rubecula*.

3.2 INTRODUCTION

Intense interspecific resource competition can shape foraging behavior. When species compete for resources that are patchily distributed, differences in foraging efficiency can determine the outcome of competition (MacArthur and Pianka, 1966; Werner and Hall 1977). Efficient foragers may spend less energy and time acquiring resources and are often able to acquire more resources than species that are less efficient (Werner and Hall, 1979; Segev & Ziv 2012; Kakareko et al., 2013; Axen et al., 2014). Among populations, the strength of selection on foraging behavior can vary with the intensity of interspecific competition (amphibians: Deitloff et al. 2008; insects: Kraaijeveld et al. 2001; Anderson and Grether 2010; birds: Grava et al. 2013; reptiles: DuFour et al. 2018).

For parasitic organisms, such as parasitic wasps (parasitoids) whose immature stages feed off of host insects, hosts are essential for reproduction. Communities of parasitoids often contain multiple species with overlapping host preferences, creating severe competition for hosts (Flanders 1966; Reitz and Trumble 2002; Van Baaren et al. 2009; Magdaraog et al. 2013). Within these communities, foraging behaviors play a significant role in determining the outcome of competitive interactions (Vet 2001; Lewis et al. 2003; Pederson & Mills 2004; Garcia-Medel et al. 2007).

Competition is broadly categorized as either interference (direct interactions) or exploitative (indirect interactions). For parasitoids, whether interference or exploitative competition predominates is correlated with the developmental stage with exploitative competition being more common among adults and interference competition more often observed among larvae (Goubault et al. 2007). Adult parasitoids are less likely to engage in agonistic interference competition (e.g., aggression) because it uses time that can be spent

looking for hosts and because the antagonism rarely guarantees offspring survival (Boivin & Brodeur 2006). Instead, adult parasitoids often win bouts of competition by being faster at finding hosts (i.e., quicker search time) or by spending less time per attack (e.g., shorter handling time) (Bajpaj et al. 2006; Xu et al. 2014; Wang et al. 2016; Bruzzone et al. 2018). Most studies attribute the outcome of interspecific competition in parasitoids to larval interactions (Harvey et al. 2013), but far less is known about how interspecific competition is resolved by the foraging behaviors of adult parasitoids.

For endoparasitoids, whose larvae develop inside the host, larval competition often results in mortality for weaker species; therefore, the host choice decisions of adult parasitoids are vital for ensuring offspring survivorship. Once an adult parasitoid enters its host's habitat, foraging consists of host finding, selection, and handling (Vinson 1976). Parasitoids can win exploitatively competitive by searching for and handling hosts more quickly (Dowell and Horn, 1977; Chua et al. 1990; Harcourt, 1990; Pijls et al. 1995). Efficient foragers have a competitive advantage in being able to sustain population growth at a lower resource levels (e.g., hosts) (Tilman 1982). An uneven distribution of hosts requires a foraging parasitoid to be adept at finding hosts at low densities, and often doing so before a stronger competitor locates the host, especially if both species tend to avoid parasitizing the same host.

Unlike predators that consume their prey and make it unavailable to competitors, a parasitoid attack leaves the host alive and vulnerable to other parasitoids. Multiparasitism occurs when a host is attacked by multiple parasitoid species. Within the multiparasitized host, different species of parasitoid larvae compete for resources and usually only one species completes development, resulting in severe costs to the weaker competitors. This creates an asymmetry in host quality for adult parasitoids while they are foraging because the multiparasitized host

becomes less valuable to the weaker parasitoid (Le Lann et al. 2011). Host discrimination and avoidance of multiparasitized hosts are behaviors that increase foraging efficiency by enabling a parasitoid to avoid selecting a host attacked by stronger competitors (McBrien and Mackauer 1990; Pijls et al. 1995; Ruschioni et al. 2015; Sithole et al. 2017). Behaviors that result in avoidance of multiparasitism may be strongly selected in locations where weaker and stronger species co-occur (van Lenteren 1981; McBrien and Mackauer 1990; Cusumano et al 2012; Harvey et al. 2013), but these behaviors may be less important in areas where stronger species are absent. The failure to recognize and avoid multiparasitized hosts can lead to competitive displacement by stronger competitors (Reitz and Trumble 2002).

In this study, we compared foraging behaviors of individuals from a region where *Cotesia rubecula* (the stronger competitor) and *C. glomerata* (the weaker competitor) coexist to those of an area where *C. glomerata* has yet to experience competition from *C. rubecula*. *Cotesia glomerata* and *C. rubecula* are the two most abundant parasitoids of the imported cabbageworm butterfly (aka cabbage white butterfly) (*Pieris rapae*) in North America (Shelton et al. 2002; Wold-Burkness et al. 2005). While much is known about these parasitoid wasps in their original habitats in Europe (Wiskerke and Vet 1991; Brodeur et al. 1998; Geervliet et al. 2000), far less is understood about their interactions in North America. While both species attack *P. rapae* in North America, *C. glomerata* has a wider host range than does *C. rubecula*, which is a strict specialist on *P. rapae* (Puttler et al. 1970; Laing and Levin 1982; Ohsaki and Sato 1990; Şengonca and Peters 1993). *Cotesia rubecula* was first documented in North America approximately 80 years after *C. glomerata* (Clausen 1978; Corrigan 1982) and has displaced *C. glomerata* in several regions where *C. glomerata* had been the dominant parasitoid of *P. rapae* (Herlihy et al. 2012). However, there remain *C. glomerata* populations that coexist

with *C. rubecula* (e.g., in Maryland) and others that have yet to encounter *C. rubecula* (e.g., in Colorado). While the mechanisms of the competitive displacement remain unclear, we know that *C. rubecula* larvae outcompete and kill *C. glomerata* in multiparasitized hosts (Laing and Corrigan 1987). The goal of this study was to determine whether the adult foraging behaviors of these two parasitoids differed by species and population, and whether these behavioral differences contribute to the outcome of competition between *C. glomerata* and *C. rubecula*.

Our objectives were to determine i) whether *C. glomerata* and *C. rubecula* differed in exploiting a range of *P. rapae* host densities, ii) if the two species differed in the time spent finding and attacking a host, iii) if *C. glomerata* foraging success and behaviors were different based on the population's association with *C. rubecula*, and iv) if interference competition occurs between the two species when they forage together. We explored these objectives using greenhouse experiments to make inter- and intraspecific comparisons in the foraging behaviors of *C. glomerata* from Colorado (CO) and Maryland (MD), and *C. rubecula* from Minnesota (MN) and MD. As a specialist on *P. rapae*, *C. rubecula* was expected to parasitize *P. rapae* more efficiently than *C. glomerata* and be unaffected by the presence of *C. glomerata*. We anticipated that MD *C. glomerata* would be more likely to avoid *C. rubecula*-parasitized hosts compared to CO *C. glomerata*. Foraging efficiency among adult *C. glomerata* could explain how some of their populations can coexist with *C. rubecula*, while others are susceptible to competitive displacement following the arrival of the stronger competitors in North America.

3.3 METHODS

3.3.1 Insects

Cotesia glomerata colonies were started from cocoon masses field-collected at the Colorado State University Agricultural Research and Education Center (CSU ARDEC; GPS: 40.652703, -104.994627) and an organic farm in Maryland (Flying Plow Farm, GPS: 39.692647, -76.099227). *Cotesia rubecula* colonies were started from cocoons collected from agricultural fields at the University of Minnesota (44.933333, -93.083333) and from Flying Plow Farm in Maryland. *Cotesia glomerata* females can lay 10-50 eggs per oviposition event that hatch into a brood of larvae. After completing development inside the host, the larvae chew their way out and each larva spins a yellow cocoon. A cocoon mass is the collection of cocoons formed by the larvae upon exiting the host. Each cocoon mass from a host was kept individually in 946ml clear plastic cup that was stored in an environmental control chamber set to a 16L:8D photoperiod and 25°C until adult wasps emerged. The containers were supplied with drops of honey and a wet cotton wick. Once the adult wasps emerged, the containers were moved to another environmental control chamber set to a 16L:8D photoperiod and 20°C. *Cotesia rubecula* lays one egg per oviposition event and cocoons are formed by the single larva that spins the cocoon after exiting the host. These cocoons were reared individually in 2.0 ml centrifuge tubes under the same conditions as the *C. glomerata* cocoon masses. After emergence, adult *C. rubecula* were kept in 473ml plastic cups under the same conditions as adult *C. glomerata*. All wasps were 5-13 days old and had yet to oviposit prior to use in the experiments. Females were housed with males, and females were presumed to be mated.

Pieris rapae colonies were started from fourth and fifth instar caterpillars that were collected from CSU ARDEC and organic farms in northern CO with a maximum distance of 83 km between any two farms. One or two caterpillars were reared on collard greens in 37ml plastic cups until pupation. Ten to 20 chrysalids (pupae) were transferred to a single 60 cm³ cage

in a greenhouse set at a 16L:8D photoperiod and 20-25°C. Adult butterflies laid eggs on live collard green plants (*Brassica oleracea*) grown in 10cm wide pots. The caterpillars from these eggs were reared in cages separate from the adults in the greenhouse prior to use in experiments. *Cotesia glomerata* and *C. rubecula* prefer to attack first and second instar caterpillars, but only second instar caterpillars were used for this study because experimental manipulation of first instars often results in high caterpillar mortality.

3.3.2 Experimental Design

Two greenhouse experiments were performed to examine the foraging behaviors of *C. glomerata* and *C. rubecula*. Each experiment was conducted under the same greenhouse conditions used for rearing the *P. rapae* adults (see above).

3.3.2.1 Experiment I: Foraging Efficiency

The first experiment assessed how individuals from the different populations of each species performed when foraging alone or with the heterospecific for a range of host densities (number of caterpillars per plant). A 6x5 factorial design was used with six levels of the wasp foraging treatment and five levels of host density treatment (one, two, four, six, or eight second instar *P. rapae* caterpillars per plant) (Table 3.1). Our field observations in Colorado and previous studies (Kobayashi 1966; Jones 1977) show that cultivated crucifer plants normally have 1-2 hosts per plant, so the selected range of host densities represented minimum to high (8-4 times) levels of infestation.

Trials were conducted in 60 cm³ cages with nylon mesh (160 µm aperture) (BugDorm-2120F, MegaView Science Co., Ltd). Each cage contained a six to seven week-old collard plant

inoculated with one of the five host densities. In order to generate herbivore-induced plant volatiles that attract the wasps (Agelopoulos and Keller 1994), the caterpillars fed on the plants in the experimental cages for 24 hours prior to the start of each trial. Thirty minutes before each trial, each wasp was placed in a 5 cm wide petri dish containing leaf material from collard greens with feeding damage from *P. rapae*. This exposure primed the wasps, increasing their interest in the hosts during the experiment (Geervliet et al. 1998; Fatouros et al. 2005). Wasps were placed into the cages and allowed to forage for four hours, after which time the wasps and hosts were collected. Hosts were dissected within 48 hours and examined for parasitism. *Cotesia glomerata* and *C. rubecula* eggs can be distinguished because *C. rubecula* eggs are at least three times larger and wider (pers. obs). New female wasps and plants were used for each trial. The number of replicates per wasp treatment differed because of the fluctuations in the availability of *C. rubecula* and *C. glomerata*.

3.3.2.2 Experiment II: Search and Handling Time

The goal of the second greenhouse experiment was to record the search and handling times of each wasp used in Experiment I. In Experiment I, we were unable to observe each individual wasp as it searched for and attacked hosts, so within 30 minutes of completing the Experiment I, the same females were used in Experiment II. Each trial was performed in a 473 ml clear plastic cup containing a leaf on which one second instar *P. rapae* was feeding. The same three levels of wasp foraging treatment from Experiment I (*C. glomerata* alone, *C. rubecula* alone, and both species together) were repeated (CO *C. glomerata* alone n=69; MN *C. rubecula* alone n=62; CO *C. glomerata* with MN *C. rubecula* n=58; MD *C. glomerata* alone n=70; MD *C. rubecula* alone n=55; MD *C. glomerata* with MD *C. rubecula* n=49). Fewer

wasps were used in Experiment II because some individuals died after Experiment I and prior to Experiment II. Wasps that foraged alone in Experiment I were kept alone and those that foraged with a heterospecific were paired with the same heterospecific individual during Experiment II.

Wasps were observed for 10 minutes starting from the moment they entered the cup. The time in seconds (secs) it took to find the host (search time) and the duration (secs) of attack (handling time) were recorded for each trial. For trials where *C. glomerata* and *C. rubecula* foraged together, the first species to attack and the occurrence of multiparasitism were also recorded. Physical contact was recorded when any part of the antennae or legs of an individual of one species touched the body of the heterospecific. Behaviors were considered aggressive if they resembled the behaviors of other parasitoids known to engage in agonistic physical interactions (Lawrence 1981; Perez-Lachaud et al., 2002; Goubault et al. 2005), namely biting, wrestling, striking, and stinging. *Cotesia glomerata* and *C. rubecula* adults can be distinguished visually because *C. rubecula* is larger. Single species trials ended after the host was attacked. Trials were omitted if wasps foraging alone failed to attack the host within 10 minutes (CO *C. glomerata* alone n=9; MN *C. rubecula* alone n=1; MD: *C. glomerata* alone n=7; MD *C. rubecula* alone n=1). For trials with both *Cotesia* species, trials ended after the second species attacked the host. Two trials were omitted because neither species attacked within 10 minutes (Colorado n=1; Maryland n=1). Hosts were dissected and examined for parasitism within 48 hours at which time the eggs of both *Cotesia* species are easily visible and distinguishable.

3.3.3 Analyses

Trials were performed on different dates and the date was treated as a block to account for any variation in greenhouse conditions across days; however, the block did not explain a

significant portion of variance and was dropped from subsequent analyses. Host density was treated as a continuous variable in all analyses for Experiment I. The numbers of hosts parasitized and multiparasitized were treated as count data and were analyzed using Poisson regressions with a log link function. The proportion of hosts parasitized and multiparasitized were treated as binomially distributed and analyzed using logistic regression with a logit link. Search time and handling time were considered continuous variables and search time was analyzed with a Welch's test for unequal means because a Levene's F test detected a lack of homogeneity of variance. Post-hoc multiple comparison tests for search time were conducted using Games-Howell tests, a non-parametric post-hoc test used when variances are not homogenous (Field 2013). Handling times for *C. glomerata* and *C. rubecula* were analyzed separately by species because *C. glomerata* attacks were consistently longer (range: 10-165 sec) than those of *C. rubecula* (range: 0.31-1.08 sec). Mean handling times were compared using one-way ANOVA followed by Tukey HSD for multiple comparisons.

Clutch size was defined as the number of eggs laid per attack; clutch size was treated as continuous response variable and analyzed with a one-way ANOVA. *Cotesia glomerata* females lay a clutch of multiple eggs per attack, whereas each *C. rubecula* female lays a single egg; therefore, we compared clutch sizes among the *C. glomerata* populations when they foraged with or without *C. rubecula*. Parameter estimates are reported with 95% confidence intervals (95% CI) and means are reported with standard errors. Except for the Welch's and Games-Howell tests completed in RStudio 1.1.453 (RStudio, Inc.), all analyses were performed in JMP version 12.0.1 (SAS Institute) or and were tested at a significance level of $\alpha=0.05$.

3.4 RESULTS

3.4.1 Experiment I: Foraging Efficiency

The number of hosts attacked by *C. glomerata* and *C. rubecula* from each population increased with increasing numbers of available hosts on a plant (Fig. 3.1A-D). With the exception of *C. rubecula* from MN, the proportion of hosts parasitized remained constant across host densities (Fig. 3.2A-D). When holding the heterospecific treatment constant, there was a 15% [2%, 23%] decrease in the odds of hosts attacked by MN *C. rubecula* for every additional caterpillar on the plant ($\chi^2_1=5.11$, $P=0.02$) (Fig. 3.2B).

The presence *C. rubecula* affected the foraging performance of MD *C. glomerata*, but not CO *C. glomerata*. MD *C. glomerata* foraging alone attacked 13% [1%, 23%] more hosts than when they foraged with MD *C. rubecula* ($\chi^2_1=4.35$, $P=0.04$) (Fig. 3.1C). There were no significant differences between the mean number of hosts attacked by wasps foraging alone or with the other species for CO *C. glomerata* ($\chi^2_1<0.001$, $P=0.99$), MN *C. rubecula* ($\chi^2_1=0.60$, $P=0.44$) or MD *C. rubecula* ($\chi^2_1=2.33$, $P=0.13$). Overall, *C. glomerata* were less successful than *C. rubecula* based on the number of hosts attacked across host densities. CO *C. glomerata* attacked 15% [8%, 22%] fewer hosts than did MN *C. rubecula* ($\chi^2_1=14.54$, $P<0.001$) and MD *C. glomerata* attacked 14% [5%, 21%] fewer hosts than did MD *C. rubecula* ($\chi^2_1=9.64$, $P=0.002$). Intraspecific comparisons showed that the number of hosts attacked was similar between the two populations of *C. glomerata* ($\chi^2_1=0.43$, $P=0.51$) and of *C. rubecula* ($\chi^2_1=1.39$, $P=0.24$).

The proportion of hosts attacked was similar for wasps foraging alone or with the heterospecific, regardless of population (Fig. 3.2A-D). When comparing between the species, the odds of a host being parasitized by CO *C. glomerata* were 34% [15%, 48%] lower than the odds of a host being attacked by MN *C. rubecula* ($\chi^2_1=10.51$, $P=0.001$), but we failed to find a

similar difference between *C. glomerata* and *C. rubecula* from MD ($\chi^2_1=2.92$, $P=0.09$). There was no evidence to show that MD *C. glomerata* or MD *C. rubecula* attacked a proportion of hosts greater or less than their conspecifics from CO or MN (MD *C. glomerata* vs. CO *C. glomerata*: $\chi^2_1=0.01$, $P=0.94$; MD *C. rubecula* vs MN *C. rubecula*: $\chi^2_1=1.71$, $P=0.19$).

When *C. glomerata* and *C. rubecula* foraged together, CO *C. glomerata* multiparasitized 21% [3%, 51%] more hosts than did MD *C. glomerata* ($\chi^2=3.06$, d.f.=1, $p=0.08$) (Fig. 3.3A). We failed to find differences in the average proportion of hosts multiparasitized between CO *C. glomerata* and MD *C. glomerata* ($\chi^2=0.84$, d.f.=1, $p=0.36$) (Fig. 3B). However, across the two populations of *C. glomerata*, the number of hosts multiparasitized increased slightly by 9% [1%, 19%] with each additional host per plant ($\chi^2_1=4.04$, $P=0.04$) (Fig. 3.3A). As host density increased, there was a marginal, 15% [1%, 30%] decrease in the proportion of multiparasitized hosts ($\chi^2_1=3.35$, d.f.=1, $P=0.07$) (Fig 3.3B).

3.4.2 Experiment II: Search and Handling Time

Since the same wasps from Experiment I were used in the second experiment, foraging experience in the cage may have affected the time taken to find a host in the 473 ml container. However, only MD *C. rubecula* foraging with MD *C. glomerata* showed a weak correlation ($R^2=0.10$, $P=0.03$) between the number of hosts attacked in Experiment I and the search time in Experiment II. No significant relationships were found between the number of hosts attacked in Experiment I and handling time in Experiment II. Therefore, the behavior of wasps in Experiment II was considered independent of their foraging experience in Experiment I.

Direct observations of *C. rubecula* and *C. glomerata* attacks showed that search times differed based on the species of *Cotesia* and its population ($F_{7, 160.64}=29.88$, $P<0.001$). Overall,

MD *C. rubecula* foraging alone were the fastest at finding the host (alone: 128.39±13.90 secs), even when compared with conspecifics from MN (MN *C. rubecula* alone: 213.49±26.41 secs; MN *C. rubecula* with CO *C. glomerata*: 190.35±24.23 secs) (Fig. 3.4). CO *C. glomerata* were significantly slower (308.88±27.29 secs) at finding a single host than were MD *C. glomerata* (217.90±24.81 secs), but only when the *C. glomerata* foraged alone ($t_{119,29}=4.83$, $P<0.001$) (Fig. 3.4). Interestingly, MD *C. glomerata* were slower than MD *C. rubecula*, but just as fast as MN *C. rubecula* at finding a host. Search times for *C. glomerata* and *C. rubecula*, from either population, were unaffected by the presence of the other species (Fig. 3.4, same colored bars). In fact, when foraging together, individuals of each species made physical contact with one another in 60 out of 106 trials with no difference between Colorado and Maryland wasps (CO *C. glomerata* with MN *C. rubecula*: 34/57; MD *C. glomerata* with MD *C. rubecula*: 26/49) (Log likelihood $\chi^2_1=0.47$, $P=0.50$). None of the bouts of physical contact resulted in aggressive interactions indicative of interference competition. The most common reaction upon contact was a change in direction by both species, followed by bouts of grooming. On 4/106 occasions, *C. rubecula* attacked a host while *C. glomerata* was ovipositing, and only once did the *C. glomerata* female immediately terminate oviposition. The short duration of *C. rubecula* attacks makes it improbable for a *C. glomerata* to attack a host while it is being attacked by *C. rubecula*.

Handling times differed across the wasp foraging treatments for *C. glomerata* ($F_{3,192}=2.63$, $P=0.05$) and for *C. rubecula* ($F_{3,212}=15.95$, $P<0.001$). Among *C. glomerata*, females from MD foraging alone spent significantly less time (24.25±2.45 secs) handling hosts than did females from CO foraging alone (34.23±7.33 secs), but no differences were found between the two populations of *C. glomerata* when they foraged with *C. rubecula* (Fig. 3.5A). *Cotesia rubecula* attacks quick lasted an average of 0.63±0.01 secs, and handling times were

faster for wasps foraging with *C. glomerata* (MD *C. rubecula* w/ MD *C. glomerata*: 0.54±0.05 secs; MN *C. rubecula* w/ CO *C. glomerata*: 0.56±0.04 secs) compared to conspecifics from the same population foraging alone (MD *C. rubecula*: 0.72±0.04 secs; MN *C. rubecula*: 0.66±0.04 secs) (Fig. 3.5B).

When *C. rubecula* and *C. glomerata* foraged together, *C. rubecula* was the first to attack in 91% (52/57) of trials when foraging with CO *C. glomerata* and in 69% (34/49) of the trials with MD *C. glomerata*. While MD and MN *C. rubecula* were first to attack more often than either population of *C. glomerata*, the odds of *C. glomerata* being first were 4.59 [1.53, 13.79] times higher for MD *C. glomerata* (15/49) compared to the CO conspecifics (5/57) (Log likelihood $\chi^2_1=8.42$, $P=0.004$). Multiparasitism was observed in 68% (39/57) of trials when CO *C. glomerata* foraged with MN *C. rubecula*, and in 61% (30/49) of trials involving MD *C. glomerata* foraging with MD *C. rubecula*, with no difference between the two *C. glomerata* populations (Log likelihood $\chi^2_1=0.60$, $P=0.44$). Both populations of *C. glomerata* attacked hosts after parasitism by *C. rubecula*, but the odds of following a *C. rubecula* attack were 9.18 [2.31, 36.53] higher for CO *C. glomerata* than for MD *C. glomerata* (Log likelihood $\chi^2_1=12.53$, $P<0.001$).

We failed to find differences in clutch sizes between CO and MD *C. glomerata*, nor did the presence of *C. rubecula* affect the number of eggs laid in a host ($F_{3,192}=0.54$, $P=0.65$). The average clutch size per *C. glomerata* attack was 21.60 (±1.10) eggs for CO *C. glomerata* foraging alone and 21.58 (±1.32) eggs for CO *C. glomerata* with MN *C. rubecula*, and 20.49 (±0.81) and 22.50 (±1.15) eggs for MD *C. glomerata* foraging alone and with MD *C. rubecula*, respectively. Previous attacks by *C. rubecula* failed to influence the clutch size of subsequent *C. glomerata* attacks ($F_{1,187}=0.66$, $p=0.42$). In bouts of multiparasitism where *C. glomerata* was the

second to attack, the mean clutch size (CO *C. glomerata*: 22.43 ± 1.18 eggs, $N=35$; MD *C. glomerata*: 22.94 ± 1.83 eggs, $N=17$) was similar to that when hosts were attacked by *C. glomerata* first or just *C. glomerata* (CO *C. glomerata*: 22.83 ± 0.91 , $N=61$; MD *C. glomerata*: 20.78 ± 0.71 , $N=78$). Since *C. rubecula* laid only one egg per attack, the number of *C. rubecula* eggs always equaled the number of attacks on a host. Multiple *C. rubecula* eggs were observed only when a female repeatedly attacked a host (MD *C. rubecula*: 9/49; MN *C. rubecula*: 10/57), which was allowed only when foraging together with a *C. glomerata* that had yet to attack the host within the 10-minute observation period.

3.5 DISCUSSION

We found that the competitive environment from which *C. glomerata* originates affected its foraging behaviors. *Cotesia glomerata* from MD compete with *C. rubecula* for *P. rapae* and the foraging performance of these *C. glomerata* decreased when foraging with *C. rubecula*. CO *C. glomerata*, which lack experience with *C. rubecula*, were less efficient than their MD conspecifics and were more likely to make the costly mistake of attacking a host previously parasitized by *C. rubecula*. Regardless of their population, *C. glomerata* had slower search and handling times than did *C. rubecula*, but contrary to our expectations, both species performed similarly at exploiting hosts across a range of host densities. Antagonistic interactions were absent, suggesting that interference competition plays a minimal, if any, role among adults of these two parasitoid wasps.

Our results on search and handling time support the idea that *C. rubecula* can use *P. rapae* hosts more efficiently than can *C. glomerata*. The faster handling time of *C. rubecula* is likely due to laying only one egg per host compared to the larger clutches of *C. glomerata*.

Wasps were given only one host in Experiment II, but when parasitizing multiple caterpillars (10-20) for our colony maintenance, we have observed *C. rubecula* attacking numerous (>5) hosts within 30 seconds, which is as long as some attack durations for *C. glomerata* (pers. obs. DV & RP).

Comparative studies of closely related species are valuable for inferring the adaptive value of species traits. *Cotesia glomerata* and *C. rubecula* are phylogenetically related (Michel-Salzat and Whitfield 2004), yet their foraging strategies in Europe diverged as each wasp evolved to exploit the distribution of its preferred host species. *Cotesia glomerata* prefer *P. brassicae*, which is found in large clusters, whereas *C. rubecula* prefer *P. rapae*, which has a dispersed distribution (Wiskerke and Vet 1991; Vos et al. 1998; Vos and Hemerik 2003). In contrast, North American *C. glomerata* rely on *P. rapae* as their main host and the wasps behave somewhat like *C. rubecula* with less explorative behaviors and a greater attack rate than their European conspecifics (le Masurier and Waage 1993; Vos and Hemerick 2003). This might explain why we found few differences between the two *Cotesia* species when they foraged across a range of *P. rapae* densities. In addition, both *Cotesia* species are attracted to the herbivore-induced plant volatiles (HIPVs) emitted from *P. rapae* herbivory (Fatouros et al. 2005). Increasing host density increases HIPVs, resulting in greater motivation for host searching in *C. glomerata* and *C. rubecula* (Kaiser and Cardé 1992; Steinberg et al. 1992; Geervliet et al. 1998), possibly explaining the observed relationship between increases in host density and parasitism.

In addition to search and handling time, foraging efficiency is also affected by choosing the highest quality host. When female parasitoids select hosts parasitized by stronger competitors, foraging efficiency is reduced since time is wasted on hosts that result in high

mortality. The reunion of *C. glomerata* and *C. rubecula* in North America likely created a selection pressure on *C. glomerata* for avoiding hosts parasitized by *C. rubecula* since these hosts are deadly for *C. glomerata* (Laing and Corrigan 1987). When faced with this costly host option, *C. glomerata* has two options: multiparasitize or expend energy to find unparasitized hosts. The absence of *C. rubecula* in a habitat should preclude selection on *C. glomerata* for avoiding multiparasitism. Indeed, when foraging with *C. rubecula*, the MD *C. glomerata* were less likely to make the mistake of multiparasitism than were CO *C. glomerata*, which are naïve to *C. rubecula*. The fewer hosts attacked by MD *C. glomerata* when foraging with *C. rubecula* suggests that these *C. glomerata* foraged less in this situation, perhaps because they were more discriminatory of the hosts they attacked. Hosts previously attacked by *C. rubecula* are of low-quality for *C. glomerata*, thus *C. glomerata* foraging success is partly defined by how well they avoid these hosts.

Foraging efficiency is a behavioral link between parasitism and its effects on population stability (Hassell 2000; Vet 2001). Within some parasitoid communities, the foraging behaviors of adults can help overcome competitive deficiencies experienced by their larvae. *Aphidius ervi* larvae outcompete *A. smithi* when both parasitoids attack the same pea aphid (*Acyrtosiphon pisum*), but adult *A. smithi* use their higher search efficiency to outcompete adult *A. ervi* (Chua et al. 1990). *Trissolcus basalis* is better than *Oencyrtus telenomicida* at using kairomones and synomones to locate stinkbug (*Nezara viridulai*) hosts, and this search efficiency helps *T. basalis* overcome poor larval competition with *O. telenomicida* (Colazza et al. 1999; Peri et al. 2014). Competitive displacement is more likely to occur when one parasitoid species has the advantage in both adult foraging efficiency and larval competition. Faster search and handling time and superior larval competitive ability allow *Bathyplectes anurus* to displace *B. curculionis* as the

primary parasitoid of the alfalfa weevil (*Hypera postica*) in Ontario (Harcourt 1990). Less efficient foraging among adult females and poor survival in larval competition are likely contributing to the competitive displacement of *C. glomerata* by *C. rubecula* in North America (Herlihy et al. 2012).

Stronger competitors can be selection pressures and cause variability in foraging strategies. The behavioral changes required to escape displacement can cause a population to be different from conspecifics that experience a weaker degree of pressure from competition. For species with wide geographical distributions, each population may inhabit a unique competitive environment in which it evolves behaviors used to mediate competition.

Table 3.1. Sample sizes (# of replicates) for five levels of the host density (# of *Pieris rapae* per plant) treatment and six levels of the wasp foraging treatment used in Experiment I.

Host Density Treatment	Wasp Foraging Treatment						Total # of hosts
	CO <i>C. glomerata</i> alone	MN <i>C. rubecula</i> alone	CO <i>C. glomerata</i> + MN <i>C. rubecula</i>	MD <i>C. glomerata</i> alone	MD <i>C. rubecula</i> alone	MD <i>C. glomerata</i> + MD <i>C. rubecula</i>	
1	15	13	12	15	11	11	77
2	15	13	12	15	11	11	154
4	15	13	12	15	11	11	308
6	15	13	12	15	11	11	462
8	15	13	12	15	11	11	616
Total # of <i>C. glomerata</i>	75	-	60	75	-	55	
Total # of <i>C. rubecula</i>	-	65	60	-	55	55	

Population of *Cotesia*: CO=Colorado, MD=Maryland, MN=Minnesota

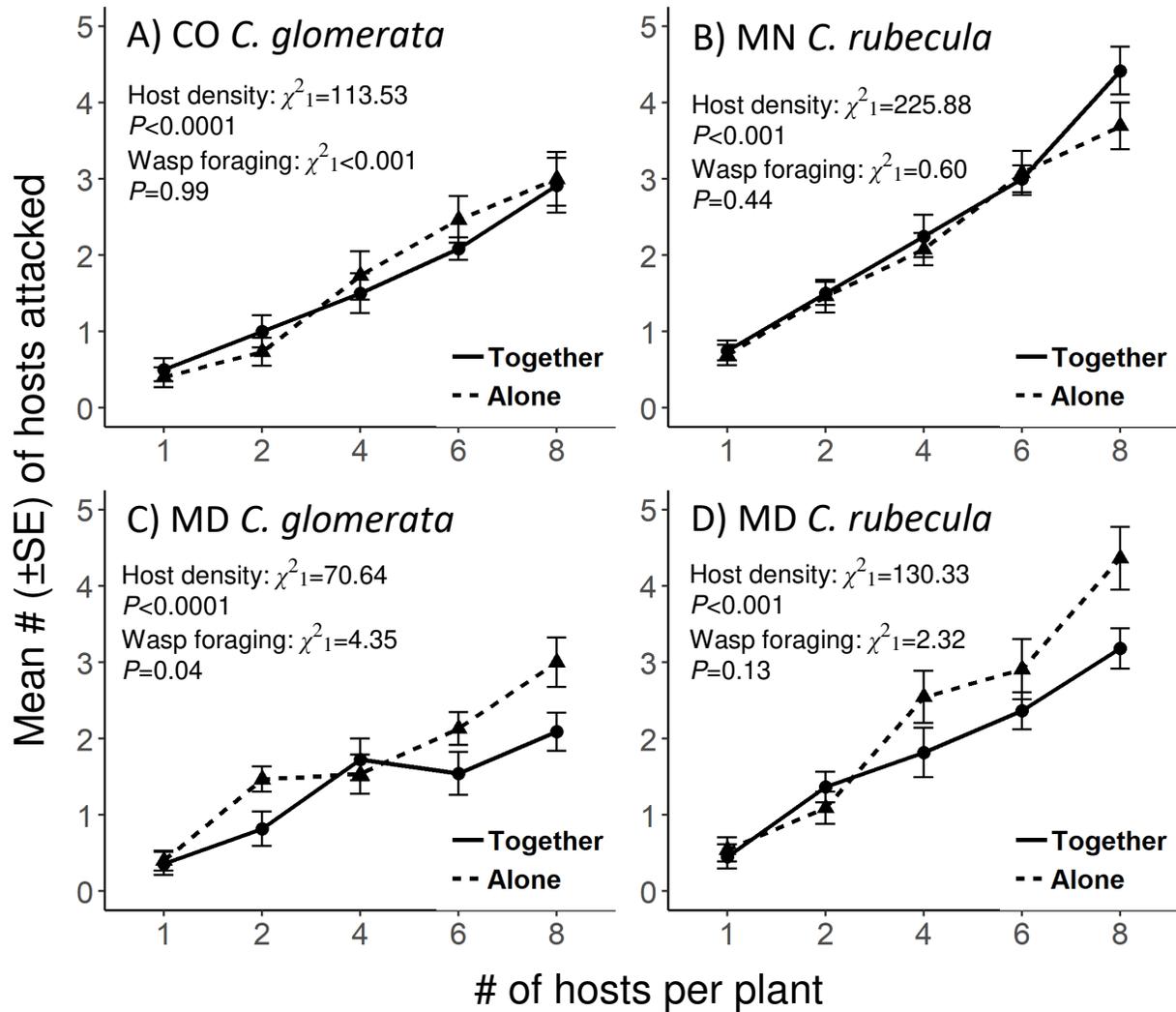


Figure 3.1. The relationship between host density (number of hosts per plant) and the mean (\pm SE) number of *Pieris rapae* hosts that were attacked by *Cotesia glomerata* from populations in Colorado (CO) (A) and Maryland (MD) (C), and by *C. rubecula* from populations in Minnesota (MN)(B) and Maryland (MD) (D), when wasps foraged alone or together with a female of the other species. CO *C. glomerata* and MN *C. rubecula* foraged together, and MD *C. glomerata* foraged with MD *C. rubecula*.

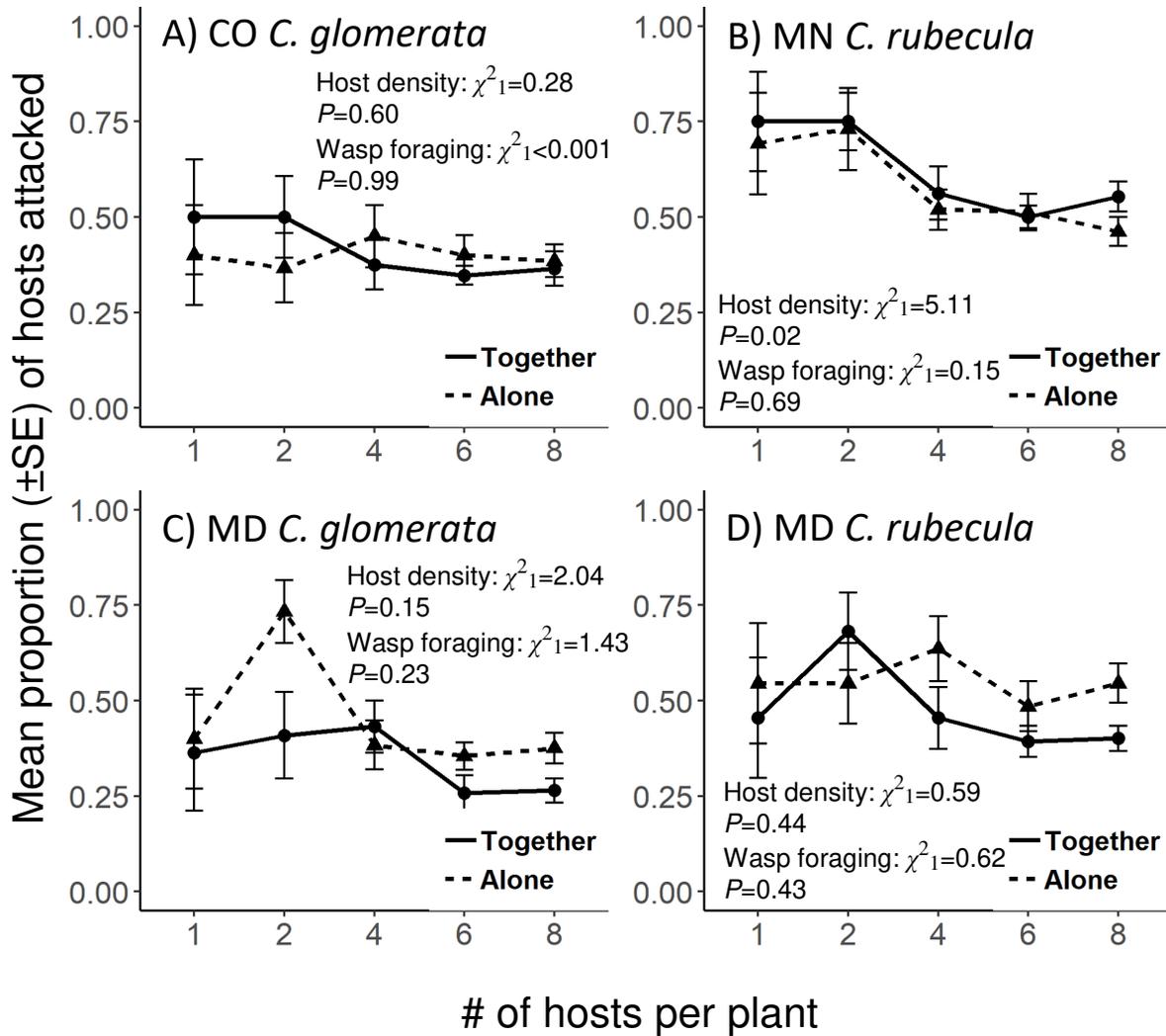


Figure 3.2. A comparison of the mean (\pm SE) proportion of *Pieris rapae* parasitized, per host density (number of hosts per plant), by *Cotesia glomerata* from populations in Colorado (CO) (A) and Maryland (MD) (C), and by *C. rubecula* from populations in Minnesota (MN) (B) and Maryland (MD) (C) when wasps foraged alone or with the other species. CO *C. glomerata* and MN *C. rubecula* foraged together, and MD *C. glomerata* foraged with MD *C. rubecula*.

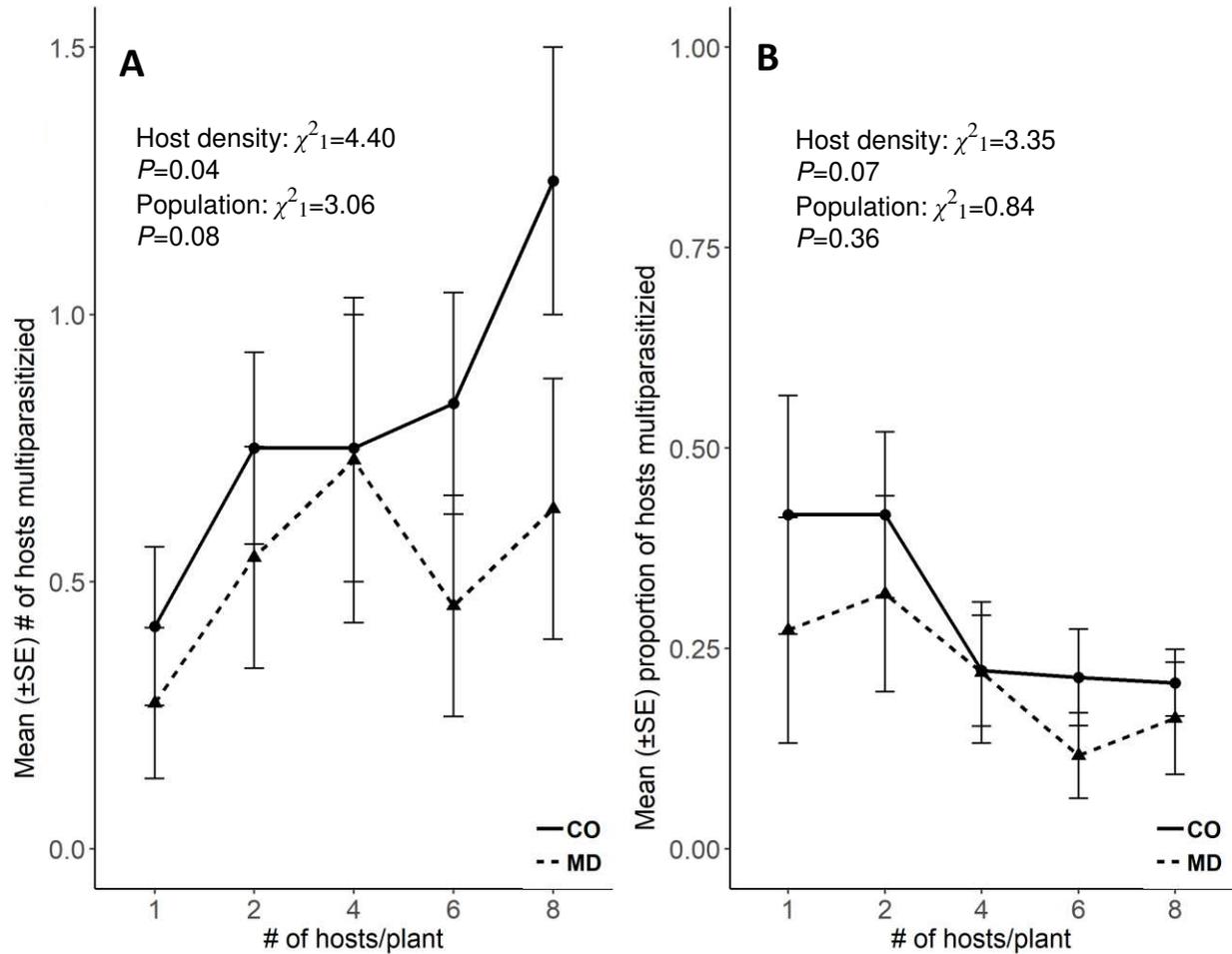


Figure 3.3. The mean (\pm SE) number (A) and proportion (B) of *Pieris rapae* hosts that were parasitized by both *C. glomerata* and *C. rubecula* (i.e., multiparasitized) as a function of host density when both *Cotesia* species foraged together. *Cotesia glomerata* were from a population in Colorado (CO; solid line) or a population in Maryland (MD; dashed line) and CO *C. glomerata* foraged with *C. rubecula* from Minnesota (MN), while MD *C. glomerata* foraged with MD *C. rubecula*. A total of 12 replicates were completed for CO *C. glomerata* and 11 for MD *C. glomerata*.

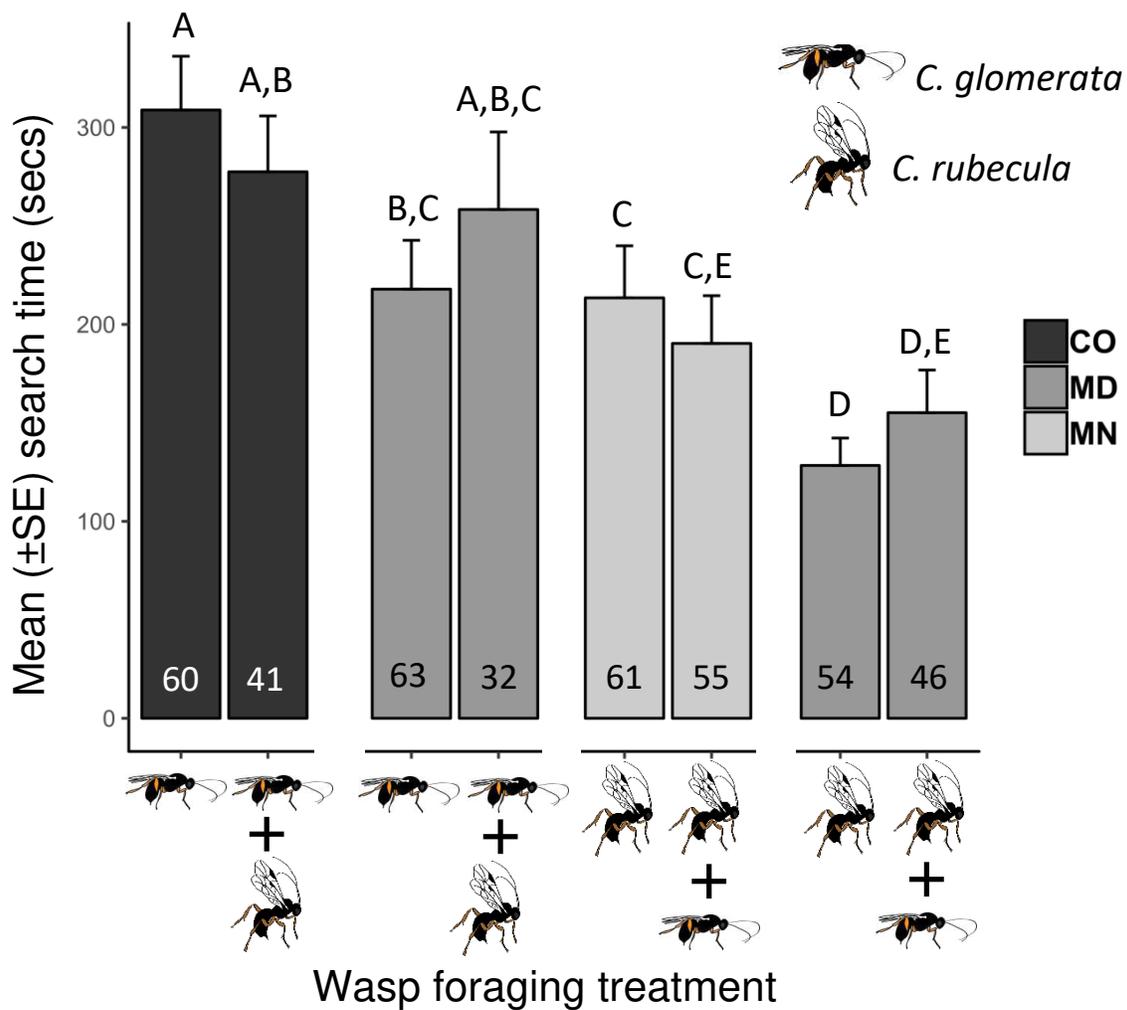


Figure 3.4. The mean (\pm SE) amount of time (sec) it took for *Cotesia glomerata* and *C. rubecula* to find and attack a single *Pieris rapae* hosts when foraging with or without the other wasp species. *Cotesia glomerata* were from populations in Colorado (CO) or in Maryland (MD), and *C. rubecula* were from populations in Maryland (MD) or Minnesota (MN). When foraging together, CO *C. glomerata* were with MN *C. rubecula* and MD *C. glomerata* were with MD *C. rubecula*. The numbers inside the bars indicate the number of females per treatment. Different letters on top of the bars indicate statistically significant differences at $p < 0.05$ (Games-Howell multiple comparison tests)

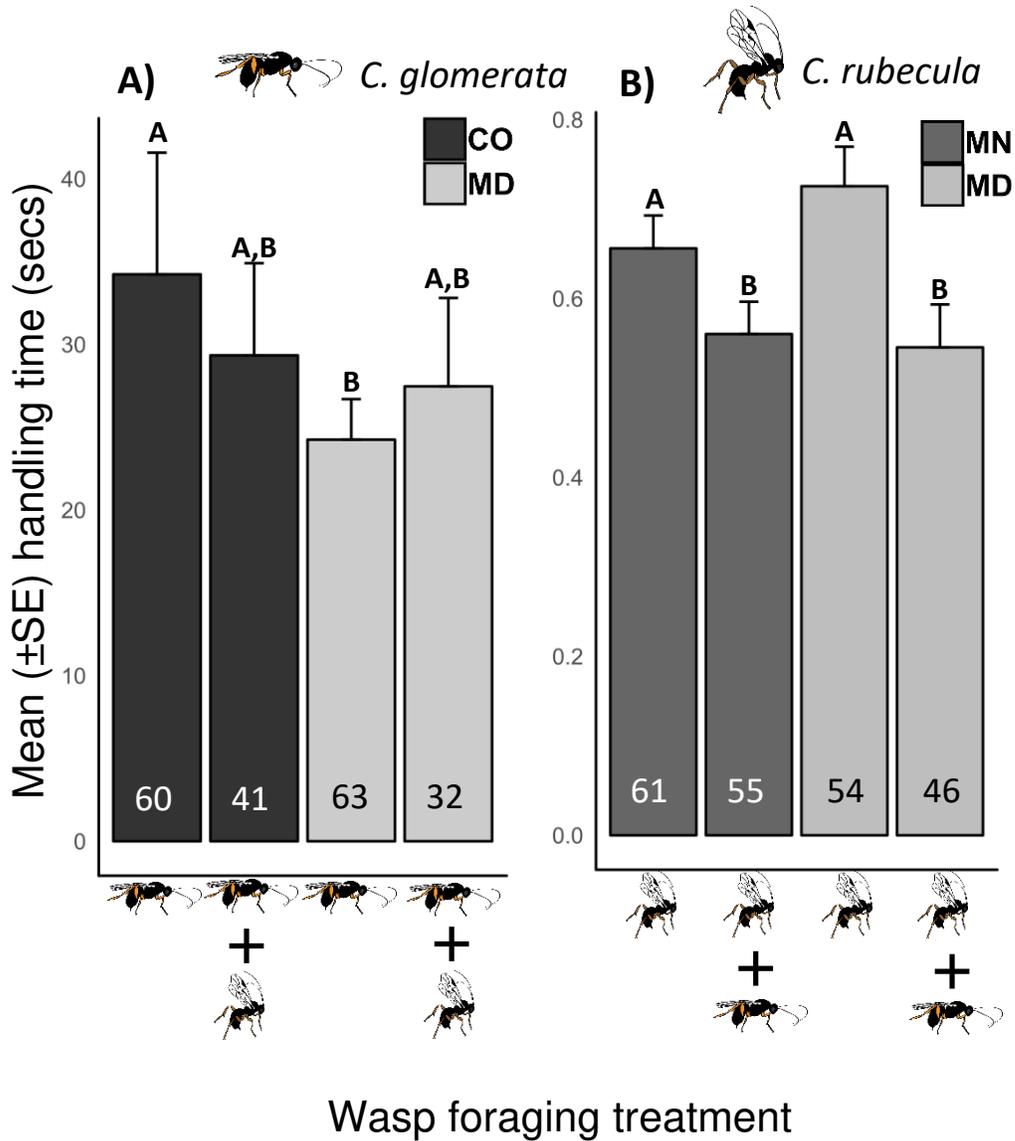


Figure 3.5. The mean (\pm SE) duration (sec) of attacks on a single *Pieris rapae* caterpillar when *Cotesia glomerata* (A) and *C. rubecula* (B) wasps foraged with or without the heterospecific in a plastic cup. *Cotesia glomerata* were from populations in Colorado (CO) or in Maryland (MD), and *C. rubecula* were from populations in Maryland (MD) or Minnesota (MN). When foraging together, CO *C. glomerata* were with MN *C. rubecula* and MD *C. glomerata* were with MD *C. rubecula*. The numbers inside the bars indicate the number of females per treatment. Different letters on top of the bars indicate statistically significant differences at $p < 0.05$ (Tukey HSD multiple comparison tests).

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CHAPTER 4: Ecological dissociation and re-association with a superior competitor alters host selection behavior in a parasitoid wasp

4.1 SYNOPSIS

Interspecific competition for limited resources can drive ecological specialization and trait expression. Organisms released from intense competition may exploit a broader range of resources, but if reunited with stronger competitors, survivorship may depend on foraging behaviors that reduce competition. We compared the host selection behavior of the parasitoid *Cotesia glomerata* from two North American populations that differ in their association with *C. rubecula*, a superior competitor. Both parasitoids originate from Europe and attack the imported cabbageworm (a.k.a. small cabbage white) *Pieris rapae*, but *C. glomerata* was introduced into North America almost a century before *C. rubecula*. After re-association in North America, *C. rubecula* has displaced *C. glomerata* in several regions, but not in other regions. Host selection was measured in female *C. glomerata* from Maryland (MD) where it coexists with *C. rubecula*, and in conspecifics from Colorado (CO) where *C. rubecula* is absent. Unparasitized and *C. rubecula*-parasitized *P. rapae* hosts were used in choice tests to examine whether *C. glomerata* host selection behavior differed based on the population's association history with *C. rubecula*. We found that *C. glomerata* from MD had a higher likelihood of avoiding hosts parasitized by *C. rubecula* (and thus avoiding competition) than did wasps from CO. The ability of *C. glomerata* to avoid hosts parasitized by *C. rubecula* may facilitate coexistence in MD; whereas, the lack of discrimination in CO populations of *C. glomerata* naïve to *C. rubecula* could contribute to the displacement of *C. glomerata* were *C. rubecula* to enter the same habitat.

4.2 INTRODUCTION

Interspecific interactions are well-known to influence the foraging decisions of individuals (e.g., predation – Milinski and Heller 1978; Brown et al. 1988; Kohler and McPeck 1989; competition – Willis 1966; Inouye 1978). In species with broad geographic ranges, foraging behaviors are expected to covary with the community structure of consumers, competitors, and/or resources (e.g., prey for predators, plants for herbivores). Changes in community structure might be expected to select for altered foraging behaviors when a species expands its range and becomes dissociated from its historical community. Such dissociation can result in relaxed selection for behaviors that had previously mitigated the effects of predation or competition (Coss 1999; Lahti et al. 2009).

When asymmetric competition exists between two species that exploit the same resource, weaker competitors can co-exist with competitively superior species by altering their foraging behaviors, typically by recognizing and avoiding the particular resources or foraging habitats used by the stronger competitor. Studies of interspecific competition in a wide range of taxa show that avoidance of a shared foraging habitat is common in communities where two or more competing species have strongly overlapping niches (e.g., mammals: Grasse et al. 2015; birds: Bjorklund et al. 2016; fish: Britton et al. 2018; insects: Janssen et al. 1995; Murdoch et al. 1996; Tamò et al. 2006; Chailleux et al. 2014). However, it is unclear how these avoidance mechanisms may change when weaker competitors have been dissociated from a stronger competitor for many generations.

Interspecific competition is a significant component of the community ecology of insect parasitoids, including many species of parasitic wasps. Parasitoids are insects whose larvae develop in, or on, the bodies of other insects whereas the adults are free living (Godfray 1994).

In nature, some host individuals are often attacked by two or more parasitoid species (i.e., multiparasitism) (Price 1972; Ueno 1999; Harvey et al. 2013), creating conditions in which there may be intense competition for host resources. Within a multiparasitized host, immature endoparasitoids (species whose larvae develop inside their insect host) employ various means of excluding competitors, such as physical attack, physiological suppression, and/or resource competition (Harvey et al. 2013). Given the severe costs (typically, death) of multiparasitism for the loser, the weaker parasitoid competitor will benefit from avoiding hosts previously parasitized by a stronger competitor. Avoidance of multiparasitism has been documented in several host-parasitoid systems (Fisher 1961; van Dijken et al. 1992; Gauthier et al. 1999; Tamò et al. 2006), yet little is known about the short-term changes in avoidance behaviors under conditions of dissociation from, and subsequent re-association with, superior competitors. Such events are likely commonplace in both natural range expansions as well as intentional introductions for biological control programs. Avoidance behaviors may be reduced, or even lost, if a weaker competitor exists in competitor-free space for a number of generations.

Here, we compare the effects of interspecific competition on the host selection decisions of *Cotesia glomerata* females from populations that have a history of interaction with a stronger competitor, *C. rubecula* (both Hymenoptera: Braconidae), with *C. glomerata* females that have been dissociated from *C. rubecula*. When *C. glomerata* and *C. rubecula* parasitize the same host, *C. rubecula* usually kills eggs or larvae of *C. glomerata* (Laing and Corrigan 1987). Both parasitoids are originally from Europe, where coexistence in the same field sites is largely mediated by their use of different host species; *C. rubecula* is a specialist on *Pieris rapae* (Lepidoptera: Pieridae) and *C. glomerata* predominately attacks *P. brassicae* and only rarely attacks *P. rapae* (Geervliet et al. 2000). In North America, *C. glomerata* was introduced

intentionally as a biological control agent against *P. rapae* in the 1880s and rapidly spread throughout much of the continent (Clausen 1978). On the other hand, *C. rubecula* was first discovered in British Columbia in the early 1960s, possibly as an unassisted introduction, and was subsequently redistributed as a biological control agent to several locations in the upper Midwest and New England (US) (Wilkinson 1966; McDonald and Kok 1992; van Driesche and Nunn 2002; Wold-Burkness et al. 2005). *Cotesia glomerata* have at least two generations per year (Laing and Levin 1982; Fei et al. 2014); therefore, prior to the arrival of *C. rubecula*, North American *C. glomerata* lived without *C. rubecula* for an approximate minimum of 160 generations. *Cotesia glomerata* continues to live without *C. rubecula* in some parts of North America where it has undergone at least an additional 100 generations since the two species were last in contact.

Pieris brassicae is absent in North America, forcing *C. rubecula* and *C. glomerata* to rely on *P. rapae* as the primary host species. While the two parasitoids exhibited considerable geographic overlap in the years after the establishment of *C. rubecula* in North America, *C. rubecula* has competitively displaced *C. glomerata* in many of these locations (Biever 1992; Herlihy et al. 2012). Nevertheless, there are areas in North America where *C. glomerata* and *C. rubecula* currently coexist using the same host species (e.g., Maryland and Delaware, US), as well as areas where *C. rubecula* has never occurred and *C. glomerata* continues to exist without any significant competition for *P. rapae* hosts (e.g., Colorado, US). Therefore, it is conceivable that Colorado populations of *C. glomerata* have lived in competitor-free space possibly long enough to have lost the ability to detect and avoid hosts previously parasitized by *C. rubecula*. Failure by female *C. glomerata* to avoid hosts parasitized by *C. rubecula* could lead to displacement of *C. glomerata* by *C. rubecula* if the two species are re-associated (Herlihy et al.

2012); conversely, avoidance of hosts parasitized by *C. rubecula* may permit coexistence of these two parasitoids.

In this study, we compared the avoidance behaviors of two North American *C. glomerata* populations, each with different histories of association with *C. rubecula*. Specifically, we tested the hypothesis that the long-term dissociation from *C. rubecula* has led to relaxed selection on the ability of *C. glomerata* to avoid hosts already parasitized by its stronger competitor. We predicted that Maryland (MD) *C. glomerata* would avoid hosts previously parasitized by *C. rubecula*, whereas Colorado (CO) *C. glomerata* would fail to discriminate between unparasitized hosts and hosts previously attacked by *C. rubecula*. We also explored whether the time elapsed since parasitism by *C. rubecula* influenced the foraging decisions of MD and CO female *C. glomerata*. Some parasitoids can successfully attack a previously parasitized host as long as the subsequent attack occurs within a safe period (Ueno 1999; de Moraes and Mescher 2005; Magdaraog et al. 2013). A *C. rubecula* egg usually hatches 48 hours after oviposition and the first instar larva uses its sharp mandibles to kill con- and heterospecific larvae (Laing and Corrigan 1987). Second, and subsequent, instars of *C. rubecula* lack enlarged, sharp mandibles. We expected that *C. glomerata* females from the MD population would be more likely than CO *C. glomerata* to avoid hosts that were parasitized by *C. rubecula* less than 24 hours earlier, because upon hatching, *C. glomerata* larvae would be exposed to the killing morph of *C. rubecula* in these hosts. Finally, if *C. glomerata* attacks hosts previously parasitized by *C. rubecula*, then they should lay smaller clutch sizes to reduce fitness losses from larval competition with *C. rubecula*.

4.3 METHODS

4.3.1 Insects

Cotesia glomerata and *C. rubecula* both prefer to oviposit in first and second instars of caterpillars in the family Pieridae (Brodeur and Geervliet 1992; Gols et al. 2019). *Cotesia rubecula* lays one egg per attack, whereas *C. glomerata* can lay 20-50 eggs per attack. The *C. rubecula* larva usually emerges from its host in the host's penultimate (4th) instar whereas *C. glomerata* larvae emerge from the host's final (5th) instar. *Cotesia* larvae complete development by egressing from their host and pupating within a single white cocoon in the case of *C. rubecula* or a group of yellow cocoons in the case of *C. glomerata*.

Cotesia glomerata colonies were initiated from field-collected broods from Colorado State University's Agricultural Research and Education Center (CSU ARDEC; GPS: 40.652703, -104.994627) and an organic farm in Maryland (Glade Link Farms, GPS: 39.569801, -77.285140). The *C. glomerata* colony initiated from Colorado-collected material was maintained separately from the colony initiated from Maryland-collected material. Field-collected *C. glomerata* broods were housed in 946 ml clear plastic cups that were placed in environmental control chambers set to a 16L:8D photoperiod and 25°C until adult wasps emerged. Adult wasps were provided with a 10% honey-water solution and kept in 946 ml plastic cups in the chambers set to a 16L:8D photoperiod and 20°C. *Cotesia rubecula* colonies were started from field-collected cocoons at sites near the University of Minnesota in St. Paul, MN, USA. Cocoons were reared individually in 2 ml plastic centrifuge tubes and adults were reared at the same conditions as *C. glomerata*. *Pieris rapae* colonies were initiated with approximately 20 pupae that were collected from CSU ARDEC. They were reared in insect

cages in the greenhouse at a 16L:8D photoperiod at 20-25°C. Adult butterflies oviposited onto live collard plants (*Brassica oleracea*) grown in 10 cm wide pots.

Cotesia rubecula is absent in Colorado, but it co-occurs, often in the same host individuals (i.e., multiparasitism), with *C. glomerata* in Maryland. In order to determine the level of multiparasitism in the field, *P. rapae* caterpillars were collected in 2017 from varieties of cabbage (*Brassica oleracea*) at five different organic farms (Calvert Farm [39.712712, -75.984629], Calvert's Gift Farm [39.584793, -76.738527], Flying Plow [39.692704, -76.089965], Gorman Farm I [39.148985, -76.866518] and Gorman Farm II [39.185325, -76.951153]) in Maryland from May to September in 2017. We collected and dissected a total of 751 caterpillars from across the 5 instars (L1-L5): 83 L1, 300 L2, 141 L3, 147 L4 and 80 L5. The farms in Maryland were located throughout the state with a minimum separation of 13.5 km. At each farm, 20-50 plants per variety were selected and each leaf was inspected for caterpillars. If a farm was visited more than once, a different row or plot of the same cabbage variety was selected to prevent repeated sampling of plants. A total of 1063 plants were surveyed for caterpillars.

Field-collected caterpillars were dissected to assess whether immature *C. glomerata* and/or *C. rubecula* were present. *Cotesia rubecula* eggs are distinguished from *C. glomerata* because *C. rubecula* eggs occur in fewer numbers (<5) and are approximately twice the size of *C. glomerata* eggs. Only *C. rubecula* larvae possess large sclerotized-mandibles and a caudal appendage. At times (28/751), the parasitoid eggs were encapsulated, which occurs when the host's immune cells surround and impair parasitoid egg development (Nappi 1975). When encapsulated eggs were observed, we were able to accurately distinguish the species of parasitoid by counting the number of eggs (>5 for *C. glomerata*) and size of the egg, characteristics that

remain observable even 3-5 days following encapsulation (Brodeur and Vet 1995; DV and JH pers. observation).

4.3.2 Experimental design

Choice tests were conducted to compare the host selection behavior of female *C. glomerata* from Colorado with females from Maryland (which are currently associated with *C. rubecula*) when presented with unparasitized caterpillars and caterpillars previously parasitized by *C. rubecula*. Each host individual was in the second instar because this stage is readily attacked by both *Cotesia* species (Brodeur and Geervliet 1992). Previously parasitized hosts were attacked by *C. rubecula* either <0.5 h, 24 h, 48 h, or 72 h prior to exposure to *C. glomerata*, allowing us to determine whether *C. glomerata* host preference was affected by the time since parasitism by *C. rubecula* (and hence, developmental stage of *C. rubecula*).

Prior to the start of each choice test, each female *C. glomerata* was 'primed' (to increase her responsiveness to hosts in choice tests; Fatouros et al. 2005) by exposing her to a collard leaf previously damaged by a *P. rapae* larva for 10 minutes in a 60 mm petri dish. Choice tests involved placing a mated, primed female *C. glomerata* with no prior oviposition experience in an arena containing an unparasitized *P. rapae* caterpillar and a caterpillar previously parasitized by *C. rubecula*. The unparasitized and *C. rubecula*-parasitized caterpillars were placed on separate collard leaves in the choice test arena prior to the introduction of the *C. glomerata* female. The collard leaves with the caterpillars were cut from the plant and the leaf petioles were placed individually in 37 ml plastic cups filled with water to prevent wilting. The cups were color-coded with a marker to indicate which leaf held the unparasitized or *C. rubecula*-parasitized caterpillar. Arenas consisted of a 946 ml clear plastic cylinder, into which the test *C. glomerata*

female was introduced above and away from the pair of leaves on which the caterpillars were feeding. Once the female ceased grooming and began to walk towards the leaves, we observed foraging behaviors for 15 minutes.

We recorded the following observations in each choice test: the first leaf (with unparasitized or *C. rubecula*-parasitized larva) on which foraging behavior was observed, first host attacked (unparasitized or *C. rubecula*-parasitized), time to attack (seconds), attack duration (seconds), and clutch size (number of eggs). Host-searching behavior was defined as either repeated contact of the distal end of the female's abdomen or her antennae with the surface of the leaf. An attack was defined as the insertion of the ovipositor into the host while the female assumed a stationary posture with wings spread and legs elevated off the substrate. Attacks ended when the female began moving and retracted her ovipositor from the host. After an attack, the *C. glomerata* female was removed and the arena containing the host caterpillars was placed in an incubator set at a 16L:8D photoperiod and 20°C for 48 hours to allow the parasitoid eggs to hatch. Forty-eight hours after a choice test, each caterpillar was dissected to verify the presence of and count the number of eggs or larvae from both *Cotesia* species. If a host caterpillar exposed to *C. rubecula* lacked a *C. rubecula* egg or larva, that choice test was excluded from the analyses as this would have meant that the foraging *C. glomerata* had been mistakenly presented with two unparasitized hosts. Each choice test ended after a female completed oviposition or if 15 minutes elapsed without a female contacting either leaf. Five CO *C. glomerata* and three MD *C. glomerata* failed to contact the leaf or to attack either host; these choice tests were discarded and repeated with new females.

A total of 18 choice tests were performed per time interval for a total of 72 replicates for CO *C. glomerata* and 72 replicates for MD *C. glomerata*. Separate female *C. glomerata* were

used for each choice test. Therefore, a total of 72 CO *C. glomerata*, 72 MD *C. glomerata*, 144 caterpillars parasitized by *C. rubecula* and 144 unparasitized caterpillars were used in this study. Multiple choice tests were completed in a day, which was treated as a block (see below).

4.3.3. Analyses

We analyzed our data with three statistical models. First, host preference (unparasitized, *C. rubecula*-parasitized) was treated as a binary response and analyzed using a multiple logistic regression with *C. glomerata* population (CO, MD), time interval since parasitism by *C. rubecula* (<0.5, 24, 48, or 72 h), and their interaction term as explanatory variables. Time interval was considered as an ordinal variable. Second, time to attack (seconds), attack duration (seconds), and clutch size (number of eggs) were treated as continuous response variables and were each analyzed using separate two-way ANOVA with parasitism status (unparasitized or *C. rubecula*-parasitized) of host and *C. glomerata* population (CO or MD) as explanatory variables. Third, we were also interested in assessing whether these three response variables were affected by the time since parasitism by *C. rubecula*. For this final model, unparasitized hosts were excluded because they were not attacked by *C. rubecula* and obviously lacked a time interval between attack by *C. rubecula* and exposure to *C. glomerata*. Time to attack, attack duration, and clutch size were each analyzed using separate two-way ANOVAs with time since oviposition by *C. rubecula* and *C. glomerata* population treated as explanatory variables. The relationship between attack duration and clutch size was measured using Pearson correlation after log transformation of attack duration to decrease skew. Five females that attacked a host but failed to lay eggs were omitted from this analysis (CO unparasitized = 1; CO *C. rubecula*-parasitized = 1; MD *C. rubecula*-parasitized = 3). Blocks were the different dates on which sets

of choice test experiments were performed and block was treated as a random effect in the regression analyses. Response variables were unaffected by the block term in any of the analyses; therefore, statistical models were rerun without the blocking variable and these results are presented. A two-tailed exact binomial test, with function `binom.test` in RStudio version 1.0.136 (R Core Team 2017), was used to compare the observed and expected probabilities of multiparasitism found in field-collected caterpillars. JMP version 12.0.1 (SAS Institute) was used for all other analyses. All means are presented as means with standard errors (mean \pm SE) and all statistical analyses were tested at a significance level of $\alpha=0.05$.

4.4 RESULTS

Multiparasitism was rarely observed in field-collected *P. rapae* caterpillars from Maryland with 10% (74/751) containing both *C. glomerata* and *C. rubecula*. Of the 751 *P. rapae* collected, 310 (41%) were attacked by *C. glomerata* and 153 (20%) were attacked by *C. rubecula*. Based on these values, randomly searching wasps in a patch of 100 hosts were expected to multiparasitize 8% [95%CI: 7%, 12%] of these hosts, which was slightly below the observed value of 10% ($p=0.08$) (Fig. 4.1).

Cotesia glomerata females from Colorado were 2.1 times [95% CI: 1.1, 4.0] more likely to attack hosts that were previously parasitized by *C. rubecula* than were *C. glomerata* females from Maryland (Fig. 4.2; likelihood ratio $\chi^2=4.73$, d.f.=1, $p=0.03$). The time interval between parasitism by *C. rubecula* and encounter by *C. glomerata* had no influence on the likelihood of attacking *C. rubecula*-parasitized hosts by either CO or MD *C. glomerata* (CO: likelihood ratio- $\chi^2=3.41$, d.f.=3, $p=0.33$; MD: likelihood ratio- $\chi^2=3.42$, d.f.=3, $p=0.33$). With time interval held constant, *C. glomerata* females from MD were significantly more likely to choose unparasitized

hosts over hosts previously parasitized by *C. rubecula* (likelihood ratio χ^2 goodness-of-fit test=4.55, d.f.=1, p=0.03), whereas *C. glomerata* females from CO failed to show a preference for unparasitized hosts over those previously parasitized by *C. rubecula* (likelihood ratio χ^2 goodness-of-fit test=1.39, d.f.=1, p=0.24). Taken together, these findings indicate that *C. glomerata* from Colorado are less likely to avoid against hosts previously parasitized by *C. rubecula* than are *C. glomerata* from Maryland.

Overall, 133 out of 144 (92.3%) *C. glomerata* attacked the host that was on the leaf first contacted by the female. The remaining 11 trials consisted of either *C. glomerata* females first contacting an unparasitized host followed by an attack of a parasitized host (n=7) or the opposite (n=4). When a *C. glomerata* first contacted an unparasitized host and ignored it to attack a *C. rubecula* parasitized host, six out of seven (85.7%) trials involved a CO *C. glomerata*. In three of the four (75%) trials where a *C. glomerata* first contacted a parasitized host, but then attacked the unparasitized host, the *C. glomerata* were from MD.

Once a female *C. glomerata* initiated rapid antennal and/or abdominal contact with the leaf in pursuit of a host, the mean time to attack an unparasitized or *C. rubecula*-parasitized host was independent of the population origin of *C. glomerata* (CO vs. MD: 305.4±27.2 sec vs. 322.1±28.8 sec; $F_{1,140}=0.15$, p=0.70) or parasitism state of the host (unparasitized vs. *C. rubecula*-parasitized: 311.4±29.0 sec vs. 315.8±27.1 sec; $F_{1,140}=0.002$, p=0.97). When *C. glomerata* females from Colorado and Maryland attacked hosts previously parasitized by *C. rubecula*, the time to attack was similar for both populations (CO vs MD: 310.5±34.1 sec vs. 312.8±52.1 sec; $F_{1,61}=0.02$, p=0.89) and was unaffected by the time since parasitism by *C. rubecula* (<0.5 h=401.8±91.5 sec, 24 h=241.1±39.3 sec, 48 h=304.6±49.5 sec, 72 h=334.9±61.6 sec; $F_{3,61}=1.51$, p=0.22).

After a host was found, the time a female spent attacking was independent of population (CO vs. MD: 67.1 ± 8.6 sec vs. 52.75 ± 5.9 sec; $F_{1,140}=1.49$, $p=0.22$) and the parasitism status of the host (unparasitized vs. *C. rubecula*-parasitized: 66.1 ± 8.8 sec vs. 54.3 ± 5.9 sec; $F_{1,140}=1.10$, $p=0.35$). When attacking hosts that were previously parasitized by *C. rubecula*, there were no significant differences in oviposition duration between population (CO vs. MD: 74.8 ± 13.1 sec vs. 53.4 ± 10.2 sec; $F_{1,61}=1.45$, $p=0.23$) or time interval since *C. rubecula* attack (<0.5 h= 97.1 ± 29.4 sec, 24 h= 48.9 ± 12.3 sec, 48 h= 54.6 ± 11.8 sec, 72 h= 75.5 ± 19.5 sec; $F_{3,61}=0.92$, $p=0.43$).

For both unparasitized and *C. rubecula*-parasitized hosts, we found a weak positive relationship between attack duration and clutch size for only *C. glomerata* from CO (Fig. 4.3; unparasitized hosts: $r=0.52$, $n=30$, $p=0.004$; *C. rubecula*-parasitized hosts: $r=0.45$, $n=40$, $p=0.004$). There was no relationship between the time it took female *C. glomerata* from MD to oviposit and the size of their clutches (Fig. 4.3). Female *C. glomerata* from MD and CO laid similar clutch sizes in unparasitized hosts and hosts previously parasitized by *C. rubecula* (CO unparasitized vs. parasitized: 26.63 ± 1.35 eggs vs. 26.02 ± 1.02 eggs; MD unparasitized vs. parasitized: 24.20 ± 1.04 vs. 23.40 ± 1.38 eggs; $F_{3,135}=1.48$, $p=0.22$). After controlling for the parasitism status of the hosts, CO *C. glomerata* had larger clutch sizes than did MD *C. glomerata* (CO vs. MD: 26.29 ± 0.82 eggs vs. 23.91 ± 0.83 eggs; $F_{1,135}=4.43$, $p=0.04$). When wasps attacked *C. rubecula* parasitized hosts, clutch size was independent of population ($F_{1,57}=2.40$, $p=0.13$) or the length of time since parasitism by *C. rubecula* (<0.5 h= 23.9 ± 1.3 eggs, 24 h= 24.2 ± 1.8 eggs, 48 h= 22.9 ± 2.7 eggs, 72 h= 23.3 ± 2.1 eggs; $F_{3,57}=0.33$, $p=0.81$). Clutch sizes ranged from 12-56 eggs; five females (two from CO and three from MD) attacked a host without laying any eggs, so these females were excluded from these analyses.

4.5 DISCUSSION

Foraging parasitoids are capable of avoiding oviposition in hosts previously parasitized by conspecific females (van Alphen and Visser 1990; Godfray 1994) and can even detect herbivore-induced volatiles at long distances to avoid hosts parasitized by a stronger competitor (Janssen et al. 1995; Tamò et al. 2006). While other studies have found between-population variability in the ability to avoid hosts attacked by conspecifics (van Baaren and Boivin 1998; Goubault et al. 2004), to our knowledge this is the first study to compare the ability of different populations to discriminate against hosts parasitized by heterospecifics. Our results support the hypothesis that host selection behavior of North American populations of *C. glomerata* differ based on whether they have become re-associated with *C. rubecula*. *Cotesia glomerata* females from CO, where *C. rubecula* is absent, appear to lack the ability to discriminate between unparasitized hosts and those previously parasitized by *C. rubecula*. *Cotesia glomerata* experience high mortality if attacking a host parasitized by *C. rubecula* (Laing and Corrigan 1987); thus, *C. rubecula* should exert strong directional selection favoring avoidance behavior in *C. glomerata* females from MD towards hosts previously parasitized by *C. rubecula*. In Europe, where both parasitoids and *P. rapae* originate, the two *Cotesia* species coexist because they almost exclusively use different host species: *C. rubecula* attacks only *P. rapae* and *C. glomerata* predominately attacks *P. brassicae* and only rarely attacks *P. rapae* (Geervliet et al. 2000).

That *C. glomerata* from MD were significantly less likely to multiparasitize a host than were *C. glomerata* from CO, yet females from both locations almost always oviposit in the first host examined by antennal contact and laid similar clutch sizes, suggests that host discrimination in MD females may occur well before the host is physically encountered. Parasitoids, including

C. glomerata and *C. rubecula* (Geervliet et al. 1998; 1998; Fatouros et al. 2005; Poelman et al. 2011), are well-known to respond to herbivore-induced plant volatiles (Geervliet et al. 1998; Baldwin et al. 2002; Dicke and Baldwin 2010; Gols 2014). Geervliet et al (2000) used a wind tunnel study to show that *C. glomerata* females avoid patches of *P. rapae* hosts that are nearby *C. rubecula*. Indeed, parasitoid species identity is known to alter herbivore-induced plant volatile profiles (Poelman et al. 2011). For instance, *Brassica oleracea* plants attacked by unparasitized *P. rapae* caterpillars, *C. rubecula*-parasitized caterpillars, and *C. glomerata*-parasitized caterpillars all differ in their volatile profiles (Poelman et al. 2012; Zhu et al. 2014). These volatile profiles are, in turn, differentially attractive to hyperparasitoids (Poelman et al. 2012) and, presumably, used as cues by MD *C. glomerata* females to assess whether a host is unparasitized, parasitized by a heterospecific competitor, or parasitized by a conspecific. When given a choice between plants fed upon by unparasitized *Pieris* caterpillars or ones parasitized by *C. rubecula* (multiparasitism) or *C. glomerata* (superparasitism), *C. glomerata* uses competitor-related cues to avoid plants with parasitized hosts (multiparasitism – Geervliet et al. 2000; superparasitism – Fatouros et al. 2005).

Previous studies with *P. rapae* as a host have found that mated and unmated *C. glomerata* reduce clutch size and attack duration when superparasitizing hosts (Kusano and Kitano 1974; Ikawa and Suzuki 1982; Tagawa 1992). Interestingly, we found that once an attack was initiated, neither CO nor MD *C. glomerata* females adjusted their clutch sizes regardless of the time elapsed between when the host was parasitized by *C. rubecula* and subsequently encountered by *C. glomerata*. It is likely that female *C. glomerata* in our study failed to adjust clutch sizes in response to the presence of *C. rubecula* because *C. rubecula* causes 100% mortality of immature *C. glomerata* regardless of the number of eggs laid. Under these

conditions, there would be no selection for altered clutch sizes since variation in clutch size fails to increase survivorship.

Where *C. glomerata* and *C. rubecula* co-occur in North America, at least three possible mechanisms may be responsible for their coexistence. First, as was the focus of this study, *C. glomerata* may have experienced strong selection pressure to avoid attacking hosts already parasitized by *C. rubecula* as seems to be the case with our MD study population. To further support the idea that multiparasitism avoidance may influence the coexistence between *C. glomerata* and *C. rubecula*, our dissections of field-collected *P. rapae* hosts in MD suggest that multiparasitism is infrequently observed. It was not possible to know the order of attack for these multiparasitized hosts; but as *C. rubecula* is known to accept hosts parasitized by *C. glomerata* (Laing and Corrigan 1987), these multiparasitized hosts could be a product of *C. rubecula* attacking after *C. glomerata*. Second, *C. glomerata* populations may persist if alternative host species are present. Whereas *C. rubecula* is an extreme specialist on *P. rapae* (Brodeur et al. 1996), *C. glomerata* has a broader host range including several North American pierid species, such as the green-veined white *P. napi*, the checkered white *Pontia protodice*, and the western white *Pontia occidentalis* (Laing and Levin 1982; Benson et al. 2003; van Driesche et al. 2004; Herlihy et al. 2012; DV pers. observation). If these alternate host populations are sufficiently high, *C. glomerata* may be able to persist even in the face of intense competition with *C. rubecula* for *P. rapae*. Third, hyperparasitism may reduce the survivorship of *C. rubecula*, thus reducing its negative impact on *C. glomerata* (McDonald and Kok 1991; Kaser and Ode 2016; Weis et al. 2016). Mortality from hyperparasitoid attacks is suspected to limit the range of *C. rubecula* in North America (McDonald and Kok 1992; Gaines and Kok 1999), therefore this top-down factor could be significant for mediating competitive interactions

between the two *Cotesia* species. The relative importance of these non-exclusive explanations is unknown and requires further research with additional *C. glomerata* populations. These parasitoid communities could be operating under different evolutionary pressures across a broad geographical landscape, creating a mosaic of communities in which different North American populations of *C. glomerata* exist (Thompson 1994).

Across taxa, separation from important heterospecifics (e.g., predators, pathogens, and pollinators) is known to alter behaviors that are important for mediating interspecific interactions. Studies on predator-free space show that relaxed selection can attenuate a wide range of behaviors, including visual (Coss 1999), acoustic (Fullard et al. 2007) and olfactory recognition of predators (Cousyn et al. 2001). Just as recognition of predators weakens in predator-free space, so should recognition of stronger competitors in competitor-free space.

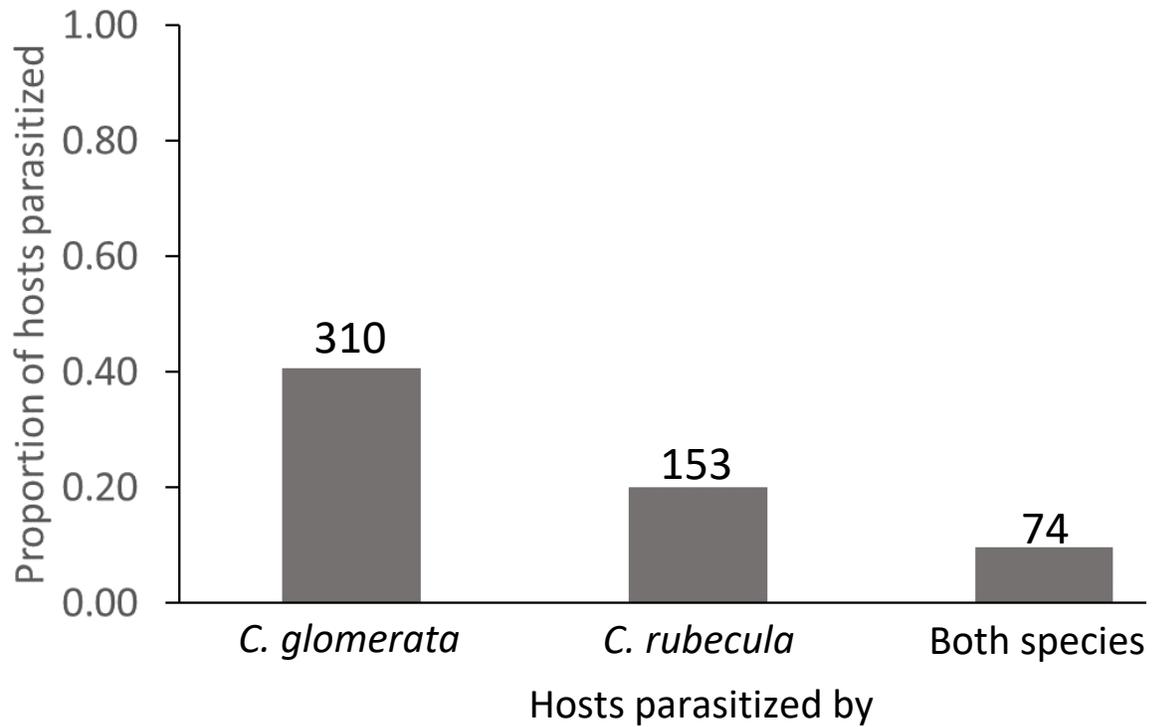


Figure 4.1. The proportion of field collected *Pieris rapae* (n=751) that were parasitized by *Cotesia glomerata*, *C. rubecula*, and by both *Cotesia* species (i.e., multiparasitized) in MD. The values above the bars are the number of *P. rapae* parasitized for each parasitism category. A binomial test indicated that the observed probability of multiparasitized hosts was above the expected value from wasps foraging randomly (0.08 [95%CI: 0.07, 0.12]).

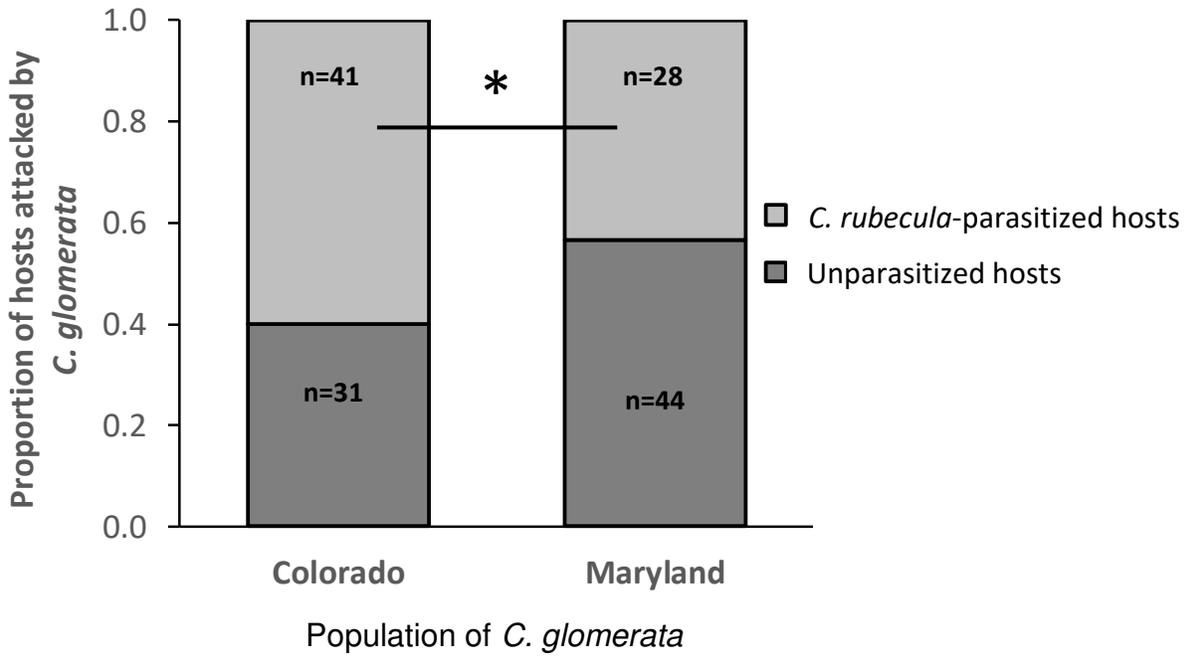


Figure 4.2. The proportion of unparasitized hosts and hosts parasitized by *Cotesia rubecula* that were attacked by Colorado (CO) and Maryland (MD) *C. glomerata* in a choice test (N = 72 females for each population). The numbers inside the graphs indicate how many females attacked each type of host. The asterisk indicates significant differences between the CO and MD *C. glomerata* when attacking each type of host.

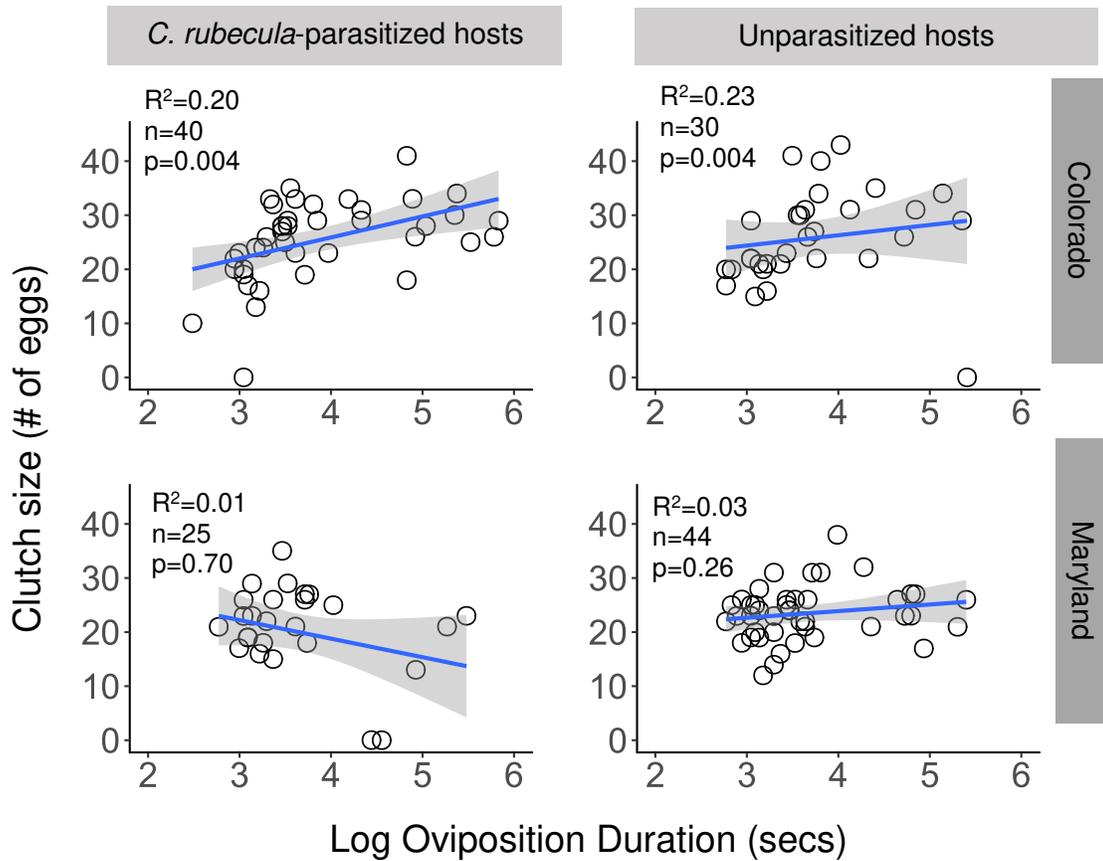


Figure 4.3. The relationship between the log of oviposition duration and the number of *Cotesia glomerata* eggs observed in unparasitized and *C. rubecula*-parasitized hosts attacked by Colorado and Maryland *C. glomerata*. The shaded area around the line is the 95% confidence interval.

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CHAPTER 5: Differential hyperparasitism of two competing parasitoids

5.1 SYNOPSIS

Hyperparasitoids are frequently implicated in the failure of biological control agents to establish and provide control of insect pests. Classical biological control programs introduce primary parasitoids to new geographic regions, often exposing them to existing hyperparasitoids. A reliable assessment of the hyperparasitoid community is needed to understand how top-down trophic interactions influence the effectiveness of introduced parasitoids. We examined the diversity of hyperparasitoids attacking *Cotesia glomerata* (Linnaeus) (Hymenoptera: Braconidae) in Colorado (USA), where the congener *C. rubecula* is a proposed biological control agent for the imported cabbageworm (*Pieris rapae*) and compared this with the hyperparasitoids of *C. glomerata* from Maryland (USA) where both wasps co-occur. Field collected *C. glomerata* broods were analyzed to determine how brood sizes and adult sex ratios were affected by the different hyperparasitoid species. A total of nine hyperparasitoid species were found in Colorado, of which four species occur in the eastern US. Larger *C. glomerata* broods increased the odds of hyperparasitism, but had higher per capita survivorship than smaller broods. The proportion of *C. glomerata* males showed a positive correlation with brood size for both unparasitized and hyperparasitized broods, suggesting that female *C. glomerata* were not preferentially parasitized. Hyperparasitoids inflicted greater mortality on *C. rubecula* than on *C. glomerata*, and this differential impact may enable the coexistence of *C. glomerata* with its competitively dominant congener *C. rubecula*, which usually outcompetes and displaces *C. glomerata*. The establishment of *C. rubecula* in Colorado could be hindered by the preexisting populations of hyperparasitoids that attack *C. glomerata*.

5.2 INTRODUCTION

A seminal paper by Price et al. (1980) argued that to better understand the dynamics of plant-herbivore interactions, it is imperative to include consideration of natural enemies of herbivores, such as predators and parasitoids, in the third trophic level. Similarly, consideration of the role of predators and hyperparasitoids in the fourth trophic level is necessary to fully understand interactions between herbivores and their natural enemies (Sullivan and Völkl 1999). Hyperparasitoids are a significant mortality factor for many parasitoids and have been implicated in the failure of several biological control introductions to establish and to provide adequate control (Sullivan 1987; Stiling 1993; Sullivan and Völkl 1999; Chacón et al. 2008; Schooler et al. 2011; Frago et al. 2012; Nofemala 2013; Kaser and Ode 2016).

There are two ways in which hyperparasitoids can influence the communities inhabited by parasitoids. First, by reducing parasitoid populations, they provide enemy-release for herbivores that can have cascading, negative effects on plant fitness through increased herbivory (Rosenheim 1998; Brodeur and McNeil 1992; Schooler et al. 2011). Secondly, hyperparasitoids can affect the outcome of apparent competition between parasitoids (van Nouhuys and Hanski 2000; Morris et al. 2001; Acebes and Messing 2013; Nofemala 2013; Kaser and Ode 2016). Furthermore, at an individual level, hyperparasitoids can influence foraging behavior and allocation decisions of adult parasitoids. Parasitoids may minimize residence times at patches containing hosts, thereby reducing mortality risks associated with predation and hyperparasitism (Mackauer and Völkl 1993; Petersen et al. 2000). Since female parasitoids are often larger than males, thus offer more resources as a host for hyperparasitoids, differential hyperparasitism of female parasitoids can result in more male-biased offspring sex ratios at adult emergence compared to the sex ratio at oviposition (e.g., Chow and Mackauer 1996; Heinz, 1996). Given

the influence that hyperparasitoids have on parasitoid population dynamics, as well as foraging behavior, characterizing the diversity and abundance of hyperparasitoids is important for understanding the success of parasitoids in regulating their host populations. Yet, despite their importance in structuring communities and in the implementation of biological control programs, in too few cases do we understand the consequences of hyperparasitoid communities that attack primary parasitoids.

Cotesia glomerata (Linnaeus) (Hymenoptera: Braconidae) and *C. rubecula* (Marshall) are parasitoid wasps that originate in Europe where their co-existence in shared habitats is mediated largely by their use of different host species. While *C. glomerata* primarily attacks *Pieris brassicae* (L.) (Lepidoptera: Pieridae) and occasionally attacks *P. rapae* (Linnaeus) (Laing and Levin 1982; Feltwell 1982; Ohsaki and Sato 1990), *C. rubecula* is a specialist on *P. rapae* (L.) (Geervliet et al. 2000). Both *Cotesia* species are koinobiont parasitoids, but *C. glomerata* is a gregarious while *C. rubecula* is solitary. As a specialist, *C. rubecula* is considered a more effective biological control agent of *P. rapae* (Puttler et al. 1970; Parker et al. 1972), but its establishment and success may be reduced because of its vulnerability to the hyperparasitoids that also attack *C. glomerata* (McDonald and Kok 1991; McDonald and Kok 1992; Gaines and Kok 1999). Whether differences in hyperparasitoid assemblages, associated with different *C. glomerata* populations, mediate host use patterns and interactions with competitors such as *C. rubecula* remain open questions.

We explored the impact of hyperparasitoids on *C. glomerata* and its stronger competitor, *C. rubecula*, in a region where they coexist (Maryland, USA [MD]) and in a habitat where *C. glomerata* has yet to experience *C. rubecula* (Colorado, USA [CO]). Our objectives included comparing the likelihood of hyperparasitism of *C. glomerata* in CO and in MD to the likelihood

of hyperparasitism *C. rubecula* populations in MD where both species compete for *P. rapae* hosts. We show that *C. rubecula* experiences higher rates of hyperparasitism, which may allow coexistence of the two *Cotesia* species in MD and, perhaps, impair establishment of *C. rubecula* in areas yet to be colonized by the latter species. We also examined the relationship between hyperparasitism and *C. glomerata* brood size and sex ratio. As a gregarious species, per capita rates of *C. glomerata* mortality due to hyperparasitism should be lower in larger broods because of a dilution effect – a larger brood will increase the chances that any given *C. glomerata* larva will escape hyperparasitism. Furthermore, if female *C. glomerata* larvae (which are larger than males; Gols et al. 2009) are more likely to be hyperparasitized, we expect that brood sex ratios of emerging *C. glomerata* will be more male-biased in populations experiencing higher rates of hyperparasitism. Lastly, we assessed the abundance of the hyperparasitoid community in relation to the abundance of the gregarious *Cotesia glomerata* in northern CO, as well as documented the overall diversity and abundance of hyperparasitoids attacked these *Cotesia* species in CO and MD.

5.3 METHODS

5.3.1 Study System

In North America, *C. glomerata* and *C. rubecula* have been introduced as biological control agents of *P. rapae*, an important pest of many *Brassica* crops (Wilkinson 1966; Clausen 1978; McDonald and Kok 1992; van Driesche and Nunn 2002; Wold-Burkness et al. 2005). *Pieris brassicae*, the preferred host of *C. glomerata*, is absent in North America where both *Cotesia* species use *P. rapae* as the primary host. When *C. glomerata* and *C. rubecula* attack the same host individual (multiparasitism), *C. rubecula* invariably wins competition by killing the

eggs or larvae of *C. glomerata* (Laing and Corrigan 1987; Geervliet et al. 2000). In many regions of North America, *C. rubecula* has largely outcompeted *C. glomerata*, resulting in the extirpation of *C. glomerata* (Herlihy et al. 2012); although, some evidence suggests that *C. glomerata* may persist by switching to pierids such as the mustard white *P. napi oleracea* (Benson et al. 2003) or the checkered white *Pontia protodice* (Boisduval & LeConte) (D.K. Vyas and R. Paul pers. obs.). There remain parts of North America (e.g., CO) where *C. glomerata* occurs without *C. rubecula* and a few areas where the two species coexist (e.g., MD). Populations of *C. glomerata* that co-occur with *C. rubecula* (e.g., in MD) exhibit strong avoidance behaviors of hosts previously parasitized by *C. rubecula* (Vyas et al. in review).

In the Netherlands, *C. glomerata* is parasitized by at least 11 species of hyperparasitoids (Laing and Levin 1982; Harvey et al. 2014; Poelman et al. 2012). North American populations of *C. glomerata* are attacked by a largely different community of hyperparasitoids; of the six hyperparasitoid species emerging from *C. glomerata* cocoons collected in Virginia (USA), only *Baryscapus* (= *Tetrastichus*) *galactopus* (Ratzeburg) (Hymenoptera: Eulophidae) has been documented to parasitize *C. glomerata* in Europe (Gaines and Kok 1999). Nevertheless, little is known about the diversity of hyperparasitoids that attack different *C. glomerata* populations across North America, especially with regards to whether *C. rubecula* is also present.

5.3.2 Field sites

Field samples from Colorado (CO) were collected from June to October 2015 from cultivated *Brassica oleracea* crops grown at six vegetable farms (Table A.1 in Appendix A). Each farm was surveyed to ensure the absence of insecticides that harm *P. rapae* and associated parasitoids. Crops were planted before June 2015 at all farms. Planting of cultivars was

determined by the owner of each farm, except at Colorado State University's Agriculture Research Development and Education Center (ARDEC) North and ARDEC South where crops were planted by D.V, R.P and P.J.O. At the commercial farms, plants were grown in rows with a range of 15 to 50 plants per row. Plants were grown in 15 rows at ARDEC North (ca. 60 plants per row) and 10 rows at ARDEC South (ca. 70 plants per row) with 0.30 m spacing between plants and 0.90 m between rows.

In MD, *C. glomerata* and *C. rubecula* pupae were collected from cultivated varieties of *B. oleracea* on seven vegetable farms in 2016 (Table A.2 in Appendix A). Field collection of *Cotesia* pupae was performed using similar methods as described above for CO, however *P. rapae* were not sampled in the same manner as in CO because we did not have access to facilities for rearing live insect specimens in MD.

5.3.3 Data collection

Sampling commenced on June 30, 2015 and ended on October 28, 2015 in Colorado with each site visited every two weeks. Field sampling in MD occurred between August 15-20, 2016, May 24-September 23, 2017, and July 6-9, 2018. Approximately 3-5 crop varieties (Tables A.1-A.2 in Appendix A) were sampled each visit and a different row of plants was randomly selected to avoid re-sampling the same plants in subsequent weeks. At each farm, individual leaves of 20-50 plants per variety were searched for *P. rapae* larvae and *Cotesia* pupae. Within a row, every third plant was searched for the presence of *C. glomerata* broods (and *C. rubecula* broods in the MD samples) that had emerged from their hosts. As each *C. glomerata* or *C. rubecula* larva finishes feeding and emerges from its host, it forms a silken cocoon. Only a single cocoon is made by the solitary *C. rubecula* larva, but in the case of *C. glomerata*, a cluster of individual

cocoons from the same host was considered a brood. Sampling concluded for the season when plants were harvested by the owners, when plants became too large to effectively detect *C. rubecula* cocoons or *C. glomerata* broods, or after the onset of freezing temperatures. *Cotesia glomerata* broods and *C. rubecula* cocoons were collected and brought back to the laboratory to rear out any hyperparasitoids.

Each *C. glomerata* brood or *C. rubecula* cocoon was kept individually in 37 ml plastic cups and placed in an environmental chamber at 25°C and 16L:8D photoperiod. *Cotesia glomerata* broods or *C. rubecula* cocoons were checked daily for the emergence of adult parasitoids and any hyperparasitoids. *Cotesia glomerata* broods and *C. rubecula* cocoons were kept in the environmental chamber for at least five months, a period sufficient to allow hyperparasitoids to complete development and emerge as adults. After this period, the contents of each brood or cocoon was analyzed under a stereomicroscope for the number of individual cocoons per brood, sex ratio (proportion male) of emerged and unemerged parasitoids, identity and number of emerged and unemerged adult hyperparasitoids. Unemerged adult *C. glomerata* and *C. rubecula* were included in the overall sex ratio if their sex could be distinguished.

At times, *C. rubecula* cocoons and *C. glomerata* broods were collected because they appeared intact in the field, but inspections under the microscope showed that they were empty. We distinguished whether the empty cocoons contained a *Cotesia* wasp or a hyperparasitoid based on the nature of the exit hole(s) on the cocoon. When *C. glomerata* or *C. rubecula* exit their cocoon, they always make a straight-lined hole at the cocoon's terminal end (D.K. Vyas, J.A. Harvey and R. Paul pers. obs.). In contrast, hyperparasitoids usually exit the cocoon through a smaller jagged-edged hole on the lateral sides of the cocoon. Species identification was not attempted for empty hyperparasitized cocoons.

Unemerged immature hyperparasitoids (both larvae and pupae) could not be reliably identified to species, but could be distinguished from unemerged immature *C. glomerata* or *C. rubecula* based on the hyperparasitoids' sizes and number of larvae per cocoon in a brood. Unemerged hyperparasitoid larvae were at least half the size and were generally found to be alive, whereas unemerged *C. glomerata* or *C. rubecula* larvae were generally dead and desiccated. In the case of *C. glomerata*, each larva spins a single cocoon, so when two or more larvae or pupae are found in an individual cocoon, this indicated that the cocoon was hyperparasitized. Only adult hyperparasitoids were identified to the species.

5.3.4 Data Analyses

Hyperparasitoid data were analyzed as presence-absence of hyperparasitoids in a brood, the number of adult hyperparasitoids found, and the number of cocoons parasitized out of the total number of *C. glomerata* cocoons in a brood. As described above, hyperparasitoids create distinct exit holes that are easily distinguished from the holes made by *C. glomerata* during eclosion (D.K. Vyas pers. obs.), thus allowing calculation of the proportion of a *C. glomerata* brood that was parasitized. A brood was scored as hyperparasitized if adult or immature hyperparasitoids were observed or if cocoons displayed exit holes indicative of hyperparasitoids. *Cotesia glomerata* brood size was calculated as the number of cocoons per brood. Proportions of male *C. glomerata* in a brood (i.e., sex ratios) and proportion of adult *C. glomerata* emerged were treated as binomial counts. *Cotesia glomerata* brood size and the proportion of emerged *C. glomerata* adults were compared between unparasitized and hyperparasitized *C. glomerata* broods using t-tests for brood size and logistic regression for proportion emerged. The relationship between *C. glomerata* brood size and sex ratio, as well as between brood size and

likelihood of hyperparasitism were examined with logistic regression (PROC LOGISTIC; SAS Institute Inc., Cary, NC). We found unequal variances when comparing the mean number of *B. galactopus* adults found in MD *C. glomerata* and MD *C. rubecula*, therefore a Welch's t-test was used for this analysis. Unless noted otherwise, all means are reported with standard errors and parameter estimates are reported with 95% confidence intervals. For all statistical analyses, the level of significance was set at $p=0.05$. Analyses were performed in JMP[®] PRO, version 12 (SAS Institute Inc., Cary, NC) or SAS[™] Studio, version 3.5 (SAS Institute Inc., Cary, NC).

5.4 RESULTS

5.4.1 Mortality from hyperparasitoid attacks

When hyperparasitoids attacked *C. rubecula* and *C. glomerata* (from CO and MD), the mortality was more severe for *C. rubecula*, likely because its cocoons develop alone and not in a brood as does *C. glomerata*. Hyperparasitoids rarely attacked the entire *C. glomerata* brood, with only 7% (10/144) of MD *C. glomerata* and 1% (4/278) of CO *C. glomerata* broods experiencing 100% mortality, whereas 66% (176/266) of *C. rubecula* cocoons died from hyperparasitoid attacks. Although 48.9% (136/278) of CO *C. glomerata* broods and 68% (98/144) of MD *C. glomerata* broods were attacked by hyperparasitoids, the majority of cocoons within these broods escaped hyperparasitism (59% [1444/2459] for CO *C. glomerata*; 72% [2607/3635] for MD *C. glomerata*). The odds of a MD *C. rubecula* cocoon being hyperparasitized were 2.78 (95% CI 2.13, 3.63) times greater than that of a CO *C. glomerata* cocoon (log likelihood $\chi^2=60.25$, d.f.=1, $p<0.001$) and 4.96 (95% CI: 3.81, 6.46) times greater than that of a MD *C. glomerata* cocoon (log likelihood $\chi^2=151.35$, d.f.=1, $p<0.001$). Among *C. glomerata*, the odds of a MD brood being hyperparasitized were 2.26 (95% CI: 1.47, 3.44) times

greater than that of a CO brood (log likelihood $\chi^2=14.84$, d.f.=1, $p<0.001$), but the cocoons in a MD brood were half as likely (odds = 0.56; 95% CI 0.50, 0.62) to be attacked as were conspecifics in CO (log likelihood $\chi^2=110.34$, d.f.=1, $p<0.001$).

5.4.2 Hyperparasitism and brood size of *C. glomerata*

Across all hyperparasitoid species that attacked CO *C. glomerata*, larger broods were more likely to be attacked with the odds of hyperparasitism increasing by 3% (95% CI 1%, 5%) with each additional cocoon in a brood (Fig. 5.1A). While larger brood sizes increased the odds of an attack, they reduced per capita hyperparasitism since the proportion of cocoons that were hyperparasitized decreased with each additional cocoon, but the relationship was only slightly above statistical significance (Fig. 5.2A). These relationships between brood size and hyperparasitism may be regional since MD *C. glomerata* failed to show an increase in hyperparasitoid attacks with larger brood size (Fig. 5.1B). As with CO *C. glomerata*, there was a no relationship between brood size and the probability of hyperparasitism for MD *C. glomerata* (Fig. 5.2B).

5.4.3 Hyperparasitism and sex ratio of *C. glomerata*

Hyperparasitoids unlikely influenced *C. glomerata* brood sex ratios as both unparasitized and hyperparasitized broods had a similar proportion of male *C. glomerata* emerging in CO (proportion of males for hyperparasitized vs unparasitized broods: 0.43 ± 0.03 vs. 0.37 ± 0.02) (log likelihood $\chi^2=0.12$, $p=0.73$, d.f.=1) and in MD (0.38 ± 0.05 vs. 0.49 ± 0.06) (log likelihood $\chi^2=0.74$, $p=0.39$, d.f.=1). For CO *C. glomerata*, increasing brood size, rather than the likelihood of hyperparasitism, was an important predictor of male-biased sex ratios. With every additional

C. glomerata cocoon in a brood, male-bias increased by 6% (95% CI: 2%,9%) for unparasitized broods and by 3% (95% CI: 0.01%, 6%) for hyperparasitized broods (Fig. 5.3A-B); but, there was no difference in the increase in male-bias between unparasitized and hyperparasitized broods ($\chi^2= 0.18$, d.f.=1, p=0.67). MD *C. glomerata* failed to show a similar increase in sex ratio with brood size for either unparasitized or hyperparasitized broods (Fig. 5.3C-D).

5.4.4 Hyperparasitoid communities attacking *C. glomerata* (in CO and MD) and *C. rubecula*

Across the six CO field sites and between June and October 2015, we collected a total of 605 *C. glomerata* broods from 2307 *B. oleracea* plants. The majority of *C. glomerata* broods were collected at ARDEC North (39.83%) and ARDEC South (45.95%) with 85% of all broods collected between August and September (Table 5.1). Of the 605 broods, 328 were excluded from the analyses because they were found to be empty (e.g., all parasitoids had already eclosed) during field sampling, lacked data on the number of cocoons, or *Cotesia* wasps were absent (e.g., escaped from container). The remaining 277 broods were analyzed for hyperparasitism and 136 (49.09%) of these broods were hyperparasitized. Adult hyperparasitoids emerged from 75% (101/136) of the total number of hyperparasitized broods (Table 5.1), whereas the remaining 35 broods contained unemerged adult hyperparasitoids (n=12) or immature hyperparasitoids (n=23) that were found during dissections. Hyperparasitism of CO *C. glomerata* was detected from July to October, but most of the CO *C. glomerata* broods that were analyzed for hyperparasitism were collected in August (49.28%) and September (42.39%) (Table 5.1).

As the abundance of *P. rapae* increased per plant, so did the odds that any one *P. rapae* caterpillar was parasitized by CO *C. glomerata*; every additional caterpillar resulting in a 6% (95% CI: 4%, 8%) increase in the likelihood that a *P. rapae* was parasitized (log likelihood

$\chi^2=23.6$ d.f.=1 $p<0.001$) (Fig. 5.4). *Cotesia glomerata* brood sizes ranged between 5 and 75 cocoons per brood, and the number of cocoons in a brood was independent of the month when the brood was collected ($r^2=0.002$, $p=0.42$). Interestingly, the probability that a CO *C. glomerata* brood was hyperparasitized increased with the percentage of *P. rapae* caterpillars per plant that were parasitized by *C. glomerata* (log likelihood $\chi^2= 35.80$, d.f.=1, $p<0.001$) (Fig. 5.5). For every additional *P. rapae* parasitized by *C. glomerata*, there was a 4% (3%, 5%) increase in the likelihood of hyperparasitism.

A total of nine hyperparasitoid species were identified (Table 5.1) from CO *C. glomerata* broods with 89% (121/136) of the samples dominated by three species: *Baryscapus galactopus* (Ratzeburg) (Hymenoptera: Eulophidae), *Catolaccus aeneoviridis* (Girault) (Hymenoptera: Pteromalidae) and *Trichomalopsis dubia* (Ashmead) (Hymenoptera: Pteromalidae). All three of these hyperparasitoids were found from a similar proportion of CO *C. glomerata* broods, but *B. galactopus* was the most abundant. In contrast to the nine hyperparasitoid species found attacking *C. glomerata* broods in CO, MD *C. glomerata* broods were attacked by four hyperparasitoid species (Table 5.1). As with CO *C. glomerata*, MD *C. glomerata* were mainly attacked by *B. galactopus* (n=83) (Table 1). *Conura torvina* (Cresson) (Hymenoptera: Chalcididae) was found in more MD broods than in CO broods (Appendix B), whereas *C. aeneoviridis* was less common in MD *C. glomerata*.

Hyperparasitoids attacked 176 of the 266 *C. rubecula* cocoons (Table 5.1), but 10 of these cocoons lacked the adult hyperparasitoids preventing species identification. As with *C. glomerata*, the most common hyperparasitoid of *C. rubecula* was *B. galactopus* (n=119), followed by *C. torvina* (n=45), which was more common in *C. rubecula* cocoons compared to *C. glomerata* broods from either MD or CO (Table 5.1). *Catolaccus aeneoviridis* was found from

only two *C. rubecula* cocoons and neither of the unknown hyperparasitoids seen attacking *C. glomerata* in CO and MD were observed in *C. rubecula* cocoons. When hyperparasitoids emerged from *C. rubecula* cocoons, it was always one individual per cocoon, with the exception of *B. galactopus*. However, the multiple cocoons of *C. glomerata* broods made the brood susceptible to attacks from more than one hyperparasitoid species. Indeed, we found that 24.2% (33/136) of CO *C. glomerata* broods and 15.3% (15/98) of MD *C. glomerata* broods had more than one species of hyperparasitoid (Tables A.3-A.4 in Appendix A).

Hyperparasitism inflicted an obvious fitness costs for *C. glomerata*, which experienced a reduction in the emergence of adults from hyperparasitized broods. The odds of adult CO *C. glomerata* emerging from broods were 1.44 (95% CI 1.10, 1.92) times higher in unparasitized cocoons (log likelihood $\chi^2=6.86$, $p=0.01$, d.f.=1), even though unparasitized broods were smaller on average (unparasitized broods $n=141$: 26.6 ± 0.9 ; hyperparasitized broods $n=121$: 31.5 ± 1.3) ($t_{260}=3.24$, $p=0.001$). MD *C. glomerata* also had higher success emerging from unparasitized broods with the odds being nearly twice (odds=1.95, 95% CI 1.33, 2.92) as high as in hyperparasitized broods (log likelihood $\chi^2=12.33$, $p<.001$, d.f.=1), however average brood sizes were similar between unparasitized ($n=46$, 24.52 ± 1.94) and hyperparasitized broods ($n=98$, 25.58 ± 1.33) ($t_{142}=0.45$, $p=0.65$).

5.5 DISCUSSION

Cotesia rubecula has been introduced into several areas of North America as a biological control agent for *P. rapae* (Puttler et al. 1970). While both *Cotesia* species parasitize *P. rapae* in North America, *C. rubecula* is usually the dominant competitor and has displaced *C. glomerata* throughout northern latitudes of North America (Herlihy et al. 2012). However, *C. rubecula* is

uncommon below a latitude of 40° N. While this geographical limit has been partially attributed to asynchronous diapause cycles between *C. rubecula* and *P. rapae* (Nealis, 1985), differential attack of *C. rubecula* by hyperparasitoids has also been proposed as an explanation (McDonald and Kok 1991; Gaines and Kok 1998).

Our results support the hypothesis that differential hyperparasitism permits the coexistence of *C. glomerata* and *C. rubecula*. While the hyperparasitoid community in MD is equally likely to attack *C. rubecula* and *C. glomerata* broods, these hyperparasitoids inflicted higher mortality on *C. rubecula* because its single offspring dies from hyperparasitoid attacks, whereas the gregarious brood of *C. glomerata* ensures some cocoons survive even if siblings become parasitized. Therefore, developing in a group reduces the per capita likelihood of attack from a hyperparasitoid. In support of this per capita dilution effect, brood size plays a significant role in influencing the likelihood of survivorship of individual *C. glomerata*. The per capita risk of hyperparasitism significantly decreases in larger broods. Indeed, gregariousness as a life-history trait may have evolved to decrease the mortality from natural enemies (Ode and Rosenheim 1998; Mayhew 1998; Pexton and Mayhew 2004). Additionally, that *C. rubecula* individuals are larger than individual *C. glomerata* may also contribute to the apparent preference of hyperparasitoids for *C. rubecula*. Furthermore, given that both *Cotesia* species largely share the same hyperparasitoid community, *C. glomerata* is potentially a reservoir for hyperparasitoids that can in turn attack *C. rubecula*, possibly preventing competitive exclusion of *C. glomerata* by *C. rubecula*. Taken together, this asymmetry in the effects of hyperparasitoids may help *C. glomerata* coexist with *C. rubecula* in locations such as MD and may even prevent the establishment of *C. rubecula* in other regions.

Hyperparasitoids are known to play a role in apparent competition within other parasitoid communities that share the same host species. For instance, introductions of *C. glomerata* in Finland led to increases in the density of the hyperparasitoid *Gelis agilis* (Fabricius) (Hymenoptera: Ichneumonidae), which attacks both *C. glomerata* and its congener *C. melitaearum* (Wilkinson) (Hymenoptera: Braconidae), a specialist of the Glanville fritillary butterfly (*Melitaea cinxia* (L.)) (Lepidoptera: Nymphalidae) (van Nouhuys and Hanski 2000). *Cotesia melitaearum* began to decline following the introduction of *C. glomerata*, presumably because *C. melitaearum* suffered differential hyperparasitism by *G. agilis*. In South Africa, the presence of hyperparasitoids may have played a role in the apparent competition between *Cotesia vestalis* (Haliday), the dominant parasitoid of diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and two other primary parasitoids. As hyperparasitism of *C. vestalis* increased, the population of *C. vestalis* declined and populations of the other two primary parasitoids, *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae), increased (Nofemela 2013). These studies demonstrate the impact hyperparasitoids can have in influencing the structure of parasitoid communities.

While significantly different from Europe (e.g., Harvey et al. 2014), the hyperparasitoid fauna attacking *C. glomerata* and *C. rubecula* are broadly similar across North America. Similar to other surveys of hyperparasitoids elsewhere in North America (McDonald and Kok 1991; Weis et al. 2016), we showed that *B. galactopus* was the most abundant hyperparasitoid of *C. glomerata* and *C. rubecula* in both CO and MD. While *B. galactopus* is the one hyperparasitoid that occurs in both Europe and North America, it plays a minor role compared to *Lysibia nana* (Gravenhorst) (Hymenoptera: Ichneumonidae) in Europe (Poelman et al. 2012). While *B.*

galactopus appears ubiquitous, regional differences in the other hyperparasitoid species could affect the outcome of interactions between *C. glomerata* and *C. rubecula*, possibly explaining the patchwork nature of *C. rubecula* – *C. glomerata* interactions including apparent competition (*C. glomerata* coexisting with *C. rubecula*), competitive exclusion (elimination of *C. glomerata* by *C. rubecula*), and regions where *C. rubecula* has yet to establish.

We conclude that by impacting *C. rubecula* more negatively than *C. glomerata*, hyperparasitoids can affect the outcome of competition between these two parasitoids. Larger brood sizes of *C. glomerata* lowered the risk of mortality from hyperparasitism, whereas the single *C. rubecula* cocoons were more vulnerable. Differences in the hyperparasitoid communities between the ancestral and introduced ranges may limit the effectiveness of the introduced parasitoid in reducing the pest host population. While some biological control programs are apparently little affected by hyperparasitoids, (Agricola and Fischer 1991; Herren and Neuenschwander 1991; Neuenschwander and Hammond 1988), other control efforts are disrupted by the mortality inflicted by hyperparasitoids (Rosenheim 1998; Frago et al. 2012; Gómez-Marco et al. 2015). Given that parasitoid survivorship can be impacted by hyperparasitoids, successful biological control initiatives must consider this guild of wasps before implementing parasitoids as a management strategy.

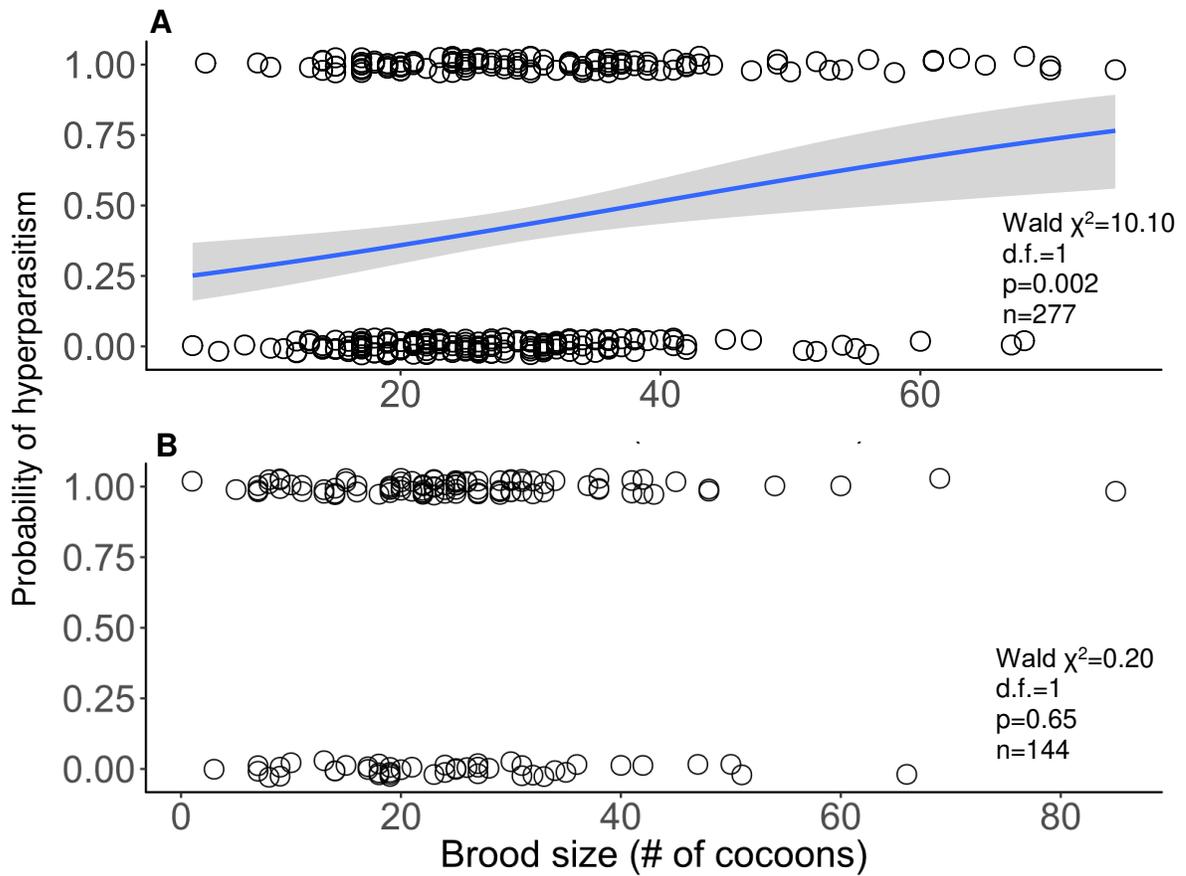


Figure 5.1. Probability that broods of *Cotesia glomerata* from Colorado (A) and from Maryland (B) would be hyperparasitized (0 = unparasitized; 1 = hyperparasitized) as a function of *C. glomerata* brood size; these data are across all hyperparasitoid species. Probability values are jittered to reduce overlap in data points. The shaded area around the regression line is the 95% confidence interval.

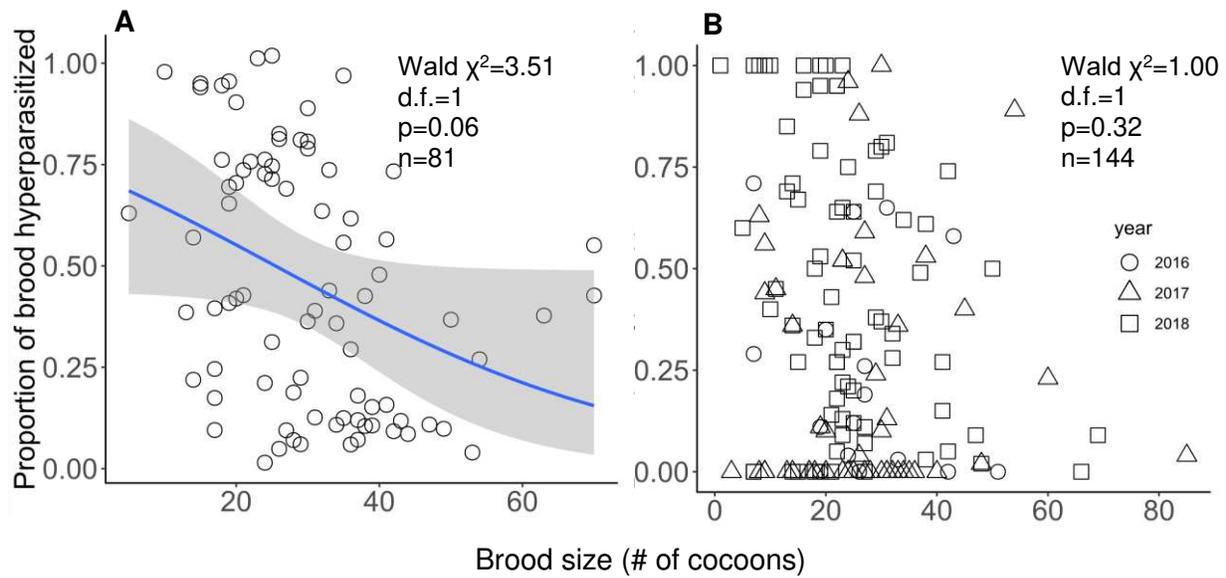


Figure 5.2. The proportion of a *C. glomerata* brood that was hyperparasitized as a function of brood size. The negative relationship between brood size and the proportion of *Cotesia glomerata* cocoons that were hyperparasitized in 2015 from Colorado (A) and across three years in Maryland (B); thus, suggesting that per capita survivorship may be higher in larger broods. Year of data collection was not significant for Maryland broods ($\chi^2=3.50$, d.f.=1, $p=0.06$). Proportion of a brood that was hyperparasitized was treated as binomial count data (number of cocoons hyperparasitized out of the total number of cocoons in the brood) and was modeled with a logistic regression. Data points (open shapes) are jittered in order to view the number of data points with the same values. The shaded area around the regression line is the 95% confidence interval.

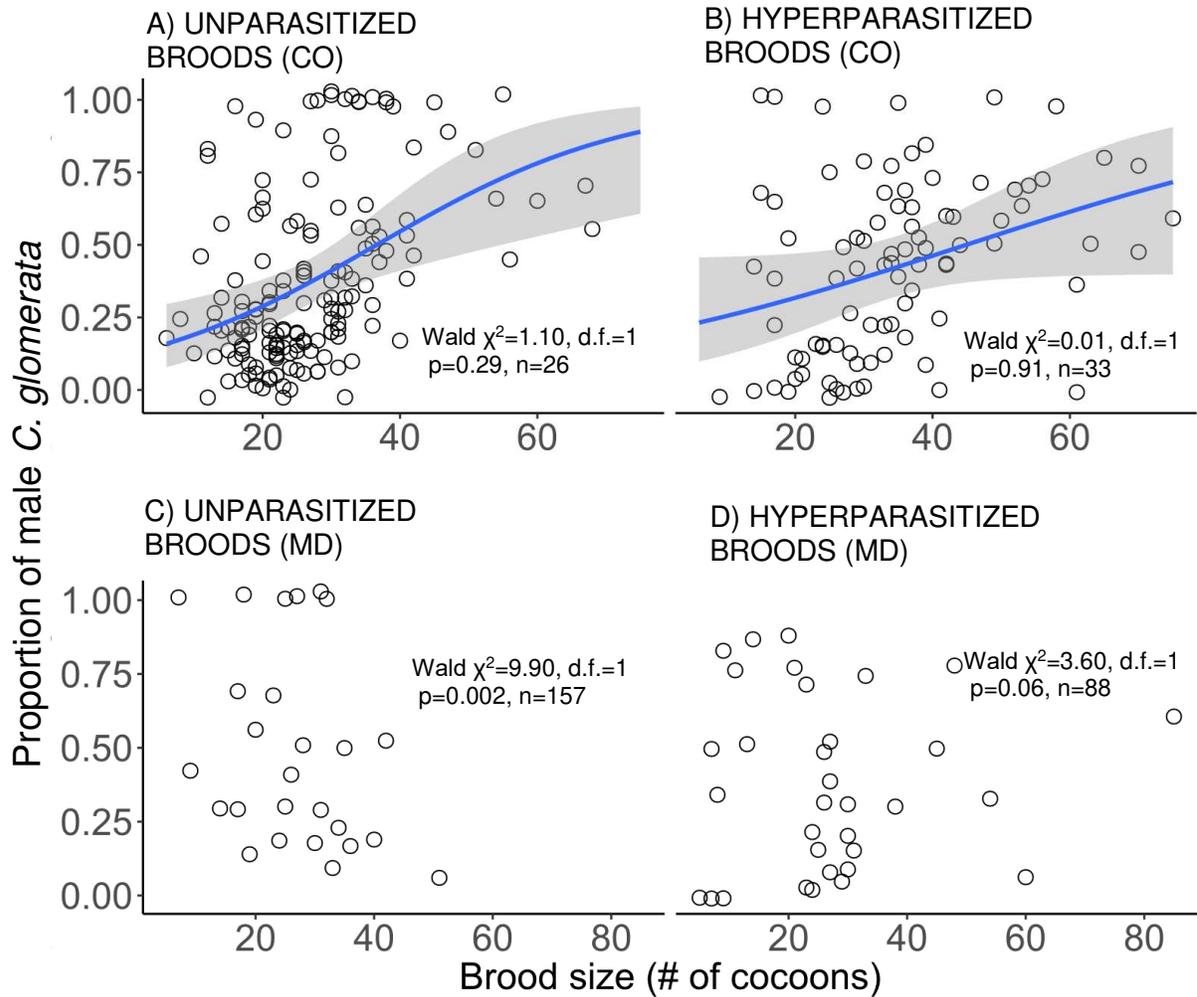


Figure 5.3. Proportion of male *Cotesia glomerata* as a function of brood size in unparasitized *C. glomerata* broods from A) Colorado (CO) and C) Maryland (MD), and in hyperparasitized broods from B) CO and D) MD. Proportion male data were modeled as binomial counts (number of males out of the total number of adults) with logistic regression. Data points (open circles) are jittered in order to view the number of data points with the same values. The shaded area around the regression line is the 95% confidence interval.

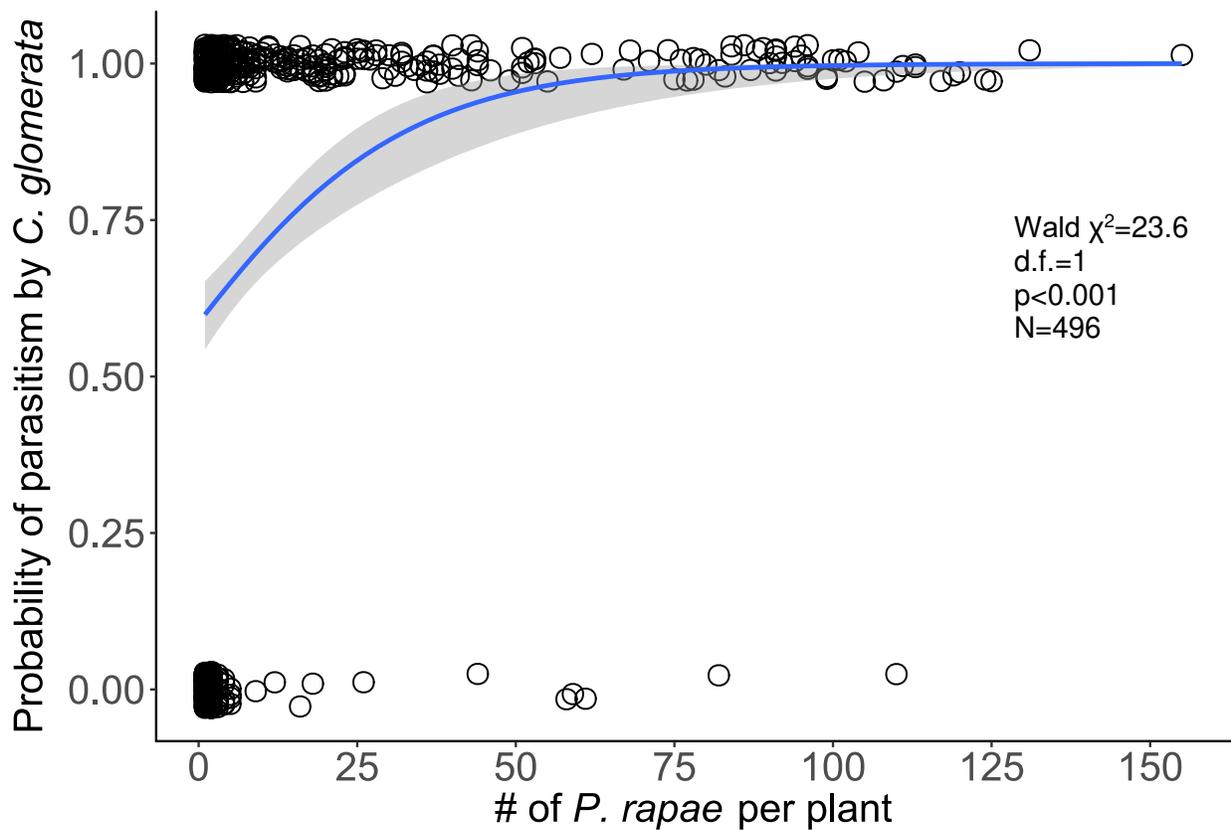


Figure 5.4. The probability (0 = unparasitized; 1 = parasitized) that any given *Pieris rapae* would be parasitized by *Cotesia glomerata* as a function of the number of *P. rapae* per plant. Data points (open circles) are jittered in order to view the number of data points with the same values. The shaded area around the regression line is the 95% confidence interval.

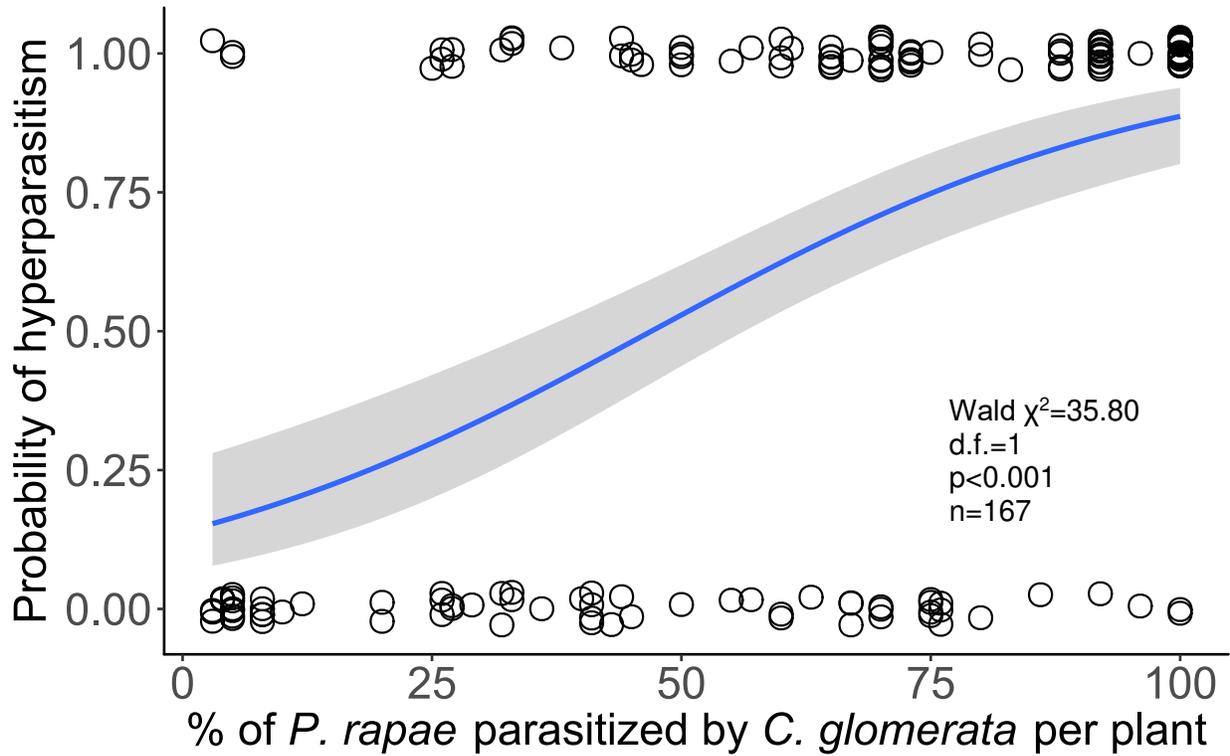


Figure 5.5. The relationship between the probability that a *Cotesia glomerata* brood in Colorado was hyperparasitized by at least one hyperparasitoid (0 = unparasitized; 1 = hyperparasitized) and the percent of *Pieris rapae* per plant that were parasitized by *C. glomerata*. Probability values are jittered to reduce overlap in data points. The shaded area around the regression line is the 95% confidence interval.

Table 5.1. The hyperparasitoid species that emerged from *Cotesia glomerata* broods (n=278) collected in Colorado from June-October 2015 and from *C. glomerata* broods (n=144) and *C. rubecula* cocoons (n=266) collected in Maryland from August 2016, May-September 2017 and July 2018.

Hyperparasitoid species	Family	Subfamily	# of Cg broods & Cr cocoons with hyperparasitoid species			Number of emerged individuals		
			CO Cg	MD Cg	MD Cr	CO Cg	MD Cg	MD Cr
<i>Baryscapus galactopus</i>	Eulophidae	Tetrastichinae	42	83	119	874	2188	1366
<i>Trichomalopsis dubia</i>	Pteromalidae	Pteromalinae	39	0	0	372	0	0
<i>Catolaccus aeneoviridis</i>	Pteromalidae	Pteromalinae	40	6	2	264	49	2
<i>Conura torvina</i>	Chalcididae	Chalcidinae	2	17	45	23	34	45
<i>Hypopteromalus tabacum</i>	Pteromalidae	Pteromalinae	3	0	0	21	0	0
<i>Dibrachys cavus</i>	Pteromalidae	Pteromalinae	1	0	0	36	0	0
<i>Gelis</i> sp.	Ichneumonidae	Cryptinae	1	0	0	1	0	0
Unknown	Encyrtidae	n/a	1	0	0	1	0	0
Unknown ^b	Pteromalidae	n/a	7	1	0	83	13	0

^aAdult hyperparasitoids that were dissected out of individual cocoons

^bSamples were unavailable for identification, but were suspected to be either *T. dubia* or *H. tabacum*
CO Cg = Colorado *C. glomerata*, MD Cg = Maryland *C. glomerata*, MD Cr = Maryland *C. rubecula*

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CHAPTER 6: Final Discussion

The main goal of this study was to investigate the proximate mechanisms that explain the outcome of competition between *C. glomerata* and its stronger competitor, *C. rubecula*. I compared the foraging strategies of *C. glomerata* from two different competitive environments: in CO, where *C. glomerata* is naïve to *C. rubecula* and in MD, where *C. glomerata* coexists with *C. rubecula*. I also examined whether top-down pressures from shared enemies had the potential to affect the competitive interactions between these two wasps.

The three main objectives of this study were to 1) compare the foraging efficiency among the two populations of *C. glomerata* and between the species, 2) assess how the two *C. glomerata* populations react to hosts parasitized by *C. rubecula* and 3) quantify the impact from hyperparasitoids that attack both *Cotesia* species. I showed that *C. rubecula* is faster at finding hosts and spends much less time handling a host per attack. While *C. glomerata* is less efficient than *C. rubecula*, *C. glomerata* from the competitive environment (MD) were more efficient than conspecifics from the less competitive environment (CO). I found that the foraging strategies of MD *C. glomerata* included avoidance of hosts previously attacked by *C. rubecula*, whereas CO *C. glomerata* had a lower tendency to avoid these costly hosts. Both *Cotesia* species were attacked by the same hyperparasitoids, but *C. rubecula* experienced much greater costs from this top-down factor. The large brood sizes of *C. glomerata* provide an escape from the complete mortality experienced by *C. rubecula* when it is hyperparasitized.

My experiments and field observations provided answers to my research questions, but there is room for further investigation. *Cotesia glomerata* and *C. rubecula* are common in agricultural fields because of their utility as biological control agents of *P. rapae*. While

agroecosystems are an important habitat for the *Cotesia* wasps and their host caterpillars, we know little about these species from less manipulated habitats. My study was based on individuals caught on varieties of cultivated cabbage, so it would be interesting to compare similar findings from the same species inhabiting wild brassicaceous plants. Wild plants are often smaller, contain higher concentrations of chemical defenses (Gols and Harvey 2009) and are more dispersed than the cultivated varieties from which my insects were collected. Natural habitats may also serve as competitor-free spaces if they provide *C. glomerata* with alternative host species that are uncommon in agroecosystems (van Driesche et al. 2003; Benson et al. 2003).

Aside from regions along the mid-Atlantic coast of the U.S.A, there are other areas where *C. glomerata* has been observed co-occurring with *C. rubecula* (Herlihy et al. 2012). There may also be additional populations of *C. glomerata* that are naïve to *C. rubecula*. It would be intriguing to replicate my experiments with wasps from these areas to create important comparisons with my data from CO and MD.

Perhaps the most significant unknown is how European *C. glomerata* react to *P. rapae* previously attacked by *C. rubecula*. The presence of *P. brassicae*, an alternate host that is preferred by European *C. glomerata*, may preclude directional selection for these *C. glomerata* to discriminate and avoid hosts parasitized *C. rubecula*. However, little is known about the frequency of multiparasitism in European populations. Future studies should attempt to fill this gap with field surveys in European where both *C. glomerata* and *C. rubecula* co-occur, and design experiments that examine the foraging behaviors that are important for mediating competitive interaction between the two species.

We may never know the exact mechanism behind the extirpation of *C. glomerata* from numerous regions of North America; however, the arrival of *C. rubecula* is surely a significant, if not the primary, contributor to this outcome. If *C. rubecula* was to establish in CO, I expect CO *C. glomerata* to be vulnerable to displacement because of their relatively poor foraging efficiency and inclination to accept hosts parasitized by *C. rubecula*. *Cotesia rubecula* would become a major source of mortality, and would in turn, create a selection pressure for *C. glomerata* to increase foraging efficiency, which includes avoiding multiparasitism. However, hyperparasitoid attacks may alter the asymmetrical outcome of interspecific competition that normally favors *C. rubecula* over *C. glomerata*. The hyperparasitoids of CO *C. glomerata* can also attack *C. rubecula*, and these attacks would be a more severe threat to *C. rubecula* than to *C. glomerata*. Colonizing populations of *C. rubecula* would face a top-down pressure that, through apparent competition, could even the competitive playing field with *C. glomerata* and allow for coexistence.

Competition presents a significant challenge for species within a community. The loss of *C. glomerata* in parts of North America is an example of how stronger competitors can alter community composition. While ecological communities have always experienced some flux, anthropogenic factors are increasing the rate of change. The loss of established species and the introductions of new ones are exposing populations to novel selection pressures. With the rapid change in ecosystems worldwide, it is increasingly important to understand the role of interspecific interactions in the survival of species at local and global scales.

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APPENDIX A: Additional data from the study on hyperparasitoids of *Cotesia glomerata* and *C. rubecula*

Table A.1. Summary of the total number of *Cotesia glomerata* broods and *Pieris rapae* caterpillars collected per site during the eight 2015 collection periods in Colorado. Proportion of *P. rapae* parasitized includes both collected *C. glomerata* broods and dissected *P. rapae* caterpillars collected from the field. The different crop varieties sampled at each site and the date corresponding to each sample are given in the footnotes. Rows with n/a indicate that the site not visited beyond the previous sample.

SITE										
# of <i>C. glomerata</i> broods collected										
# of collected <i>C. glomerata</i> broods w/ hyperparasitoids										
# of collected <i>P. rapae</i> caterpillars										
# of <i>P. rapae</i> caterpillars dissected										
Prop. of <i>P. rapae</i> parasitized by <i>C. glomerata</i>										
	GPS Location ^a	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	TOTAL
		0	0	12	0	22	81	10		125
		0	0	1	0	5	63	5		74
ARDEC North (AR) ^{1,A}	40.653176°, -104.997239°	28	31	122	282	242	275	54	n/a	1034
		14	13	92	114	92	9	10		344
		0.07	0.10	0.25	0.34	0.58	0.70	0.89		--

	# of <i>C. glomerata</i> broods collected	
	# of collected <i>C. glomerata</i> broods w/ hyperparasitoids	136
	# of <i>P. rapae</i> collected	4203
	# of <i>P. rapae</i> caterpillars dissected	2053

^a Degrees latitude and longitude

¹ collard greens, green kale, broccoli, green cabbage, Brussel's sprouts,

² green cabbage, deacon cabbage, red cabbage, kohlrabi

³ broccoli, Brussel's sprouts, green cabbage, red cabbage, green kale, red kale, dino kale

⁴ green cabbage, red cabbage, purple cauliflower, Romanesco cauliflower, white cauliflower, broccoli, collard greens, green kale, dino kale, red Russian kale

⁵ dino kale, green kale, kohlrabi

⁶ red cabbage, mini cabbage, green cabbage, savoy cabbage, deacon cabbage, broccoli, winterbor kale, hybrid kale, kalettes, Brussels sprouts

^A AR (sample #) dates: (1) 7/15, (2) 7/22, (3) 8/1, (4) 8/26, (5) 9/9, (6) 9/23, (7) 10/14

^B BP (sample #) dates: (1) 6/30, (2) 7/21, (3) 7/31, (4) 8/30, (5) 9/18, (6) 10/9

^C HF (sample #) dates: (1) 7/13, (2) 7/23, (3) 8/1, (4) 8/20, (5) 8/31, (6) 9/14, (7) 10/7, (8) 10/28

^D IF (sample #) dates: (1) 7/20, (2) 7/28, (3) 8/3, (4) 8/28, (5) 9/11, (6) 9/25, (7) 10/16

^E RG (sample #) dates: (1) 7/17, (2) 7/29, (3) 8/14

^F SC (sample #) dates: (1) 7/9, (2) 7/24, (3) 8/6, (4) 8/24, (5) 9/2, (6) 9/16, (7) 10/12

Table A.2. Summary of the total number of *Cotesia glomerata* (Cg) broods and *C. rapae* (Cr) cocoons collected per site during the sampling visits in Maryland. The different crop varieties sampled at each site and the date corresponding to each sample are given in the footnotes. Rows with n/a indicate that the site was not visited beyond the previous sample.

SITE # of broods/cocoons collected # of collected broods/cocoons w/ hyperparasitoids	GPS Location ^a	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8		Sample 9		TOTAL		
		Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	Cg	Cr	
		Brandon's Farm (BF) ^{1,A}	39.571646°, -76.739242°	1 1	1 0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Calvert Farm (CF) ^{2,B}	39.712993°, -75.984275°	0 0	38 37	1 0	0 --	2 0	0 --	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	3 0	38 37
Calvert's Gift Farm (CGF) ^{3,C}	39.584740°, -76.738560°	2 0	2 0	1 0	0 --	2 0	4 2	7 5	1 0	8 2	5 1	2 2	11 4	n/a	22 9	23 7						
Flying Plow (FP) ^{4,D}	39.692647°, -76.099227°	0 --	2 2	7 5	9 5	4 1	13 4	4 1	5 0	8 4	4 2	4 3	4 1	17 16	38 26	19 16	49 42	40 29	74 50	103 77	198 132	
Glade Link Farm (GL) ^{5,E}	39.570300° -77.285373°	7 6	2 0	7 4	3 0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	14 10	5 0
Gorman Farms (GF) ^{6,F}	39.148199°, -76.863064°	1 1	0 --	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1 1	0 --
University of Delaware Student Gardens (UD) ^{7,G}	39.668151° -75.751258°	0 --	1 0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0 --	1 --
TOTAL																						

# of <i>C. glomerata</i> broods analyzed	144	266
# of collected broods/cocoons w/ hyperparasitoids	98	176

^a Degrees latitude and longitude

¹ green kale, dino kale

² green kale

³ cauliflower, Brussel's sprouts, broccoli, dino kale, green kale

⁴ broccoli, savoy cabbage, cauliflower, brussels sprouts

⁵ broccoli, collard greens, green cabbage, cauliflower

⁶ red cabbage, mini cabbage, green cabbage, savoy cabbage, deacon cabbage, broccoli, winterbor kale, hybrid kale, kalettes, Brussels sprouts

⁷ collard greens,

^A BF (sample #) dates: (1) 8/17/16

^B CF (sample #) dates: (1) 8/19/16, (2) 8/11/2017, (3) 9/20/17

^C CGF (sample #) dates: (1) 8/17/16, (2) 7/6/17, (3) 7/13/17, (4) 8/10/17, (5) 9/22/17, (6) 7/6/18

^D FP (sample #) dates: (1) 8/19/16, (2) 7/15/2017, (3) 7/17/2017, (4) 8/11/2017, (5) 8/14/17, (6) 9/21/17, (7) 7/7/2018, (8) 7/8/2018, (9) 7/9/2018

^E GL (sample #) dates: (1) 8/15/16, (2) 8/17/16

^F GF (sample #) dates: (1) 8/8/17

^G UD (sample #) dates: (1) 8/19/16

Table A.3. Characteristics of *Cotesia glomerata* broods attacked by a single hyperparasitoid or by different combinations of three of the most common hyperparasitoids of *C. glomerata* in Colorado*.

	Broods with only <i>Baryscapus galactopus</i>	Broods with only <i>Catolaccus aeneoviridis</i>	Broods with only <i>Trichomalopsis dubia</i>	Broods with <i>B. galactopus</i> and <i>C. aeneoviridis</i>	Broods with <i>B. galactopus</i> and <i>T. dubia</i>	Broods with <i>C. aeneoviridis</i> and <i>T. dubia</i>	Broods with <i>B. galactopus</i> , <i>C. aeneoviridis</i> , and <i>T. dubia</i>	Unparasitized broods
# of broods collected	22	21	17	6	9	11	2	141
Total # of <i>C. glomerata</i> cocoons in broods	623	429	399	203	186	327	106	3746
Avg. # of cocoons per brood ($\bar{x} \pm SE$)	35.1 \pm 3.13	28.6 \pm 4.2	24.9 \pm 1.7	33.8 \pm 7.3	26.6 \pm 6.9	32.7 \pm 5.3	53 \pm 16	26.6 \pm 0.891
Avg. # of cocoons per brood from which <i>C. glomerata</i> emerged ($\bar{x} \pm SE$)	12.7 \pm 2.6	16.5 \pm 4.6	11.8 \pm 2.5	18.8 \pm 7.8	10.6 \pm 3.3	16.7 \pm 2.6	9 \pm 3	20.8 \pm 0.860
Avg. Proportion of total cocoons from which adult <i>C. glomerata</i> emerged	0.359 \pm 0.075	0.372 \pm 0.129	0.204 \pm 0.079	0.387 \pm 0.112	0.144 \pm 0.043	0.254 \pm 0.094	0.168 \pm 0.006	0.787 \pm 0.018
Avg. Proportion of male <i>C. glomerata</i> per brood ($\bar{x} \pm SE$)	0.482 \pm 0.081	0.541 \pm 0.101	0.29 \pm 0.114	0.513 \pm 0.056	0.410 \pm 0.175	0.327 \pm 0.126	0.79 \pm 0.04	0.366 \pm 0.0239

* Six other hyperparasitoid species were excluded because they emerged from a small number of broods (Table 2).

Table A.4. Characteristics of *Cotesia glomerata* broods attacked by a single hyperparasitoid or by different combinations of two of the most common hyperparasitoids of *C. glomerata* in Maryland*.

	Broods with only <i>Baryscapus galactopus</i>	Broods with only <i>Conura torvina</i>	Broods with <i>B. galactopus</i> and <i>C. torvina</i>	Unparasitized broods
# of broods collected	68	6	11	46
Total # of <i>C. glomerata</i> cocoons in broods	1769	197	267	1128
Avg. # of cocoons per brood ($\bar{x} \pm$ SE)	26.01 \pm 1.67	32.83 \pm 7.49	24.27 \pm 2.07	24.52 \pm 1.92
Avg. # of cocoons per brood from which <i>C. glomerata</i> emerged ($\bar{x} \pm$ SE)	13.28 \pm 1.66	26.50 \pm 7.14	10.82 \pm 2.51	20.09 \pm 2.06
Avg. Proportion of total cocoons from which adult <i>C. glomerata</i> emerged	0.45 \pm 0.04	0.80 \pm 0.06	0.42 \pm 0.09	0.75 \pm 0.05
Avg. Proportion of male <i>C. glomerata</i> per brood ($\bar{x} \pm$ SE)	0.37 \pm 0.06	0.50 \pm 0.00	0.67 \pm 0.18	0.49 \pm 0.06

* Two other hyperparasitoid species were excluded because they emerged from a small number of broods (Table 1). Four broods included *B. galactopus* and *C. aeneoviridis* and one brood had *C. aeneoviridis* and an unknown hyperparasitoid.

APPENDIX B: Additional descriptions of the four main hyperparasitoids that attacked *Cotesia glomerata* in Colorado (CO), as well as *C. glomerata* and *C. rubecula* in Maryland (MD).

Baryscapus galactopus

Baryscapus galactopus occurred in a greater percentage of CO *C. glomerata* (42/136) and MD *C. glomerata* (83/98) broods than did any other hyperparasitoid. This species was also the most abundant with individuals accounting for 52.1% (874/1675), 95.8% (2188/2284) and 96.7% (1366/1413) of hyperparasitoids collected from CO *C. glomerata*, MD *C. glomerata* and *C. rubecula*, respectively. Given its predominance over other hyperparasitoids, *B. galactopus* was largely responsible for the relationship between the probability of hyperparasitism and brood size for CO *C. glomerata* (log likelihood $\chi^2=9.30$ d.f.=1, $p=0.002$). *Baryscapus galactopus* was the only gregarious hyperparasitoid with a range of 1 to 59 adult *B. galactopus* emerging from a single *C. glomerata* brood and 2 to 26 adults emerged from a *C. rubecula* cocoon. On average, more individuals were observed in broods of CO *C. glomerata* (20.81 ± 1.53 , $n=42$) and MD *C. glomerata* (26.36 ± 2.03 , $n=83$) than in MD *C. rubecula* cocoons (11.48 ± 0.44 , $n=119$) (CO *C. glomerata* and *C. rubecula*: $t_{43.14}=3.36$, $p=0.002$; MD *C. glomerata* and *C. rubecula*: $t_{85.30}=4.73$, $p<0.001$).

Catolaccus aeneoviridis

Catolaccus aeneoviridis was the second most common hyperparasitoid species collected in CO based on the number of *C. glomerata* broods hyperparasitized. A total of 40 broods (29.1%) out of 136 broods were parasitized by *C. aeneoviridis*. The number of *C. aeneoviridis* from a single brood varied from 1 to 19 adults with an average of 6.5 ± 0.8 adults per brood.

Compared to CO, *C. aeneoviridis* was a weaker threat to either MD *C. glomerata* or MD *C. rubecula* based on the smaller number of broods and cocoons from which it was collected (Table 1).

Trichomalopsis dubia

Trichomalopsis dubia was the second most abundant hyperparasitoid in CO accounting for 22.2% (372/1675) of all individual hyperparasitoids. Between 1 and 29 adult *T. dubia* emerged from a single brood with an average of 9.3 ± 1.1 adults emerging per brood. Neither MD *C. glomerata* or *C. rubecula* were attacked by this hyperparasitoid.

Conura torvina

Conura torvina was more common in *C. rubecula* cocoons than in *C. glomerata* broods from either CO or MD. This hyperparasitoid accounted for 25.6% (45/176) of hyperparasitized *C. rubecula* cocoons. Each of the 45 *C. rubecula* cocoons attacked by *C. torvina* produced only one adult *C. torvina*, suggesting that this hyperparasitoid is solitary. *Conura torvina* was uncommon in CO *C. glomerata*, and the hyperparasitoid was in only 11.8% (17/144) of MD *C. glomerata* broods with a range of 1 to 6 and an average of 2.0 ± 0.35 *C. torvina* emerging from a brood.