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GENETIC DIFFERENTIATION OF THE PARASITOID, *COTESIA CONGREGATA* (SAY), BASED ON HOST-PLANT COMPLEX

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Host races of the parasitoid, *Cotesia congregata* (Say) on two host-plant complexes

Abstract

GENETIC DIFFERENTIATION OF THE PARASITOID, *COTESIA CONGREGATA* (SAY), BASED ON HOST-PLANT COMPLEX

By Georgia M. Karns, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2009

Directors: Karen M. Kester, Ph.D. Associate Professor, Department of Biology
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Endoparasitoids of herbivorous lepidopterans have intimate relationships with their host species as well as the plant on which their host develops. Characteristics of both hosts and plants can affect parasitoid success in tri-trophic systems and thus, drive diversification. Genetic differentiation was estimated for *Cotesia congregata* (Say) collected from two distinct host-plant complexes, *Manduca sexta* L. on tobacco (*Nicotiana*

tabacum L.) and *Ceratomia catalpae,* (Haworth) on catalpa (*Catalpa bignonioides* Walker), in the mid-Atlantic region of North America using seven microsatellite loci and the mitochondrial COI locus. Microsatellite allele frequencies were differentiated based on host-plant complex, and COI haplotypes from individuals on the same host-plant were identical despite geographic distances between catalpa sites of up to 830 km and distances between tobacco sites of up to 294 km. Results indicate genetic differentiation of subpopulations of *C. congregata* based on host-plant complex and not geographic distance, and were designated as host races.

Cotesia congregata is a gregarious parasitoid, meaning that many individuals develop in a single host larva. Superparasitism, or repeated egg-laying events in the same host larva, is likely to occur in gregarious species. Brood size was not a good predictor of superparasitism in *C. congregata,* but within-brood male allele diversity indicates either superparasitism or multiple mating by female wasps.

Host races of the parasitoid, *Cotesia congregata* (Say), on two host-plant complexes

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Abstract

Endoparasitoids of herbivorous lepidopterans have intimate relationships with their host species as well as the plant on which their host develops. Characteristics of both hosts and plants can affect parasitoid success in tri-trophic systems and thus, drive diversification. Genetic differentiation was estimated for *Cotesia congregata* (Say) collected from two distinct host-plant complexes, *Manduca sexta* L. on tobacco (*Nicotiana tabacum* L.) ("MsT") and *Ceratomia catalpae* (Haworth) on catalpa (*Catalpa bignonioides* Walker) ("CcC"), in the mid-Atlantic region of North America using seven microsatellite loci and the mitochondrial COI locus. Microsatellite allele frequencies were differentiated based on host-plant complex, and COI haplotypes from individuals on the same host-plant were identical despite geographic distances between catalpa sites of up to 830 km and distances between tobacco sites of up to 294 km. Results indicate genetic differentiation of subpopulations of *C. congregata* based on host-plant complex, and were designated as host races, MsT and CcC.

Keywords

Host-plant complex differentiation, host associated differentiation, host races, cryptic species, parasitoid, Braconidae, *Cotesia congregata* (Say)

Introduction

Hymenopterous parasitoids represent an extremely diverse group of organisms, and the potential mechanism for their diversification is a topic of increasing interest to

entomologists and evolutionary biologists. Parasitoids, particularly endoparasitoids, have intimate relationships with their host species as well as the plant on which their hosts develop. As reviewed in Price *et al.* (1980), both host and plant related factors, such as host life history strategy and patterns of host abundance and distribution (Gross 1993), as well as plant structure (Andow 1990), apparency, chemistry (Vet and Dicke 1992, Benrey 1997, Eben *et al.* 2000), and distribution patterns (Andow 1991), can affect parasitoid success in tri-trophic systems. Specialization on different hosts and/or host-plants can result from disruptive selection due to population differences in habitat selection (Smith 1966, Rice 1984, 1985, 1987, Rice and Salt 1988).

Habitat-related variation in behavioral responses to hosts and/or host-plants has been documented for a few species of parasitoids. For example, *Cotesia congregata* (Say) (Hymenoptera: Braconidae) collected from a single host species on tobacco and tomato exhibit differential searching responses (length of searching time) to the allelochemical nicotine applied to leaf discs, and show differential ovipositional preference for the two host-plant complexes (Kester and Barbosa 1994). Likewise, differential behavioral responses to two host-plant complexes have been demonstrated for the braconid, *Diachasma alloeum* (Muesebeck), which attacks two congeneric host species on blueberry and hawthorn (Stelinski and Liburd 2005). Another braconid, *Cotesia glomerata* (L.) exhibits distinct differences in behavioral responses to a single host species on wild and cultivated crucifers (Van Nouhuys and Via 1999). This variability in parasitoid responses to host-plant characteristics, along with host fidelity, may play an important role in parasitoid population divergence.

Host fidelity in parasitoids may be promoted by learning behavior, which in turn may lead to local adaptation to plant or host-plant complexes, and population diversification. For example, post-emergence experience (i.e., early adult learning) increases searching responses of females of *C. congregata* to the plant experienced at emergence and often inhibits responses to alternative host foodplants (Kester and Barbosa 1991, 1992). A similar increase in flight response to plant volatiles due to prior experience was observed in *D. alloeum* for uninfested and newly-infested blueberries (Stelinski *et al.* 2006). Assuming that learning is not energetically costly, such learned habitat preferences can theoretically promote sympatric speciation more effectively than genetic habitat preference (Beltman and Metz 2005).

Because parasitoids are attracted to and tend to mate on or near their natal host-plants, reproductive isolation with respect to host-plant complex may limit gene flow among different host-plant associated populations. Limited gene flow among populations due to assortative mating and habitat selection can lead to genetic differentiation, host-race formation, and even sympatric speciation (Smith 1966, Bush 1975, Berlocher and Feder 2002). In addition, exposure to host-ingested plant chemicals that negatively affect the development and fitness of larval endoparasitoids can exert directional selective pressure that can facilitate greater population differentiation, as reported for *C. congregata* originating from *Manduca sexta* L. on tobacco compared to tomato (Kester 1991).

Genetic differentiation associated with host or host-plant has been documented for several parasitoid species. Microsatellite allele frequency distributions, mitochondrial COI sequences, and differential behavioral observations indicate that the braconid, *Cotesia*

melitaearum (Wilkinson), is an aggregate of five cryptic species associated with specific nymphalid hosts across Europe (Kankare, *et al.* 2005). Genetic differentiation has also been reported in two species of parasitoids, the platygastrid, *Platygaster variabilis,* and the encyrtid, *Copidosoma gelechiae*, attacking gallmakers on two sympatric species of goldenrod (Stireman *et al.* 2006). Most recently, analysis of developmental characters, microsatellites and mitochondrial COI sequences has revealed the presence of incipient species of the braconid, *D. alloeum,* associated with incipient sympatric speciation of the tephritid host, *Rhagoletis pomonella*, on apple and hawthorn (Forbes *et al.* 2009). However, genetic differentiation of parasitoids with respect to host or host foodplants cannot be assumed for all species. For example, host-associated differentiation has not been supported in the aphid parasitoid, *Aphidius transcaspicus* Telenga (Braconidae), or in *Eusandalum* spp. that attack yucca moth (*Prodoxus* spp.). For these species, genetic structure is determined by geography rather than host or plant association (Althoff 2008, Lozier *et al.* 2009).

Host races of phytophagous insects are defined by several criteria (Dres & Mallet 2002). First, populations must use and exhibit host fidelity on different host taxa in sympatry. Second, populations on different hosts must be genetically differentiated at more than one locus regardless of geographic distance. Third, populations must have higher fitness on natal host species than on other sympatric host species. Lastly, assortative mating must occur within populations on the same host and exhibit gene flow "at an appreciable rate" among populations on different hosts. If gene flow among populations is very low or absent, populations utilizing sympatric host-plants may be

considered cryptic species (Dres and Mallet 2002). If geographic proximity influences genetic structure, local adaptation, rather than host association, may be responsible for population differences (reviewed in Kawecki and Ebert 2004). The same criteria can also be used to determine the predominant influence on genetic differentiation among populations of parasitoids.

The gregarious larval endoparasitoid, *C. congregata,* offers an ideal system for investigation of genetic differentiation based on host-plant complex. This species is native to North America and is reported to attack numerous species in the Sphingidae (Krombein, *et al.* 1979), most of which are specialists on one to a few plant families (Tietz 1972). This parasitoid, with host, *Manduca Sexta (L.) (Lepidoptera: Sphingidae)*, has served as a model system for increasingly in-depth investigations of host-parasite interactions (Beckage 2008). In the USA mid-Atlantic region, *C. congregata* attacks *M. sexta*, a specialist on plants in the family Solanaceae, and *Ceratomia catalpa* (Boisduval) (Lepidoptera: Sphingidae), which feeds exclusively on *Catalpa* spp. (Bignoniaceae). The work reported herein investigates genetic differentiation of *C. congregata* originating from *M. sexta* ("tobacco hornworm") on tobacco (*Nicotiana tabacum* L.) and *C. catalpae* ("catalpa sphinx") on southern catalpa (*Catalpa. bignonioides* Walker) from the mid-Atlantic region of North America. These two hosts of *C. congregata* differ with respect to life history traits and distribution. The tobacco hornworm is solitary and until late in the growing season, larvae are distributed one to a few larvae on each plant. In contrast, the catalpa sphinx is gregarious and when present, occurs in much larger numbers. The two plants differ in both apparency and distribution; herbaceous tobacco is generally cultivated

in large stands in agricultural settings, whereas catalpa, which is native to the south and also has been introduced as an ornamental in urban and suburban landscapes, tends to be more widely dispersed and present as one to a few trees. Most importantly, these plants differ greatly in plant chemistry. Cultivated tobacco plants produce the alkaloid, nicotine (Sisson & Saunders, 1982), which has been shown to directly affect development time and survival of *C. congregata* (Barbosa *et al.* 1991, Bentz and Barbosa 1992). In contrast, catalpa produces iridoid glycosides (Boros & Stermitz 1990) that are sequestered by *C. catalpae* (Bowers 2003) yet have no effect on brood size or survival of *C. congregata* (Bowers & Lampert, pers. comm.). In addition to host-plant-specific behavioral responses of *C. congregata* discussed previously, preliminary results of genetic analyses reveal microsatellite allele frequency distributions that differ according to these two host-plant complexes (Jensen *et al.* 2002).

The present study was designed to further investigate genetic differences in *C. congregata* collected from these two host-plant complexes at a regional scale, using seven microsatellite loci and the mitochondrial COI locus. We hypothesized that genetic differentiation based on host-plant complex would be associated with restricted gene flow among populations of *C. congregata* that utilize different host-plant complexes. Depending on measurements of gene flow, that may range from none to little, among populations that utilize different host-plant complexes, these populations can be designated as host races or cryptic species. The investigation of natural populations was complemented by the experimental introduction of tobacco at one “catalpa site” for two consecutive years to test the hypothesis that host-plant races of *C. congregata* could exist

in sympatry. If host-plant complex is a primary factor determining population structure of *C. congregata*, genetic data from wasps collected from the newly introduced tobacco should show similarities to wasps collected from other tobacco sites. Otherwise, panmixia or local adaptation due to isolation by distance would be implicated if host plant is not an important factor determining genetic structure of *C. congregata.*

Materials and Methods

Collection sites, experimental site, sampling, and rearing

Larvae of *M. sexta* and *C. catalpae* were collected from their respective host plants, tobacco (*N. tabacum*) and catalpa *C. bignonioides* at a total of eight sites across the mid-Atlantic region over the summers of 2006 and 2007 (Table 1, Figure 1). Sites were chosen on the basis of prior knowledge and availability of larvae. Ultimately, the following subpopulations were defined for comparison, with the host-plant complex defined as "MsT" for *M. sexta/N. tabacum* and "CcC" for *C. catalpae/ C. bignonioides* and a subscript representing the collection site: MsT_{NEWTON}, and $MsT_{BLACKSTONE;}$ $MsT_{MARLBORO}$, CcC_{TYSON}, CcC_{NEWTON}, $CcC_{LAKESIDE}$, $CcC_{CLEMSON}$, CcC_{COLMAR}, and $CcC_{ROANOKE}$

At one site designated NEWTON, where tobacco had never been cultivated, a stand of tobacco ('NC95) was planted in 2006 and 2007 within 100 m of a group of four catalpa trees. Tobacco plants were grown from seed and treated with Admire Pro (Imidacloprid) for aphid control at 10mL per 1000 plants three weeks prior to transplant each May. Plots contained four rows of 50-60 plants, spaced 0.75 m apart.

Both parasitized (cocoons attached) and apparently unparasitized third through fifth-instar larvae were collected and transferred to the laboratory. Larvae without egressed parasitoids were grouped by collection site and reared in plastic boxes on leaves of their respective host plant; fresh leaves were provided daily. Two to three days after egression, parasitoid cocoons were removed from hosts and stored in small covered plastic cups until emergence, after which they were counted, sexed, and frozen at -20ºC. Each brood was stored separately. Broods were counted and sexed.

Molecular analyses

DNA was extracted from wasps using the Geneclean® *Turbo* 96 kit (Q-BIOgene, Carlsbad, CA) according to the manufacturer's instructions. Individual specimens were analyzed for variation at nuclear and mitochondrial loci. Seven microsatellite loci were assayed: *cco1A, cco5A, cco27, cco65A, cco65B,* and *cco68* isolated from *C. congregata* (Say) (Jensen et al. 2002), and *cot1*, isolated from *C. glomerata* (Zhou et al. 2005). Amplification was performed as described by Li, *et al* (2006) with three primers to facilitate fluorescent detection. Amplification products were resolved using the MegaBACE 1000 DNA Analysis System (Amersham Bioscience, Norway) and alleles were scored using Fragment Profiler Ver 1.2 (Amersham Bioscience, Norway).

Because *C. congregata* has haplodiploid sex determination, only diploid females were assayed for heterozygosity. To ensure independent sampling of alleles, only one female was randomly selected from each brood for analyses. The probability of null alleles was assessed using MICROCHECKER© (University of Hull, England). To

analyze population structure, GENETICSTUDIO (Dyer 2009) was used to calculate allele frequencies and deviation from Hardy-Weinberg equilibrium in each population, as well as genetic variance (Φ_{ST}), and Nei's genetic distance among populations (Nei 1972). The Manteller routine (Dyer 2009) was used to determine the likelihood of isolation by distance as described by Mantel (1967). To quantify and compare the levels of inbreeding within subpopulations, the multi-locus inbreeding coefficient *F* (Ayres and Balding 1998) was calculated using GENETICSTUDIO (Dyer 2009). To estimate the rate of effective migration among subpopulations, N_em, the multilocus private alleles method of estimation (Slatkin 1985), and a test for linkage disequilibrium were implemented through GENEPOP (Raymond and Rousset 1995). A phylogeny was resolved from microsatellite data using NEIGHBOR and CONSENSE, in PHYLIP v. 3.6 (Felsenstein 2005) to construct neighbor-joining trees and to compute consensus trees from the Nei's genetic distance matrix generated by GENETICSTUDIO. Microsatellite data from a cryptic species complex of *C. melitaearum* (CMEL) collected from four different hosts including *Euphydryas aurinia* (EA), *Euphydryas desfontainii* (ED), *Melitaea deione* (MDE), and *Melitaea phoebe* (MP), as described in Kankare *et al.* (2005) were used to represent the outgroup in the distance tree (Kankare *et al.,* pers. comm.).

The mitochondrial COI gene was amplified using a forward primer 5'TGAACGTATATTTAAAATAGTAGT3' and a reverse primer 5'NTGGTTTTGGAAATTGATTAA3' designed from a consensus sequence obtained by aligning COI accessions from Genbank (DQ538817, DQ538815, DQ232332, DQ232331, AY934823, AY333870, AY333888, DQ232338, DQ232337; MegAlign and Primer,

Lasergene, Madison, WI). Mitochondrial sequences of *C. congregata* were aligned using the Clustal V method in MegAlign and inspected to identify single nucleotide polymorphisms (SNPs) associated with the host-plant complex. A bootstrapped 50% majority rule consensus tree was constructed using maximum parsimony in PAUP (Swofford 2003). *Cotesia melitaearum* (CMEL; GenBank Accession number AY333882), and *Cotesia plutellae* (CPLU; GenBank Accession number AY934821) were used to represent outgroups.

Results

Sampling

The number of collections made at each site varied from one to many. Multiple collections of larvae of *M. sexta* on tobacco were made at the BLACKSTONE and NEWTON sites during mid-July through September of both years. A single collection of *M. sexta* larvae was made at the MARLBORO site in October in 2006. Multiple collections of larvae of *C. catalpae* were made at the TYSON site, July and August in 2006, and late June through September in 2007, as well as at the NEWTON site in July, 2006, and from July to September in 2007. Single collections of *C. catalpae* were made at the LAKESIDE, CLEMSON, and ROANOKE sites in September, 2007, and at the COLMAR site in October, 2006. The only primary parasitoid species reared from the two sphingids was *C. congregata.* The number of broods collected from each site ranged from 2 to 150 (Table 1); however, due to high rates of hyperparasitism, some broods did not yield any samples of *C. congregata*. Excluding hyperparasitized larvae and those host larvae with cocoons

present at the time of collection, the mean brood sizes and mean sex ratios across both years ranged from 53 – 63 individuals (51% - 59% female) in tobacco subpopulations and from 45 – 46 individuals (55% - 74% female) in catalpa subpopulations.

Microsatellite analyses

Only the TYSON, NEWTON, BLACKSTONE, and LAKESIDE sites yielded sufficient sample sizes to be included in microsatellite analyses for *C. congregata.* None of the subpopulations were found to be in Hardy-Weinberg equilibrium. Null alleles were detected in at least one subpopulation for six out of seven loci examined, but not across all subpopulations, implying that the deficiency of heterozygotes, although possibly a consequence of amplification failure, was more likely due to high levels of inbreeding. Linkage was not detected at any loci across all populations, and was not detected in samples from BLACKSTONE or from catalpa at NEWTON. Inbreeding (multilocus estimates of F_{IS}) was lowest in the $CcC_{LAKESIDE}$ subpopulation (F_{IS}=0.14), and highest in the CcC_{NEWTON} (F_{IS}=0.40) and CcC_{TYSON} (F_{IS}=0.34) subpopulations. Intermediate values were observed for the MsT_{NEWTON} (F_{IS}=0.20) and $MsT_{BLACKSTONE}$ (F_{IS}=0.21) subpopulations. An F_{IS} value of 0.25 or higher indicates sib-mating (Hedrick2005).

Microsatellite allele frequency distributions differed significantly (P = 0.000) among populations utilizing different host-plant complexes. With the following exceptions that do detect significant differences, MsT_{NEWTON} *vs.* $MsT_{BLACKSTONE}$ (X^2 = 24.19, P = 0.043), CcC_{NEWTON} *vs.* CcC_{TYSON} (X^2 = 35.59, P = 0.001) and CcC_{TYSON} *vs.* $CcC_{LAKESIDE}$ (X^2 = 51.62, P < 0.000), subpopulations utilizing the same host-plant complex did not

differ. Genetic differentiation was not correlated with geographic distance among subpopulations (Mantel test, $P = 0.84$). Instead, microsatellite allele frequency distributions exhibited host-plant specific variation (Table 2), though differences were more striking for some loci than for others (Figure 2). Measures of population differentiation (Φ_{ST}) were 20 times higher among subpopulations collected from different host-plant complexes (mean $\Phi_{ST} = 0.41$) at a site than similar measures estimated for *C. congregata* collected from the same host-plant complex at different locations (mean $\Phi_{ST} = 0.02$). Effective migration rates were a factor of 6.7 higher between subpopulations from the same host-plant complexes at different locations (average $N_em = 3.58$) than were observed for *C. congregata* collected from different complexes at the same location (average $N_em = 0.53$; Table 3). The neighbor-joining analysis using Nei's genetic distance (Figure 3) grouped subpopulations of *C. congregata* based on host-plant complex rather than site.

Mitochondrial DNA analysis

A 214 basepair fragment was sequenced from the COI region of mitochondrial DNA from 25 individuals collected from catalpa and 19 individuals collected from tobacco (Genbank accession numbers GQ412122-412130); multiple representatives from all sites. Six informative nucleotide sites were identified, and there was a single deletion of one nucleotide in one individual from MARLBORO. Including the sequence with the deletion and excluding the outgroup sequences, a total of eight haplotypes were identified. The six informative characters typical of individuals collected from catalpa, CGCAAC (hereafter

referred to as the “catalpa haplotype”), were unique to and present in 72% of all CcC samples. The haplotype typical of individuals collected from tobacco, GATCAT (hereafter referred to as “tobacco haplotype”), was present in 89% of MsT samples. The tobacco haplotype had an approximately 2% sequence divergence from the catalpa haplotype.

All MsT individuals collected from the BLACKSTONE and MARLBORO sites had the tobacco haplotype only, and with the exception of one nucleotide deletion in an individual collected from COLMAR, all individuals collected from four catalpa sites, including CLEMSON, ROANOKE, TYSON, and COLMAR had the catalpa haplotype only. One haplotype, AATCAT, was observed exclusively in individuals collected from both CcC and MsT at the NEWTON site. The haplotype, CGCCAC, was unique to samples from CcC_{NEWTON} and the haplotype, CACAAC, was unique to $CcC_{LAKESIDE}$. At the NEWTON site, where tobacco was introduced experimentally, eight of ten MsT individuals had the tobacco haplotype and two had haplotypes that differed by only one nucleotide. Five different haplotypes were sequenced from eight individuals collected from $CcC_{NEWTON.}$ Four of eight individuals collected from CcC had the catalpa haplotype, however, three individuals had haplotypes either identical or similar (one nucleotide substitution) to the tobacco haplotype. Phylogenetic analysis grouped all CcC-collected wasps in one clade, with the exception of two individuals collected at the NEWTON site in 2007. All but one MsT-collected wasp were also grouped together (the exception was collected at the NEWTON site in 2007; Figure 4).

Discussion

Ecological specialization leading to host-plant associated genetic differentiation of phytophagous insects is thought to be quite common (e.g., Funk *et al.* 2002, Dres and Mallet 2002, Stireman *et al.* 2005) and has also been documented for a few species of hymenopterous parasitoids (e.g., Forbes *et al.* 2009, Stireman *et al.* 2006). Populations of *C. congregata* utilizing different host-plant complexes may experience differential selective pressures related to numerous biotic factors, and in the present study we are concerned mainly with the influence of differences in host-plant chemistry on population structure. Learning behavior in parasitoids promotes host fidelity, which can allow genetic differentiation in different habitats to occur via both stochastic population processes and selective forces acting in each habitat. Haplodiploid sex determination may allow adaptation to new habitats to occur at a faster rate than in diploid organisms, since deleterious alleles are exposed and eliminated in haploid males each generation (reviewed in Werren 2003), and because male genotypes are inherited solely from mothers that have successfully developed on a specific host-plant complex. The life history of *C. congregata* is ideal for radiation and adaptation to novel habitats. The present study illustrates the extent of genetic differentiation among subpopulations of *C. congregata* on two common host-plant complexes in the USA mid-Atlantic region, and provides strong evidence that host-plant complex differentiation is a likely explanation for genetic diversification in this species.

Microsatellite alleles in *C. congregata* had host-plant complex specific allele distributions consistent across loci, even at a newly introduced experimental tobacco site.

Mitochondrial COI haplotypes also exhibit host-plant complex specific variation across a wide geographic area, but in the experimental plot, tobacco haplotypes were found in individuals collected from catalpa, indicating the possibility of migration across host-plant complexes. Similar genetic differentiation and phylogenetic grouping based on host-plant is evident in another braconid, *D. alloeum*, on apple, blueberry, and hawthorn (Forbes *et al.* 2009). Kankare *et al.* (2005) found genetic differentiation based on host species. Hosts co-occurred on the same host-plant species, therefore host-plant complexes were not unique in regards to host-plant distribution and chemistry as in the present study. Stireman *et al.* (2006), using the mitochondrial COI region and allozymes, found varying degrees of host-plant associated genetic differentiation in two species of parasitoids on each of two cryptic species of gall-makers, that have very specific and intimate relationships with sympatric host-plants. Host associated clades of *P. variabilis* had approximately 8% COI sequence divergence, while the mitochondrial COI sequences from *C. gelechiae* showed no differentiation; estimates of F_{ST} between populations from different hosts for *C. gelechiae* were low. Further investigation, using amplified fragment length polymorphisms (AFLPs), by Kolaczan *et al.* (2009) found very weak host-plant associated genetic differentiation in *C. gelechiae,* and also no patterns of geographic population structure. It is possible that *C. gelechiae* is in the early stages of host associated differentiation (Stireman *et al.* 2006, Kolaczan *et al.* 2009). Values of Φ_{ST} comparing subpopulations of *C. congregata* on different host-plant complexes were 20 times higher than F_{ST} values estimated for populations of *C. gelechiae*, which were similar to F_{ST} values comparing *C. congregata* at distant locations.

The differences in microsatellite allele and COI haplotype distributions for *C. congregata* could be explained by comparisons of behavioral and developmental responses to tobacco and catalpa. In a companion study, searching responses of *C. congregata* were higher on catalpa than tobacco, irrespective of host-plant complex origin, and MsT wasps searched longer on tobacco than CcC wasps, and MsT wasps were generally more responsive to plant cues (Crocker and Kester, unpublished data). Likewise, duration of larval development and survival to adulthood was higher for MsT than CcC wasps reared in *M. sexta* fed a laboratory diet with added tobacco-leaf diet or dietary nicotine. Further, sex ratio allocation is also affected by post-emergence learning in *C. congregata*, in that wasps allocate more females to hosts offered with the plant experienced at emergence (Lentz and Kester 2008). Ultimately, behavioral and fitness responses of *C. congregata* to host-plant chemistry appear to play an important role in the diversification of populations on different host-plant complexes.

Braconid hymenopterans utilize a symbiotic polydnavirus, located on chromosome 5 in *C. congregata* (Belle *et al.* 2002) that suppresses the immune system of larval hosts and allows developmental of offspring within the host (Beckage *et al.* 1994). The phylogeny of the polydnavirus found in the genus *Cotesia* directly mirrors species diversification of *Cotesia* (Whitfield 2000). Though not explored in the current study, the microsatellite locus *cco51,* amplified from *C. congregata* from *M. sexta* on tobacco, was partially homologous to the *C. congregata* polydnavirus, and failed to amplify in wasps collected from *C. catalpae* on catalpa (Jensen *et al.* 2002). Amplification of this locus from individuals collected from tobacco and catalpa across a wider geographic range may

provide evidence that host-plant associated populations may also have divergent polydnavirus sequences.

Geographic distance does not appear to play an important role in genetic differentiation of subpopulations of *C. congregata.* Individual COI haplotypes were identical despite geographic distances between catalpa sites of up to 830 km and distances between tobacco sites of up to 294 km. Measures of population differentiation were much higher among populations on different host-plant complexes at a site than between subpopulations from distant locations of the same host-plant complex. Other recent studies of parasitoids, including the aphid parasitoid, *Aphidius transcaspicus,* on congeneric host-plant associated species in the genus *Hyalopterus* (Lozier *et al.* 2009) and the yucca moth parasitoid *Eusandalum* spp. on congeneric generalist species in the genus *Prodoxus* (Althoff 2008) found little or no host or host-plant associated genetic differentiation, but rather geographic population structure. The two host species and their respective host-plants considered in the present study are more distantly related and therefore a greater degree of host and/or host-plant associated genetic differentiation would be expected for *C. congregata*. In addition, the geographic scale and variability of the sampling sites may affect the results of population analyses. For example, *A. transcaspicus* was sampled from sites in Greece and Spain, which are separated by the Mediterranean Sea (Lozier *et al.* 2009), and *Eusalandum* spp. were collected from sites across more than 1200 km of the southwest US. Larger scale sampling of *C. congregata* may reveal a more complex population structure.

The data presented here indicate that the host-plant complex associated populations of *C. congregata* can, with a high degree of certainty, be designated as host-races that utilize *M. sexta* on solanaceous plants ("MsT race") and *C. catalpae* on catalpa ("CcC race"). It also is possible that a reproductive barrier due to differences in host-plant chemistry and behavioral adaptations of *C. congregata* may have led to cryptic species divergence within this species. However, further research including more extensive sampling from additional host-plant complexes across a wider geographic range, in addition to comparisons of polydnavirus sequences from both host-plant complexes, would be necessary to further resolve the species status of host races of *C. congregata*.

Acknowledgements

We thank everyone who made this work possible. Dr. Maaria Kankare shared microsatellite allele frequency data for *Cotesia melitaearum.* Dr. Paul Semtner, Southern Piedmont Agricultural Research and Experimental Station in Blackstone, Virginia provided tobacco for field collections. Dr. Scott Newton donated his land and time to establish an experimental planting of tobacco. Mr. and Mrs. Buster Tyson provided unlimited access to their catalpa grove. Mr. John Kemper collected samples at Upper Marlboro and Colmar Manor, and Dr. Evan Lampert provided samples from Roanoke and Clemson. Laboratory analyses were funded in part by Ecogen, Inc. and field components of this project were supported largely though personal funding (KMK). We also thank the Virginia Commonwealth University School of Graduate Studies for support in the form of a summer thesis assistantship to GMK.

List of References

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

Andow, DA, DR Prokrym. 1990. Plant structural complexity and host finding by a parasitoid. *Oecologia* 82: 162-165.

Andow, DA. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36: 561-586.

Ayres, KL, DJ Balding. 1998. Measuring departures from Hardy-Weinberg: a Markov chain Monte Carlo method for determining the inbreeding coefficient. *Heredity* 80: 769-777.

Barbosa, P, P Gross, J Kemper. 1991. Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology* 72: 1567-1575.

Beckage, NE., FF Tan, KW. Schleifer, RD. Lane, LL. Cherubin. 1994. Characterization and biological effects of *C. congregata* polydnavirus on host larvae of the tobacco hornworm, *Manduca sexta*. Arch. *Insect Biochemistry and Physiology* 26:165–195.

Beckage, NE, 2008. Parasitoid polydnaviruses and insect immunity, IN (NE Beckage, ed.) *Insect Immunology*, Academic Press/Elsevier, pp. 243-277.

Belle, E, N, Beckage, J Rousselet, M Poirié, F Lemeunier, JM Drezen. 2002. Visualization of polydnavirus sequences in a parasitoid wasp chromosome. *Journal of Virology* 76(11): 5793–5796.

Beltman, JB, JAJ Metz. 2005. Speciation: More likely through a genetic or through a learned habitat preference? *Proceedings: Biological Sciences* 272: 1455-1463.

Benrey, B, RF Denno, L Kaiser. 1997. The influence of plant species on attraction and host acceptance in *Cotesia glomerata* (Hymenoptera: Braconidae). *Journal of Insect Behavior* 10: 619-630.

Bentz, JA, P Barbosa. 1992. Effects of dietary nicotine and partial starvation of tobacco hornworm, *Manduca sexta*, on the survival and development of the parasitoid *Cotesia congregata. Entomologia Experimentalis et Applicata* 65: 241-245.

Berlocher, SH, JL Feder. 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annual Review of Entomology*. 47: 773-815.

Boros, CA, FR Stermitz. 1990. Iridoids. An updated review, part I. J. Nat. Prod. 53:1055–1147.

Bowers, MD. 2003. Hostplant suitability and defensive chemistry of the catalpa sphinx, *Ceratomia catalpae. Journal of Chemical Ecology* 29: 2359-2367.

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

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

Dyer, RJ. 2009. GeneticStudio: A suite of programs for spatial analysis of genetic-marker data. *Molecular Ecology Resources* 9: 110-113.

Eben, A, B Benrey, J Sivinski, M Aliya. 2000. Host species and host plant effects on preference and performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Environmental Entomology* 29: 87-94.

Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. *Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.*

Forbes, AA, THQ Powell, LL Stelinski, JJ Smith, JL Feder. 2009. Sequential sympatric speciation across trophic levels. *Science* 323: 776-779.

Funk, DJ, KE Filchak, JL Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* 116: 251-267.

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

Hedrick, PW. 2005. *Genetics of Populations.* Sudbury, MA: Jones and Bartlett Publishers.

Jensen, MK, KM. Kester, M Kankare, BL Brown. 2002. Characterization of microsatellite loci in the parasitoid *Cotesia congregata* (Say) (Hymenoptera Braconidae). *Molecular Ecology Notes* 2: 346-348.

Kankare, M, S Van Nouhuys, I Hanski. 2005. Host specialization by *Cotesia* wasps (Hymenoptera: Braconidae) parasitizing species-rich Melitaeini (Lepidoptera: Nymphalidae) communities in north-eastern Spain. *Biological Journal of the Linnean Society* 86: 45-65.

Kawecki, TJ, D Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters* 7: 1225-1241.

Kester, KM. Behavioral and ecological constraints imposed by plants on the insect parasitoid, *Cotesia congregata* (Say), University of Maryland, 1991, 287 p., pp. 1-55.

Kester, KM, P Barbosa. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae). *Journal of Insect Behavior*. 4(6): 727-742.

Kester, KM, P Barbosa. 1992. Effects of postemergence experience on searching and landing responses of the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae), to plants. *Journal of Insect Behavior*. 5(3): 301-320.

Kester, KM, P Barbosa. 1994. Behavioral responses to host foodplants of two populations of the insect parasitoid *Cotesia congregata* (Say). *Oecologia* 99:151-157.

Kolaczan, CR, SB Heard, KA Seagraves, DM Althoff, JD Nason. 2009. Spatial and genetic structure of host associated differentiation in the parasitoid *Copidosoma gelechiae. Journal of Evolutionary Biology* 22: 1275-1283.

Krombein, KV, PD Hurd, DR Smith, BD Burks. 1979. Catalog of Hymenoptera in America North of Mexico. Smithsonian Institution, Washington, D. C.

Lentz, AJ, KM Kester. 2008. Postemergence learning affects sex ratio allocation in a gregarious insect parasitoid, *Journal of Insect Behavior* 21:34-45.

Li, L, HP Wang, C Givens, S Czesnys, B Brown. 2006. Isolation and characterization of microsatellites in yellow perch (*Perca flavescens*). *Molecular Ecology Notes* 7: 600-603.

Lozier, JD, GK Roderick, NJ Mills. 2009. Molecular markers reveal strong geographic, but not host-associated, genetic differentiation in *Aphidius transcaspicus,* a parasitoid of the aphid genus *Hyalopterus. Bulletin of Entomological Research* 99: 83-96.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res 27:209-220.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283.

Price, PW, CE Bouton, P Gross, BA McPheron, JN Thompson, AE Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11: 41-65.

Raymond M, F Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248-249.

Rice, WR. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. *Evolution* 38: 1251-1260.

Rice, WR. 1985. Disruptive selection on habitat preference and the evolution of reproductive isolation: an exploratory experiment. *Evolution* 39: 645-656.

Rice, WR. 1987. Speciation via habitat specialization. *Evolutionary Ecology* 1: 301-314.

Rice, WR, GW Salt. 1988. Speciation via disruptive selection on habitat preference: experimental evidence. *The American Naturalist* 131: 911-917.

Sisson VA, JA Saunders. 1982. Alkaloid composition of the USDA tobacco (*Nicotiana tabacum* L.) introduction collection. Tob Sci 26:117–120

Slatkin M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16:393-430.

Smith, JM. 1966. Sympatric speciation. *The American Naturalist* 100: 637-650.

Stelinski, LL OE Liburd. 2005. Behavioral evidence for host fidelity among populations of the parasitic wasp, *Diachasma alloeum* (Muesebeck). *Naturwissenschaften* 92: 65-68.

Stelinski, LL, KS Pelz-Stelinski, OE Liburd, LJ Gut. 2006. Control strategies for *Rhagoletis mendax* disrupt host-finding and ovipositional capability of its parasitic wasp, *Diachasma alloeum*. *Biological Control* 36: 91-99.

Stireman, JO, III, JD Nason, SB Heard. 2005. Host associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution* 59: 2573-2587.

Stireman, JO, III, JD Nason, SB Heard, JM Seehawer. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. *Proceedings of the Royal Society B* 273: 523-530.

Swofford, DL. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusets.

Tietz, HM. 1972. "An Index to the Described Life Histories, Early Stages and Hosts of the Macrolepidoptera of the of the Continental United States and Canada." Allyn Museum, Sarasota, Vol. 1 and 2, 10412 pp.

Van Nouhuys, S, S Via. 1999. Natural selection and genetic differentiation of behavior between parasitoids from wild and cultivated habitats. *Heredity* 83: 127-137.

Werren, JH. 2003. The evolution of inbreeding in haplodiploid organisms. In NW Thornhill (Ed.), *The Natural History of Inbreeding and Outbreeding* (pp. 42-59). Chicago, IL: The University of Chicago Press.

Whitfield, JB. 2000. Phylogeny of microgastroid braconid wasps, and what it tells us about polydnavirus evolution. In A Austin and M Dowton (Eds.) *Hymenoptera: Evolution, Biodiversity, and Biological Control* (pp. 97-105). Australia: CSIRO Publishing.

Zhou, Y, H Gu, and S Dorn (2005) Polymorphic microsatellite loci in the parasitic wasp *Cotesia glomerata* (Hymenoptera: Braconidae). *Molecular Ecology Notes*. 5: 475-477.

Table 1. Collection data for *Cotesia congregata* from *Manduca sexta* on *Nicotiana tabacum* (MsT) and *Ceratomia catalpae* on *Catalpa bignonioides* (CcC) in the USA mid-Atlantic region in 2006 and 2007. The number of broods collected at each site is indicated in parenthesis.

Site name (# broods)	**Site location**	**Host-Plant Complex**	**Latitude, Longitude**
BLACKSTONE (150)	Blackstone, Virginia	*M. sexta/N. tabacum*	37.081707, -77.975566
CLEMSON (24)	Clemson, South Carolina	*C. catalpae/C. bignonioides*	34.686809, -82.814076
COLMAR (11)	Colmar Manor, Maryland	*C. catalpae/C. bignonioides*	38.936136, -76.948164
LAKESIDE (27)	Richmond, Virginia	*C. catalpae/C. bignonioides*	37.622114, -77.462325
MARLBORO (12)	Upper Marlboro, Maryland	*M. sexta/N. tabacum*	38.861582, -76.774438
NEWTON (120)	Columbia, Virginia	*C. catalpae/C. bignonioides* and *M. sexta/N. tabacum*	37.672979, -78.219928
ROANOKE (2)	Roanoke, Virginia	*C. catalpae/C. bignonioides*	37.504691, -80.109341
TYSON (148)	Columbia, Virginia	*C. catalpae/C. bignonioides*	37.712802, -78.163481

Table 2. Allele frequency distributions across subpopulations of *Cotesia congregata* (MsT_{NEWTON}, and $MsT_{BLACKSTONE}$, CcC_{TYSON}, CcC_{NEWTON}, $CcC_{LAKESIDE}$) and *Cotesia melitaearum* (EA, ED, MDE, MP).

		Subpopulations								
		Cotesia congregata					***Cotesia melitaearum***			
Locus	**Allele length**	**BLACKSTONE MsT**	**NEWTON MsT**	**NEWTON CcC**	**TYSON CcC**	**LAKESIDE CcC**	**EA**	**ED**	**MDE**	**MP**
cco1A	**125**						**89**	**93**	**92**	**12**
	128						**5**	**6**		
	131									
	134						**6**	**1**	**7**	**17**
	137									
	149									
	152									**52**
	155	**1**								
	158	**2**	**2**	**29**	**33**	**44**				**3**
	161		**1**	**50**	**50**	**44**				**7**
	164		**1**	**21**	**12**	**7**				
	167	**3**								**9**
	170	**91**	**95**		**5**	**4**				
	173	**2**	**1**							
cco5a	**83**						**1**			
	86									
	89									**100**
	92									
	95									
	98						**99**	**100**	**100**	
	101									
	104									
	107					**2**				
	110	**1**								
	113	**4**		**6**						
	116	**1**	**3**	**92**	**94**	**87**				
	119	**70**	**81**	**3**	**5**	**7**				
	122	**20**	**12**		**1**	**4**				
	125	**2**	**4**							
	128	**1**								

Table 2, continued.

		Subpopulations								
		Cotesia congregata					*Cotesia melitaearum*			
Locus	Allele length	BLACKSTONE MsT	NEWTON MsT	NEWTON CcC	TYSON CcC	LAKESIDE CcC	EA	ED	MDE	MP
cco27	106						1			
	109						1			100
	112								12	
	115				1					
	118									
	121									
	124						91	100		
	127		2	3						
	130	1	1		1				15	
	133	7	4							
	136	1	1	34	20	48	7		73	
	139									
	142	70	67		3	4				
	145	2	2			2				
	148		5	50	52	28				
	151	10	9	13	22	19				
	154	9	8		1					
cco65A	125								71	
	128								13	
	131									3
	134								16	
	137						2			
	140									
	143						15	19		72
	146						50	34		4
	149						24	38		
	152						4	3		
	155									
	158						5	7		20
	161	2								
	164			8						
	167	1								
	170	32	46			7				
	173	31	32			2				
	176	14	19		2					
	179	11	3		2					
	182			4		12				
	185	3		25	49	38				
	188			4		2				
	191	1		59	43	4				
	194	5			1	2				
	197				3					

Table 2, continued.

Locus	Allele length	Subpopulations								
		Cotesia congregata					*Cotesia melitaearum*			
		BLACKSTONE MsT	NEWTON MsT	NEWTON CcC	TYSON CcC	LAKESIDE CcC	EA	ED	MDE	MP
cco65B	113						2			
	116									
	119									
	122						5			40
	125						2			
	128						33	14	63	
	131						54	86	3	
	134						4			60
	137									
	140									
	143								32	
	146								3	
	149									
	173									
	176		4							
	179									
	182									
	185	6	5		2					
	188	40	43		12	23				
	191	40	29	12	18	7				
	194	10	12	13		5				
	197	4	5	50	50	36				
	200			19	17	7				
	203					7				
	206				1	7				
	209		2	6		4				
	212					2				
	215									
	218					2				

Table 2, continued.

		Subpopulations								
		Cotesia congregata					*Cotesia melitaearum*			
Locus	Allele length	BLACKSTONE MsT	NEWTON MsT	NEWTON CcC	TYSON CcC	LAKESIDE CcC	EA	ED	MDE	MP
cco68	152									22
	155						23	18	100	18
	158						68	70		80
	161						1	7		
	164									
	167									
	170						7	5		
	173									
	176	2	4							
	179	8	4							
	182	3	7		1		2			
	185		1							
	188	13	1	46	39	64				
	191	8	1	36	44	25				
	194	2			15	4				
	197	18	17	7		6				
	200	24	24			2				
	203	11	29	4	13					
	206	5		7						
	209	3	1							
	212	2	8							
	215	2	1							
cot1	294					2				
	297					2				
	300	12	25	11						
	303									
	306	6	2	11						
	309	22	7		1					
	312	20	18							
	315	17	24		1					
	318	5	3		1	2				
	321	2	3		7	2				
	324	2	6	17	20	17				
	327	15	9	17	6	14				
	330		4	11	11	33				
	333				37	10				
	336			33	10	13				
	339				6	6				

Table 3. Measures of population differentiation among subpopulations of *Cotesia congregata* collected from *Manduca sexta* on *Nicotiana tabacum* (MsT_{NEWTON}, $MsT_{BLACKSTONE}$) or *Ceratomia catalpae* on *Catalpa bignonioides* (CcC_{TYSON}, $CcC_{LAKESIDE}$, CcC_{NEWTON}), Φ_{ST} above diagonal (bolded values are significant at $P < 0.001$ and N_em below diagonal (number of migrants per generation based on private alleles method; Slatkin 1985).

	$MsT_{BLACKSTONE}$	MsT_{NEWTON}	CcC_{NEWTON}	CcC_{TYSON}	$CcC_{LAKESIDE}$
$MsT_{BLACKSTONE}$	--	0.01	**0.41**	**0.36**	**0.44**
MsT_{NEWTON}	6.07	--	**0.43**	**0.39**	**0.48**
CcC_{NEWTON}	0.36	0.3	--	0.01	0.01
CcC_{TYSON}	0.52	0.46	1.72	--	0.07
$CcC_{LAKESIDE}$	0.81	0.75	2.64	3.87	--

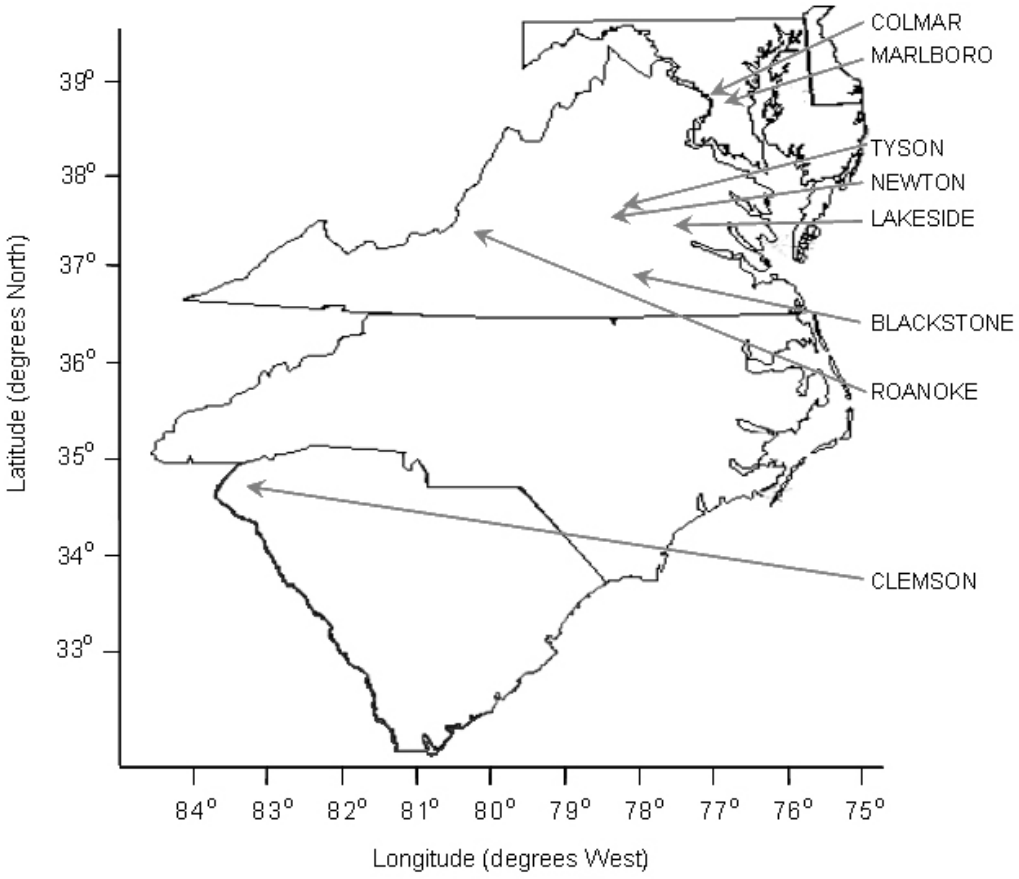
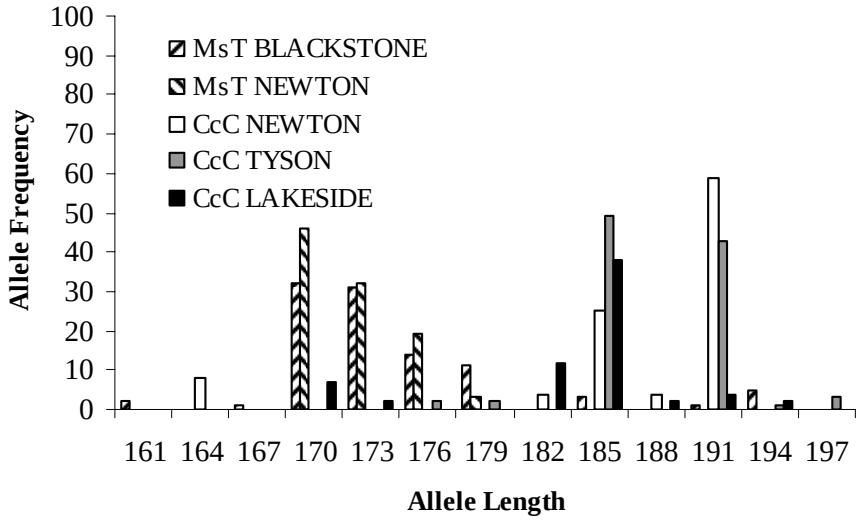
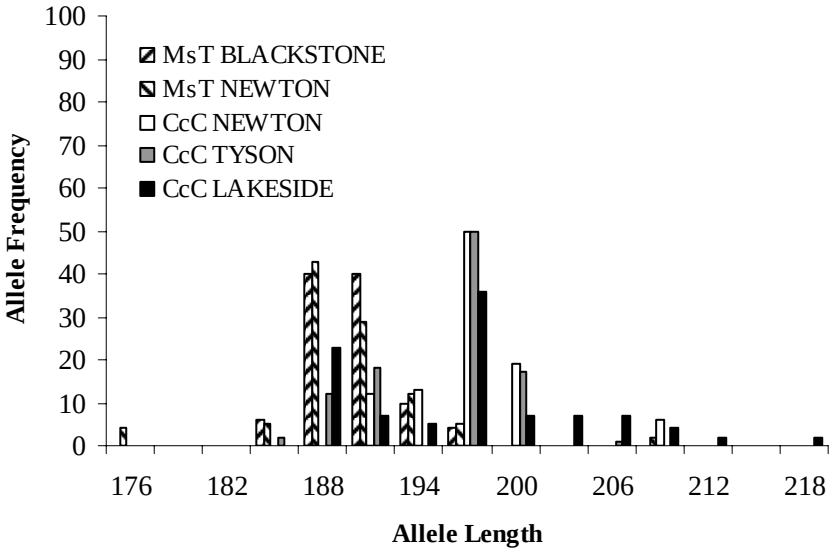


Figure 1. Geographic locations of eight populations of *Cotesia congregata* sampled in Maryland, Virginia, and South Carolina. Details are given in Table 1.



A



B

Figure 2. Allele frequency distribution for (A) locus *cco65A* and (B) locus *cco65B* for *Cotesia congregata* collected from *Ceratomia catalpae* on *Catalpa bignonioides* at three locations and *Manduca sexta* on *Nictoiana tabacum* at two locations.

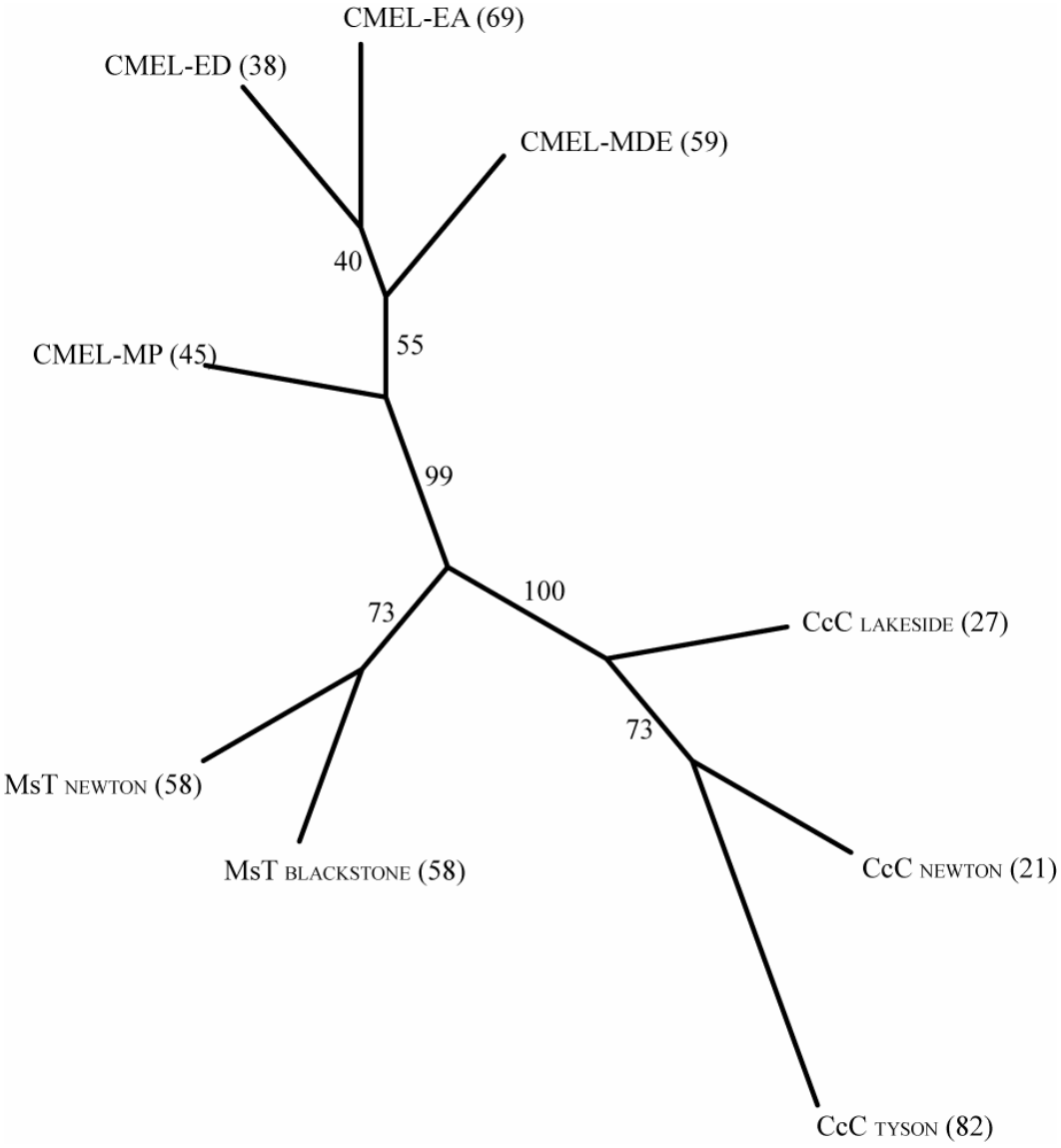


Figure 3. Neighbor-joining consensus tree based on microsatellite data for subpopulations of *Cotesia congregata* collected from *Manduca sexta* on *Nicotiana tabacum* ($MsT_{BLACKSTONE,}$ $MsT_{NEWTON,}$) and *Ceratomia catalpae* on *Catalpa bignonioides* (CcC_{NEWTON}, and CcC_{TYSON}, $CcC_{LAKESIDE}$), and four populations of *Cotesia melitaearum* (CMEL) widely-distributed across Europe (see Kankare *et al.* 2005). Bootstrap values are indicated. Sample sizes are in parentheses. Because data for the *cot1* locus were not available from *C. melitaearum,* this locus was excluded from the phylogenetic analyses.

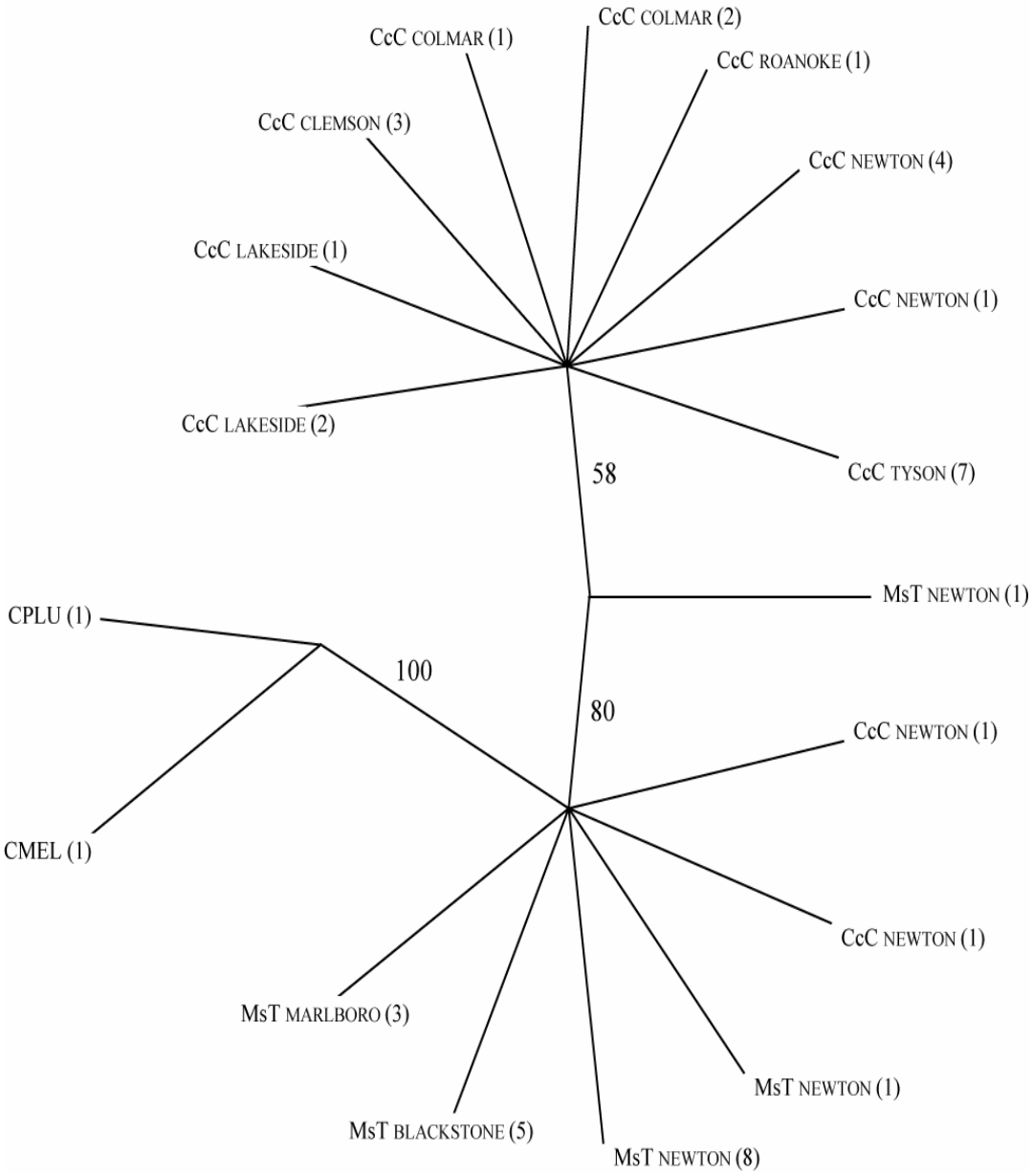


Figure 4. Bootstrapped 50% majority rule consensus tree constructed from mtDNA COI sequences using maximum parsimony for *Cotesia congregata* collected from *Manduca sexta* on *Nicotiana tabacum* (MsT) and *Ceratomia catalpae* on *Catalpa bignonioides* (CcC) at multiple locations across the USA mid-Atlantic region (abbreviations as shown in Table 1). COI sequences from *Cotesia melitaearum* (CMEL) and *Cotesia plutellae* (CPLU) obtained from GenBank were used to represent outgroups.

Are broods of *Cotesia congregata* full-sibling?

Georgia M. Karns

Abstract

Superparasitism occurs when a female lays eggs in a previously parasitized host. The gregarious lifestyle of *C. congregata* may allow for multiple parasitism of the same host larvae in the field. In order to test for the occurrence of superparasitism, or rather whether broods of *C. congregata* are full-sibling, we compared sizes of broods with known superparasitism to field-collected broods. We also looked at allele diversity among males within broods. Though we can not distinguish between superparasitism and multiple mating, broods of *C. congregata* are not full-sibling.

Introduction

Superparasitism occurs when a female lays eggs in a previously parasitized host. The gregarious lifestyle of *C. congregata* may allow for multiple parasitism of the same host larvae in the field (Dorn and Beckage 2003). In fact, superparasitism is regularly observed in the laboratory colony of *C. congregata* maintained at Virginia Commonwealth University. Previously, Gu *et al.* (2003) compared brood sizes of *Cotesia glomerata* (broods are defined as all individuals developing inside a single host larva) from field-collected host larvae and larvae that were parasitized in the laboratory one, two, three, four, or five times. The distribution of field-collected brood sizes was significantly different from the distribution of brood sizes from singly parasitized host larvae in the laboratory, and the field-collected brood sizes exceeded the maximum brood size from singly parasitized hosts (Gu *et al.* 2003). We compared the brood sizes, with additional genetic

data, to determine if broods of *Cotesia congregata* (Say) were full-sibling, or if superparasitism or multiple mating had occurred.

Materials and Methods

Cotesia congregata were collected from study sites and reared as described in Chapter 1. Broods that had not egressed prior to collection or had not been hyperparasitized were counted and sexed. In addition, a laboratory colony of *C. congregata* provided individuals for parasitizing third instar *Manduca sexta* larvae. Third instar larvae of *M. sexta* were parasitized once and twice by a single female, and twice by two different females. Resulting broods, defined as all individual wasps emerging as adults from a single host larva, were counted and sexed. In addition, five microsatellite loci (*cco5A, cco27, cco65A, cco68, cot1)* were amplified as described in Chapter 1 from varying numbers of male and female individuals within each brood. The number of alleles present was counted and compared among broods. If males originating from the same brood have more than two alleles, then we can infer either the occurrence of superparasitism or multiple mating by female *C. congregata.*

Results and Discussion

For *C. congregata,* the median brood size from singly parasitized hosts in the laboratory was 113, while the median brood size from field-collected individuals was 34

for 2006 and 53 for 2007. Hosts parasitized in the laboratory two and three times had median brood sizes of 145 and 135, respectively. Hosts parasitized by two different females had a median brood size of 134. The maximum brood size from a single oviposition was 248 and from two females ovipositing was 278. The field-collected wasp brood sizes did not exceed the maximum brood sizes from the laboratory regardless of how many times the larvae were parasitized. Therefore, we can not make any inferences regarding brood sizes and superparasitism in *C. congregata*.

As is predicted by the theory of local mate competition (Hamilton 1967), the sex ratio (% female) decreased with multiple parasitism. The median sex ratio of broods from a single oviposition was 71% and for broods from two females ovipositing was 46%. Therefore it is possible that the second ovipositing female is laying a higher proportion of males.

At all loci, more than two alleles amplified from males across multiple field-collected broods (Table 4), therefore we can conclude that either superparasitism or multiple mating is occurring in wild populations of *C. congregata*. Though multiple mating is possible, superparasitism is more likely, in that for most *Cotesia* spp. studied, females mate once immediately after emergence before dispersing and this has been previously observed for *C. congregata* (Kester and Barbosa 1991). In any case, it is unlikely that broods of *C. congregata* are full-sibling.

List of References

Dorn, S and N Beckage. 2003. Superparasitism in gregarious hymenopteran parasitoids: ecological, behavioral and physiological perspectives. *Physiological Entomology* 32: 199-211.

Gu, H, Q Wang, and S Dorn. 2003. Superparasitism in *Cotesia glomerata*: response of hosts and consequences for parasitoids. *Ecological Entomology* 28: 422-431.

Hamilton, WD. 1967. Extraordinary sex ratios. *Science* 156: 477-488.

Kester, KM, P Barbosa. 1991. Postemergence learning in the insect parasitoid, *Cotesia congregata* (Say) (Hymenoptera: Braconidae). *Journal of Insect Behavior*. 4(6): 727-742.

Table 4. Numbers of alleles of five microsatellite loci amplified from males in broods collected from *Manduca sexta* on *Nicotiana tabacum* and *Ceratomia catalpae* on *Catalpa bignonioides.* Numbers in parentheses are the total number of males used.

	Host	*M. sexta*			*M. sexta*				*C. catalpae*			
	Host-plant	*N. tabacum*			*N. tabacum*				*C. bignonioides*			
	Site	BLACKSTONE			NEWTON				NEWTON			
	Brood Number	202	220	235	202	218	223	244	54	206	210	215
Locus	*cco5a*	3 (13)	--	--	2 (8)	--	4 (10)	--	--	--	--	1 (13)
	cco27	--	3 (15)	2 (13)	--	3 (15)	--	--	2 (13)	1 (7)	1 (8)	--
	cco65a	3 (13)	3 (15)	--	2 (3)	--	3 (11)	--	--	2 (5)	2 (8)	2 (10)
	cco68	5 (9)	--	--	--	--	4 (12)	--	--	--	--	1 (16)
	cot1	5 (9)	2 (7)	--	1 (6)	--	3 (15)	6 (13)	--	2 (6)	2 (3)	2 (10)

Table 4, continued.

	Host	*C. catalpae*			*C. catalpae*		
	Host-plant	*C. bignonioides*			*C. bignonioides*		
	Site	TYSON			LAKESIDE		
	Brood Number	272	277	287	221	224	225
Locus	*cco5a*	--	1 (16)	--	1 (16)	--	--
	cco27	2 (14)	--	3 (15)	3 (15)	3 (15)	2 (9)
	cco65a	2 (12)	--	--	--	--	2 (10)
	cco68	--	2 (15)	--	1 (14)	--	--
	cot1	3 (13)	6 (11)	--	3 (14)	--	3 (8)

VITA

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