Parasitoid Communities And Genetic Structure: Host Plant Does Not Matter

PhD thesis presented by

Violaine Jourdie

Université de Neuchâtel, Institut de Biologie

PhD supervisor:
Prof Ted Turlings

Defended on September 24, 2008,
in front of a jury composed of:

Dr. Nadir Alvarez, Université de Neuchâtel
Dr. Pierre Franck, INRA Avignon, France
Prof. Beatrice Lanzrein, Universität Bern
Dr. Betty Benrey, Université de Neuchâtel

Photo: M. Held

Université de Neuchâtel unine

FACULTE DES SCIENCES
Secrétariat-Décanat de la faculté
- Rue Emile-Argand 11
- CP 158
- CH-2009 Neuchâtel

IMPRIMATUR POUR LA THESE

Parasitoid communities and genetic structure: host plant does not matter

Violaine JOURDIE

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel,
sur le rapport des membres du jury

Mmes B. Benrey, B. Lanzrein (Berne),
MM. T. Turlings (directeur de thèse), N. Alvarez
et P. Franck (Avignon F)

autorise l'impression de la présente thèse.

Neuchâtel, le 7 novembre 2008

Le doyen :
F. Kessler

UNIVERSITE DE NEUCHATEL
FACULTE DES SCIENCES
Secrétariat - décanat de la faculté
Rue Emile-Argand 11 - CP 158
CH-2009 Neuchâtel
Felix Kessler

■ Téléphone : +41 32 718 21 00 ■ E-mail : secretariat.sciences@unine.ch ■ www.unine.ch/sciences

Keywords: parasitoid, Hymenoptera, Braconidae, Ichneumonidae, population genetics, communitie structure, genetic structure, *Spodoptera frugiperda*, fall armyworm, *Chelonus insularis*, *Campoletis sonorensis*, host plant, maize, sorghum, microsatellites, phylogeography

Mots clés: parasitoïde, Hymenoptera, Braconidae, Ichneumonidae, génétique des populations, structuration des communautés, *Spodoptera frugiperda*, légionaire d'automne, *Chelonus insularis*, *Campoletis sonorensis*, plante-hôte, maïs, sorgho, microsatellites, phylogéographie

Abstract

Plant-insect interactions have long been studied and reveal intricate mechanisms. Plants are capable of defending themselves both directly by poisoning insect herbivores and indirectly by emitting volatile compounds that are used by the natural enemies to localize their host. In response, insects have evolved strategies to defeat plant defense mechanisms. Because insect pests are affected by plant signals, their natural enemies also bear these effects. As host plant can affect the physiology and behavior of parasitoids, it may also contribute to shaping their population genetic structure. This thesis mainly aimed to investigate the effects of host plant on the population genetics of parasitoids of the fall armyworm (FAW), *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), using microsatellite markers. The FAW is one of the New World's most devastating pests and it attacks several economically important crops as well as grasses. It is commonly controlled by chemical insecticides. However, as it is attacked by numerous parasitoids species, and in order to limit the use of toxic pesticides, biological control is a safer alternative mean of management for this pest. The success of biological control relies on a good knowledge of the system, hence the importance of investigating population genetics and communities structure. This study focused on two primary parasitoids of *S. frugiperda*, *Chelonus insularis* Cresson (Hymenotera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae). It was conducted on two host plants, maize and sorghum, in Mexico where maize originated and where sorghum was introduced barely over a century ago.

Due to difficulties encountered during sampling, whereby immature parasitoids did not complete their life cycle and therefore could not be morphologically identified, a technique was first developed, as a cheaper and faster alternative to sequencing, to molecularly assign parasitoid larvae to species. This simple but nonetheless efficient technique consists in amplifying DNA through polymerase chain reaction and digesting it with a cocktail of restriction endonucleases in order to obtain a species specific pattern when the digestion product is run on an agarose gel. With this technique, we could get an accurate estimation of which species were collected and in what proportions, which allowed to study parasitoid community structure.

The study of population genetics first required the development and optimization of reliable molecular markers. Fifteen and 13 highly polymorphic microsatellites were respectively isolated from *C. sonorensis* and from *C. insularis*. These markers were used to investigate fine-scale genetic structure in Mexican populations. We could discern a regional effect, but host plant seems to play no role in shaping the populations genetic structure. High levels of admixture indicate that gene flow between populations is considerable.

Finally, genetic structure was investigated at larger scale through a phylogeography using sequences of mitochondrial and nuclear marker genes. The lack of local structure was confirmed for both species. We found however evidence for North-South migration through a single colonization event in *C. insularis*, and a cryptic species distributed in Canada was discovered. Dispersal of these insects seems to be largely driven by wind as suggested by genetic similarities between geographically very distant individuals.

Remerciements

Après plus d'un quart de siècle passé ''à l'école'', j'arrive enfin au bout d'un long chemin! Une telle entreprise ne peut s'accomplir seul et la liste des personnes qui ont de près ou de loin contribué à cette expérience est longue.

Je remercie Ted Turlings de m'avoir accueillie dans son laboratoire et de m'avoir permis de mener à bien ce projet. Merci également à Betty Benrey d'avoir initié ce projet.

J'éprouve une immense gratitude envers Nadir Alvarez qui m'a fait preuve d'un soutien technique et moral inconditionnel durant les trois dernières années de ma thèse. Nadir, tu as été le roc sur lequel j'ai pu m'appuyer dans ce laboratoire et je t'en suis immensément reconnaissante. A tes côtés, j'ai appris une multitude de choses. Sans ton aide précieuse, ce projet n'aurait probablement jamais abouti.

Je remercie Beatrice Lanzrein et Pierre Franck d'avoir accepté de faire partie de mon jury. Je remercie également Pierre pour son aide et ses conseils quant à l'analyse des données.

Un grand merci également aux personnes au Mexique ayant collaboré avec moi: Trevor Williams, Jaime Molina-Ochoa, David Bergvinson qui m'ont assitée dans l'organisation et la logistique des voyages de terrain, mais aussi tous les étudiants sur place qui ont participé aux récoltes. Je remercie aussi Silverio Garcia Lara pour son accueil chaleureux et d'avoir été mon interprète au tout début avant que je ne me familiarise avec l'espagnol.

Un merci spécial à Ivan Hiltpold qui m'a accompagnée sur le terrain. Nous avons supporté ensemble les affres des méchantes petites bactéries mexicaines qui nous compliquaient tant la vie!

Merci à Natacha Schneiter et Brigitte Cattin, nos fidèles secrétaires.

Je n'oublie pas non plus Josiane Pont, notre bibliothécaire, toujours tellement efficace pour nous trouver les publications et ce toujours avec le sourire.

Je garderai un excellent souvenir de toutes ces heures passées avec mes collègues de bureau, Yann Triponez et Nicolas Margraf. Merci les garçons pour les rigolades et les moments tellement agréables passés ensemble qui aidaient largement à supporter le stress de la thèse! Gros bisous!

Merci à toute l'équipe du laboratoire d'Entomologie Evolutive (qui a changé de nom et s'est scindé depuis, mais c'est plus simple comme ça!), plus spécialement Anahi avec sa bonne humeur perpétuelle et contagieuse, Matthias Held pour son aide avec les statistiques… et les ordinateurs! Merci à Neil pour son aide en laboratoire pendant les derniers mois de cette thèse. Merci à Sarah, pour les bons moments passés ensemble.

Merci Marie-Eve pour ta présence et ton soutien.

Je tiens à remercier Karina Mohr, Virginie Matera et Mary-Alix Kaczmarek pour les heures passées ensemble les mains dans l'argile. La poterie aura été pour moi plus qu'un loisir, une vraie thérapie qui me permettait d'évacuer le stress et le trop plein d'énervement. Merci aussi, Virginie, pour ton chaleureux accueil et tous les agréables moments passés ensemble durant cette fin de thèse.

Je remercie aussi mes fidèles amis, Marie-Hélène Mignerat et Guillaume Blanchard. Même si l'on se voit moins souvent maintenant, rien ne change et ça fait chaud au cœur de savoir que vous êtes là.

Je remercie mes parents de m'avoir soutenue tout au long de mes études et d'avoir toujours cru en moi.

Merci à Elvire, ma sœur, à Bertrand, mon beau-frère, à Andéol, mon neveu, et à Aziadé, ma nièce, pour tous les week-ends passés ensemble loin des pipettes et des parasitoïdes!

Un grand merci à mes beaux-parents, Charles et Marguerite, pour les agréables week-ends dépaysants et reposants passés chez vous, en Alsace.

Le dernier mais non le moindre, merci à toi Stéphane pour ton amour, ton soutien et ta compréhension.

Contents

General introduction

Introduction

For over fifty years, the use of toxic chemicals has prevailed as a pest control strategy (Lewis *et al.,* 1997). Safety problems and ecological disruptions continue to ensue, and there are renewed appeals for effective, safe and economically acceptable alternatives. Among alternative methods for controlling pests is biological control, which uses natural enemies to manage populations of pest organisms. The success of biological control heavily relies on a good knowledge of the system, more specifically on a good understanding of the interactions within the system. Most insect species are attacked by a number of natural enemies, among which parasitoids play a major role. Parasitoid insects, mostly wasps, lay their eggs on or into the body of preimaginal stages (eggs, larvae or pupae) of other insects (Godfray, 1994b; Quicke, 1997). When early stages of endoparasitoids have escaped or outcome immune rejection of the host, they develop as parasites and consume the tissues of their host until it dies, then pupate and emerge as free living adults. Because successful parasitization always results in host death, parasitoids constitute an important (and perhaps the first) regulator of insect populations (Hawkins *et al.,* 1997). Thus, they behave as powerful biocontrol agents and have stimulated extensive research in population dynamics (Murdoch and Briggs, 1996; Hassell, 2000), behavioral ecology (Godfray, 1994b; Godfray and Shimada, 1999) and evolutionary biology (Boulétreau, 1986; Kraaijeveld *et al.,* 1998; Dupas *et al.,* 2003).

Considering that crop plants are active components of multitrophic level interactions, a total systems approach to pest management is crucial. It has long been known that plant traits have important impacts on both herbivores and their natural enemies. The increasing number of studies on the interactions between plants and the natural enemies of herbivores attacking these plants has revealed an astonishing sophistication. In fact, when under attack by a herbivore, plants start emitting volatiles through a systemic response, which attract the natural enemies of these herbivores. This mechanism is known as indirect defense. This sophistication is most apparent in the specificity of the interactions as plants may respond differently to different herbivores and the natural enemies are able to distinguish among these differences (Sabelis and Vandebaan, 1983; Takabayashi *et al.,* 1995; De Moraes *et al.,* 1998; Powell *et al.,* 1998). Not only herbivory, but also egg deposition by herbivores can lead to the emission of volatile signals that attract natural enemies (Meiners *et al.,* 2000; Hilker and Meiners, 2002). There is even evidence to suggest that plants selectively employ direct and indirect defenses depending on which herbivore feeds on them (Kahl *et al.,* 2000). These plant signals can be expected to affect multiple interactions within the entire food webs (Janssen *et al.,* 1998; Sabelis *et al.,* 1999).

The importance of plants for parasitoids performance

Several behavioral studies have shown cues emanating from the plant on which their host feeds are more informative and more frequently used by parasitoids than cues emanating from their host itself (Turlings *et al.,* 1991; McCall *et al.,* 1993; De Moraes and Mescher, 1999). Moreover, studies conducted with lima beans, corn, cabbage and cotton have demonstrated that plants are actively involved in the production and release of the chemicals used by parasitoids to localize their host (Dicke and Sabelis, 1988; Dicke and Sabelis, 1989; Turlings *et al.,* 1990; Takabayashi *et al.,* 1991; Turlings *et al.,* 1991; Agelopoulos and Keller, 1994; Mattiacci *et al.,* 1994; Dicke, 1995; Turlings *et al.,* 1995). Studies on the mechanisms of volatile emission revealed that chemical compounds of the herbivore's regurgitant were responsible for the induction of volatiles emitted by the host plant (i.e. the plant fed upon by the herbivore host) (Mattiacci *et al.,* 1995; Alborn *et al.,* 1997). Apart from

being emitted in large quantities through a systemic response (Turlings and Tumlinson, 1992; Dicke *et al.,* 1993; Cortesero *et al.,* 1997), the released odor cues are very informative for parasitoids. Indeed, the blend of herbivore-induced volatiles is specific to the plant species (Turlings *et al.,* 1993), to the herbivore species (Dicke, 1995; De Moraes *et al.,* 1998) or to the different instars of the same herbivore (Takabayashi *et al.,* 1995).

Plants have evolved a wide array of chemical defenses that can strongly affect herbivores. When attacking a host plant, insect herbivores are confronted with plant secondary compounds that can affect them in various ways. Some compounds have been shown to repel insects or inhibit feeding, whereas others poison insects or reduce their ability to digest food (Giamoustaris and Mithen, 1995; van Dam *et al.,* 2000). In turn, herbivores have evolved various strategies to overcome these defense mechanisms. Many toxic compounds are detoxified by physiological means (Nitao, 1989; Berenbaum and Zangerl, 1992; Ode *et al.,* 2004), but the negative effect of defense compounds can also be avoided by means of behavioral adaptations (Futuyma, 1983; Evans *et al.,* 2000). Some specialized insects have even evolved a strategy of sequestration of toxic compounds produced by plants for their own defense against natural enemies (Futuyma, 1983; Rowell-Rahier *et al.,* 1991; Ehmke *et al.,* 1999; Ode *et al.,* 2004).

Because of the intimate trophic interactions between immature parasitoids and their hosts, plant chemistry indirectly affects the fitness of parasitoids (Bottrell *et al.,* 1998; Turlings and Benrey, 1998). The survival, development, size, fecundity and sex ratio of herbivores parasitoids are affected by plant chemistry (Vinson and Iwantsch, 1980; Godfray, 1994a). The effects of sequestered plant secondary compounds on the parasitoids have been studied in the case of cultivated tomato and tobacco and their associated herbivores and parasitoids (Thurston and Fox, 1972; Campbell and Duffey, 1979, 1981; Barbosa and Saunders, 1985; Barbosa *et al.,* 1986; Duffey *et al.,* 1986; Kester and Barbosa, 1991). The development time, survival and adult size of the ichneumonid *Hyposoter exiguae* is altered by the concentration of tomatin, an allelochemical present in tomato plants and sequestered by the noctuid *Heliothis zea* (Campbell and Duffey, 1979, 1981). Similarly, high levels of nicotine in the diet of the tobacco hornworm, *Manduca sexta*, decrease the survivorship of the parasitoid *Cotesia congregata* (Thurston and Fox, 1972)

The quality of an herbivore as a host for a parasitoid depends heavily on the plants or plant parts that they feed on. Variation in plant nutritional quality due to genetic or environmental factors may also affect parasitoids indirectly by affecting the host's suitability and vulnerability (Benrey and Denno, 1997; Benrey *et al.,* 1998). There is also evidence that variation in plant nutritional and chemical quality can affect the performance of interacting organisms across several trophic levels (Harvey *et al.,* 2003). For example, in a study of a four-trophic level system consisting of the solitary secondary hyperparasitoid *Lysibia nana* (fourth trophic level), its primary endoparasitoid host, *Cotesia glomerata* (third trophic level) and the herbivore host *Pieris brassicae* (second trophic level) feeding on two cruciferous plants, *Brassica oleracae* and *B. negra* (first trophic level), which vary in glucosinolates concentrations, plant variability was found to have no effect on the second and third trophic levels but drastically affected the performance of the fourth trophic level (Harvey *et al.,* 2003). The poor correspondence between the performance of the second, third and fourth trophic levels can be explained by their host specialization. Indeed, *L. nana* has a broad host range (Harvey *et al.,* 2003) while *Cotesia glomerata* only attacks pierid butterflies feeding on plants containing glucosinolates (van Loon and Schoonhoven, 1999), and *P. brassica* is highly specialized on crucifers (Schoonhoven, 1967). Consequently, *L. nana* is likely to be less adapted to the highly chemically defended *B. nigra* than are the specialized *P. brassicae* and *C. glomerata*. Another explanation for

the poor correspondence reported between the performance of parasitoids and their host on certain plant species (Englishloeb *et al.*, 1993) could be that variation in plant quality can interfere with the herbivore's immune response thereby affecting the host's ability to encapsulate parasitoid eggs (Cheng, 1970; Rhoades, 1983; Benrey and Denno, 1997). Indeed, the effectiveness of the encapsulation reaction depends on the physiological condition of the host, which can be weakened by poor nutritive quality or the presence of toxins (Muldrew, 1953; Salt, 1956, 1964; van den Bosch, 1964; Vinson and Barbosa, 1987).

Herbivore's growth rate and development depend on the nutritional quality of the plant they feed on. Since prolonged development may result in greater mortality caused by natural enemies (Clancy and Price, 1987), plant nutritional quality can indirectly affect parasitoids as herbivores feeding on different plants that vary in nutritional quality may grow at different rates.

As described above, plants both have positive and negative effects on insects. As food webs are not restricted to three trophic levels, plant toxic compounds could in the end benefit parasitoids in some cases. Indeed, parasitoids are often attacked by hyperparasitoids and may therefore benefit from sequestered secondary plant compounds for their own defense. Sequestration of plant secondary compounds by parasitoids through their herbivore host was first proposed by Meiners and Hilker (1997) and evidence for such process was reported for the first time by Harvey *et al*. (2003).

Coevolutionary processes seem to have largely shaped plant-insect interactions. Plant traits can affect the performance and behavior of parasitoids in many ways. Differential performance and behavior may reflect on the genetic structure of parasitoids. Therefore, parasitoid population genetic structure may be affected by the plant fed upon by the herbivorous host.

Such effects can be investigated by means of molecular tools to measure several population genetics parameters.

Population genetics and its use for studies on parasitoid ecology

Population genetics represents a synthesis of Mendelian genetics and Darwinian evolution, and is concerned with the mechanisms that cause allele frequencies to change from one generation to the next. The Hardy-Weinberg equilibrium principle is a null model that provides the conceptual framework for population genetics (Hardy, 1908; Weinberg, 1908). It shows that under simple assumptions (no selection, no mutation, no migration, no genetic drift and random mating) allele frequencies do not change.

When any one of the first four assumptions is violated, allele frequencies may change across generations. Selection, mutation, migration and genetic drift are thus the four mechanisms of evolution. Nonrandom mating does not cause allele frequencies to change, and is thus not a mechanism of evolution. It can, however, alter genotype frequencies and thereby affect the course of evolution.

Allele and genotype frequencies can be measured in real populations. Thus, one can tests whether allele frequencies change across generations and whether the genotype frequencies conform to the Hardy-Weinberg equilibrium expectations. If either of the conclusions of the Hardy-Weinberg analysis is violated, it means that one or more of the assumptions does not hold. The nature of the deviation from Hardy-Weinberg expectations does not, by itself, identify the faulty assumption. We can, however, often infer which mechanisms of evolution are at work based on other characteristics of the population under study.

Selection occurs when individuals with different genotypes differ in their success at getting copies of their genes into future generations. It is a powerful force of evolution. Selection can cause allele frequencies to change from one generation to the next, and can take genotype frequencies away from Hardy-Weinberg equilibrium. Some patterns of selection tend to drive some alleles to fixation and

others to loss; other patterns of selection serve to maintain allelic diversity in the populations.

Alone, mutation is a weak evolutionary force. Mutation does, however provide the genetic variation that is the raw material for evolution. In some cases, a steady supply of new mutant alleles can balance selection against some same alleles, and thereby serve to hold allele frequencies at equilibrium.

Migration, in its evolutionary meaning, is the movement of alleles from one population to another. When allele frequencies are different in the source population than in the recipient population, migration causes the recipient population to evolve. As a mechanism of evolution, migration tends to homogenize allele frequencies across populations. In doing so, it may tend to eliminate adaptive differences between populations that have been produced by natural selection.

Genetic drift is evolution that occurs as a result of sampling error in the production of a finite number of zygotes from a gene pool. Just by chance, allele frequencies can change from one generation to the next in finite populations. Genetic drift is more dramatic in smaller populations than in larger ones. Over many generations, drift results in an inexorable loss of genetic diversity. If some of the alleles that become fixed are deleterious recessive, genetic drift can result in a reduction of the fitness of individuals in the population.

Nonrandom mating does not directly change allele frequencies and is thus not strictly speaking a mechanism of evolution. However, nonrandom mating does influence genotype frequencies. For example, inbred populations have more homozygotes and fewer heterozygotes than otherwise comparable populations, in which mating is random. An increase in homozygosity often exposes deleterious recessive alleles, and results in a reduction in fitness know as inbreeding depression.

Molecular genetic markers, initially protein based (especially allozymes) in the 1970s, and more recently DNA-based markers, have become more and more widely used in the field of molecular ecology (Avise, 1994). They have proved very useful in investigating biological parameters such as basic population structure (Nunez *et al.*, 2006), movement and migration (Meagher and Nagoshi, 2004), overwintering (Kimura *et al.*, 2002), reproduction (Nagoshi *et al.*, 2006), sex ratio (Cheng *et al.*, 2006) and host preference (Yourman and Luster, 2004). These markers have provided novel information on founder effects, bottlenecks, migratory range, host switches and preference to name only a few of the applications. Many of these molecular genetic markers, if not most, would have been unimagined prior to the advent of the polymerase-chain reaction (PCR) (Saiki *et al.*, 1985; Saiki *et al.*, 1988) which allows marker amplification from a very small amount of DNA template.

Molecular markers allow fine resolution of ecological interactions to be elucidated. In addition, and accepting that ecological interactions represent the first level of evolutionary process, the organisms concerned are continually subject to the likelihood, if not the reality, of selection and genetic drift and hence adaptation at one or more trophic levels, so that these markers also have taxonomic significance. They allow discrimination of recently diverged taxa (e.g. biotypes, races, subspecies, sibling species) cryptic species, and immature life stages that present intractable morphological differences (Claridge *et al.*, 1997).

The molecular genetic markers at disposal are numerous and various. These include protein markers, principally allozymes (Richardson *et al.*, 1986; Berlocher, 1999), but more commonly nowadays DNA markers, including DNA sequencing, microsatellites, AFLPs, RFLPs, RAPDs and SNPs.

Sequencing usually relies on regions of the nuclear ribosomal RNA cluster (rDNA), e. g., 18S (Sanchis *et al.*, 2000) and 28S (Mardulyn and Whitfield, 1999; Sanchis *et al.*, 2000), and mitochondrial DNA genes (mtDNA), including 12S and 16S (Jarvis and Whiting, 2006; Triponez *et al.*, 2007), cytochrome oxidase subunit I (COI) (Machado

et al., 1996), cytochrome oxidase subunit II (COII) (Despres *et al.*, 2002) and cytochrome B (Kerdelhue *et al.*, 1999). Less commonly, other DNA markers such as nuclear DNA coding genes, e.g., the elongation factor 1α (Belshaw and Quicke, 1997) and the DNA internal transcribed spacer region (ITS) (Thomson *et al.*, 2003). Sequencing is most commonly used in phylogenetics. In the case of parasitic Hymenoptera, a number of studies have been performed primarily using rDNA (18S, 28S, and 16S) and mtDNA markers (COI, Cytochrome *b* and nicotinamide adenine dinucleotide dihydrogenase subunit 1 (NADH1)) (Dowton and Austin, 1998; Quicke and Belshaw, 1999; Smith *et al.*, 1999; Belshaw *et al.*, 2000; Kambhampati *et al.*, 2000; Sanchis *et al.*, 2000; Babcock *et al.*, 2001; Schmidt *et al.*, 2001). The use of mitochondrial DNA has also been popular in population genetic studies due to the extensive intraspecific polymorphism it exhibits. This has allowed the spatial distribution of genealogical lineages to be analyzed and has led to the birth of "phylogeography" as a formal discipline (Avise *et al.*, 1987; Avise, 1998, 2000). Numerous such studies have recently been reported, including braconid parasitoids (Althoff and Thompson, 2001; Hufbauer *et al.*, 2004), honeybees, *A. mellifera* (de la Rua *et al.*, 2001), bumblebees, *Bombus* spp. (Estoup *et al.*, 1996; Widmer *et al.*, 1998), and the yucca moth, *Prodoxus quiquepunctellus* (Althoff and Thompson, 2001).

Microsatellites are codominant genetic markers consisting of tandem repeats of 1-6 nucleotides found at high frequency in the nuclear genomes of most taxa. These regions, which are often referred to as simple sequence repeat (SSR) or simple tandem repeat (STR) loci, have long been recognized as a major source of genetic variation (Tautz *et al.*, 1986; Tautz, 1989; Weber and May, 1989). As a result of the widespread use of microsatellites, our understanding of their mutational behavior, function, evolution and distribution in the genome and across taxa is increasing rapidly (Ellegren *et al.*, 1997; Li *et al.*, 2002). SSRs constitute a large fraction of noncoding DNA and are relatively rare in protein-coding regions. A microsatellite locus typically varies in length between 5 and 40 repeats, but longer strings of repeats are possible. Microsatellites can be classified on the basis of the repeat motif length (i.e. dinucleotide, trinucleotide, tetranucleotide, etc.) and motif contiguity (e.g. perfect or interrupted). Dinucleotide, trinucleotide and tetranucleotide repeats are the most common choices for molecular genetic studies. Dinucleotide repeats account for the majority of microsatellites for many species (Wang *et al.*, 1994; Schug *et al.*, 1998). Trinucleotides and hexanucleotides are the most likely repeat classes to occur in coding regions because they do not cause a frameshift (Toth *et al.*, 2000). Mononucleotide repeats are less reliable because of problems with amplification. In contrast to the triplet SSRs, di- and tetranuclotide repeats are much less frequent in coding regions than in non-coding regions. A number of genes, however, was found with dinucleotide SSRs in the untranslated 5' and/or 3' ends (e.g. (Liu *et al.*, 1999). The potential size expansion of di- or tetranucleotide SSRs at the 3' or 5' regions and introns could lead to disruption of the original protein and/or formation of new genes by frame shift (Bachtrog *et al.*, 1999; Liu *et al.*, 1999). The differences between coding and non-coding SSR frequencies seem to arise from specific selection against frame-shift mutations in coding regions resulting from length changes in nontriplet repeats (Liu *et al.*, 1999; Dokholyan *et al.*, 2000). Nevertheless, 14% of all proteins contain repeated sequences, with a three times higher abundance of repeats in eukaryotes compared to prokaryotes (Marcotte *et al.*, 1999). Prokaryotic and eukaryotic repeat families are clustered to nonhomologous proteins. This indicates that repeated sequences emerged after these two kingdoms had split. The eukaryotes incorporating more repeats may have an evolutionary advantage of faster adaptation to new environments (Kashi *et al.*, 1997; King and Soller, 1999; Marcotte *et al.*, 1999; Wren *et al.*, 2000).

Characteristics of the repeat motif (type, length, and contiguity) appear to affect the rate of mutation and levels of allelic variation. Interruptions within the core sequence seem to stabilize arrays of repeats, rendering levels of polymorphism for microsatellites with interrupted repeats less variable than loci with pure repeats (Richards and Sutherland, 1994; Pepin *et al.*, 1995; Petes *et al.*, 1997). Levels of allelic diversity are also correlated with repeat length (Weber, 1990), as loci with longer repeats are generally more polymorphic than loci composed of short motifs (Beckmann and Weber, 1992).

It is assumed that the great majority of microsatellite loci lies outside of genes and are selectively neutral. However, loci occurring within or adjacent to expressed gene regions might be under selection. For loci present within noncoding regions (i.e. introns), the extent to which these loci are under selection is unclear. Moreover, for a given number of repeats, the tetranucleotide locus is longer than the dinucleotide. This may affect the selective pressure if the stability of meiotic processes depends on the absolute size (in base pairs) of the target region. Loci with longer repeat units seem to experience stronger selection against the difference in size, especially in genome regions with high recombination rates (Samadi *et al.*, 1998).

Mutation rates for microsatellites are among the highest reported, with rates estimated at 10^{-2}-10^{-6} event per locus per generation (Dallas, 1992; Weber and Wong, 1993; Dib *et al.*, 1996). Many changes of repeat numbers at SSR loci are caused by slip-strand mispairing errors during DNA replication (Eisen, 1999) which escape proof reading and mismatch DNA repair. A mutation model specific to microsatellites, the stepwise mutation model (SMM), was proposed to explain SSRs mutational mechanisms. As opposed to the traditional infinite allele model (IAM) in which every mutation event creates a new allele (whose size is dependent from the progenitor allele), the SMM adds or subtracts one or more repeat units from the string of repeats at some constant rate to mimic the process of errors during DNA replication that generates mutations, creating a Gaussian-shaped allele frequency distribution (Ellegren, 2004). However, non-stepwise mutation processes are also known to occur, including point mutations and recombination events such as unequal crossing over and gene conversion (Richard and Paques, 2000). While debate continues about the prevalence of non-stepwise mutation for microsatellites, the current consensus is that the frequency and effects are usually low, and stepwise mutation appears to be the dominant force in creating new alleles in the few model organisms studied to date (Eisen, 1999; Ellegren, 2004), and microsatellite mutation models are not strict SMM nor IAM, as the mutation model also depends on the microsatellite sequence.

The study system and the aim of the study

The fall armyworm (FAW), *Spodoptera frugiperda*, is a voracious polyphagous pest in the Americas and it attacks many economically important crops such as alfalfa, cotton, cowpea, tomato, maize and sorghum (Knipling, 1980; Pashley, 1986; Lu and Adang, 1996). The value of parasitoids in reducing larval populations of FAW has long been recognized (Luginbill, 1928; Vickery, 1929), and FAW parasitoids have been extensively surveyed in the Americas (Virla *et al.*, 1999; Molina-Ochoa *et al.*, 2000; Molina-Ochoa *et al.*, 2003; Molina-Ochoa *et al.*, 2004; Murúa *et al.*, 2006; Wyckhuys and O'Neil, 2006; Zenner *et al.*, 2006). *Chelonus insularis* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) are two major representatives of the FAW natural enemies. The first is an egg-larval parasitoid while the latter attacks its hosts at the larval stage. These two species co-occur in Central-, South- and North-America (Cave, 1995), and both attack a wide range of Noctuidae species, many of which belong to the genus *Spodoptera*.

Since *Spodoptera* species are polyphagous, its parasitoids should be able

to locate their hosts on a wide range of different plant species. Nevertheless, some plant species are more likely to offer more hosts, hence, in order to optimize host location, parasitic wasps would benefit from adapting to and learning odours emitted by plants attacked by a herbivore. Learning of such odours can lead to specialization, which in turn may favour local adaptation, leading to populations of parasitoids having higher fitness on the host plant they have learned to focus on. Local adaptation could translate into genetic differentiation of populations of parasitoids. Maize originated in Mexico, so parasitoids in this part of the world have always coevolved with this plant. In contrast, sorghum was introduced just over a century ago, which is quite recent on an evolutionary scale. Therefore, this system is ideal to test hypotheses on local adaptation.

This thesis mainly aimed to investigate the effects of host plant on the population genetics of parasitoids of *S. frugiperda*, both at a small scale using microsatellites and at a large scale using DNA sequencing. The community structure of parasitoids and how it can be influenced by different biotic and abiotic factors was also studied.

In the first chapter of this thesis, I describe a method developed to molecularly identify seven species of parasitoids. The second chapter is dedicated to parasitoid community structure and how they are influenced by geographic and biotic parameters (such as host plant). In the third section, I present the microsatellites which I had to develop and optimize in order to investigate fine scale population structure, which is introduced in chapter 4. Finally, the last section is devoted to a large scale phylogeography of *C. insularis* and *Campoletis* sp.

References

Agelopoulos, N. G., and M. A. Keller. 1994. Plant natural enemy association in the tritrophic system *Cotesia rubecula-Pieris rapae-Brassicaceae* (Cruciferae). 2. Preference of *C. rubecula* for landing and searching. *Journal of Chemical Ecology* 20:1735-1748.

Alborn, T., T. C. J. Turlings, T. H. Jones, G. Stenhagen, J. H. Loughrin, and J. H. Tumlinson. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276:945-949.

Althoff, D. M., and J. N. Thompson. 2001. Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches. *Journal of Evolutionary Biology* 14:406-417.

Avise, J. C. 1994. Molecular Markers Natural History and Evolution. Chapman and Hall, USA.

Avise, J. C. 1998. The history and purview of phylogeography: a personal reflection. *Molecular Ecology* 7:371-379.

Avise, J. C. 2000. Phylogeography: the history and formation of species. USA: Harvard University Press, Cambridge, Massachussetts.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific Phylogeography - the Mitochondrial-DNA Bridge between Population-Genetics and Systematics. *Annual Review of Ecology and Systematics* 18:489-522.

Babcock, C. S., J. M. Heraty, P. J. De Barro, F. Driver, and S. Schmidt. 2001. Preliminary phylogeny of *Encarsia forster* (Hymenoptera : Aphelinidae) based on morphology and 28S rDNA. *Molecular Phylogenetics and Evolution* 18:306-323.

Bachtrog, D., S. Weiss, B. Zangerl, G. Brem, and C. Schlotterer. 1999. Distribution of dinucleotide microsatellites in the *Drosophila melanogaster* genome. *Molecular Biology and Evolution* 16:602-610.

Barbosa, P., and J. A. Saunders. 1985. Plant allelochemicals: linkage between herbivores and their natural enemies. *Recent Advances in Phytochemistry*

19:107-137.

Barbosa, P., J. A. Saunders, J. Kemper, R. Trumbule, J. Olechno, and P. Martinat. 1986. Plant allelochemicals and insect parasitoids effects of nicotine on *Cotesiacongregata*(Say)(Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology* 12:1319-1328.

Beckmann, J. S., and J. L. Weber. 1992. Survey of human and rat microsatellites. *Genomics* 12:627-631.

Belshaw, R., M. Dowton, D. L. J. Quicke, and A. D. Austin. 2000. Estimating ancestral geographical distributions: a Gondwanan origin for aphid parasitoids? *Proceedings of the Royal Society London Series B - Biological Sciences* 267:491-496.

Belshaw, R., and D. L. J. Quicke. 1997. A molecular phylogeny of the aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution* 7:281-293.

Benrey, B., A. Callejas, L. Rios, K. Oyama, and R. F. Denno. 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. *Biological Control* 11:130-140.

Benrey, B., and R. F. Denno. 1997. The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* 78:987-999.

Berenbaum, M. R., and A. R. Zangerl. 1992. Genetics of physiological and behavioral resistance to host furanocoumarins in the parsnip webworm. *Evolution* 46:1373-1384.

Berlocher, S. H. 1999. Host race or species? Allozyme characterization of the 'flowering dogwood fly', a member of the Rhagoletis pomonella complex. *Heredity* 83:652-662.

Bottrell, D. G., P. Barbosa, and F. Gould. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology* 43:347-367.

Boulétreau, M. 1986. The genetic and coevolutionary interactions between parasitoids and their hosts. Pp. 169–200 *in* J. Waage, and D. Greathead, eds. Insect Parasitoids. Academic Press, London.

Campbell, B. C., and S. S. Duffey. 1979. Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science* 205:700-702.

Campbell, B. C., and S. S. Duffey. 1981. Alleviation of alpha-tomatine-induced toxicity to the parasitoid, *Hyposoter exiguae*, by phytosterols in the diet of the host, *Heliothis zea*. *Journal of Chemical Ecology* 7:927-946.

Cave, R. D. 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en America Central. Zamorano Academic Press, Zamorano, Honduras.

Cheng, L. 1970. Timing of attack by *Lypha dubia* Fall (Diptera:Tachinidae) on winter moth *Operophtera brumata* (L) (Lepidoptera: Geometridae) as a factor affecting parasite success. *Journal of Animal Ecology* 39:313-and.

Cheng, Y. H., T. F. Kuo, D. N. Lee, and C. F. Weng. 2006. Sex identification of the Black-Faced Spoonbill (Platalea minor). *Zoological Studies* 45:104-113.

Clancy, K. M., and P. W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. *Ecology* 68:733-737.

Claridge, M. F., H. A. Dawah, and M. R. Wilson, eds. 1997. Species: The Units of Biodiversity. Chapman and Hall, London.

Cortesero, A. M., C. M. De Moraes, J. O. Stapel, J. H. Tumlinson, and W. J. Lewis. 1997. Comparisons and contrasts in host-foraging strategies of two larval parasitoids with different degrees of host specificity. *Journal of Chemical*

Ecology 23:1589-1606.

Dallas, J. F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. *Mammalian Genome* 3:452-456.

de la Rua, P., J. Galian, J. Serrano, and R. F. A. Moritz. 2001. Molecular characterization and population structure of the honeybees from the Balearic islands (Spain). *Apidologie* 32:417-427.

De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570-573.

De Moraes, C. M., and M. C. Mescher. 1999. Interactions in entomology: plant-parasitoid interaction in tritrophic systems. *Journal of Entomological Science* 34:31-39.

Despres, L., E. Pettex, V. Plaisance, and F. Pompanon. 2002. Speciation in the globeflower fly Chiastocheta spp. (Diptera : Anthomyiidae) in relation to host plant species, biogeography, and morphology. *Molecular Phylogenetics and Evolution* 22:258-268.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun, M. Lathrop, G. Gyapay, J. Morissette, and J. Weissenbach. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152-154.

Dicke, M. 1995. Why do plants talk? *Chemoecology* 6:159-165.

Dicke, M., and M. Sabelis. 1989. Does it pay plants to advertise for bodyguards? Toward a cost-benefit analysis of induced synomone production *in* H. Lambers, M. L. Cambridge, H. Konings, and T. L. Pons, eds. Causes and consequences of variation in growth rate and productivity of higher plants. SPB Academic Publications, The Hague, The Netherlands.

Dicke, M., and M. W. Sabelis. 1988. How plants obtain predatory mites as bodyguards. *Netherland Journal of Zoology* 38:148-165.

Dicke, M., P. Vanbaarlen, R. Wessels, and H. Dijkman. 1993. Herbivory induces systemic production of plant volatiles that attract predators of the herbivore: extraction of endogenous elicitor. *Journal of Chemical Ecology* 19:581-599.

Dokholyan, N. V., S. V. Buldyrev, S. Havlin, and H. E. Stanley. 2000. Distributions of dimeric tandem repeats in non-coding and coding DNA sequences. *Journal of Theoretical Biology* 202:273-282.

Dowton, M., and A. D. Austin. 1998. Phylogenetic relationships among the microgastroid wasps (Hymenoptera : Braconidae): Combined analysis of 16S and 28S rDNA genes and morphological data. *Molecular Phylogenetics and Evolution* 10:354-366.

Duffey, S. S., K. A. Bloem, and B. C. Campbell. 1986. Consequences of sequestration of plant natural products in plant-insect-parasitoids interactions. Pp. 31-60 *in* D. J. Boethel, and R. D. Eikenbary, eds. Interactions of plant resistance and parasitoids and predators of insects. John Wiley and Sons, New York.

Dupas, S., Y. Carton, and M. Poirié. 2003. The genetic dimension of the coevolution of virulence resistance in *Drosophila*-parasitoid wasp relationships. *Heredity* 90:84-89.

Ehmke, A., M. Rahier, J. M. Pasteels, C. Theuring, and T. Hartmann. 1999. Sequestration, maintenance, and tissue distribution of pyrrolizidine alkaloid N-oxides in larvae of two *Oreina* species. *Journal of Chemical Ecology* 25:2385-2395.

Eisen, J. A. 1999. Mechanistic basis for microsatellite instability. Pp. 34-38 *in* D. B. Goldstein, and C. Schlötterer, eds. Microsatellites: Evolution and Applications. Oxford University Press, Oxford.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Rev. Genetics* 5:435-445.

Ellegren, H., S. Moore, N. Robinson, K. Byrne, W. Ward, and B. C. Sheldon. 1997. Microsatellite evolution: a reciprocal study of repeat lengths at homologous loci in cattle and sheep. *Molecular Biology and Evolution* 14:854-860.

Englishloeb, G. M., A. K. Brody, and R. Karban. 1993. Host-plant-mediated interactions between a generalist folivore and its tachinid parasitoid. *Journal of Animal Ecology* 62:465-471.

Estoup, A., M. Solignac, J. M. Cornuet, J. Goudet, and A. Scholl. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Molecular Ecology* 5:19-31.

Evans, P. H., J. X. Becerra, D. L. Venable, and W. S. Bowers. 2000. Chemical analysis of squirt-gun defense in *Bursera* and counterdefense by chrysomelid beetles. *Journal of Chemical Ecology* 26:745-754.

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pp. 207-231 *in* D. J. Futuyma, and M. Slatkin, eds. Coevolution. Sinauer Associates Inc., Sunderland, Massachussets.

Giamoustaris, A., and R. Mithen. 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. o*leifera*) on its interaction with specialist and generalist pests. *Annals of Applied Biology* 126:347-363.

Godfray, H. C. J. 1994a. Parasitoids behavioural and evolutionary ecology. Princeton University Press, New Jersey.

Godfray, H. C. J. 1994b. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ.

Godfray, H. C. J., and M. Shimada. 1999. Parasitoids: a model system to answer questions in behavioral, evolutionary and population ecology. *Research in Population Ecology* 41:3-10.

Hardy, G. H. 1908. Mandelian proportions in a mixed population. *Science* 28:49-50.

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

Hassell, M. P. 2000. Host–parasitoid population dynamics. *Journal of Animal Ecology* 69.

Hawkins, B. A., H. V. Cornell, and M. E. Hochberg. 1997. Predators, parasitoids, and pathogens as mortality agents in phytophagous insect populations. *Ecology* 78:2145-2152.

Hilker, M., and T. Meiners. 2002. Induction of plant responses to oviposition and feeding by herbivorous arthropods: a comparison. *Entomologia Experimentalis et Applicata* 104:181-192.

Hufbauer, R. A., S. M. Bogdanowicz, and R. G. Harrison. 2004. The population genetics of a biological control introduction: mitochondrial DNA and microsatellie variation in native and introduced populations ofAphidus ervi, a parisitoid wasp. *Molecular Ecology* 13:337-348.

Janssen, A., A. Pallini, M. Venzon, and M. W. Sabelis. 1998. Behaviour and indirect interactions in food webs of plant-inhabiting arthropods. *Experiments in Applied Acarology* 22:497-521.

Jarvis, K. J., and M. F. Whiting. 2006. Phylogeny and biogeography of ice crawlers (Insecta : Grylloblattodea) based on six molecular loci: Designating conservation status for Grylloblattodea species. *Molecular Phylogenetics and Evolution* 41:222-237.

Kahl, J., D. H. Siemens, R. J. Aerts, R. Gabler, F. Kuhnemann, C. A. Preston, and I. T. Baldwin. 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta* 210:336-342.

Kambhampati, S., W. Volkl, and M. Mackauer. 2000. Phylogenetic relationships among genera of Aphidiinae (Hymenoptera : Braconidae) based on DNA sequence of the mitochondrial 16S rRNA gene. *Systematic Entomology* 25:437-445.

Kashi, Y., D. King, and M. Soller. 1997. Simple sequence repeats as a source of quantitative genetic variation. *Trends in Genetics* 13:74-78.

Kerdelhue, C., I. Le Clainche, and J. Y. Rasplus. 1999. Molecular phylogeny of the *Ceratosolen* species pollinating *Ficus* of the subgenus *Sycomorus sensu stricto*: Biogeographical history and origins of the species-specificity breakdown cases. *Molecular Phylogenetics and Evolution* 11:401-414.

Kester, K. M., and P. Barbosa. 1991. Behavioral and ecological constraints imposed by plant on insect parasitoids: implication for biological control. *Biological Control* 1:64-106.

Kimura, M., S. M. Clegg, I. J. Lovette, K. R. Holder, D. J. Girman, B. Mila, P. Wade, and T. B. Smith. 2002. Phylogeographical approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic-Neotropical warbler (Wilsonia pusilla). *Molecular Ecology* 11:1605-1616.

King, D. G., and M. Soller. 1999. Variation and fidelity: the evolution of simple sequence repeats as functional elements in adjustable genes. Pp. 65-82 *in* S. P. Wasser, ed. Evolutionary Theory and Processes: Modern Perspectives. Kluwer Academic Press, The Netherlands.

Knipling, E. F. 1980. Regional management of the fall armyworm — a realistic approach? *Florida Entomologist* 63:468-480.

Kraaijeveld, A. R., J. J. M. Van Alphen, and H. C. J. Godfray. 1998. The coevolution of host resistance and parasitoid virulence. *Parasitology* 116:S29-S45.

Lewis, W. J., J. C. van Lenteren, S. C. Phatak, and J. H. Tumlinson. 1997. A total system approach to sustainable pest management. *Proceedings of the National Academy of Science U. S. A.* 94:12243-12248.

Li, Y. C., A. B. Korol, T. Fahima, A. Beiles, and E. Nevo. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Molecular Ecology* 11:2453-2465.

Liu, Z., G. Tan, P. Li, and R. A. Dunham. 1999. Transcribed dinucleotide microsatellites and their associated genes from channel catfish *Ictalurus puctatus*. *Biochemistry and Biophysics ResearchCommunication* 259:190-194.

Lu, Y. J., and M. J. Adang. 1996. Distinguishing fall armyworm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Florida Entomologist* 79:48-55.

Luginbill , P. 1928. The fall armyworm. *U. S. Department of Agriculture Technical Bulletin* 34:92.

Machado, C. A., E. A. Herre, S. McCafferty, and E. Bermingham. 1996. Molecular phylogenies of fig pollinating and non-pollinating wasps and the implications for the origin and evolution of the fig-fig wasp mutualism. *Journal of Biogeography* 23:531-542.

Marcotte, E. M., M. Pellegrini, T. O. Yeates, and D. Eisenberg. 1999. A census of protein repeats. *Journal of Molecular Biology* 293:151-160.

Mardulyn, P., and J. B. Whitfield. 1999. Phylogenetic signal in the COI, 16S, and 28S genes for inferring relationships among genera of Microgastrinae (Hymenoptera; Braconidae): Evidence of a high diversification rate in this group of parasitoids. *Molecular Phylogenetics*

and Evolution 12:282-294.

Mattiacci, L., M. Dicke, and M. A. Posthumus. 1994. Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *Journal of Chemical Ecology* 20:2229-2247.

Mattiacci, L., M. Dicke, and M. A. Posthumus. 1995. Beta-glucosidase: an elicitor of herbivore-nduced plant odor that attracts host searching parasitic wasps. *Proceedings of the National Academy of Science U. S. A.* 92:2036-2040.

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

Meagher, R. L., and R. N. Nagoshi. 2004. Population dynamics and occurrence of Spodoptera frugiperda host strains in southern Florida. *Ecological Entomology* 29:614-620.

Meiners, T., and M. Hilker. 1997. Host location in *Oomyzus gallerucae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112:87-93.

Meiners, T., C. Westerhaus, and M. Hilker. 2000. Specificity of chemical cues used by a specialist egg parasitoid during host location. *Entomologia Experimentalis et Applicata* 95:151-159.

Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs, and J. E. Foster. 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomologist* 86:254-289.

Molina-Ochoa, J., J. E. Carpenter, R. Lezama-Gutierrez, J. E. Foster, M. Gonzalez-Ramirez, C. A. Angel-Sahagun, and J. Farias-Larios. 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in Mexico. *Florida Entomologist* 87:461-472.

Molina-Ochoa, J., J. J. Hamm, R. Lezama-Gutiérrez, M. López-Edwards, M. González-Ramírez, and A. Pescador-Rubio. 2000. A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the mexican states of Michoacán, Colima, Jalisco, and Tamaulipas. *Florida Entomologist*, 84: 31-36

Muldrew, J. A. 1953. The natural immunity of the larch sawfly (*Pristiphora erichsoni* (Htg.)) to the introduced parasite *Mesoleius tenthredinis* Morley, in Manitoba and Saskatchewan. *Canadian Journal of Zoology* 31:313-332.

Murdoch, W. W., and C. J. Briggs. 1996. Theory for biological control: recent developments. *Ecology* 77:2001-2013.

Murúa, G., J. Molina-Ochoa, and C. Coviella. 2006. Population dynamics of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and its parasitoids in northwestern Argentina. *Florida Entomologist* 89:175-182.

Nagoshi, R. N., R. L. Meagher, G. Nuessly, and D. G. Hall. 2006. Effects of fall armyworm (Lepidoptera : Noctuidae) interstrain mating in wild populations. *Environmental Entomology* 35:561-568.

Nitao, J. K. 1989. Enzymatic adaptation in a specialist herbivore for feeding on furanocoumarin-containing plants. *Ecology* 70:629-635.

Nunez, Y., J. Gallego, F. Ponz, and R. Raposo. 2006. Analysis of population structure of Erysiphe necator using AFLP markers. *Plant Pathology* 55:650-656.

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

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Annals of the Entomological Society of America* 79:898-904.

Pepin, L., Y. Amigues, A. Lepingle, J. L. Berthier, A. Bensaid, and D. Vaiman. 1995. Sequence conservation of microsatellites between *Bos Taurus* (cattle), *Capra hircus* (goat) and related species: examples of use in parentage testing and phylogeny analysis. *Heredity* 74:53-61.

Petes, T. D., P. W. Greenwell, and M. Dominska. 1997. Stabilization of microsatellite sequences by variant repeats in the yeast *Saccharomyces cerevisiae*. *Genetics* 146:491-498.

Powell, W., F. Pennacchio, G. M. Poppy, and E. Tremblay. 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae). *Biological Control* 11:104-112.

Quicke, D. L. J. 1997. Parasitic Wasps. Chapman and Hall, London.

Quicke, D. L. J., and R. Belshaw. 1999. Incongruence between morphological data sets: An example from the evolution of endoparasitism among parasitic wasps (Hymenoptera : Braconidae). *Systematic Biology* 48:436-454.

Rhoades, D. F. 1983. Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. *Acs Symposium Series* 208:55-68.

Richard, G. F., and F. Paques. 2000. Mini- and microsatellite expansions: the recombination connection. *Embo Reports* 1:122-126.

Richards, R. I., and G. R. Sutherland. 1994. Simples repeat DNA is not replicated simply. *Nature Genetics* 6:114-116.

Richardson, B. J., P. R. Baverstock, and M. Adams. 1986. Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies. Academic Press, London.

Rowell-Rahier, M., M. Witte, A. Ehmke, T. Hartmann, and J. M. Pasteels. 1991. Sequestration of plant pyrrolizidine alkaloids by chrysomelid beetles and selective transfer into the defensive secretions. *Chemoecology* 2:41-48.

Sabelis, M. W., M. van Baalen, F. M. Bakker, J. Bruin, B. Drukker, M. Egas, A. R. M. Janssen, I. K. Lesna, B. Pels, P. C. J. van Rijn, and P. Scutareau. 1999. The evolution of direct and indirect plant defence against herbivorous arthropods. Pp. 106-109 *in* H. Olf, V. K. Brown, and R. H. Drents, eds. Herbivores: between Plants and Predators. Blackwell, Oxford.

Sabelis, M. W., and H. E. Vandebaan. 1983. Location of distant spider-mite colonies by *Phytoseiid* predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomologia Experimentalis et Applicata* 33:303-314.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA-Polymerase. *Science* 239:487-491.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim. 1985. Enzymatic Amplification of Beta-Globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle-Cell Anemia. *Science* 230:1350-1354.

Salt, G. 1956. Experimental studies in insect parasitism. IX. The reactions of a stick insect to an alien parasite. *Proceedings of the Royal Society London* 146:93-108.

Salt, G. 1964. The ichneumonid parasite *Nemeritis canescens* (Gravenhorst) in relation to the wax moth *Galleria mellonella* (L.). *Transactions of the Royal Entomological Society* 116:1-14.

Samadi, S., F. Erard, A. Estoup, and P. Jarne. 1998. The influence of mutation, selection and reproductive systems on microsatellite variability: a simulation approach. *Genetical Research* 71:213-222.

Sanchis, A., A. Latorre, F. Gonzalez-Candelas, and J. M. Michelena. 2000. An 18S rDNA-based molecular phylogeny of Aphidiinae (Hymenoptera : Braconidae). *Molecular Phylogenetics and Evolution* 14:180-194.

Schmidt, S., I. D. Naumann, and P. J. De Barro. 2001. *Encarsia* species (Hymenoptera : Aphelinidae) of Australia and the Pacific Islands attacking *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera : Aleyrodidae) - a pictorial key and descriptions of four new species. *Bulletin of Entomological Research* 91:369-387.

Schoonhoven, L. M. 1967. Chemoreception of mustard oil glucosides in larvae of *Pieris brassicae*. *Proc. Koninklijke Nederlandse Akademie Van Wetenschappen Series C-Biological and Medical Sciences* 70:556-and.

Schug, M. D., K. A. Wetterstrand, M. S. Gaudette, R. H. Lim, C. M. Hutter, and C. F. Aquadro. 1998. The distribution and frequency of microsatellite loci in *Drosophila melanogaster*. *Molecular Ecology* 7:57-70.

Smith, P. T., S. Kambhampati, W. Volkl, and M. Mackauer. 1999. A phylogeny of aphid parasitoids (Hymenoptera : Braconidae : Aphidiinae) inferred from mitochondrial NADH 1 dehydrogenase gene sequence. *Molecular Phylogenetics and Evolution* 11:236-245.

Takabayashi, J., M. Dicke, and M. A. Posthumus. 1991. Induction of indirect defense against spider mites in uninfested Lima bean leaves. *Phytochemistry* 30:1459-1462.

Takabayashi, J., S. Takahashi, M. Dicke, and M. A. Posthumus. 1995. Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by corn plants. *Journal of Chemical Ecology* 21:273-287.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* 17:6463-6471.

Tautz, D., M. Trick, and G. A. Dover. 1986. Cryptic simplicity in DNA is a major source of genetic variation. *Nature* 322:652-656.

Thomson, L. J., B. J. Rundle, M. E. Carew, and A. A. Hoffmann. 2003. Identification and characterization of Trichogramma species from south-eastern Australia using the internal transcribed spacer 2 (ITS-2) region of the ribosomal gene complex. *Entomologia Experimentalis et Applicata* 106:235-240.

Thurston, R., and P. M. Fox. 1972. Inhibition by nicotine of emergence of *Apanteles congregatus* Hymenoptera: Braconidae from Its host, tobacco hornworm lepidoptera Lepidoptera: Sphingidae. *Annals of the Entomological Society of America* 65:547-and.

Toth, G., Z. Gaspari, and J. Jurka. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. *Genome Research* 10:967-981.

Triponez, Y., R. E. Naisbit, J. B. Jean-Denis, M. Rahier, and N. Alvarez. 2007. Genetic and environmental sources of variation in the autogenous chemical defense of a leaf beetle. *Journal of Chemical Ecology* 33:2011-2027.

Turlings, T. C. J., and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5:321-333.

Turlings, T. C. J., J. H. Loughrin, P. J. McCall, U. S. R. Rose, W. J. Lewis, and J. H. Tumlinson. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Science U. S. A.* 92:4169-4174.

Turlings, T. C. J., P. J. McCall, H. T. Alborn, and J. H. Tumlinson. 1993. An elicitor in

caterpillar oral secretions that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *Journal of Chemical Ecology* 19:411-425.

Turlings, T. C. J., and J. H. Tumlinson. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Science U. S. A*. 89:8399-8402.

Turlings, T. C. J., J. H. Tumlinson, R. R. Heath, A. T. Proveaux, and R. E. Doolittle. 1991. Isolation and identification of allelochemicals that attract the larval parasitoid, *Cotesia marginiventris* (Cresson), to the microhabitat of one of its hosts. *Journal of Chemical Ecology* 17:2235-2251.

Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251-1253.

van Dam, N. M., K. Hadwich, and I. T. Baldwin. 2000. Induced responses in *Nicotiana attenuata* affect behavior and growth of the specialist herbivore *Manduca sexta*. *Oecologia* 122:371-379.

van den Bosch, R. 1964. Encapsulation of the eggs of *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) in larvae of *Hypera brunneipenis* (Boheman) and *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). *Journal of Insect Pathology* 6:343-367.

van Loon, J. J. A., and L. M. Schoonhoven. 1999. Specialist deterrent chemoreceptors enable Pieris caterpillars to discriminate between chemically different deterrents. *Entomologia Experimentalis et Applicata* 91:29-35.

Vickery, R. A. 1929. Studies on the fall armyworm in the Gulf coast district of Texas. *Technical Bulletin* 138:1-63.

Vinson, S. B., and P. Barbosa. 1987. Interrelationships of nutritional ecology of parasitoids. Pp. 673-695 *in* F. Slansky, and J. G. Rodriguez, eds. Nutritional ecology of insects, mites, and spiders and related invertebrates. John Wiley and Son, New York.

Vinson, S. B., and G. F. Iwantsch. 1980. Host regulation by insect parasitoids. The *Q Reviews in Biology* 55:143-165.

Virla, E. G., M. V. Colomo, C. Berta, and L. Valverde. 1999. El complejo de parasitoides del "gusano cogollero" del maíz, *Spodoptera frugiperda*, en la República Argentina (Insecta: Lepidoptera). *Neotrópica* 45:3-12.

Wang, Z., J. L. Weber, G. Zhong, and S. D. Tanksley. 1994. Survey of plant short tandem DNA repeats. *Theoretical and Applied Genetics* 88:1-6.

Weber, J. L. 1990. Informativeness of human$(dC\text{-}dA)_N.(dG\text{-}dT)_N$ polymorphisms. *Genomics* 7:524-530.

Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction. *American Journal of Human Genetics* 44:388-396.

Weber, J. L., and C. Wong. 1993. Mutations of human short tandem repeats. *Human Molecular Genetics* 2:1123-1128.

Weinberg, W. 1908. Ueber den Nachweis der Vererbung beim Menschen. *Jahreshefte des Vereins für Vaterländische Naturkunde in Württenburg* 64:368-382.

Widmer, A., P. Schmid-Hempel, A. Estoup, and A. Scholl. 1998. Population genetic structure and colonization history of *Bombus terrestris* s.l. (Hymenoptera : Apidae) from the Canary Islands and Madeira. *Heredity* 81:563-572.

Wren, J. D., E. Forgacs, J. W. Fondon, A. Pertsemlidis, S. Y. Cheng, T. Gallardo, R. S. Williams, R. V. Shohet, J. D. Minna, and H. R. Garner. 2000. Repeat polymorphisms within gene regions: phenotypic and evolutionary implications. *American Journal of Human Genetics* 67:345-356.

Wyckhuys, K. A. G., and R. J. O'Neil. 2006. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. *Crop Protection* 25:1180-1190.

Yourman, L. F., and D. G. Luster. 2004. Generation of molecular markers for the identification of isolates of Puccinia jaceae, a potential biological control agent of yellow starthistle. *Biological Control* 29:73-80.

Zenner, I., A. Alvarez, and S. Barreto. 2006. Influence of parasitism by *Chelonus insularis* Cresson (Hymenoptera: Braconidae) on the susceptibility of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) to insecticides. *Neotropical Entomology* 35:818-822.

Chapter 1:

Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), using PCR amplification and restriction enzyme digestion

Identification of seven species of hymenopteran parasitoids of Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), using PCR amplification and restriction enzyme digestion

Violaine Jourdie, Nadir Alvarez and Ted C. J. Turlings

Université de Neuchâtel, Institut de Biologie, Laboratoire d'Entomologie Evolutive, Case Postale 158, 2009 Neuchâtel, Switzerland

Published in ***Agricultural and Forest Entomology*** (2008), 10, 129–136

Abstract

1. The fall armyworm, *Spodoptera frugiperda*, is a voracious pest of numerous crops of economic importance throughout the New World. In its native Mexico, larvae can be attacked by several species of parasitic wasps, which are candidate biological control agents against this and other lepidopteran pests.
2. We attempted to survey the parasitoid fauna on *S. frugiperda* in maize and sorghum fields throughout Mexico. However, our efforts have been hampered by the incomplete development of parasitoid larvae emerging from collected *Spodoptera* caterpillars.
3. Here we describe how we solved this problem by developing a method to identify seven species of parasitic wasps using PCR amplification and restriction enzyme digestion. This method enables the precise determination of the species of parasitoid larvae that are usually not morphologically identifiable.

Keywords: *Spodoptera frugiperda*; hymenoptera; parasitoid; restriction enzyme digestion; species identification

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith (Lepidoptera: Noctuidae), is a devastating pest throughout the Americas (Kranz *et al.*, 1977), inflicting costly damage to several crops of great economic importance such as corn, rice, sorghum, peanuts, soybeans, alfalfa and forage grasses (Knipling, 1980; Pashley, 1986; Lu and Adang, 1996). Synthetic insecticides are commonly used to control this pest. However, resistance has been observed and is a concern (Yu, 1991, 1992).

As FAW is attacked by many different species of hymenopteran parasitoids, biological control should be considered as an alternative to insecticides (Gross and Pair, 1986). Parasitoids have long been recognized as excellent candidates for reducing larval populations of this noctuid (Luginbill, 1928; Vickery, 1929). Moreover, it has been documented that parasitization of the caterpillar by a parasitoid reduces its feeding rate and has a direct effect on the damage it inflicts (Rahman, 1970; Guillot and Vinson, 1973; Brewer and King, 1978; Parkman and Shepard, 1981; Powell, 1989; Fritzsche Hoballah and Turlings, 2001).

Determining which parasitoids are most successful at attacking a pest species in a particular habitat may help in optimizing biological control. In order to develop a better understanding of the natural distribution of the FAW parasitoid complex, several surveys have been conducted in

different regions of Mexico, where FAW is likely to have originally evolved on wild crop (Hoballah *et al.*, 2004; Molina-Ochoa *et al.*, 2000, 2004). So far these surveys have revealed the occurrence of seven species of braconids attacking FAW (*Aleoides laphygmae* Viereck, *Cotesia marginiventris* Cresson, *Chelonus insularis* Cresson, *Chelonus cautus* Cresson, *Homolobus truncator* Say, *Meteorus laphygmae* Viereck and *Glyptapanteles militaris* Walsh), four species of ichneumonids (*Campoletis sonorensis* Cameron, *Ophion flavidus* Brulle, *Pristomerus spinator* Fabricius and *Eiphosoma vitticolle* Cresson), two eulophid species (*Euplectrus plathypenae* Howard and *Aprostocetus* sp. Westwood), and one trichogrammatid species (*Trichogramma atopovirilia* Oatman AND Platner). These surveys have been limited to the states of Michoacán, Colima, Jalisco, Nayarit, Sinaloa, Veracruz and Tamaulipas. Additional surveys and ecological studies will be necessary to assess the full potential of the various parasitoids for the control of FAW.

We have been collecting naturally occurring larvae of FAW throughout Mexico for a study that aims to compare the genetic population structure of several parasitoid species occurring on maize with those on sorghum. Rearing the collected larvae until parasitoid emergence in the laboratory conditions is not always successful and it commonly happens that the larvae do not spin a cocoon. These individuals cannot complete their life cycle to adulthood. Whereas adult parasitoids can be identified morphologically, this is virtually impossible for the larval stages. Molecular techniques may achieve such identification (Hebert *et al.*, 2003a; Blaxter, 2004; Hebert *et al.*, 2004; Barrett and Hebert, 2005; Hebert and Gregory, 2005; Monaghan *et al.*, 2005; Hajibabaei, 2007). Gene sequencing is one possibility but it is expensive and time-consuming. As an alternative to sequencing, we here introduce a fast, easy and relatively cheap molecular diagnosis to identify seven commonly found species of wasp larvae using PCR amplification and restriction endonucleases digestion.

Methods

Insects

The species considered were four braconids (*Cotesia marginiventris*, *Chelonus cautus*, *Chelonus insularis* and *Meteorus laphygmae*) and three ichneumonids (*Pristomerus spinator*, *Campoletis sonorensis* and *Eiphosoma vitticolle*). These parasitoids all exit the host as larvae and form cocoons externally. Naturally occurring *S. frugiperda* larvae were collected in 23 sites in Mexico between June and September 2005 (Table 1). The caterpillars were reared individually on artificial diet in 24-well ELISA plates to prevent cannibalism. The parasitoid larvae were placed on a piece of absorbing paper in a plastic container upon emergence from the caterpillars. The parasitoid larvae that spun a cocoon were left in the plastic container until the emergence of the adults. The parasitoid larvae that did not spin a cocoon within 24 hours following emergence from the caterpillars were preserved in 100% ethanol. The adult parasitic wasps that emerged from the cocoons were also preserved in 100% ethanol upon emergence. Adults were taxonomically assigned to different species following Cave (1995).

DNA amplification and sequencing

Total genomic DNA was extracted using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For adult wasps, extraction was conducted on the abdomen, whereas for larvae half of the body was used. Total DNA was re-suspended in 200 µl of elution buffer (two elutions of 100 µl each). The mtDNA *COI* gene (Cytochrome *c* oxidase subunit 1) was partially amplified using C1-J-1859 (forward) and C1-N-2191 (reverse) primers adapted for the bee (Simon *et al.*, 1994). Final volume was 30 µl, and contained 3 µl of extracted DNA, 1.8 µl of 25 mM $MgCl_2$, 3 µl of 1.5 mM dNTPs, 3 µl of PCR buffer (Promega, Madison, USA), 3 units of Taq DNA polymerase (Promega, Madison, USA),

Table 1: Sampled locations. Insects were collected from 23 locations in 6 mexican states in June, August and September 2005.

STATE	CODE	LOCATION	MUNICIPIO	GPS COORD.
Colima	C1	Agua Zarca	Coquimatlán	N 19°13'12.0", W 103°56'10.1"
	C2	El Colomo, Coquimatlán	Coquimatlán	N 19°13'58.3", W 103°57'17.4"
	C3	Pueblo Juarez, Coquimatlán	Coquimatlán	N 19°09'45.9", W 103°53'44.4"
	C4	Los Mezcales	Comala	N 19°19'57.3", W 103°46'07.6"
	C6	La Caja	Comala	N 19°22'31.3", W 103°48'12.8"
	C7	Villa de Alvarez	Minatitlán	N 19°16'57.9", W 103°46'41.0"
	C8	Villa de Alvarez	Minatitlán	N 19°17'01.2", W 103°46'41.0"
Chiapas	CH1	Jaritas	Tapachula	N 14°42'53.3", W 92°18'24.9"
	CH2	Jaritas	Tapachula	N 14°43'01.5", W 92°18'26.6"
	CH3	Jaritas	Tapachula	N 14°44'53.7", W 92°20'06.2"
	CH4	Jaritas	Tapachula	N 14°43'32.3", W 92°18'58.1"
	CH5	Jaritas	Tapachula	N 14°43'20.0", W 92°19'09.1"
Jalisco	J1	Usmajac	Sayula	N 19°52'08.9", W 103°33'16.7"
	J2	Usmajac	Sayula	N 19°52'18.4", W 103°33'45.6"
Nayarit	N1	Carretera Jala	Ahuacatlán	N 21°03'39.6", W 104°27'09.8"
	N2	Ejido Mexpan	Ixtlán del Rio	N 21°02'35.0", W 104°28'03.1"
	N3	Ejido Mexpan	Ixtlán del Rio	N 21°02'38.3", W 104°27'42.6"
	N4	El Humedo	Ahuacatlán	N 21°01'35.9", W 104°28'17.7"
Puebla	P1	CIMMYT tropical field station	Agua Fría	N 20°27'18.3", W 97°38'28.8"
	P2	CIMMYT tropical field station	Agua Fría	N 20°27'10.3", W 97°38'28.7"
	P3	CIMMYT tropical field station	Agua Fría	N 20°27'19.6", W 97°38'26.3"
	P4	CIMMYT tropical field station	Agua Fría	N 20°27'17.4", W 97°38'26.3"
Veracruz	V1	Lindero	Lindero	N 20°29'31.9", W 97°32'17.2"

1.5 µl of 10 µM forward primer, and 1.5 µl of 10 µM reverse primer. PCR amplification was conducted in a Uno II thermal cycler (Biometra, Goettingen, Germany) using the following cycling conditions: initial denaturation at 94°C (1 min 30 s); 35 cycles of 94°C for 1 min, 50°C for 1 min, 70°C for 1 min; final elongation at 70°C for 5 min. The PCR products were purified using the Qiaquick® PCR Purification Kit (Qiagen, Hilden, Germany) and re-suspended in 30 µl. Sequencing was carried out by Macrogen Inc. (Seoul, South Korea) with the forward primer under BigDyeTM terminator cycling conditions, purifying the reacted products using Ethanol Precipitation and running them using an Automatic Sequencer 3730xl (Applied Biosystem, Foster City, USA). A total of 195 adults and 256 larvae were sequenced.

Sequence analysis

The sequences were manually corrected using Chromas 2.23 (Technelysium Pty. Ltd, Queensland, Australia) and further aligned using ClustalW 1.4 (Thompson *et al.*, 1994) implemented in BioEdit (Hall, 1999). However, since all sequenced fragments were of the same size, alignment was trivial. BLAST searches were conducted on all sequences to check for possible contamination. Based on sequences obtained in adult wasps, a consensus sequence was generated for each species.

Phylogenetic reconstruction

In order to confirm that *COI* sequences were variable enough to allow clear distinction between species, a neighbor joining analysis was performed on adult

sequences using the DNAdist program (Phylip 3.5 package; Felsenstein, 1993) implemented in BioEdit, with a Kishino-Hasegawa model of substitution (Kishino and Hasegawa, 1989). The genetic distances between and within species were also computed in Mega (Kumar *et al.*, 2004) with a Tamura-Nei model of substitution (Tamura and Nei, 1993).

Selection of restriction enzymes

A restriction map was created for each consensus sequence using the online restriction map software Molecular Toolkit (Colorado State University, http://arbl.cvmbs.colostate.edu/molkit/mapper/index.html). Simulations were run to find the combination of enzymes that maximizes differences in restriction patterns among species. In order to test whether this tool would also allow the identification of other closely related parasitoid species, simulations were run on sequences from *Cotesia plutellae* and *Cotesia flavipes* found on GenBank (accession numbers AM087129 and DQ232330 respectively).

Digestion protocol and electrophoresis

Since not all enzymes had a similar optimal temperature, a two-step digestion was performed, following indications provided by the manufacturers. Enzymatic digestion was performed according to the following conditions: 10µl of Tango buffer 1X (Fermentas, St. Leon-Rot, Germany) were added to 10µl of non-purified PCR product and 2U of each enzyme active at 37°C. This mix was incubated overnight at the indicated temperature and 2.5µl of Tango buffer 10X and 2U of Bcl I were then added to the mix and incubated for 2 hours at 55°C. The reaction was stopped by incubating the preparation 15 min at 65°C. The samples were then loaded on a 2% agarose gel and run at 90V for 4 hours. Due to the long-lasting migration, a large quantity of Ethidium Bromide (EtBr) needed to be added to the gel (15µl of EtBr for a 150ml gel).

Results

Insects

Seven species of parasitoids were reared out of *S. frugiperda* caterpillars. Not all the species were present at all locations and the occurrence of parasitoids and the level of parasitism varied greatly among locations (Table 2a). Total level of parasitism varied from 2% (location N4) to 58.9% (location CH5). *Chelonus insularis* was overall the most abundant parasitoid species (Table 2 b).

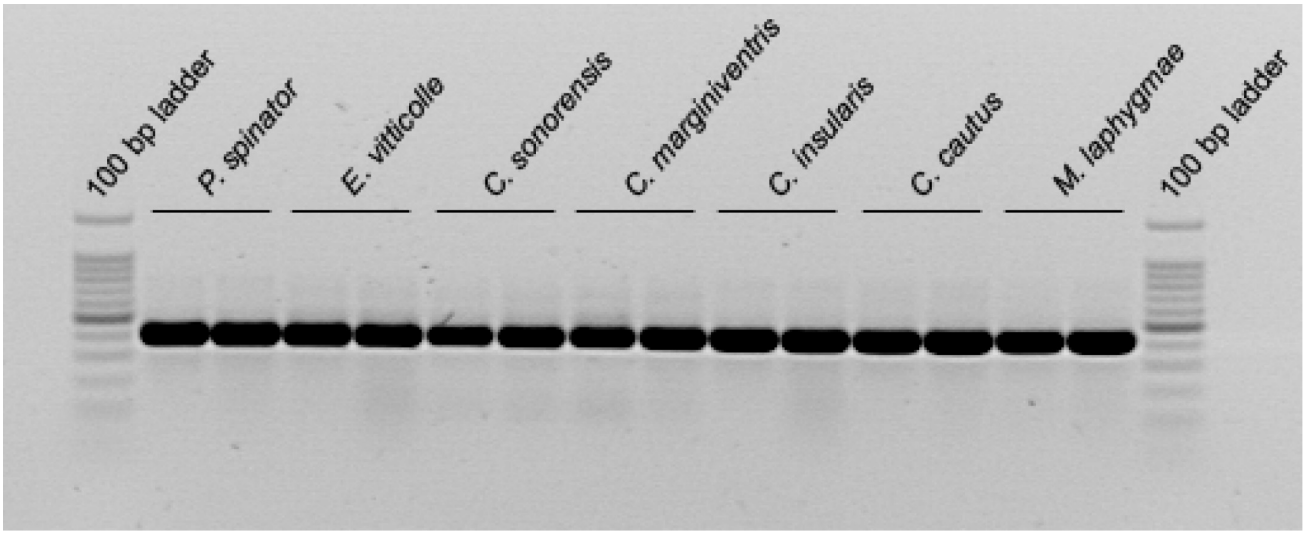


Figure 1: PCR amplification pattern. Equal size amplification of *COI* fragment was obtained for all seven species.

DNA amplification and sequencing

As a first step to molecularly distinguish seven species of parasitoids collected from *S. frugiperda* throughout Mexico (Table 1), their *COI* gene was partially amplified using primers compiled by Simon *et al.* (1994). The amplified *COI* fragment was of equal size for all seven species (375 bp) (Fig. 1).

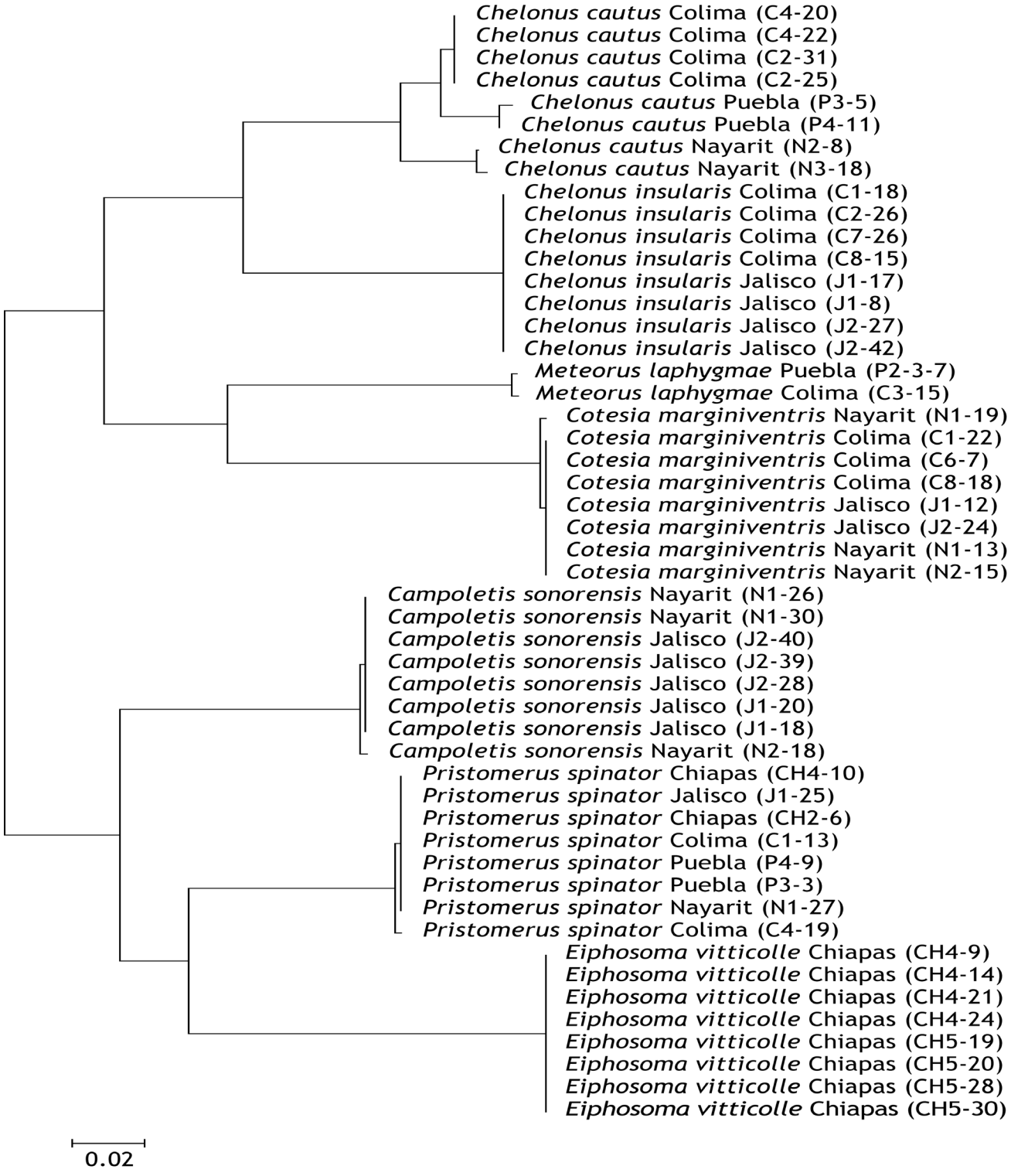


Figure 2: N-J Tree. Using the *COI* sequences with a Kishino-Hasegawa model of substitution, each individual could clearly be assigned to its corresponding species, in the mid-point rooted phylogenetic tree. Eight adult individuals per species from different locations were included in this tree, except for *Meteorus laphygmae* for which only two adults were collected.

Table 2: Number of parasitoids collected at each location (a) and relative abundance of each species in observed parasitism (b). The proportion of each species and its ranking abundance are given for adults, larvae, and both cumulated (total).

a)

Sampling location	Number of *Spodoptera* collected	Parasitoid stage	*Chelonus insularis*		*Pristomerus spinator*		*Cotesia marginiventris*		*Chelonus cautus*		*Campoletis sonorensis*		*Eiphosoma vitticolle*		*Meteorus laphygmae*		Total number of parasitoids	Total % parasitism
			Number collected	% parasitism	Number collected	% parasitism	Number collected	% parasitism	Number collected	% parasitism	Number collected	% parasitism	Number collected	% parasitism	Number collected	% parasitism		
C1	119	adults	2	11.8	3	2.5	1	0.8	-	-	-	-	-	-	-	-	18	15.1
		larvae	12		-		-		-		-		-		-			
C2	120	adults	1	15.8	5	7.5	-	-	2	1.7	-	-	-	-	-	-	30	25.0
		larvae	18		4		-		-		-		-		-			
C3	93	adults	2	11.8	-	1.1	-	-	-	-	-	-	-	-	1	1.1	13	14.0
		larvae	9		1		-		-		-		-		-			
C4	150	adults	1	5.3	3	3.3	-	-	2	5.3	-	-	-	-	-	-	21	14.0
		larvae	7		2		-		6		-		-		-			
C6	120	adults	2	3.3	1	0.8	2	1.7	-	-	-	-	-	-	-	-	7	5.8
		larvae	2		-		-		-		-		-		-			
C7	150	adults	7	18.0	1	0.7	-	-	-	-	-	-	-	-	-	-	28	18.7
		larvae	20		-		-		-		-		-		-			
C8	150	adults	3	11.3	-	0.0	1	0.7	-	-	-	-	-	-	-	-	18	12.0
		larvae	14		-		-		-		-		-		-			
CH1	150	adults	-	1.3	-	0.0	-	-	-	-	-	-	-	-	-	-	6	4.0
		larvae	2		-		-		-		-		4	2.7	-			
CH2	150	adults	3	2.0	13	9.3	-	-	-	-	-	-	-	-	-	-	17	11.3
		larvae	-		1		-		-		-		-		-			
CH3	150	adults	3	3.3	1	0.7	-	-	-	-	-	-	-	-	-	0.7	7	4.7
		larvae	2		-		-		-		-		-		1			
CH4	150	adults	8	9.3	2	2.0	-	-	-	-	-	-	6	4.7	-	-	24	16.0
		larvae	6		1		-		-		-		1		-			
CH5	56	adults	12	48.2	-	0.0	-	-	1	1.8	-	-	5	8.9	-	-	33	58.9
		larvae	15		-		-		-		-		-		-			
J1	125	adults	10	12.0	2	2.4	4	3.2	-	-	2	1.6	-	-	-	-	24	19.2
		larvae	5		1		-		-		-		-		-			
J2	165	adults	10	19.4	2	1.2	1	0.6	-	-	7	4.2	-	-	-	-	42	25.5
		larvae	22		-		-		-		-		-		-			
N1	160	adults	-	4.4	6	4.4	14	8.8	-	-	3	1.9	-	-	-	-	31	19.4
		larvae	7		1		-		-		-		-		-			
N2	158	adults	1	3.2	-	0.0	9	5.7	1	0.6	1	0.6	-	-	-	-	16	10.1
		larvae	4		-		-		-		-		-		-			
N3	150	adults	4	4.7	-	0.0	12	8.0	1	0.7	1	0.7	-	-	-	-	21	14.0
		larvae	3		-		-		-		-		-		-			
N4	150	adults	-	-	-	0.0	3	2.0	-	-	-	-	-	-	-	-	3	2.0
		larvae	-		-		-		-		-		-		-			
P1	70	adults	-	1.4	-	0.0	-	-	-	-	-	-	-	-	-	-	1	1.4
		larvae	1		-		-		-		-		-		-			
P2		adults	-	-	-	-	-	-	-	-	-	-	-	-	1	-	36	-
		larvae	29		-		1		1		-		-		4			
P3		adults	2	-	8	-	-	-	1	-	-	-	-	-	-	-	20	-
		larvae	3		6		-		-		-		-		-			
P4	80	adults	-	17.5	-	-	-	-	-	6.3	-	-	-	-	-	-	19	23.8
		larvae	14		-		-		5		-		-		-			
V1	212	adults	-	1.4	3	2.8	-	-	1	0.9	-	-	-	-	-	-	11	5.2
		larvae	3		3		-		1		-		-		-			

b)

Species	Adults			Larvae			Total		
	Number	%	Rank	Number	%	Rank	Number	%	Rank
C. insularis	71	34.8	1	198	81.8	1	269	60.3	1
P. spinator	50	24.5	2	20	8.3	2	70	15.7	2
C. marginiventris	47	23	3	1	0.4	5	48	10.8	3
C. cautus	9	4.4	6	13	5.4	3	22	4.9	4
E. vitticolle	11	5.4	5	5	2.1	4	16	3.6	5
C. sonorensis	14	6.9	4	0	0	6	14	3.1	6
M. laphygmae	2	1	7	5	2.1	4	7	1.6	7

Sequence analysis

After manually correcting the sequences, a neighbor joining analysis was performed on sequences of adult wasps, and the inter- and intra-specific genetic distances were computed. The phylogenetic analyses showed that the *COI* sequence allows clear distinction between the collected species (Fig. 2). Indeed, genetic distances within species were negligible compared to inter-species distances (Table 3). Sequences of the fifty individuals included in the phylogenetic analysis are available in GenBank under Accession No. EF555594 to EF555643.

Selection of restriction enzymes

Restriction maps were generated and different combinations of restriction endonucleases were tested. The combinations providing the clearest species-specific patterns were retained. Sequences were not polymorphic within species at the restriction sites chosen. The four enzymes selected were Bcl I (5'…T▫GATCA…3'), HpyCH4 V (5'…TG▫CA…3'), Xho II (5'…R▫GATCY…3') and Xmn I (5'…GAANN▫NNTTC…3'). Bcl I, Xho II and Xmn I enzymes were purchased from Fermentas (St. Leon-Rot, Germany). HpyCH4 V enzyme was purchased from New England Biolabs (Ipswich, MA, USA).

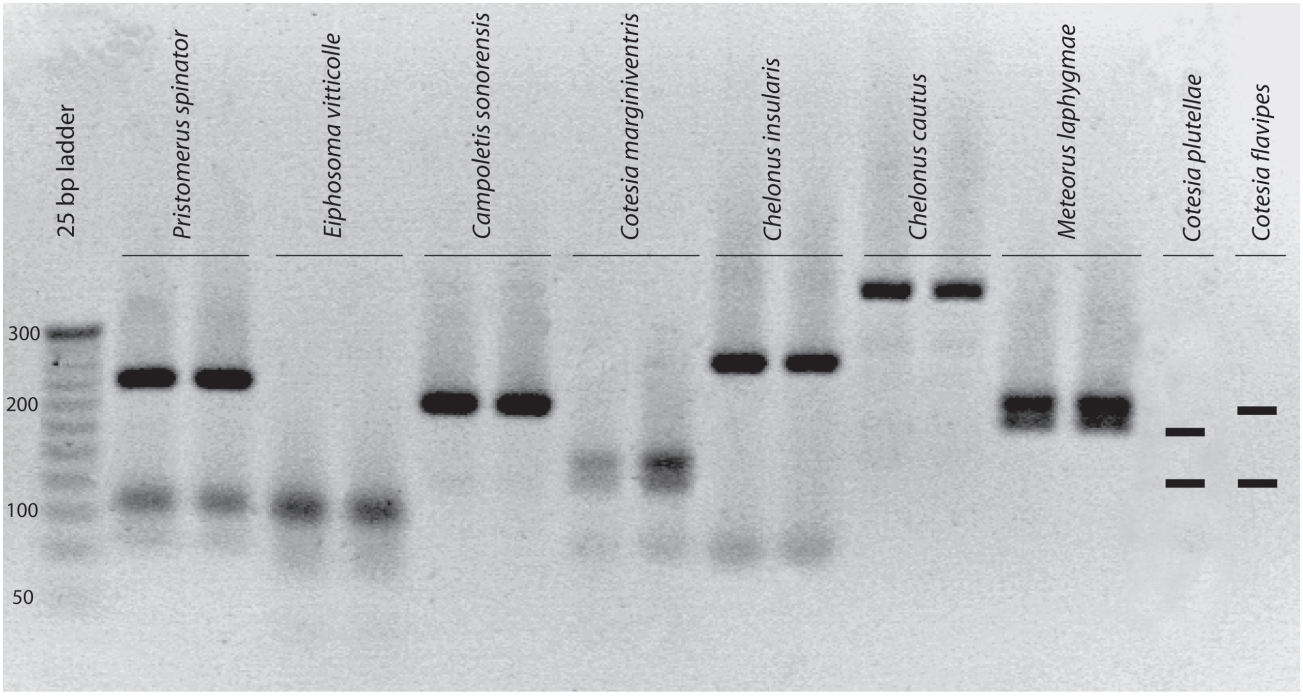


Figure 3: Digestion pattern. A species specific pattern was obtained upon digestion with the combination of restriction endonucleases Bcl I, Xho II, Xmn I and HpyCH4 V. Two individuals from different populations were used for each species. For *Cotesia plutellae* and *Cotesia flavipes*, simulated results of *COI* digestions are shown at the right of the gel.

Table 3: Genetic distances within (lower half) and between (diagonal) species. The genetic distances were computed with a Tamura-Nei model of substitution. Intraspecific genetic distances are much lower than interspecific distances, indicating that the *COI* fragment allows clear distinction between species.

	P. spinator	*M. laphygmae*	*E. vitticolle*	*C. marginiventris*	*C. insularis*	*C. cautus*	*C. sonorensis*
Pritomerus spinator	0.001	-	-	-	-	-	-
Meteorus laphygmae	0.276	0.003	-	-	-	-	-
Eiphosoma vitticolle	0.159	0.283	0	-	-	-	-
Cotesia marginiventris	0.261	0.17	0.278	0.002	-	-	-
Chelonus insularis	0.246	0.242	0.267	0.191	0	-	-
Chelonus cautus	0.227	0.239	0.288	0.213	0.145	0.027	-
Campoletis sonorensis	0.146	0.235	0.217	0.255	0.236	0.23	0.001

With this combination of enzymes, a specific pattern was obtained for adult wasps of each species when the digested product was run on an agarose gel (Fig. 3). *Pristomerus spinator* displayed two distinct bands at 230 bp and 110 bp respectively. *Eiphosoma vitticolle* exhibited only one band at 100 bp. One band at 210 bp characterized *Campoletis sonorensis*. *Cotesia marginiventris* was characterized by two close bands at 140 bp and 125 bp. *Chelonus insularis* could be recognized by one band at 250 bp. *Chelonus cautus* DNA was not digested and therefore exhibited a single band at 400 bp. *Meteorus laphygmae* could be easily identified with its two close bands at 220 bp and 180 bp. Bands shorter than 100 bp were barely distinguishable and were therefore not considered. These patterns observed for each species corresponded to the predictions made by the restriction maps and they were congruent with morphological species classification. The pattern obtained from each larva could be assigned to one of the specific patterns obtained from the adults, thereby allowing us to reliably identify the larvae.

The usefulness of the method was further confirmed with simulations run on *COI* sequences published for *C. plutellae* and *C. flavipes*. The simulations showed distinct patterns for the two species allowing easy discrimination from the other species (Fig. 3).

Discussion

The presented method allows the accurate identification of seven species of parasitoids of *S. frugiperda*. Similar techniques have been employed to identify parasitoid species (Tilmon *et al.*, 2000; Mowry and Barbour, 2004), but never for this many species with only a single test. Moreover, the results from simulations with published sequences of two additional closely related species of *Cotesia* parasitoids show that the method can be broadly employed.

With *S. frugiperda* being one of the most devastating pests on the American continent, easy and cost effective surveys of parasitoid communities attacking this noctuid may facilitate efforts to understand the distribution and biology of these potential biocontrol agents. Previous surveys conducted by Hoballah *et al.* (2004) and Molina-Ochoa *et al.* (2000, 2004) suggest that the seven species identified in this study represent 60% of the parasitoid species and account for 90% of total detected parasitism of *S. frugiperda* in the sampled locations. Not all parasitoid species are equally successful at parasitizing *S. frugiperda* and there may be considerable differences between seasons and locations. Indeed, in Hoballah *et al.*'s survey (2004), *C. sonorensis* and *C. marginiventris* were responsible for at least 85% of the observed parasitism. According to Molina-Ochoa *et al.*'s observations (2000, 2004), *C. insularis*, *C. cautus* and *P. spinator* accounted for over 80% of the observed parasitism at some of the same locations

as sampled in the current study. These five dominant species are among the seven that we sampled, which means that the chances of correctly identifying a parasitoid of *S. frugiperda* using this test are very high.

Results of a parasitoid inventory may be biased if only emerging adults are taken into account (Table 2). In fact, our sampling revealed that *C. insularis* accounted for 34.8% of parasitism when only adults were considered. However, almost 82% of the larvae that did not spin a cocoon belonged to this species, implying that *C. insularis* was actually responsible for 60.3% of total parasitism. Similarly, *C. sonorensis* was ranked fourth (out of seven) in abundance before identifying the larvae, but it was moved back to the sixth position when the larvae were included, switching positions with *C. cautus*. The other species remained at the same rank, although the proportion that they represented decreased due to the large number of *C. insularis* present among the larvae. It is important to note that *E. vitticolle* accounts for 3.60% and is ranked fifth, but it was actually only found in the state of Chiapas. This parasitoid is therefore a relatively abundant species in that area where it was responsible for 18.6% of the parasitism.

Chelonus insularis was the most abundant species in all the states, except for the locations sampled in the state of Nayarit, where *C. marginiventris* represented 53.5% of all parasitoids collected. The collections made in the state of Veracruz yielded only two parasitoid species: *C. insularis* and *C. cautus*. This is very different from the collections made by Hoballah *et al*. (2004). They sampled a very short distance away from our sampling location and found that *C. sonorensis* and *C. marginiventris* accounted for 85% of the observed parasitism. They also observed a total of 10 species. This discrepancy seems to indicate that there are strong seasonal variations. They sampled in January and February 2000 and 2001, whereas we sampled in June 2005. The rainy season usually starts in June, however, it was delayed that year. Consequently, few crops had been planted yet and the plants suffered much from drought. This undoubtedly affected the conditions for the insects living on the plants and may have favored a different species complex.

This study confirms the efficiency of *COI* as a barcode for animal taxa. Hebert *et al*. (2003a) demonstrated that *COI*-based information was sufficient to assign organisms to higher taxonomic ranks (e.g. genus, family). Moreover, *COI* can also distinguish species while showing only a low percentage of intraspecific divergence. Mallet and Willmott (2003) objected that DNA sequence differences among closely related species will be too small to allow their discrimination, due to gene introgression. This issue has never been studied comprehensively, however, it has been found that closely related species of vertebrates regularly show more than 2% divergence at another mitochondrial gene, *cytB* (Johns and Avise, 1998). Hebert *et al*. (2003b) further addressed this by examining the extent of sequence diversity at *COI* among congeneric taxa in the major animal phyla. They established that congeneric species of animals regularly possess substantial sequence divergence in their *COI* gene. More than 98% of the 13,320 species pairs analyzed by Hebert *et al*. (2003b) showed sequence divergence greater than 2%, the mean divergence value being 11.3%, indicating that most pairs were separated by more than 50 diagnostic substitutions in every 500 bp of their *COI* gene. The less than 2% divergence in the remaining species pairs may reflect short history of reproductive isolation (Hebert *et al*. 2003b). Our results support the notion that *COI* can reliably discriminate species. In fact, the inter-specific divergence ranged from 14.5% (between *C. insularis* and *C. cautus*) to 28.8% (between *C. cautus* and *E. vitticolle*) with a mean value of 22.9%, while the intra-specific divergence never exceeded 2.7% (*C. cautus*) with a mean value of 0.5%. The high intra-specific distance observed in *C. cautus* may be explained by a strong geographic structure. Indeed, groups of individuals coming from

distant geographic regions clearly form distinct clades (Fig. 2). This suggests that *C. cautus* has evolved in Mexico for a very long time and may even have originated in the Mesoamerican region.

It can be concluded that the presented method is reliable for the identification of the studied species. The method should also allow recognition of new species if novel patterns appear, as was confirmed with the simulations made on *C. plutellae* and *C. flavipes* sequences. In case a new pattern is found in future surveys, subsequent sequencing and comparison with published sequences might be necessary to determine exactly which species we are dealing with.

References

Barrett, R. D. H., and P. D. N. Hebert. 2005. Identifying spiders through DNA barcodes. *Canadian Journal of Zoology* 83(3):481-491.

Blaxter, M. L. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society B* 359:669-679.

Brewer, F. D., and E. G. King 1978. Effects of parasitism by a tachinid, Lixophaga diatraeae, on growth and food consumption of sugarcane borer larvae. *Annals of the Entomolomological Society of America* 71:19–22.

Cave, R. D. 1995. Manual para el reconocimiento de Parasitoides de Plagas Agrícolas en America Central. Primera Edicíon, Zamorano, Honduras: Zamorano Academic Press. 202 p.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package), Version 3.5c. Department of Genome Sciences, University of Washington, Seattle.

Fritzsche Hoballah, M. E., and T. C. J. Turlings. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research* 3:1-13.

Gross, Jr., H. R., and S. D. Pair. 1986. The fall armyworm: status and expectations of biological control with parasitoids and predators. *Florida Entomologist* 69(3):502-515.

Guillot, F. S., and S. B. Vinson. 1973. Effect of parasitism by Cardiochiles nigriceps on food consumption and utilization by Heliothis virescens. *Journal of Insect Physiology* 19:2072–2082.

Hajibabaei, M., G. A. C. Singer, P. D. N. Hebert, and D. A. Hickey. 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23(4):167-172.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003a. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B* 270:313-321.

Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London (Suppl.)* 270:S96-S99.

Hebert, P. D. N., M. Y. Stoeckle, T. S. Zemlak, and C. M. Francis. 2004. Identification of birds through DNA barcodes. *PLoS Biology* 2(10):1657-1663.

Hebert, P. D. N., and T. R. Gregory. 2005. The promise of DNA barcoding for taxonomy. *Systematic Biology* 54(5):852-859.

Hoballah, M. E., T. Degen, D. Bergvinson, A. Savidan, C. Tamó, and T. C. J. Turlings. 2004. Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agricultural and Forest Entomology* 6:83-88.

Johns, G. C., and J. C. Avise. 1998. A comparative summary of genetic distances in the vertebrates from the

mitochondrial cytochrome b gene. *Molecular Biology and Evolution* 15:1481-1490.

Kishino, H., and M. Hasegawa. 1989. Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA-sequence data, and the branching order in hominoidae. *Journal of Molecular Evolution* 29(2):170-179.

Knipling, E. F. 1980. Regional management of the fall armyworm — a realistic approach? *Florida Entomologist* 63:468-480.

Kranz, J., H. Schmutterer, and W. Koch 1977. Diseases, Pests and Weeds in Tropical Crops. Verlag Paul Parey, Berlin.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Briefings in Bioinformatics 5:150-163.

Lu, Y., and M. J. Adang. 1996. Distiguishing fall armyworm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Florida Entomologist* 79:48-55.

Luginbill, P. 1928. The fall armyworm. *U. S. Department of Agriculture Technical Bulletin* No. 34.

Mallet, J., and K. Willmott. 2003. Taxonomy: renaissance of Tower of Babel? *Trends in Ecology and Evolution* 18(2):57-59.

Molina-Ochoa, J., J. J. Hamm, R. Lezama-Gutiérrez, M. López-Edwards, M. González-Ramírez, and A. Pescador-Rubio. 2000. A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican States of Michoacán, Colima, Jalisco, and Tamaulipas. *Florida Entomologist* 84(1):31-36.

Molina-Ochoa, J., J. E. Carpenter, R. Lezama-Gutiérrez, J. E. Foster, M. González-Ramírez, C. A. Angel-Sahagún, and J. Farías-Larios. 2004. Natural distribution of hymenopteran parasitoids of Spodoptera frugiperda (Lepidoptera: Noctuidae) larvae in Mexico. Florida Entomologist 87(4):461-472.

Monaghan, M. T., M. Blake, T. R. Gregora, and A. P. Vogler. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society B* 360:1925-1933.

Mowry, T. M., and J. D. Barbour. 2004. Distinguishing the parasitic wasp, Peristenus howardi, from some of its cogeners using polymerase chain reaction and restriction endonuclease digestion. *Journal of Insect Science* 4:25.

Parkman, P., and M. Shepard. 1981. Foliage consumption by yellowstriped armyworm larvae after parasitization by Euplectrus plathypenae. *Florida Entomologist* 64:192–194.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Annals of the Entomological Society of America* 79:898-904.

Powell, J. E. 1989. Food consumption by tobacco budworm (Lepidoptera: Noctuidae) larvae reduced after parasitization by Microplitis demolitor or M. croceipes (Hymenoptera: Braconidae). *Journal of Economical Entomology* 82:408–411.

Rahman, M. 1970. Effect of parasitism on food consumption of P. rapae larvae. *Journal of Economical Entomology* 63:820–821.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87(6):651-701.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial-

DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.

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

Tilmon, K. J., B. N. Danforth, W. H., Day, and M. P. Hoffmann. 2000. Determining parasitoid species composition in a host population: a molecular approach. *Annals of the Entomological Society of America* 93(3):640-647.

Vickery, R. A. 1929. Studies of the fall armyworm in the Gulf Coast district of Texas. *U. S. Department of Agriculture Technical Bulletin* No. 138.

Yu, S. J. 1991. Insecticide resistance in the fall armyworm, Spodoptera frugiperda (J. E. Smith). *Pesticide Biochemistry and Physiology* 39:84-91.

Yu, S. J. 1992. Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). *Journal of Economical Entomology* 85:675-682.

Acknowledgments

We are grateful to Trevor Williams, Jaime Molina-Ochoa, and David Bergvinson for their assistance in the organization of field work. We also thank the Mexican students who helped us with field collections. Pierre Franck provided insightful comments on an earlier version of the manuscript. This study was supported by the Swiss National Center of Competence in Research “Plant Survival” and grant 3100A0-107974 from the Swiss National Science Foundation.

Chapter 2:

Environmental factors affecting parasitoid communities of the fall armyworm, *Spodoptera frugiperda*, on maize and sorghum

Environmental factors affecting parasitoid communities of the fall armyworm, *Spodoptera frugiperda*, on maize and sorghum

Violaine Jourdie and Ted C. J. Turlings

Université de Neuchâtel, Institut de Biologie, Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), Case Postale 158, 2009 Neuchâtel, Switzerland

Abstract

The role of plants in host location by parasitoids has long been investigated and is relatively well understood. However, successful biological control strategies also rely on a good knowledge about parasitoids communities.

In this paper, we investigate whether a relatively newly introduced host plant could affect community structure of parasitoids of the fall armyworm. The study was conducted in Mexico on maize and sorghum. Maize originated in Mesoamerica, hence parasitoids have coevolved with this plant for tens of thousands of years in this part of the world, whereas sorghum was introduced just over a century ago. Extrapolating from the "species-pool" hypothesis, we suggested that species richness would be greater on maize than on sorghum, and that species distribution would differ between the two host plants, as insects species may not have enough time yet to fully adapt to the recently introduced species. Collections were made in Central and South-Mexico. A canonical correspondence analysis revealed no significant effect of the host plant on parasitoid species distribution. However, we observed significant regional differences.

Keywords: host plant, latitude, elevation, parasitoid communities, Braconidae, Ichneumonidae, fall armyworm

Introduction

Plants often provide the first cue in the sequence of events that lead to host location by parasitoids of phytophagous insects, and parasitism levels of different parasitoid species on the same host species have been reported to vary from one host plant species to another (Vinson, 1976). Price *et al.* (1980) list and discuss some of the host plant characteristics that influence parasitism levels and parasitoid species composition in a host population. These include plant-secreted attractants, differences in parasitoid search behavior on different host plants, structural refuges that conceal the host and plant toxins sequestered by the host insect that adversely affect parasitoid survival.

All these plant signals are emitted upon attack by insect herbivores, such as the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), one of the New World's most devastating pest. The FAW is a serious pest of a wide variety of economically important crops in the Americas (Kranz *et al.*, 1977). Insecticides are commonly used to kill its larvae which feed on plant leaves, causing great damage. However, the use of toxic chemicals is unsafe for both environmental and health issues. It is therefore crucial to resort to safer alternatives methods. Biological control is a good candidate (Gross and Pair, 1986) as the FAW is attacked by many different species of hymenopteran parasitoids (Molina-Ochoa *et al.*, 2000; Molina-Ochoa *et al.*, 2003; Molina-Ochoa *et al.*, 2004). It is essential to have good knowledge of the system in order to develop strategies that

optimize biological control.

Many basic questions remain to be answered concerning the tritrophic system that comprises the FAW, the plants it attacks and its parasitoids. Although many studies have looked at how plants can affect parasitoids behavior and development (Barbosa *et al.*, 1986; El-Heneidy *et al.*, 1988; Turlings *et al.*, 1989; Turlings *et al.*, 1990; Vet and Groenewold, 1990; Whitman and Nordlund, 1994; Turlings and Benrey, 1998), to our knowledge, the effect of host plant on parasitoids communities has only barely been investigated. In this study, we aimed to determine whether host plant influences parasitoid species distribution. The study was conducted in Mexico where maize originated and has evolved there for tens of thousands of years (Smith, 1968; Heiser, 1979). In contrast, sorghum originated in Africa (Harlan, 1976) and was first introduced in America around mid 19th century (Smith and Fredriksen, 2000). FAW larvae were collected on maize and sorghum fields and reared out until emergence of parasitoids.

According to the "species-pool" hypothesis (Taylor *et al.*, 1990), the older and the larger the local and/or global area of a habitat type, the greater the past opportunity for speciation and hence, the greater the number of available species that are adapted to that particular habitat type. Similarly, in a system like ours, we could expect that there has yet not been enough time for all insect species to adapt to the introduced plant species. We can then suppose insect species diversity would be higher on host plants that have been around for longer. Our hypothesis was therefore that species diversity would be greater on maize than on sorghum.

Table 1: Collections were made in 33 locations in Central and South Mexico on maize and sorghum. State, name of location, exact GPS coordinates, elevation and host plant are listed for each sampled site.

STATE	CODE	LOCATION	MUNICIPIO	GPS COORD.	ELEVATION (m)	CROP
Colima	C1	Agua Zarca	Coquimatlán	19°13'12.0"N, 103°56'10.1"W	273	m
	C2	El Colomo, Coquimatlán	Coquimatlán	19°13'58.3"N, 103°57'17.4"W	322	m
	C3	Pueblo Juarez, Coquimatlán	Coquimatlán	19°09'45.9"N, 103°53'44.4"W	234	s
	C4	Los Mezcales	Comala	19°19'57.3"N, 103°46'07.6"W	637	m
	C6	La Caja	Comala	19°22'31.3"N, 103°48'12.8"W	636	m
	C7	Villa de Alvarez	Minatitlán	19°16'57.9"N, 103°46'41.0"W	520	s
	C8	Villa de Alvarez	Minatitlán	19°17'01.2"N, 103°46'41.0"W	520	m
Chiapas	CH1	Jaritas	Tapachula	14°42'53.3"N, 92°18'24.9"W	16	s
	CH2	Jaritas	Tapachula	14°43'01.5"N, 92°18'26.6"W	23	m
	CH3	Jaritas	Tapachula	14°44'53.7"N, 92°20'06.2"W	27	s
	CH4	Jaritas	Tapachula	14°43'32.3"N, 92°18'58.1"W	17	s
	CH5	Jaritas	Tapachula	14°43'20.0"N, 92°19'09.1"W	24	s
Guanajuato	G1	Los Lobos	Valle de Santiago	20°27'52.9"N, 101°12'01.7"W	1720	s
	G2	Rancho Seco	Salamanca	20°27'30.9"N, 101°12'16.7"W	1723	m
	G3	Rancho Seco	Salamanca	20°27'30.9"N, 101°12'16.7"W	1723	s
	G4	Lucero de Ramales	Silao	20°55'15.6"N, 101°27'38.9"W	1771	m
	G5	Lucero de Ramales	Silao	20°55'15.6"N, 101°27'38.9"W	1771	s
	G6	Puerta Chica	Silao	20°52'10.3"N, 101°29'20.9"W	1752	m
Jalisco	J1	Usmajac	Sayula	19°52'08.9"N, 103°33'16.7"W	1374	s
	J2	Usmajac	Sayula	19°52'18.4"N, 103°33'45.6"W	1374	s
	J3	La Sierrita	Degollado	20°28'40.1"N, 102°12'26.5"W	1734	m
	J4	Rancho Nuevo	Degollado	20°28'22.3"N, 102°12'06.4"W	1739	s
Nayarit	N1	Carretera Jala	Ahuacatlán	21°03'39.6"N, 104°27'09.8"W	1027	m
	N2	Ejido Mexpan	Ixtlán del Rio	21°02'35.0"N, 104°28'03.1"W	1016	s
	N3	Ejido Mexpan	Ixtlán del Rio	21°02'38.3"N, 104°27'42.6"W	1035	s
	N4	El Humedo	Ahuacatlán	21°01'35.9"N, 104°28'17.7"W	1024	m
	N5	La Iguerita	Jala	21°05'29.0"N, 104°26'04.9"W	1054	m
	N6	La Iguerita	Jala	21°05'20.9"N, 104°26'05.0"W	1048	s
Puebla	P1	CIMMYT tropical field station	Agua Fría	20°27'18.3"N, 97°38'28.8"W	95	m
	P2	CIMMYT tropical field station	Agua Fría	20°27'10.3"N, 97°38'28.7"W	102	m
	P3	CIMMYT tropical field station	Agua Fría	20°27'19.6"N, 97°38'26.3"W		m
	P4	CIMMYT tropical field station	Agua Fría	20°27'17.4"N, 97°38'26.3"W		m
Veracruz	V1	Lindero	Lindero	20°29'31.9"N, 97°32'17.2"W	66	m

Materials and methods

Insects were collected from 33 locations in seven Mexican states on both maize and sorghum (Table 1) in June, August and September 2005 and July 2006. Insects were reared as described earlier (Jourdie *et al.*, 2008). Adult parasitoids were morphologically identified following a manual (Cave, 1995), whereas parasitoid larvae were molecularly assigned to species using the technique described in Jourdie *et al*. (2008).

A Canonical Correspondence Analysis (CCA) using the program CANOCO for Windows version 4.52 (ter Braak and Smilauer, 1998) was used to analyze the relationship between species distribution and environmental variables, namely latitude/longitude, elevation and host plant. A logarithmic transformation (ter Braak and Smilauer, 1998) was applied to the data. A Monte Carlo (ter Braak and Smilauer, 1998) permutation test with unrestricted permutations was used to evaluate if both ordination axes were related to the environmental variables. Graph was drawn using CANODRAW 4.12 (Smilauer, 1992). Forward selections were performed to determine the importance of the different factors.

Results

A total of ten parasitoid species emerged from FAW larvae: five Braconidae (*Cotesia marginiventris*, *Cotesia plutellae*, *Chelonus insularis*, *Chelonus cautus*, and *Meteorus laphygmae*), three Ichneumonidae (*Pristomerus spinator*, *Campoletis sonorensis* and *Eiphosoma vitticolle*) and two Trichogrammatidae (*Trishogramma exiguum* and *Trichogramma* sp.). Parasitoid species distributions per region and per host plant are presented in Figure 1. Only maize is present in the Poza Rica area (collections from Puebla-Veracruz), hence no data are available on sorghum for this region. *Chelonus insularis* was by far the most abundant species in all regions, ranging from 58.6% of total collections in Chiapas to 72.3% in Nayarit. The highest frequency of *C. sonorensis* was observed in Nayarit, where it accounted for 22.3% of all parasitoids collected. It was however absent in our collections done on the eastern coast of Mexico, in the states of Puebla and Veracruz. Highest diversity was observed in the states of Nayarit and Colima where 8 species were collected in each state (*C. insularis*, *P. spinator*, *C. marginiventris*, *C. cautus*, *M. laphygmae*, *C. sonorensis*, *E. vitticolle* and *C. plutellae* in Nayarit; and *C. insularis*, *P. spinator*, *C. cautus*, *C. marginiventris*, *C. sonorensis*, *Trichogramma* sp., *M. laphygmae* and *T. exiguum* in Colima, by decreasing order of abundance). Collections in the state of Chiapas were characterized by the presence of *E. vitticolle* in relatively high proportion (18.4%). It occurred only on sorghum.

Slight differences were observed between collections made on maize and collections made on sorghum (Figure 1). The major difference was observed in Chiapas, where the species collected on maize and their abundance [*P. spinator* (71.4%), *C. insularis* (23.8%) and *M. laphygmae* (4.8%)] differed from those collected on sorghum [*C. insularis* (69.7%), *E. vitticolle* (24.2%), *P. spinator* (4.5%) and *C. cautus* (1.5%)]. The fact that *E. vitticole* was only found on sorghum may only be an artifact of sampling conditions. Indeed, weather conditions were not favorable that season and *Spodoptera* eggs were dislodged from the leaves by heavy rains, hence difficulties of finding suitable patches for collections. Therefore, only one maize field was visited versus four sorghum fields. In Nayarit, *M. laphygmae* was observed on maize, but not on sorghum. Conversely, in the state of Colima, *M. laphygmae* was present on sorghum, but absent on maize, whereas *C. cautus* and *T. exiguum* were collected on maize, but not on sorghum. *Chelonus insularis* was slightly less dominant on maize than on sorghum.

CCA showed a strong correlation between environmental variables and species distribution (Figure 2). The eigenvalues for the first two ordination axis were 0.269 and 0.199, explaining 14.6 and 25.3% of the species variance and 51.1

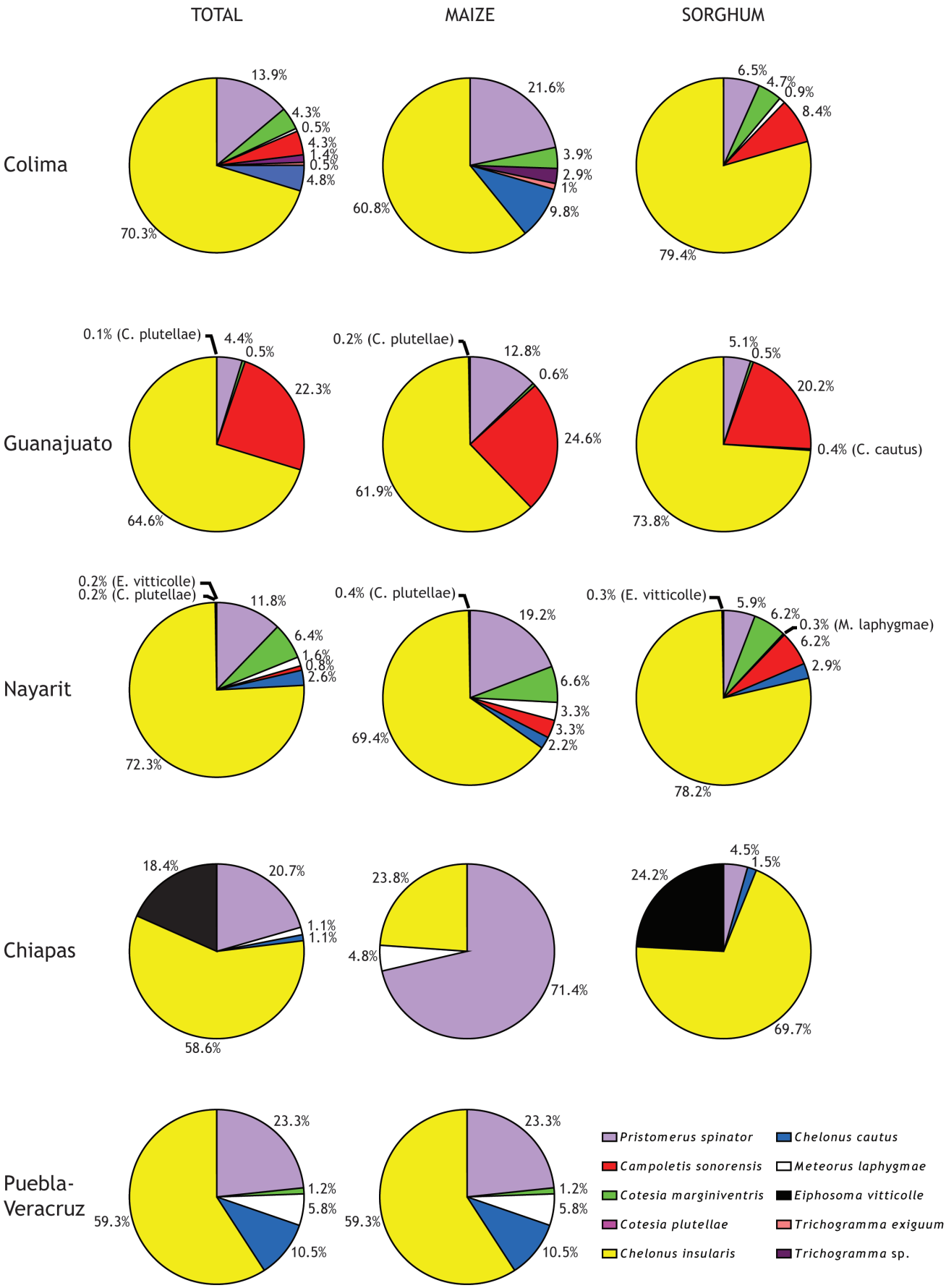


Figure 1: Species distribution per region. *Chelonus insularis* was largely predominant in all regions. The highest species diversity was observed in the state of Nayarit. Diversity was low in the state of Guanajuato compared to surrounding states: this could be explained by the fact that collections were made in areas of intensive agriculture.

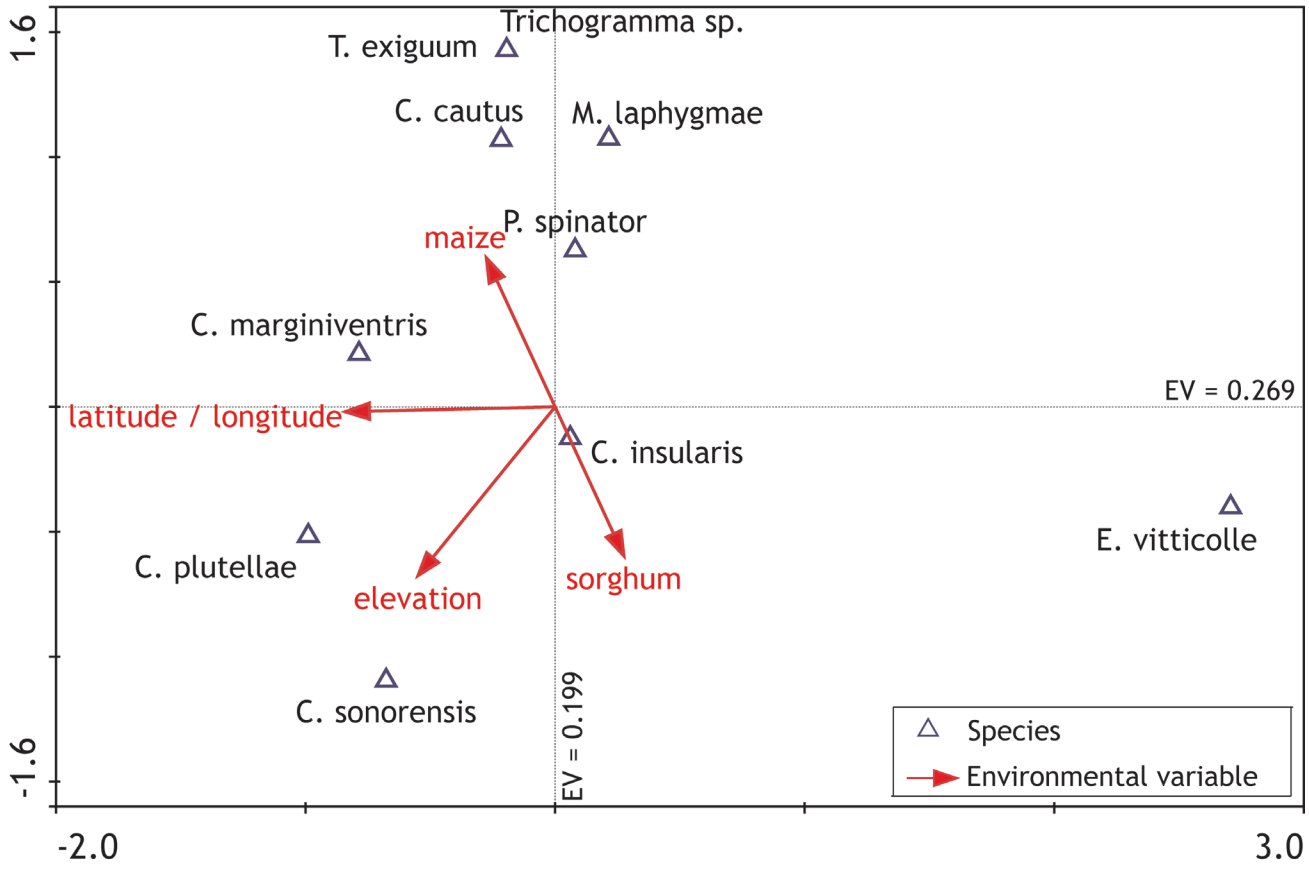


Figure 2: Canonical Correspondence Analysis showed correlation between environmental variables and species distribution. Elevation and geographic coordinates significantly influenced species distribution ($P = 0.002$), as opposed to host plant which had no significant impact ($P = 0.114$).

and 88.9% of the cumulative variance of the relationship species x environmental variables. Forward selections revealed that geographic coordinates (i.e. latitude and longitude) and elevation significantly influenced species distribution (F-ratio = 4.97, P = 0.002; and F-ratio = 3.83, P = 0.002 respectively for geographic coordinates and elevation). Indeed, species richness tended to be higher at higher latitudes, though some species were found mainly in southernmost locations (e.g. *E. vitticolle*). *Campoletis sonorensis*, *C. marginiventris* and *C. plutellae* were encountered mostly at higher elevations and at higher latitudes. Host plant, however, had no significant effect on species distribution (F-ratio = 1.67, P = 0.114) despite the slight differences observed on maize versus sorghum.

Discussion

Our results do not support the hypothesis that species richness would be higher on maize than on sorghum, as host plant seems to have no effect on species distribution. The parasitoid species that occur on maize apparently had enough time to adapt to fairly recently introduced sorghum, or their flexibility in foraging and development on different diets preclude the need for major adaptations. It might be worthwhile to compare species distribution on host plants that are taxonomically more distant than maize and sorghum, which both belong to the Poaceae. *S. frugiperda* attacks a variety of newly introduced crop plants species, including peanut, rice or alfalfa (Knipling, 1980; Pashley, 1986; Lu and Adang, 1996), which are more likely to require adaptations in its parasitoids.

We found a significant effect of latitude on species distribution. *Campoletis sonorensis*, *C. marginiventris* and *C. plutellae* were collected at higher latitudes, while *E. vittivolle* was only/

mainly present at lower latitudes (Figure 2). All other species seemed to be less influenced by this factor. Our collections in the state of Nayarit, which was our northernmost sampling location, yielded the highest species richness. This is congruent with results from previous studies which showed that entomophagous parasitoid species richness does not increase along the increasing latitudinal gradient of increasing species richness of all insects (Owen and Chanter, 1970; Owen and Owen, 1974; Janzen and Pond, 1975; Rathcke and Price, 1976; Janzen, 1977, 1981). Indeed, the expectation that parasitoid species richness will increase when prey species richness increases (Price, 1977) ignores the fact, as Janzen (1981) points out, that if there are more species of prey, there must be fewer of each kind if the aggregate insect biomass or turnover rate does not increase. As the parasitoid resource base becomes ever more finely divided, there must come a point where the carrying capacity of the habitat for parasitoid species is reduced by further resource fragmentation (Janzen, 1981).

Species richness in this study was relatively low compared to previous studies that provided an inventory of hymenopteran parasitoids of the FAW (Molina-Ochoa *et al.,* 2000; Molina-Ochoa *et al.,* 2003; Hoballah *et al.,* 2004; Molina-Ochoa *et al.,* 2004; Wyckhuys and O'Neil, 2006). Species richness was particularly low in the state of Guanajuato and Chiapas. This may be due to the fact that we collected in areas of intense agricultural. During agricultural intensification, the overall complexity of the landscape is reduced, natural non-crop habitat is fragmented and the non-crop landscape becomes a mosaic of relatively discrete habitat types (Daily *et al.,* 2001; Tscharntke *et al.,* 2002; Tscharntke *et al.,* 2005a). Habitat fragmentation is associated with low density of individuals of a given species and high rates of local and regional extinction, leading to low overall species diversity (Hanski, 1994; Harrison and Bruna, 1999; Tscharntke and Brandl, 2004). In an agricultural landscape, however, habitat fragmentation may be a greater problem for specialist than for generalist parasitoids, as the latter can use prey resources in a greater variety of habitat types (Golden and Crist, 1999; Tscharntke *et al.,* 2005a; Tscharntke *et al.,* 2005b; Rand and Tscharntke, 2007). However, generalist parasitoids may suffer as much as specialist from habitat fragmentation as non-crop habitat types such as forest, hedgerows, field margins, fallows and meadows, which provide a number of important resources for parasitoids such as permanent vegetation cover suitable for overwintering, refuges from disturbances, as well as resources such as alternatives prey, pollen and nectar (Landis *et al.,* 2000; Cronin and Reeve, 2005; Bianchi *et al.,* 2006), become more scarce.

The effect of altitude we observed cannot be clearly distinguished from region effect. Indeed, each elevation corresponds to one region, with the exception of Chiapas and Puebla-Veracruz which are both at sea level. Altitude has been shown to have effects on many physiological and morphological traits in insects as well as on insect community structure [review in Hodkinson (2005)]. Therefore, we should be cautious with interpreting on the apparent altitude effect. More replicates for each elevation, latitude, host plant and other factors are needed in order to safely draw any conclusion. More studies of this kind also may help to identify key ecological factors that determine the success or failure of biological control of crop pests.

Host plant is obviously not the most important factor shaping parasitoid communities. Therefore, more environmental variables need to be taken into consideration. Information on agricultural practices in the past and recent history in each region would undoubtedly be useful to try and understand patterns of species distribution. Data on landscape structure surrounding each sampled field could be used to build a GIS model on which the collected species could then be mapped. Data on phytosanitary treatments may also contribute to explaining parasitoid species distribution.

References

Barbosa, P., J. A. Saunders, and J. E. A. Kemper. 1986. Plant allelochemicals and insect parasitoids: effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology* 12:1319-1328.

Bianchi, F., C. J. H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B* 273:1715-1727.

Cave, R. D. 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en America Central. Zamorano Academic Press, Zamorano, Honduras.

Cronin, J. T., and J. D. Reeve. 2005. Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proceedings of the Royal Society B* 272:2225-2235.

Daily, G. C., P. R. Ehrlich, and G. A. Sánchez-Azofeifa. 2001. Countryside biogeography: utilization of human-dominated habitats by the avifauna of southern Costa Rica. *Ecological Applications* 11:1-13.

El-Heneidy, A. H., P. Barbosa, and P. Gross. 1988. Influence of dietary nicotine on fall armyworm, *Spodoptera frugiperda* and its parasitoid, the ichneumonid wasp *Hyposoter annulipes*. *Entomologia Experimentalis et Applicata* 46:227-232.

Golden, D. M., and T. O. Crist. 1999. Experimental effects of habitat fragmentation on old-field canopy insects: community, guild and species responses. *Oecologia* 118:371-380.

Gross, H. R., and S. D. Pair. 1986. The fall armyworm: status and expectations of biological control with parasitoids and predators. *Florida Entomologist* 69:502-515.

Hanski, I. 1994. Spatial scale, patchiness and population dynamics on land. *Philosophical Transactions of the Royal Society London B* 343:19-25.

Harlan, J. R. 1976. Plant and animal distribution in relation to domestication. Pp. 13-25 *in* J. Hutchinson, ed. The Early History of Agriculture. Oxford University Press, Oxford.

Harrison, S., and E. Bruna. 1999. Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* 22:225-232.

Heiser, C. B. 1979. Origins of some cultivated New World plants. *Annual Review of Ecology and Systematics* 10:309-326.

Hoballah, M. E., T. Degen, D. Bergvinson, A. Savidan, C. Tamo, and T. C. J. Turlings. 2004. Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agriculatural and Forest Entomology* 6:83-88.

Hodkinson, I. D. 2005. Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* 80:489-513.

Janzen, D. H. 1977. Why are there so many species of insects? Pp. 84-94 *in* D. White, ed. Proceedings of The Fourth International Congress on Entomology. Available from the Entomological Society of America, College Park, Maryland.

Janzen, D. H. 1981. The peak in North American Ichneumonid species richness lies between 38 degrees and 42 degrees N. *Ecology* 62:532-537.

Janzen, D. H., and C. M. Pond. 1975. A comparison, by sweep sampling, of the arthropod fauna of secondary vege- tation in Michigan, England and Costa Rica. *Transactions of the Royal Entomological Society London* 127:33-50.

Jourdie, V., N. Alvarez, and T. C. J. Turlings. 2008. Identification of seven species of

hymenopteran parasitoids of *Spodoptera frugiperda*, using polymerase chain reaction amplification and restriction enzyme digestion. *Agriculatural and Forest Entomology* 10:129-136.

Knipling, E. F. 1980. Regional management of the fall armyworm — a realistic approach? *Florida Entomologist* 63:468-480.

Kranz, J., H. Schmutterer, and W. Koch. 1977. Diseases, Pests and Weeds in Tropical Crops. Verlag Paul Parey, Berlin.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:175-201.

Lu, Y. J., and M. J. Adang. 1996. Distinguishing fall armyworm (Lepidoptera: Noctuidae) strains using a diagnostic mitochondrial DNA marker. *Florida Entomologist* 79:48-55.

Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs, and J. E. Foster. 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomologist* 86:254-289.

Molina-Ochoa, J., J. E. Carpenter, R. Lezama-Gutierrez, J. E. Foster, M. Gonzalez-Ramirez, C. A. Angel-Sahagun, and J. Farias-Larios. 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in Mexico. *Florida Entomologist* 87:461-472.

Molina-Ochoa, J., J. J. Hamm, R. Lezama-Gutiérrez, M. López-Edwards, M. González-Ramírez, and A. Pescador-Rubio. 2000. A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican states of Michoacán, Colima, Jalisco, and Tamaulipas. *Florida Entomologist* 84(1):31-36.

Owen, D. F., and D. O. Chanter. 1970. Species diversity and seasonal abundance in tropical Ichneumonidae. *Oikos* 21:142-144.

Owen, D. F., and J. Owen. 1974. Species diversity in temperate and tropical Ichneumonidae. *Nature* 249:583-584.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Annals of the Entomological Society of America* 79:898-904.

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

Price, P. W., C. E. Bouton, and P. E. A. Gross. 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.

Rand, T. A., and T. Tscharntke. 2007. Contrasting effects of natural habitat loss on generalist and specialist aphid natural enemies. *Oikos* 116:1353-1362.

Rathcke, B. J., and P. W. Price. 1976. Anomalous diversity of tropical ichneumonid parasitoids: a predation hypothesis. *American Naturalist* 110:889-893.

Smilauer, P. 1992. CANODRAW: User's guide., version 3.0. Microcomputer Power, Ithaca, New York.

Smith, C. E. 1968. New Wrold centers of origin of cultivated plants ans archeological evidence. *Economical Botany* 22:253-and.

Smith, C. W., and R. A. Fredriksen, eds. 2000. Sorghum: origin, history, technology, and production. John Wiley and Sons, New York.

Taylor, R. D., L. W. Aarssen, and C. Loehle. 1990. On the relationship between r/K selection and environmental carrying capacity: a new habitat templet for plant life history strategies. *Oikos* 58:239-250.

ter Braak, C. J. F., and P. Smilauer. 1998. CANOCO Reference manual and user's guide to Canoco for Windows: software for canonical community ordination, version 4. Microcomputer Power, Ithaca, New York.

Tscharntke, T., and R. Brandl. 2004. Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology* 49:405-430.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005a. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* 8:857-874.

Tscharntke, T., T. A. Rand, and F. J. J. A. Bianchi. 2005b. The landscape context of trophic interactions: insect spillover across the crop-noncrop interface. Annales Zoologici Fennici 42:421-432.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. *Ecological Applications* 12:354-363.

Turlings, T. C. J., and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5:321-333.

Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251-1253.

Turlings, T. C. J., J. H. Tumlinson, W. J. Lewis, and L. E. M. Vet. 1989. Beneficial arthropods behavior mediated by airborne semiochemicals. VIII. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *Journal of Insect Behavior* 2:217-225.

Vet, L. E. M., and A. W. Groenewold. 1990. Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16:3119-3135.

Vinson, S. B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21:109-133.

Whitman, D. W., and D. A. Nordlund. 1994. Plant chemicals and the location of herbivorous arthropods by their natural enemies. Pp. 133-159 *in* T. N. Anathakrishnan, ed. Functional dynamics of phytophagous insects. Oxford and IBH Publishing.

Wyckhuys, K. A. G., and R. J. O'Neil. 2006. Population dynamics of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. *Crop Protection* 25:1180-1190.

Acknowledgements

The authors wish to thank Trevor Williams, Jaime Molina-Ochoa, and David Bergvinson for their assistance in the organization of field work. We also thank the Mexican students who helped us with field collections. Matthias Held provided help with the statistical analyses. This study was supported by the Swiss National Center of Competence in Research "Plant Survival" and grant 3100A0-107974 from the Swiss National Science Foundation.

Chapter 3:

Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of the noctuid *Spodoptera frugiperda*: *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera)

Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of the noctuid *Spodoptera frugiperda*: *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera)

Violaine Jourdie[1], Nadir Alvarez[1], Ted C. J. Turlings[1] and Pierre Franck[2]

[1]*Institut de Biologie, Université de Neuchâtel, Emile-Argand 11, 2009 Neuchâtel, Switzerland*

[2] *UR1115, Plantes and Systèmes de culture Horticoles, INRA, 84914 AVIGNON Cedex 9, France*

Accepted in ***Molecular Ecology Resources*** on June 9, 2008

Abstract

Fifteen and 13 microsatellite loci were isolated respectively from *Campoletis sonorensis* Cameron and from *Chelonus insularis* Cresson. These two parasitic Hymenoptera are primary parasitoids of Lepidoptera in North, Central and South America, including the important agricultural pest *Spodoptera frugiperda*. Allelic diversity and heterozygosity were quantified in samples from Mexico. Each locus was polymorphic, with the number of alleles ranging from two to 16 in *C. sonorensis* and from four to 18 in *C. insularis*. Heterozygosity ranged from 0.088 to 0.403 in *C. sonorensis* and from 0.106 to 0.458 in *C. insularis*.

Keywords: *Chelonus*, *Campoletis*, microsatellites, parasitoids, population structure

Introduction

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is one of the most devastating pests in the Americas (Kranz *et al.*, 1977) attacking numerous crops of great economic importance (Knipling, 1980; Pashley, 1986; Sparks, 1979). This Lepidoptera is in turn attacked by a number of parasitoid species (Molina-Ochoa *et al.*, 2003; Molina-Ochoa *et al.*, 2004; Molina-Ochoa *et al.*, 2000). A good understanding of the patterns of genetic variability within and among parasitoid populations may help us to determine the selection forces that drive their population dynamics and evolution. *Chelonus insularis* Cresson (Hymenoptera: Braconidae: Cheloninae) is an egg-larval parasitoid of *Spodoptera* sp. while *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae: Campoleginae) is a larval parasitoid. Both species are distributed over South, Central and North America and they were the two predominant parasitoid species collected in Mexico during the summers of 2005 and 2006. Microsatellite markers were developed to investigate a potential effect of the host plant on the population genetic structure of parasitoids.

Materials and Methods

Total genomic DNA was isolated from four individuals of *C. insularis* and from three individuals of *C. sonorensis*, using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Enriched DNA library with microsatellite sequences and sequencing of 192 clones per species were performed by the SREL DNA Lab, University of Georgia, USA following

Table 1: Attributes of the *Campoletis sonorensis* and *Chelonus insularis* microsatellite loci: *N* indicates the number of females scored for each species, *N*a the number of alleles and T_m the melting temperature of the primer pair. Results of Hardy-Weinberg exact test (HW) comparing observed (H_O) and expected (H_E) heterozygosities are reported.

Locus	Repeat	Size range (bp)	N_a	Primer sequence (5'-3') (F = forward, R = reverse)	Fluorescent dye	[$MgCl_2$] (mM)	T_m (°C)	H_E	H_O	HW†	GenBank Accession no.
Campoletis sonorensis *N* = 68											
Cs6	$(TAGA)_6$	230-258	8	F: CGA GAA TCG AGA GAC ACG R: GAA CAT TCG CGT GAG C	6-FAM	1.5	55	0.348	0.279	**	EU678871
Cs9	$(TCTA)_{10}$	98-122	6	F: GCA TTC TCA CGC ATC G R: AGC GAA ACA CCT TAC CG	6-FAM	1.5	55	0.284	0.228	NS	EU678872
Cs14	$(CTT)_5$	150-162	5	F: AGC CAC GTA ATA CAA AGT CG R: TGT GTG TGT CAG AGG ATC G	6-FAM	1.5	55	0.289	0.321	NS	EU678873
Cs15	$(TG)_{11}$	149-165	5	F: AGA GGA AGC TCT GTG ACG R: AGC TCA ACC CAC TCT CG	6-FAM	1.5	54	0.136	0.156	NS	EU697897
Cs19	$(TGA)_{21}$	235-279	16	F: CTG GCA TTG TGT GTC G R: CTT CTA ACT TTG CGT CTC G	6-FAM	1.5	53	0.459	0.403	*	EU697898
Cs20	$(TGA)_8$	209-227	7	F: GAG TGT CTG CGT GAA AGG R: GAT CCG TTT ATC GTG TAT CG	NED	1.5	55	0.355	0.324	NS	EU678874
Cs21	$(GA)_{10}$	214-254	16	F: ATG GAA GCT GGT TGA AGG R: CCT CAT CAT CGT CTG TGG	NED	1	55	0.4	0.338	***	EU678875
Cs22	$(GA)_8$	232-264	11	F: ACG ATC ACG AGA CAG TGA G R: TAT TTG TGT GCC ATG GTG	NED	1.5	54	0.334	0.375	NS	EU678876
Cs23	$(CAAA)_6$	178-198	6	F: GAT AGC TGC ACG AAA ACG R: GAT ACC TGT CTC GCT ATT GG	HEX	1.5	55	0.173	0.144	*	EU697899
Cs37	$(TTGA)_8$	92-104	4	F: CAT AAC CGA GAG GAA TCG R: GCC CTA AGG ACT TAT CCA G	6-FAM	1.5	54	0.276	0.161	***	EU697900
Cs42	$(CAAA)_6$	153-165	4	F: GAG TGT CAC CCT GTC TCG R: GAT GTT GAT GAT TAA TTG TTG C	NED	1	55	0.281	0.294	NS	EU678877
Cs44	$(TGA)_5$	209-212	2	F: TGG GGT TGG TAT ATT TGG R: TGT TGG GTG ATA GTT CAG G	HEX	1.5	54	0.206	0.213	NS	EU678878
Cs47	$(GTCT)_4$	204-216	3	F: CAA CAC GTG CCA ATC G R: AAT GGC TTT TCT CCA AGC	HEX	1.5	56	0.083	0.088	NS	EU678879
Cs48	$(GTT)_7$	150-174	8	F: CCA ATG AGG TCA GTT TCG R: ACG AAT GAA CCA CAG AGC	6-FAM	1.5	55	0.373	0.368	NS	EU678880
Cs49	$(TGAG)_4$	221-237	5	F: AAA ACC ACC AAC TAC TAC GG R: TGC TAA AGC GAG AAA AGC	HEX	1	55	0.175	0.14	NS	EU678881

Chelonus insularis											
N = 59											
Ci1	$(CAA)_{26}$	304-373	18	F: GGC TTC CTG AAT CAA ATG R: GAC AAT GGT GAA TTT GGA C	6-FAM	1	54	0.451	0.415	NS	EU678882
Ci9	$(TGTA)_{16}$	298-350	12	F: CCA GTG GAA AAG TAT CG R: TGA GAA CAG AAT AGT TTA AGG	6-FAM	1	49	0.435	0.226	***	EU697893
Ci10	$(TGA)_7$	165-174	4	F: CAA AAG ACG AGT ATT GAT GG R: CCA GCA TAA GTT GGA GAA G	6-FAM	1.5	54	0.245	0.237	NS	EU678883
Ci11	$(CAA)_{11}$	182-212	9	F: ATC GAC GTC AAC ATA CTG G R: TGA AGG TGG TGG TAG TGG	NED	1	55	0.394	0.415	NS	EU678884
Ci12	$(TGA)_9$	247-265	7	F: TGC CTA GGT CAT TTG C R: TGA GTT AAT ACT CTC GCT TG	6-FAM	1	51	0.331	0.345	NS	EU678885
Ci13	$(GT)_{14}$	261-279	6	F: CCT TCG ATT ATT CCT TTC G R: CCT TTG AAA CCT GTA GAT AAC C	6-FAM	1	55	0.294	0.106	***	EU697894
Ci15	$(GTT)_7$	164-173	4	F: TGA TTT GGT TGC TCT TGC R: TCC TCG AAC TCT TCA CAC C	6-FAM	1.5	56	0.257	0.167	*	EU697895
Ci16	$(GA)_9$	214-228	5	F: TAG CTA CAT TGG GGA TCG R: GGG TGA AAT TCC CTT CC	NED	1.5	55	0.183	0.195	NS	EU678886
Ci17	$(TCA)_7$	187-208	7	F: GAA GCA AAG GGC AAC G R: GCG AAG GAC ATA CTC ATG G	HEX	1	57	0.375	0.356	*	EU678887
Ci28	$(TGA)_{21}$	251-314	18	F: CAG ACT GGT TGG TTA CAG G R: AGC AGA ATC ACC AAC AGC	6-FAM	1	54	0.459	0.42	*	EU697896
Ci30	$(GA)_{11}$	225-233	5	F: AGC CCA AGC AAT ATT ATC C R: CTT TCT TCG TCA TTC AGT CC	NED	1.5	55	0.309	0.328	NS	EU678888
Ci31	$(CT)_{15}$	256-284	12	F: CCT TAA TTG TAT TAG GCT CTG G R: GCC AAT TGT CTG AAA AGC	6-FAM	1.5	55	0.436	0.458	NS	EU678889
Ci33	$(GA)_9$	167-173	4	F: TGG TGA GTG TGT GTG AGC R: GTA TGA GCG GAC AAA AGG	6-FAM	1.5	54	0.173	0.203	NS	EU678890

[†]NS = non significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Glenn and Schable's protocol (Glenn and Schable, 2005). Cloned microsatellite sequences were edited using the ChromasPro 1.41 software (Technelysium Pty. Ltd, Queensland, Australia). Microsatellite loci which offered a flanking region long enough to design primers were selected. Seventy-six and 98 primer pairs were then designed respectively for *C. insularis* and *C. sonorensis* with Primer3 (v. 0.4.0) (Rozen and Skaletsky, 2000) using stringent conditions (i.e. Primer T*m*: 50-60°C (optimal:55°C), max. T_m difference: 1°C, GC content: 40-60%, max. self complementarity: 6, max. 3' self complementarity: 2, max. poly-X: 4, GC clamps: 2).

PCR amplifications were carried out in 10-µl volumes containing 1 µl of extracted DNA, 1.5 mM $MgCl_2$ depending on the locus (Table 1), 1 µl of 1.5 mM dNTPs, 1 µl of PCR buffer (Promega, Madison, USA), 1 unit of Taq DNA polymerase (Promega, Madison, USA), 0.5 µl of 10 µM forward primer, and 0.5 µl of 10 µM reverse primer. Microsatellites were amplified in Biometra Uno II thermocyclers (Biometra, Goettingen, Germany) using the following touch-down program: initial denaturation at 94°C for 1 min 30 sec; five cycles of 94°C for 45 sec, 60°C for 45 sec, 70°C for 45sec; five cycles of 94°C for 45 sec, 57°C for 45 sec, 70°C for 45 sec; 10 cycles of 94°C for 45 sec, 55°C for 45 sec, 70°C for 45 sec; 20 cycles of 94°C for 45 sec, 52°C for 45 sec, 70°C for 45 sec; final elongation at 70°C for 5 min. PCR products were run on 0.6% agarose gels to check for amplification. These first tests were carried out on four females coming from the same population and on three females coming from three different locations. Among the 76 and 98 loci tested for the two species respectively, only 13 and 15 provided satisfactory amplification (amplified bands of expected size with few supernumery bands) respectively for *C. insularis* and *C. sonorensis*. Amplifying loci were then genotyped to check for polymorphism eventually adjusting PCR conditions by reducing $MgCl_2$ concentration to 1 mM to eliminate supernumery bands preliminary observed at some loci. For all loci, the forward primer was labelled with either 6-FAM, HEX or NED. Loci were multiplexed by size and by color when possible to decrease the number of genotyping reactions. Genotyping of PCR products was performed by Macrogen Inc. (Seoul, Republic of Korea) on 59 and 68 females (males were not genotyped because of the haplodiploid nature of inheritance in Hymenoptera), respectively for *C. insularis* and *C. sonorensis*, from one population collected in the state of Guanajuato (Mexico), on an ABI Prism 3100 sequencer (Applied Biosystem, Foster City, USA) (Table 1). Genotypes were scored using Peak Scanner™ Software v1.0 (Applied Biosystem, Foster City, USA). All loci were then checked for the presence of null alleles using Micro-Checker (Van Oosterhout *et al.*, 2004). Eight loci showed evidence for the presence of null alleles (*Ci9*, *Ci13*, *Ci15*, and *Ci28* in *C. insularis*, and *Cs15, Cs19, Cs23* and *Cs37* in *C. sonorensis*).The expected heterozygosity (H_E) and the observed heterozygosity (H_O) were calculated using GenAlEx (available at http://www.anu.edu.au/BoZo/GenAlEx/) (Peakall, Smouse, 2006). The number of alleles, genotypic disequilibrium and deviation from Hardy-Weinberg equilibrium were estimated using Genepop 4.0 (Raymond, Rousset, 1995).

Results

Allelic diversity ranged from four to 18 alleles in *C. insularis* and from two to 16 alleles in *C. sonorensis*. Heterozygosities were between 0.106 to 0.458 in *C. insularis* and between 0.088 to 0.403 in *C. sonorensis*. Five loci showed significant deviation from Hardy-Weinberg equilibrium (HWE) in both *C. insularis* and *C. sonorensis* when exact tests (Genepop 4.0; Raymond, Rousset, 1995) were performed. No linkage disequilibrium was observed for any loci pair in *C. insularis*. In *C.* sonorensis, the exact test for genotypic disequilibrium showed four loci pairs with significant *p* values after Bonferroni corrections: *Cs15-Cs19* ($P < 0.01$), *Cs19-Cs21* ($P < 0.01$), *Cs-19-Cs48* ($P < 0.01$) and *Cs20-Cs21* ($P < 0.01$).

Discussion

These results showed that the microsatellite DNA loci described here are highly polymorphic, and that these markers will be useful in the investigation of population genetic structure, parentage and mating system of *C. insularis* and *C. sonorensis*. Having reliable molecular tools at hand is one of the keys to a good understanding of the interactions between parasitoids and their hosts, and can help to develop novel strategies in managing populations of major pests, such as *S. frugiperda*.

References

Glenn, T. C., and N. A. Schable. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* 395:202-222.

Knipling, E.F. 1980. Regional management of the fall armyworm — a realistic approach? *Florida Entomologist* 63:468-480.

Kranz, J., H. Schmutterer, and W. Koch. 1977. Diseases, Pests and Weeds in Tropical Crops. Verlag Paul Parey, Berlin.

Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs, and J. E. Foster. 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomologist* 86:254-289.

Molina-Ochoa, J., J. E. Carpenter, R. Lezama-Gutierrez, J. E. Foster, M. Gonzalez-Ramirez, C. A. Angel-Sahagun, and J. Farias-Larios. 2004. Natural distribution of hymenopteran parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae in Mexico. *Florida Entomologist* 87:461-472.

Molina-Ochoa, J., J. J. Hamm, R. Lezama-Gutiérrez, M. López-Edwards, M. González-Ramírez, and A. Pescador-Rubio. 2000. A survey of fall armyworm (Lepidoptera: Noctuidae) parasitoids in the Mexican states of Michoacán, Colima, Jalisco, and Tamaulipas. *Florida Entomologist* 84(1):31-36.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Annals of the Entomological Society of America* 79:898-904.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6:288-295.

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

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds. Krawetz S, Misener S), pp. 365-386. Humana Press, Totowa.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. *Florida Entomologist* 62:82-87.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535-538.

Acknowledgments

We are very grateful to Trevor Williams, Jaime Molina-Ochoa and David Bergvinson for their assistance in the organization of field work. We also warmly thank the Mexican students who helped us with field collections. This study was financed by the Swiss National Center of Competence in Research 'Plant Survival' and grant 3100A0-107974 from the Swiss National Science Foundation.

Chapter 4:

Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidopotera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): is host plant important?

Population genetic structure of two primary parasitoids of *Spodoptera frugiperda* (Lepidopotera), *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera): is host plant important?

Violaine Jourdie[1†], Nadir Alvarez[1†], Jaime Molina-Ochoa[2], Trevor Williams[3], David Bergvinson[4], Betty Benrey[1], Ted C. J. Turlings[1], and Pierre Franck[5]

1Université de Neuchâtel, Institut de Biologie, Case Postale 158, 2009 Neuchâtel, Switzerland

2Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Apartado postal no. 36, Tecomán, Colima 28100, Colima, México

3ECOSUR, Apdo Postal 36, Tapachula 30700, Chiapas, México

Present address: INECOL, Apartado Postal 63, Xalapa 91070, Veracruz, México

4CIMMYT Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

Present address : Gates Foundation, 1551 Eastlake Ave. East, Seattle, WA 98102, USA

5INRA, AgroParc, Domaine St-Paul, 84914 AVIGNON Cedex 9, France

†These two authors contributed equally to this paper.

Abstract

Plant chemistry can strongly influence interactions between herbivores and their natural enemies, either by directly affecting fitness and development correlates of parasitoids and predators, but also because plant compounds are involved in host finding and acceptance behavior of foraging natural enemies. It has only recently been recognized that through these effects plants may influence parasitoid population genetic structure. Crop plants that are grown in monocultures may be particularly important in this context. We tested for a possible crop plant specialization in *Chelonus insularis* and *Campoletis sonorensis*, two primary parasitoids of the fall armyworm, *Spodoptera frugiperda*. Mexican populations collected from maize and sorghum plants were compared. Genetic variations at eight and eleven microsatellites were respectively assayed for *C. insularis* and *C. sonorensis* to examine population structure according to isolation by distance, host plant and region effect. Kinship analyses were also performed to assess locally female migration among host-plants. Analysis of molecular variance showed considerable within population variation and revealed significant region effect. No difference was observed in population structure due to host plant in any of the two species. Isolation by distance was observed at the individual level, but not at the population level. Kinship analyses revealed significantly more pairs of related individuals on the same plant species than pairs of individuals that came from different plant species, suggesting that locally, mothers seem to preferentially stay on the same plant species. Although no effect of plant species on population structure was found for standard population genetics parameters, the kinship analyses revealed that mothers exhibit plant species preferences, which may speed up divergence if adaptation were to occur.

Keywords: *Chelonus insularis*, *Campoletis sonorensis*, *Spodoptera frugiperda*, population genetic structure, microsatellites, host plant.

Introduction

Host plants have been shown to affect both insect herbivores and their parasitoids in many ways. Insect pest are directly targeted by plant toxic secondary compounds (Giamoustaris and Mithen, 1995; van Dam *et al.,* 2000) and they have evolved various strategies to defeat plant defense mechanisms (Futuyma, 1983; Nitao, 1989; Berenbaum and Zangerl, 1992; Evans *et al.,* 2000; Ode *et al.,* 2004). Because of the intimate trophic interactions between immature parasitoids and their hosts, plant chemistry also indirectly affects the fitness of parasitoids (Bottrell *et al.,* 1998; Turlings and Benrey, 1998). Tritrophic effects of host plant chemistry on natural enemy fitness correlates such as survivorship, body size, and clutch size have been frequently demonstrated (Campbell and Duffey, 1979, 1981; Barbosa *et al.,* 1986; Thorpe and Barbosa, 1986; Barbosa *et al.,* 1991; Bourchier, 1991; Gauld and Gaston, 1994; Roth *et al.,* 1997).

Although studies on the effects of specific plant chemicals on parasitoid fitness are limited, it is generally concluded that plant defense chemicals mostly have a negative impact on natural enemy traits such as development time, survivorship, and body size (Thurston and Fox, 1972; Campbell and Duffey, 1979, 1981; Barbosa *et al.,* 1986; El-Heneidy *et al.,* 1988; Barbosa *et al.,* 1991; Thaler, 1999). These effects can strongly vary with plant species (Smith, 1957; Altahtawy *et al.,* 1976; Bhatt and Singh, 1989; Senrayan and Annadurai, 1991; Werren *et al.,* 1992; Fox *et al.,* 1996; Kruse, 1997; Eben *et al.,* 2000; Harvey *et al.,* 2003; Zvereva and Rank, 2003) and even cultivar (Kauffman and Flanders, 1985; Orr and Boethel, 1985; Hare and Luck, 1991; Reed *et al.,* 1991; Rogers and Sullivan, 1991; Riggen *et al.,* 1992; Stark *et al.,* 1992) can.

These differential effects on parasitoid performance and fitness among host plants may lead to behavioral and physiological adaptations and thereby to specificity and plant fidelity in parasitoids. If enough divergence among parasitoid populations on different plant species occurs, this could eventually lead to host race formation and even speciation (Aebi, 2004). Behavioral adaptation could be translated into mothers ovipositing preferentially in hosts on a certain plant species and focusing its foraging efforts on this species.

Genetic divergence among plant-specialized populations can be demonstrated with phylogeographic studies. Indeed, individuals emerging on different plants would cluster in different clades, and individuals collected in distant areas but on the same plant would cluster in the same clade. However, iterative local adaptation is more likely to occur, whereby females locally show fidelity to a given host plant, but adaptation may not lead to the same genotype in all locations.In such a case, if reproductive isolation is too weak to be shown through classical population genetics, it may be possible to observe it by studying the mothers' foraging and oviposition preferences.

In the current study, we investigated whether host plant population genetic structure in two species simultaneously: *Chelonus insularis* Cresson (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae). These two wasps parasitize noctuids. They are two primary parasitoids of the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Americas, a major subtropical polyphagous pest (Kranz *et al.,* 1977; Sparks, 1979; Knipling, 1980; Pashley, 1986) notably attacking maize and sorghum (Molina-Ochoa *et al.,* 2003). The principle aim was to determine if there are distinguishable genotypes of the wasps that have adapted to a specific host plant. In other words, do parasitoids keep foraging on a plant species to the point that they become genetically distinguishable from congeners foraging on a different host plant species? We measured genetic variability through standard population genetics parameters such as heterozygosity, allelic richness, gene diversity and deviation from Hardy-Weinberg equilibrium. Isolation by distance, as well as host plant and region effect were tested. Finally, kinship

analyses were performed to identify related individuals and thus investigate possible oviposition preferences by mothers on a particular plant species.

Materials and Methods

Biological material

Larvae of the FAW were collected at 33 locations in Central and Southern Mexico (Table 1, Figure 1) in June, August and September 2005 and in July 2006. They were reared and stored as described in Jourdie *et al*. (2008). At each location, they were sampled on either maize or sorghum, and locations where these two crops occurred next to one another were sampled whenever possible. Adult parasitoids were taxonomically assigned to species following Cave (1995), whereas parasitoid larvae which did not complete their life cycle were molecularly identified as described by Jourdie *et al*. (2008). Ten species of parasitoids emerged from the collected larvae, but *C. insularis* and *C. sonorensis* considered for this study were most abundant.

DNA isolation, PCR and genotyping

DNA extractions were carried out from the abdomen of parasitoid adults or from half of the body of larvae, using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Total DNA was re-suspended in 200 µl of elution buffer (two elutions of 100 µl each).

Table 1: Insects were collected at 33 locations in South Central and South Mexico on maize (m) and sorghum (s) in June, August and September 2005 and in July 2006.

CODE	LOCATION	MUNICIPIO	GPS COORD.	ELEVATION (m)	CROP
C1	Agua Zarca	Coquimatlán	19°13'12.0"N, 103°56'10.1"W	273	m
C2	El Colomo, Coquimatlán	Coquimatlán	19°13'58.3"N, 103°57'17.4"W	322	m
C3	Pueblo Juarez, Coquimatlán	Coquimatlán	19°09'45.9"N, 103°53'44.4"W	234	s
C4	Los Mezcales	Comala	19°19'57.3"N, 103°46'07.6"W	637	m
C6	La Caja	Comala	19°22'31.3"N, 103°48'12.8"W	636	m
C7	Villa de Alvarez	Minatitlán	19°16'57.9"N, 103°46'41.0"W	520	s
C8	Villa de Alvarez	Minatitlán	19°17'01.2"N, 103°46'41.0"W	520	m
CH1	Jaritas	Tapachula	14°42'53.3"N, 92°18'24.9"W	16	s
CH2	Jaritas	Tapachula	14°43'01.5"N, 92°18'26.6"W	23	m
CH3	Jaritas	Tapachula	14°44'53.7"N, 92°20'06.2"W	27	s
CH4	Jaritas	Tapachula	14°43'32.3"N, 92°18'58.1"W	17	s
CH5	Jaritas	Tapachula	14°43'20.0"N, 92°19'09.1"W	24	s
G1	Los Lobos	Valle de Santiago	20°27'52.9"N, 101°12'01.7"W	1720	s
G2	Rancho Seco	Salamanca	20°27'30.9"N, 101°12'16.7"W	1723	m
G3	Rancho Seco	Salamanca	20°27'30.9"N, 101°12'16.7"W	1723	s
G4	Lucero de Ramales	Silao	20°55'15.6"N, 101°27'38.9"W	1771	m
G5	Lucero de Ramales	Silao	20°55'15.6"N, 101°27'38.9"W	1771	s
G6	Puerta Chica	Silao	20°52'10.3"N, 101°29'20.9"W	1752	m
J1	Usmajac	Sayula	19°52'08.9"N, 103°33'16.7"W	1374	s
J2	Usmajac	Sayula	19°52'18.4"N, 103°33'45.6"W	1374	s
J3	La Sierrita	Degollado	20°28'40.1"N, 102°12'26.5"W	1734	m
J4	Rancho Nuevo	Degollado	20°28'22.3"N, 102°12'06.4"W	1739	s
N1	Carretera Jala	Ahuacatlán	21°03'39.6"N, 104°27'09.8"W	1027	m
N2	Ejido Mexpan	Ixtlán del Rio	21°02'35.0"N, 104°28'03.1"W	1016	s
N3	Ejido Mexpan	Ixtlán del Rio	21°02'38.3"N, 104°27'42.6"W	1035	s
N4	El Humedo	Ahuacatlán	21°01'35.9"N, 104°28'17.7"W	1024	m
N5	La Iguerita	Jala	21°05'29.0"N, 104°26'04.9"W	1054	m
N6	La Iguerita	Jala	21°05'20.9"N, 104°26'05.0"W	1048	s
P1	CIMMYT tropical field station	Agua Fría	20°27'18.3"N, 97°38'28.8"W	95	m
P2	CIMMYT tropical field station	Agua Fría	20°27'10.3"N, 97°38'28.7"W	102	m
P3	CIMMYT tropical field station	Agua Fría	20°27'19.6"N, 97°38'26.3"W		m
P4	CIMMYT tropical field station	Agua Fría	20°27'17.4"N, 97°38'26.3"W		m
V1	Lindero	Lindero	20°29'31.9"N, 97°32'17.2"W	66	m

Figure 1: Sampled locations were distributed in South- and Central-Mexico.

The genotypes were assessed according to (Jourdie *et al.* in press) at eight microsatellite loci for *C. insularis* (*Ci1*, *Ci10*, *Ci11*, *Ci16*, *Ci17*, *Ci30*, *Ci31* and *Ci33*) and at eleven microsatellite loci for *C. sonorensis* (*Cs6*, *Cs9*, *Cs14*, *Cs20*, *Cs21*, *Cs22*, *Cs42*, *Cs44*, *Cs47*, *Cs48* and *Cs49*). Multiplex PCRs were run for combinations of the loci *Ci1-Ci10-Ci16-Ci17*, *Ci30-Ci31-Ci33* and *Ci11-Ci12* in *C. insularis* and for combinations of the loci *Cs6-Cs20-Cs47*, *Cs14-Cs22-Cs44*, *Cs48-Cs21-Cs49* and *Cs9-Cs42* in *C. sonorensis*. PCR reactions were carried out in a final volume of 10 µl. The reaction mixtures contained 1 µl of total DNA, 0.5 µM of each primer, 1.5 mM of each nucleotide, 1.0 or 1.5 mM $MgCl_2$, 1 µl of PCR buffer (Promega, Madison, USA) and 1 U of Taq DNA polymerase (Promega, Madison, USA). PCR was conducted in a Uno II thermal cycler (Biometra, Goettingen, Germany) using the following touchdown cycling conditions: initial denaturation at 94°C for 1 min 30 sec; five cycles of 94°C for 45 sec, 60°C for 45 sec, 70°C for 45sec; five cycles of 94°C for 45 sec, 57°C for 45 sec, 70°C for 45 sec; 10 cycles of 94°C for 45 sec, 55°C for 45 sec, 70°C for 45 sec; 20 cycles of 94°C for 45 sec, 52°C for 45 sec, 70°C for 45 sec; final elongation at 70°C for 5 min.

Electrophoresis of PCR product was performed by Macrogen Inc. (Seoul, Republic of Korea) on an ABI Prism 3100 sequencer (Applied Biosystem, Foster City, USA). Genotypes were scored using Peak Scanner™ Software v1.0 (Applied Biosystem, Foster City, USA). Size values were sorted by lengths and plotted using the Excel software in order to determine round integer sizes for each allele.

Genetic variability

Genetic variability was investigated for *C. insularis* population samples with more than 15 females (locations G1, G2, G3, G4, G5, G6, J2, J3, J4, N6 and P2) and for *C. sonorensis* populations with more than 10 females (locations G4, G5, J3, J4 and N6). Number of alleles, allele frequencies, allelic richness and genotypic linkage disequilibrium were assessed using the Fstat 2.9.3.2 software (Goudet, 1995). Observed and expected heterozygosities, gene diversity and departure from Hardy-Weinberg equilibrium (HWE) were calculated for each population separately, using the software package GENEPOP 4.0 (Raymond and Rousset, 1995). Two-way ANOVAs were performed to test for an effect of plant species as well as for effects of interactions between the locus and plant species on gene diversity, allelic richness and heterozygosity.

On these 16 population samples, we tested population size variation with the BOTTLENECK 1.2.02 program (Cornuet and Luikart, 1996). This program assumes that the number of alleles is reduced faster than heterozygosity during a significant drop in population size. As a consequence, observed heterozygosities are higher than expected at mutation-drift equilibrium (Cornuet and Luikart, 1996) and a shift in mode of the frequency distribution of alleles from rarest alleles being the most frequent to more common alleles being less frequent (Luikart *et al.*, 1998). We used the stepwise mutation model (SMM) and the infinite allele model (IAM). These two extreme opposite models were chosen to get the best view of

the putative demographic fluctuations.

Isolation by distance

Due to the high variability in sizes of sampling population, we chose to analyze isolation by distance between individuals (Rousset, 2000) instead of between populations. Indeed, some populations used for this analysis had as few as two individuals, which induces a serious bias in the calculation of F_{ST}-values or other genetic distances (e.g. Nei's minimum distance, distance of Cavalli-Sforza). Isolation by distance between individuals was assessed using GENEPOP 4.0 (Raymond and Rousset, 1995). In this analysis, the a-statistic described by Rousset (2000) is computed, which is somewhat analogous to $F_{ST}/(1-F_{ST})$. The program then implements a Mantel's test (Mantel, 1967) to examine the relationship between genetic and geographic distances. The GPS coordinates were recorded using the WGS 84 system. These coordinates were further projected and transformed in meters using the ArcGIS 9.0 software (ESRI, 2004).

Since *C. sonorensis* was only collected from the states of Colima, Jalisco, Guanajuato and Nayarit (as opposed to *C. insularis*, which was found in all states we visited) and in order to look for a potential difference between the two species, the same analysis was also performed with *C. insularis* individuals coming only from these four states.

Isolation by distance was also investigated at the population level considering only population samples with more than 15 individuals, using the online GENEPOP version (GENEPOP ON THE WEB at http://genepop.curtin.edu.au/; (Raymond and Rousset, 1995). Pairwise F_{ST} (Weir and Cockerham, 1984) among population samples were computed, and the geographic distances were calculated from the GPS coordinates recorded at each sampled location. Then $F_{ST}/1-(F_{ST})$ was tested against Ln(distance) using a Mantel's test (Mantel, 1967).

Host plant and region effect

To investigate any potential host plant effect and regional effect, a locus by locus analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) was performed with the software Arlequin 3.1.1.1 (Schneider *et al.*, 2000), using the hierarchical model for genotypic data with several groups of populations and no within-individual level. For this analysis, only populations presenting more than 15 individuals were considered and both males and females were included. To circumvent the problem of haplodiploidy, we assumed we knew the females gametic phase. This way, the software partitions the genotypes into haplotypes, which has no effect on the calculation of fixation indices.

We did not have enough degree of freedom to test for interaction between plant species and region.

Kinship analyses

Lod scores (Morton, 1995) were

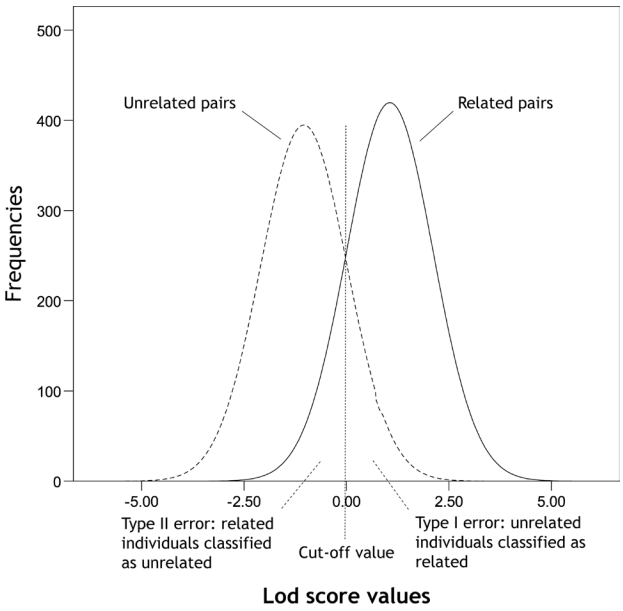


Figure 2: Distribution of lod score frequencies. Distributions of related and unrelated individuals always overlap. To determine the cut-off value, one needs to choose which error is more acceptable: shifting the cut-off value to the left increases the risk of type I error, while shifting the cut-off value to the right increases the risk of type II error.

considered to perform kinship analyses. This statistical method is based on likelihood ratios. Let p be the probability that two individuals share the same allele if they are related and q if they are unrelated. The ratio of these two probabilities ($R_s = p/q$) is the "likelihood ratio" of the two individuals being related as opposed to being unrelated, knowing that they have the same genotype at a given locus. For several loci, the likelihood ratio is the product of likelihood ratios at each locus. Lod scores are the decimal logarithms of likelihood ratios, so that multilocus lod scores are obtained by adding lod score values over all loci.

Analyses were performed with the Kinship 1.3.1 software (freely available at http://www.gsoftnet.us/GSoft.html) on populations with more than 15 individuals (males and females). Population pairs were selected in which one population was collected from maize and the other from sorghum in two adjacent fields. For each population pair, we wanted to determine whether there were related individuals that came from both populations. If so, this would indicate that the mothers do migrate from one plant species to the other to find a host, whereas in case of rare relatedness this would indicate that mothers would preferentially forage on one plant species. To use lod scores to determine relationships between individuals taken pairwise, the distribution of lod scores among related individuals and among unrelated individuals must first be determined. For each population pair, simulations were run to randomly generate diploid-diploid, diploid-haploid and haploid-haploid pairs of unrelated individuals. The same type of simulations was run to generate pairs of related individuals. Each time, 5000 simulated pairs were generated. Lod score values are lower for unrelated pairs than for related pairs of individuals, but the two distributions always overlap (Figure 2). Therefore, we have to determine a cut-off value above which we consider individuals as related. Any cut-off value generates two kinds of erroneous assignment: shifting the cut-off value to the left increases the risk of misclassifying unrelated individuals as related (type I error), while shifting the cut-off value to the right increases the risk of misclassifying related individuals

Table 2: Genetic variability is presented for A) *Chelonus insularis* and B) *Campoletis sonorensis*. For each species, the number of individuals collected (N), gene diversity (GD), the number of alleles (N_a), allelic richness (AR), expected (H_E) and observed (H_O) heterozygosity, results of Hardy-Weinberg exact test (HWE) per population over all loci (parts A) and B)) and per locus over all populations (part C)), and heterozygosity at mutation-drift equilibrium (Heq) under the infinite allele model (IAM) and under the stepwise mutation model (SMM) are provided.

B)

	G4	G5	J3	J4	N6	Total
	(39)	(29)	(25)	(11)	(10)	—
GD	0.566	0.566	0.576	0.592	0.572	—
N_a	6.182	5.273	5.818	4.727	4.182	—
AR	4.181	4.09	4.252	4.567	4.182	—
H_E	0.563	0.556	0.573	0.572	0.545	—
H_O	0.55	0.535	0.514	0.636	0.555	—
HWE (p-value)	0.31	0.571	0.393	0.622	0.588	—
Heq (IAM)	0.553	0.519	0.571	0.6	0.547	—
Heq (SMM)	0.669	0.632	0.672	0.669	0.607	—

A)

	G1	G2	G3	G4	G5	G6	J2	J3	J4	N6	P2	Total
	(32)	*(23)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(16)*	*(18)*	*(20)*	*(18)*	—
GD	0.667	0.671	0.659	0.689	0.69	0.651	0.682	0.65	0.68	0.684	0.66	—
N_a	6.875	6.125	6.75	7.5	7	6.375	6.25	5.375	5.875	6.25	6.375	—
AR	5.192	5.306	5.71	5.706	5.718	5.875	5.78	5.158	5.351	5.538	5.491	—
H_E	0.632	0.639	0.614	0.653	0.632	0.591	0.652	0.609	0.641	0.656	0.635	—
H_O	0.545	0.541	0.595	0.652	0.63	0.517	0.569	0.574	0.577	0.608	0.571	—
HWE (p-value)	0.225	0.209	0.636	0.36	0.582	0.38	0.392	0.584	0.414	0.448	0.147	—
Heq (IAM)	0.561	0.582	0.609	0.614	0.614	0.622	0.594	0.592	0.55	0.592	0.644	—
Heq (SMM)	0.657	0.676	0.691	0.739	0.698	0.688	0.663	0.666	0.612	0.675	0.731	—

C)

Chelonus insularis												
Locus	*Ci1*	*Ci10*	*Ci11*	*Ci16*	*Ci17*	*Ci30*	*Ci31*	*Ci33*				
HWE (p-value)	0.142	0.662	0.321	0.545	0.272	0.395	0.201	0.623				
Campoletis sonorensis												
Locus	*Cs6*	*Cs9*	*Cs14*	*Cs20*	*Cs21*	*Cs22*	*Cs42*	*Cs44*	*Cs47*	*Cs48*	*Cs49*	
HWE (p-value)	0.316	0.392	0.474	0.52	0.236	0.253	0.532	0.832	1	0.481	0.411	

as unrelated (type II error). We chose to decrease type I error to a maximum, and therefore, we set the cut-off value to be equal to the highest lod score value observed for unrelated pairs. From related pairs of individuals, the number of families (i.e. more than two related individuals) was also inferred. A binomial analysis was performed in R 2.6.2 software (R Development Core Team, 2008) using a generalized linear model (GLM) to test for an effect of the plant species and of the sex on the number of related pairs observed.

Results

Genetic variability

Sample size, gene diversity, number of alleles, allelic richness, observed and expected heterozygosity and results of exact test for Hardy-Weinberg equilibrium are presented per population in Table 2 for both species (Complete table showing these parameters per locus and per population is presented in Appendix 1). All loci were polymorphic in all populations. Allelic richness varied from 2.5 (*Ci33*) to 11.7 (*Ci1*) in *C. insularis* (Table 2A), and from 2 (Cs44) to 8.1 (*Cs21*) in *C. sonorensis* (Table 2B). No deviation from Hardy-Weinberg equilibrium was observed by locus over all the populations (Table 2A for *C. insularis* and Table 2B for *C. sonorensis*), nor by population over all the loci (Table 2C). Permutation tests for each locus pair did not indicate significant linkage disequilibrium after correcting for multiple tests.

The two-way ANOVAs showed no effect of the host plant on gene diversity ($F = 0.822$, $P = 0.368$), nor on allelic richness ($F = 0.065$, $P = 0.799$) or on observed heterozygosity ($F = 0.488$, $P = 0.487$) in *C. insularis*. These analyses also excluded any effect of the interaction between locus

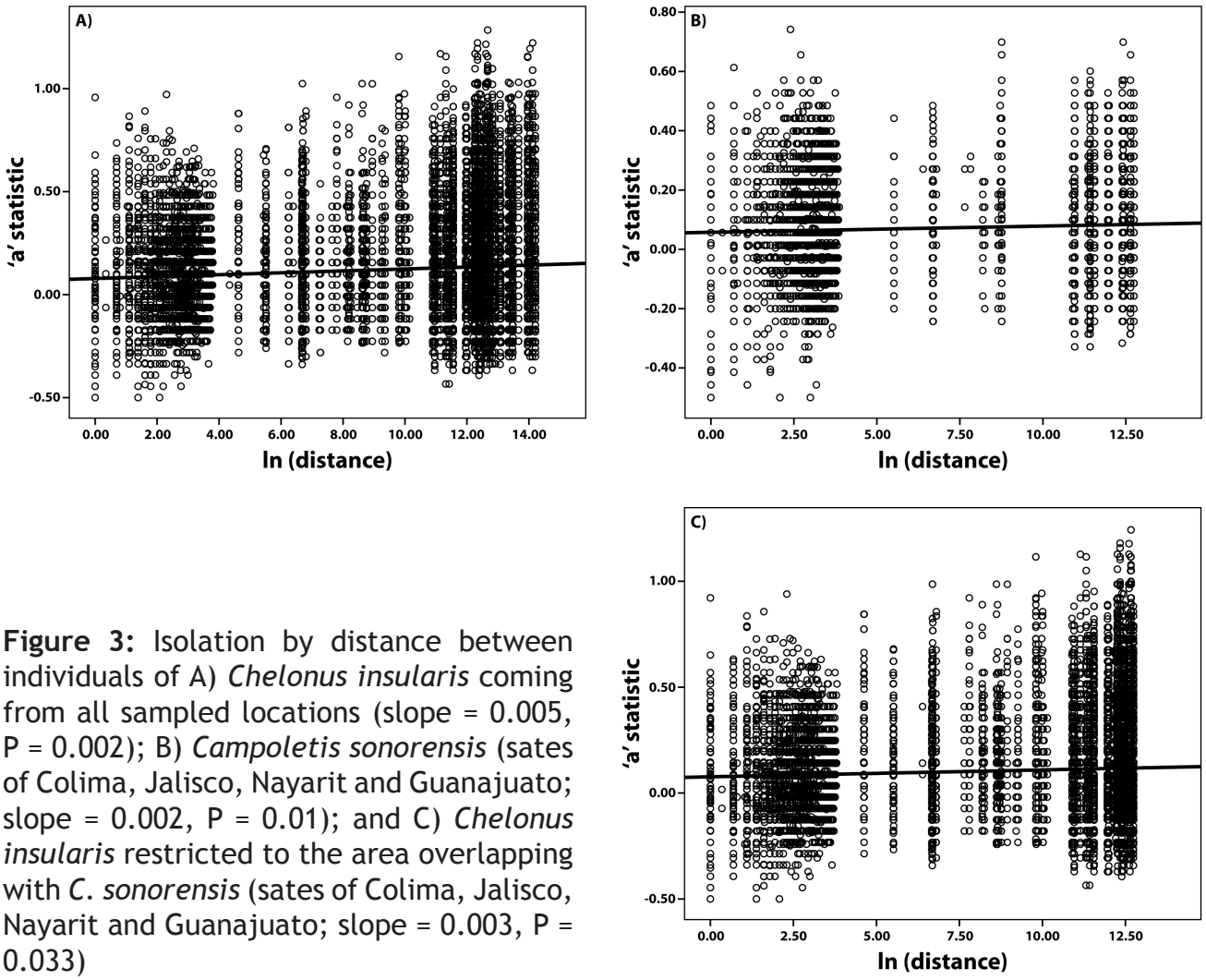


Figure 3: Isolation by distance between individuals of A) *Chelonus insularis* coming from all sampled locations (slope = 0.005, P = 0.002); B) *Campoletis sonorensis* (sates of Colima, Jalisco, Nayarit and Guanajuato; slope = 0.002, P = 0.01); and C) *Chelonus insularis* restricted to the area overlapping with *C. sonorensis* (sates of Colima, Jalisco, Nayarit and Guanajuato; slope = 0.003, P = 0.033)

and host plant (gene diversity: F = 0.253, *P* = 0.959; allelic richness: F = 1.756, *P* = 0.11; observed heterozygosity: F = 0.817, *P* = 0.576). Similar results were obtained for *C. sonorensis*: an effect of the host plant on these parameters can be excluded (gene diversity: F = 0.0538, *P* = 0.818; allelic richness: F = 0.0918, *P* = 0.764; observed heterozygosity: F = 1.768, *P* = 0.193), as well as a crossed effect of the locus and the host plant (gene diversity: F = 0.258, *P* = 0.986; allelic richness: F = 0.376, *P* = 0.949; observed heterozygosity: F = 0.222, *P* = 0.992).

Analyses with BOTTLENECK indicated that all populations in both species showed no significant excess of heterozygotes after Bonferroni correction (cut off value for α = 0.05: P = 0.0045) under both mutation models, thus showing no evidence for recent bottleneck in population size.

Isolation by distance

A Mantel test showed that geographic distances and genetic distances between individuals were significantly positively correlated in both species (*C. insularis*: slope = 0.005, *P* = 0.002 (Figure 2A); *C. sonorensis*: slope = 0.002, *P* = 0.01 (Figure 2B)). Analyses performed on individuals of *C. insularis* from specific states showed that for Colima, Jalisco, Guanajuato and Nayarit the effect of isolation by distance was still present in this species at a smaller geographic scale (slope = 0.003, *P* = 0.033) (Figure 3C). However, this could have been an artefact of small population sizes, as analyses run on populations with more than 15 individuals revealed no significant correlation between geographic distances and F_{ST} values (*C. insularis*: slope = 0.0006, *P* = 0.21; *C. sonorensis*: slope = 0.001, *P* = 0.11).

Table 3: AMOVA showed significant differences between regions but not between host plants.

Parameter	Source of variation	d.f.	Sum of squares	Variance component	Percentage variation	*P*-value
Chelonus insularis						
Host plant	Between maize and sorghum	1	5.368	-0.003	-0.119	0.486
	Within populations within maize and sorghum	29	146.237	0.084	3.253	0.000
	Within populations	1235	2364.289	2.507	96.866	0.000
Region	Between groups[1]	4	39.581	0.032	0.747	0.000
	Among populations within groups[1]	26	112.024	0.060	2.536	0.000
	Within populations	1235	2364.289	2.507	96.717	0.000
Campoletis sonorensis						
Host plant	Between maize and sorghum	1	5.506	-0.005	-0.125	0.626
	Within populations within maize and sorghum	13	61.991	0.034	0.888	0.000
	Within populations	535	2030.763	3.796	99.236	0.000
Region	Among groups[2]	2	12.996	0.038	0.986	0.000
	Among populations within groups[2]	12	54.501	0.021	0.553	0.000
	Within populations	535	2030.763	3.796	98.462	0.000

[1]groups = Colima, Nayarit, Guanajuato, Chiapas and Puebla-Veracruz

[2]groups = Colima, Nayarit and Guanajuato

Table 4: Results of kinship analyses per population pair. In each population pair, one population was collected on maize and the other on sorghum. For each population pair, the number of females (f) and males (m) collected in given. The number of female-female (f-f), male-male (m-m) and female-male (f-m) pairs of related individuals observed on maize, on sorghum and across fields (maize-sorghum) is indicated.

Population pair	Nb pairs tested	Sex	Sample size		Pair sex	Number of related pairs (maize)	Number of related pairs (sorghum)	Number of related pairs (maize-sorghum)
			maize	sorghum				
Chelonus insularis								
C7-C8	861	f	10	11	f-f	0	8	0
		m	6	15	m-m	1	0	1
					f-m	0	3	0
		total	16	26	total	1	11	1
G1-G2	4851	f	23	32	f-f	1	4	1
		m	22	22	m-m	0	16	2
					f-m	1	4	1
		total	45	54	total	2	24	4
G2-G3	3486	f	23	22	f-f	4	1	1
		m	22	17	m-m	2	1	0
					f-m	3	1	1
		total	45	39	total	9	3	2
G4-G5	5565	f	40	23	f-f	6	1	1
		m	21	22	m-m	2	0	3
					f-m	5	1	1
		total	61	45	total	13	2	5
J3-J4	1431	f	16	18	f-f	6	4	0
		m	8	12	m-m	4	0	0
					f-m	4	1	0
		total	24	30	total	14	5	0
N5-N6	1653	f	11	20	f-f	0	1	1
		m	15	12	m-m	2	0	1
					f-m	2	0	1
		total	26	32	total	4	1	3
TOTAL	17847		217	226		43	46	15
Campoletis sonorensis								
G4-G5	9180	f	39	29	f-f	8	6	3
		m	27	41	m-m	3	0	0
					f-m	7	1	1
		total	66	70	total	18	7	4
J3-J4	2080	f	25	11	f-f	2	1	3
		m	15	14	m-m	0	2	0
					f-m	2	5	1
		total	40	25	total	4	8	4
TOTAL	11260		106	95		22	15	8

Host plant and region effect

The same pattern was observed in both species when an AMOVA was run on genotypic data from both males and females (Table 3): although the percentage of variation was always much higher within populations than among groups, we found a significant effect of the region. There was no significant effect of plant species at the whole geographical scale ($P = 0.486$ and $P = 0.626$ respectively for *C. insularis* and *C. sonorensis*).

Kinship analyses

Results for kinship analyses are summarized in Table 4. In both species, there were significantly less pairs of related individuals in which each individual came from a different plant species (*C. insularis*: $P < 0.001$; *C. sonorensis*: $P = 0.01$). There was no significant difference between the number of pairs of related males, the number of pairs of related females and the number of male-female pairs in any of the two species. In *C. insularis*, 7 and 8 families were observed respectively only on sorghum and only on maize, while only 4 families were observed on both plants. In *C. sonorensis*, no family was observed on sorghum, 4 families were observed on maize, and 5 families were observed on both plants.

Discussion

Overall, there was no genetic differentiation for the two parasitoids between populations collected on maize and populations collected on sorghum. Parameters used to investigate genetic diversity (i.e. allelic richness, gene diversity, heterozygosity) showed no effect of plant species on which the parasitoids were collected. In other words, wasps collected on maize were not genetically distinguishable from wasps collected on sorghum. We found some regional differentiation; however, disregard of the region, within-population variation was huge. No strong evidence of a pattern of isolation by distance was detected, probably because of important gene flow over long distances. Absence of isolation by distance was previously observed in the parasitoid *Neotypus melanocephalus* (Anton *et al.*, 2007). In this study, the genetic structures of populations of *N. melanocephalus* and of its lepidopteran host *Maculinea nausithous* were investigated in parallel. In contrast to the parasitoid, the host exhibited strong genetic differentiation increasing with geographic distance. Genetic differentiation with increasing geographic distance arises from the joint effect of gene flow, genetic drift, and adaptation to local conditions (Wright, 1943; Peterson and Denno, 1998; Hutchison and Templeton, 1999). The fact that no pattern of isolation by distance was observed at the population level in *C. insularis* and in *C. sonorensis* may indicate that gene flow is more important than drift in these two parasitoid species. Absence of isolation by distance in beans and associated bruchids has previously been explained by frequent long-distance transportation by humans (Gepts, 1998; Papa and Gepts, 2003; Alvarez *et al.*, 2007). This explanation can be ruled out in our system, as two parasitoids attack either eggs or second instar larvae (Cave, 1995), which are only found on immature plants. The parasitoids emerge from the host within a week, long before the plants are harvested and before plants may migrate through human trade. Absence of isolation by distance between parasitoid populations is more likely explained by a large number of migrating individuals, which would also contribute to high within-population variation. Although migration of these minute insects has not been investigated, it seems reasonable to propose that they can be carried by winds over rather large distances.

Kinship analyses revealed that more pairs of related individuals occur on the same plant species than on different species. Both parasitoids exhibited the same patterns, which indicates that locally, the mothers tend to stay in the same field (i.e. on the same plant species) to lay their eggs.

The same patterns of genetic diversity, isolation by distance, population differentiation and kinship were observed in both species despite their different biologies. *Chelonus insularis* is an egg larval parasitoid, whereas *C. sonorensis* is a larval parasitoid. As an egg-larval parasitoid, *C. insularis* encounters patches of eggs, so it can parasitize tens of hosts in a very limited area. Conversely, *C. sonorensis* will have to search considerably more to parasitize comparable numbers of hosts. Indeed, due to the highly cannibalistic nature of *S. frugiperda*, only a few caterpillars remain per plant soon after their emergence. *C. sonorensis* therefore might need to visit many more plants relative to *C. insularis*. These behavioral differences seem not to result in differences in genetic structure. The only parameter differing between the two species, which may reflect these behaviors, is the number of families observed. In *C. insularis*, we observed more families on either of the two plants than on both plants simultaneously. In *C. sonorensis*, no families occurred on sorghum, and we observed almost the same numbers of families on maize as on mixed plants. This may indicate that *C. sonorensis* indeed searches its hosts on more different plants than does *C. insularis*. Although there are few caterpillars per plant, *S. frugiperda* is often present at extremely high frequencies, and it is not unusual that, in the absence of pesticide, every single plant in a field is infested (V. Jourdie, personal observation).

Hence, even if *C. sonorensis* has to visit many different plants, it can do so within a quite limited area.

Previous studies provide evidence for host race formation in phytophagous insects (Berlocher and Feder, 2002; Dres and Mallet, 2002; Malausa *et al.*, 2005). Evidence of formation of a host race was also found in parasitoids (Aebi, 2004), although this was not confirmed by subsequent study on the same system with a more extensive sampling (Espindola, 2006). Systems in which both the herbivore and the parasitoid may specialize may be more suitable for a host race to evolve at the third trophic level. In our study, there was no sign that the parasitoids studied are genetically differentiated on the two crop plants. However, given the pattern of oviposition and migration observed with kinship analyses, if an adaptation to host plant were to evolve, and this individual fidelity could speed up differentiation and may eventually lead to a host race formation.

References

Aebi, A. 2004. The influence of host plants on the ecological genetics of the third trophic level: the case of beans, bruchids and their parasitoids. Pp. 169. Department of Zoology. University of Neuchâtel, Neuchâtel.

Altahtawy, M. M., S. M. Hammad, and E. M. Hegazi. 1976. Studies on the dependence of *Microplitis rufiventris* Kok. (Hym., Braconidae) parasitizing *Spodoptera littoralis* (Boisd.) on own food as on food of its host. Zeitschrift für Angewandte Entomologie 81:3-13.

Alvarez, A., M. Hossaert-McKey, G. Restoux, A. Delgado-Salinas, and B. Benrey. 2007. Anthropogenic effects on population genetics of phytophagous insects associated with domesticated plants. *Evolution* 61:2986-2996.

Anton, C., I. Zeisset, M. Musche, W. Durka, J. J. Boomsma, and J. Settele. 2007. Population structure of a large blue butterfly and its specialist parasitoid in a fragmented landscape. *Molecular Ecology* 16:3828-3838.

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

Barbosa, P., J. A. Saunders, and J. E. A. Kemper. 1986. Plant allelochemicals and insect parasitoids: effects of nicotine on *Cotesia congregata* (Say) (Hymenoptera: Braconidae) and *Hyposoter annulipes* (Cresson) (Hymenoptera: Ichneumonidae). *Journal of Chemical Ecology* 12:1319-1328.

Berenbaum, M. R., and A. R. Zangerl. 1992. Genetics of physiological and behavioral resistance to host furanocoumarins in the parsnip webworm. *Evolution* 46:1373-1384.

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

Bhatt, N., and R. Singh. 1989. Bionomics of an aphidiid parasitoid *Trioxys indicus*. 30. Effect of host plants on reproductive and developmental factors. *Biological Agriculture and Hortscience* 6:149-157.

Bottrell, D. G., P. Barbosa, and F. Gould. 1998. Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology* 43:347-367.

Bourchier, R. S. 1991. Growth and development of *Compsilura concinnata* (Meigan) (Diptera: Tachinidae) parasitizing gypsy moth larvae feeding on tannin diets. *Canadian Entomology* 123:1047-1055.

Campbell, B. C., and S. S. Duffey. 1979. Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science* 205:700-702.

Campbell, B. C., and S. S. Duffey. 1981. Alleviation of α-tomatine-induced toxicity to the parasitoid, *Hyposoter exiguae*, by phytosterols in the diet of the host, *Heliothis zea*. *Journal of Chemical Ecology* 7:927-946.

Cave, R. D. 1995. Manual para el reconocimiento de parasitoides de plagas agrícolas en America Central. Zamorano Academic Press, Zamorano, Honduras.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001-2014.

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

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

El-Heneidy, A. H., P. Barbosa, and P. Gross. 1988. Influence of dietary nicotine on fall armyworm, *Spodoptera frugiperda* and its parasitoid, the ichneumonid wasp *Hyposoter annulipes*. *Entomologia Experimentalis et Applicata* 46:227-232.

Espindola, A. 2006. Interactions between *Horismenus butcheri* Hannsson and Aebi (Hymenoptera : Eulophidae) and two *Phaseolus* (Fabaceae) bean species in Central Mexico. Biology, population genetics and geospatial studies. Department of Zoology. University of Neuchâtel, Neuchâtel.

ESRI, ed. 2004. ArcGIS 9 − Getting started with ArcGIS. ESRI Press.

Evans, P. H., J. X. Becerra, D. L. Venable, and W. S. Bowers. 2000. Chemical analysis of squirt-gun defense in *Bursera* and counterdefense by chrysomelid beetles. *Journal of Chemical Ecology* 26:745-754.

Excoffier, L., P. Smouse, and J. Quattro. 1992. Analysis of molecular variance infered from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

Fox, L. R., K. M. Kester, and J. Eisenbach. 1996. Direct and indirect responses of parasitoids to plants: sex ratio plant quality and herbivore diet breadth. *Entomologia Experimentalis et Applicata* 80:289-292.

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pp. 207-231 *in* D. J. Futuyma, and M. Slatkin, eds. Coevolution. Sinauer Associates Inc., Sunderland, Massachussets.

Gauld, I. D., and K. J. Gaston. 1994. The taste of enemy-free space: parasitoids and nasty hosts. Pp. 279-299 *in* B. A. Hawkins, and W. Sheehan, eds. Parasitoid community ecology. Oxford University Press.

Gepts, P. 1998. Origin and evolution of common bean: past events and recent trends. *Hortscience* 33:1124-1130.

Giamoustaris, A., and R. Mithen. 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. o*leifera*) on its interaction with specialist and generalist pests. *Annals of Applied Biology* 126:347-363.

Goudet, J. 1995. Fstat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity* 86:485-486.

Hare, J. D., and R. F. Luck. 1991. Indirect effects of citrus cultivars on life history parameters of a parasitic wasp. *Ecology* 72:1576-1585.

Harvey, J. A., N. M. van Dam, and R. Gols. 2003. Interactions over four trophic levels: foodplant quality affects

development of a hyperparasitoid as mediated through a herbivore and its primary parasitoid. *Journal of Animal Ecology* 72:520-531.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53:1898-1914.

Jourdie, V., N. Alvarez, and T. C. J. Turlings. 2008. Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda*, using polymerase chain reaction amplification and restriction enzyme digestion. *Agriculatural and Forest Entomology* 10:129-136.

Jourdie, V., N. Alvarez, T. C. J. Turlings, and P. Franck. in press. Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of the noctuid *Spodoptera frugiperda*: *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera). *Molecular Ecology Resources*.

Kauffman, W. C., and R. V. Flanders. 1985. Effects of variably resistant soybean and lima bean cultivars on *Pediobius foveolatus* (Hymenoptera: Eulophidae), a parasitoid of the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae). *Environmental Entomology* 14:678-682.

Knipling, E. F. 1980. Regional management of the fall armyworm — a realistic approach? *Florida Entomologist* 63:468-480.

Kranz, J., H. Schmutterer, and W. Koch. 1977. Diseases, Pests and Weeds in Tropical Crops. Verlag Paul Parey, Berlin.

Kruse, J. J. a. R., K. F. . 1997. Effects of selected midwestern larval host plants on performance by two strains of the gypsy moth (Lepidoptera: Lymantriidae) parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). *Environmental Entomology* 26:1155-1166.

Luikart, G., F. W. Allendorf, J. M. Cornuet, and W. B. Sherwin. 1998. Distortion of allele frequency distribution provides a test for recent population bottlenecks. *Journal of Heredity* 89:238-247.

Malausa, T., M.-T. Bethenod, A. Bontemps, D. Bourguet, J. M. Cornuet, and S. Ponsard. 2005. Assortative mating in sympatric host races of the european corn borer. *Science* 308:258-260.

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

Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs, and J. E. Foster. 2003. Parasitoids and parasites of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomologist* 86:254-289.

Morton, N. E. 1995. LODs past and present. *Genetics* 140:7-12.

Nitao, J. K. 1989. Enzymatic adaptation in a specialist herbivore for feeding on furanocoumarin-containing plants. *Ecology* 70:629-635.

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

Orr, D. B., and D. J. Boethel. 1985. Comparative development of *Copidosoma truncatellum* (Hymenoptera: Encyrtidae) and its host, *Pseudoplusia includens* (Lepidoptera: Noctuidae), on resistant and susceptible soybean genotypes. *Environmental Entomology* 14:612-616.

Papa, R., and P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. *Theoretical and Applied*

Genetics 106:239-250.

Pashley, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? *Annals of the Entomological Society of America* 79:898-904.

Peterson, M. A., and R. F. Denno. 1998. The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *American Naturalist* 152:428-446.

R Development Core Team. 2008. R: A Language and Environment for Statistical Computing, Vienna, Austria.

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

Reed, D. K., J. A. Webster, and B. G. E. A. Jones. 1991. Tritrophic relationships of Russian wheat aphid (Homoptera: Aphididae), a hymenopterous parasitoid (*Diaeretiella rapae* McIntosh), and resistant and susceptible small grains. *Biological Control* 1.

Riggen, T. M., D. J. Isenhour, and K. E. Espelie. 1992. Effect of *Cotesia marginiventris* (Hymenoptera: Braconidae) when rearing host fall armyworm (Lepidoptera: Noctuidae) on meridic diet containing foliage from resistant or susceptible corn genotypes. *Environmental Entomology* 21:214-219.

Rogers, D. J., and M. J. Sullivan. 1991. Development of *Brachymeria ovata* (Hymenoptera: Chalcididae) in three noctuid hosts reared on artificial diet and insect-resistant and susceptible soybean genotypes. *Entomophaga* 36:19-27.

Roth, S., C. Knorr, and R. L. Lindroth. 1997. Dietary phenolics affects performance of the gypsy moth (Lepidoptera: Lymantriidae) and its parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). *Environmental Entomology* 26(3):668-671.

Rousset, F. 2000. Genetic differentiation between individuals. *Journal of Evolutionary Biology* 13:58-62.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin: a software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Senrayan, R., and R. S. Annadurai. 1991. Influence of host's food plant and habitat on *Anastatus ramakrishnae* (Mani) (Hym., Eupelmidae), an egg parasitoid of *Coridius obscurus* (Fab.) (Het., Pentatomidae). *Journal of Applied Entomology* 112:237-243.

Smith, J. M. 1957. Effects of the food plant of California red scale, *Aonidiella aurantii* (Mask.) on reproduction of its hymenopterous parasites. *Canadian Entomology* 89:219-230.

Sparks, A. N. 1979. A review of the biology of the fall armyworm. *Florida Entomologist* 62:82-87.

Stark, J. D., T. T. Y. Wong, and R. I. e. a. Vargas. 1992. Survival, longevity, and reproduction of tephritid fruit fly parasitoids (Hymenoptera: Braconidae) reared from fruit flies exposed to azadirachtin. *Journal of Economical Entomology* 85:1125-1129.

Thaler, J. S. 1999. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* 399:686-688.

Thorpe, K. W., and P. Barbosa. 1986. Effects of comsumption of high and low nicotine tobacco by *Manduca sexta* (Lepidoptera: Sphingidae) on survival of gregarious endoparasitoid *Cotesia congregata* (Hymenoptera: Braconidae). *Journal of Chemical Ecology* 12(6):1329-1337.

Thurston, R., and P. M. Fox. 1972. Inhibition by nicotine of emergence of *Apanteles congregatus* from its host, the tobacco hornworm. *Annals of the Entomological Society of America* 65:547-550.

Turlings, T. C. J., and B. Benrey. 1998. Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience* 5:321-333.

van Dam, N. M., K. Hadwich, and I. T. Baldwin. 2000. Induced responses in *Nicotiana attenuata* affect behavior and growth of the specialist herbivore *Manduca sexta*. *Oecologia* 122:371-379.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Genetics* 133:737-749.

Werren, J. H., M. J. Raupp, and C. S. e. a. Sadoff. 1992. Host plants used by gypsy moths affect survival and development of the parasitoid *Cotesia melanoscela*. *Environmental Entomology* 21:173-177.

Wright, S. 1943. Isolation by distance. *Genetics* 28:114-138.

Zvereva, E. L., and N. E. Rank. 2003. Host plant effects on parasitoid attack on the leaf beetle *Chrysomela lapponica*. *Oecologia* 135:258-267.

Acknowledments

We are grateful to the Mexican students and to Ivan Hiltpold who helped us with field collections. This study was supported by the Swiss National Center of Competence in Research “Plant Survival” and grant 3100A0-107974 from the Swiss National Science Foundation.

Chapter 5:

Molecular phylogeography of *Chelonus insularis* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), two primary neotropical parasitoids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Molecular phylogeography of *Chelonus insularis* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), two primary neotropical parasitoids of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Violaine Jourdie[1], Eduardo Virla[2], Henry Murillo[3], Mauricio Bento[4], Ted C. J. Turlings[1] and Nadir Alvarez[1]

[1]*Université de Neuchâtel, Institut de Biologie, Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland*

[2]*PROIMI-Biotecnología, Av. Belgrano y Pje. Caseros (T4001 MBV), Tucumán, Argentina*

[3]*University of Windsor, Department of Biological Sciences, 401 Sunset Avenue, Windsor, ON Canada N9B 3P4*

[4]*University of São Paulo, LEF - ESALQ/USP, Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, Av. Pádua Dias, 11, 13418-900 Piracicaba-SP, Brazil*

Abstract

In a previous study, we observed no genetic structure in Mexican populations of the parasitoids *Chelonus insularis* and *Campoletis sonorensis* using microsatellite markers (Jourdie *et al*. in prep). In the current study, we investigated whether there is any potential structure at a larger scale. Insects of both species were collected across the American continent and their phylogeography was investigated using both nuclear and mitochondrial markers. Our results suggest an ancient north-south migration of *C. insularis*, while no clear pattern could be determined for *C. sonorensis*. The absence of a local fine scale structure was also found at a larger scale for both species. Dispersal of these insects may be partly driven by wind as suggested by genetic similarities between individuals coming from very distant locations.

Keywords: *Chelonus insularis, Campoletis sonorensis, 16S, COI, ITS, 28S, CytB.*

Introduction

Patterns of intraspecific spatial genetic structure (SGS) reflect both historical and contemporaneous levels of gene flow among populations. Although it can be difficult to evaluate which factor, or combination of factors, best explains the observed SGS pattern (Barton and Wilson, 1996; Waser and Strobeck, 1998), it is important to combine our understandings of both the biology of a studied organism and its phylogeographical history in order to interpret dispersal patterns correctly. This is particularly true when working with species of economical importance.

In order to interpret dispersal patterns correctly, an understanding of the historic biogeography of a region as well as the biology of the organism are often necessary. Genetic structure can result from past or current barriers to dispersal, density fluctuations, dispersal patterns and mating systems (Chesser, 1991a, b). However, it can be often difficult to judge which factor, or combination of factors, best explains the observed pattern (Barton and Wilson, 1996;

Waser and Strobeck, 1998).

In this study, we looked at the phylogeography of *Chelonus insularis* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), two important parasitoids of the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The FAW is one of the major pest of several economically important crops throughout the American continent (Kranz *et al.*, 1977). It is attacked by a number of parasitoids (Molina-Ochoa *et al.*, 2003; Murúa *et al.*, 2006; Wyckhuys and O'Neil, 2006) and its management through biological control could be enhanced with knowledge on the population dynamics of these parasitoids and on their past and present genetic history.

The two parasitoids show different ecologies and behaviors. *Chelonus insularis* is an egg-larval parasitoid, whereas *C. sonorensis* attacks larvae. They can be expected to have adapted their searching behavior to the specific stage they attack. Indeed, *C. insularis* encounters patches of physically defenseless eggs, so it can lay tens of eggs at once without having to move much. In contrast, *C. sonorensis* can lay rarely more than one egg per plant visited, as *S. frugiperda* larvae are often solitary due to the highly cannibalistic their behavior. It will therefore have to travel much more than *C. insularis* in order to find a comparable number of hosts to parasitize. These different life histories may reflect in the phylogeographic patterns of the two species and we might expect more structuring at a large scale in *C. insularis* than in *C. sonorensis*. *Chelonus insularis* might tend to disperse less than *C. sonorensis*, and therefore, more local structure may arise in this species.

The current study was also motivated by a proximate conclusion from a previous study (Jourdie *et al.*, in prep) in which we documented the absence of genetic structure in these two species when performing small-scale analyses [using microsatellite loci developed in Jourdie *et al.* (in press)] for populations of parasitoids collected in Mexico. To go one step further and to resolve patterns of dispersal throughout the New World (at the continental scale), we investigate in this paper whether this lack of structure at a small-scale also occurs at a larger scale (including samples from the neotropical and nearctic areas) using both nuclear and mitochondrial DNA sequences.

Table 1: Sequenced individuals and their geographic origins.

Individual	Geographic coordinates
Chelonus insularis	
Argentina 1	27°18'49.78"S, 58°55'03.43"W
Argentina 2	27°18'49.78"S, 58°55'03.43"W
Argentina 3	27°18'49.78"S, 58°55'03.43"W
Argentina 4	27°18'49.78"S, 58°55'03.43"W
Argentina 5	27°18'49.78"S, 58°55'03.43"W
Argentina 6	27°18'49.78"S, 58°55'03.43"W
Argentina 7	27°18'49.78"S, 58°55'03.43"W
Brazil 1	19°28'4.47"S, 44°14'51.54"W
Brazil 2	19°28'4.47"S, 44°14'51.54"W
Mexico, Colima 1	19°16'57.9"N, 103°46'41.0"W
Mexico, Colima 2	19°16'57.9"N, 103°46'41.0"W
Mexico, Jalisco 1	19°52'18.4"N, 103°33'45.6"W
Mexico, Jalisco 2	19°52'18.4"N, 103°33'45.6"W
Mexico, Chiapas 1	14°43'01.5"N, 92°18'26.6"W
Mexico, Chiapas 2	14°43'32.3"N, 92°18'58.1"W
Mexico, Chiapas 3	14°43'20.0"N, 92°19'09.1"W
Mexico, Chiapas 4	14°43'20.0"N, 92°19'09.1"W
Mexico, Veracruz 1	20°29'31.9"N, 97°32'17.2"W
Mexico, Veracruz 2	20°29'31.9"N, 97°32'17.2"W
Mexico, Puebla 1	20°27'10.3"N, 97°38'28.7"W
Mexico, Puebla 2	20°27'10.3"N, 97°38'28.7"W
Mexico, Puebla 3	20°27'10.3"N, 97°38'28.7"W
Campoletis sonorensis	
Canada 1	42°18'50.80"N, 83° 2'12.57"W
Canada 2	42°18'50.80"N, 83° 2'12.57"W
Canada 3	42°18'50.80"N, 83° 2'12.57"W
Canada 4	42°18'50.80"N, 83° 2'12.57"W
Canada 5	42°18'50.80"N, 83° 2'12.57"W
Canada 6	42°18'50.80"N, 83° 2'12.57"W
Canada 7	42°18'50.80"N, 83° 2'12.57"W
Mexico, Jalisco 1	19°52'08.9"N, 103°33'16.7"W
Mexico, Jalisco 2	19°52'18.4"N, 103°33'45.6"W
Mexico, Jalisco 3	20°28'22.3"N, 102°12'06.4"W
Mexico, Jalisco 4	20°28'22.3"N, 102°12'06.4"W
Mexico, Nayarit 1	21°05'29.0"N, 104°26'04.9"W
Mexico, Nayarit 2	21°05'20.9"N, 104°26'05.0"W
Campoletis flavicincta	
Brazil 1	19°28'4.47"S, 44°14'51.54"W
Brazil 2	19°28'4.47"S, 44°14'51.54"W
Campoletis grioti	
Argentina 1	26°15'14.86"S, 65°16'27.90"W
Argentina 2	26°15'14.86"S, 65°16'27.90"W

Materials and methods

Sampling

Insects were collected across the American continent (Table 1). *Chelonus insularis* specimens from Mexico, Brazil and Argentina were included in the study. Individuals of *C. sonorensis* came from Canada and Mexico. As no sequences are published for *Campoletis* except for *C. sonorensis*, other taxons of the same genus were included as well in order to determine the monophyletic status of the species and their relative position. Individuals of *C. grioti* from Argentina and individuals of *C. flavicincta* from Brazil were thus included. All individuals were obtained from *Spodoptera frugiperda*, except for three Canadian individuals (individuals 5, 6 and 7), which emerged from *Trichoplusia ni* (Lepidoptera: Noctuidae).

Table 2: Primers used in this study.

Gene	Sequence	Reference
28S		
F: 28S D2 f	5'-AGA GAG AGT TCA AGA GTA CGT G-3'	Belshaw and Quicke, 1997
R: 28S D2 r	5'-TTG GTC CGT GTT TCA AGA CGG G-3'	Campbell *et al.*, 1993
ITS		
F: ITS 4	5'-TCC TCC GCT TAT TGA TAT GC-3'	White *et al.* 1990
R: ITS 5	5'-GGA AGT AAA AGT CGT AAC AAG G-3'	
cytochrome *b*		
F: CytB f	5'-TCT TTT TGA GGA GCW ACW GTW ATT AC-3'	Belshaw and Quicke, 1997
R: CytB r	5'-AAT TGA ACG TAA AAT WGT RTA AGC AA-3'	
16S		
F: 16S f	5'-CAC CTG TTT ATC AAA AAC AT-3'	Dowton and Austin 1994
R: 16S r	5'-CTT ATT CAA CAT CGA GGT C-3'	Whitfield 1997
COI		
F: C1-J-1859	5'-GGA ACT GGA TGA ACA GTA TAT-3'	Simon *et al.* 1994
R: C1-N-2191	5'-CCA GGT AAA ATT AAA ATA TAA ACT TC-3'	

Laboratory protocols

Total genomic DNA was extracted from the wasps' abdomen using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Total DNA was re-suspended in 200 µl of elution buffer (two elutions of 100 µl each).

Two mitochondrial markers (*16S* and *COI*) were amplified in both genera. An additional nuclear gene (cytochrome *b*) was amplified in *C. insularis* and two nuclear markers (*28S* and *ITS*) were amplified in *Campoletis* (PCR amplification was not satisfying for any nuclear marker in *C. insularis*). Primer information are summed up in Table 2.

28S – The forward primer (28S D2 f) from Belshaw and Quicke (1997) and the reverse primer (28S D2 r) from Campbell *et al.* (1993) were used to obtain an amplified fragment of 427 to 510 bp. The PCR conditions were 30 cycles of 98°C denaturation (15 s), 49°C annealing (30 s) and 72°C elongation (40 s) with an initial denaturation of 3 min at 93°C and a final elongation at 72°C for 3 min.

Internal Transcribed Spacer (ITS) – The forward primer ITS 4 and the reverse primer ITS 5 (White *et al.*, 1990) amplified a 1102 to 1517 bp-long fragment under the following PCR conditions: initial denaturation of 1 min 30 s at 95°C; 35 cycles of 35 s at 95°C, 1 min at 53°C and 2 min at 72°C; final extension of 8 min at 72°C.

Cytochrome b –The wobble primers designed by Belshaw and Quicke (1997) were used to amplify a 231-424 bp fragment. The PCR conditions were 35 cycles of 92°C denaturation (1 min), 53°C annealing (1 min) and 72°C extension (1 min) with an initial denaturation of 1 min 30 s at 94°C and a final extension of 3 min at 72°C.

16S – The forward primer from Whitfield (1997) and the reverse primer from Dowton and Austin (1994) yielded an amplified fragment of 409 to 459 bp. The following PCR conditions were used: intitial denaturation of 1 min 30 s at 94°C, followed by 35 cycles of 94°C denaturation (1 min), 53°C annealing (1 min) and 72°C

extension (1 min), and a final extension of 3 min at 72°C.

Cytochrome c oxidase subunit 1 – The mtDNA Cytochrome *c* oxidase subunit 1 gene (*COI*) was partially amplified using C1-J-1859 (forward) and C1-N-2191 (reverse) primers adapted for the bee (Simon *et al.*, 1994). The amplified fragment was between 364 and 392 bp. It was obtained through the following PCR conditions: 1 min 30 s at 94°C; 35 cycles of 94°C denaturation (1 min), 50°C annealing (1 min) and 70°C extension (1 min); final extension of 5 min at 70°C.

Data analysis

Sequence alignment – The sequences were manually corrected using ChromasPro 1.41 (Technelysium Pty. Ltd, Queensland, Australia) and further aligned using ClustalW 1.4 (Thompson *et al.*, 1994) implemented in BioEdit (Hall, 1999). Alignment was straightforward for all genes as there were no indels. BLAST searches were conducted on all sequences to check for possible contamination.

Selection of outgroups – Sequences from closely related taxa to both *C. insularis* and *Campoletis* sp. were compared to determine which species were more suitable as outgroups. GenBank accession numbers of the sequences used are provided in Table 3. Sequences were obtained in Fasta format from GenBank and converted into Phylip format with ForCon 1.0 (Raes and Van de Peer, 1999). Further alignment with *C. insularis* and *Campoletis* sp. matrices was performed using ClustalW 1.4 (Thompson *et al.*, 1994) implemented in BioEdit (Hall, 1999). A phylogenetic tree was then reconstructed using RAxML (Stamatakis, 2006; Stamatakis *et al.*, 2008) to determine the closest related outgroup taxa. Once the outgroup was picked, a supermatrix composed of three and four nucleotide sequences respectively for *C. insularis* and *C. sonorensis* was built using CONCATENATE (Alexis Criscuolo, http://www.lirmm.fr/~criscuol/). In the supermatrix, taxa in which no sequences were gathered for a given partition were coded as missing values for the corresponding cells (Wiens

Table 3: Taxa used to determine outgroups and GenBank Accession numbers.

	CytB	*16S*	*COI*
Chelonus cautus 1	-	-	EF555604
Chelonus cautus 2	-	-	EF555605
Chelonus cautus 3	-	-	EF555606
Chelonus cautus 4	-	-	EF555607
Chelonus cautus 5	-	-	EF555608
Chelonus cautus 6	-	-	EF555609
Chelonus cautus 7	-	-	EF555610
Chelonus cautus 8	-	-	EF555611
Chelonus sp. 1	-	EU107068	EU106961
Chelonus sp. 2	-	EU107069	EU106962
Chelonus sp. 3	-	EU107070	EU106963
Chelonus sp. 4	-	DQ538554	DQ538844
Chelonus sp. 5	-	-	AF102723
Chelonus sp. 6	-	U68150	-
Chelonus sp. 7	-	AY004037	-
Chelonus sp. 8	-	AY004038	-
Chelonus sp. 9	-	AF003512	-
Chelonus sp. 10	-	AF029115	-
Chelonus sp. 11	-	-	AY165727
Chelonus sp. 12	Z83639	-	-
Chelonus inanitus 1	-	DQ538562	DQ538853
Chelonus inanitus 2	-	AJ535933	-
Sathon falcatus	Z83638	AF102764	-
Alphomelon sp.	-	AF102752	AF102707
Apanteles canarsiae	-	AF102750	-
Apanteles galleriae	-	-	DQ538812
Apanteles nephoptericis	-	AF102763	DQ538813
Campoletis sonorensis 1	-	DQ538558	DQ538849
Campoletis sonorensis 2	-	DQ538559	DQ538850
Cardiochiles floridanus	-	DQ538553	DQ538843
Chelonus sp.	-	-	DQ538844
Choeras sp.	-	DQ538526	-
Cotesia congregata	-	DQ538527	DQ538815
Cotesia electrae	-	DQ538529	DQ538817
Cotesia flaviconchae	-	DQ538531	DQ538819
Cotesia hyphantriae	-	DQ538532	DQ538820
Cotesia melanoscela	-	DQ538533	DQ538821
Cotesia obscuricornis	-	DQ538534	DQ538822
Cotesia rubecula	-	DQ538535	DQ538823
Cotesia sesamiae	-	AF110827	-
Deuterixys rimulosa	-	DQ538537	-
Diolcogaster bakeri	-	DQ538538	-
Diolcogaster schizurae	-	AF102759	DQ538825
Dolichogenidea lacteicolor	-	AF102761	DQ538826
Epsilogaster sp.	-	DQ538555	DQ538845
Fornicia sp.	-	AY044195	AY044210
Glyptapanteles indiensis	-	AF102757	DQ538827
Glyptapanteles porthetriae	-	AF102758	DQ538828
Hypomicrogaster sp.	-	DQ538539	DQ538829
Hypomicrogaster ecdytolophae	-	AF102756	AF102712
Microgaster canadensis	-	U68154	AF102708
Microplitis demolitor	-	DQ538540	DQ538830
Mirax sp.	-	DQ538556	DQ538846
Parapanteles sp.	-	AF102753	DQ538831
Phanerotoma sp.	-	DQ538557	DQ538847
Pholetesor bedelliae	-	U68153	AF102715
Prasmodon sp. 1	-	DQ538541	DQ538832
Prasmodon eminens	-	AF102748	AF102700
Promicrogaster sp. 1	-	DQ538542	DQ538833
Promicrogaster sp. 2	-	DQ538543	DQ538835
Pseudapanteles sp.	-	DQ538545	-
Rhygoplitis sp. 1	-	DQ538546	DQ538836
Rhygoplitis sp. 2	-	DQ538547	DQ538837
Sendaphne sp.	-	DQ538548	DQ538838
Snellenius sp. 1	-	AF102749	DQ538839
Snellenius sp. 2	-	DQ538549	DQ538840
Toxoneuron nigriceps	-	U68151	AF102724
Venanides sp.	-	DQ538550	-
Venanus sp.	-	DQ538551	DQ538841
Venturia canescens	-	DQ538560	DQ538851
Xanthomicrogaster sp.	-	DQ538552	-

and Reeder, 1995).

Phylogeography Estimation

Phylogenetic analyses and their corresponding bootstrap analyses were performed using the three following criteria: maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. All trees generated were edited with FigTree v. 1.1.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Maximum parsimony analyses – MP analyses were performed using parsimony ratchet (Nixon, 1999) as implemented in PAUPrat (Sikes and Lewis, 2001). Based on recommendations by Nixon (1999), ten independent searches were performed with 200 iterations and 15% of the parsimony informative characters perturbed. The shortest equally most parsimonious trees were combined to produce a majority-rule consensus tree. To assess the support at each node, non parametric bootstrap analyses were performed using PAUP* version 4.0b10 (Swofford, 2002) with 1000 replicates, TBR branch swapping, simple sequence addition, MULTREES and holding 10 trees per replicate.

Maximum Likelihood – A maximum likelihood (ML) analysis was performed using the RAxML web-server (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis, 2006; Stamatakis *et al.,* in press). The model used by this software is by default GTR+G. *Treefinder* (Jobb, 2008) was also used to perform a ML analysis and to check for congruency between the two softwares. MrAIC 1.4.3 (Nylander, 2004) was run to select the appropriate model of evolution for each gene separately.

Bayesian inference analyses – A Bayesian inference analysis (Nylander *et al.,* 2004) was performed using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). Two simultaneous Monte Carlo Markov Chains were run for 5×10^5 generations, saving one tree every 100 generations. Stationarity was determined by looking at the average standard deviation of split frequencies. Trees recovered prior to stationarity being reached were discarded and Bayesian posterior probabilities (BPP), representing the percentage of times each node was recovered, were calculated from a consensus of the remaining trees. Effective sampling size (ESS) for MCMC analyses in both species were calculated with Tracer 1.3 (Rambaut and Drummond 2005).

Results

Samples

Chelonus insularis – Seven individuals from northern Argentina (Chaco province), two individuals from Brazil (Sete Lagoas), three individuals from central western Mexico (states of Colima and Jalisco), four individuals from Central Eastern Mexico (states of Puebla and Veracruz) and four individuals from Southern Mexico (state of Chiapas) were included in the analyses.

Campoletis sp. – Seven individuals of *C. sonorensis* from Ontario, Canada (four and three individuals emerging respectively from *S. frugiperda* and from *T. ni*), six individuals of *C. sonorensis* from central western Mexico (states of Jalisco and Nayarit), two individuals of *C. flavicincta* from Brazil and two individuals of *C. grioti* from Argentina were used for the analyses.

Alignments

Chelonus insularis – Two individuals failed to amplify *16S*, so 90.9% individuals were represented in this marker. All individuals amplified *COI* and all individuals but one (95.5%) were represented by Cyt *b*.

Campoletis sp. – Sixteen individuals (94.1%) provided satisfactory amplification for both 16S and 28S. All individuals were represented by *COI*, while only 82.4% of individuals correctly amplified ITS.

Sequences are available in GenBank under Accession numbers XXX to XXX.

Selection of outgroups

From the phylogenies obtained with the sequences published online, *Chelonus inanitus* (Hymenoptera: Braconidae) and *Venturia canescens* (Hymenoptera: Ichneumonidae) were chosen as outgroups

for *C. insularis* and *Campoletis* respectively (tree not shown). No Cyt *b* sequence was available for *C. inanitus*, nor was the *ITS* sequence for *V. canescens*.

Phylogenetic analyses

Chelonus insularis – Among 38 variable characters (of 1190 in total), 36 characters were parsimony-informative. Under the MP criterion, the heuristic search resulted into 188 equally most parsimonious trees of 83 steps (CI= 0.904; RI= 0.897; RC= 0.811; HI=0.096). Considering probabilistic criteria (i.e. ML and Bayesian inference) the best-fit model was the general time reversible (GTR). The topologies produced during the two ML searches (i.e. RAxML and TreeFinder) were identical and fully congruent with the Bayesian Inference partitioned-analysis, in which the best-fit model for individual DNA partitions was F81 for all partitions. In this latter analysis, average standard deviation of split frequencies reached 1% after 150,000 MCMC generations, thus the first 1500 first trees were discarded (burn-in). ESS was above 500 for all parameters.

Campoletis sp. – Among 98 variable characters (of 2591 in total), 43 characters were parsimony-informative. Under the MP criterion, the heuristic search resulted into 201 equally most parsimonious trees of 154 steps (CI= 0.948; RI= 0.929; RC= 0.880; HI=0.051). Considering probabilistic criteria (i.e. ML and Bayesian inference) the best-fit model was the Hasegawa-Kishino-Yano (HKY) with an alpha parameter for the shape of the gamma distribution to account for among-site rate heterogeneity and a proportion of invariable site. The topologies produced during the two ML searches were identical and fully congruent with the Bayesian Inference partitioned-analysis, in which the best-fit model for individual DNA partitions were HKY+G for 16S, HKY for 28S, HKY+I for COI and K2P for *ITS*. The fact that the best model of evolution for *ITS* is a much more simple one than for the other genes may lie in the fact that no outgroup information was available for this marker. In this latter analysis, average standard deviation of split frequencies reached 1% after 100,000 generations, thus discarding the first 1000 trees. ESS was above 200 for all parameters.

Topologies

Since the topologies unraveled with the different methods were highly similar, only the phylogenetic trees resulting from the Baysian inference analyses are shown (see Figure 1A for *C. insularis* and Figure 1B for *Campoletis* sp.). Topologies resulting from other criteria are presented in Appendix 1 (*C. insularis*) and Appendix 2 (*Campoletis*).

Chelonus insularis – The group including individuals coming from Argentina and Brazil is monophyletic and nested in the Mexican group (BPP = 0.99). Within the South-American group, no clear structure can be observed. The Mexican group shows no clear structure either and exhibits paraphyletic origins together with the South-American group. However, this paraphyly is not well supported (BPP = 0.38).

Campoletis sp. – *Campoletis sonorensis* forms a paraphyletic group with well supported clades (BPP = 1). Similarly, *C. grioti* exhibits paraphyly (BPP = 1). In contrast, *C. flavicincta* is monophyletic (BPP = 1). No clear geographic structure is observed in *C. sonorensis*. However, the three Canadian individuals collected on *T. ni* come out in an entirely separate clade (BPP = 0.98).

Discussion

Chelonus insularis

The group of individuals coming from South America is clearly nested into the Mexican group, implying that South American wasps derive from Mesoamerican ancestors. The monophyly of the South American group is in contrast with the paraphyletic origins of the Mexican group. South American populations may therefore have arisen from a single colonization event. Moreover, the Mexican group is as diversified as the South American group, which, in conjunction with the previous

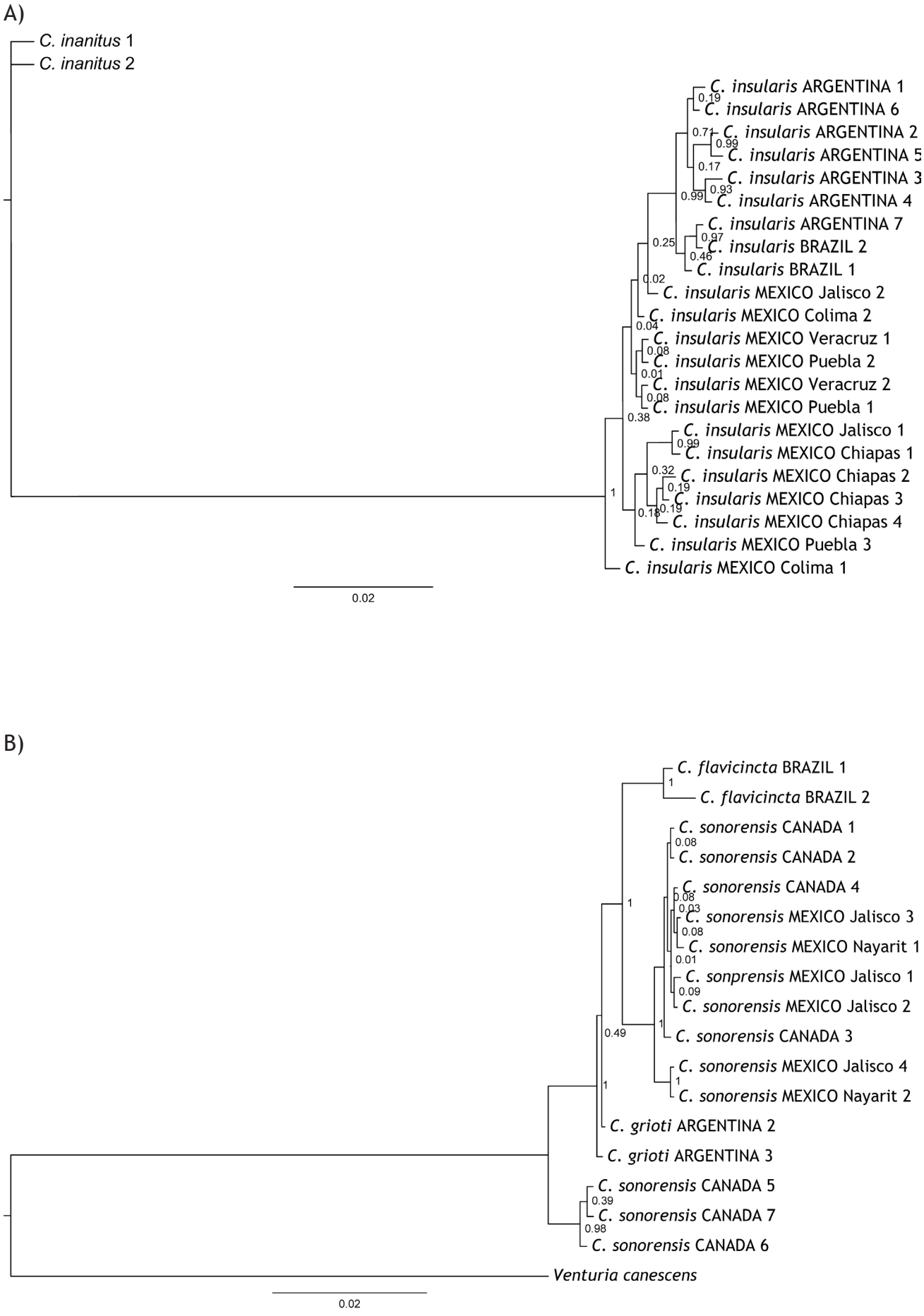


Figure 1: Phylogenetic trees resulting from Bayesian inference analyses for A) *Chelonus insularis* and B) *Campoletis* sp.

notion, leads us to suppose a north-south migration. Yet, a more extensive sampling in North America would be necessary to determine the exact geographic origins of this species.

The high level of admixture and the very weak local fine structure seem to indicate considerable gene flow between populations. For instance, individual 1 from Jalisco and individual 1 from Chiapas come out together in a very well supported clade (BPP = 0.99) (i.e. sequences from the two individuals are identical in all partitions) despite being geographically distant from more than1000 km. Similarly, individual 7 from Argentina and individual 2 from Brazil are identical for all partitions even though they come from two distant locations separated by over 1300 km.

Campoletis

The data strongly support *Campoletis sonorensis* to be paraphyletic. The apparent paraphyly of *C. grioti* might be mostly due to a small sampling size. Indeed, it is not supported by Bayesian posterior probability (BPP<0.5).

There was a striking difference between the three Canadian individuals (5, 6 and 7), which were collected on *T. ni* differ substantially from the other Canadian individuals which emerged from *S. frugiperda* (BPP = 1), implying that differential host may strongly affect evolutionary processes and may have lead to genetic differentiation. Molecular tools once again proved useful to bring cryptic species to the fore, as it was the case in several previous studies (Hebert *et al.*, 2004; Smith *et al.*, 2006; Burns *et al.*, 2008).

There was, however, no indication of local fine structure. Indeed, *C. sonorensis* individuals coming from close geographic locations (i.e. individuals 3 and 4 from Jalisco (Mexico), and individuals 1 and 2 from Nayarit (Mexico)) pair-up in different very well-supported clades (BPP =1). Moreover, if we only take into consideration individuals of *C. sonorensis* emerging from *S. frugiperda*, there is no evidence for structure at the scale of the North-American continent.

Hence, the absence of genetic structure observed at the local Mexican scale using microsatellites (Jourdie *et al.*, in prep) was confirmed at a larger scale with the use of sequence markers. Interpopulation movements appear to be very important even over very large distances. Wind is a likely mode of dispersal for these insects as they are too small and fragile to fly over such distances. Wind is known to play an important role in dispersal of small flying insects (Compton, 2002). Dispersal by moving air largely dictates direction and distance of migration to small insects, which can be expected to reflect in the genetic population structure (Dudley, 2000).

The observed absence of genetic structure might also result from a keen ability of these insects to adapt to environmental changes and therefore to readily invade new environments (Hengeveld, 1989; Kareiva, 1996). This adaptability enhances their potential as effective biological control agents in new areas of release. Finally, cryptic taxa brought to evidence in this study may also have revealed new candidates for biological control.

References

Barton, N. H., and I. Wilson. 1996. Genealogies and geography. Pp. 23-56 in P. H. Harvey, A. J. L. Brown, J. Maynard Smith, and S. Nee, eds. New Uses for New Phylogenies. Oxford University Press, Oxford.

Belshaw, R., and D. L. J. Quicke. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution* 7:281-293.

Burns, J. M., D. H. Janzen, M. Hajibabaei, W. Hallwachs, and P. D. N. Hebert. 2008. DNA barcodes and cryptic species of skipper butterflies in the genus Perichares in Area de Conservacion Guanacaste, Costa Rica. *Proceedings of the National Academy of Science USA* 105:6350-6355.

Campbell, B. C., J. D. Steffen-Campbell, and J. H. Werren. 1993. Phylogeny of the Nasonia species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Molecular Biology* 2:225-237.

Chesser, R. K. 1991a. Gene diversity and female philopatry. *Genetics* 127:437-447.

Chesser, R. K. 1991b. Influence of gene flow and breeding tactics on gene diversity within populations. *Genetics* 129:573-583.

Compton, S. G. 2002. Sailing with the wind: dispersal by small flying insects. Pp. 113-153 in J. M. Bullock, R. E. Kenward, and R. Hails, eds. Dispersal ecology: the 42nd symposium of the British Ecological Society held at the University of Reading, UK, on 2-5 April 2001. Blackwell Science, Oxford, UK.

Dowton, M., and A. D. Austin. 1994. Molecular phylogeny of the insect order Hymenoptera: Apocritan relationships. *Proceedings of the National Academy of Science USA* 91:9911-9915.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proceedings of the National Academy of Science USA* 101:14812-14817.

Hengeveld, R. 1989. Caught in an ecological web. *Oikos* 54:15-22

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754-755.

Jobb, G. 2008. TREEFINDER version of June 2008. Pp. Distributed by the author at www.treefinder.de, Munich, Germany.

Jourdie, V., A. Alvarez, J. Molina-Ochoa, T. Williams, D. Bergvinson, B. Benrey, T. C. J. Turlings, and P. Franck. in prep. Population genetic structure of two primary parasitoids of Spodoptera frugiperda (Lepidopotera), Chelonus insularis and Campoletis sonorensis (Hymenoptera): is host plant important?

Kareiva, P. 1996. Contributions of ecology to biological control. *Ecology* 77:1963-1964.

Kranz, J., H. Schmutterer, and W. Koch. 1977. Diseases, Pests and Weeds in Tropical Crops. Verlag Paul Parey, Berlin.

Molina-Ochoa, J., J. E. Carpenter, E. A. Heinrichs, and J. E. Foster. 2003. Parasitoids and parasites of Spodoptera frugiperda (Lepidoptera: Noctuidae) in the Americas and Caribbean Basin: an inventory. *Florida Entomologist* 86:254-289.

Murúa, G., J. Molina-Ochoa, and C. Coviella. 2006. Population dynamics of the fall armyworm, Spodoptera frugiperda (Lepidoptera: Noctuidae) and its parasitoids in northwestern Argentina. *Florida Entomologist* 89:175-182.

Nixon, K. C. 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15:407-414.

Nylander, J. A. A. 2004. MrAIC. pl. Program distributed by the author, Evolutionary Biology Center, Uppsala University.

Nylander, J. A. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53:47-67.

Raes, J., and Y. Van de Peer. 1999. ForCon: a software tool for the conversion of sequence alignments. *Embnet. News* 6:10-12.

Rambaut, A., and A. J. Drummond. 2005. Tracer version 1.3: MCMC Trace Analysis Tool. Distributed by the authors: Evolutionary Biology Group, Department of Zoology, University of Oxford, UK. Available at http://evolve.zoo.ox.ac.uk.

Sikes, D. S., and P. O. Lewis. 2001. PAUPRat: a tool to implement parsimony ratchet searches using PAUP*, beta software, version 1. Sinauer Associates, Sunderland, MA.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America* 87:651-701.

Smith, M. A., N. E. Woodley, D. H. Janzen, W. Hallwachs, and P. D. N. Hebert. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Science USA* 103:3657-3662.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688-2690.

Stamatakis, A., P. Hoover, and J. Rougemont. in press (2008). A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology*.

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

Waser, P. M., and C. Strobeck. 1998. Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution* 13:43-44.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 in M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. PCR protocols: a guide to methods and applicaction. Academic Press, Inc, New York.

Whitfield, J. B. 1997. Molecular and morphological data suggest a common origin for the polydnaviruses among braconid wasps. *Naturwissenschaften* 84:502-507.

Wiens, J. J., and T. W. Reeder. 1995. Combining data sets with different numbers of taxa for phylogenetic analysis. *Systematic Biology* 44:548-558.

Wyckhuys, K. A. G., and R. J. O'Neil. 2006. Population dynamics of Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) and associated arthropod natural enemies in Honduran subsistence maize. *Crop Protection* 25:1180-1190.

Acknowledments

The authors wish to thank Neil Villard who carried out laboratory work. We are also grateful to the Mexican students and to Ivan Hiltpold who helped us with field collections. This study was supported by the Swiss National Center of Competence in Research “Plant Survival” and grant 3100A0-107974 from the Swiss National Science Foundation.

General conclusions

Molecular species identification

A two-step technique using PCR amplification and restriction enzyme digestion was successfully developed to identify 7 species of parasitoids. This technique was very helpful as it enabled to include in the population genetic study all the parasitoid larvae that did not complete their life cycle. Without this technique, sequencing would have been necessary to assign the larvae to their species. The technique presented in this manuscript is much faster and cheaper than sequencing, it requires basic laboratory equipment and it is as reliable as sequencing. It however has its limitations: if we are dealing with a species other than the 7 species it was designed for, a new pattern will appear. Sequencing would then be the only way to determine the species.

Microsatellite development

Fifteen and 13 polymorphic hypervariable microsatellites were developed respectively for *Campoletis sonorensis* and *Chelonus insularis*. Allelic diversity ranged from two to 16 in *C. sonorensis* and from four to 18 in *C. insularis*. Heterozygosity ranged from 0.088 to 0.403 in *C. sonorensis* and from 0.106 to 0.458 in *C. insularis*. No linkage disequilibrium was observed for any of the loci in *C. insularis* while four pairs of loci showed significant linkage. These highly polymorphic markers will be very useful for whoever wishes to study population genetic structure, parentage and mating system of these two species.

Fine scale population genetic study

The use of microsatellites at small scale (Mexico) revealed no clear genetic structure in any of the two parasitoid species, *C. insularis* and *C. sonorensis*. Gene flow in these two species appears to be considerable. The fact that plants seem to have no effect in shaping populations genetic structure is not really surprising considering conditions wasps are confronted with in the field. Indeed, *Spodoptera frugiperda*, their lepidopteran host, attacks numerous plant species and is abundant no matter the environment. The parasitoids thus do not need to search much for a host as they encounter at least one on about any plant they land on. The absence of pattern of isolation by distance is however more surprising. Indeed, such small insects are not expected to have enough resources to fly over long distance, yet no clear distinction can be made between insects coming from location apart from over 1000 km. As passive dispersion through commercial exchanges can be ruled out for these species, wind seems to be the major actor in dispersal of these minute insects.

Large scale phylogeography

Sequencing of mitochondrial and nuclear DNA confirmed the absence of genetic structure observed at small scale. The analyses reveal an ancient North-South migration in *C. insularis*. The monophyly of the South-American group is clearly opposed to the paraphyletic origins of the species, indicating that colonization of the South-American continent may have occurred through a single event. No clear local structure was detected either on the North- or on the South-American continent, and the high level of admixture suggests that gene flow between populations is considerable.

The group of *C. sonorensis* is paraphyletic. No local structure was observed in this species either. However, the analyses revealed a cryptic species in Canada which comes from a different host (*Trichoplusia ni* instead of *S. frugiperda*). These results suggest that it would be worthwhile to rather look for an effect of the second trophic level instead of the first one on the population genetic structure of parasitoids.

The lack of structure in these insects might confer (or be due to) great abilities of adaptation to new conditions. The cryptic species brought to the fore may constitute

a new candidate for biological control.

The study of parasitoids population genetics is not easy in our system. Indeed, large sampling sizes are needed for the analyses, but unfortunately, collecting large numbers of individuals from the same species in one population is rare. This is due to the fact that the host is attacked by a number of parasitoids, therefore, we are not assured, by far, that all individuals collected in the same field will be conspecific. The level of parasitism is also a reason why obtaining large sample sizes of wasps is complicated. Indeed, I have observed very high levels of parasitism in some areas (e.g. aroung 70% in some fields in Guanajuato), but most of the time, parasitism barely reaches 20%. Therefore, huge numbers of caterpillars are needed to yield satisfying population sampling sizes. If a similar study were to be carried out in the future, a different sampling strategy should be considered. Maybe it would be better to focus on only a few fields and sample them over and over until enough parasitoids have been collected, rather than sample many fields in many different locations.

Moreover, this system is after all not the best suited to this type of study. Indeed, if the first trophic level (i.e. the host plant) does have an affect on the third one (i.e. the parasitoid), it is highly diluted in such a system where neither the pest nor the parasitoid is a specialist. In my opinion, the only way to bring evidence for a host plant effect on parasitoids population genetics is to work on a highly specialized system where a single species of insect herbivore feeds on only one host plant, and is in turn attacked by a single parasitoid species. In such a case, one may be able to show some effect of the plant on the third trophic level, provided that enough is known about the ecology and life history of all the organisms in the system. In this project, I was confronted with too many unknowns, which delayed progress and rendered interpretation of the data chaotic in some parts. A lot of work should have been done before this project was started, to first answer basic ecological questions about the system. Instead, unfortunately, corners were cut. These results will however bring an additional stone in building a bridge between chemical ecology and population genetics.

Appendix 1:

Genetic variability

Chelonus insularis

Locus		G1	G2	G3	G4	G5	G6	J2	J3	J4	N6	P2	Total
Ci1	*(N)*	*(32)*	*(18)*	*(22)*	*(40)*	*(23)*	*(15)*	*(14)*	*(12)*	*(17)*	*(17)*	*(18)*	—
	GD	0.875	0.884	0.923	0.894	0.907	0.917	0.896	0.811	0.934	0.888	0.825	—
	N_a	16	12	14	15	15	13	14	7	13	12	12	25
	AR	9.952	10.076	11.668	10.395	11.056	11.852	12.681	7.000	11.532	10.324	9.765	11.665
	H_E	0.874	0.878	0.921	0.892	0.907	0.915	0.897	0.812	0.927	0.884	0.824	—
	H_O	0.781	0.667	0.818	0.775	0.913	0.867	0.929	0.833	0.706	0.765	0.778	—
	HWE (p-value)	0.026	0.000*	0.106	0.13	0.387	0.004*	0.086	0.13	0.000*	0.204	0.07	—
	*H*eq (IAM)	0.871	0.865	0.879	0.845	0.887	0.897	0.919	0.763	0.888	0.870	0.863	—
	*H*eq (SMM)	0.917	0.901	0.913	0.905	0.920	0.921	0.935	0.822	0.914	0.905	0.901	—
Ci10	*(N)*	*(32)*	*(23)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(16)*	*(18)*	*(20)*	*(18)*	—
	GD	0.503	0.546	0.528	0.492	0.531	0.433	0.442	0.488	0.435	0.526	0.559	—
	N_a	2	3	3	4	4	2	2	2	2	3	4	6
	AR	2.000	2.777	2.545	3.025	3.043	2.000	2.000	2.000	2.000	2.600	3.333	2.550
	H_E	0.506	0.549	0.527	0.492	0.529	0.434	0.444	0.484	0.437	0.529	0.554	—
	H_O	0.688	0.652	0.500	0.525	0.478	0.467	0.500	0.375	0.500	0.650	0.389	—
	HWE (p-value)	0.073	0.699	0.811	0.943	0.877	1.000	1.000	0.591	1.000	0.254	0.032	—
	*H*eq (IAM)	0.199	0.373	0.388	0.447	0.491	0.237	0.235	0.238	0.227	0.380	0.518	—
	*H*eq (SMM)	0.255	0.490	0.480	0.597	0.615	0.289	0.283	0.283	0.280	0.487	0.624	—
Ci11	*(N)*	*(32)*	*(22)*	*(21)*	*(40)*	*(23)*	*(15)*	*(16)*	*(14)*	*(17)*	*(20)*	*(18)*	—
	GD	0.737	0.797	0.743	0.799	0.761	0.829	0.898	0.832	0.800	0.822	0.850	—
	N_a	9	8	8	9	8	8	9	7	7	9	7	13
	AR	6.177	6.902	6.588	7.221	6.609	7.559	8.612	6.839	6.597	7.725	6.825	7.521
	H_E	0.734	0.796	0.742	0.799	0.760	0.823	0.889	0.836	0.795	0.818	0.840	—
	H_O	0.563	0.773	0.714	0.825	0.739	0.667	0.625	0.929	0.647	0.650	0.500	—
	HWE (p-value)	0.000*	0.157	0.785	0.131	0.540	0.040	0.005	0.871	0.040	0.002*	0.001*	—
	*H*eq (IAM)	0.736	0.739	0.741	0.714	0.738	0.780	0.805	0.752	0.722	0.784	0.718	—
	*H*eq (SMM)	0.833	0.820	0.823	0.831	0.819	0.838	0.860	0.812	0.801	0.851	0.800	—
Ci16	*(N)*	*(32)*	*(23)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(16)*	*(18)*	*(19)*	*(18)*	—
	GD	0.403	0.244	0.326	0.387	0.346	0.395	0.442	0.235	0.203	0.322	0.307	—
	N_a	5	4	5	5	4	4	3	4	2	3	4	8
	AR	4.000	3.197	3.854	3.771	3.631	3.600	2.750	3.444	1.992	2.867	3.531	3.572
	H_E	0.403	0.243	0.327	0.386	0.347	0.395	0.433	0.236	0.203	0.323	0.303	—
	H_O	0.406	0.174	0.364	0.350	0.391	0.400	0.188	0.250	0.222	0.368	0.167	—
	HWE (p-value)	0.346	0.042	1.000	0.489	1.000	0.078	0.017	1.000	1.000	1.000	0.028	—
	*H*eq (IAM)	0.553	0.486	0.575	0.527	0.493	0.534	0.415	0.529	0.236	0.391	0.511	—
	*H*eq (SMM)	0.682	0.612	0.694	0.675	0.611	0.632	0.507	0.626	0.285	0.495	0.625	—
Ci17	*(N)*	*(32)*	*(22)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(15)*	*(18)*	*(18)*	*(18)*	—
	GD	0.758	0.748	0.577	0.786	0.728	0.512	0.585	0.679	0.775	0.768	0.691	—
	N_a	7	6	7	7	7	7	5	6	6	7	7	9
	AR	5.875	5.444	5.648	6.139	5.837	6.297	4.444	5.590	5.325	6.311	5.864	5.828
	H_E	0.754	0.746	0.576	0.785	0.728	0.510	0.581	0.676	0.775	0.724	0.698	—
	H_O	0.531	0.682	0.545	0.725	0.696	0.467	0.438	0.600	0.778	0.667	0.944	—
	HWE (p-value)	0.003*	0.193	0.249	0.117	0.274	0.382	0.419	0.378	0.306	0.133	0.000*	—
	*H*eq (IAM)	0.664	0.646	0.693	0.645	0.689	0.741	0.607	0.688	0.662	0.726	0.717	—
	*H*eq (SMM)	0.780	0.753	0.792	0.773	0.788	0.807	0.707	0.769	0.762	0.803	0.799	—

Chelonus insularis

Ci30	*(N)*	*(32)*	*23)*	*(22)*	*(39)*	*(23)*	*(15)*	*(16)*	*(16)*	*(18)*	*(20)*	*(18)*	—
	GD	0.525	0.607	0.616	0.600	0.660	0.569	0.646	0.592	0.770	0.583	0.621	—
	N_a	3	3	5	5	5	4	4	4	5	3	6	6
	AR	2.763	2.981	4.077	3.871	4.291	3.600	3.932	3.932	4.665	2.943	4.895	4.024
	H_E	0.524	0.602	0.615	0.600	0.660	0.561	0.647	0.591	0.763	0.581	0.622	—
	H_O	0.500	0.391	0.591	0.641	0.652	0.333	0.688	0.563	0.556	0.500	0.667	—
	HWE (p-value)	0.671	0.009	0.728	0.712	0.142	0.050	0.791	0.651	0.014	0.130	0.454	—
	H eq (IAM)	0.350	0.380	0.574	0.520	0.575	0.531	0.524	0.516	0.605	0.381	0.666	—
	H eq (SMM)	0.471	0.487	0.693	0.675	0.697	0.635	0.630	0.633	0.704	0.497	0.759	—
Ci31	*(N)*	*(30)*	*(22)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(15)*	*(17)*	*(19)*	*(18)*	—
	GD	0.867	0.874	0.900	0.868	0.896	0.900	0.867	0.914	0.846	0.882	0.768	—
	N_a	10	10	10	11	11	10	10	10	10	10	8	17
	AR	8.379	8.551	9.303	8.412	9.283	9.325	9.069	9.520	8.699	8.903	7.011	9.425
	H_E	0.863	0.870	0.900	0.869	0.897	0.894	0.865	0.906	0.841	0.879	0.770	—
	H_O	0.667	0.682	0.864	0.900	0.913	0.733	0.813	0.667	0.706	0.789	0.833	—
	HWE (p-value)	0.000*	0.015	0.413	0.215	0.440	0.108	0.143	0.052	0.012	0.413	0.001*	—
	H eq (IAM)	0.773	0.802	0.798	0.771	0.821	0.840	0.832	0.841	0.829	0.815	0.757	—
	H eq (SMM)	0.855	0.865	0.866	0.864	0.878	0.883	0.878	0.882	0.875	0.870	0.830	—
Ci33	*(N)*	*(31)*	*(23)*	*(22)*	*(40)*	*(23)*	*(15)*	*(16)*	*(16)*	*(18)*	*(19)*	*(17)*	—
	GD	0.400	0.429	0.303	0.396	0.231	0.190	0.465	0.329	0.382	0.512	0.474	—
	N_a	3	3	2	4	2	3	3	3	2	3	3	4
	AR	2.387	2.522	1.999	2.812	1.992	2.766	2.750	2.942	2.000	2.632	2.706	2.491
	H_E	0.397	0.426	0.304	0.397	0.232	0.191	0.462	0.331	0.386	0.511	0.469	—
	H_O	0.226	0.304	0.364	0.475	0.261	0.200	0.375	0.375	0.500	0.474	0.294	—
	HWE (p-value)	0.010	0.350	1.000	0.140	1.000	1.000	0.673	1.000	0.524	1.000	0.152	—
	H eq (IAM)	0.345	0.368	0.223	0.443	0.220	0.418	0.411	0.412	0.233	0.390	0.398	—
	H eq (SMM)	0.465	0.483	0.265	0.590	0.258	0.502	0.504	0.504	0.275	0.495	0.512	—

Campoletis sonorensis

Locus		G4	G5	J3	J4	N6	Total
Cs6	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.697	0.691	0.732	0.559	0.728	—
	N_a	8	5	7	4	5	8
	AR	5.024	4.638	5.599	3.909	5.000	5.139
	H_E	0.719	0.677	0.715	0.541	0.720	—
	H_O	0.641	0.552	0.640	0.727	0.700	—
	HWE (p-	0.020	0.153	0.096	0.812	0.501	—
	H eq (IAM)	0.691	0.553	0.684	0.565	0.669	—
	H eq (SMM)	0.804	0.686	0.786	0.657	0.734	—
Cs9	*(N)*	*(39)*	*(29)*	*825)*	*(11)*	*(10)*	—
	GD	0.542	0.586	0.683	0.595	0.783	—
	N_a	6	5	5	6	5	8
	AR	4.230	3.798	4.189	5.723	5.000	4.389
	H_E	0.534	0.574	0.668	0.562	0.740	—
	H_O	0.436	0.483	0.600	0.455	0.700	—
	HWE (p-	0.244	0.485	0.156	0.383	0.692	—
	H eq (IAM)	0.592	0.551	0.565	0.730	0.671	—
	H eq (SMM)	0.732	0.686	0.695	0.789	0.735	—
Cs14	*(N)*	*(39)*	*(28)*	*(25)*	*(11)*	*(10)*	—
	GD	0.566	0.593	0.590	0.614	0.522	—
	N_a	5	4	5	5	2	5
	AR	3.489	3.801	3.690	4.727	2.000	3.594
	H_E	0.575	0.583	0.677	0.640	0.495	—
	H_O	0.641	0.643	0.440	0.818	0.500	—
	HWE (p-	0.495	0.803	0.046	0.027	1.000	—
	H eq (IAM)	0.524	0.465	0.569	0.665	0.279	—
	H eq (SMM)	0.676	0.600	0.692	0.732	0.317	—
Cs20	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.753	0.635	0.618	0.777	0.661	—
	N_a	7	6	6	5	5	8
	AR	5.067	4.635	4.081	4.905	5.000	4.714
	H_E	0.743	0.624	0.606	0.731	0.630	—
	H_O	0.692	0.586	0.640	0.545	0.700	—
	HWE (p-	0.116	0.532	1.000	0.257	0.695	—
	H eq (IAM)	0.639	0.615	0.638	0.658	0.673	—
	H eq (SMM)	0.775	0.737	0.748	0.734	0.740	—
Cs21	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.798	0.806	0.810	0.841	0.867	—
	N_a	12	13	13	8	8	19
	AR	7.279	8.550	8.521	7.719	8.000	8.136
	H_E	0.786	0.790	0.794	0.806	0.820	—
	H_O	0.692	0.655	0.800	0.909	0.800	—
	HWE (p-	0.069	0.000*	0.049	0.322	0.503	—
	H eq (IAM)	0.801	0.840	0.852	0.818	0.832	—
	H eq (SMM)	0.878	0.897	0.901	0.860	0.867	—

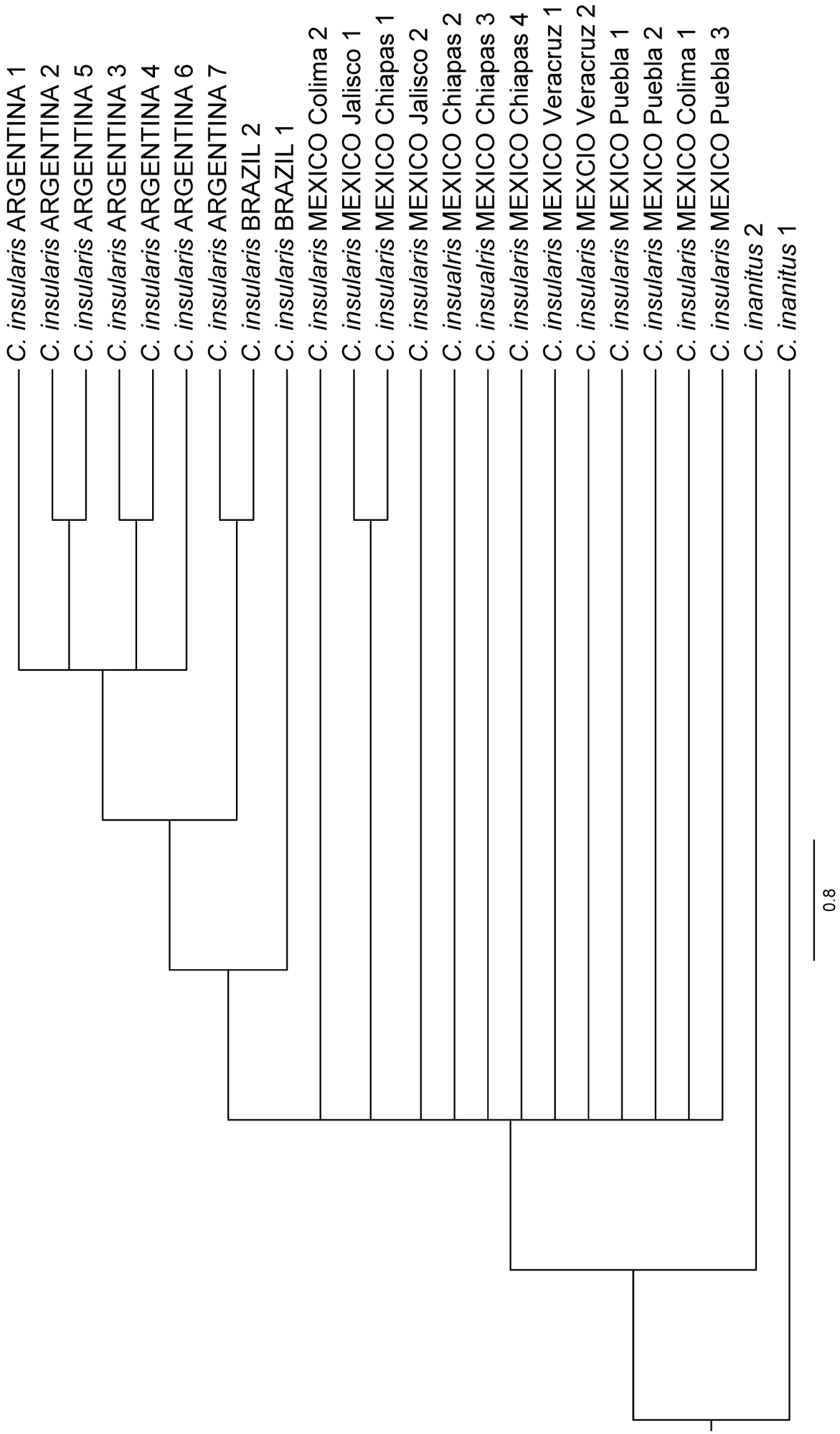
Campoletis sonorensis

Cs22	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.741	0.544	0.625	0.800	0.567	—
	N_a	10	5	7	7	4	16
	AR	5.591	3.034	4.490	6.805	4.000	5.065
	H_E	0.732	0.537	0.610	0.769	0.545	—
	H_O	0.795	0.690	0.480	0.909	0.700	—
	HWE (p-	0.201	0.164	0.021	0.845	0.031	—
	H eq (IAM)	0.752	0.550	0.682	0.776	0.583	—
	H eq (SMM)	0.848	0.685	0.788	0.826	0.659	—
Cs42	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.568	0.557	0.559	0.600	0.622	—
	N_a	4	3	4	4	4	4
	AR	3.099	2.826	3.045	3.818	4.000	3.138
	H_E	0.561	0.547	0.546	0.579	0.585	—
	H_O	0.615	0.552	0.480	0.727	0.500	—
	HWE (p-	0.061	1.000	0.846	0.625	0.130	—
	H eq (IAM)	0.445	0.359	0.483	0.571	0.588	—
	H eq (SMM)	0.591	0.476	0.613	0.656	0.662	—
Cs44	*(N)*	*(39)*	*(29)*	*(259*	*£(11)*	*(10)*	—
	GD	0.373	0.459	0.413	0.509	0.100	—
	N_a	2	2	2	2	2	2
	AR	1.999	2.000	2.000	2.000	2.000	1.999
	H_E	0.369	0.452	0.403	0.483	0.095	—
	H_O	0.385	0.483	0.320	0.455	0.100	—
	HWE (p-	1.000	1.000	0.329	1.000	¾	—
	H eq (IAM)	0.202	0.202	0.222	0.266	0.267	—
	H eq (SMM)	0.230	0.252	0.259	0.310	0.311	—
Cs47	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.123	0.223	0.153	0.177	0.189	—
	N_a	3	3	3	3	2	4
	AR	2.044	2.553	2.193	2.818	2.000	2.258
	H_E	0.122	0.219	0.150	0.169	0.180	—
	H_O	0.128	0.241	0.160	0.182	0.200	—
	HWE (p-	1.000	1.000	1.000	1.000	1.000	—
	H eq (IAM)	0.352	0.363	0.368	0.443	0.270	—
	H eq (SMM)	0.466	0.470	0.482	0.532	0.316	—
Cs48	*(N)*	*(39)*	*(29)*	*(25)*	*(11)*	*(10)*	—
	GD	0.722	0.769	0.675	0.718	0.767	—
	N_a	7	7	8	5	6	9
	AR	5.670	5.578	5.733	4.818	6.000	5.653
	H_E	0.713	0.755	0.662	0.694	0.725	—
	H_O	0.744	0.724	0.720	0.909	0.700	—
	HWE (p-	0.195	0.405	0.549	0.576	0.680	—
	H eq (IAM)	0.647	0.671	0.727	0.660	0.737	—
	H eq (SMM)	0.775	0.782	0.819	0.732	0.796	—
Cs49	*(N)*	*(39)*	*(29)*	*(24)*	*(11)*	*(10)*	—
	GD	0.341	0.364	0.483	0.327	0.483	—
	N_a	4	5	4	3	3	5
	AR	2.503	3.578	3.226	2.991	3.000	3.076
	H_E	0.336	0.356	0.470	0.314	0.460	—
	H_O	0.282	0.276	0.375	0.364	0.500	—
	HWE (p-	0.011	0.165	0.235	1.000	0.646	—
	H eq (IAM)	0.437	0.544	0.487	0.449	0.453	—
	H eq (SMM)	0.589	0.686	0.614	0.530	0.539	—

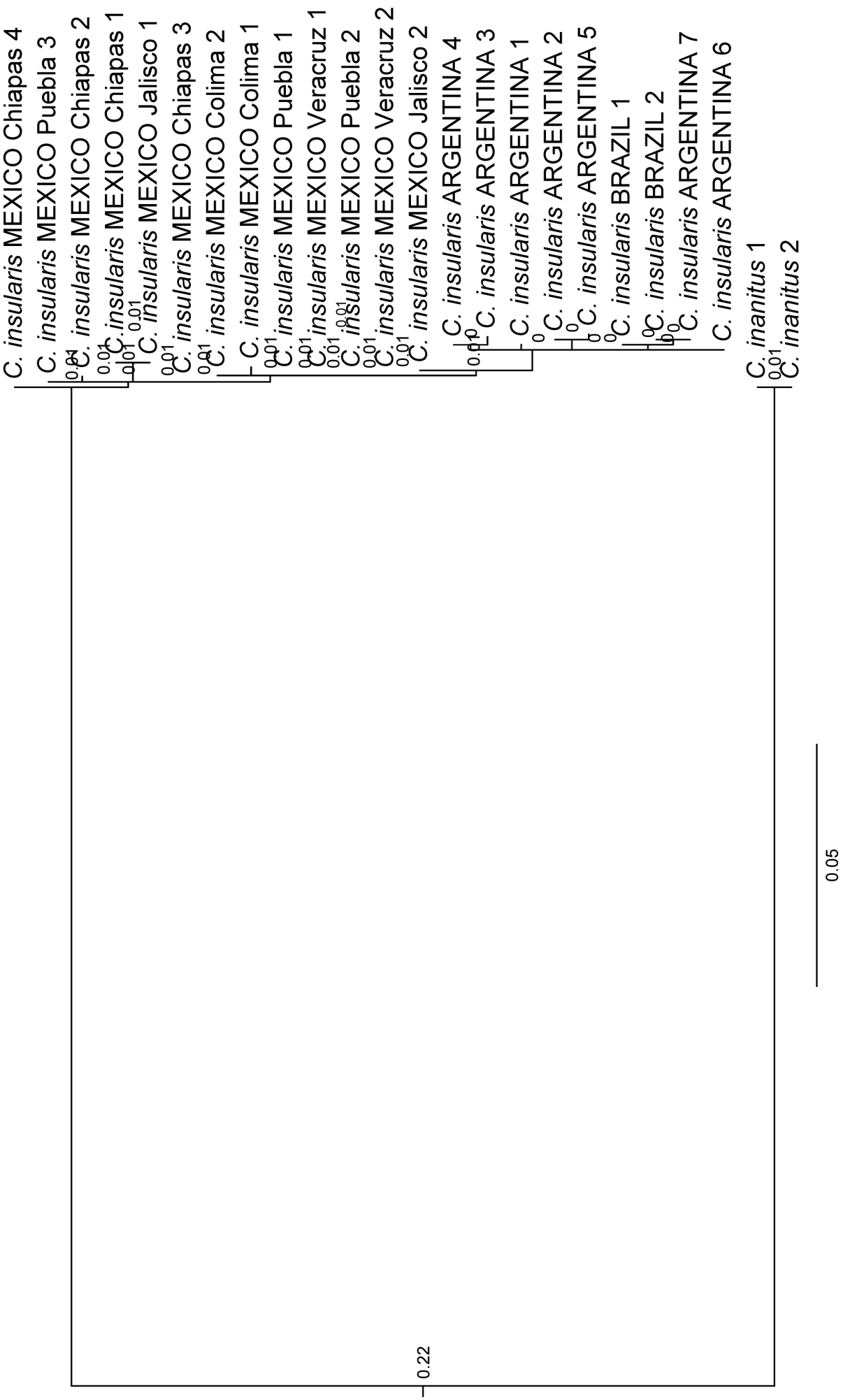
Appendix 2:

Phylogenetic trees for *Chelonus insularis*

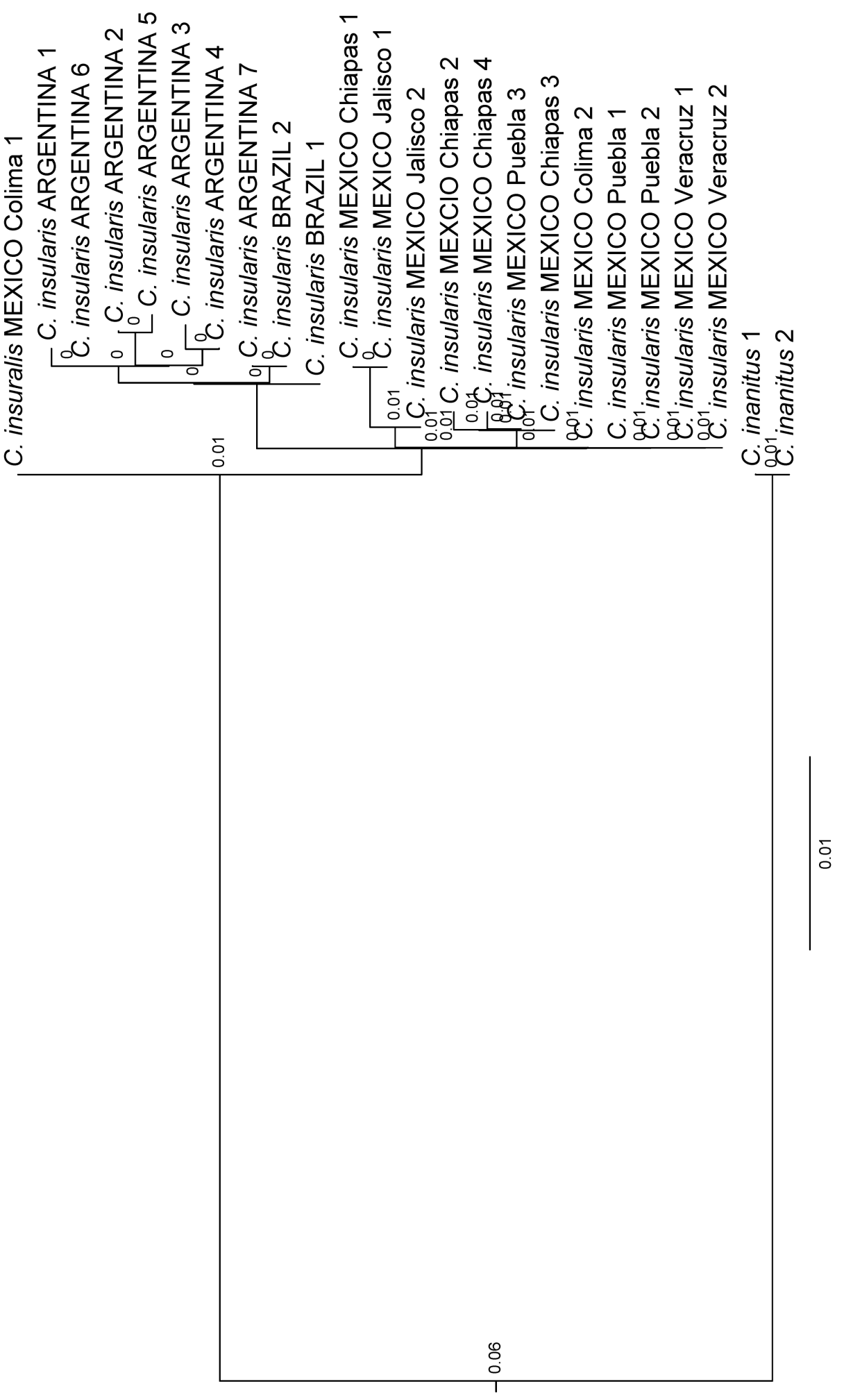
Phylogenetic trees for *Chelonus insularis* resulting from maximum parsimony analyses.



Phylogenetic trees for *Chelonus insularis* resulting from maximum likelihood analyses with RAxML.



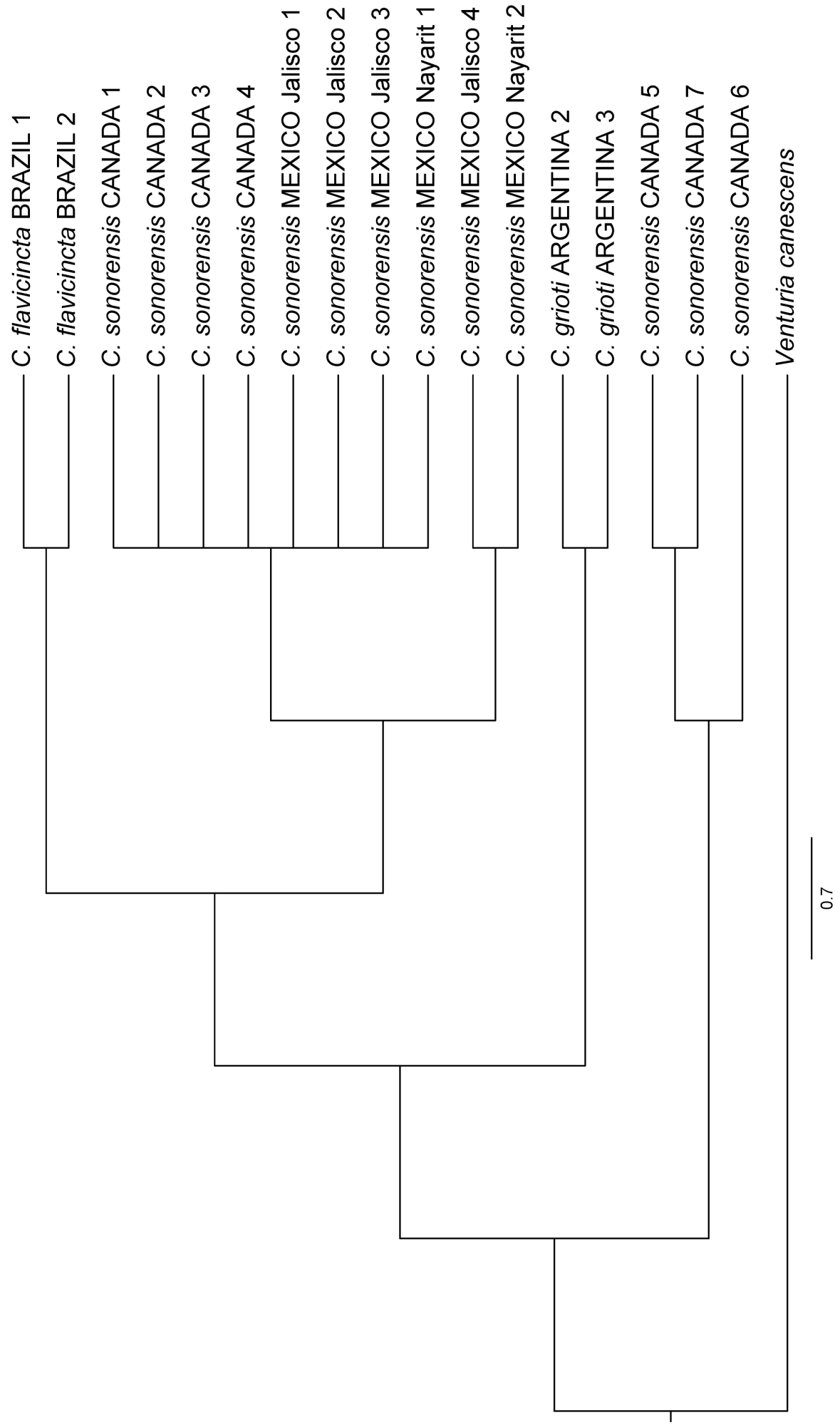
Phylogenetic trees for *Chelonus insularis* resulting from maximum likelihood analyses with TreeFinder.



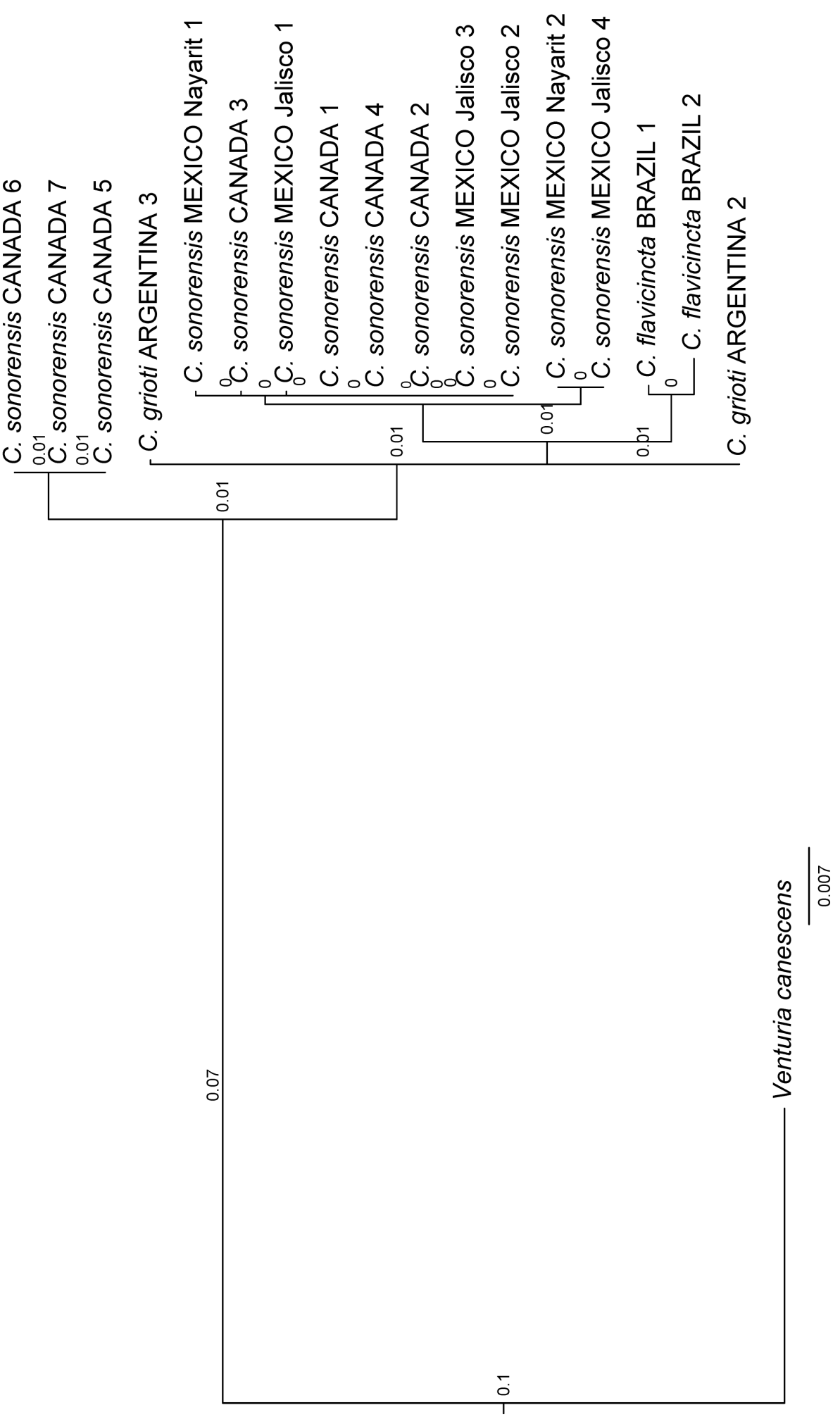
Appendix 3:

Phylogenetic trees for *Campoletis* sp.

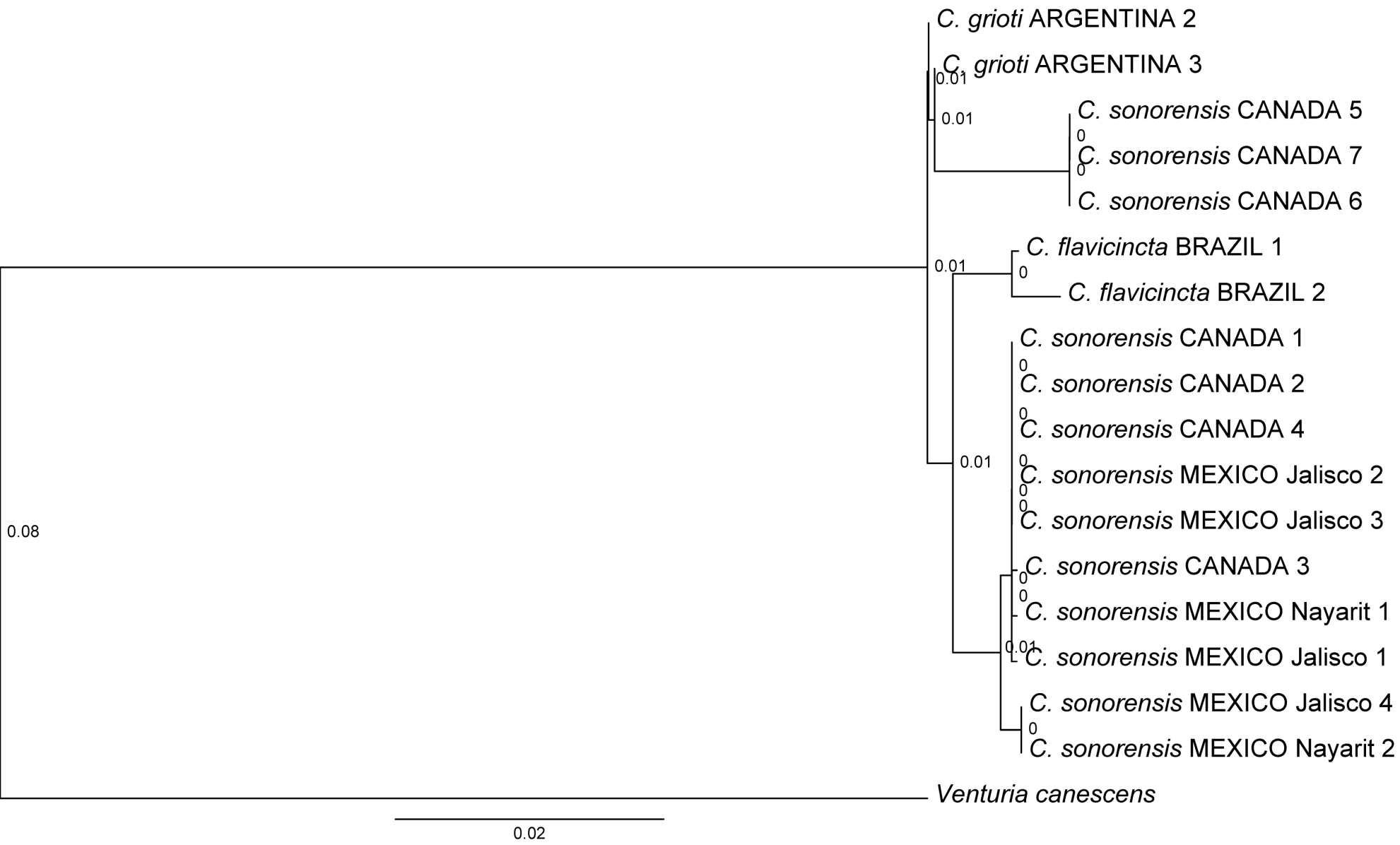
Phylogenetic trees for *Campoletis* sp. resulting from maximum parsimony analyses.



Phylogenetic trees for *Campoletis* sp. resulting from maximum likelihood analyses with RAxML.



Phylogenetic trees for *Campoletis* sp. resulting from maximum likelihood analyses with TreeFinder.



Curriculum vitae

Violaine Jourdie

Born February 27, 1979

Flat 9, The Mayfair
59 Palatine Road
Manchester
M20 3SL
United Kingdom

violaine.jourdie@unine.ch

Nationality: French
Status: Married

EDUCATION

2008 **PhD in Biology.** Laboratory of Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Switzerland.

2002 **Master of Science in Biology.** Laboratory for Sensory Ecology. Bowling Green State University, Bowling Green, OH, USA.

1999 **Bachelor of Science in Biology.** Bowling Green State University, Bowling Green, OH, USA.

1996 **Scientific "Baccalauréat"**, major in Physics and Chemistry. Lycée Saint Louis, Pont l'Abbé d'Arnoult, France.

RESEARCH EXPERIENCE

October 2004 – September 2008

Laboratory of Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Switzerland.
PhD under the supervision of Prof. Ted Turlings.

"Parasitoid communities and genetic structure: host plant does not matter"

June - September 2004

Laboratory of Evolutionary Entomology, University of Neuchâtel, Switzerland.
Scientific collaborator supervised by Prof. Ted Turlings and Dr. Jurriaan Ton.

Explored the molecular basis for the induced defence response of maize plants upon herbivore attack and a possible priming of the genes involved.

November 2003 - May 2004

Laboratory of Sensory Physiology, University of Neuchâtel, Switzerland.
Supervisor: Dr. Patrick Guerin.

Identification of a blend of key compounds used by mosquitoes in orientation in the aim to develop an odour-baited trap to control yellow fever and malaria vectors.

April – September 2003

Department of Ecology and Evolution, University of Lausanne, Switzerland.
Internship under the supervision of Prof. Philipp Heeb.

Research on the role of nestlings UV reflectance in parental food provisioning in birds.
Research on communities of feather degrading bacteria in starlings.

August 2000 – August 2002

Laboratory for Sensory Ecology, Bowling Green State University, OH, USA.
MSc under the supervision of Dr. Paul A. Moore.

"Effects of habitat perturbation on the social structure of a population of captive crayfish"

January – July 2000

Laboratory for Animal Species Conservation, Museum of Natural History, France.
Internship under the supervision of Dr. Claude-Anne Gauthier.

Behavioural study on lemurs: "Ontogeny of the Crowned Sifaka, (*Propithecus verreauxi coronatus*)"

September – December 1998

Laboratory for Molecular Neurobiology, Bowling Green State University, OH, USA.
Supervisor: Dr. Lakshmidevi Pulakat.

Assisted a MSc student in her research on the communication pathways between different subtypes of angiotensin II receptors.

TEACHING EXPERIENCE

October 2005 – February 2007

Protists and Invertebrates practical classes (2nd year students), University of Neuchâtel. Person in charge: Prof. Bruno Betschart.

April 2005 and 2008

Statistics practical classes (2nd and 3rd year students), University of Neuchâtel. Person in charge: Dr. Betty Benrey.

October 2004 – February 2005

Evolutionary Ecology practical classes (3rd year students), University of Neuchâtel. Person in charge: Prof. Ted Turlings.

October 2003 – February 2004

Sensory Physiology practical classes (3rd year students), University of Neuchâtel. Person in charge: Dr. Patrick Guerin.

August 2000 – August 2002

General Biology practical classes (1st and 2nd year students), Bowling Green State University. Person in charge: Tamera Wales.

STUDENT ADVISING

Valentina SALA: During my PhD thesis, I supervised this MSc student whose project consisted in developing and optimizing a transorganismal-transgenerational labelling technique for parasitoids, using trace elements. She also explored the toxicological effects of these elements on the development and fitness of parastoids.

ORGANISATION EXPERIENCE

September 2004

Member of the organization committee for the workshop **"Host recognition by parasites and parasitoids"** held in Neuchâtel (Switzerland).
Contact person for invited speakers.
Responsible for organization details (meals, housing, registration…).

March 2002

Member of the organization committee for the **Midwest Ecology and Evolution Conference** held in Bowling Green (USA).
Responsible for publicity.
This conference recruited graduate students and postdoctoral fellows from all Midwest states.

PUBLICATIONS

Jourdie V., Alvarez N., Turlings T.C.J., and Franck P. (In press) Isolation and characterization of polymorphic microsatellite loci in two primary parasitoids of *Spodoptera frugiperda*: *Chelonus insularis* and *Campoletis sonorensis* (Hymenoptera). **Molecular Ecology Resources.**

Jourdie V., Alvarez N., and Turlings T.C.J. (2008) Identification of seven species of hymenopteran parasitoids of *Spodoptera frugiperda* using PCR amplification and restriction enzyme digestion. **Agricultural and Forest Entomology** 10(2): 129-136

Fero K., Simon J.L., **Jourdie V.**, and Moore P.A. Consequences of social dominance on crayfish resource use. **Behaviour** 144(1): 61-82.

Ton J., D' Alessandro M., **Jourdie V.**, Jakab G., Karlen D., Held M., Mauch-Mani B., and Turlings T. (2007) Priming by air-borne signals boosts direct and indirect resistance in maize. **The Plant Journal** 49(1): 16-26

Lucas F.S., Moureau B., **Jourdie V.**, and Heeb P. (2005) Brood size modifications affect plumage bacterial assemblages of European starlings. **Molecular Ecology** 14(2): 639-647

Jourdie V., Moureau B., Bennett A.T.D., and Heeb P. (2004) Ultraviolet reflectance by the skin of nestlings. **Nature** 431(7006): 262

PRESENTATIONS AT CONFERENCES

September 2007

Xth European Workshop on Insect Parasitoids, Erice, Sicily, Italy. "Do plant odours affect the population genetic structure of parasitic wasps?" (Poster)

February 2006

Biology07, Zurich, Switzerland. "Identification of seven species of parasitoids of *Spodoptera frugiperda*, using PCR amplification and restriction enzyme digestion." (Oral presentation)

June 2006

XIVth International Entomophagous Insect Workshop, University of Delaware, Newark, DE, USA. "Identification of seven species of parasitoids of *Spodoptera frugiperda*, using PCR amplification and restriction enzyme digestion." (Oral presentation)

March 2005

NCCR Plant Survival International Conference, Leysin, Switzerland. "Herbivory-induced volatiles of maize prime neighbouring plants for defence gene expression." (Poster)

February 2005

Biology 05, Basel, Switzerland. "Herbivory-induced volatiles of maize prime neighbouring plants for defence gene expression." (Poster)

November 2004

IOBC Workshop on Methods in Research on Induced Resistance against Insects and Diseases, Delémont, Switzerland. "Herbivory-induced volatiles of maize prime neighbouring plants for defence gene expression." (Poster)

SKILLS

Molecular biology

DNA extraction from blood and insect tissue
RNA extraction from plant tissue
PCR
Electrophoresis
Sequencing
Genotyping (microsatellites)
Northern blot

Bioinformatics

Primer3
PeakScanner, BioEdit, Chromas, Mega
Population genetics softwares (Genepop, Arlequin, FSTAT, genetix, populations, Bottelneck, Geneland, Structure…)
SPSS, R, Canoco

LANGUAGES

French	Mother tongue
English	Fluent
Spanish	Good command
German, Chinese	Basic knowledge

HOBBIES

Cultural Pottery, jewellery making, music: practiced the oboe for 13 years, choir singing, theatre

Sports Hiking, climbing, volleyball, horse riding, skiing

REFERENCES

Prof. Ted Turlings
University of Neuchâtel
Institute of Biology
Laboratory of Fundamental and Applied
Research in Chemical Ecology
Emile-Argand 11
2009 Neuchâtel, Switzerland
ted.turlings@unine.ch
+41 (0)32 718 3158

Dr. Paul A. Moore
Bowling Green State University
Department of Biological Sciences
Laboratory for Sensory Ecology
Bowling Green, OH 43403, USA
pmoore@bgnet.bgsu.edu
+1 419 372 8556

Dr. Pierre Franck
UR1115
Plantes & Systèmes de Culture Horticoles
INRA
84914 AVIGNON Cedex 9, France
pfranck@avignon.inra.fr
+33 (0)4 32 72 26 73

Dr. Nadir Alvarez
University of Neuchâtel
Institute of Biology
Laboratory of Evolutionary Entomology
Emile-Argand 11
2009 Neuchâtel, Switzerland
nadir.alvarez@unine.ch
+41 (0)32 718 3103

Prof. Philipp Heeb
Université Paul Sabatier III
Laboratoire Evolution et Diversité Biologique
UMR 5174 CNRS/UPS
Bâtiment IVR3
118 Route de Narbonne
31062 Toulouse, CEDEX 9, France
heeb@cict.fr
+33 (0)5 61 55 64 50