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

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Abstract

Plant chemistry can strongly influence interactions between herbivores and their natural enemies, either by providing volatile compounds that serve as foraging cues for parasitoids or predators, or by affecting the quality of herbivores as hosts or prey. Through these effects plants may influence parasitoid population genetic structure. We tested for a possible specialization on specific crop plants in *Chelonus insularis* and *Campoletis sonorensis*, two primary parasitoids of the fall armyworm, *Spodoptera frugiperda*. Throughout Mexico, *S. frugiperda* larvae were collected from their main host plants, maize and sorghum and parasitoids that emerged from the larvae were used for subsequent comparison by molecular analysis. Genetic variation at eight and 11 microsatellites were respectively assayed for *C. insularis* and *C. sonorensis* to examine isolation by distance, host plant and regional effects. Kinship analyses were also performed to assess female migration among host-plants. The analyses showed considerable within population variation and revealed a significant regional effect. No effect of host plant on population structure of either of the two parasitoid species was found. Isolation by distance was observed at the individual level, but not at the population level. Kinship analyses revealed significantly more genetically related—or kin—individuals on the same plant species than on different plant species, suggesting that locally, mothers preferentially stay on the same plant species. Although the standard population genetics parameters showed no effect of plant species on population structure, the kinship analyses revealed that mothers exhibit plant species fidelity, which may speed up divergence if adaptation were to occur.

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

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Introduction

Host plants have been shown to affect both insect herbivores and their parasitoids in many ways. Insect pests are directly targeted by plant toxic secondary compounds (Giamoustaris & Mithen 1995; van Dam *et al.*

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2000; Rausher 2001; van Dam 2009) and they have evolved various strategies to circumvent plant defense mechanisms (Futuyma 1983; Nitao 1989; Berenbaum & Zangerl 1992; Evans *et al.* 2000; Ode *et al.* 2004; Zhu-Salzman & Zeng 2008). 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 & 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 & Duffey 1979, 1981; Barbosa *et al.* 1986; Thorpe & Barbosa 1986; Barbosa *et al.* 1991; Bourchier 1991; Gauld & Gaston 1994; Roth *et al.* 1997; Ode 2006).

Although studies on the effects of specific plant chemicals on parasitoid fitness are limited, it is generally concluded that plant defence chemicals mostly have a negative impact on natural enemy traits such as development time, survivorship and body size (Thurston & Fox 1972; Campbell & Duffey 1979, 1981; Barbosa *et al.* 1986; El-Heneidy *et al.* 1988; Barbosa *et al.* 1991; Thaler 1999, 2002; Dicke 2006). These effects can strongly vary among plant species (Smith 1957; Altahtawy *et al.* 1976; Bhatt & Singh 1989; Senrayan & Annadurai 1991; Werren *et al.* 1992; Fox *et al.* 1996; Kruse & Raffa 1997; Eben *et al.* 2000; Harvey *et al.* 2003; Zvereva & Rank 2003; Lu *et al.* 2004) and even among cultivars of a same plant species (Kauffman & Flanders 1985; Orr & Boethel 1985; Hare & Luck 1991; Reed *et al.* 1991; Rogers & Sullivan 1991; Riggin *et al.* 1992; Stark *et al.* 1992; Dosdall & Ulmer 2004; Kahuthia-Gathu *et al.* 2008).

These differential effects on parasitoid performance and fitness among host plants may lead to behavioural 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). Behavioural adaptation could be translated into mothers ovipositing preferentially in hosts on a certain plant species and focusing their foraging efforts on this species.

Genetic divergence among parasitoid populations specialized on different plants can be demonstrated by performing population genetic studies. In the case of host race formation, one would find that individuals emerging on different plants would be more distantly related than individuals collected in distant areas but on the same plant. Such differences can only arise as long as adaptation does not occur iteratively at a local scale. Indeed, if local adaptation—whereby females locally show fidelity to a given host plant—were to occur repeatedly, establishment of the same genotype on a same host could be prevented. Finally, if local adaptation were to be recent and/or in cases where dispersal abilities of the parasitoids are high, reproductive isolation among regions might be too weak to be shown through classical population genetics; in such cases it may be possible to observe the potential for local adaptation by studying the mothers' foraging and oviposition preferences.

Here, we investigated whether the population genetic structure of two parasitoid species, *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) and *Chelonus insularis* Cresson (Hymenoptera: Braconidae), is interconnected to two major domesticated cereals in Mexico, *Zea mais* (maize) and *Sorghum bicolor* (sorghum). The larval parasitoid *C. sonorensis*, like many others of its kind, relies on volatiles emitted by caterpillar-damaged plants to locate hosts (Elzen *et al.* 1983; McAuslane *et al.* 1991; Tamò *et al.* 2006). *Chelonus insularis* is an egg-larval parasitoid and could use cues coming directly from the eggs and traces of pheromones or other chemicals left by ovipositing females to locate host eggs, but evidence is mounting that eggs, once deposited on plants, may also trigger the emission of volatiles by plants that attract egg parasitoids (reviewed by Fatouros *et al.* 2008). This dependence on plant-provided signals could facilitate plant specialization in both species. The two parasitoids are generalists and can potentially attack a broad range of noctuid Lepidoptera (Lingren *et al.* 1970; Cave 1995), but with the massive cultivation of cereals throughout Mexico, they can be expected to most frequently parasitize the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae), which is a major subtropical pest of maize and sorghum (Kranz *et al.* 1977; Sparks 1979; Knipling 1980; Pashley 1986). *Spodoptera frugiperda* has a broad potential host range of more than 60 plant species, mainly grasses (Luginbill 1928). However, if available, it preferentially attacks maize and sorghum (Luginbill 1928; Ashley *et al.* 1989; Molina-Ochoa *et al.* 2003). These crops are abundantly present throughout Mexico and indeed heavily attacked by the pest, therefore providing an ideal setting for specialization. Such specialization was for instance found for the pathogen sorghum head smut, *Sphacelotheca reiliana* (Al-Sohaily *et al.* 1963), which has distinctly different pathotypes on the two plants (Naidoo & Torres-Montalvo 2002). *Spodoptera frugiperda* itself also shows evidence for host race formation, specializing on either maize or rice races in the USA (Nagoshi & Meagher 2008). Maize is a crop on which *S. frugiperda* and its parasitoids have co-occurred for several thousand years in Mexico (Matsuoka *et al.* 2002), thus representing one of the longest associations between a crop plant and associated insects. Sorghum was introduced during the mid-1800s (Smith & Frederiksen 2000), giving ample time for the insects to adapt and specialize. This makes it an ideal system for the current study,

Table 1 Sampling list of the 33 locations where insects were collected in Mexico, on maize (M) and sorghum (S) in June, August and September 2005 and July 2006

Code	Location	Municipio	GPS Co-ordinates.	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	100	M
P4	CIMMYT tropical field station	Agua Fría	20°27′17.4′′N, 97°38′26.3′′W	100	M
V1	Lindero	Lindero	20°29′31.9′′N, 97°32′17.2′′W	66	M

which aimed to address the question whether parasitoid lineages, by focusing their foraging efforts on a particular plant species, reach the point that they become genetically distinguishable from congeners foraging on a different host plant species.

Materials and methods

Biological material

Larvae of *S. frugiperda* were collected at 33 locations in Central and Southern Mexico (Table 1) in June, August and September 2005 and July 2006. They were reared and stored as described in Jourdie *et al.* (2008). At each location, they were sampled for one or two days on either maize or sorghum and, whenever possible, we sampled locations where these two crops occurred next to one another, whereby field edges were not more than 5 m apart and at most separated by a road. Larvae were collected in the middle of each field in order to avoid edge effects. Therefore, depending on the size of each field, distance between sampling locations was no more than a couple hundred meters. Adult parasitoids were taxonomically assigned to species following Cave (1995), whereas parasitoid larvae that did not complete their life cycle were molecularly identified as described in Jourdie *et al.* (2008). Ten species of parasitoids emerged from the collected larvae, but *C. insularis* and *C. sonorensis* considered for this study were by far the 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).

The genotypes were assessed according to Jourdie *et al.* (2009) 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*, *Cs21-Cs48-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 s; five cycles of 94 °C for 45 s, 60 °C for 45 s, 70 °C for 45s; five cycles of 94 °C for 45 s, 57 °C for 45 s, 70 °C for 45 s; 10 cycles of 94 °C for 45 s, 55 °C for 45 s, 70 °C for 45 s; 20 cycles of 94 °C for 45 s, 52 °C for 45 s, 70 °C for 45 s; 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 Biosystems, Foster City, CA, USA). Genotypes were scored using Peak Scanner™ Software v1.0 (Applied Biosystems). Size values were sorted by lengths and plotted 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 Fstat 2.9.3.2 (Goudet 1995). Observed and expected heterozygosities, gene diversity and departure from Hardy–Weinberg equilibrium (HWE) were calculated for each population separately, using GENEPOP 4.0 (Raymond & 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.

For these 16 population samples, we tested population size variation with BOTTLENECK 1.2.02 (Cornuet & 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 & 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

Overall F_{ST} was calculated for both species considering populations with more than six females, using the software package GENEPOP 4.0 (Raymond & Rousset 1995). Due to the high variability in sampling sizes of populations, we chose to analyse isolation by distance between individuals (Rousset 2000) instead of between populations. Indeed, some sampled populations harboured 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). Isolation by distance between individuals was assessed using GENEPOP 4.0 (Raymond & Rousset 1995). Here, we computed the $\hat{a}$ statistic, which is somewhat analogous to $F_{ST}/(1 - F_{ST})$, as described by Rousset (2000). We further performed Mantel's tests (Mantel 1967) to examine the relationship between genetic and geographic distances. The GPS coordinates that were recorded using the WGS 84 system were further projected and transformed in meters using the ArcGIS 9.0 software (ESRI 2004).

Campoletis sonorensis was only collected from the states of Colima, Jalisco, Guanajuato and Nayarit (in contrast to *C. insularis*, which was found in all states we visited). Therefore, we also performed an analysis comprising only *C. insularis* individuals from these four states in order to look for a potential difference between the two species.

Isolation by distance was also investigated at the population level considering only population samples with more than 15 individuals, using GENEPOP version 3.2 (Raymond & Rousset 1995). Pairwise F_{ST} (Weir & 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) by performing a Mantel's test (Mantel 1967).

Host plant and region effects

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 posed by 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 (L. Excoffier, personal communication). Interactions between plant species and region could not be tested because of limited sampling size.

Kinship analyses

Kinship analyses were performed by computing lod scores (Morton 1995), following methods used by Baudry *et al.* (1998) and later by Cameron *et al.* (2004). Calculations were done using KINSHIP 1.3.1 (Goodnight & Queller 1999; http://www.gsoftnet.us/GSoft.html) on populations with more than 15 individuals (males and females). Population pairs were selected among neighbouring populations collected in two adjacent maize and sorghum fields. For each population pair, we wanted to determine the level of relatedness of individuals coming from both fields. If related individuals were to be found in different fields, this would indicate that the mothers do migrate from one plant species to the other to find a host. The opposite would indicate that mothers would preferentially search for hosts on one single plant species. To establish relationships between individuals taken pairwise, the distribution of lod scores among related individuals and among unrelated individuals was first 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 (Fig. 1). Therefore, we determined a cut-off value above which we consider individuals as related. Any cut-off value generates two kinds of erroneous assignments: 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 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. Based on our results for 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 CRAN 2.6.2 (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.

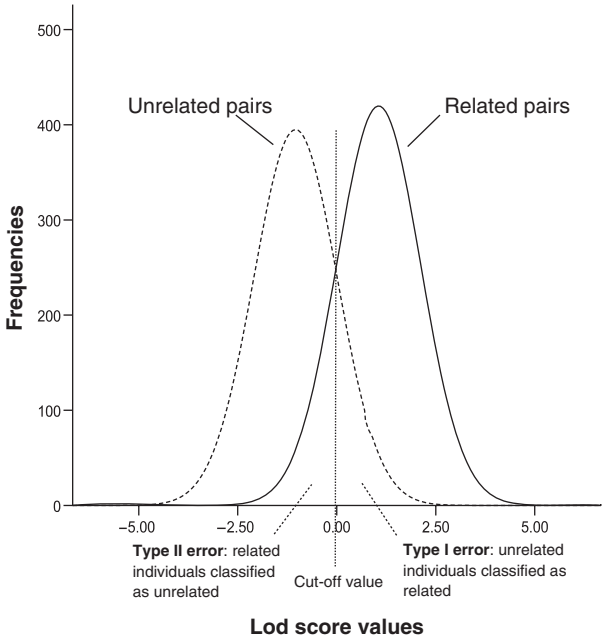

Fig. 1 Distribution of lod score frequencies. Distributions of related and unrelated individuals always overlap. To determine the cut-off value, one needs to choose which error is more acceptable: shifting the cut-off value to the left increases the risk of type I error, while shifting the cut-off value to the right increases the risk of type II error.

Results

Genetic variability

Sample size, gene diversity, number of alleles, allelic richness, observed and expected heterozygosity and results of exact tests for Hardy–Weinberg equilibrium are presented per population in Table 2 for both species. 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 (data not shown). 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

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), number of alleles (N_a), allelic richness (AR), expected (H_E) and observed (H_O) heterozygosity, results of Hardy-Weinberg exact test (HWE), heterozygosity at mutation-drift equilibrium (*H*eq) under the infinite allele model (IAM) and under the stepwise mutation model (SMM) are provided

(A) *Chelonus insularis*	G1	G2	G3	G4	G5	G6	J2	J3	J4	N6	P2
	(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
*H*eq (IAM)	0.561	0.582	0.609	0.614	0.614	0.622	0.594	0.592	0.55	0.592	0.644
*H*eq (SMM)	0.657	0.676	0.691	0.739	0.698	0.688	0.663	0.666	0.612	0.675	0.731

(B) *Campoletis sonorensis*	G4	G5	J3	J4	N6
	(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
*H*eq (IAM)	0.553	0.519	0.571	0.6	0.547
*H*eq (SMM)	0.669	0.632	0.672	0.669	0.607

between locus 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 the populations of both species showed no significant excess of heterozygotes after Bonferroni correction (cut off value for $\alpha = 0.05$: $P = 0.0045$) under both mutation models, thus showing no evidence for recent bottlenecks in population size.

Isolation by distance

The overall F_{ST} were 0.0304 and 0.0120 respectively for *C. insularis* and for *C. sonorensis*. A Mantel test showed that geographic distances and genetic distances between individuals were significantly positively correlated in both species [*C. insularis*: slope = 0.005, $P = 0.002$ (Fig. 2A); *C. sonorensis*: slope = 0.002, $P = 0.01$ (Fig. 2B)] although slope values were very low. 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, $P = 0.033$) (Fig. 2C). However, this could have been an artefact of small population sizes, since analyses ran 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, $P = 0.11$).

Host plant and region effect

Both species demonstrated an identical pattern 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 on the structure of the genetic variation. 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 specimen came from a different plant species than those including specimans from the same species (*C. insularis*: $P < 0.001$; *C. sonoren-*

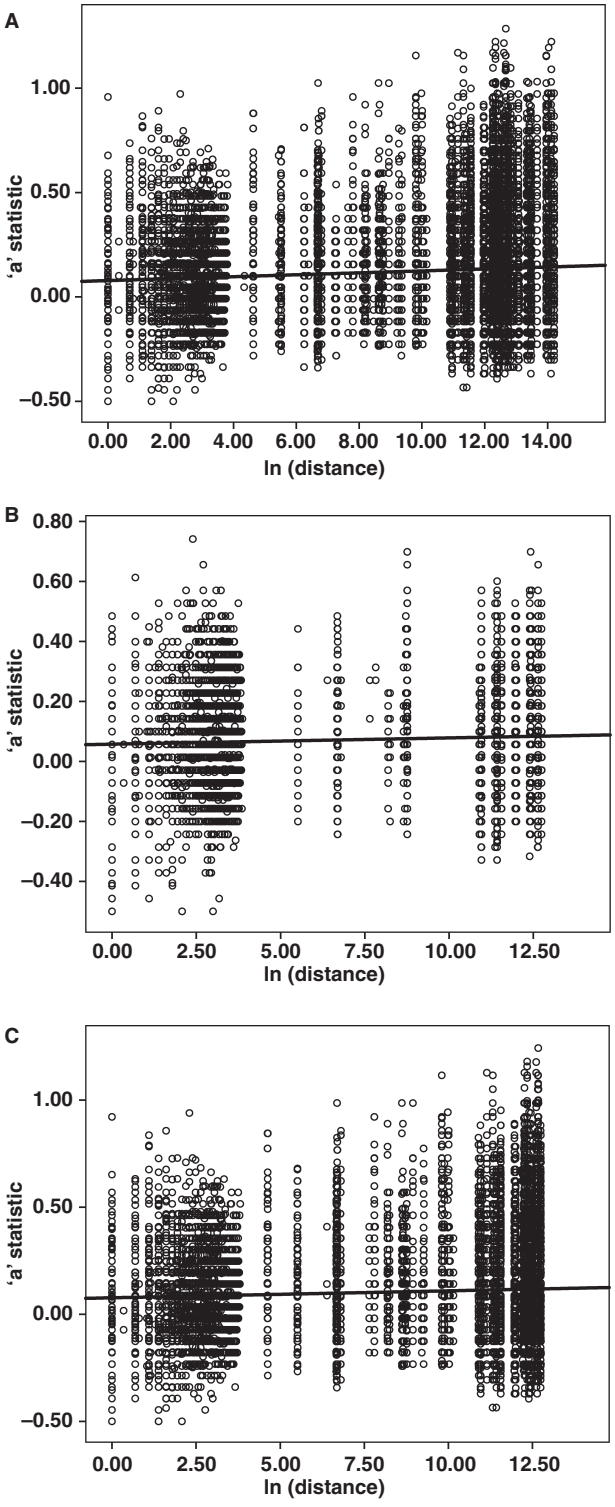

Fig. 2 Isolation by distance between individuals of (A) *Chelonus insularis* coming from all sampled locations (slope = 0.005, $P = 0.002$); (B) *Campoletis sonorensis* (states of Colima, Jalisco, Nayarit and Guanajuato; slope = 0.002, $P = 0.01$); and (C) *Chelonus insularis* restricted to the area overlapping with *C. sonorensis* (states of Colima, Jalisco, Nayarit and Guanajuato; slope = 0.003, $P = 0.033$).

sis: $P = 0.01$). 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*, seven and eight families were observed respectively only on sorghum and only on maize, while only four families spread over both plants were observed. In *C. sonorensis*, no family was observed on sorghum, four families were observed on maize and five families were spread over both plants.

Discussion

For both *C. sonorensis* and *C. insularis* we found no genetic differentiation between individuals collected on maize and individuals collected on sorghum. The overall F_{ST} indicates very low levels of genetic differentiation between all populations in both parasitoid species. The genetic diversity (estimated with parameters such as allelic richness, gene diversity and heterozygosity) did not correlate with the plant species on which the parasitoids were collected. The AMOVA analyses detected no significant differentiation by host plant despite the fact that genetic variation within populations was very high. Despite a considerable sampling effort, the numbers of insects obtained per population for the analyses was limited. Yet, the numbers should be adequate to detect differences between the plant species if these differences are sufficiently strong to be ecologically relevant. Although significant, isolation by distance was characterized by a very small value of its slope, probably because of substantial gene flow over long distances and because of large population sizes.

Genetic differentiation with increasing geographic distance arises from the joint effect of gene flow, genetic drift and adaptation to local conditions (Wright 1943; Peterson & Denno 1998; Hutchison & Templeton 1999). The fact that isolation by distance was detectable only when considering a large spatial scale may indicate that gene flow is more important than drift in these two parasitoid species and that the chance of local adaptation will be limited. Interestingly, absence of isolation by distance in cultivated beans and associated bruchid beetles has previously been explained by frequent long-distance transportation by humans of beans for consumption (Gepts 1998; Papa & Gepts 2003; Alvarez *et al.* 2007). However, this explanation can be ruled out in our system, as the 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 they may be dispersed through human trade. The pattern observed in the present study is more likely explained by a large number of migrants, which could also

Table 3 Results of the AMOVA testing the geographic structure of populations in *Chelonus insularis* (A) and *Campoletis sonorensis* (B). For each level are represented the number of freedom degrees (d.f.), the sum of squares, the variance components (i.e. the variance explained by a given level) and the proportion of variance (in %) explained by the level in the global model. Values presented in the table are averaged to three decimals.

Parameter	Source of variation	d.f.	Sum of squares	Variance components	Percentage variation	*P* value
(A) *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	Among groups*	4	39.581	0.032	0.747	0.000
	Among populations within groups*	26	112.024	0.060	2.536	0.000
	Within populations	1235	2364.289	2.507	96.717	0.000
(B) *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	12.996	0.038	0.986	0.000
	Among populations within groups†	12	54.501	0.021	0.553	0.000
	Within populations	535	2030.763	3.796	98.462	0.000

*Groups: Colima, Nayarit, Guanajuato, Chiapas and Puebla-Veracruz.
†Groups: Colima, Nayarit and Guanajuato.

have contributed to the observed high level of within-population genetic variation. Although migration of these minute insects has not been investigated empirically, it seems reasonable to propose that they can be carried by winds over rather large distances.

Kinship analyses revealed that the two species differed in the frequency with which females would switch foraging between maize and sorghum. More pairs of related individuals of *C. insularis* occurred on the same plant species than on the two different plant species. This indicates that locally the mothers tend to stay in the same field (i.e. on the same plant species) to lay their eggs. *Campoletis sonorensis* showed a contrasting pattern, in which migration between fields does frequently occur (i.e. the numbers of families found on one plant species and those found on both plant species were similar). To our knowledge, this is the first time that a kinship analysis was applied to parasitoids. The method is usually employed to study social organisms (e.g. Rossiter *et al.* 2002; Darvill *et al.* 2004; Tóth *et al.* 2008), but here is shown that the technique can be readily used to study host selection and foraging in insects.

In general, comparable patterns of genetic diversity, isolation by distance, population differentiation and kinships were observed for both species despite the fact that they differ in some major life-history traits: *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 numerous hosts in a very limited area. *Campoletis sonorensis* will need to forage considerably more to find an equal number of host because, due to the highly cannibalistic nature of *S. frugiperda*, there will only be one or a few caterpillars per plant soon after their emergence. *Campoletis sonorensis* therefore might visit many more plants relative to *C. insularis*. These likely differences in foraging behaviour—which seem not to have resulted in discrepancies regarding the genetic structure of the two parasitoids—might have driven the differences in the patterns and the numbers of families observed in the two species: in *C. insularis*, we observed more families on either of the two plants than on both plants simultaneously, whereas in *C. sonorensis*, no families occurred on sorghum and we observed almost the same numbers of families on maize as on both plants. This may indirectly reflect different foraging strategies, with *C. sonorensis* searching its hosts on more plants than *C. insularis*. *Spodoptera frugiperda* is often present at extremely high frequencies in cultivated fields and it is not unusual that, in the absence of pesticides, 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 & Feder 2002; Dres & Mallet 2002; Malausa *et al.* 2005). Evidence of formation of a host race was also found in a parasitoid (Aebi 2004), although this could not be confirmed in a subsequent study on the same system with a more extensive sampling effort (Espindola 2006). Systems in which both the herbivore and the parasitoid could specialize may be more suitable for

Table 4 Results of kinship analyses per population pair in (A) *Chelonus insularis* and (B) *Campoletis sonorensis*. 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 is 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				
(A) *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
(B) *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 races to evolve at the third trophic level. In our study, there was no sign that the parasitoids studied are genetically differentiated in function of the host plant. However, given the pattern of oviposition and the limited field-to-field migration observed with the kinship analyses in *C. insularis*, specialization could evolve if plant traits affect the parasitoid's foraging behaviour and performance.

Plant volatiles emitted in response to herbivory play an important role in the foraging behaviour of larval parasitoids (Turlings & Wäckers 2004). Egg parasitoids have been shown to use cues left by ovipositing females, but evidence is mounting that egg deposition also induces volatile emissions in plants and that egg parasitoids use these volatiles as cues to locate hosts (Fatouros *et al.* 2008). If this is the case for *C. insularis* as well, the individual fidelity for a plant species could speed up differentiation and may eventually lead to host race formation in this species.

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This work was part of the PhD thesis of V.J., who was supervised by T.C.J.T. and N.A. T.C.J.T. is a chemical ecologist whose research group studies mechanistic and ecological aspects of herbivore-induced plant volatiles and their attraction to parasitic wasps and entomopathogenic nematodes. N.A. is interested in the evolution of plant-insect interactions at different scales of time and space. J.M.-O.'s research interests lie in biological control of insect pests and plant resistance to insects. T.W. conducts research on the biology and ecology of insect natural enemies with an emphasis on insect pathogenic viruses, as well as on biological control of insect pests. D.B. was working on insect pests in maize while at CIMMYT and has now joined the Gates Foundation. B.B. is interested in the evolution of tri-trophic interactions and the relative importance of proximal factors that may shape it. P.F. is a population geneticist investigating the population dynamics of insect pests.
