

Inconsistent genetic structure among members of a multitrophic system: did bruchid parasitoids (*Horismenus* spp.) escape the effects of bean domestication?

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Abstract

Anthropogenic range expansion and cultural practices have modified the distribution, abundance and genetic diversity of domesticated organisms, thereby altering multitrophic assemblages through space and time. The putative Mesoamerican domestication centre of the common bean, *Phaseolus vulgaris* L., in Mexico allows investigating the effects of plant domestication on the genetic structure of members of a multitrophic system. The aim of this study was to compare the evolutionary history of *Horismenus* parasitoids (Hymenoptera: Eulophidae) to those of their bruchid beetle hosts (Coleoptera: Bruchidae) and their domesticated host plant (*P. vulgaris*), in the context of traditional agriculture in Mexico. We analyzed the population genetic structure of four *Horismenus* species in Mexico using mitochondrial *COI* haplotype data. The two most abundant parasitoid species were *Horismenus depressus* and *Horismenus missouriensis*. *Horismenus missouriensis* were infected by *Wolbachia* endosymbionts and had little to no population differentiation ($F_{ST}=0.06$). We suspect the mitochondrial history of *H. missouriensis* to be blurred by *Wolbachia*, because differentiation among infected vs. non-infected individuals exists ($F_{ST}=0.11$). Populations of *H. depressus* were found to be highly differentiated ($F_{ST}=0.34$), but the genetic structuring could not be explained by tested spatial components. We then compared the genetic structure observed in this parasitoid species to previously published studies on bruchid beetles and their host plants. Despite extensive human-mediated migration and likely population homogenization of its two *Acanthoscelides* bruchid beetle hosts, *H. depressus* populations are structured like its host plant, by a recent dispersal from a diverse ancestral gene pool. Distinct evolutionary dynamics may explain inconsistent patterns among trophic levels. Parasitoids likely migrate from wild bean populations and are poorly adapted to bean storage conditions similar to their bruchid beetle hosts. Integrating several trophic levels to the study of evolutionary history has proven to be fruitful in detecting different ecological responses to human-mediated disturbances and host parasite interactions.

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Introduction

Interactions among community members are important in creating the selective pressures that drive the evolution of natural and human-modified ecosystems (Tschamtké & Hawkins, 2002). Comparative studies using crops and their wild relatives have revealed that trophic interactions among plants, herbivores and their natural enemies can be altered by selection of plant attributes during the domestication process (Macfadyen & Bohan, 2010). Anthropogenic range expansion and cultural practices have modified the distribution, abundance and genetic diversity of domesticated organisms (Doebly et al., 2006), thereby altering multitrophic assemblages through space and time (Macfadyen & Bohan, 2010). Microorganisms associated with members of a biological system represent additional layers of interactions and are strongly linked to the evolutionary outcome of multitrophic assemblages (Colwell et al., 1996).

Strategies for conservation and plant breeding sometimes consider the use of the genetic diversity of wild-weedy-domesticated complexes to improve plant productivity by maintaining the genetic diversity of natural enemies. The study of the genetic structure of pests and their natural enemies can infer recent and ancient events that have shaped the evolution of multitrophic assemblages and the evolutionary consequences of plant domestication on an ecosystem.

The putative Mesoamerican domestication centre of the common bean, *Phaseolus vulgaris* L., is in a region of Mexico between the Transverse Neovolcanic Axis to the south and the southern edges of the Sierra Madre Occidental and Altiplano to the north (Kwak et al., 2009). It provides an ideal system to investigate the effects of plant domestication on the genetic structure of members of a multitrophic system. Human-mediated migration has been identified as an important evolutionary factor influencing the genetic structure of the bean plants and their associated beetle populations (Gonzalez-Rodriguez et al., 2002; Papa & Gepts, 2003; Alvarez et al., 2007; Kwak & Gepts, 2009; Restoux et al., 2010). In the present study, we examined the evolutionary history of a higher trophic level, the parasitoids. We compared these results to those from the plant and herbivorous hosts to determine if population genetic structures are congruent across trophic levels. We predicted that because parasitoids complete their development within the beetle host, the genetic structure of the host and the parasitoid would be similar or equally influenced by human-mediated migration. The intracellular endosymbiont alpha-proteobacteria *Wolbachia* is commonly found in numerous arthropod species (Hilgenboecker et al., 2008). Thus, as a secondary hypothesis, we predicted a bias in the genetic structure of parasitoid populations when infected by *Wolbachia* due to both the hitchhiking effect of maternally inherited mtDNA haplotypes and the reproductive manipulation strategies used by the bacteria to enhance and spread infection in host populations.

The multitrophic system

Beans (Leguminosae: *Phaseolus* spp.) are the most important grain legumes for direct human consumption in the world (Broughton et al., 2003). Common beans, *Phaseolus vulgaris* L., are the primary source of protein in Mexico, with 67% of the crop being produced on small farms (<5 ha) (Broughton et al., 2003). Common beans are predominantly autogamous (self-fertilizing), unlike their close relative, the scarlet runner beans, *Phaseolus coccineus* L., which are allogamous (cross-fertilizing). Present-day domesticated bean populations from the Mesoamerican gene pool are highly differentiated (Zizumbo-Villarreal et al., 2005; Kwak & Gepts, 2009).

Bruchid beetles (Coleoptera: Bruchidae) are among the most important pests of bean seeds in Mexico. Bruchids develop inside the bean from the first instar to the adult stage. Two *Acanthoscelides* species feed on both *P. vulgaris* and *P. coccineus* bean seeds in Mexico. *Acanthoscelides obvelatus* Bridwell is univoltine (Alvarez et al., 2005a) and mostly found on wild beans and at higher altitudes than its sibling species *A. obtectus* Say (Alvarez et al., 2005b). The multivoltine trait of *A. obtectus* and its ability to exploit the year-round fruiting of wild beans is likely to have favored its adaptation to granaries (Alvarez et al., 2005b). Two *Zabrotes* species, *Z. subfasciatus* Boheman and *Z. sylverstris*, also feed on *P. vulgaris* in Mexico (Romero & Johnson, 1999), with *Z. sylverstris* being predominant between altitudes of 1200 m to 2000 m (Aebi et al., 2008).

Members of the genus *Horismenus* Walker (Hymenoptera: Chalcidoidea: Eulophidae: Entedoninae) are the most abundant parasitoids associated with wild and domesticated beans in Mexico (Hansson et al., 2004). This genus includes parasitoids and hyperparasitoids known to attack larvae of Lepidoptera, Coleoptera and Hymenoptera. Four *Horismenus* species (*H. butcheri* Hansson; Aebi & Benrey, *H. depressus* Gahan; *H. missouriensis* Ashmead; and *H. productus* Ashmead) have been identified from beans infested by *A. obvelatus*, *A. obtectus*, *Z. subfasciatus* and *Z. sylverstris* in Mexico (Bonet, 2009). A fourth rare species is currently being described using both morphological and genetic characters (Hansson, Kenyon and Benrey, unpublished data).

Wolbachia endosymbionts are known to express different lifestyles, from mutualism to parasitism (Werren et al., 2008). They can affect the reproductive success of their host through male-killing (Hurst et al., 1999), male feminization (Rigaud et al., 1991), cytoplasmic incompatibility (Yen & Barr, 1973) and induction of thelytokous parthenogenesis (Stouthamer & Luck, 1993). Because endosymbiotic bacteria are transmitted maternally (much like mitochondria), the hitchhiking effect associated with *Wolbachia* tends to alter the frequency distribution of mtDNA haplotypes in infected host populations (e.g. Charlat et al., 2009; Yu et al., 2011).

Methods

Sampling

With the goal of identifying historical and contemporary factors structuring the genetic diversity of *Horismenus* spp. at the population scale, three regions were targeted for sampling (A, B and C; *fig. 1*). Small fields of local bean varieties (*P. vulgaris* and *P. coccineus*) were sampled to maximize the probability of finding beans infested with beetles since the use of pesticides is common in larger commercial fields.

Sixty sites were sampled during the dry season from January to March 2008, ranging from latitude 18°33'38.4" to 20°03'47.8"N and from longitude 97°28'11.3" to 101°49'43.8"W, in the southern altiplano of Mexico characterized by high altitudes (see table S1). Farmers typically harvest their crops from August to February. Beans were either sampled in the field, collected from plants left behind following harvest or purchased directly from farmers or at local markets. GPS coordinates and altitude of each sampling site were either recorded in the field or deduced from information provided by farmers. One to two kilograms of dry beans per site were brought back to the laboratory, placed into 1-l plastic containers with holes for ventilation, and stored at room temperature (18–25°C). Emerged parasitoids and beetles were removed every two to three days and observations continued for 14 days after the last emergence. Parasitoids were individually preserved in vials with 100% ethanol for DNA extraction and beetles were identified to genus. Only samples from sites that produced six or more individuals from a single *Horismenus* species were retained for population level analyses.

Sixteen of the 60 sample sites had enough *Horismenus* specimens for analysis (table S1). Field collections from previous and ongoing studies (Benrey & Kenyon, unpublished data), indicate that beans collected in the southern altiplano of Mexico are infested primarily by *Acanthoscelides* beetles (Alvarez *et al.*, 2005a).

Community structure

Individuals from the *Horismenus* complex, the most abundant parasitoids emerging from beetle-infested beans, were identified using a taxonomic key based on morphological traits (Hansson *et al.*, 2004). Prior to the population genetics analyses, we ensure that DNA sequences of *COI* clustered in monophyletic groups, validating the morphological identification of *Horismenus* species we previously made. Principal components analysis (PCA) was used to examine differences in *Horismenus* species abundances among sampling sites. Hellinger transformations (Legendre & Gallagher, 2001) were first applied to the matrix of species abundance before PCA analysis using the R language 'Vegan' package (Oksanen *et al.*, 2008).

Genetic analyses

Genomic DNA was extracted from the individual *Horismenus* abdomens using standard proteinase K digestion, phenol-chloroform extraction and precipitation with ethanol (Sambrook & Russell, 2001). Extractions were screened for mitochondrial DNA variation using single strand conformation polymorphism (SSCP) (Orita *et al.*, 1989; Sheffield *et al.*, 1993), of a 278 bp segment of cytochrome c oxidase subunit I

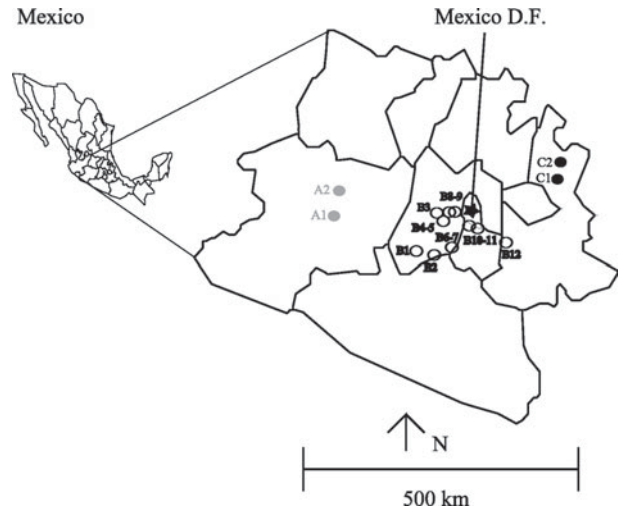


Fig. 1. Sampling sites in the southern altiplano of Mexico. Circles represent sites that were used in this study. Three regions are identified with letters A, B, and C.

(*COI*). SSCP is known to be 99% accurate for differentiating sequence fragments between 100 and 300 bp (Smith & Wayne, 1996). Primers 130F (5' CGGGGGTTAGTTCIATTATAGG 3') and 430R (5' TTCATAGACTCATTACAAATTATATGAG 3'), were designed from sequences obtained from GenBank to amplify the *COI* segment nested in the standard 'COI barcoding' fragment and to avoid the amplification of *Wolbachia* endosymbionts. Amplification reactions were carried out in 12.5 µl volumes, including 1.25 µl of 10× reaction buffers (GenScript USA Inc., Piscataway, NJ, USA), 1.25 µl of 10% trehalose (Fisher Scientific, Ottawa, Canada) solution, 1.5 mM MgCl₂ (Genscript USA Inc.), 0.2 mM of each dNTP (Genscript USA Inc.), 0.4 µM of each primer, 0.5 U of Taq DNA polymerase (Genscript USA Inc.) and 2 µl of DNA template. Conditions used with a Bio-Rad thermocycler were as follows: 92°C for 30 s, 35 cycles of 48°C for 15 s, 68°C for 30 s and one final cycle of 68°C for 1 min. Amplified products were electrophoresed on a nondenaturing polyacrylamide gel (6%) (Angers & Bernatchez, 1998) and visualized using silver nitrate staining (Bassam *et al.*, 1991). Conformers were first scored within a population on a gel and then different conformers from different populations were compared on a new gel.

DNA sequencing

Each different conformer detected on SSCP was reamplified for sequencing in 50 µl volumes, and PCR products were purified using the QIAquick purification kit (Qiagen Inc., Mississauga, Canada). PCR sequencing was performed with the Big Dye terminator cycle sequencing kit version 1.1 (Applied Biosystems, Inc., Foster City, CA, USA). Sequencing was performed with an Applied Biosystem ABI 3730 DNA analyzer. Chromatograms of all sequences were checked with FinchTV 1.4.0 (Geospiza Inc., Seattle, WA, USA). Sequences were then checked with BioEdit (Hall, 1999) and aligned using Clustal X version 1.83 (Thompson *et al.*, 1997). Nucleotide sequence data were deposited in GenBank database under the accession numbers (JX068543-JX068599).

Screening of *Wolbachia* endosymbionts

The presence of *Wolbachia* bacteria in samples was detected using a PCR assay. A maximum of 30 individuals was tested for each population. Specific primers, *wbcF* (5' GCACTTCATGTTGCTGGTATGCATCAATT 3') and *wbcR* (5' CTTGGATGACCAAAAATCAAACAGGTG 3'), were designed to amplify a 280bp fragment of *COI*. We ensured specificity of primers by designing them from conserved regions of aligned *Wolbachia* *COI* sequences taken from GenBank (*Drosophila melanogaster*; *Deceania* sp.; *Culex quinquefasciatus*: (AE017196.1; AY800177.1; AM999887.1)) and the one obtained in this study (GenBank: JX068600) with the 2154-*bee* and 2492-*bee* primers (Simon *et al.*, 1994). This guarantees that the primers are not specific to a *Wolbachia* strain. The same conditions as described above were used for the amplification, with the exception of an adjusted annealing temperature of 51°C. *Wolbachia* occurrence was inferred from the amplification product as revealed by electrophoresis on a 1.5% agarose gel.

Genetic analyses

Phylogenetic relationships and molecular identification

Phylogenetic analyses were used to test morphological parasitoid identification by ensuring that different taxa fall into the appropriate clade. Phylogenetic relationships among *Horismenus* species were inferred using Bayesian (BI) and maximum likelihood (ML) methods as implemented in the Phylobayes 3 (Lartillot *et al.*, 2009) and Phylml (Guindon & Gascuel, 2003) programs, respectively. The best-fit substitution model (GTR + Γ) was determined using MODELTEST 3.7 (Posada & Crandall, 1998). Sequences from *Entedon* sp. (GenBank: DQ149194), *Torymus benefices* (GenBank: AB070504.1) and *Megastigmus transvaalensis* (GenBank: AF420405) were used as outgroups. Each BI analysis was initiated with a random starting tree, included two parallel Markov chain Monte Carlo runs and with 400,000 iterations sampled every 1000 iterations, until convergence (maximum discrepancy across all bipartitions <0.1). A 50% majority rule consensus tree was constructed from the remaining trees after discarding the first 40,000 iterations (burn-in) of sampled trees. ML analysis was conducted with five ML tree searches with 100 bootstrapping replicates. The ML tree with the highest likelihood value was used to plot bootstrap values.

Genetic diversity, structure and differentiation

We first characterized the molecular diversity of parasitoid populations to investigate population genetic structure and differentiation under different geographic models or *Wolbachia* infection status. Measures of population genetic diversity, including the number of haplotypes (*n*), the number of sequenced haplotypes (*s*), haplotype diversity (*hd*) (Nei & Tajima, 1981), nucleotide diversity (π) (Nei, 1987) and the number of substitutions (*ns*) were estimated from mitochondrial DNA polymorphism using the computer program Arlequin 3.0 (Excoffier *et al.*, 2005). A matrix of mutational differences among haplotypes was calculated to produce a minimum length spanning network of haplotypes using the same software. Nodes of the network represent the haplotypes, while branch length is proportional to the number of mutations between them.

Genetic diversity found within versus among populations was estimated by computing global and pairwise F_{ST} (Weir & Cockerham, 1984) using Arlequin 3.0 (Excoffier *et al.*, 2005). This was also quantified by using global and pairwise Φ_{ST} (Excoffier *et al.*, 2005) to take into account the mutational differences among haplotypes. In order to determine if the mutations contributed significantly to genetic differentiation, the difference between global F_{ST} and Φ_{ST} values was tested using a randomization procedure implemented in SPAGeDi 1.1 (Hardy & Vekemans, 2002). Hierarchical analyses of the genetic diversity (Excoffier *et al.*, 1992) were also performed using Arlequin 3.0 (Excoffier *et al.*, 2005). *A priori* criteria for grouping were tested according to the three different geographical regions sampled (A, B and C; fig. 1). Grouping by *Wolbachia* infection status was used to test differentiation by estimating global F_{ST} among infected vs. non-infected individual.

Spatial structure was also investigated by autocorrelation analysis based on the Moran's *II* coefficient calculated using the AIDA software (Bertorelle & Barbujani, 1995). For *H. depressus* and *H. missouriensis*, the Moran's *II* coefficient was calculated for four and three distance classes (table S2), respectively, as implemented in AIDA software (Bertorelle & Barbujani, 1995). The shape of the correlogram (Moran's *II* coefficient plotted against geographic distance) represents the pattern of genetic similarity over space.

Results

Community structure

Horismenus depressus and *H. missouriensis* were the most abundant species parasitizing beetles on seeds of *P. vulgaris*, while *H. butcheri* and *H. sp.* were the predominant species on *P. coccineus* (table 1). *Horismenus* species represent 87% of all emerging parasitoids. The two most abundant *Horismenus* species tended to segregate among sample sites, clustering, respectively, in the first and second quadrant of the PCA projection (fig. S1), except for three sites (B3, B10 and A2) where the two species were collected together.

Genetic diversity

Within species diversity

We were unable to amplify any DNA fragment from 17 individuals. The 600 remaining extractions revealed, by SSCP screening, 80 distinct *COI* haplotypes for over the four *Horismenus* species. Fifty-eight haplotypes were detected in *H. depressus* (*N*=332), nine in *H. missouriensis* (*N*=194), 11 in *H. butcheri* (*N*=59) and two in *Horismenus* sp. (*N*=15). Fifty-seven of these were sequenced: 42/58 for *H. depressus*, 7/9 for *H. missouriensis*, 6/11 for *H. butcheri* and 2/2 for *H. sp.* (table 2). These sequences clustered into four monophyletic clades where *H. depressus*, *H. missouriensis* and *H. butcheri* were well-supported by BI and ML analyses (fig. 2). The *H. sp.* clade was poorly supported. Each cluster was found to correspond to a given morphological species. Intraspecific divergence was high for *H. depressus* (6.5%), *H. butcheri* (6.0%) and *H. sp.* (3.2%), but low for *H. missouriensis* (0.4%).

Population diversity

The SSCP survey identified between two and 12 haplotypes per population of *H. depressus*. For *H. missouriensis*, one

Table 1. Abundances of parasitoids, bruchid beetles (*Acanthoscelides* and *Zabrotes*), and the bean species from which studied insects emerged.

Trophic level	Species	West (A)		Central (B)												East (C)	
		A1	A2	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	C1	C2
3	<i>H. depressus</i>	16	17	27	124	3	–	0	0	36	14	21	31	18	–	34	4
3	<i>H. missouriensis</i>	–	106	2	1	6	9	24	30	5	1	–	24	–	–	–	–
3	<i>H. butcheri</i>	–	–	–	22	–	–	–	–	–	2	–	2	–	–	–	39
3	<i>H. sp</i>	–	–	–	–	–	–	–	–	–	–	–	4	–	11	–	–
?	<i>Others parasitoids</i>	1	–	–	36	–	1	–	–	31	–	5	1	21	–	–	–
2	<i>Acanthoscelides</i>	856	989	148	2331	209	119	459	553	2410	203	1295	587	186	32	815	2850
2	<i>Zabrotes</i>	26	–	37	3	1	–	–	10	374	–	–	–	–	–	–	–
1	Beans species	v	v	v	v	v	v	v	v	v	v	v	v	v	c	v	v & c

v: *Phaseolus vulgaris*; c: *Phaseolus coccineus*.

Table 2. Genetic diversity of mtDNA fragments. Sample sites are indicated in the top row and the number of haplotypes (*n*), number of sequenced haplotypes (*s*), haplotype diversity (*hd*), nucleotide diversity (π), and number of substitutions (*ns*) are indicated for each species.

	A1	A2	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	C1	C2	Total	Means	SD
<i>H. depressus</i>																			
<i>n</i>	2	3	9	12					9	5	3	4	3		8		58	5.8	3.4
<i>s</i>	2	1	7	9					6	3	3	4	3		4		42	4.2	2.4
<i>hd</i>	0.40	0.49	0.88	0.83					0.78	0.54	0.49	0.31	0.41		0.62		–	0.6	0.2
π	0.92	0.00	5.07	6.55					5.05	2.07	1.61	3.34	0.66		4.55		–	3.0	2.2
<i>ns</i>	2	0	19	22					16	9	6	20	3		20		42	11.6	8.4
<i>H. missouriensis</i>																			
<i>n</i>		3			2	1	1	3				4					9	2.3	1.2
<i>s</i>		1			1	1	1	1				2					7	1.2	0.4
<i>hd</i>		0.11			0.33	0.0	0.0	0.20				0.61					–	0.2	0.2
π		0.00			0.00	0.0	0.0	0.00				0.13					–	0.0	0.1
<i>ns</i>		0			0	0	0	0				1					1	0.2	0.4
<i>H. butcheri</i>																			
<i>n</i>			1						2			1			7		11	2.8	2.9
<i>s</i>			0						2			1			3		6	1.5	1.3
<i>hd</i>			0.00						1.00			0.00			0.64		–	0.4	0.5
π			0.00						1.00			0.00			0.28		–	0.3	0.5
<i>ns</i>			–						2			0			8		23	2.5	3.8

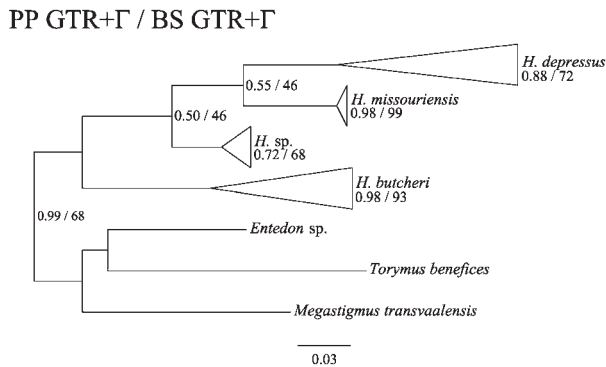


Fig. 2. Monophyletic group differentiation by Bayesian and maximum likelihood analysis of the observed COI sequences of the SSCP survey. Bayesian posterior probabilities (PP) and ML bootstrap support values (BS) are shown in this order for each clade on the tree obtained by BI analysis.

to four haplotypes were found, with two populations (B4 and B5) having a unique fixed haplotype. For *H. butcheri*, one to seven haplotypes were identified, with one population (B2) having a fixed unique haplotype. The two *H. sp.* populations (B10 and B12) each had distinct fixed haplotypes.

Haplotype networks revealed contrasting patterns between *H. depressus* and *H. missouriensis* (fig. 3A–B). *Horismenus depressus* displayed a large number of private haplotypes (detected in a single population), with only haplotype h03 detected in more than one population. In addition, the nucleotide diversity was generally very high within most populations ($\pi = 3.0$; table 2) due to the coexistence of highly divergent haplotypes. For instance, haplotypes h05 and h06 detected in the C1 population differed by ten mutations (fig. 3A). In contrast, one unique haplotype was detected in five of six *H. missouriensis* populations, and the additional haplotype differed by only a single mutation (fig. 3B). As a result, nucleotide diversity was very low for populations of *H. missouriensis*.

Genetic structure

The estimated values of F_{ST} and Φ_{ST} for *H. depressus* were 0.3872 and 0.4376, respectively ($P = 0.0349$; $N_{ST} = 0.6069$). Both estimators were calculated using only the 42 haplotypes for which sequencing was performed, corresponding to 87% of sampled individuals. However, the difference between F_{ST} and Φ_{ST} , is driven by only two populations (B10 and B11) that are characterized by very low diversity. When these sites are removed from the analyses, F_{ST} (0.3403) and Φ_{ST} (0.3191) become similar and statistically not different ($P = 0.9674$), which assess the absence of molecular information.

Geographical grouping by region A, B, and C (fig. 1), does not result in a significant F_{CT} estimate (table 3) for *H. depressus*. Another way to test the genetic structure is by computing Moran's I autocorrelation coefficient values for different distance classes. For *H. depressus*, all Moran's I coefficients were significant ($P < 0.005$) and ranged from low positive values transitioning to increasingly negative values with added distance (fig. S2). Except for the first distance class (within populations) where low autocorrelation was detected, the set of coefficients shown in the correlogram can be

attributed to the random spatial distribution of haplotypes since they are close to zero. For *H. missouriensis*, no significant Moran's I values were detected. This also indicates the absence of spatial patterns of DNA diversity (Bertorelle & Barbujani, 1995).

The estimations of F_{ST} (0.9317) and Φ_{ST} (0.9317) for *H. missouriensis* were higher than for *H. depressus*. However, most of the inter-population diversity is driven by a single population (B4), which was not infected by *Wolbachia* and was fixed for a distinct haplotype. Removing this population decreased F_{ST} to 0.0604 and Φ_{ST} to 0.0604, reflecting an insignificant level of population differentiation.

Wolbachia

Screening for the presence of *Wolbachia* revealed that most of the *H. missouriensis* populations were infected (5/6; table 1). The bacteria were not detected in the other *Horismenus* species. Up to 83% of tested individuals within a population were infected by *Wolbachia* (table 4). Infection status was restricted to only one mtDNA haplotype (A2:B3:B5:B6:B10.h59; fig. 3B). Grouping individuals from *H. missouriensis* populations by *Wolbachia* infection status leads to a significant differentiation ($F_{ST} = 0.10914$; table 3).

Discussion

The aim of this study was to compare the evolutionary history of parasitoids to those of their bruchid beetle hosts and their domesticated host plant in Mexico. Consistent structure among members of different trophic levels is expected in the presence of strong ecological interactions. Consequently, we predicted that the genetic structure of host and parasitoid populations would be congruent because they have been similarly influenced by human-mediated migration since the beginning of the domestication process. On the other hand, parasitoid structure could be biased by the presence of *Wolbachia* endosymbionts.

We found four *Horismenus* species that emerged from *Acanthoscelides* spp. that had infested domesticated beans, while a previous study found only one species, *H. missouriensis* (Aebi *et al.*, 2008). The two most abundant species, *H. depressus* and *H. missouriensis*, tended to segregate among sampled sites and emerged predominantly from infested *P. vulgaris* seeds. Although uncommon, *H. butcheri* and *H. sp.* were the most abundant species on *P. coccineus* seeds. However, populations of these two species were too few to be meaningfully analyzed.

A blurred evolutionary history

Populations of *H. depressus* and *H. missouriensis* exhibited different patterns of mitochondrial diversity. *Horismenus missouriensis* showed very low within-species diversity, with one haplotype shared by nearly all populations. This low diversity appears to be linked to the presence of *Wolbachia*, which was detected exclusively in this species. Both *Wolbachia* and mtDNA are transmitted maternally, and the reproductive manipulation by the bacteria (e.g. cytoplasmic incompatibility) tends to alter the frequency distribution of mtDNA haplotypes within host populations (Charlat *et al.*, 2009; Yu *et al.*, 2011). This also may explain the marked difference between infected and uninfected individuals of *H. missouriensis* (table 4), and this would be the first report that we are aware of suggesting that *Wolbachia* infection lowers mtDNA

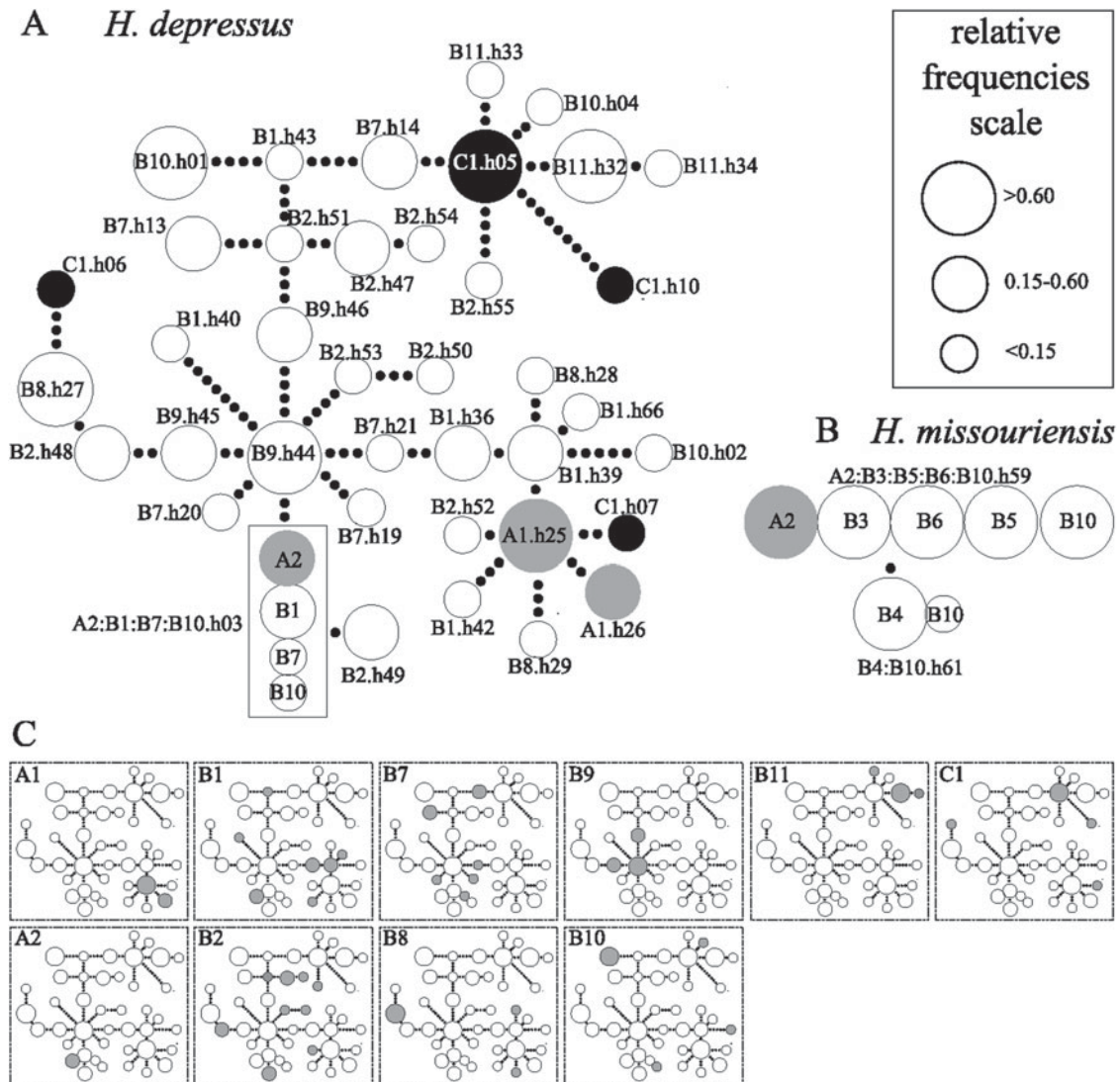


Fig. 3. Minimum spanning network of mtDNA haplotypes for the (A) ten populations of *H. depressus* (291 individuals) and (B) six populations of *H. missouriensis* (175 individuals). Colors correspond to the three sampling regions: grey for A, white for B and black for C (fig. 1). For each circle representing an observed haplotype, there is a sample site and haplotype identifier. The size of each circle is scaled to its relative haplotype frequency according to the SSCP survey. Small black circles indicate unsampled intermediate haplotypes. The haplotype (A2:B1:B7:B10:h03) contained in a box have been detected in four populations. (C) Representation of the distribution of the 42 haplotypes among the ten *H. depressus* populations studied within the minimum spanning network.

diversity of parasitoid populations. The genetic diversity of spider mite (*Tetranychus urticae*) populations was also reported to be lowered by *Wolbachia* infection in China (Yu *et al.*, 2011). Another explanation for the low genetic diversity of *H. missouriensis* and its infection by *Wolbachia* would be that a bottleneck before the dispersal of this haplotype coincided with the presence of *Wolbachia* by chance.

Therefore, it is not possible to determine on the basis of the mitochondrial data, whether *H. missouriensis* has evolved in parallel with other *Horismenus* species, since its historical signal has likely been blurred by the effects of *Wolbachia* infection. Interestingly, the broad geographic range of a single infected haplotype may indicate at least one relatively recent historical dispersal event (table 5). Future studies should

include more data on the genetic diversity of *Wolbachia* that could enhance the understanding of the evolutionary history of its hosts (Lozier *et al.*, 2007).

Inconsistent genetic structure among trophic levels

Populations of *H. depressus*, were found to be strongly differentiated even over short geographic distances. Unlike *H. missouriensis*, high levels of sequence divergence were detected within *H. depressus*. However, relatively low F_{ST} and Φ_{ST} estimates were observed due to the very high intra-population diversity, with a large number of distinct haplotypes and abundant mutations among haplotypes. The polyphyly characterizing lineages within *H. depressus*

Table 3. Hierarchical analyses of the genetic diversity of *H. depressus* and *H. missouriensis* populations. Groups were defined by geographic region (A, B, and C; fig. 1) for *H. depressus* without the B10 and B11 sites (see Results: Population diversity section). Two groups were defined by infection status of *H. missouriensis* by *Wolbachia*.

Dataset	Group	<i>n</i> *	Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	<i>P</i> -value
<i>H. depressus</i> ♂	West	33	Among groups	2	11.787	0.02288	4.38	$F_{CT}=0.04381$	$P=0.12877$
	Central	220	Among populations	5	22.973	0.13669	26.17	$F_{SC}=0.27370$	$P<0.00000$
	East	33	Within populations	278	100.835	0.36271	69.45	$1-F_{ST}=0.69448$	
		286	Total	285	135.594	0.52228		$F_{ST}=0.30552$	$P<0.00000$
<i>H. missouriensis</i>	Infected	33	Among populations	1	1.185	0.02105	10.91		
	Non-infected	89	Within populations	120	20.618	0.17182	89.09		
		122	Total	121	21.803	0.19287		$F_{ST}=0.10914$	$P=0.00079$

♂ Group 1: A1, A2; Group 2: B1, B2, B7, B8, B9; Group 3: C1.

Table 4. Number of *H. missouriensis* individuals infected with *Wolbachia*.

Sample sites	Number of individuals infected/tested (%)
A2	6/30 (20%)
B3	5/6 (83%)
B4	0/9 (0%)
B5	8/24 (33%)
B6	9/30 (30%)
B10	5/24 (21%)

populations (fig. 3C) suggest that the actual population structure is recent (i.e. Karlin & Nevo, 1986). The analyses also suggest that the absence of historical structure in the system results from the random dispersal of haplotypes among regions and populations as illustrated in fig. 3C. The level of genetic differentiation found for *H. depressus* is similar to that reported for one of its bruchid hosts, *Zabrotes subfasciatus* (Shani & Benrey, unpublished data; Gonzalez-Rodriguez *et al.*, 2002; see table 5). However, human-mediated migration cannot be considered as a relevant contemporary factor for *H. depressus* parasitoids, as it has been assumed to be the case for its two *Acanthoscelides* beetle hosts (Alvarez *et al.*, 2007; Restoux *et al.*, 2010; table 5). The two *Acanthoscelides* species, the most abundant bruchids, have low levels of differentiation even over long distances (Alvarez *et al.*, 2007; Restoux *et al.*, 2010). Negligible genetic differentiation among *Acanthoscelides* populations suggests at least one recent historical dispersal event, which was presumed to be a consequence of human seed trade (Alvarez *et al.*, 2007; Restoux *et al.*, 2010). It also suggests that there has been insufficient time since dispersal to allow for differentiation among populations. Interestingly, *Z. subfasciatus* populations were found to be well differentiated and organized by dispersal (Shani & Benrey, unpublished data; Gonzalez-Rodriguez *et al.*, 2002). The spatial genetic structure of *H. depressus* is also similar to that of its host plant, consisting of random historical dispersal from a highly diversified ancestral gene pool (Papa & Gepts, 2003; Zizumbo-Villarreal *et al.*, 2005; Kwak & Gepts, 2009; Rossi *et al.*, 2009).

Present-day domesticated bean populations from the Mesoamerican gene pool are highly differentiated and are now structured by dispersal, while their wild relatives are structured by vicariance (Papa & Gepts, 2003;

Zizumbo-Villarreal *et al.*, 2005; Kwak & Gepts, 2009; Rossi *et al.*, 2009). Human-mediated seed dispersal, in addition to genetic drift, selection by growers and environmental pressures, have affected the genetic diversity of insects since the beginning of domestication (Papa & Gepts, 2003; Zizumbo-Villarreal *et al.*, 2005; Blair *et al.*, 2009; Kwak & Gepts, 2009; Rossi *et al.*, 2009).

Evolutionary dynamics

Inconsistent patterns among trophic levels could be explained by distinct evolutionary dynamics. In spite of an extensive human-mediated migration that homogenized the population structure of beetle hosts, parasitoid populations (*H. depressus*) remain highly differentiated, and therefore parasitoids likely migrate from wild-weedy bean populations adjacent to crop fields.

The relatively high nucleotide and haplotype diversity within *H. depressus* populations indicates the existence of a large and stable population with a long evolutionary history and subsequent contacts among differentiated lineages associated with wild beans. This corresponds with the fourth category, defined by Grant & Bowen (1998), to infer on the likelihood of mtDNA lineages survival under peculiar demographic scenarios. The maintenance of ancestral lineages in the *Horismenus* parasitoid complex suggests past fragmentation and restricted gene flow among parasitoid populations. The complex topography of the southern altiplano of Mexico (i.e. a large range of altitudes), together with the limited effect of the last glaciations (i.e. altitudinal effect: Bryant & Holloway, 1985; Ramamoorthy, 1998), may constitute important factors favoring the conservation of mtDNA lineages in subdivided populations. Furthermore, subdivided populations have the capacity to retain more diversity as drift may operate distinctly within each subpopulation. However, genetic drift within extinction-recolonization dynamics could be responsible for the absence of a large number of intermediate haplotypes with respect to limited sampling effort.

Parasitoids may not have a large enough capacity for dispersal between crop fields and houses where seeds are stored. Furthermore, they may not be able to survive storage conditions since they need other sources of nutrition (e.g. nectar and pollen). On the other hand, the bruchid beetle hosts are well pre-adapted to storage conditions since they consume dry seeds and show polymorphism in dispersal and

Table 5. Comparison within and among trophic levels, information on the third trophic level were obtained in this study and information on the first and second trophic levels came from previously published studies.

Trophic level	Species	Region of study	Number of individuals	Markers	Differentiation (F_{ST})	Inferred patterns	Putative mechanisms	References
3	<i>H.depressus</i>	SAM	286	mtDNA	0.3403	dispersal	HMM	This study
3	<i>H.missouriensis</i>	SAM	194	mtDNA	0.0604	blurred by <i>Wolbachia</i>	Wolbachia RMS	This study
2	<i>A.obvelatus</i>	SAM	135	microsatellites	0.107	dispersal	HMM	(Alvarez <i>et al.</i> , 2007)
2	<i>A.obtectus</i>	SAM	199	microsatellites	0.0409	dispersal	HMM	(Restoux <i>et al.</i> , 2010)
2	<i>Z.subfasciatus</i>	SAM	150	allozymes	0.305	dispersal	HMM	(Gonzalez-Rodriguez <i>et al.</i> , 2002)
1	<i>P.vulgaris</i>	Mexico	134	AFLP	0.2275–0.4150	dispersal	HMM & E&GS	(Papa & Gepts, 2003)

SAM: southern altiplano of Mexico.

HMM: human-mediated migration.

RMS: reproductive manipulation strategies.

E&GS: environmental and grower selection.

reproductive traits (Tuda *et al.*, 2006). Furthermore, *A. obtectus* has been found to exhibit resistance to several types of pesticides (Alvarez *et al.*, 2005b). This species is now cosmopolitan while *Horismenus* parasitoids have a much more restricted distribution.

The surrounding environment could be responsible for the population genetic structure observed in *Horismenus* parasitoids since *H. depressus* populations are, even over short distances, highly differentiated (table 5). Traditional crop fields in Mexico are commonly surrounded by wild-weedy bean populations (Papa & Gepts, 2003; Zizumbo-Villarreal *et al.*, 2005) known to support far higher numbers of *Horismenus* parasitoids compared to domesticated bean populations (Laurin-Lemay, personal observations). In addition, local adaptation to highly differentiated wild-weedy-domesticated bean complexes (Campan *et al.*, 2005; Zizumbo-Villarreal *et al.*, 2005) might also prevent gene flow.

Our data revealed inconsistent genetic structure among members of different trophic levels, as well as within a trophic level. Future research should focus on two main questions aimed to elucidate the microevolutionary processes occurring in this multitrophic system. First, what is the role of parasitoid populations associated with wild-weedy beans in structuring the genetic diversity of parasitoids attacking bruchids in crop fields? Second, what is the origin of migrants detected among all members of the system? Additional molecular data should then be obtained for a higher-resolution assessment of the phylogeographical inferences and the putative role of bean domestication on insect population structure.

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Supplementary material

The online figures and tables can be viewed at <http://journals.cambridge.org/ber>.

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