

## GENETIC STRUCTURE OF POPULATIONS OF *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE) IN MEXICO

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### ABSTRACT

The wide geographic range of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in Mexico and its ability to use various taxonomically unrelated host plant species suggests that this species has considerable evolutionary potential and represents a high risk pest. The genetic diversity and structure of *A. ludens* populations from 7 Mexican states (Chiapas, Yucatán, Morelos, Veracruz, San Luis Potosí, Tamaulipas and Durango) were investigated. Flies were collected as larvae from infested citrus fruits in each state, and sent as pupae to the Genetic Sexing Laboratory at the "Moscafrut" facility in Metapa, Chiapas, where adults emerged and were used in isoenzymatic analysis. Genetic diversity was estimated based on expected and observed heterozygosity, mean number of alleles and polymorphism obtained from allelic and genotypic frequencies of 6 enzyme loci revealed in cellulose acetate. Expected heterozygosity ( $H_e$ ) ranged from 0.199 to 0.330, and percentage of polymorphic loci ( $P$ ) was between 50 and 67%. We found a high level of inbreeding ( $F_{is} = 0.393$ ,  $F_{it} = 0.456$ ) and moderate genetic differentiation among populations ( $F_{st} = 0.105$ ). A negative correlation was found between elevation and  $H_e$ . We conclude that *A. ludens* populations are genetically diverse with moderate levels of differentiation. Genetic structure could not be attributed to the geographic distance among populations. Differentiation could be the result of natural selection associated with the colonization process. Genetic drift and pest management practices may have contributed to this differentiation to a lesser extent.

Key Words: Mexican fruit fly, genetic diversity, gene flow, population genetics

### RESUMEN

La amplia distribución de *Anastrepha ludens* (Loew) (Diptera: Tephritidae) en México y el uso de diferentes plantas hospederas taxonómicamente no relacionadas sugiere que esta es una especie con alto potencial evolutivo y representa una plaga de alto riesgo. Se investigó la diversidad y estructura genética de poblaciones de *Anastrepha ludens* en siete estados de la República Mexicana (Chiapas, Yucatán, Morelos, Veracruz, San Luis Potosí, Tamaulipas y Durango). Las moscas se colectaron como larvas dentro de frutos de cítricos en cada estado, y se enviaron como pupas al Laboratorio de Sexado Genético de la planta "Moscafrut" en Metapa, Chiapas, en donde emergieron los adultos y se analizaron genéticamente. La diversidad genética de las poblaciones se estimó con base en la heterocigosidad observada y esperada, se obtuvo el número promedio de alelos y el polimorfismo, con base en las frecuencias alélicas y genotípicas de seis loci enzimáticos revelados en acetatos de celulosa. La heterocigosidad esperada ( $H_e$ ) fue de 0.199 a 0.330, y el porcentaje de loci polimórficos ( $P$ ) fue entre 50 y 67%. Se encontró un alto coeficiente de endogamia ( $F_{is} = 0.393$ ,  $F_{it} = 0.456$ ) y una diferenciación genética moderada entre poblaciones ( $F_{st} = 0.393$ ,  $F_{it} = 0.456$ ). La correlación entre la altitud y  $H_e$  fue negativa. Concluimos que las poblaciones de *A. ludens* son genéticamente diversas y con nivel de diferenciación moderado. La estructura genética no pudo ser atribuida a la distancia geográfica entre poblaciones. Probablemente, la diferenciación pueda ser resultado de la selección asociada al proceso de colonización. La deriva genética y las prácticas de manejo posiblemente han contribuido a esta diferenciación en menor grado.

Palabras Clave: Mosca mexicana de la fruta, diversidad genética, flujo genético, genética de poblaciones

The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is widely distributed in Mexico, and it has also been recorded in the southern United States, Belize and Central America (Hernández-Ortíz 1992). Twenty two plant species have been reported as *A. ludens* hosts (Norrbom & Kim 1988). The species is native to México and *Sargentia greggii* Wats and *Cassimiroa edulis* Llave & Lex of the Rutaceae family have been proposed as its native hosts (Plummer et al. 1941). Among commercial crops, *Citrus* spp. L. and *Mangifera indica* L. (Sapindales: Anacardiaceae) are the most economically important ones (Norrbom & Kim 1988; Hernández-Ortíz 1992).

The wide range of environmental conditions in which this species is found and its ability to use different taxonomically unrelated plant species as hosts, suggests that this species has considerable evolutionary potential and represents a high risk pest. A species' evolutionary potential is closely related to its population genetic variation (Futuyma 1986; Gould 1991). Greater genetic diversity increases the possibility to respond to natural and human induced environmental changes (Kim 1993).

Genetic diversity and structure of insect populations is determined by natural selection, by the differential movement of individuals between populations, the type of reproduction, random events (demographic and environmental) and the effective population size (Hedrick 2000; Zúñiga et al. 2006; Demirci et al. 2011). The level of genetic diversity and how it is distributed within and among populations depends on the intensity of each factor and how they interact (Slatkin 1994; Hedrick 2000). Population genetic studies with tephritid species have shown a wide range of genetic diversity ( $H_e$ ). In *Ceratitis capitata* (Wied.), heterozygosity values based on isoenzymes have been determined between  $H_e = 0.005$  and  $H_e = 0.186$  (Huettel et al. 1980; Gasperi et al. 1987; Vilardi et al. 1990). This variation has been attributed mainly to the geographic origin of the populations sampled.

Knowledge on genetic variability and its distribution among populations of tephritid species has been useful for pest management strategies; for example, to determine pest origin and to recognize migration routes (Reyes & Ochando 1998; Davies et al. 1999; Gilchrist et al. 2006). In the case of *A. ludens*, there is limited information on the genetic diversity of its populations. Pecina-Quintero et al. (2009) found moderate genetic diversity (index = 0.30) in a population in northeast Mexico using the AFLP technique. Our goals in this study were to describe the genetic diversity of *A. ludens* populations from 7 states in México, and to estimate by means of enzymatic markers if these populations are genetically structured.

## MATERIALS AND METHODS

### Collection Sites

The samples of *A. ludens* were collected as larvae from infested citrus fruits (*Citrus aurantium* L., *C. sinensis* Osbeck, and *C. paradisi* Makfad.) in Chiapas, Durango, Morelos, San Luis Potosí, Tamaulipas, Veracruz and Yucatán from Sep 2008 to Jan 2009. Figure 1 shows collection sites and Table 1 shows the different climate types, mean annual temperatures, main natural vegetation at each site, altitudes and decimal coordinates, and the hosts collected at each location. Collections were made in small orchards of 500 to 10,000 m<sup>2</sup>. Flies collected at one farm or orchard in 3 states were considered as a population. In the case of Veracruz, Chiapas, Morelos and Durango, we grouped the flies collected in different sites in order to have a representative sample of each state and to avoid sampling biases with respect to the other 3 States.

### Sampling

Fruits infested with third instar larvae were collected. These fruits were taken to the local laboratory of the national fruit fly program (SENASICA-SAGARPA) in each State, and where larval development was completed. The larvae ready to pupate were placed in containers with vermiculite. The insects were shipped as pupae from the different locations to the Genetic Sexing Laboratory of the "Moscafrut" facility in Metapa, Chiapas, Mexico. Here, the pupae were maintained at 26 °C and 70% RH until adult emergence. *Anastrepha ludens* is by far the most common tephritid species found in citrus in Mexico. When adults emerged, their taxonomic identity was confirmed following Hernández-Ortíz (1992).

### Genetic Analysis

When adults were 15 days-old, a random sample of 40 individuals (20 males and 20 females) from each location was taken. Adult flies were individually placed in vials, frozen at -70 °C temperature and maintained at these conditions until electrophoresis. Six enzymatic loci were used as biochemical genetic markers, i.e., 6-Phosphogluconate Dehydrogenase (6PGDH, EC1.1.1.44), Glutamate-oxaloacetate transaminase (also known as Aspartate Amino Transferase; GOT, EC 2.6.1.1), Glucose-6-Phosphate Isomerase (GPI, EC 5.3.1.5), Isocitrate Dehydrogenase (IDH, EC 1.1.1.42), Malate Dehydrogenase (MDH, EC 1.1.1.37), and Malic Enzyme (ME, EC 1.1.1.40); using CAMMP to pH 7.0 as electrophoresis buffer (see Herbet & Beaton 1993 for details). Each individual adult was macerated in 250 mL of a solution of CAAMP- pH 7.0 and centrifuged at 13,000 rpm during 3 min. The supernatant was

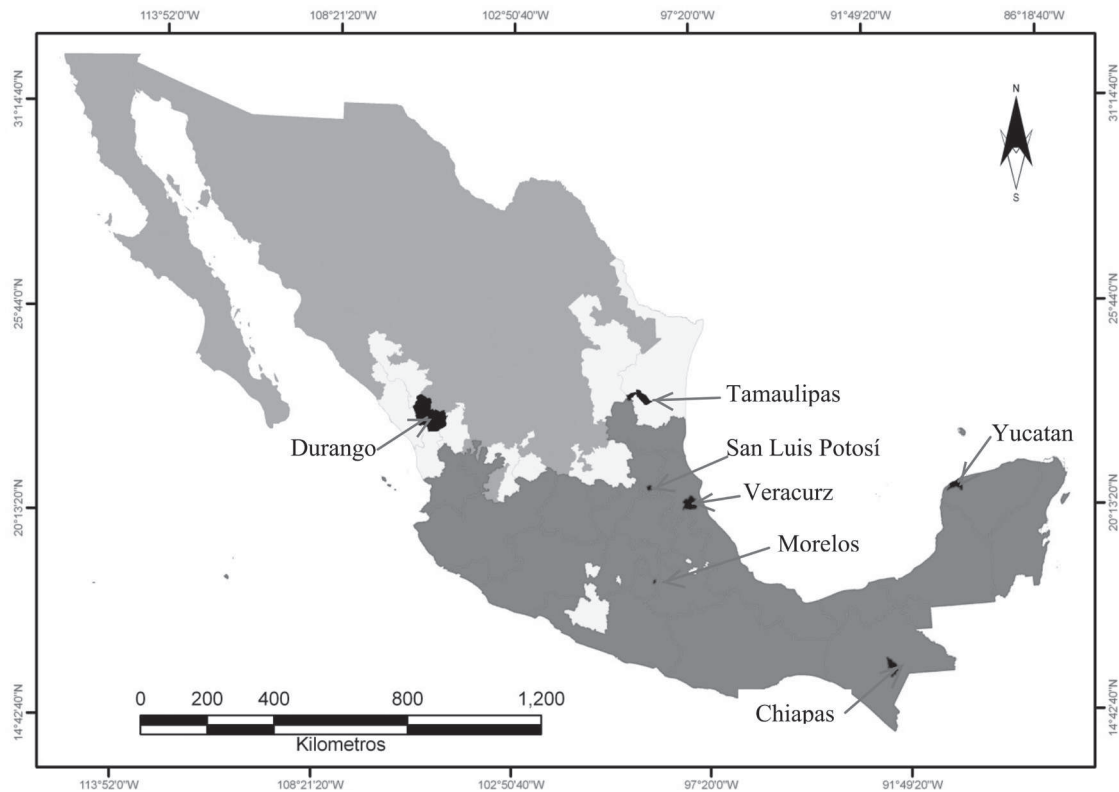


Fig. 1. Geographic location of collection sites of *Anastrepha ludens*. In black are collection sites, the medium gray area toward the north is the fruit fly free zone; the light grey areas are the low prevalence areas, and the dark gray southern zone is under phytosanitary control (SENASICA 2009).

used immediately for enzyme separation by electrophoresis in cellulose acetate.

Electrophoretic separation was carried out at room temperature, at 55 V and 30 mA during 150 min. We visualized the loci using the staining procedure indicated by Herbert & Beaton (1993). The number of individuals for each enzyme-genotype was recorded (Richardson et al. 1986; Herbert & Beaton 1993). We used only reproducible, clearly legible and interpretable electro-morphs. The percent of polymorphisms, and genotypic and allelic frequencies were estimated for each population. Expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity of each population were used as a measure of genetic diversity. We used all loci scored in this analysis. The average of all populations represented the diversity of *A. ludens* in Mexico. Chi-square tests for goodness fit were carried out to test if each of the loci was in Hardy-Weinberg equilibrium. Genetic diversity was compared within and among populations by Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) using GeneALEX software (Peakall & Smouse 2006). The relationship between genetic diversity ( $H_e$ ) with altitude was analyzed by linear regression with arcsine( $x$ )<sup>1/2</sup> transformed data for  $H_e$  and log<sub>10</sub> transformed data for altitude. To have ad-

ditional statistical criteria on the significance of the relationship, the 95% confidence interval was calculated for the regression slope (Quinn & Keough 2002) using the packages mod.lm and boot.ci of software Statistical Data Analysis R version 2.13.2 (R Development Core Team 2012).

Wright's F-statistics (Wright 1951),  $F_{is}$ ,  $F_{it}$  and  $F_{st}$ , were calculated to estimate the level of inbreeding by ancestry in each population and the whole total population, and to estimate the level of differentiation among populations, respectively. F-statistics were calculated by AMOVA, and its framework allows for statistical testing by random permutation (Peakall & Smouse 2006; GenALEX 6.4-Appendix 1-Methods and Statistics).  $F_{is}$  is the inbreeding coefficient within individuals in a given population and can be interpreted as a measure of the reduction in heterozygosity due to nonrandom mating within each population.  $F_{it}$  is the inbreeding coefficient within individuals in relation to the population (and accounts for both nonrandom mating and genetic differentiation among populations), and  $F_{st}$  provides a measure of genetic differentiation among populations (Peakall & Smouse 2006). All genetics parameters and AMOVA were obtained by using GenALEX software (Peakall & Smouse 2006).

TABLE 1. CHARACTERISTICS OF THE COLLECTION SITES OF *ANASTREPHA LUDENS* IN 7 MEXICAN STATES. MAT, MEAN ANNUAL TEMPERATURE; NSV, NATURAL SURROUNDING VEGETATION; M ASL, METERS ABOVE SEA LEVEL; DECIMAL COORDINATES OF MUNICIPALITY: LW, LONGITUDE WEST; LN, LATITUDE NORTH.

State	Municipality	Collection sites	Host	Climate <sup>a</sup>	MAT <sup>a</sup> (°C)	NSV <sup>b</sup>	Altitude (m asl)	Decimal coordinates LW, LN
Yucatán Chiapas	Hunucmá	Hunucmá	<i>C. aurantium</i>	Warm-semidry	25.5	Tropical dry forest	8	-89.899, 21.026
	Comitán	Tzimol, Comitán	<i>C. aurantium</i>	Warm temperate-sub humid	17.6	Pine-Oak forest	1660	-92.134, 16.251
Veracruz	Temapache	Sombrerete, Camelia, Nuevo Jalisco, Morallillo	<i>C. aurantium</i> , <i>C. paradiisi</i>	Warm-sub humid	22.4	Tropical semi-ever-green forest	40	-97.639, 21.065
Morelos	Ocuituco	Ocuituco	<i>C. sinensis</i>	Warm-humid	15.7	Pine-Oak forest	1920	-98.773, 18.872
San Luis Potosí	Axtla de Terrazas	Casas Viejas	<i>C. paradiisi</i>	Warm-humid	22.5	Tropical semi-ever-green forest	100	-98.875, 21.438
Tamaulipas	Villa de Guémez	Guémez	<i>C. sinensis</i>	Warm-semidry	17.6	Tropical dry forest	145	-99.007, 23.919
Durango	Pueblo Nuevo	Pie de Cuesta, La Presa	<i>C. aurantium</i> , <i>C. paradiisi</i>	Warm-sub humid	14.6	Thorn scrub forest	2500	-108.385, 27.462

<sup>a</sup>Source CONABIO (web site consulted in 2010), <sup>b</sup>Source INAFED (web site consulted in 2010)

To analyze whether there was genetic differentiation between pairs of populations and isolation by distance pattern, a linear regression analysis was done between the  $F_{st}/(1-F_{st})$  ratio and the geographic distances (km) calculated for all pairs of subpopulations. Pairwise  $F_{st}$  were calculated via AMOVA in GenAlex software (Peakall & Smouse 2006); the geographic distances were previously transformed to  $\log_{10}$  (Slatkin 1994; Rousset 1997). The regression analysis was done with the software Statistical Data Analysis R and was used to calculate the 95% confidence interval of the regression slope.

We estimated by Nei's genetic distance among populations (Nei 1972), after an UPGMA (unweighted pair-group method with arithmetic mean) analysis, and the reliability of groups was evaluated by bootstrap analysis. UPGMA was carried out by the TFGA genetic analysis program (Miller 1997). We include an analysis of population structures with a Bayesian approach implemented in the Software Structure 2.3.4 (Pritchard et al. 2000). Cluster analysis was based on the assignment of individuals to  $K$  clusters or populations inferred by a probabilistic estimation of the proportion of the genome that belongs to each  $K$  population. To obtain the value of  $K$  Structure we used a Bayesian approach and Markov Chain Monte Carlo (Pritchard 2000). We ran the program with the admixture option with a burn-in period of 10,000 iterations and a subsequent period of 10,000 independent runs of 1-7 populations were performed. The most likely  $K$  was recognized by the Evanno et al. (2005) method, and after the genome mapping of each of the individuals corresponding to the inferred cluster they were plotted to each population.

## RESULTS

### Genetic Diversity

We recorded 6 loci of which 2 (GPI and MDH) were monomorphic in all populations. We recorded 2 alleles each for 6PGDH and GOT, and 3 alleles each for IDH and ME (Table 2). The test to estimate bias in the Hardy Weinberg Equilibrium of genotype frequencies revealed that none of the loci was under equilibrium in Morelos (Table 3). Only one locus was under equilibrium in each of Chiapas (IDH), Veracruz (ME) and Durango (IDH). Two loci were in equilibrium in each of Yucatán (GOT and ME) and San Luis Potosí (6PGDH and ME), and 3 in Tamaulipas (6PGDH, GOT and ME) (Table 3). The average number of alleles per population was 2, only the samples from San Luis Potosí and Durango had less than 2 (Table 4). The percentage of polymorphism, based on monomorphic and polymorphic loci, was the same in 6 of the populations (66.7%), but in Durango it



TABLE 2. ALLELIC FREQUENCY OF SIX ENZYMATIC LOCI AT 7 MEXICAN POPULATIONS OF *ANASTREPHA LUDENS*. N, SAMPLE SIZE; N, ALLELE (FAST = 1, SLOW = 2 OR 3); DGO, DURANGO; CHIS, CHIAPAS; MOR, MORELOS; YUC, YUCATÁN; TAM, TAMAULIPAS; VER, VERACRUZ; AND SLP, SAN LUIS POTOSÍ.

Locus	Allele/n	Dgo	Chis	Mor	Yuc	Tam	Ver	SLP
6PGDH	N	26	20	40	36	20	40	34
	1	1.0	0.950	0.900	0.917	0.875	0.763	0.706
	2	0	0.050	0.100	0.083	0.125	0.238	0.294
GOT	N	40	40	35	39	32	40	36
	1	0.138	0.525	0.357	0.423	0.578	0.638	0.375
	2	0.863	0.475	0.643	0.577	0.422	0.363	0.625
GPI	N	40	39	40	40	32	40	36
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0
MDH	N	40	40	40	35	32	40	36
	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ME	N	40	40	40	40	32	40	36
	1	0.213	0.238	0.100	0.163	0.063	0.063	0
	2	0.625	0.625	0.475	0.475	0.563	0.313	0.403
	3	0.163	0.138	0.425	0.363	0.375	0.625	0.597
IDH	N	40	40	40	40	32	40	36
	1	0.225	0.225	0.713	0.263	0.359	0.550	0.278
	2	0.725	0.688	0.263	0.563	0.484	0.325	0.514
	3	0.050	0.088	0.025	0.175	0.156	0.125	0.208

was 50% (Table 4). The expected heterozygosity ( $H_e$ ) ranged from 0.199 to 0.330. The lowest  $H_e$  value was recorded from the Durango population and the greatest from San Luis Potosí. The observed heterozygosity ( $H_o$ ) was always lower than  $H_e$ .  $H_o$  ranged from 0.111 to 0.302 (Table 4). Linear regression analysis showed a negative correlation between genetic diversity ( $H_e$ ) and altitude, genetic diversity decreased as altitude increased ( $F = 6.35$ ;  $df = 1,5$ ;  $P = 0.055$ ; Fig. 2).

#### Population Genetic Structure

The AMOVA revealed at least 10 % genetic differentiation among populations; 35% among individuals from each population and 54 % among

individuals of total sample (Table 5).  $F_{is}$  and  $F_{it}$  values were 0.393 and 0.456, respectively; and they were positively significant in 9,999 permutations for the polymorphic loci ( $P < 0.0001$ ). Genetic differentiation ( $F_{st}$ ) value was 0.105, indicating a moderate genetic differentiation among populations. Pairwise  $F_{st}$  statistics were significantly different from zero, ranging from 0.02 to 0.253 (Table 6). Linear regression analysis did not detect a significant relationship between genetic differentiation and geographic distance (Fig. 3).

Cluster analysis (UPGMA) showed low genetic distances between groups of populations, ranging from 0.017 to 0.043. This analysis revealed two groups (Fig. 4): one formed by the populations from Yucatán, Tamaulipas and Chiapas (genetic

TABLE 3.  $\chi^2$  (CHI-SQUARE) STATISTICS TO TEST THE HARDY-WEINBERG EQUILIBRIUM FOR ENZYMATIC LOCI IN MEXICAN POPULATIONS OF *ANASTREPHA LUDENS*.

State	Enzymatic loci					
	GPI	MDH	6PGDH	GOT	ME	IDH
Chiapas	Fix	Fix	20.0**	32.38**	9.90*	3.40 <sup>NS</sup>
Morelos	Fix	Fix	40.0 **	16.61*	27.30**	10.47**
Yucatán	Fix	Fix	36.0**	1.75 <sup>NS</sup>	6.46 <sup>NS</sup>	11.40**
Veracruz	Fix	Fix	25.15**	28.07**	0.32 <sup>NS</sup>	12.52**
San Luis Potosí	Fix	Fix	0.89 <sup>NS</sup>	17.82**	0.34 <sup>NS</sup>	15.59**
Tamaulipas	Fix	Fix	1.98 <sup>NS</sup>	0.049 <sup>NS</sup>	0.62 <sup>NS</sup>	15.05**
Durango	Fix	Fix	Fix	32.01**	18.97**	7.71 <sup>NS</sup>

<sup>NS</sup>, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$

TABLE 4. GENETIC DIVERSITY OF *ANASTREPHA LUDENS* POPULATIONS IN MÉXICO. *N*, SAMPLE SIZE; *P*, PERCENTAGE OF POLYMORPHISM; *H<sub>e</sub>*, OBSERVED HETEROZYGOSITY; *H<sub>e</sub><sup>p</sup>*, EXPECTED HETEROZYGOSITY. DGO, DURANGO; CHIS, CHIAPAS; MOR, MORELOS; YUC, YUCATÁN; TAM, TAMAULIPAS; VER, VERACRUZ; AND SLP, SAN LUIS POTOSÍ.

Population	Locus	<i>N</i>	<i>N<sub>a</sub></i>	<i>H<sub>e</sub></i>	<i>H<sub>e</sub><sup>p</sup></i>	<i>P</i>
Dgo	6PGDH	26	1.0	0	0	
	GOT	40	2.0	0.025	0.240	
	GPI	40	1.0	0	0	
	MDH	40	1.0	0	0	
	ME	40	3.0	0.375	0.545	
	IDH	40	3.0	0.275	0.427	
	Average	37.6	1.83	0.113	0.199	50.0
Chis	6PGDH	20	2.0	0	0.097	
	GOT	40	2.0	0.050	0.505	
	GPI	39	1.0	0	0	
	MDH	40	1.0	0	0	
	ME	40	3.0	0.500	0.541	
	IDH	40	3.0	0.475	0.475	
	Average	36.5	2.0	0.171	0.266	66.7
Mor	6PGDH	40	2.0	0	0.182	
	GOT	35	2.0	0.143	0.466	
	GPI	40	1.0	0	0	
	MDH	40	1.0	0	0	
	ME	40	3.0	0.250	0.591	
	IDH	40	3.0	0.275	0.428	
	Average	39.2	2.0	0.111	0.274	66.7
Yuc	6PGDH	36	2.0	0	0.155	
	GOT	39	2.0	0.385	0.495	
	GPI	40	1.0	0	0	
	MDH	35	1.0	0	0	
	ME	40	3.0	0.750	0.624	
	IDH	40	3.0	0.675	0.591	
	Average	38.3	2.0	0.302	0.307	66.

TABLE 4. (CONTINUED) GENETIC DIVERSITY OF *ANASTREPHA LUDENS* POPULATIONS IN MEXICO.  $N$ , SAMPLE SIZE;  $P$ , PERCENTAGE OF POLYMORPHISM;  $H_o$ , OBSERVED HETEROZYGOSITY;  $H_e$ , EXPECTED HETEROZYGOSITY. DGO, DURANGO; CHIS, CHIAPAS; MOR, MORELOS; YUC, YUCATÁN; TAM, TAMAULIPAS; VER, VERACRUZ; AND SLP, SAN LUIS POTOSÍ.

Population	Locus	$N$	$N_a$	$H_o$	$H_e$	$P$
Tam	6PGDH	20	2.0	0.150	0.224	
	GOT	32	2.0	0.469	0.496	
	GPI	32	1.0	0	0	
	MDH	32	1.0	0	0	
	ME	32	3.0	0.531	0.548	
	IDH	32	3.0	0.531	0.622	
	Average	30	2.0	0.280	0.310	66.7
Ver	6PGDH	40	2.0	0.075	0.367	
	GOT	40	2.0	0.075	0.468	
	GPI	40	1.0	0	0	
	MDH	40	1.0	0	0	
	ME	40	3.0	0.500	0.514	
	IDH	40	3.0	0.550	0.584	
	Average	40	2.0	0.200	0.318	66.7
SLP	6PGDH	34	2.0	0.294	0.421	
	GOT	36	2.0	0.139	0.475	
	GPI	36	1.0	0	0	
	MDH	36	1.0	0	0	
	ME	36	2.0	0.528	0.488	
	IDH	36	3.0	0.750	0.624	
	Average	35.6	1.830	0.285	0.330	66.7
Grand Mean over Loci and Populations		36.762	1.952	0.209	0.290	64.3

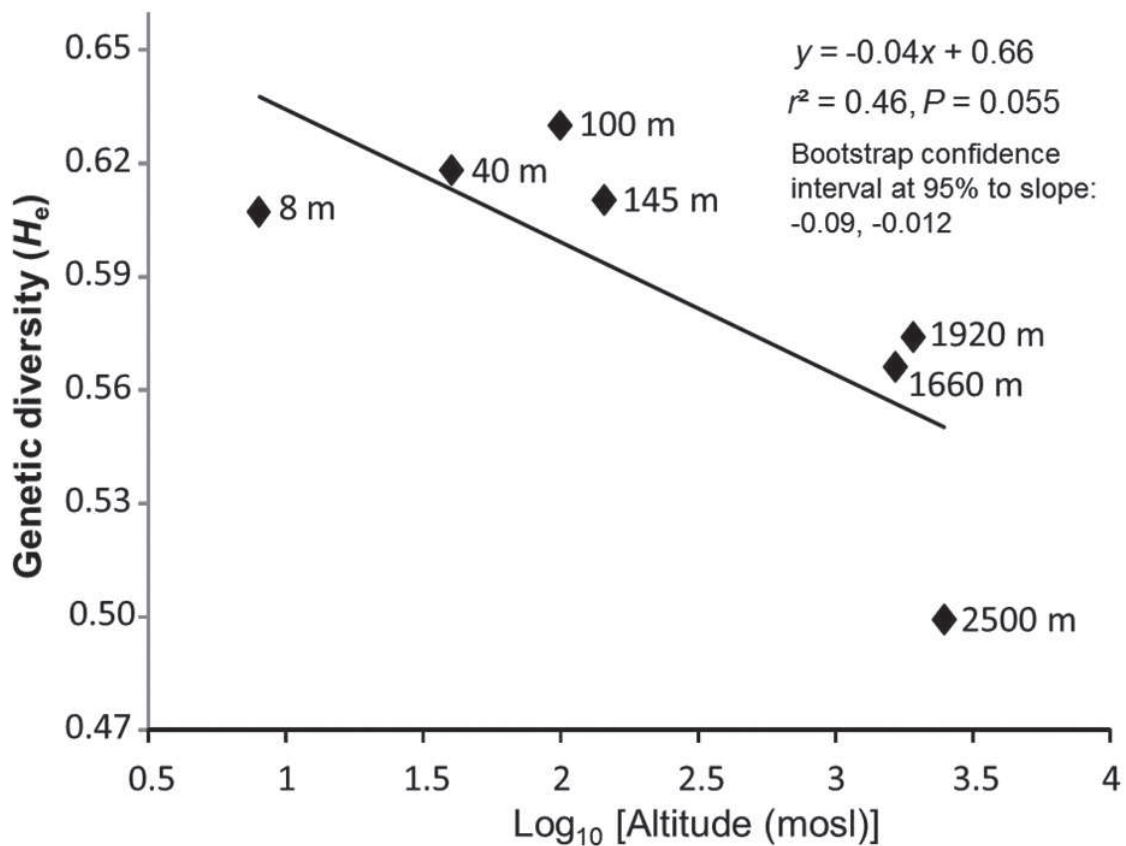


Fig. 2. Regression analysis of expected heterozygosity ( $H_e$ ) at elevation (m) of sites occupied by populations of *Anastrepha ludens* in Mexico. Numbers on the graph are altitude (m asl) without transformation.

distance = 0.017), with the population from Durango attached to this group (genetic distance = 0.042). The other group was formed by the populations from Veracruz, San Luis Potosí and Morelos (genetic distance 0.043). The Bootstrap values were below 45% for all clusters; this indicates a low reliability of node or cluster formation (Fig. 4).

The Bayesian approach indicated the probability of 2 populations (cluster inferred) that could be represented by Tamaulipas and Durango. The other populations showed an admixture genome composition (Fig. 5).

## DISCUSSION

Our study based on 4 polymorphic loci, suggests that populations of *A. ludens* had high levels of genetic diversity ( $H_e \geq 0.200$ ) and moderate genetic differentiation ( $F_{st} = 0.105$ ) (Hartl & Clark 1997). The  $H_e$  recorded values were greater than those reported for *Anastrepha fraterculus* Wiedemann (1830) from Brazil (mean  $H_e = 0.03$ ; Morgante & Malavasi 1985) but lower than those found by Alberti et al. (2002) for that same species in Argentina ( $H_e$ , 0.353 - 0.492). The Mexican populations of *A. ludens* showed a genetic diversity within the range

TABLE 5. RESULTS FROM THE ANALYSIS OF MOLECULAR VARIANCE (AMOVA) ON 268 INDIVIDUALS FROM 7 POPULATIONS OF *ANASTREPHA LUDENS* IN MEXICO. SS, SUM OF SQUARES;  $P$ , TEST THE HYPOTHESIS THAT THE OBSERVED VALUES WERE SMALLER OR EQUAL TO RANDOM VALUES BASED ON 9,999 PERMUTATIONS.

Source of variation	d.f.	SS	Variance component	% Variation explained	Inbreeding coefficient	$P$
Among populations	6	62.26	0.117	10	$F_{st} = 0.105$	0.0001
Among individuals	261	365.21	0.395	35	$F_{st} = 0.393$	0.0001
Within individuals	268	163.50	0.610	54	$F_{st} = 0.456$	0.0001



TABLE 6. PAIRWISE  $F_{st}$  (GENETIC DIFFERENTIATION) STATISTIC FOR MEXICAN POPULATIONS OF *ANASTREPHA LUDENS* BELOW OF DIAGONAL AND NM (GENE FLOW) VALUES OVER THE DIAGONAL.

Populations	Chis	Mor	Ver	Yuc	SLP	Tam	Dgo
Chis		1.002	1.084	2.982	1.653	10.87	3.325
Mor	0.200**		3.942	3.181	2.592	2.019	1.064
Ver	0.187**	0.060**		3.08	4.7	3.144	0.737
Yuc	0.077**	0.073**	0.075**		8.772	6.022	2.936
SLP	0.131**	0.088**	0.051**	0.028**		4.304	1.655
Tam	0.022*	0.110**	0.074**	0.040**	0.055**		1.983
Dgo	0.070**	0.190**	0.253**	0.078**	0.131**	0.112**	

<sup>NS</sup>, no significant; \*,  $P = 0.02$ ; \*\*,  $P = 0.01$ ;  $P$  was calculated as the probability of a random value greater or equal to the observed data value in 1,000 permutations.

reported for *Ceratitis capitata* based on enzymes (from 0.022 to 0.48; Milani et al. 1989). Bonnizzoni et al. (2001) and Meixner et al. (2002) reported lower genetic diversity for *C. capitata* in California, but this is a case of an recently adventive species and this lower diversity can be explained as the bottleneck of the colonization process (Carey 2010).

A possible explanation for the high genetic diversity in *A. ludens* is its origin in Mexico.

Ancestral populations tend to maintain high levels of genetic diversity and low genetic differentiation (Gilchrist et al. 2006, 2012). Also, its demographic characteristics, such as large size populations and high fecundity rates (Leyva et al. 1991; Liedo et al. 1992; Carey et al. 2005), could contribute to overcome the loss of genetic diversity associated with genetic drift and natural selection.

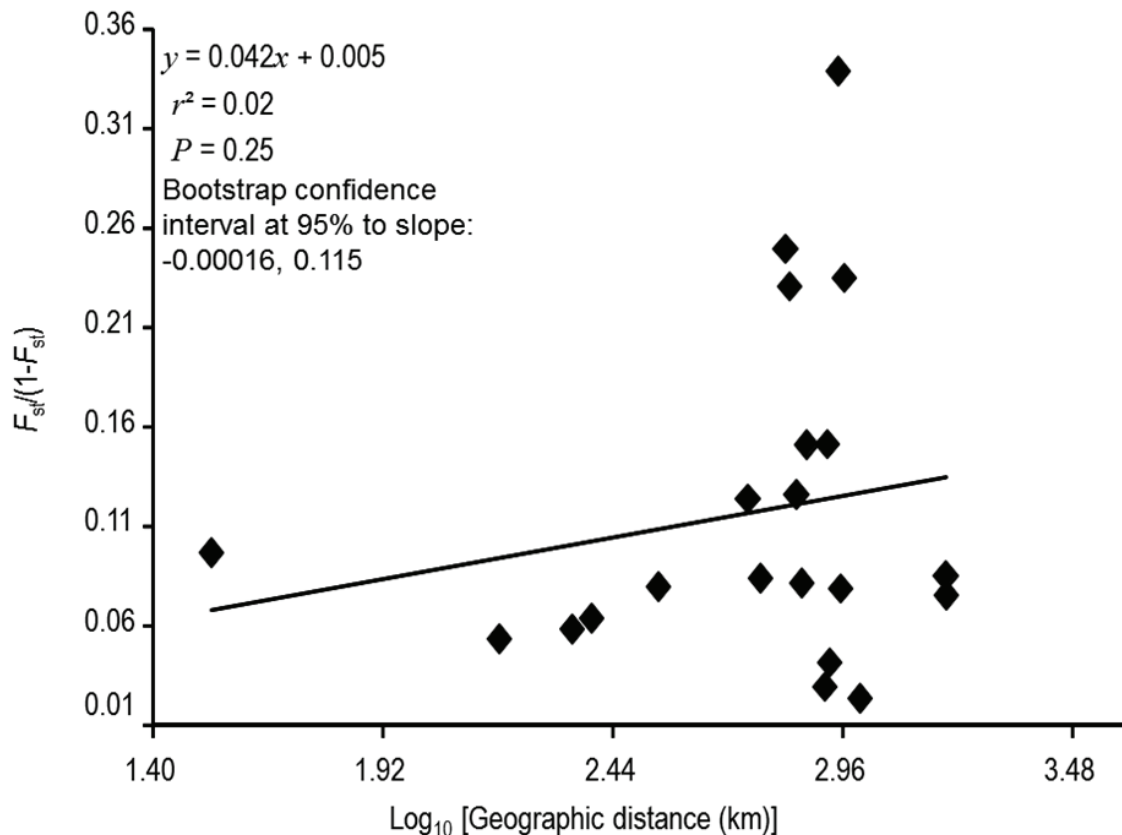


Fig. 3. Regression analysis of genetic differentiation  $F_{st}/(1-F_{st})$  and  $\log_{10}$  (km) of geographic distances separating the various populations.

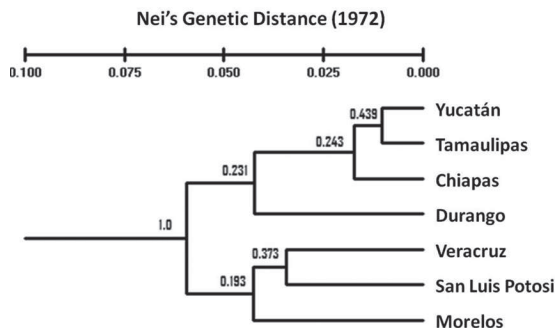


Fig. 4. UPGMA tree based on Nei's genetic distance. The number at base (node) of each cluster is the bootstrap probability based on 1,000 iterations.

Inbreeding coefficients ( $F_{is}$  and  $F_{it}$ ) were positive, indicating a deficiency of heterozygotes, probably caused by nonrandom mating both within populations and among populations. High levels of average inbreeding were also found in *A. ludens* at a smaller geographic scale in Chiapas ( $f = 0.682$ , R.M.L. unpublished data). Assortative mating is the most parsimonious explanation of high inbreeding coefficients; however, some studies have documented an absence of assortative mating among populations of *A. ludens* (Orozco et al. 2007; Aluja et al. 2009). Nonrandom inverse frequency-dependent mating could also explain high inbreeding coefficients. The lek mating behavior of this species, where one dominant male could account for a large fraction of the total number of matings (Burk 1981; Robacker & Hart 1985; Sivinski & Burk 1989), could explain our findings. Another possible cause of inbreeding could be that adults coming from the same fruit are likely to mate, as have been suggested for *A. fraterculus* (Alberti et al. 1999). The possibility of sex-linked loci and a sub-structuring of female and male populations may lead to an expected excess of homozygotes and deficiency of heterozygotes compared with Hardy-Weinberg proportions (Hedrick & Parker 1997). We did not detect significant differences in the allelic frequencies of any loci between sexes, and a possible substructure cannot be revealed with the present data due to the small number of localities sampled in each State. The null alleles also could account for the excess of homozygotes.

We found moderate genetic differentiation ( $F_{st} = 0.105$ ) among populations and from moderate to high genetic differentiation in pairwise comparisons, which were done as suggested by Hartl & Clark (1997, page 118) for qualitative interpretation of  $F_{st}$  values. A similar pattern was observed in populations of *A. ludens* from northeast Mexico (Pecina-Quintero et al. 2009). The effect of gene flow on the population genetic structure depends on the species' movement capacity and its ability to overcome geographic and ecological barriers. Isolation due to distance is one of the most common

and simple mechanisms to decrease gene flow. As distance between population increases, gene flow decreases to produce genetic differentiation (Slatkin 1994; Rousset 1997). Our results are not in agreement with this isolation-by-distance model, but we recognize that the samples from distant locations without intermediately located populations do not allow us to accurately estimate gene flow ( $N_m$ ). However, their structure could be explained by their geographical origin. The populations from Veracruz, Morelos and San Luis Potosí showed little differentiation among them ( $F_{st}$  values ranged from 0.050 to 0.088) and they conform to a group in the UPGMA analysis. The Bayesian approach showed that they have a similar genome content as in the 2 inferred clusters, which is in partial agreement with the proposed ancestral origin of this species from the Morelos region in central Mexico (Plummer et al. 1941). The gene pool of the ancestral Morelos populations would be expected to have much in common with those of the derived populations. This is supported by the fact that the Yucatan, Chiapas and Tamaulipas populations constituted a group slightly differentiated from the ancestral Morelos population and the population from Durango was further differentiated. The Bayesian approach also indicated Durango to be a single population. A genetic study of populations of northeast Mexico based on amplified fragment length polymorphism (AFLP markers) showed genetic differentiation between a population from San Luis Potosí and those from Tamaulipas and Nuevo León (Pecina-Quintero et al. 2009). Thus, we hypothesize a historical dispersion process from the Morelos region to Tamaulipas, where *A. ludens* could enlarge its population using its abundant host *S. greggi* (Plummer et al. 1941; Hernández-Ortiz 1992; Baker et al. 1944), afterwards it moved to other parts of the country facilitated by human activities, such as the establishment of *Citrus* crops, which in some cases covered large areas (García-Dessommes 2009; SIAP 2009).

Our results do not rule out the possibility that populations distributed over a smaller geographic scale might have a genetic structure consistent with the isolation-by-distance model or relating to use of different host species, considering that the typical range of movement of this species is around 240 m (Thomas & Loera-Gallardo 1998; Hernández et al. 2007).

Genetic differentiation by natural selection and genetic drift has been reported for other tephritid species, such as *C. capitata* and *A. fraterculus* (Morgante et al. 1985; Reyes & Ochando 1997, 1998; Alberti et al. 1999, 2002; Gilchrist et al. 2006). The variation in the presence and abundance of host species and the local climatic conditions may constitute a wide range of environments with selectively distinct effects on *A. ludens* populations, and thereby promote genetic differentiation. The negative relationship found between altitude and genetic diversity ( $H_e$ ) could be the result of selec-

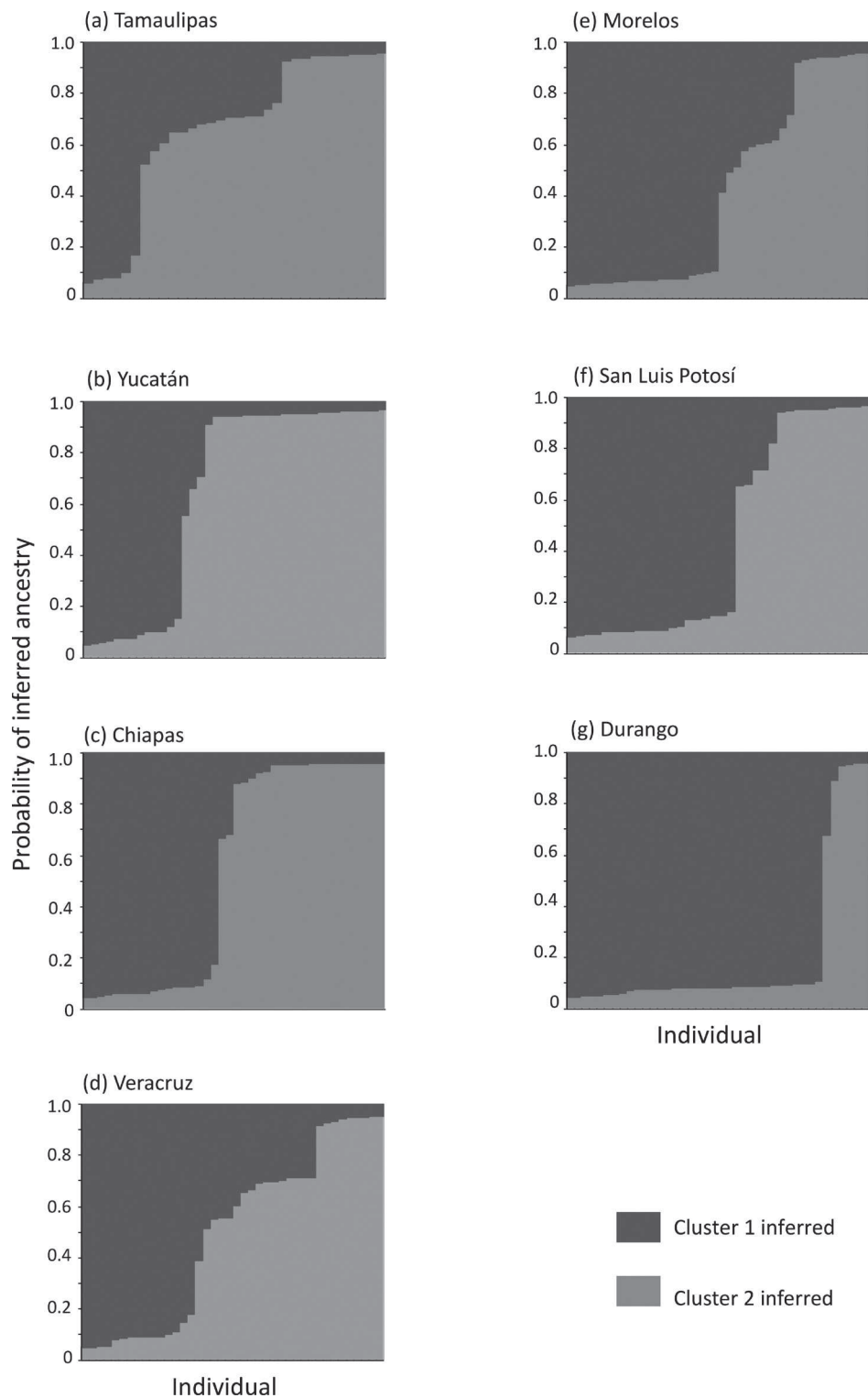


Fig. 5. Admixture analysis of *Anastrepha ludens* computed by Structure software 2.3.4 with  $K = 7$ . Each individual is represented by a vertical line broken into  $K = 2$  segments whose length is proportional to the estimated memberships in the 2 inferred populations or cluster.

tion. The altitude factor itself is not selective, but it is associated with other factors that could exert selection (Wang et al. 2008; Demirici et al. 2011). For example, temperature is known to influence developmental time and reproductive rates of many insect species, including tephritid fruit flies (Leyva 1988; Fletcher 1989; Bale et al. 2002). Developmental time and reproductive rates are important fitness components; their variation will invariably affect the genetic structure of populations. Other selective factors could be associated with the type, availability and nutritional quality of hosts, and the presence of natural enemies (Malavasi & Morgante 1981; Carey 1984; Krainacker et al. 1987; Leyva et al. 1991; Aluja et al. 2003; Díaz-Fleischer & Aluja 2003; Silva et al. 2010). The relationships between genetic diversity, temperature, presence and abundance of host species and natural enemies remain unresolved.

Finally, genetic structure could be factored into pest management strategies. The Mexican Fruit Fly Campaign divides the national territory in 3 pest management regions, according to fruit fly presence and population densities: (1) fruit fly free zones, (2) low prevalence zones, and (3) pest control zones (Fig. 1). Durango has a surface area of 121,134.0 km<sup>2</sup> of which 77.4% is fruit fly free and the rest (22.6%) is part of the low prevalence zone. San Luis Potosí comprises 62,450.0 km<sup>2</sup> of which 52.3% is fruit fly free, and 29.4% is part of the low prevalence zone, and the rest (18.3%) belongs to the areas under phytosanitary control. Tamaulipas comprises 136,063.4 km<sup>2</sup> of which 82.7% is part of the low prevalence zone, and the rest (17.3%) belongs to the areas under phytosanitary control. The other 4 states (Chiapas, Yucatán, Morelos and Veracruz) belong to the areas under phytosanitary control. These categories represent different management strategies and fruit movement is only allowed within areas of the same category (SENASICA 2009). Thus there is a possibility that the gene flow patterns could be mediated by human activities (Oliver 2006).

To obtain a better understanding of the genetic diversity and structure of *A. ludens* populations, it will be necessary to sample the species entire geographical range. Sampling on a smaller geographic scale (hundreds of meters) and the use of nuclear DNA markers such as ITS or SSR (microsatellites), and mitochondrial DNA could contribute to a better understanding of the pathway and intensity of genetic interchange among subpopulations.

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