Limited Gene Flow Among Cydia pomonella (Lepidoptera: Tortricidae) Populations in Two Isolated Regions in China: Implications for Utilization of the SIT

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Limited gene flow among *Cydia pomonella* (Lepidoptera: Tortricidae) populations in two isolated regions in China: Implications for utilization of the SIT

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Abstract

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a highly invasive species, recently became established in the Hexi Corridor, which is a long narrow passage area with many oases surrounded by deserts and tall mountains in Gansu province, China. The corridor is an important temperate fruit growing region in northwestern China as well as a natural barrier to prevent *C. pomonella* from invading other fruit growing areas of the country. Since the codling moth was firstly reported, pome fruit damage in this corridor has been severe. The sterile insect technique (SIT) is considered a possible effective control tactic for integration in a future area-wide integrated pest management (AW-IPM) program against *C. pomonella* in the corridor. Knowledge of population genetics and more specifically of genetic differentiation and gene flow patterns may be important for developing AW-IPM strategies that include the SIT. In the current study, we collected *C. pomonella* samples from 8 populations distributed across 2 adjacent regions in the Hexi corridor that are geographically separated by stone deserts and high mountains. Eight microsatellite loci were used to investigate the genetic diversity, structure and differentiation of these 8 populations. Significant genetic differentiation was found between populations of each of the 2 regions, whereas populations within each region showed a similar genetic structure, demonstrated by higher N_m and lower F_{ST} values for population pairs within the same region than in pairs between the regions. Our findings indicate limited gene flow of *C. pomonella* between the 2 regions, which suggests that SIT can be implemented to control the pest in the Hexi Corridor of China.

Key Words: codling moth; fixation index (F_{ST}); migrants; molecular markers; genetic differentiation; genetic structure

Resumen

El gusano de la manzana, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), una especie altamente invasora, recientemente se estableció en el Corredor Hexi, que es una área larga y estrecha de paso con muchos oasis rodeado de desiertos y montañas altas en la provincia de Gansu, China. El corredor es una región templada importante donde siembran frutas en el noroeste de China, así como una barrera natural para prevenir la invasión de *C. pomonella* en otras áreas donde siembran frutales en el país. Desde que se informó de la presencia del gusano de la manzana, el daño de frutas de hueso en este corredor ha sido grave. Se considera la técnica del insecto estéril (TIE) una posible táctica de control eficaz para la integración de un programa de manejo integrado de plagas en un área amplia (MIP-AA) en el futuro contra el *C. pomonella* en el corredor. El conocimiento de la genética de poblaciones, y más específicamente de los patrones de diferenciación genética y de flujo de genes pueden ser importantes para el desarrollo de estrategias MIP-AA que incluyen el TIE. En el presente estudio, se recolectaron muestras de *C. pomonella* de 8 poblaciones distribuidas en 2 regiones adyacentes al corredor de Hexi que están separados geográficamente por los desiertos de piedra y las altas montañas. Se utilizaron ocho loci de microsatélites para investigar la diversidad genética, la estructura y la diferenciación de estas 8 poblaciones. Se encontró una diferenciación genética significativa entre las poblaciones de cada una de las 2 regiones, mientras que las poblaciones dentro de cada región mostraron una estructura genética similar, demostrado por un valor mayor N_m y un valor menor de F_{ST} para los pares de la población dentro de la misma región que en pares entre las regiones. Nuestros hallazgos indican un flujo genético limitado de *C. pomonella* entre las 2 regiones, lo que sugiere que la TIE puede ser implementada para controlar la plaga en el Corredor de Hexi de China.

Palabras Clave: gusano de la manzana; índice de fijación (IDF); migrantes; marcadores moleculares; diferenciación genética; estructura genética

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is one of the most destructive pests of pome fruits (apple, pear and quince) and walnut orchards in most temperate regions of the world (Barnes 1991; Willett et al. 2009; Vreysen et al. 2010). It is generally assumed that the codling moth originated from southeastern Europe from where it spread around the world as a result of expanding apple and pear culture (Shel′Deshova 1967; Boivin et al. 2004; Franck et al. 2007; Meraner et al. 2008; Thaler et al. 2008). Codling moth is a serious invasive pest in China causing 20%–30% damage to pome fruit in those areas where it is distributed (Zhang et al. 2012). In 1957, codling moth was first detected in Xinjiang Province in Western China, which covers an area of more than 1.6 million km² consisting mostly of moun-

tains and deserts (Zhang 1957). Although *C. pomonella* has been the object of both internal and external quarantines in China, it still managed to spread from Xinjiang Province to the neighboring Hexi Corridor of Gansu Province in 1989 as a direct result of dense traffic and commercial transport (Shi et al. 2008). Thereafter, it took 20 years for codling moth to spread along the Hexi Corridor, which is composed of a1,000 km long string of oases along the northern edge of the high Tibetan Plateau, and is bordered on the north by the Gobi Desert and the grasslands of Outer Mongolia (Fig. 1). Irrigated by water from the snow-capped mountains, the oases of the Hexi Corridor are the main farming regions that supply most of the food including fruit to the arid Gansu Province. Apple and pear trees are planted widely in the oases and in the last several decades approximately 40% apple losses due to the codling moth have been reported in the corridor (Zhai et al. 2010).

Intensive spraying of chemical insecticides during the growing season is the main measure for controlling *C. pomonella* in China. However, the continuous use of broad-spectrum insecticides has not only become a serious concern for the environment and human health, but has also resulted in increased insecticide resistance of codling moth and in the loss of natural pest control (Bloem & Carpenter 2001; Reyes et al. 2009; Vreysen et al. 2010). The use of more environment-friendly pest management strategies such as the sterile insect technique (SIT) would have several advantages for the control of *C. pomonella* in China (Liu et al. 2012). The SIT is highly species-specific and friendly to the environment (Klassen 2005; Vreysen et al. 2010), and has been used successfully against a number of pest species, such as the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), the New World screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) and the tsetse fly *Glossina austeni* Newstead (Diptera: Glossinidae) (Tan 2000; Vreysen et al. 2000; Wyss 2000; Bloem & Carpenter 2001; Hendrichs et al. 2005; Klassen & Curtis 2005; Parker & Mehta 2007). The successful suppression of the codling moth in the Okanagan Valley of British Columbia, Canada for the last 20 years is arguably one of the most successful AW-IPM programs that integrated the SIT with other control tactics (Klassen 2005; Bloem et al. 2007 b; Vreysen et al. 2010). Given that the invasion of the Hexi Corridor by the codling moth is fairly recent, and that the oases of the corridor are isolated, the SIT could be considered as a potential part of an effective strategy of managing codling moth in the Hexi Corridor (Li 2013).

Population genetics data and more specifically genetic differentiation and gene flow patterns of target insect populations can provide sound baseline information to develop efficient insect pest-management strategies including the SIT (Klassen 2005; Krafsur 2005; Timm et al. 2006; Chen & Dorn 2010; Vreysen et al. 2010; Zheng et al. 2013). These data can reveal the distance over which members of a given species typically disperse, estimate the degree of genetic isolation of target populations from each other and from untargeted populations, determine whether a sub-population or sibling species exists, and establish the origin of outbreaks or reintroductions (Krafsur 2005; Chen & Dorn 2010). In practice, if immigration of gravid female moths into a given agro-ecosystem can be kept to a minimum, the SIT would be more effective. As moths released in AW-IPM programs with a SIT component should be capable of mating with females from different geographic regions, it is important to know to which degree an insect species migrates between different field populations (Krafsur 2005; Chen & Dorn 2010). Moreover, knowledge on the genetic differentiation and rate of gene flow among populations can provide an estimate of the rate of invasion or reinvasion of eradicated areas by unchallenged populations (Krafsur 2005).
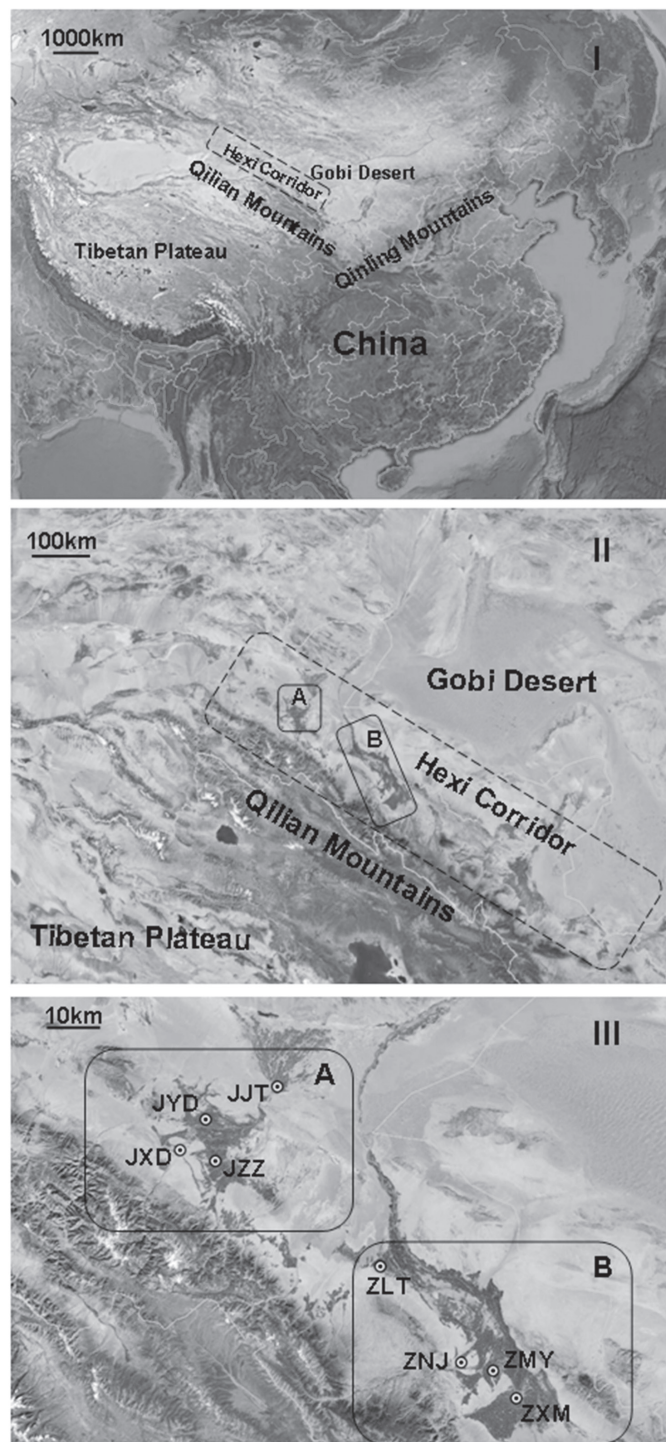
Fig. 1. Sampling regions and locations of *Cydia pomonella* in Hexi Corridor of China. (I) map of China, the Hexi Corridor is indicated in the dash box; (II) enlarged map of Hexi Corridor, the 2 sampling regions are indicated as (A) Jiuquan region and (B) Zhangye region; (III) enlarged map of the sampling regions and sampling locations. The sampling locations includes JinTa (JJT), YinDa (JYD), XiDong (JXD), and ZongZai (JZZ) of the Jiuquan Region, and Luo Tuocheng (ZLT), NiJiaying (ZNJ), MinYong (ZMY) and XiaoMan (ZXM) of the Zhangye Region.

Although the Hexi Corridor has always been considered an important natural barrier that prevents *C. pomonella* from invading the more southerly major fruit growing areas, *C. pomonella* has been established in several oases of the Hexi Corridor. However, the genetic differen-

tiation and gene flow of the different codling moth populations is still unknown. In this study, we collected *C. pomonella* samples from 2 geographically adjacent regions (Jiuquan and Zhangye) in the Hexi Corridor separated by unpopulated tall mountains and stone deserts serving as a geographic barrier. Using microsatellites and the method developed by Men et al. (2013) we aimed to characterize the genetic structure and gene flow pattern of the C. *pomonella* populations in the 2 regions. The findings are of interest for the development and implementation of a rational AW-IPM strategy that includes a SIT component.

Materials and Methods

INSECT SAMPLING

Cydia pomonella was sampled in 2 isolated adjacent regions (Jiuquan and Zhangye) of the Hexi Corridor (Fig. 1). Adult moths were sampled with 6 pheromone traps deployed for 5 consecutive days in apple orchards. Traps were replaced every 24 h and relocated to a new site within each sublocation. This resulted in a total of 30 traps per sublocation. To avoid sibling sampling, only one individual was taken from each trap and preserved in a 10 mL Falcon tube filled with ethanol. The same sampling procedure was repeated 3 times in each sublocation during the apple growing season giving a total of 45–65 moths collected in each sublocation. Around half of the samples of each region were randomly selected for genotyping in the current study. We use the term "population" for *C. pomonella* moths sampled from a single orchard (sampling unit). Samples from 4 locations in each of the 2 regions were used (Fig. 1).

DNA EXTRACTION AND MICROSATELLITE GENOTYPING

Genomic DNA was extracted from 8–10 mg of insect material using the Easy Pure ™ Genomic DNA Kit (TransGen Biotech Co., Ltd. Beijing, China) according to the manufacturer's protocol for animal tissues. DNA was eluted in 200 µL of ultra pure water and stored at −20 °C. All samples were genotyped at 8 polymorphic microsatellite loci (*Cp1.62*, *Cp2.39*, *Cp2.P*, *Cp3.56*, *Cp3.169*, *Cp3.K*, *Cp4.56* and *Cp4.129*) isolated from *Cydia pomonella* (Franck et al. 2005). Three primers, which included the forward primer synthesized with an M13 (−21) at the 5' end, the reverse primer and the FAM fluorescent dye labeled M13 (−21) primer, were used for amplification each microsatellite locus in a PCR reaction (Schuelke 2000). PCR reactions for the 8 loci were carried out in a mix of 12.5 µL 2 × *Taq* Master Mix (containing 0.05 U/µL *Taq* DNA Polymerase, 2 × *Taq* PCR Buffer, 3 mM MgCl$_2$ and 400 µM dNTP mix) (Beijing CoWin Biotech Co, Ltd. Beijing, China), 0.2 µM of each forward primer, 0.8 µM of each reverse primer, 0.8 µM M13 primer, and 1 µL genomic DNA (10–30 ng/µL). The PCR involved denaturation at 95 °C for 10 min, followed by 30 amplification cycles consisting of 95 °C for 30 s, 45 s at the primer-specific annealing temperature (57 °C for *Cp1.62*, 60 °C for *Cp2.39*, 58 °C for *Cp2.P*, 60 °C for *Cp3.56*, 54 °C for *Cp3.169*, 48 °C for *Cp3.K*, 60 °C for *Cp4.129* and 56 °C for *Cp4.56*), 72 °C for 45 s, then 8 cycles consisting of 95 °C for 30 s, 53 °C for 45 s and 72 °C for 45 s, and a final step at 72 °C for 10 min. Genotyping was carried out on an ABI3730XL automated DNA sequencer (Applied Biosystems, Foster City, California, USA) and GENEMAPPER version 4.0 (Applied Biosystems, Foster City, California, USA).

DATA ANALYSIS

Null allele and frequency were checked with Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004). FSTAT Version 2.9.3.2 (Goudet 2001) was utilized for calculating the number of alleles (*Na*), and for calculating the mean allelic richness per locus (*R*) for all the sampling populations with a rarefaction method that adjusted the observed number of alleles to a common size for each population sample. Observed heterozygosity (*H$_o$*) and expected heterozygosity (*H$_e$*) were calculated using microsatellite analyzer (MSA) version 3.15 (Dieringer & Schlötterer 2003). The Hardy-Weinberg equilibrium (HWE) and *P* value for Hardy-Weinberg equilibrium (*HWE-P*) were calculated using GENE-POP version 4.0.1 (Rousset 2008).

Analysis of molecular variance (AMOVA), inbreeding coefficients (*F$_{IS}$*), fixation index among groups (*F$_{CT}$*), fixation index among populations within groups (*F$_{SC}$*) and fixation index within populations (*F$_{ST}$*) were performed using ARLEQUIN version 3.5.1.2 (Weir & Cockerham 1984; Excoffier &Lischer 2010). We calculated the number of migrants moving between each pair of populations (*N$_m$*) per generation as $(1/F_{ST} - 1)/4$ (Excoffier &Lischer 2010; Munshi-South 2012). For AMOVA, samples were arbitrarily grouped according to the collection regions: (1) Jiuquan and (2) Zhangye. The matrices of genetic distance $F_{ST}/(1 - F_{ST})$ and the geographic distance (ln) between 8 *C. pomonella* populations were used to test isolation by distance (IBD) with 10,000 permutations (Mantel 1967). The IBD analysis was performed using the ZT software package (Bonnet &Van der Peer 2002).

The Bayesian clustering approach was used to estimate the population structure in STRUCTURE version 2.3.3 (Pritchard et al. 2000) with a burn-in period of 50,000 iterations and 1 million Markov chain Monte Carlo (MCMC) repetitions. We set the number of the cluster (*K*) from 1 to 10 and repeated 10 times, and the most likely number of clusters was calculated by delta *K* methods (Evanno et al. 2005). The graphical display of genetic structure was produced by DISTRUCT (Rosenberg 2004). A neighbor-joining (NJ) tree based on Nei's genetic distances among populations was constructed by MEGA version 5 (Tamura et al. 2011).

Results

GENETIC DIVERSITY

A total of 214 *C. pomonella* individuals were genotyped across all 8 microsatellite loci. All 8 loci were polymorphic and the number of alleles at each locus ranged from 7 to 25. The frequency of null alleles ranged from 0.049 to 0.241, which was similar to previous reports of *C. pomonella* (Franck et al. 2007; Men et al. 2013) and is typical for Lepidoptera (Dakin & Avise 2004; Meglécz et al. 2004). The average number of alleles per population ranged from 4.3 to 6.9 (Table 1). The allele richness value per locus per population was between 4.07 and 6.64. The value of observed heterozygosity (*H$_o$*) was between 0.190 and 0.534, whilst the expected heterozygosity (*H$_e$*) values ranged from 0.311 to 0.569. Three populations revealed significant departures from HWE, together with large mean values of the inbreeding coefficient, *F$_{IS}$,* ranging from 0.422 to 0.543. This indicates the existence of heterozygote deficiencies in these 3 populations. The genetic indices indicated that the 8 populations showed similar genetic diversity over the 8 microsatellite loci.

GENETIC DIFFERENTIATION

AMOVA results (Table 2) indicated significant genetic differentiation at the 3 hierarchical levels (*F$_{CT}$* = 0.097, *P* < 0.001; *F$_{SC}$* = 0.085, *P* < 0.001; *F$_{ST}$* = 0.173, *P* < 0.001) with populations grouped according to the 2 sampling regions. Although the proportion of explained variance was low (9.7%), significant genetic differentiation existed among populations from the 2 regions.

Gene flow over generations as revealed by *N$_m$* is shown in Table 3. The results indicate that *N$_m$* values within populations from Jiu-

Table 1. Sample information and population statistics for *Cydia pomonella* screened with 8 microsatellites.

Region	Sublocation	Coordinates	PC	N	N_a	R	H_o	H_e	F_{IS}	HWE-P
Jiuquan	JinTa	N 39°59' E 98°54'	JJT	22	4.4	4.38	0.534	0.512	-0.043	0.598
	YinDa	N 39°48' E 98°34'	JYD	25	4.9	4.69	0.305	0.502	0.393	0.073
	XiDong	N 39°39' E 98°28'	JXD	32	5.3	4.66	0.305	0.475	0.358	0.204
	ZongZai	N 39°38' E 98°39'	JZZ	30	5.1	4.64	0.350	0.508	0.311	0.522
Zhangye	LuoTuocheng	N 39°21' E 99°37'	ZLT	30	5.6	5.26	0.325	0.562	0.422	**0.038**
	NiJiaying	N 39°01' E 100°08'	ZNJ	25	4.3	4.07	0.195	0.311	0.374	0.128
	MinYong	N 39°00' E 100°18'	ZMY	25	6.9	6.64	0.260	0.569	0.543	**0.048**
	XiaoMan	N 38°55' E 100°27'	ZXM	25	5.0	4.84	0.190	0.406	0.533	**0.004**

Abbreviations: *PC*, population code; *N*, number of individuals successfully genotyped; N_a, average number of alleles per locus; *R*, allele richness; H_o, the observed heterozygosity; H_e, the expected heterozygosity; F_{IS}, the multilocus estimate of inbreeding coefficient and *HWE-P*, Hardy-Weinberg equilibrium *p*-values (significant departures from HW equilibrium are given in bold, *P* < 0.05). N_a, H_o, H_e, F_{IS}, and *HWE-P* are all indicated by mean values over 8 loci. The significances were tested for multi comparisons by the Bonferroni method for *k* = 8; *P* < 0.05.

quan region were between 2.103 and 133.276, with an average value of 25.561, whilst N_m values within populations from Zhangye region ranged from 1.108 to 4.765, with an average value of 2.495. The N_m values of the population pairs from the 2 different regions (Jiuquan and Zhangye) averaged 1.386, with the values ranging between 0.649 and 1.915.

Pairwise F_{ST} values for each pair of populations over the 8 loci ranged from 0.002 to 0.259. The results show significant genetic variance between all pairs of populations except one pair (JJT and JYD) that was sampled from 2 neighboring sublocations in Jiuquan region (Table 3). The average F_{ST} value of population pairs from the Jiuquan region was 0.059, versus 0.110 for population pairs from the Zhangye region. The average F_{ST} value of the population pairs from the 2 different regions (Jiuquan and Zhangye) was 0.178. The Mantel test for the 8 *C. pomonella* populations from the 2 regions revealed no correlation between genetic distances and geographic distances (*r* = 0.315, *P* = 0.133).

GENETIC STRUCTURE

In the Bayesian analysis performed with the software STRUCTURE 2.3.3, the most likely value of *K* was 2 (Pritchard et al. 2000; Evanno et al. 2005), indicating that the 8 populations included in this study can be assigned to 2 distinct clusters, hereafter referred to as cluster 1 and cluster 2. The proportions of each population that contributed to each of the 2 clusters are shown in Fig. 2. Cluster 1 was mainly made up of individuals sampled from 4 sublocations of the Jiuquan region (JJT, 0.981 implying that 98.1% of the individuals from the JJT sublocation contributed to this cluster, and that corresponding contributions from the remaining 3 sublocations in this region were: JYD, 0.966; JXD, 0.944 and JZZ, 0.774). Likewise, cluster 2 consisted largely of individuals of the 4 populations collected in the 4 sublocations of Zhangye region and with corresponding contributions as follows: ZLT, 0.885; ZNJ, 0.936; ZMY, 0.906; and ZXM, 0.938. Populations within the same region showed similar genetic structures, but were different from the genetic structures of populations in the other region.

The NJ tree based on the Nei's genetic distances is shown in Fig. 3. The 8 populations from the 2 separate regions were divided into 2 clades

in the NJ tree. All 4 populations (JJT, JYD, JXD and JZZ) from the Jiuquan region made up one clade (clade 1), and the 4 populations (ZLT, ZNJ, ZMY and ZXM) from the Zhangye region constituted the other clade (clade 2). Both the results of the NJ tree and the STRUCTURE analysis consistently and clearly indicated a distinct clustering according to region.

Discussion

We analyzed the genetic variation at 8 microsatellite loci in 214 individuals from the Jiuquan and Zhangye regions of the Hexi Corridor that were separated by mountains and stone deserts. There was significant genetic differences between the various populations of the 2 regions. Populations within the same region showed similar genetic structures, but their genetic structures differed from those of the populations from the other region. When compared with population pairs from the other region, population pairs within a region showed greater gene flow (N_m) and smaller F_{ST} values. Our results suggest only limited gene flow between populations of the Jiuquan region and the Zhangye region.

The flight capacity of a large proportion of a *C. pomonella* population is low, and only a small proportion of a population is capable of long distance flights (Schumacher et al. 1997; Dorn et al. 1999). In the current study, AMOVA analyses among the 8 populations from the 2 sampling regions showed significant genetic differentiation. Based on cluster analysis, populations sampled in the 2 regions differed in genetic structure, and they formed 2 distinct clades in the NJ tree. The codling moth has traditionally been regarded as a sedentary insect (Keil et al. 2001; Timm et al. 2006; Thaler et al. 2008; Chen & Dorn 2010), and the limited gene flow implied by the significantly different population genetic characteristics of the 2 separate regions is likely the result of the limited dispersal capacity of *C. pomonella*. This was verified by mark-release-recapture studies in the field and flight mill experiments in the laboratory (Schumacher et al. 1997; Dorn et al. 1999; Keil et al. 2001). Our results are consistent with findings in South Africa, northern Italy and Switzerland, where limited gene flow and significant genetic differentiation between different local geographic regions were found (Timm et al. 2006; Thaler et al. 2008; Chen & Dorn 2010).

Table 2. Analysis of molecular variance of populations from Jiuquan and Zhangye regions of the Hexi Corridor.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation Indices	P value
Among groups	1	59.502	0.22430	9.69	F_{CT} = 0.097	P < 0.001
Among populations within groups	6	68.072	0.17712	7.65	F_{SC} = 0.085	P < 0.001
Within populations	420	803.793	1.91379	82.66	F_{ST} = 0.17	P < 0.001

Table 3. Pairwise population differentiation estimates (F_{ST}) averaging 8 loci between 8 populations of *Cydia pomonella* with *P* value (below the diagonal) and gene flow (*Nm*) values (above the diagonal).

	JJT	JYD	JXD	JZZ	ZLT	ZNJ	ZMY	ZXM
JJT		133.276	3.532	5.603	1.915	0.654	1.838	0.845
JYD	0.002[NS]		2.103	6.682	1.782	0.649	1.942	0.962
JXD	0.066[**]	0.106[**]		2.167	1.493	0.715	1.032	0.775
JZZ	0.043[**]	0.036[*]	0.103[**]		2.736	0.862	2.322	1.647
ZLT	0.115[**]	0.123[**]	0.143[**]	0.084[**]		2.444	4.765	3.286
ZNJ	0.277[**]	0.278[**]	0.259[**]	0.225[**]	0.093[**]		1.108	1.837
ZMY	0.120[**]	0.114[**]	0.195[**]	0.097[**]	0.050[**]	0.184[**]		1.531
ZXM	0.228[**]	0.206[**]	0.244[**]	0.132[**]	0.071[**]	0.120[**]	0.140[**]	

*$P < 0.05$, **$P < 0.001$; NS = non-significant. The significances were tested for multi comparisons by the Bonferroni method for *k* = 28; *P* < 0.05.
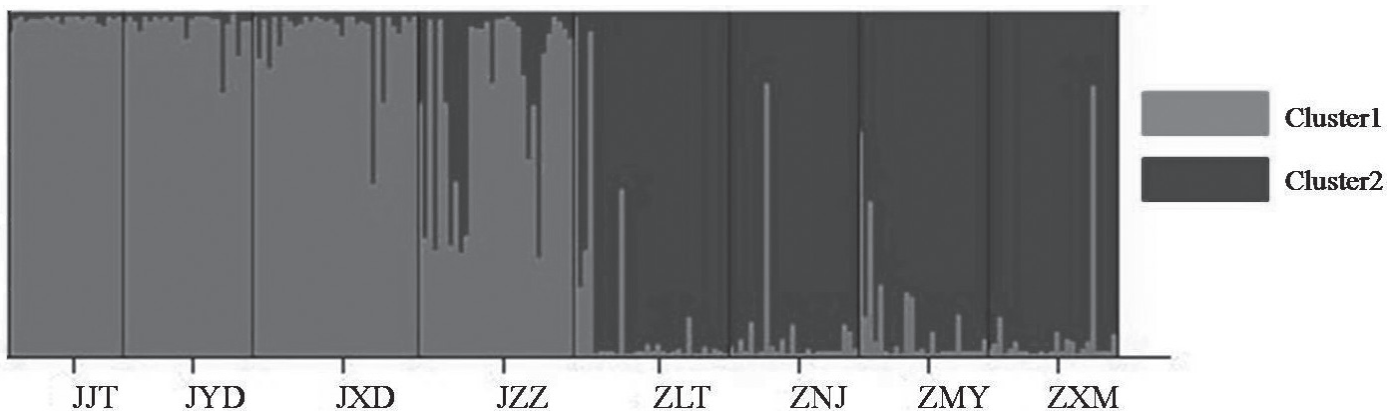

Fig. 2. Bayesian clustering analysis by use of STRUCTURE, which indicates the presence of 2 clusters. Proportion of membership coefficient for 8 *Cydia pomonella* populations falling into the 2 clusters is depicted by 2 different shades of gray, respectively. The sampling locations include JinTa (JJT), YinDa (JYD), XiDong (JXD) and ZongZai (JZZ) of Jiuquan Region, and Luo Tuocheng (ZLT), NiJiaying (ZNJ), MinYong (ZMY) and XiaoMan (ZXM) of Zhangye Region.

Similar results were also reported in the closely related species with small flight capacity, *Grapholita molesta* (Timm et al. 2008; Torriani et al. 2010; Kirk et al. 2013 a, b; Zheng et al. 2013), as well as in aphids (Loxdale et al. 1993; Miller et al. 2003).

We found similar genetic diversities and similar genetic structures within each region in this study. In the current analysis, *Nm* values were greater and F_{ST} values were smaller within population pairs from the same region in comparison to those found in population pairs from the 2 regions, indicating limited gene flow between the regions. Dispersal caused by humans might have contributed to these results (Mazzi & Dorn 2012; Kirk et al. 2013 a, b). Population genetics of local popula-

Fig. 3. Dendrogram generated by NJ analysis representing the genetic distance among populations of *Cydia pomonella*. The topology was tested by bootstrap analysis with 1,000 replicates. The scale bar represents 2.0% genetic distance. The sampling locations include JinTa (JJT), YinDa (JYD), XiDong (JXD) and ZongZai (JZZ) of Jiuquan Region, and Luo Tuocheng (ZLT), NiJiaying (ZNJ), MinYong (ZMY) and XiaoMan (ZXM) of Zhangye Region.

tions of sedentary species such as *C. pomonella* and *G. molesta* can obviously be affected by anthropogenic influences including neurotoxic pesticide spraying, regional fruit transportation, the recent spread of modern fruit culture and regional integrated production and marketing programs (Chen & Dorn 2008; Torriani et al. 2010; Mazzi & Dorn 2012). Anthropogenic influences are considered important reasons for the small genetic differentiation of *C. pomonella* as found in populations in France, Chile and Croatia (Franck et al. 2007; Fuentes-Contreras et al. 2008; Voudouris et al. 2012). Actually, local Chinese governments promote centralized purchasing of fruit and distribution of apple and pear cultivars that might promote gene flow and facilitate genetic exchange of *G. molesta* populations within regions, as is also the case for cooperatives in Italy (Torriani et al. 2010). Two populations (JJT and JYD) from the Jiuquan region were very similar in genetic diversity and showed very large N_m values, which can be explained by the frequent fruit transportation between the 2 regions offering ample opportunity for interbreeding between the populations of the 2 sublocations.

The SIT has been successfully used to suppress *C. pomonella* in the fruit growing Okanagan Valley of British Columbia in Canada (Klassen 2005; Bloem et al. 2007b; Vreysen et al. 2010). The Hexi Corridor is considered an important natural barrier that prevents the codling moth from invading into the more southerly major fruit growing areas of China. Our results show that gene flow between the 2 investigated regions is limited, which creates the possibility of developing and implementing a sequential eradication strategy for the sustainable removal of the codling moth populations in the 2 regions. The use of the SIT is considered a possibility for use in an AW-IPM strategy in view of the absence of mating barriers between codling moth populations worldwide (Taret et al. 2010; Liu et al. 2012; Li 2013).
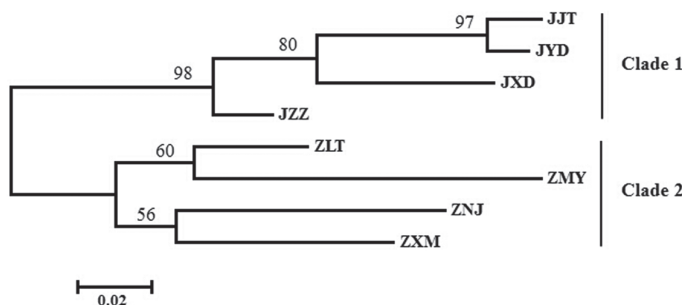
Acknowledgments

References Cited

Barnes MM. 1991. Codling moth occurrence, host race formation and damage, pp. 313-327 *In* van der Geest LPS, Evenhuis HH [eds.], Tortricid Pests: Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, The Netherlands.

Bloem S, Carpenter JE. 2001. Evaluation of population suppression by irradiated Lepidoptera and their progeny. Florida Entomologist 30: 165-171.

Bloem S, Carpenter J, Hofmeyr H. 2007a. Area-wide control tactics for the false codling moth *Thaumatotibia leucotreta* in South Africa: a potential invasive species, pp. 351-359 *In* Vreysen MJB, Robinson AS, Hendrichs J [eds.], Area-Wide Control of Insect Pests. From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Bloem S, McCluskey A, Fugger R, Arthur R, Wood S, Carpenter J. 2007b. Suppression of the codling moth *Cydia pomonella* in British Columbia, Canada using an area-wide integrated approach with an SIT component, pp. 591-602 *In* Vreysen MJB, Robinson AS, Hendrichs J [eds.], Area-Wide Control of Insect Pests. From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Boivin T, Bouvier JC, Beslay D, Sauphanor B. 2004. Variability in diapause propensity within populations of a temperate insect species: interactions between insecticide resistance genes and photoperiodism. Biological Journal of the Linnean Society 83: 341-351.

Bonnet E, Van der Peer Y. 2002. ZT: a software tool for simple and partial Mantel tests. Journal of Statistical Software 7: 1-12.

Chen MH, Dorn S. 2010. Microsatellites reveal genetic differentiation among populations in an insect species with high genetic variability in dispersal, the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Bulletin of Entomological Research 100: 75-81.

Dakin EE, Avise JC. 2004. Microsatellite null alleles in parentage analysis. Heredity 93: 504-509.

Dieringer D, Schlötterer C. 2003. Microsatellite Analyser (MSA): A platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3: 167-169.

Dorn S, Schumacher P, Abivardi C, Meyhöfer R. 1999. Global and regional pest insects and their antagonists in orchards: spatial dynamics. Agriculture, Ecosystems and Environment 73: 111-118.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14: 2611-2620.

Excoffier L, Lischer HL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564-567.

Franck P, Guérin F, Loiseau A, Sauphanor B. 2005. Isolation and characterization of microsatellite loci in the codling moth *Cydia pomonella* L. (Lepidoptera, Tortricidae). Molecular Ecology Notes 5: 99-102.

Franck P, Reyes M, Olivares J, Sauphanor B. 2007. Genetic architecture in codling moth populations: comparison between microsatellite and insecticide resistance markers. Molecular Ecology 16: 3554-3564.

Fuentes-Contreras E, Espinoza JL, Lavandero B, Ramírez CC. 2008. Population genetic structure of codling moth (Lepidoptera: Tortricidae) from apple orchards in central Chile. Journal of Economic Entomology 101: 190-198.

Goudet J. 2001. FSTAT, version 2.9.3, A program to estimate and test gene diversities and fixation indices. Lausanne University, Lausanne, Switzerland.

Hendrichs J, Vreysen MJB, Enkerlin WR, Cayol JP. 2005. Strategic options in using sterile insects for area-wide integrated pest management, pp. 563-600 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Keil S, Gu HN, Dorn S. 2001. Response of *Cydia pomonella* to selection on mobility: laboratory evaluation and field verification. Ecological Entomology 26: 495-501.

Kirk H, Dorn S, Mazzi D. 2013a. Worldwide population genetic structure of the oriental fruit moth (*Grapholita molesta*), a globally invasive pest. BMC Ecology 13: 12.

Kirk H, Dorn S, Mazzi D. 2013b. Molecular genetics and genomics generate new insights into invertebrate pest invasions. Evolutionary Applications 6: 842-856.

Klassen W. 2005. Area-wide integrated pest management and the sterile insect technique, pp. 43-76 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Klassen W, Curtis CF. 2005. History of the sterile insect technique, pp. 3-36 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Krafsur ES. 2005. Role of population genetics in the sterile insect technique, pp. 389-406 *In* Dyck VA, Hendrichs J, Robinson AS [eds.], Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Li Y. 2013. Genetic diversity and invasion source of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) populations in China inferred from mtDNA gene sequences. MSc Dissertation, Northwest A&F University, Yangling (In Chinese).

Liu W, Xu J, Zhang RZ. 2012. Advances in the sterile insect technique for controlling codling moth. Chinese Journal of Applied Entomology 49: 268-274 (In Chinese).

Loxdale HD, Hardie JIM. Halbert S, Foottit R, Kidd NA, Carter CI. 1993. The relative importance of short- and long-range movement of flying aphids. Biological Reviews 68: 291-311.

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

Mazzi D, Dorn S. 2012. Movement of insect pests in agricultural landscapes. Annals of Applied Biology 160: 97-113.

Meglécz E, Péténian F, Danchin E, Coeur d'Acier A, Rasplus JY, Faure E. 2004. High similarity between flanking regions of different microsatellites detected within each of two species of Lepidoptera: *Parnassius apollo* and *Euphydryas aurinia*. Molecular Ecology 13: 1693-1700.

Men QL, Chen MH, Zhang YL, Feng JN. 2013. Genetic structure and diversity of a newly invasive species, the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in China. Biological Invasions 15: 447-458.

Meraner A, Brandstätter A, Thaler R, Aray B, Unterlechner M. 2008. Molecular phylogeny and population structure of the codling moth (*Cydia pomonella*) in Central Europe: I. Ancient clade splitting revealed by mitochondrial haplotype markers. Molecular Phylogenetics and Evolution 48: 825-837.

Miller NJ, Birley AJ, Overall ADJ, Tatchell GM. 2003. Population genetic structure of the lettuce root aphid, *Pemphigus bursarius* (L.), in relation to geographic distance, gene flow and host plant usage. Heredity 91: 217-223.

Munshi-South J. 2012. Urban landscape genetics: Canopy cover predicts gene flow between white-footed mouse (*Peromyscus leucopus*) populations in New York City. Molecular Ecology 21: 1360-1378.

Parker A, Mehta K. 2007. Sterile insect technique: A model for dose optimization for improved sterile insect quality. Florida Entomologist 90: 88-95.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Reyes M, Franck P, Olivares J, Margaritopoulos J, Knight A, Sauphanor B. 2009. Worldwide variability of insecticide resistance mechanisms in the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae). Bulletin of Entomological Research 99: 359-369.

Rosenberg NA. 2004. DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137-138.

Rousset F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8: 103-106.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233-234.

Schumacher P, Donald C, Weber C, Hagger C, Dorn S. 1997. Heritability of flight distance for *Cydia pomonella*. Entomologia Experimentalis et Applicata 85: 169-175.

Shi HL, Wang PX, Li PF, Ren YC. 2008. Risk analysis for *Cydia pomonella* invasion to Shaanxi Province. Shaanxi Forest Science and Technology 4: 76-80 (In Chinese).

Shel Deshova GG. 1967. Ecological factors determining distribution of the codling moth *Laspeyresia pomonella* L. (Lepidoptera, Tortricidae) in the northern and southern hemispheres. Entomological Review 46: 349-361.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739.

Tan KH. 2000. Sex pheromone components in defense of melon fly, *Bactrocera cucurbitae* against Asian house gecko, *Hemidactylus frenatus*. Journal of Chemical Ecology 26: 697-704.

Taret G, Sevilla M, Wornoayporn V, Islam I, Ahmad S, Caceres C, Robinson AS, Vreysen MJB. 2010. Mating compatibility among populations of codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) from different geographic origins. Journal of Applied Entomology 134: 207-215.

Thaler R, Brandstätter A, Meraner A, Chabicovski M, Parson W, Zelger R, Dalla Via J, Dallinger R. 2008. Molecular phylogeny and population structure of the codling moth (*Cydia pomonella*) in Central Europe: II. AFLP analysis reflects human-aided local adaptation of a global pest species. Molecular Phylogenetics and Evolution 48: 838-849.

Timm AE, Geertsema H, Warnich L. 2006. Gene flow among *Cydia pomonella* (Lepidoptera: Tortricidae) geographic and host populations in South Africa. Journal of Economic Entomology 99: 341-348.

Timm AE, Geertsema H, Warnich L. 2008. Population genetic structure of *Grapholita molesta* (Lepidoptera: Tortricidae) in South Africa. Annals of the Entomological Society of America 101: 197-203.

Torriani MVG, Mazzi D, Hein S, Dorn S. 2010. Structured populations of the oriental fruit moth in an agricultural ecosystem. Molecular Ecology 19: 2651-2660.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Voudouris CC, Franck P, Olivares J, Sauphanor B, Mamuris Z, Tsitsipis JA, Margaritopoulos JT. 2012. Comparing the genetic structure of codling moth *Cydia pomonella* (L.) from Greece and France: long distance gene-flow in a sedentary pest species. Bulletin of Entomological Research 102: 185-198.

Vreysen MJB, Saleh KM, Ali MY, Abdulla AM, Zhu ZR, Juma KG, Dyck VA, Msangi AR, Mkonyi PA, Feldmann, HU. 2000. *Glossina austeni* (Diptera: Glossinidae) eradicated on the island of Unguja, Zanzibar, using the sterile insect technique. Journal of Economic Entomology 93: 123-135.

Vreysen MJB, Carpenter JE, Marec F. 2010. Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. Journal of Applied Entomology 134: 165-181.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population-structure. Evolution 38: 1358-1370.

Willett MJ, Neven L, Miller CE. 2009. The occurrence of codling moth in low latitude countries: validation of pest distribution reports. HortTechnology 19: 633-637.

Wyss JH. 2000. Screwworm eradication in the Americas. Annals of the New York Academy of Sciences 916: 686-193.

Zhai XW, Liu WW, Wan FH, Pu CJ. 2010. Occurrence dynamics of *Cydia pomonella* in Jiuquan. Chinese Bulletin of Entomology 47: 715-719 (In Chinese).

Zhang RZ, Wang FX, Zhang YL, Chen HJ, Luo JC, Wang QY, Liu WX, Ainiwaer M, Pu CJ, Yan YG, Guo JM, Liu XY, Chen JG, Zhang ZF, Yang S, Xu JJ, Cui GZ, Xu J. 2012 Progress on monitoring and control of the codling moth, *Cydia pomonella* (L.). Chinese Journal of Applied Entomology 4: 37-42 (In Chinese).

Zhang XZ. 1957. Taxonomic notes on the codling moth, *Carpocapsa pomonella* L. in Sinkiang. Acta Entomologica Sinica 7: 467-472 (In Chinese).

Zheng Y, Peng X, Liu GM, Pan HY, Dorn S, Chen MH. 2013 High genetic diversity and structured populations of the oriental fruit moth in its range of origin. PLoS ONE 8(11): e78476. doi: 10.1371/journal.pone.0078476.