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Mitochondrial DNA revealed the extent of genetic diversity and invasion origin of populations from two separate invaded areas of a newly invasive pest, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in China

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Abstract

Cydia pomonella is a serious invasive insect pest in China, and has caused severe damage to the production of apple and pear in its invaded areas. This species is distributing in the northwest and northeast of China, but no occurrence of it has been recorded in the large areas (about 3000–5000 km away) between the invaded northwestern and northeastern regions despite continuous monitoring. As yet the genetic diversity and invasion origin of the *C. pomonella* populations in Northwestern and Northeastern China is obscure. In this study, we investigate the genetic diversity of 14 populations of *C. pomonella* sampled throughout the main distribution regions in Northwestern (Xinjiang and Gansu Provinces) and Northeastern (Heilongjiang Province) China and compared them with nine populations from Europe and other continents using the mitochondrial COI, COII and Cytb genes. Both the populations from Northeastern and Northwestern China shared some haplotypes with populations from other countries. Haplotypes of the three mitochondrial genes had a different distribution in Northeastern and Northwestern China. The northeastern populations had more private haplotypes than the northwestern populations. A large number of the individuals from northwestern populations shared a few haplotypes of each of the three genes. The haplotype numbers and haplotype diversities of the northeastern populations were similar to those of field populations in other countries, but were higher than those of the northwestern populations. Populations from the Northwestern China showed similar haplotype number and haplotype diversity. We conclude that the population genetic background of *C. pomonella* populations in Northeastern and Northwestern China varies due to different invasion sources and that this should be considered before the application of new pest control tactics.

Keywords: genetic diversity, mtDNA marker, invasion source, codling moth

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Introduction

Invasive exotic species threaten native biodiversity worldwide and cause significant economic losses in agriculture, forestry and other industries (Vitousek *et al.*, 1996). Genetic diversity of a newly invasive species is affected by a variety

of factors including invasion origin, invasive history and passive human-aided dispersal (Ramstad *et al.*, 2004; Dlugosch & Parker, 2008; Watts *et al.*, 2010; Inoue *et al.*, 2013). Generally, genetic diversity of an established invasive species is lower in the newly invaded region compared with its native distribution areas (Grapputo *et al.*, 2005; Ficetola *et al.*, 2008; Zheng *et al.*, 2013). However, several studies have found that the genetic diversity of invasive species was not reduced due to invasion from multiple sources, or to new colonization of a given area from a large initial introduction (Johnson & Starks, 2004; Cognato *et al.*, 2005; Wan *et al.*, 2011; Inoue *et al.*, 2013).

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a key fruit pest in temperate areas worldwide (Shel'Deshova, 1967; Barnes, 1991). It infests pome fruits (apple and pear), stone fruits (apricot, plum, peach, nectarine and cherry) and quince, as well as walnut (Barnes, 1991). Larvae of the codling moth attack various fruits or nuts and can damage a high percentage of the crops if not managed, leading to substantial economic losses (Barnes, 1991). Due to its broad host range, relatively ample climate tolerance, and developed resistance to varied types of insecticides, the codling moth has achieved a nearly global distribution and is considered to be one of the most successful and the most important pest insects in the world (Bues *et al.*, 1995; Reyes *et al.*, 2009; Chen & Dorn, 2010).

In China, *C. pomonella* is a serious new invasive species that is mainly distributed in two provinces in the northwest (Xinjiang and Gansu Provinces) and one province in the northeast of the country (Heilongjiang Province). The first reported sightings of the codling moth in China were in 1957 in Korla in the Xinjiang Province, in the northwestern region of the country (Zhang, 1957). It then took about 30 years for the pest to cross the mountains, deserts and unpopulated areas and reach Dunhuang of the Hexi Corridor in Gansu Province, spreading along the string of oases of this corridor to the adjacent sparsely distributed fruit-growing regions of Inner Mongolia and Ningxia Provinces. In 2006, *C. pomonella* was first reported in MudanJiang, Heilongjiang Province in Northeastern China (Qin *et al.*, 2006). Heilongjiang is about 3000 and 5000 km away from Gansu and Xinjiang, respectively. In China, 90% of the apple trees are planted in seven provinces located between Heilongjiang and Xinjiang Provinces, yet Gansu Province is the only major apple-growing province in which *C. pomonella* is found. In spite of the considerable distance from Heilongjiang to Xinjiang and Gansu, no occurrence of this pest has been recorded in the large area in between, despite continuous monitoring with pheromone traps in apple, plum, pear, walnut and other fruit orchards, as well as along the highways (Zhang *et al.*, 2012).

Despite the economic and ecological threats of the codling moth in the northwestern and northeastern parts of China, the genetic diversity and the origin of the pest in these separate areas of China is still obscure. Populations with different invasion origin may vary in genetic diversity and genetic structure. This is an important aspect to be considered in *C. pomonella* management. For example, the sterile moths released in area-wide integrated pest management with sterile insect technique (SIT) should have good mating compatibility with females from different origins (Taret *et al.*, 2010; Vreysen *et al.*, 2010). Populations with similar genetic structure should be considered as the same management unit for effective control (Ayres *et al.*, 2010). Furthermore, populations with different invasion origin may differ in response to the pheromone used in the mating disruption technique, and may differ in response to the isolates of *Cydia pomonella* granulosis viruses (CpGV) used to control the pest (Gan *et al.*, 2011). A previous microsatellite analysis by our group (Men *et al.*, 2013) observed sequential loss of genetic diversity in *C. pomonella* populations from northwestern China, and found that populations from Northwestern and Northeastern China had a different genetic structure. However, additional research was needed to detail the extent of genetic diversity and the invasion sources of the codling moth populations in China as well as their spread through China. Mitochondrial gene haplotypes were shown to be effective for direct comparison of genetic diversity and invasion sources of an invasive species distributing in different invaded regions (Avise, 2000; Roderick & Navajas, 2003; Roderick, 2004; Triapitsyn *et al.*, 2008; De León *et al.*, 2011). In this study, we used three mitochondrial genes to analyze a total of 14 *C. pomonella* populations collected throughout the main distribution regions in Northwestern and Northeastern China, and samples from nine other countries on different continents were used for comparison. Our objective was to compare the genetic diversity and characterize the origin of populations of this new invasive species from the northwest and northeast of China and to provide basic population genetic knowledge for controlling this pest in the country.

Materials and methods

Insect sampling

Samples of codling moth were collected from apple orchards in all the three main *C. pomonella* distribution areas (Xinjiang and Gansu Provinces in the northwest, and Heilongjiang Province in the northeast) of China from 2008 to 2010 (table 1, fig. 1), as done by Men *et al.* (2013) and Li *et al.* (2013). In Xinjiang Province, one infested fruit was collected from each fruit tree per orchard and one larva per fruit was used, the distance between sampled fruit trees was at least 5 m, only one 2nd to 3rd instar larvae per tree was used to minimize sibling collection. In the newly invaded Gansu and Heilongjiang Provinces, samples of adults were collected on 5 days each time, with six pheromone traps exposed for 24 h in each orchard (>5 ha) every day, adding up to a total of 30 traps which were hanged on different trees. The distance between each of the six traps was at least 100 m, and only one moth per trap was used for further analysis to minimize collecting sibling samples. Samples collected with the aforementioned methods were used in previous genetic diversity analysis of the *C. pomonella* populations (Li *et al.*, 2013; Men *et al.*, 2013). For comparison, *C. pomonella* samples collected in nine other countries were included in the analysis. Most samples from these countries were obtained from field pheromone trap captures, except the samples from laboratory colonies that originated from Oregon in the USA and Tel Aviv in Israel (table 2). We use the term 'population' for *C. pomonella* specimens sampled from one and the same orchard (sampling unit). All the samples of 14 populations from China and nine populations from other countries were preserved in 10 ml Falcon tubes filled with ethanol and stored at −20°C prior to analyses.

DNA extraction

Genomic DNA was extracted from 8 to 10 mg of insect material using the genomic DNA kit (QIAGEN Company, Basel, Switzerland) according to the manufacturer's instructions. The extracted DNA was eluted in TE buffer and stored at −20°C.

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Table 1. Sampling information of *Cydia pomonella* populations from China and other countries.

Country	Province (code)	Location	Population code	Sample size	Latitude	Longitude	Sample date
China	Heilongjiang (HLJ)	DongNing	DN	18	44°06′N	131°14′E	2010.08
		MuDanJiang	MDJ	20	44°35′N	129°34′E	2010.08
		JiDong	JD	15	45°15′N	131°07′E	2010.08
	Xinjiang (XJ)	YiLi	YL	17	43°57′N	81°19′E	2008.08
		JingHe	JH	18	44°67′N	82°92′E	2008.08
		KuiTun	KT	18	44°27′N	84°56′E	2008.08
		Urumqi	WL	15	43°54′N	87°28′E	2008.08
		Kumul	HM	18	42°50′N	93°40′E	2008.08
		Korla	KE	11	41°36′N	86°08′E	2008.08
		Kashgar	KS	21	39°32′N	76°01′E	2008.08
	Gansu (GS)	DunHuang	DH	18	41°35′N	95°30′E	2009.07
		JiuQuan	JQ	19	39°46′N	98°28′E	2010.05
		ZhangYe	ZY	19	38°55′N	100°27′E	2010.05
		WuWei	WW	15	37°55′N	102°38′E	2010.05
Austria		Haschhof	AUS	14			2008.05
Byelorussia		Minsk	BYE	14			2008.07
Germany		Karlsruhe	GER	15			2008.07
Italy		Rolise	ITA	14			2007.08
Switzerland		Chur	SWI	20			2009.06
Israel		Tel Aviv	ISR	11			2011.09
South Africa		Cape Town	SA	14			2010.11
UK		Kent	UK	6			2008.07
USA		Oregon	USA	20			2008.07

PCR amplification and sequencing of mitochondrial genes

Three mitochondrial genes were used in the analysis. The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified with the primer pairs C1-J-1751 (5′-GGATCACCT GATATAGCATTCCC-3′) and C1-N-2191 (5′-CCCGGTAA AATTAAAATATAAACTTC-3′) (Simon *et al*., 1994), the complete mitochondrial COII region was amplified with primer pairs TL2-J-3037 (5′-ATGGCAGATTATATGTA ATGG-3′) and TK-N-3785 (5′-GTTTAAGAGACCAGTAC TTG-3′) (Simon *et al*., 1994), while the mitochondrial cytochrome b (Cytb) gene was amplified by forward primer 5′-TATGTTTTACCATGAGG TCAAATATC-3′ and reverse primer 5′-TATTTCTTTCTTAAG TTTTCAAAAC-3′.

All PCR reactions were carried out in a total volume of 50 μl, containing 60 ng template DNA, 0.4 μM each primer, 100 μM each dNTP, 4 mM Mg^{2+}, 10 × PCR reaction buffer with 500 mM KCl and 100 mM Tris–HCl (PH 8.3 at 20°C), distilled water and 2 unit of Taq DNA polymerase (5 U μl^{-1}, Sangon Biotech Co., Ltd., Shanghai, China). PCR reactions were performed on a Bio-Rad S1000TM Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) and consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, with annealing temperatures of 48°C (COI), 57°C (COII) or 52°C (Cytb) for 1 min and 72°C for 1 min, with a final 10 min extension at 72°C. PCR products were visualized on 1.0% agarose gels under UV light and were directly sequenced with forward PCR primers. All the sequences were read on an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster city, CA, USA).

Data analysis

The obtained mitochondrial gene sequences were aligned by CLUSTALX version 2.0 (Thompson *et al*., 1994). All population genetic parameters including number of haplotypes (N_h), nucleotide diversity (π) and haplotype diversity (H_d) were calculated using ARLEQUIN version 3.5 (Excoffier *et al*., 2005). The haplotype diversity was based on the formula $H_d = (1 - \Sigma xi^2)\, n/(n-1)$, where xi is the frequency of a haplotype and n is the sample size (Nei, 1987). The nucleotide diversity (π) was calculated by $\hat{\pi} = 2\sum_{i<j} \hat{d}_{xj}/[n(n-1)]$ where $\hat{d}_{xj}$ is an estimate of the number of nucleotide substitutions per site between gene sequences i and j (d_{xj}) and n is the number of gene sequences examined (Tajima, 1983; Nei, 1987). A two-tailed t test at the significance level 0.05 was used to test whether the differences of haplotype diversity (H_d), nucleotide diversity (π), proportion of individuals with different haplotypes, proportion of individuals with private haplotypes and proportion of private haplotypes were significant between populations from the three provinces of China and other countries.

Analysis of molecular variance (AMOVA) was performed using the ARLEQUIN version 3.5 software based on the combination of the three gene sequences (Excoffier *et al*., 2005) according to the following three models. (A) 'comparison of variance among populations from the two northwestern provinces of China'. In this model, 11 *C. pomonella* populations from the two neighboring provinces in Northwestern China were separated into two groups according to province: (1) Gansu (DH, JQ, ZY and WW); (2) Xinjiang (YL, JH, KT, WL, HM, KE and KS). (B) 'comparison of variance among populations from Northwestern and Northeastern China'. In this model, all the 14 *C. pomonella* populations from China were divided into two groups according to the separate distribution areas: (1) Northwestern China (DH, JQ, ZY, WW, YL, JH, KT, WL, HM, KE and KS); (2) Northeastern China (DN, MDJ and JD). (C) 'comparison of variance among populations from Northwestern China (Gansu, Xinjiang), Northeastern China (Heilongjiang) and European countries (Austria, Byelorussia,

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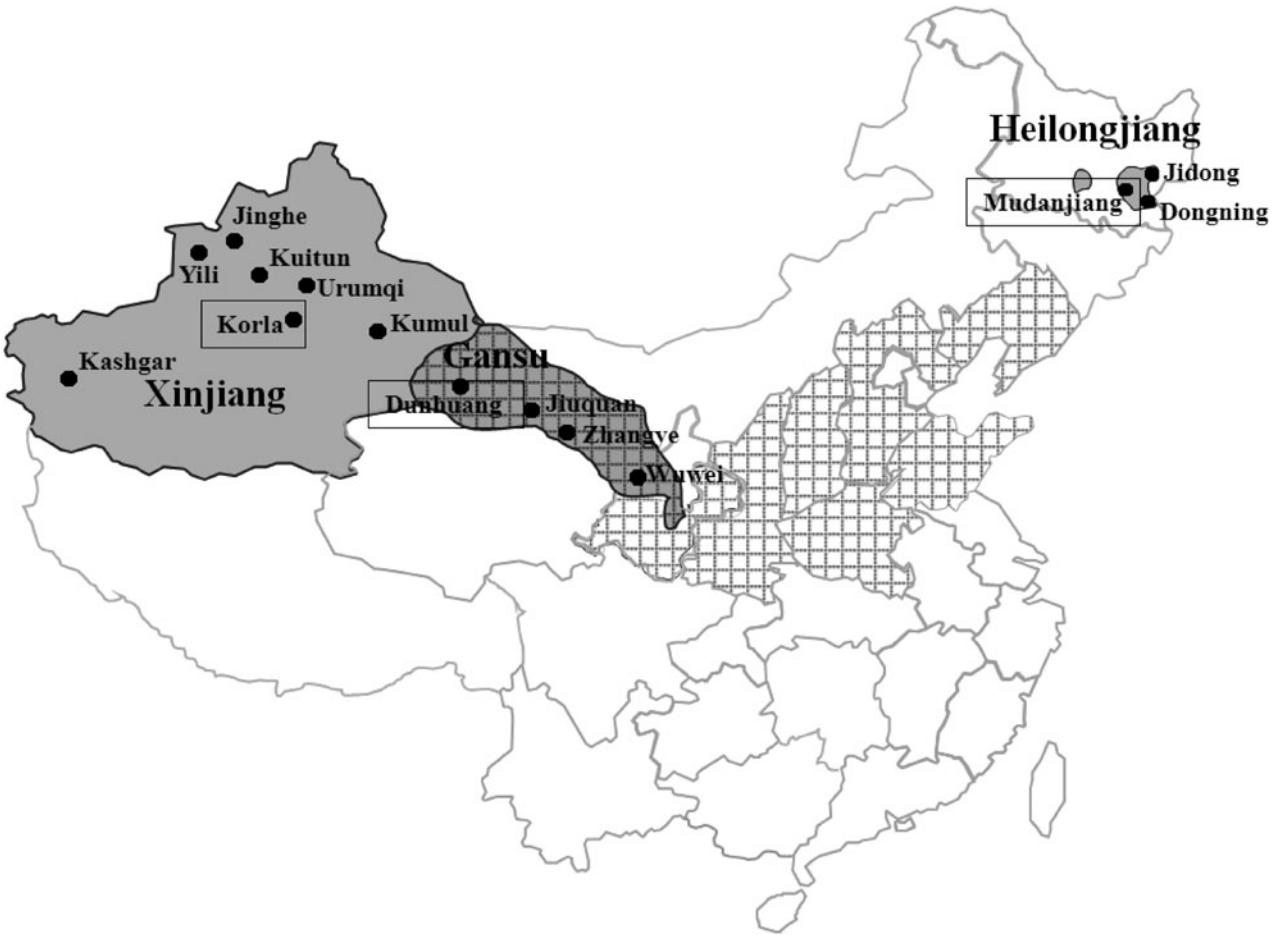

Fig. 1. Sampling regions of *Cydia pomonella* in Xinjiang, Gansu and Heilongjiang Provinces of China. The main distribution areas of *Cydia pomonella* are indicated in dark gray color, the major apple-growing areas of China are indicated with a grid square, and the overlapping regions of major apple-growing areas and *C. pomonella* distribution areas are indicated with a grid square in dark gray color. The name of the first reported site of *C. pomonella* in each of the three provinces is indicated in box.

Germany, Italy, Switzerland and UK)'. In this model, the 20 *C. pomonella* populations were divided into three groups: (1) Northwestern China (DH, JQ, ZY, WW, YL, JH, KT, WL, HM, KE and KS); (2) Northeastern China (DN, MDJ and JD); (3) European countries (AUS, BYE, GER, ITA, SWI and UK)'. The program NETWORK 4.6.1 to construct the Median-joining networks of mitochondrial DNA (mtDNA) haplotypes based on statistical parsimony (Bandelt *et al.*, 1999).

Results

Sequence variation

We obtained the COI (435 bp), COII (682 bp) and Cytb (710 bp) sequences from all the 370 individuals analyzed. No insertions or deletions were observed in either of the three gene regions. The COI gene contained 27 variable sites, 18 of which were parsimony informative. The COII gene covered 43 variable sites, 31 of which were parsimony informative sites, while 29 of the 52 polymorphic sites in Cytb gene were parsimony informative. Populations from China contained a total of 20 variable sites in the COI sequences, 27 variable sites in the COII sequences and 29 variable sites in the Cytb sequences, while *C. pomonella* populations from other countries exhibited 19 variable sites in the COI sequences, 33 variable sites in the COII sequences and 42 variable sites in the Cytb sequences.

Mitochondrial gene haplotypes

We observed 42 haplotypes for the COI gene (GenBank accession numbers are KJ789183 to KJ789224), 43 haplotypes for the COII gene (GenBank accession numbers are KJ789279 to KJ789321) and 53 haplotypes for Cytb gene (GenBank accession numbers are KJ789225 to KJ789278).

Four COI gene haplotypes (H2, H3, H4 and H8) were shared by samples from Northeastern China, Northwestern China and other countries. One COI haplotype (H1) was shared by populations from Northeastern China, Europe and South Africa. One COI haplotype (H11) was shared by populations from Northwestern China, Europe and South Africa. Three COI haplotypes (H29, H30 and H31) were shared by populations from Northeastern China. One COI haplotype (H35) was shared by Populations from Northwestern China. For the populations from northwestern China, Gansu

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Table 2. Distribution of *Cydia pomonella* mitochondrial haplotypes shared by different populations.

Gene	Haplotype	Number of individuals from populations of each region												Total (370)
		HLJ (53)	XJ (118)	GS (71)	AUS (14)	BYE (14)	GER (15)	ITA (14)	SWI (20)	ISR (11)	SA (14)	UK (6)	USA (20)	
COI	H1	4			3			1	1		6			15
	H2	1	25	2	1	2	4		5			1		41
	H3	5	1		4		3	6	6		2	2	19	48
	H4	13	3		2		1	1		1				21
	H7				1				2					3
	H8	9	2	1		2	1							15
	H11		59	44		2	2		2		1	2		112
	H13						1	4			1			6
	H29	5												5
	H30	5												5
	H31	8												8
	H35		12	22										34
	H37		2											2
COII	H2	1	87	34	1	2	4		8			1		138
	H4				3		1	1	2					7
	H7	13	5	2	1	5		7	3	1	6			43
	H8	8			5	5	1	1						20
	H10						2		3		1	2		8
	H13	5					2	4	1		1			13
	H14	6					2							8
	H22	4							1					5
	H29	2												2
	H30	3												3
	H32	2												2
	H33	2												2
	H38	1	2											3
	H40		13	24										37
	H42		7											7
Cytb	H2				1		4		6					11
	H5	27	16	25	7	10	1	7	3	3	8			107
	H6				1				1					2
	H10					1			2		1	2		6
	H12	3	3			1								7
	H16						3	4	2		5			14
	H17	3					2							5
	H24	1						1						2
	H29	5							1					6
	H45	1	35	2										38
	H47		60	34										94
	H51		1	1										2

The region and population codes are explained in Table 1, number in brackets indicates sample size of each region.

populations share one COI haplotype (H37), whereas no COI haplotype was only found in Xinjiang populations. Two COI haplotypes (H7 and H13) were shared by populations from other countries, but were not found in Chinese populations (table 2). The variable sites of haplotype H1 were included in haplotype AF497838 (GenBank accession number) obtained in European populations, while the variable sites of H2 were included in AF497841, of H3 in haplotype AF497836, of H4 in haplotype AF497842, of H13 in haplotype AF497837, of H16 in haplotype AF497844 and of H28 in haplotype AF497839 (Meraner *et al.*, 2008).

Two *C. pomonella* COII gene haplotypes (H2 and H7) were shared by samples from Northeastern China, Northwestern China and other countries. Four COII haplotypes (H8, H13, H14 and H22) were shared by populations of Northeastern China and other countries. Seven COII haplotypes were only reported in Chinese populations, of which two (H40 and H42) were only found in populations from Northwestern China, four (H29, H30, H32 and H33) were only found in northeastern Chinese populations, one (H38) was shared by northeastern and northwestern Chinese populations. Two COII haplotypes (H4 and H10) were only obtained in populations from other countries (table 2).

Two *C. pomonella* Cytb gene haplotypes (H5 and H12) were shared by populations from Northeastern China, Northwestern China and other countries. Three Cytb gene haplotypes (H17, H24 and H29) were found in northeastern Chinese and European populations. Four Cytb gene haplotypes (H2, H6, H10 and H16) were only found in populations from other countries. Three Cytb gene haplotypes (H45, H47 and H51) were only found in Chinese populations, of which two Cytb gene haplotypes (H47 and H51) were only reported in northwestern Chinese populations (table 2).

A total of 121 haplotypes were obtained with the combination of COI, COII and Cytb genes. Four haplotypes (H2, H3, H4 and H9) of the combined gene were shared among

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Table 3. Number of haplotypes of the combined COI, COII and Cytb gene shared between populations.

	DN (18)	MDJ (20)	JD (15)	YL (17)	JH (18)	KT (18)	WL (15)	HM (18)	KE (11)	KS (21)	DH (18)	JQ (19)	ZY (19)	WW (15)	AUS (14)	BYE (14)	GER (15)	ITA (14)	SWI (20)	ISR (11)	SA (14)	UK (6)	USA (20)
DN		4	2													1							
MDJ			2																				
JD				1	1	1				1				1			1						
YL					3	3	2	1		1	1	1	2	2									
JH						2	1	1	1	1	1	1	1	2									
KT							2	2	2	1	1	1	1	2									
WL								1	1		1	1	1	1									
HM									2		2	2	1	1									
KE											1	1	1										
KS																							
DH												2	1	1									
JQ													1										
ZY														1									
WW																							
AUS																	1		1				
BYE																							
GER																		1	2				
ITA																			1		2		
SWI																					2		
ISR																							
SA																						1	
UK																							
USA																							

The region and population codes are explained in Table 1, number in brackets indicates sample size of each region.

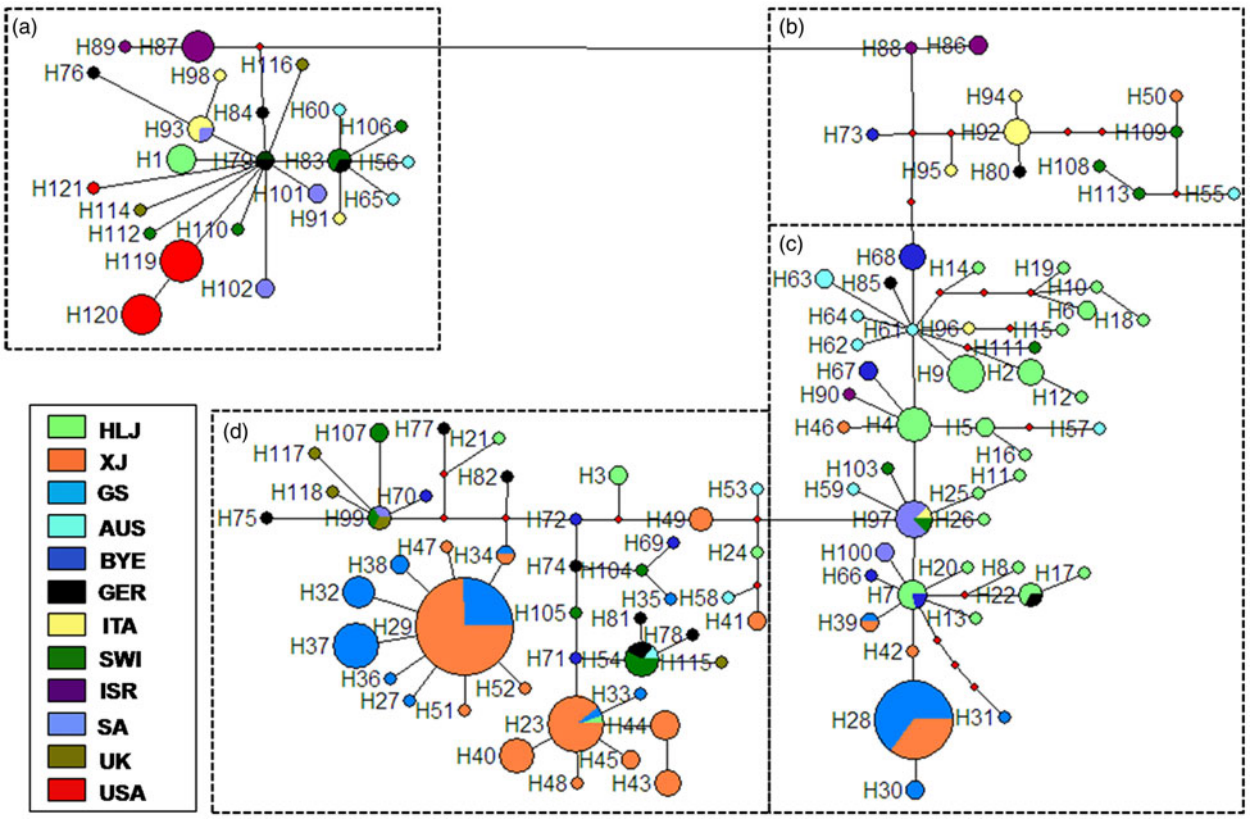

Fig. 2. Median-Joining network based on the combination of *C. pomonella* COI, COII and Cytb mtDNA haplotypes. Each circle represents a haplotype, and the area of a circle is proportional to the number of observed individuals. Colors within the nodes refer to the *C. pomonella* sampling regions. A, B, C and D indicate the four clades obtained.

populations from northeastern China, six haplotypes (H23, H28, H29, H34 H40 and H49) were shared among populations from Northwestern China, four haplotypes were shared among populations from different European countries (H54, H79, H83 and H97) (table 3 and fig. 2). One combined gene haplotype (H23) was shared by populations from Northeastern and Northwestern China, two haplotypes (H7 and H22) were shared by populations from Northeastern China and European countries, whereas no haplotype was shared by populations from Northwestern China and other countries. Samples from South Africa shared three haplotypes (H93, H97 and H99) with samples from Europe (table 3 and fig. 2).

Genetic diversity

The average number of COI haplotypes over the three populations from Northeastern China was 6.7, ranging from 6 to 7, while it was 3.3 over the 11 northwestern populations, ranging from 2 to 6. For COII, the mean number of haplotypes over the three northeastern Chinese populations was 9.3, ranging from 9 to 10, whereas it was 2.5 over all the 11 northwestern populations, ranging from 2 to 3. The mean number of haplotypes for Cytb over the three northeastern Chinese populations was 6.7, ranging from 5 to 9, whereas it was 3.3 for all the 11 northwestern populations, ranging from 2 to 6 (table 4). For the combined gene of COI, COII and Cytb, the average number of haplotypes for the three northeastern Chinese populations was 10.67, and for the 11 northwestern Chinese populations 4.45 (table 4). Among the 11 populations from Northwestern China, the population from Yili region, which was the first site where the codling moth was found in China, showed the highest number of haplotypes (six for COI, three for COII and six for Cytb) for the three genes (table 4). The seven field populations from the other countries also showed high numbers of gene haplotypes, with an average number of 6.7, ranging from 4 to 9 for COI, an average number of 6.4, ranging from 4 to 9 for COII, an average number of 6.4, ranging from 3 to 10 for Cytb, an average number of 10.29, and ranging from 6 to 15 for the combination of COI, COII and Cytb genes (table 4). Compared with the field populations from other countries, the two laboratory populations from Israel and the USA showed lower numbers of haplotypes for each of the three genes (table 4).

The haplotype diversity (H_d) ranged from 0.105 to 0.905 for COI, from 0.133 to 0.933 for COII and from 0.000 to 0.933 for Cytb (table 4). Similarly, compared with the northwestern Chinese populations, the three northeastern Chinese populations had higher haplotype diversity for the three genes. The average haplotype diversity for COI over the three northeastern Chinese populations was 0.815, and that over the 11 northwestern Chinese *C. pomonella* populations was 0.448. The mean COII haplotype diversity over the three northeastern Chinese populations was 0.859, and which over the

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Table 4. Measurement of genetic variation of 23 *Cydia pomonella* populations as revealed by three mitochondrial genes.

PC	*N*	COI				COII				Cytb				Combined gene			
		V	N_h	H_d	π(%)	*V*	N_h	H_d	π(%)	*V*	N_h	H_d	π(%)	*V*	N_h	H_d	π(%)
DN	18	7	7	0.784	0.350	18	10	0.850	0.458	8	9	0.797	0.175	29	13	0.928	0.302
MDJ	20	10	6	0.842	0.795	19	9	0.879	0.873	15	5	0.700	0.783	44	10	0.900	0.823
JD	15	7	7	0.819	0.438	9	9	0.848	0.258	7	6	0.648	0.180	23	9	0.848	0.271
YL	17	8	6	0.700	0.593	2	3	0.279	0.052	7	6	0.574	0.259	16	7	0.692	0.223
JH	18	7	4	0.471	0.319	3	3	0.216	0.061	5	3	0.464	0.232	15	4	0.471	0.188
KT	18	4	4	0.471	0.219	2	3	0.307	0.045	6	6	0.562	0.229	12	7	0.569	0.158
WL	15	7	4	0.371	0.241	1	2	0.133	0.019	2	2	0.133	0.038	10	4	0.371	0.079
HM	18	6	3	0.529	0.609	3	3	0.529	0.175	5	3	0.529	0.232	14	3	0.529	0.300
KE	11	1	2	0.436	0.100	1	2	0.432	0.061	4	2	0.426	0.246	6	2	0.436	0.142
KS	21	2	3	0.514	0.118	1	2	0.324	0.045	0	1	0.000	0.000	3	4	0.705	0.055
DH	18	6	3	0.621	0.724	2	3	0.582	0.149	3	2	0.529	0.224	11	4	0.673	0.315
JQ	19	6	2	0.351	0.484	2	2	0.351	0.099	3	2	0.351	0.148	11	2	0.351	0.210
ZY	19	5	2	0.105	0.121	2	3	0.556	0.088	5	3	0.292	0.087	12	4	0.614	0.096
WW	15	6	3	0.362	0.232	2	2	0.133	0.038	7	5	0.752	0.276	15	8	0.828	0.176
AUS	14	9	7	0.879	0.740	16	8	0.857	0.824	17	8	0.769	0.792	42	13	0.989	0.778
BYE	14	7	6	0.890	0.851	4	4	0.769	0.210	6	5	0.539	0.690	17	9	0.910	0.295
GER	15	10	9	0.905	0.771	17	9	0.914	0.746	21	9	0.905	0.942	48	14	0.990	0.832
ITA	14	7	6	0.769	0.541	12	5	0.659	0.786	15	5	0.703	0.952	34	9	0.901	0.797
SWI	20	12	9	0.858	0.818	17	8	0.816	0.697	17	10	0.889	0.801	45	15	0.958	0.764
ISR	11	5	3	0.600	0.961	11	2	0.182	0.283	14	4	0.673	0.917	35	5	0.709	0.598
SA	14	10	6	0.802	0.887	14	6	0.802	0.821	12	3	0.582	0.845	36	6	0.802	0.864
UK	6	5	4	0.867	0.582	13	5	0.933	0.905	15	5	0.933	1.005	33	6	1.000	0.873
USA	20	1	2	0.100	0.023	3	2	0.100	0.045	4	3	0.574	0.116	8	3	0.574	0.066

Combined gene, the combination of COI, COII and Cytb gene sequences; *N*, sample size of each population; *PC*, population code; *V*, polymorphic sites of all the sequences of a gene obtained in samples of each population; N_h, number of haplotypes; H_d, haplotype diversity; and π, nucleotide diversity.

11 northwestern Chinese *C. pomonella* populations was 0.349. For the Cytb haplotype diversity, the average value of haplotype diversity was 0.715 for the three northeastern Chinese populations, and 0.419 for the 11 northwestern Chinese *C. pomonella* populations. For the combined gene of COI, COII and Cytb genes, the average number of haplotype diversity for the three northeastern Chinese populations was 0.892, and for the 11 northwestern Chinese populations 0.567. The average haplotype diversity of the seven field populations from other countries showed the mean value of 0.853, 0.821, 0.760 and 0.936 for the COI, COII, Cytb and the combination of the three genes, respectively. However, the two laboratory populations from Israel and the USA showed lower average haplotype (table 4).

Using a two-tailed *t* test at the significance level 0.05, we tested whether the differences of number of haplotypes (N_h), haplotype diversity (H_d), nucleotide diversity (π), proportion of individuals with different haplotypes, proportion of individuals with private haplotypes and proportion of private haplotypes were significant between populations from the three provinces of China and the filed populations from other countries (table 5). The results showed that the mean number of haplotypes obtained in populations of Heilongjiang Province in Northeastern China was significantly higher than the respective mean number of haplotypes found in populations from the two northwestern Chinese Provinces, Gansu Province ($t = 2.06$, df = 26, $P < 0.001$) and Xinjiang Province ($t = 2.02$, df = 38, $P < 0.001$). Heilongjiang Province showed similar number of haplotypes to the field populations of the other countries ($t = 2.02$, df = 38, $P = 0.720$). Results of the two-tailed *t* test at the significance level 0.05 indicated that the northeastern Chinese populations showed significant higher number of haplotype diversity (H_d) ($t = 2.00$, df = 54, $P < 0.001$), nucleotide diversity (π) ($t = 2.00$, df = 54, $P < 0.001$), proportion of individuals with different haplotypes ($t = 2.23$, df = 10, $P = 0.001$), proportion of individuals with private haplotypes ($t = 2.23$, df = 10, $P = 0.031$) and proportion of private haplotypes ($t = 2.23$, df = 10, $P = 0.024$) than the northwestern populations.

Haplotype network of the combined mtDNA

The Median-Joining network of the haplotypes can be divided into four major clades (Clade A, B, C and D) (fig. 2). It is interesting that all the haplotypes in Clade A were obtained from samples of the other countries except that one (H1) was found in samples from northeastern China, while all the haplotypes in Clade B were from Central Europe except that one (H50) was in a sample from northwestern China. Most haplotypes from Northeastern China were included in Clade C, whereas most haplotypes from Northwestern China were deposited in Clade D.

Analysis of molecular variance

The AMOVA results based on the combination of the three gene sequences showed that there was no significant genetic variance among populations from the two northwestern provinces (Xinjiang and Gansu Provinces). However, significant genetic variances were found among populations from Northeastern and Northwestern China, with around 21.75% of the overall molecular variation explained by the two separate distribution areas of *C. pomonella*. Furthermore, in the model with populations grouped according to Northeastern

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Table 5. Number and proportion of haplotypes obtained from *Cydia pomonella* populations.

C	N	COI					COII					Cytb					Combined gene				
		N_h	P_1 (%)	N_p	P_2 (%)	P_3 (%)	N_h	P_1 (%)	N_p	P_2 (%)	P_3 (%)	N_h	P_1 (%)	N_p	P_2 (%)	P_3 (%)	N_h	P_1 (%)	N_p	P_2 (%)	P_3 (%)
HLJ	53	11	20.75	3	5.66	0.51	17	32.08	6	11.32	0.67	14	26.42	8	15.09	1.08	26	49.06	20	37.74	1.45
XJ	118	11	9.32	5	4.24	0.39	6	5.08	1	0.85	0.14	8	6.78	3	2.54	0.32	18	15.25	10	8.47	0.47
GS	71	5	7.04	1	1.41	0.28	4	5.63	1	1.41	0.35	7	9.86	3	4.23	0.60	14	19.72	9	12.68	0.91
AUS	14	7	50.00	2	14.29	2.04	8	57.14	4	28.57	3.57	8	57.14	5	35.71	4.46	13	92.86	12	85.71	6.59
BYE	14	6	42.86	3	21.43	3.57	4	28.57	3	21.43	5.36	5	35.71	2	14.29	2.86	9	64.29	8	57.14	6.35
GER	15	9	60.00	3	20.00	2.22	9	60.00	1	6.67	0.74	9	60.00	5	33.33	3.70	14	93.33	10	66.67	4.76
ITA	14	6	42.86	2	14.29	2.38	5	35.71	1	7.14	1.43	5	35.71	2	14.29	2.86	9	64.29	6	42.86	4.76
SWI	20	9	45.00	4	20.00	2.22	8	40.00	2	10.00	1.25	10	50.00	4	20.00	2.00	15	75.00	11	55.00	3.67
ISR	11	3	27.27	2	18.18	6.06	2	18.18	1	9.09	4.55	4	36.36	3	27.27	6.82	5	45.45	5	45.45	9.09
SA	14	6	42.86	2	14.29	2.38	6	42.86	3	21.43	3.57	3	21.43	0	0.00	0.00	6	42.86	3	21.43	3.57
UK	6	4	66.67	1	16.67	4.17	5	83.33	3	50.00	10.00	5	83.33	4	66.67	13.33	6	100.00	5	83.33	13.9
USA	20	2	10.00	1	5.00	2.50	2	10.00	2	10.00	5.00	3	15.00	3	15.00	5.00	3	15.00	3	15.00	5.00

Combined gene, the combination of COI, COII and Cytb gene sequences; N, number of *C. pomonella* individuals; N_h, number of haplotypes; $P_1 = N_h / N$, proportion of individuals with different haplotypes; N_p, number of private haplotypes; $P_2 = N_p / N$, proportion of individuals with private haplotypes; $P_3 = N_p / N_h$, proportion of private haplotypes.

China, Northwestern China and European countries, the genetic variance was significant (table 6).

Discussion

Using three mitochondrial genes, we investigated the genetic diversity of 242 *C. pomonella* individuals sampled throughout the main distribution areas in China, and 128 samples from nine other countries. Both the populations from Northeastern and Northwestern China shared most of the common haplotypes of the three genes with populations from Europe and other continents. Populations from Northeastern and Northwestern China showed a different population genetic structure, implying different invasion source of *C. pomonella* in the separate northeastern and northwestern distribution regions.

The geographic origin of the codling moth is presumably Europe, from where it subsequently spread throughout the world along with the culturing of apple and pear (Shel'Deshova, 1967; Boivin *et al*., 2004; Franck *et al*., 2007; Meraner *et al*., 2008; Thaler *et al*., 2008). The first report of this species in China was in Xinjiang Province in 1957 (Zhang, 1957), but its origin was unclear. In the present study, we found that Chinese codling moth populations shared most of the common haplotypes of COI (H1, H2, H3, H4, H8 and H11), COII (H2, H7 and H8) and Cytb (H5) genes with populations from Europe and other continents. Interestingly, the variable sites of seven COI haplotypes in our study were exactly the same as the overlapping regions of the respective seven COI haplotypes obtained in European populations (Meraner *et al*., 2008). Together with the shared haplotypes of the three genes between the Chinese and European populations in our analysis, the same variable sites of the COI haplotypes in our Chinese population and in the previously reported European populations indicate that *C. pomonella* populations in China have the same ancestor as European populations.

We found that the haplotypes of the three mitochondrial genes have a different distribution in Northeastern and Northwestern China. Considering the haplotypes found in Chinese populations, a total of 14 haplotypes (three COI haplotypes, eight COII haplotypes and three Cytb haplotypes) were shared by populations from northeastern, but were not found in northwestern populations. However, seven haplotypes (three COI haplotypes, two COII haplotypes and two Cytb haplotypes) were shared among northwestern populations, but were not obtained in northeastern populations. Moreover, a large number of the individuals from northwestern populations represented the same few haplotypes shared in this region (71 of the 118 individuals from Xinjiang and 66 of the 71 individuals from Gansu had COI H11 and H35 haplotypes, 100 individuals from Xinjiang and 58 individuals from Gansu had COII H2 and H40 haplotypes, and 95 individuals from Xinjiang and 36 individuals from Gansu had Cytb H45 and H47 haplotypes). On the other hand, our results demonstrated that *C. pomonella* populations from Northeastern China showed significantly higher numbers of haplotypes (N_h), haplotype diversities (H_d), nucleotide diversities (π), proportions of individuals with different haplotypes, proportions of individuals with private haplotypes and proportions of private haplotypes than populations from Northwestern China.

The distance between the northeastern and northwestern distribution regions of *C. pomonella* in China is 3000–5000 km. Although apple, pear, plum, walnut and other host plants

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Table 6. AMOVA based on the combination of COI, COII and Cytb genes to compare the genetic variation among *C. pomonella* populations using three models.

Model	Source of variation	Percentage of variation	Fixation indices	*P* value
(A) Populations from two northwestern provinces of China	Among groups	5.52	$F_{CT} = 0.05519$	$P = 0.180$
	Among populations within groups	33.42	$F_{SC} = 0.35370$	$P < 0.001$
	Within populations	61.06	$F_{ST} = 0.38937$	$P < 0.001$
(B) Populations from Northwestern and Northeastern China	Among groups	21.75	$F_{CT} = 0.21749$	$P < 0.05$
	Among populations within groups	22.99	$F_{SC} = 0.29385$	$P < 0.001$
	Within populations	55.26	$F_{ST} = 0.44743$	$P < 0.001$
(C) Populations from Northeastern China, Northwestern China and European countries	Among groups	29.81	$F_{CT} = 0.29805$	$P < 0.001$
	Among populations within groups	14.58	$F_{SC} = 0.20775$	$P < 0.001$
	Within populations	55.61	$F_{ST} = 0.44388$	$P < 0.001$

of *C. pomonella* are widely growing in the area considered to be the potential distribution area of the pest (Wan *et al.*, 2009; Zhang *et al.*, 2012), so far, we did not find the species in spite of intensive monitoring in different fruit orchards (apple, pear, plum, walnut, etc.) and along the highways. The weak flight capacity, geographical barriers and strictly enforced quarantine measures are considered the major factors responsible for having slowed down the expansion of *C. pomonella* from the northeastern and northwestern distribution region to the areas in-between (Wan *et al.*, 2009; Zhang *et al.*, 2012). Together with the different distribution pattern of the shared and private haplotypes in the northeastern and northwestern populations, the phenomenon that a large proportion of individuals from Northwestern China shared some haplotypes implied that the northeastern populations had a different invasion source than the northwestern populations. There is an important land port (Dongning Port) in the *C. pomonella* distribution regions in Northeastern China. This land port is near the far eastern region of Russia, where the codling moth has been documented (Willett *et al.*, 2009). The frequent trade between Dongning and the Russian far eastern region may aid the codling moth to invade to the neighboring apple-growing region of Northeastern China. However, both our previous microsatellite analysis and the current mitochondrial analysis show that *C. pomonella* populations in Xinjiang and Gansu Provinces have a similar population genetic background (Men *et al.*, 2013), but which differs from that in Heilongjiang Province. We expect that *C. pomonella* populations in the northwestern region of China came from Central Asia and spread from the Xinjiang to the Gansu Province (Men *et al.*, 2013). Different geographical populations of another serious invasive species, the wooly apple aphid *Eriosoma lanigerum*, which is damaging apple in different regions of China, showed the same invasion resource based on microsatellite data (Wu, 2009).

As in the previous microsatellite analysis (Men *et al.*, 2013), we found that the genetic diversity of populations from Northeastern China was similar to that of the native European *C. pomonella* populations. This can be explained by the more recent invasion history in the northeastern region. Populations in the northeastern regions were first found in 2006 around 9 years ago, whereas the northwestern populations were reported in 1957 around 60 years ago. The new invasive populations could retain fairly high levels of genetic diversity that still reflect that of the source population compared with older populations (Wan *et al.*, 2011; Inoue *et al.*, 2013). With time populations of an invasive species often lose their genetic diversity under selection or drift with range expansion and colonization of new areas (Ramachandran *et al.*, 2005; Herborg *et al.*, 2007; Dlugosch & Parker, 2008).

The codling moth has caused severe damage to apple and pear in the Chinese areas it invaded (Qin *et al.*, 2006; Wan *et al.*, 2009). In China, control of the codling moth relied on the use of broad spectrum insecticides, including organophosphate, carbamates and pyrethroids (Bahatiguli, 2009; Zhao, 2011), which are known to select for resistance to several insecticide groups (Knight *et al.*, 1994; Sauphanor *et al.*, 1998; Dunley & Welter, 2000; Fuentes-Contreras *et al.*, 2008; Reyes *et al.*, 2009). In addition, the frequent reliance and use of these insecticides are a constant threat to the environment and to human health. As food safety is becoming a major concern for consumers, the use of environment friendly tactics such as the SIT, mating disruption and the granulovirus will be increasingly required for the integrated pest management of *C. pomonella* (Bloem & Carpenter, 2001; Krafsur, 2005; Vreysen *et al.*, 2010). In this study, we found that the population genetic background of *C. pomonella* populations in the Northeastern and Northwestern China varies due to different invasion sources. This should be considered before applying new control tactics such as SIT (Bloem *et al.*, 2007; Vreysen *et al.*, 2010; Taret *et al.*, 2010).

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