Population genetic diversity and genetic structure of *Spodoptera exigua* around the Bohai Gulf area of China based on mitochondrial DNA signatures

L.-H. Zhou[1,2]*, X.-Y. Wang[3]* and J.-J. Lei[1]

[1]Department of Horticulture, Shenyang Agricultural University, Shenyang, China
[2]Liaoning Academy of Agricultural Sciences, Shenyang, China
[3]Wuxi Entry-Exit Inspection and Quarantine Bureau, Wuxi, China

*These authors contributed equally to this study.
Corresponding author: J.-J. Lei
E-mail: jiajunleisy@163.com

ABSTRACT. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is an economically important pest that causes major losses in some main crop-producing areas of China. To control this pest effectively, it is necessary to investigate its population genetic diversity and genetic structure around the Bohai Gulf area of China. In this study, we used two mitochondrial genes, *COI* (578 bp) and *Cytb* (724 bp), to investigate its genetic diversity. We obtained 622 *COI* sequences and 462 *Cytb* sequences from 23 populations, and 28 and 73 haplotypes, respectively, were identified. Low to moderate levels of genetic diversity (*COI*: Hd = 0.267 ± 0.023, Pi = 0.00082 ± 0.00010; *Cytb*: Hd = 0.689 ± 0.018, Pi = 0.00255 ± 0.00029) for the total populations were observed. Phylogenetic

and median-joining network analyses indicated no distinct geographical distribution pattern among the haplotypes. Overall, this study revealed that there was significant differentiation among the populations (*COI*: F_{ST} = 0.158, P < 0.001; *Cytb*: F_{ST} = 0.148, P < 0.001). F_{ST} values for Shenyang, Baoding, and Funing were significantly different to those for most of the other populations. Finally, unimodal mismatch distribution analysis, combined with negative neutrality test results, showed a recent population expansion of the beet armyworm around the Bohai Gulf area of China.

INTRODUCTION

The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is a worldwide polyphagous pest of crops that include cotton, soybean, maize, potato, tobacco, and many flowers (Adamczyk et al., 2003; Rizwan-ul-Haq et al., 2009). To the best of our knowledge, this species is native to Asia, but has spread worldwide and is now found almost anywhere its many host crops are grown (Wei et al., 2010). Generally, *S. exigua* larvae feed on plant foliage, leading to reduced yields and the eventual death of the host plant. In China, it was first recorded in Beijing in the 1890s. In recent years, with global warming and the adjustment of the agricultural planting structure, this pest has spread over most of the important crop-producing areas of China, and annual outbreaks cause severe economic losses to Chinese agricultural production (Luo et al., 2000). For example, the species seriously damaged Welsh onion production by infesting a total area of over 8000 hm^2 in Tianjin, reducing annual yields by 30% (Zheng et al., 2009; Zhu et al., 2010). *S. exigua* has a long history of insecticide exposure, and has developed resistance to many compounds, including chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids (Meinke and Ware, 1978; Chaufaux and Ferron, 1986). Therefore, it is difficult to effectively control this destructive pest.

It is evident that a good estimation of the level of genetic diversity and genetic structure can aid in the understanding of this pest's biology and ultimate control in an integrated pest management system (Timm et al., 2005). In recent years, many molecular markers have been used to investigate the phylogeny and biogeography of insect populations (Chatterjee and Tanushree, 2004; Behura, 2006). Due to their strictly maternal inheritance, high evolutionary rates, and lack of recombination, mitochondrial DNA genes have been a popular choice as markers for studying population genetic structures (Avise, 1991; Sunnucks, 2000; Behura, 2006; Feng et al., 2016). Both of the mitochondrial DNA genes cytochrome oxidase subunit I (*COI*) and cytochrome b (*Cytb*) have a moderate evolutionary rate, and a clear evolutionary pattern that is suitable for studies of phylogenetic evolution at the intra- and interspecific levels (Hebert et al., 2003). Previous studies have found low genetic variation, little genetic differentiation, and no significant population genetic structure among the different geographical populations identified for this species. However, these earlier studies used a limited number of molecular markers (Niu et al., 2006; Wang et al., 2014; Wang and Zhou, 2016). The area around the Bohai Gulf is one of the main crop-producing areas of China, and is a hotspot for the study of biological geography. However, little research has been conducted on the genetic

structure and demographic history of *S. exigua*, and to date, no population-level genetic studies have been conducted on this destructive pest in this region.

Our specific objectives for this study were the following: i) to investigate the genetic variability and genetic structure, and the extent of gene flow, among *S. exigua* populations around the Bohai Gulf area of China and ii) to elucidate its ecological adaptation and population history. The results of this study will help to establish a theoretical framework for the development of appropriate management strategies for this species.

MATERIAL AND METHODS

Sample collection

S. exigua populations were collected through an extensive field survey combined with trapping, using sex pheromone traps from 23 locations in five provinces around the Bohai Gulf area of China between June and September 2012. Additional samples of *S. exigua* from Shenyang were obtained between 2012 and 2015 (Table 1). Samples of *S. exigua* from Changdao were collected using a suction trap (Keyun ST-1B), which was developed by the Institute of Zoology, Chinese Academy of Sciences. To prevent the overrepresentation of siblings from each location, each larva was collected at least one meter from the neighboring one. All of the samples were preserved and stored at -20°C in 95% ethanol and deposited in the Horticultural Branch, Liaoning Academy of Agricultural Sciences in Shenyang, China. *Spodoptera depravata* (JX509780) and *Spodoptera exempta* (HQ177659) were used as outgroup species in the phylogenetic analysis.

Table 1. Sampling information of different geographical populations of *Spodoptera exigua* around the Bohai Gulf area of China.

Pop. No.	Pop. code	Sampling location	Sampling date	Latitude/Longitude	Insect stage	Altitude (m)
1	BD	Baoding, Hebei	September 2012	38.54°N, 115.51°E	Larva	21
2	FN	Funing, Qinhuangdao, Hebei	August 18, 2012	40.19°N, 119.61°E	Larva	19
3	HD	Cixian Handan, Hebei	September 2, 2012	36.44°N, 114.34°E	Larva	75
4	XL	Pengjia, Xinle, Hebei	August 20, 2012	38.37°N, 114.77°E	Adult	68
5	XT	Longyao, Xingtai, Hebei	September 1, 2012	37.38°N, 114.72°E	Larva	41
6	DAL	Jinzou, Dalian, Liaoning	August 2012	39.29°N, 121.95°E	Adult	37
7	FX	Xinqiu, Fuxin, Liaoning	August 22, 2012	42.07°N, 121.77°E	Larva	184
8	HLD	Lianshan, Huludao, Liaoning	August 20, 2012	40.88°N, 120.68°E	Larva	17
9	LY	Lingyuan, Liaoning	August 26, 2012	41.02°N, 119.06°E	Larva	453
10	SY2012	Shenyang, Liaoning	July 2012	41.82°N, 123.55°E	Adult	52
11	SY2013	Shenyang, Liaoning	July 2013	41.82°N, 123.55°E	Adult	52
12	SY2014	Shenyang, Liaoning	July 2014	41.82°N, 123.55°E	Adult	52
13	SY2015	Shenyang, Liaoning	July 2015	41.82°N, 123.55°E	Adult	52
14	AS	Taian, Anshan, Liaoning	August 17, 2012	41.34°N, 122.44°E	Adult	8
15	AQ	Anqiu, Shandong	August 2012	36.47°N, 119.33°E	Larva	48
16	BZ	Boxing, Binzhou Shandong	August 2012	37.19°N, 118.22 °E	Adult	7
17	CD	Changdao, Penglai, Shandong	August 2012	38.07°N, 120.75°E	Adult	8
18	HZ	Mudan, Heze, Shandong	August 4, 2012	35.18°N, 115.34°E	Larva	55
19	TA	Daiyuen, Taian, Shandong	August 2012	36.22°N, 117.38°E	Adult	145
20	TZ	Tengzhou, Shandong	August 4, 2012	35.09°N, 117.08°E	Larva	70
21	ZQ	Zaoyuan, Zhangqiu, Shandong	August 2012	36.70°N, 117.52°E	Adult	67
22	BJ	Haidian, Beijing	June 25, 2012	40.01°N, 116.27°E	Adult	68
23	TJ	Baodi, Tianjin	September 2012	39.58°N, 117.22°E	Larva	8

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Total DNA was extracted using a DNeasy® extraction kit (Qiagen, Valencia, CA, USA)

according to the manufacturer protocol, with a slight modification. A 700-bp *COI* fragment was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Favret and Voegtlin, 2004). PCR amplification of approximately 1000 bp of *Cytb* used the primers CP1 (5'-GATGATGAAATTTTGGATC-3') (Sezonlin et al., 2006) and TRs (5'-TATTTCTTTATTATGTTTTCAAAAC-3') (Simon et al., 1994). Each PCR mixture contained 0.25 µL *EasyTaq*® DNA polymerase (5 U/µL), 2.5 µL 10X *EasyTaq*® buffer, 0.5 µL dNTP mixture (2.5 mM of each), 0.5 µL of each primer (10 pM), 1 µL DNA template, and 19.75 µL distilled water, making a final volume of 25 µL. The reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the following conditions: 94°C for 5 min; 35 cycles at 94°C for 30 s, 54°C (*COI*)/46°C (*Cytb*) for 30 s, and 72°C for 45 s, with a final extension at 72°C for 5 min. The verification of a successful PCR amplification was assessed by agarose gel electrophoresis, and the PCR products of the partial *COI* and *Cytb* genes were purified and directly sequenced using the PCR primers. All of the specimens were sequenced on an Applied Biosystems ABI 3730 DNA sequencer (Applied Biosystems). Sequence chromatograms, including sense and antisense, were edited and assembled using DNASTAR 5.0 (DNASTAR Inc., Madison, WI, USA) to obtain a single consensus sequence.

Data analyses

Sequence variation and genetic diversity

The sequences were preliminarily aligned using the CLUSTAL X 1.81 software program (Thompson et al., 1997), and subsequently aligned manually. Nucleotide compositions, conserved sites, variable sites, parsimony informative sites, and distances between DNA sequences based on Kimura's two-parameter method were calculated using MEGA 5.2 (Tamura et al., 2011). Nucleotide diversity (*Hd*) and haplotype diversity (*Pi*) were estimated using the program DnaSP 4.0 (Rozas et al., 2003).

Phylogenetic and network relationships among haplotypes

Bayesian inference (BI) analyses were used to identify major clades, and to evaluate the relationships among the haplotypes separately. The analysis was performed using PAUP* 4.10b (Swofford, 2003). ModelTest 3.7 (Posada and Crandall, 2001) and the Akaike information criterion (Posada and Buckley, 2004) were used to identify the appropriate nucleotide substitution models, and the selected models of sequence evolution were used for BI phylogeny reconstruction. The analysis used a random starting tree and proceeded for one million Markov chain Monte Carlo generations. Trees were sampled every 100 generations. The first 2500 generations (25% of the total) were later discarded as burn-in. Fifty-percent majority-rule consensus trees were generated from the remaining trees, and the posterior probabilities were computed. Compared with conventional phylogenetic trees, haplotype networks can identify the relationships among haplotypes and are preferable for intraspecific analyses. Unrooted networks for the *COI* and *Cytb* haplotypes were constructed using NETWORK 2.0 by the median-joining method (Bandelt et al., 1999).

Genetic differentiation and population genetic structure

The levels of genetic differentiation between pairs of populations were estimated using pairwise F_{ST} values (Weir and Cockerham, 1984) computed after 10,000 permutations in Arlequin 3.0 (Excoffier et al., 2007). Analysis of molecular variance (AMOVA) was also conducted using Arlequin. The significance of the variance component estimates for the different hierarchical subdivisions were determined after 1000 non-parametric permutations.

Population demographic history and neutrality test

Demographic history changes were analyzed for *S. exigua* using two neutrality tests, Tajima's *D* (Tajima, 1989) and Fu's *FS* (Fu, 1997), which were calculated from the total number of segregating sites. They were used to assess the evidence for population expansion, when negative values would be expected (Aris-Brosou and Excoffier, 1996). Estimation and testing were performed by bootstrap resampling (10,000 replicates) using Arlequin. Neutrality tests were used as an indication of recent population expansion, when the null hypothesis of neutrality is rejected due to significant negative values ($P < 0.05$ for Tajima's *D* and Fu's *Fs*). According to coalescent theory, a population usually exhibits a unimodal mismatch distribution following a recent population demographic or range expansion. Mismatch distributions that were based on the difference between the observed and expected mismatch distributions were used as a test statistic.

RESULTS

Sequence variation and genetic diversity

A region of 518 bases from the mtDNA *COI* gene was isolated from 622 individuals, which were collected from 23 locations around the Bohai Gulf area of China during 2012-2015. Of a total of 578 characters, 549 sites were conserved and 29 sites were variable (5.60% of the total length), including 12 singleton polymorphic sites and 17 parsimonious-informative sites. On average, the *COI* sequences were found to be A-T rich: 39.1% T, 15.8% C, 31.1% A, and 14.0% G. In addition, the analysis identified 28 haplotypes (Hap_1-Hap_28), which were deposited into the GenBank database (accession Nos. KX576873-KX576900). The average number of haplotypes in a single population was 2.91 ± 2.13, with a range of 1-10 (Table 2). The highest number of haplotypes was found in Shenyang in 2015, with 10 haplotypes in 28 individuals. Twelve haplotypes were shared by at least two populations. Hap_12 was both the most widespread and the most common haplotype, and was shared by 531 samples in the analyzed populations. Hap_3 and Hap_19 were shared by 9 and 23, respectively, of the populations analyzed.

For the *Cytb* sequence, a region of 712 bases was sequenced from 462 individuals, which were collected from 23 locations. This region contained 584 conserved sites and 128 variable sites (17.98% of the total length), including 82 singleton polymorphic sites and 46 parsimonious-informative sites; therefore, the *Cytb* gene was more variable than the *COI* gene. These sequences were similarly heavily biased towards A and T nucleotides, and averaged 33.1% T, 11.0% C, 43.0% A, and 13.1% G. We defined 73 haplotypes (HAP_1-HAP_73), which were deposited in the GenBank database (accession Nos. KX576901-KX576973).

Table 2. Genetic diversity and neutrality test among different populations of *Spodoptera exigua* around the Bohai Gulf area of China based on mtDNA *COI* and *Cytb* sequences.

Code	mtDNA *COI* sequencing					mtDNA *Cytb* sequencing				
	COI (N/H)	*Hd* ± SD	*Pi* ± SD	Tajima's *D*	Fu's *F$_S$*	*Cytb* (N/H)	*Hd* ± SD	*Pi* ± SD	Tajima's *D*	Fu's *F$_S$*
BD	23/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	15/11	0.905 ± 0.072	0.00942 ± 0.00244	-2.396***	-2.156
FN	18/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	22/8	0.775 ± 0.068	0.00222 ± 0.00060	-1.794	-2.758
HD	26/2	0.077 ± 0.070	0.00013 ± 0.00012	-1.156	-1.094	25/7	0.487 ± 0.121	0.00151 ± 0.00070	-2.095*	-2.750
XL	16/2	0.125 ± 0.106	0.00022 ± 0.00018	-1.162	-0.700	11/2	0.182 ± 0.144	0.00025 ± 0.00020	-1.129	-0.410
XT	31/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	19/2	0.105 ± 0.092	0.00015 ± 0.00013	-1.165	-0.838
DAL	31/2	0.065 ± 0.059	0.00011 ± 0.00010	-1.145	-1.239	16/2	0.400 ± 0.114	0.00055 ± 0.00016	0.650	0.872
FX	35/3	0.215 ± 0.089	0.00058 ± 0.00026	-1.226	-0.621	27/7	0.507 ± 0.115	0.00237 ± 0.00095	-2.134*	-1.104
HLD	32/5	0.290 ± 0.103	0.00084 ± 0.00084	-1.629	-2.500	30/5	0.492 ± 0.100	0.00103 ± 0.00029	-1.450	-1.406
LY	8/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	6/4	0.800 ± 0.172	0.00691 ± 0.00249	-1.472	1.179
SY2012	36/3	0.475 ± 0.064	0.00089 ± 0.00015	0.122	0.229	12/3	0.318 ± 0.164	0.00046 ± 0.00025	-1.451	-1.325
SY2013	43/4	0.218 ± 0.082	0.00117 ± 0.00058	-2.078	-0.229	36/5	0.389 ± 0.098	0.00073 ± 0.00022	-1.461	-2.145
SY2014	37/4	0.206 ± 0.087	0.00072 ± 0.00033	-1.381	-1.477	31/4	0.346 ± 0.104	0.00077 ± 0.00028	-1.497	-0.930
SY2015	57/10	0.500 ± 0.079	0.00158 ± 0.00032	-1.616	-5.630	27/7	0.595 ± 0.104	0.00669 ± 0.00257	-1.026	2.456
AS	24/4	0.239 ± 0.113	0.00058 ± 0.00031	-1.884*	-2.331	19/8	0.614 ± 0.130	0.00230 ± 0.00094	-2.287**	-3.011
AQ	14/2	0.143 ± 0.119	0.00049 ± 0.00041	-1.481	0.296	18/5	0.641 ± 0.097	0.00126 ± 0.00032	-1.173	-1.485
BZ	24/4	0.543 ± 0.085	0.00204 ± 0.00037	-0.349	0.582	16/6	0.733 ± 0.102	0.00152 ± 0.00033	-0.890	-2.383
CD	25/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	20/6	0.747 ± 0.074	0.00147 ± 0.00028	-0.752	-2.075
HZ	19/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	9/3	0.417 ± 0.191	0.00184 ± 0.00114	-1.728*	0.909
TA	30/4	0.251 ± 0.102	0.00067 ± 0.00030	-1.585	0.963	28/4	0.323 ± 0.108	0.00084 ± 0.00030	-1.074	-0.832
TZ	20/2	0.100 ± 0.088	0.00017 ± 0.00015	-1.164	-0.879	19/4	0.323 ± 0.108	0.00084 ± 0.00030	-1.074	-0.832
ZQ	29/1	0.000 ± 0.000	0.00000 ± 0.00000	0.000	0.000	24/10	0.822 ± 0.061	0.00319 ± 0.00081	-1.980	-3.202
BJ	14/6	0.791 ± 0.089	0.00510 ± 0.00152	-0.586	0.047	17/7	0.765 ± 0.094	0.00498 ± 0.00191	-0.925	0.154
TJ	30/3	0.131 ± 0.082	0.00035 ± 0.00023	-1.732	-1.627	14/7	0.802 ± 0.094	0.00331 ± 0.00149	-1.846*	-1.530
Total	622/28	0.267 ± 0.023	0.00082 ± 0.00010	-2.291**	-30.900	462/73	0.689 ± 0.018	0.00255 ± 0.00029	-2.729***	-105.229

N, Number of samples; H, number of haplotypes; *Hd*, haplotype diversity; *Pi*, nucleotide diversity; *Pi*, nucleotide diversity; SD, standard deviation; *P < 0.05, **P < 0.01, ***P < 0.001.

The average number of haplotypes per population was 8.33 ± 2.47, with a range of 2-11 (Table 2). The highest number of haplotypes was found in Baoding, where we defined 11 haplotypes in 15 individuals. Twelve haplotypes were shared by at least two populations, and HAP_13 was both the most widespread and the most common. HAP_13 was shared by 224 samples in the 23 populations analyzed. HAP_41 was found in 127 individuals. The HAP_9 haplotype was shared by 12 individuals in five populations. HAP_30, HAP_38, HAP_56, and HAP_61 were also found frequently, each being shared by 2, 7, 7, and 7 individuals in 2, 3, 5, and 5, respectively, of the populations analyzed.

Population genetic diversity was also estimated in terms of *Hd* and *Pi* in the 23 locations surveyed (Table 2). In contrast to some other Noctuidae pest species, such as *Helicoverpa armigera* (Behere et al., 2013), *Lampides boeticus* (Lohman et al., 2008), and *Busseola segeta* (Ong'amo et al., 2012), all of the 23 populations exhibited relatively high *Hd* and low *Pi*, based on the *COI* and *Cytb* genes obtained. Overall, the average *Hd* based on the *COI* gene was 0.267, and ranged from 0 for Baoding, Funing, Xingtai, Lingyuan, Changdao, Heze, and Zhangqiu to 0.791 for Beijing. The average *Pi* was 0.00082, and ranged from 0 to 0.00510 in Beijing. In contrast, the *Cytb* sequence exhibited moderate genetic diversity in all of the populations surveyed (*Hd* = 0.689 ± 0.018; *Pi* = 0.00255 ± 0.00029). The highest genetic diversity estimate was obtained for Baoding (*Hd* = 0.905 ± 0.072; *Pi* = 0.00942 ± 0.00244), and the lowest for Xingtai (*Hd* = 0.105 ± 0.092; *Pi* = 0.00015 ± 0.00013). The population genetic diversity of *S. exigua* in Shenyang during 2012-2015, based on the two mtDNA genes, is shown in Figure 1. For the *COI* sequence, the *Hd* ranged from 0.206 in 2014 to 0.500 in 2015, while the *Pi* ranged from 0.00072 in 2014 to 0.00158 in 2015. For the *Cytb* sequence, the *Hd* ranged from 0.318 in 2012 to 0.595 in 2015, and the *Pi* ranged from 0.00046 in 2012 to 0.00669 in 2015. Therefore, during the four-year study period, the highest genetic diversity was found in 2015.
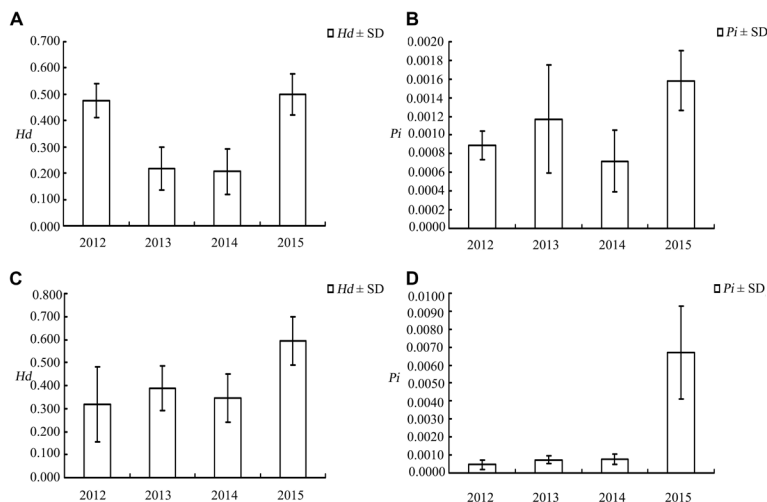

Figure 1. Population genetic diversity of *Spodoptera exigua* in Shenyang, North China during 2012-2015. mtDNA *COI*: **A.** haplotype diversity; **B.** nucleotide diversity; mtDNA *Cytb*: **C.** haplotype diversity; **D.** nucleotide diversity. Mitochondrial DNA (mtDNA); Cytochrome oxidase subunit I (*COI*); *Cytb* (Cytochrome *b*).

Genetic distance among populations

In a pairwise comparison, the genetic distances between the populations were calculated using the Kimura two-parameter method, and are shown in Table 3. For the *COI* sequence, the mean pairwise sequence difference between the populations was 0.1%, and ranged from 0 to 0.4%. The average pairwise sequence difference between the haplotypes was 0.9%, and ranged from 0.2 to 2.5%. For the *Cytb* sequence, the mean pairwise sequence difference between the populations was 0.3%, and ranged from 0.1 to 0.5%. The average pairwise sequence difference between the haplotypes was 0.19%, and ranged from 0 to 8.6%.

Overall, there were no statistically significant differences in genetic distance or small pairwise sequence between pairs of the populations. Figure 2 shows the unweighted pair group method with arithmetic mean dendrogram of the different geographical populations of *S. exigua* around the Bohai Gulf area of China, based on mtDNA *COI* and *Cytb* sequences. The data indicate that there was a discrepancy between the genetic and geographical distances. The data generated two clades, with one clade only containing Beijing, Binzhou, and Baoding.

Phylogenetic and network relationships among haplotypes

A phylogenetic analysis was conducted to determine the relationships between the 28 *COI* and 73 *Cytb* haplotypes of *S. exigua*, and to identify any discernible groups in terms of their geographical distributions. The results from ModelTest indicated that the best substitution models were TVM + I for *COI* and HKY + I + G for *Cytb*. For the *COI* sequences, a Bayesian analysis generated two clades, and simultaneously generated many unresolved branches (Figure 3A). The resulting median-joining network was similar to the topology of the phylogenetic tree, with 15 haplotypes connected to the common Hap_12 by only one mutation. The network exhibited a star-shaped genealogy, which is often associated with demographic expansion. This result is similar to the topology of the phylogenetic tree (Figure 3B). Among the populations, the largest clade was composed of haplotypes from seven locations, and these locations covered more than 1000 km. These results suggest that a random association of *COI* haplotypes of *S. exigua* exists, indicating high gene flow in this species.

The phylogenetic trees generated by the BI method for the 73 *Cytb* haplotypes were generally compatible, with the only difference being the relative positions and the statistical support possibilities of some branches. The topology based on *Cytb* is presented in Figure 4. The phylogenetic results showed that one discernible clade was detected among the 73 haplotypes, despite the low bootstrap percentages from the BI analysis (Figure 4A). It also simultaneously generated many unresolved branches. The median-joining network was similar to the topology of the phylogenetic tree based on the *Cytb* haplotypes (Figure 4B). In all cases, there was no distinct geographical distribution pattern among the haplotypes.

Genetic differentiation among populations

The pairwise F_{ST} values between populations for the two mtDNA genes are presented in Table 4. For the *COI* gene, the F_{ST} values ranged from -0.06 to 0.61, with statistically significant genetic differentiation detected in 46 comparisons ($P < 0.05$). The F_{ST} values for Shenyang (SY2012) and Beijing were significantly different to those for the other 21 locations, suggesting low gene flow between these two populations and the remaining populations.

Table 3. Kimura's two-parameter genetic distance between populations of *Spodoptera exigua* around the Bohai Gulf area of China based on *COI* sequences (below diagonal) and *Cytb* sequences (above diagonal).

	BD*	FN	HD	XL	XT	DAL	FX	HLD	LY	SY2012	SY2013	SY2014	SY2015	AS	AQ	BZ	CD	HZ	TA	TZ	ZQ	BJ	TJ
BD		0.006	0.006	0.005	0.005	0.005	0.006	0.005	0.008	0.006	0.006	0.006	0.009	0.006	0.006	0.006	0.006	0.006	0.005	0.006	0.007	0.008	0.007
FN	0.000		0.002	0.001	0.002	0.001	0.003	0.002	0.005	0.002	0.002	0.002	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.004	0.003
HD	0.001	0.001		0.001	0.001	0.001	0.002	0.001	0.004	0.002	0.002	0.002	0.005	0.002	0.001	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.002
XL	0.000	0.001	0.001		0.001	0.000	0.001	0.004	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.003	0.002
XT	0.000	0.002	0.001	0.000		0.001	0.001	0.001	0.001	0.002	0.002	0.002	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.003	0.002
DAL	0.000	0.001	0.001	0.000	0.000		0.002	0.001	0.004	0.003	0.002	0.001	0.004	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.004	0.003
FX	0.000	0.000	0.001	0.000	0.000	0.000		0.002	0.005	0.003	0.002	0.003	0.006	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.004	0.003
HLD	0.000	0.000	0.000	0.001	0.000	0.000	0.001		0.004	0.002	0.003	0.002	0.005	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.002
LY	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.005	0.006	0.005	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.004	0.005
SY2012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.001		0.001	0.001	0.004	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.004	0.003
SY2013	0.001	0.001	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.002		0.001	0.004	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003
SY2014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.002	0.001		0.004	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003
SY2015	0.001	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001		0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.006	0.005	0.006
AS	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001		0.002	0.002	0.002	0.002	0.002	0.001	0.003	0.002	0.003
AQ	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.001	0.000	0.001	0.001	0.001	0.001	0.001		0.001	0.001	0.002	0.001	0.001	0.002	0.001	0.002
BZ	0.000	0.000	0.000	0.001	0.001	0.001	0.002	0.001	0.000	0.003	0.002	0.002	0.002	0.002	0.002		0.001	0.002	0.001	0.002	0.003	0.003	0.002
CD	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.005	0.000	0.001	0.001		0.002	0.001	0.002	0.002	0.003	0.003
HZ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.000		0.002	0.002	0.006	0.004	0.003
TA	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.002	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000		0.001	0.002	0.003	0.002
TZ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000		0.003	0.003	0.003
ZQ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.003		0.004	0.003
BJ	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003	0.004	0.003	0.003	0.004	0.003	0.003	0.003	0.003	0.003		0.004
TJ	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.003	0.001	0.000	0.000	0.003	

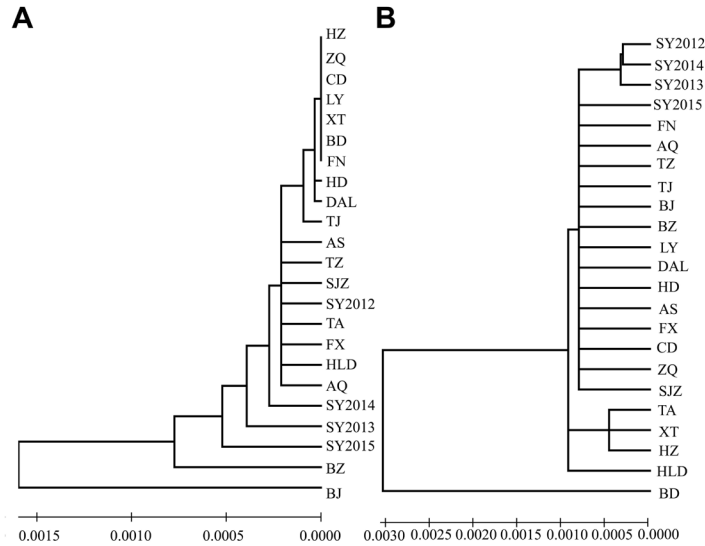*Population codes are given in Table 1.

Figure 2. Unweighted pair group method with arithmetic mean dendrogram of different geographical populations of *Spodoptera exigua* around the Bohai Gulf area of China based on mtDNA *COI* sequences (**A**) and mtDNA *Cytb* sequences (**B**). Mitochondrial DNA (mtDNA); Cytochrome oxidase subunit I (*COI*); *Cytb* (Cytochrome *b*).
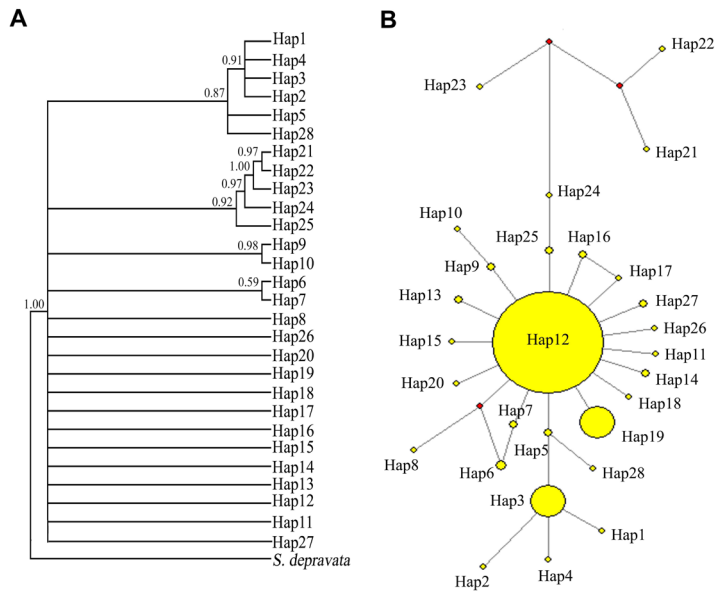

Figure 3. Bayesian phylogeny (**A**) and haplotype network (**B**) among haplotypes of mtDNA *COI* sequences. Posterior probabilities of Bayesian analysis are shown above the branch (best-fit model TVM + I). Only posterior probability values greater than 0.5 are indicated at each node. *Spodoptera depravata* (JX509780) was used as an outgroup species in the phylogenetic analysis. Each haplotype is represented by a circle, the size of which indicates the relative frequency of the corresponding haplotypes in the complete dataset. Missing haplotypes in the network are represented by dots.

Figure 4. Bayesian phylogeny (**A**) and haplotype network (**B**) among haplotypes of mtDNA *Cytb* sequences. Posterior probabilities of Bayesian analysis are shown above the branch (best-fit model HKY + I + G). Only posterior probability values greater than 0.5 are indicated at each node. *Spodoptera depravata* (HQ177659) was used as an outgroup species in the phylogenetic analysis. Each haplotype is represented by a circle, the size of which indicates the relative frequency of the corresponding haplotypes in the complete dataset. Missing haplotypes in the network are represented by dots.

For the *Cytb* gene, the F_{ST} values ranged from -0.017 to 0.345, and statistically significant genetic differentiation was detected in 142 of 253 comparisons (P < 0.05). The F_{ST} values for Baoding, Funing, and Shenyang (SY2012-SY2015) were significantly different to those for most of the other locations.

Population genetic structure

The results of the AMOVA are shown in Table 5. Variation within populations (84.17-85.23%) was greater than that among populations (14.77-15.83%), and the level of population differentiation was highly significant after 1000 random permutations (*COI*: F_{ST} = 0.158, P < 0.001; *Cytb*: F_{ST} = 0.148, P < 0.001).

Neutrality test and demographic history

Neutrality tests were conducted using Tajima's *D* and Fu's *Fs* statistics. Tajima's *D* is a selective neutrality test that decides whether the mean number of differences between pairs of DNA sequences is consistent with the observed number of segregating sites in a sample.

Table 4. Population pairwise F_{ST} values based on *COI* sequences (below diagonal) and *Cytb* sequences (above diagonal) between populations of *Spodoptera exigua* around the Bohai Gulf area of China.

|	BD^	FN	HD	XL	XT	DAL	FX	HLD	LY	SY2012	SY2013	SY2014	SY2015	AS	AQ	BZ	CD	HZ	TA	TZ	ZQ	BJ	TJ
BD		0.09*	0.04*	-0.01	0.03*	0.03*	0.04*	0.05*	-0.01	0.20*	0.29*	0.28*	0.14*	0.02*	0.05*	0.03*	0.05*	-0.02	0.06*	0.05*	0.05*	0.05*	0.02*
FN	0.00		0.09*	0.09	0.21*	0.03	0.10*	0.18*	0.10*	0.16*	0.14*	0.14*	0.07	0.08*	0.00	0.08*	0.08*	0.13*	0.20*	0.01	0.10*	0.06*	0.03
HD	0.00	-0.01		-0.04	0.01	-0.01	0.01	0.03*	0.10	0.47*	0.43*	0.43*	0.17*	-0.01	0.02	0.00	0.03	0.01	0.04*	0.02	0.04*	0.04	0.02
XL	0.02	0.01	0.01		0.01	0.00	-0.02	0.01	0.08	0.76*	0.58*	0.58*	0.13*	-0.04	0.04	0.04	0.02	0.02	0.01	0.01	0.01	0.04	0.01
XT	0.00	0.00	0.01	0.04		0.18*	0.01	0.04	0.20*	0.84*	0.67*	0.68*	0.21*	0.01	0.17*	0.13*	0.07	0.07	0.02	0.12*	0.04*	0.11*	0.09*
DAL	-0.01	-0.02	0.00	0.01	0.00		0.02	0.08*	0.12	0.60*	0.44*	0.45*	0.11*	0.00	-0.03	0.04*	0.02	0.09	0.11*	-0.03	0.04	0.04	0.00
FX	0.01	0.00	0.02	0.01	0.02	0.02		0.01	0.08	0.39*	0.40*	0.39*	0.17*	-0.02	0.04	0.01	0.02	0.01	0.03*	0.03	0.01	0.06*	-0.01
HLD	0.01	0.00	0.01	0.00	0.02	0.02	-0.01		0.17*	0.60*	0.54*	0.54*	0.23*	0.00	0.10*	0.06*	0.09*	0.06	0.04	0.09*	0.07*	0.10*	0.08*
LY	0.00	0.00	-0.06	-0.05	0.00	-0.06	-0.04	-0.05		0.33*	0.42*	0.40*	0.10	0.06	0.10	0.06	0.11	0.04	0.20*	0.09*	0.08	0.03	0.03
SY2012	0.58*	0.56*	0.57*	0.47*	0.61*	0.59*	0.51*	0.47*	0.49*		0.00	-0.01	0.03	0.40*	0.38*	0.47*	0.45*	0.57*	0.65*	0.34*	0.32*	0.19*	0.26*
SY2013	0.01	0.00	0.01	0.00	0.02	0.02	-0.01	-0.01	-0.05	0.42*		-0.02	0.07*	0.40*	0.31*	0.42*	0.42*	0.55*	0.59*	0.30*	0.35*	0.24*	0.28*
SY2014	0.02	0.01	0.02	0.01	0.03	0.03	-0.01	-0.01	-0.04	0.49*	-0.02		0.07*	0.40*	0.31*	0.41	0.41*	0.54*	0.59*	0.30*	0.34*	0.23*	0.27*
SY2015	0.03	0.02	0.04*	0.02	0.04*	0.04*	0.01	-0.01	-0.02	0.38*	0.00	0.00		0.15*	0.09	0.13	0.14*	0.15*	0.24*	0.10*	0.16*	0.10*	0.08*
AS	0.00	-0.01	0.00	-0.03	0.01	0.01	-0.01	-0.01	-0.06	0.46*	-0.01	-0.01	0.01		0.02	0.01	0.02	0.00	0.03*	0.01	0.02	0.05*	-0.01
AQ	0.04	0.02	0.02	0.00	0.06	0.03	-0.04	-0.04	-0.05	0.49*	-0.04	-0.05	-0.03	-0.04		0.01	0.03	0.10	0.13*	-0.02	0.05*	0.03	0.00
BZ	0.25	0.22	0.25	0.20	0.29	0.28*	0.17	0.14	0.14	0.45*	0.11	0.13	0.06	0.16	0.10		0.06	0.06	0.10*	0.04	0.06	0.02	0.02
CD	0.00	0.00	0.00	0.03	0.00	-0.01	0.02	0.01	0.00	0.59*	0.01	0.02	0.04	0.00	0.04	0.26		0.06*	0.07*	0.01	-0.01	0.07*	0.02
HZ	0.00	0.00	-0.01	0.01	0.00	-0.02	0.00	0.00	0.00	0.56*	0.00	0.01	0.03	-0.01	0.02	0.23	0.00		0.06	0.07*	0.02*	0.05*	0.03*
TA	0.02	0.01	0.02	0.01	0.04	0.03	0.01	0.01	-0.04	0.49*	0.01	0.01	0.03	0.00	-0.01	0.19	0.03	0.01		0.11	0.05	0.12	0.09
TZ	0.01	-0.01	0.00	-0.06	0.02	0.00	0.01	0.01	-0.06	0.50*	0.00	0.02	0.03	-0.02	0.01	0.22	0.01	0.00	0.02		0.03	0.04	-0.01
ZQ	0.00	0.00	0.00	0.04	0.00	0.00	0.02	0.02	0.00	0.60*	0.01	0.03	0.04	0.01	0.06	0.28	0.00	0.00	0.03	0.02		0.07*	0.01
BJ	0.21*	0.17*	0.21*	0.15*	0.25*	0.24*	0.19*	0.17*	0.08*	0.38*	0.13*	0.16*	0.14*	0.16*	0.10*	0.09	0.22*	0.18*	0.19*	0.18*	0.24*		0.02
TJ	-0.01	-0.02	0.00	-0.01	0.00	0.00	-0.01	-0.02	-0.06	0.54*	-0.01	-0.01	0.01	-0.02	-0.04	0.20	-0.01	-0.02	0.01	-0.01	0.00	0.20*	

Population codes are given in Table 1; *P < 0.05.

Table 5. Analysis of molecular variance of different geographical populations of *Spodoptera exigua* around the Bohai Gulf area of China based on mtDNA *COI* and *Cytb* sequences.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation (%)	*F*-statistic
mtDNA *COI*					
Among populations	22	26.818	0.038 Va	15.83	$F_{ST} = 0.158$***
Within populations	599	120.656	0.201 Vb	84.17	
Total	621	147.475	0.239		
mtDNA *Cytb*					
Among populations	22	79.132	0.138 Va	14.77	$F_{ST} = 0.148$***
Within populations	439	349.759	0.799 Vb	85.23	
Total	461	428.891	0.937		

d.f., degrees of freedom; Va, variance components among populations; Vb, variance components within populations; ***P < 0.001.

       A negative Tajima's *D* signifies an excess of low frequency polymorphisms, indicating population size expansion. Correspondingly, large negative *Fs* values are interpreted as evidence of population expansion. The results of the neutrality tests (*COI*: Tajima's *D* = -2.291, P < 0.01, Fu's F_S = -30.900; *Cytb*: Tajima's *D* = -2.729, P < 0.001, Fu's F_S = -105.229), combined with the unimodal mismatch distribution, indicate a recent population expansion of *S. exigua* around the Bohai Gulf (Figure 5). In addition, the median-joining network analysis of *COI* and *Cytb* revealed "star-like" networks, which also indicate population expansion.
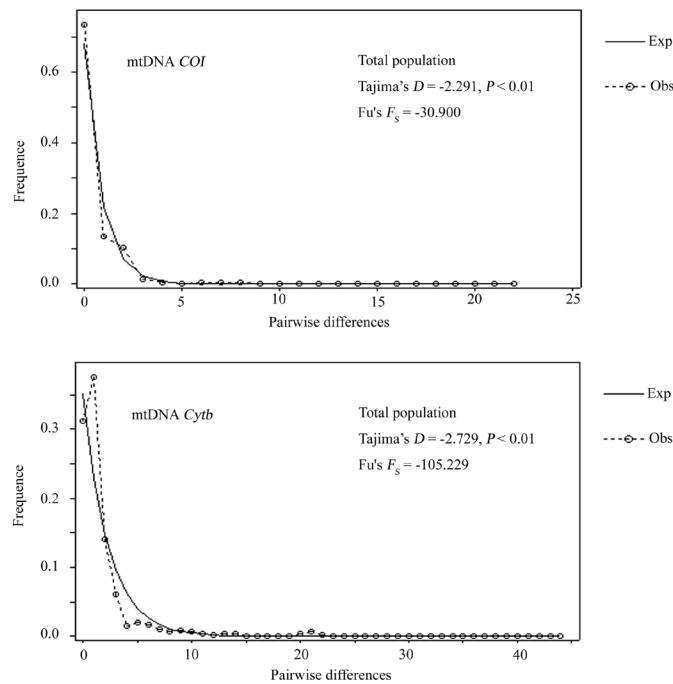
Figure 5. Mismatch distribution and neutrality test results of Tajima's *D* and Fu's F_S tests for sampling locations of *Spodoptera exigua* around the Bohai Gulf area of China. Gray line, expected; dashed line with circles, observed.

DISCUSSION

Genetic variability is an adaptive process, and one of the fundamental mechanisms upon which evolution depends. The investigation of intraspecific genetic variation can uncover new putative species, while providing insights into the evolutionary origin and history of extant populations (Lohman et al., 2008). In the present study, a low to moderate level of genetic diversity of *S. exigua* around the Bohai Gulf, based on mtDNA *COI* and *Cytb* gene sequences, was established. This result is consistent with those of previous studies (Niu et al., 2006; Wang et al., 2014; Wang and Zhou, 2016). Several studies have also reported low genetic diversity in other Lepidoptera species, such as *Arctia caja* and *Lampides boeticus* (Anderson et al., 2008; Lohman et al., 2008). This may be due to a short history of population expansion that has not accumulated more genetic variation within the short diffusion history of this region. This pest was originally found in South Asia, and quickly spread to the main crop-producing areas of Hebei, Henan, Shandong, and Shaanxi before the 1980s. Since the late 1980s, it has become widely distributed in more than 20 provinces of China. In 2000, it was first reported on vegetables and soybean plants in Liaoning Province, Northeast China. The highest level of genetic diversity was found in Beijing and Baoding, probably because the species has not experienced serious genetic bottlenecks or founder effects, or there has been a lack of selection pressure (e.g., insecticides).

Although the phylogenetic analysis revealed the presence of some primary inclusive groups based on the two mtDNA gene sequences, there was no distinct geographical distribution pattern among the mitochondrial haplotypes. Compared to conventional phylogenetic trees, haplotype networks can identify relationships among haplotypes and are preferable for intraspecific analyses, because ancestral haplotypes may still be extant (Posada and Crandall, 2001). The median-joining haplotype networks did not reveal any geographical clustering of the haplotypes, which indicates that *S. exigua* haplotypes exhibit a random association. Shared haplotypes were observed among the regions, particularly the most common Hap_12 (mtDNA *COI*) and HAP_13 (mtDNA *Cytb*) haplotypes, which were distributed in most local populations, indicating that gene flow has occurred among this species' populations in recent history. This finding blurs the original relationships between the different populations.

Understanding the dispersal ability of pest species is critical for theoretical aspects of evolutionary and population biology and for practical applications, including implementing effective forecasting systems. The beet armyworm is an economically important pest, but there is little information available on its dispersal ability. The results of this study may be particularly applicable to other migratory insects, such as *Spodoptera litura* and other noctuid pests. Our results show that beet armyworm populations in these regions lack significant differences, suggesting that extensive gene flow has occurred. *S. exigua* is a particularly polyphagous species that has been observed on almost 300 different host plant species from various botanical families, indicating that it has a strong adaptive capacity. However, finding a lack of genetic differentiation prevented us from quantifying migration rates between the populations, and the frequency of long-distance migration events. In general, species capable of active dispersal will show little genetic differentiation among populations. Again, this may be particularly true for migratory insects, such as *S. litura* and other noctuid pests. In this study, there was no statistically significant genetic differentiation among some populations, as evidenced by the high values of N_m (*COI*: $N_m = 1.332$; *Cytb*: $N_m = 1.439$). This is consistent with the results of Niu et al. (2006) and Wang et al. (2014), and may be the result of either recent

or rapid population expansion after a population bottleneck, or selection acting on some part of the mitochondrial genome.

A thorough understanding of the population genetic organization of pests can provide important biological information that can be directed toward their eventual control by broadening our knowledge regarding their modes of occurrence, migrations among locations, and the patterns of geographical variation among locations (Miller et al., 2003). Dispersal capability, geographical barriers, and other factors can also affect the population structure of a species (Avise et al., 1987). There is no obvious habitat loss, habitat fragmentation, host discontinuous distribution, or climatic barrier in the main crop-producing areas around the Bohai Gulf. Therefore, this species is predisposed to limited genetic differentiation in this region. AMOVA based on mtDNA *Cytb* showed that most of the genetic variation was within populations with the reminder occurring between populations, suggesting that there is significant genetic differentiation within the populations. Overall, the interpopulation and interindividual variation significantly affected the total variability.

The values of Tajima's *D* and Fu's *Fs* in a selective neutrality test decide whether the mean number of differences between pairs of DNA sequences is compatible with the observed number of segregating sites in a sample. Significantly negative values of these statistics indicate an excess of low-frequency variants, which can result from population expansion, weak negative selection, or positive selection. In contrast, significantly positive values of these statistics reflect an excess of intermediate-frequency alleles, which can result from population bottlenecks, structure, and/or balancing selection. A demographic analysis of the populations revealed a unimodal mismatch distribution and a significantly negative Fu's *Fs* value, indicating that there was a single expansion of the *S. exigua* range around the Bohai Gulf.

This is the first study to investigate the population genetics of *S. exigua* around the Bohai Gulf area of China using two mitochondrial DNA gene sequences, and the results may provide useful data for forecasting outbreaks and managing this species in China. Because of the large amount of damage inflicted on agricultural crops and the high risk of the pest spreading, particularly given how extensively *S. exigua* is distributed throughout China, immediate action is needed to monitor and formulate strategies for its management.

Resistance to insecticides by insects is a prime example of evolutionary adaptation to environmental change, and a superb demonstration of natural selection being affected by humans. Although this species rapidly develops resistance to insecticides, the application of synthetic insecticides is currently the most commonly used control strategy, and insecticide resistance alleles that arise in the southern region might spread quickly into the northern region by migration. Over the past several decades, pest management programs, such as cultural controls, biological controls, and the use of insecticides, have been conducted in China, and have partially prevented the spread of *S. exigua*. However, the species has developed resistance to several groups of insecticides (Meinke and Ware, 1978; Chaufaux and Ferron, 1986). High gene flow among beet armyworm populations may explain the rapid spread of insecticide-resistant genes, and many resistance characteristics also accumulate through long-distance migration (Bouvier et al., 2001). Therefore, identifying populations of *S. exigua* that exhibit resistance to insecticides at large temporal and spatial scales, investigating the distribution of resistance genes relative to genetic structure and the level of gene flow between populations of *S. exigua* in China, and quantifying the level of resistance to insecticides are important for guiding the application of chemical pesticides. Increasing our understanding of the population dynamics of *S. exigua* may improve predictions of its outbreaks and enhance management

efforts. In general, population scouting is an important component of *S. exigua* management, and field surveys with sex pheromone traps are the standard sampling method for *S. exigua*. In the future, we will focus on monitoring the population dynamics of this species, and determine rules for its seasonal occurrence in crop-producing areas of China. In addition, intensive local sampling is necessary to correctly estimate genetic diversity, and detailed investigations of the phylogenetic and phylogeographical patterns of *S. exigua* need to be undertaken. More robust inferences of the evolutionary history of *S. exigua* will require additional samplings throughout China. Longer, or more, gene sequences, as well as more rapidly evolving markers, such as microsatellites, should be used in future, in order to better understand the population genetic structure and demographic history of *S. exigua* (Yin and Ji, 2013; Zhao et al., 2015).

Conflicts of interest

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

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