RESEARCH

Carposina sasakii (Lepidoptera: Carposinidae) in its Native Range Consists of Two Sympatric Cryptic Lineages as Revealed by Mitochondrial *COI* Gene Sequences

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Subject Editor: Maria Gabriela Murúa

J. Insect Sci. *(2015)* 15(1): 85; DOI: 10.1093/jisesa/iev063

ABSTRACT. The genetic differentiation and genetic structure of the peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), was investigated in China, where the moth is native. The mitochondrial cytochrome c oxidase I (*COI*) gene of 180 individuals from 16 collections were sequenced and analyzed. The results showed that two sympatric and cryptic mtDNA lineages existed within *C. sasakii* in China. The genetic differentiation has significant correlation with the geographical distance, but has no evidence for host plant associations. Our results of haplotype distribution suggest that the *C. sasakii* individuals can naturally move between areas, while the movement of individuals between long-distance locations may be associated with human activities such as the transport of fruit. Finally, an mitochondrial *COI* gene PCR-RFLP method was developed to differentiate the two cryptic mtDNA lineages within *C. sasakii*, which provides rapid and reliable tool for the future research of the two lineages.

Key Words: *Carposina sasakii* Matsumura, genetic differentiation, cryptic mtDNA lineages, mitochondrial *COI* gene

The peach fruit moth, *Carposina sasakii* Matsumura, is an important fruit pest in Korea, Japan, China, and Russia Far East (Liu et al. 1997; Kim et al. 2000). The moth larvae can damage more than 20 kinds of fruit including peach, apricot, hawthorn, apple, pear, jujube, and wild jujube (Kim et al. 2001; Kim and Lee 2002; Ishiguri and Shirai 2004; Xu and Hua 2004).

In China, *C. sasakii* has been recorded from 24 provinces (Liu et al. 1997). Some collections of this pest were considered host biotypes (Hua and Hua 1995) because emergence time, oviposition habitat, and damage characteristics seemed to be specific to the *C. sasakii* associated with particular hosts. The genetic differentiation of these purported host biotypes has been studied using esterase isozyme patterns (Hua and Hua 1995) and random amplified polymorphic DNA (RAPD) (Xu and Hua 2004). Using RAPD method, Xu and Hua (2004) documented genetic differentiation among *C. sasakii* collected from apple, hawthorn, peach, apricot, jujube, and wild jujube. The specimens associated with apricot differed the most markedly from other host races, and the authors suggested that it should be considered as a good species. However, the genetic differentiation of the pest throughout China has not been well studied, which is indispensable to understand the evolution of the pest in its native ranges.

The aim of this research was to investigate the genetic structure of the peach fruit moth and its relationship with geographical distance and host plants by using the mitochondrial cytochrome c oxidase I (*COI*) gene as a marker. The mitochondrial *COI* gene marker has been widely used for species identification, for determining the genetic structure and differentiation (De Barro et al. 2011). In this article, we first analyze the genetic differentiation and haplotype distribution of *C. sasakii* in China, the native area of the pest, using samples collected from a range of host plants. Second, we calculate the genetic diversity of different host collections. Third, the correlation between genetic distance and geographical distance was analyzed. Finally, an mitochondrial *COI* gene PCR-RFLP method was developed to distinguish between the lineages within *C. sasakii.*

Materials and Methods

Insect Samples. In the autumn of 2010, *C. sasakii* larvae were collected from a range of orchards in 16 main fruit-growing regions in China (Fig. 1). Collected specimens were first identified morphologically followed the description by Liu et al. (2011). In total, there were 16 collections (Table 1). The larvae were put in 95% ethanol and then stored at −20°C prior to DNA extraction.

DNA Extraction, PCR, and Mitochondrial Sequencing. DNA was extracted from a single larva using the DNAzol kit (Molecular Research Center, Inc., Cincinnati, OH) following the manufacturer's specifications. *COI* fragments were amplified from 180 individuals using the primers LCO1490 (5′- GGTCAACAAATCATAAA GATATTGG -3′) and HCO2198 (5′- TAAACTTCAGGGTGACCA AAAAATCA -3′) (Folmer et al. 1994). Each PCR reaction (25 µl) contained 2 µl of template DNA, 1 unit of *Taq* polymerase (extracted from Thermus Aquaticus) (Biomed biotechnology Co. Ltd), 2.5 µl of $MgCl_2$ (2.5 mmol/l), 2 µl of dNTPs (10 mmol/l), 1 µl of 20 µmol/l of each primer, and 2.5 µl of 10 × PCR buffer. PCR amplifications began with 94°C denaturation for 5 min; followed by 35 cycles of 94°C denaturation for 1 min, 52°C annealing for 1 min, and 72°C extension for 2 min; and a final 72°C extension for 10 min. The PCR product was checked by agarose gel electrophoresis. PCR should have yielded an ∼710 bp fragment (Folmer et al. 1994). The PCR product was purified and then sequenced directly. The 180 sequences were aligned with Clustal W (Thompson et al. 1994) and were then checked for insertion and deletion (indel). The final 588-bp sequences (the alignment length) were used to analyze the lineage-specific restriction enzyme sites and the genetic structure of collection.DNA identification

As precise identification of young and/or old larvae of *C. sasakii* and similar fruits-infesting species are difficult or may be impossible practically (Sony et al. 2009; Hada and Sekine 2011), we conducted a phylogenetic analysis using the *COI* sequences collected in the present study and the homologous GenBank sequences of *C. sasakii* as well allied species available by 1 July 2012. Based on the resulting

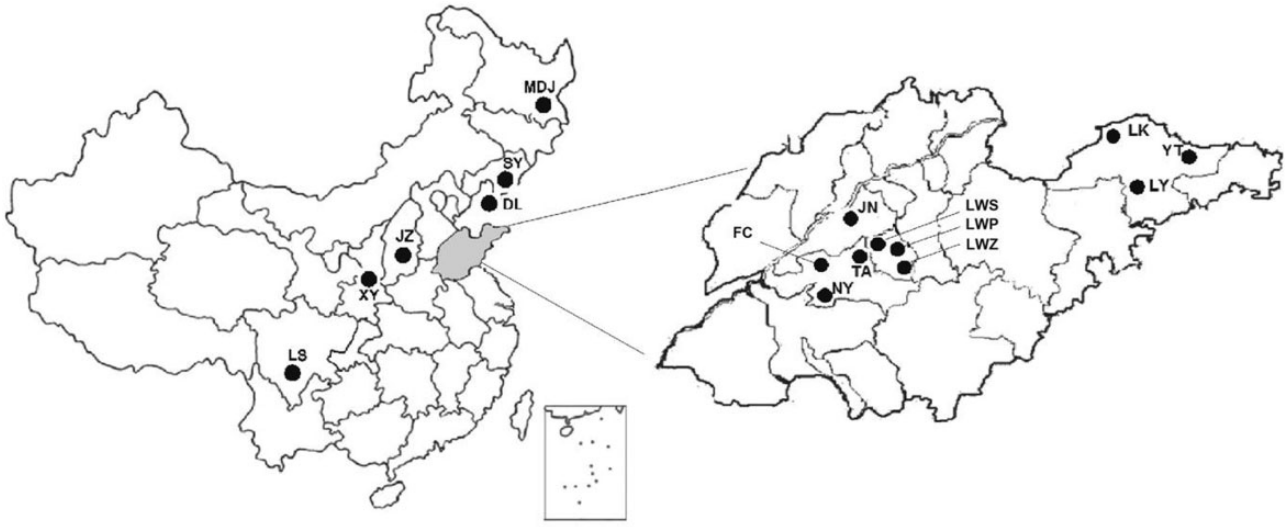

Fig. 1. The 16 collection sites of *Carposina sasakii* in China.

Table 1. *Carposina sasakii* collections from China

Collection code	Collection site	Host plant	Collection date	Lineages markers					
				Individuals of *COI* sequences			Individuals of *COI* PCR-RFLP		
				Total	Lineage I	Lineage II	Total	Lineage I	Lineage II
DL	DaLian, LiaoNing	Apple	8/2010	12	10	2	12	10	2
SY	ShenYang, LiaoNing	Apple	9/2010	6	4	2	6	4	2
MDJ	MuDanJiang, HeiLongJiang	Apple	8/2010	8	2	6	8	2	6
XY	XianYang, ShaanXi	Apple	9/2010	8	0	8	8	0	8
JZ	JinZhong, ShanXi	Jujube	9/2010	5	0	5	5	0	5
LS	LiangShan, SiChuan	Apple	9/2010	5	0	5	5	0	5
LK	LongKou, ShanDong	Apple	8/2010	17	17	0	17	17	0
NY	NingYang, ShanDong	Jujube	9/2010	11	11	0	11	11	0
FC	FeiCheng, ShanDong	Peach	8/2010	19	18	1	19	18	1
LY	LaiYang, ShanDong	Apple	9/2010	18	8	10	18	8	10
JN	JiNan, ShanDong	Apple	9/2010	11	7	4	11	7	4
TA	TaiAn, ShanDong	Jujube	9/2010	4	4	0	4	4	0
YT	YanTai, ShanDong	Apple	9/2010	16	16	0	16	16	0
LWP	LaiWu, ShanDong	Apple	9/2010	13	0	13	13	0	13
LWZ	LaiWu, ShanDong	Jujube	9/2010	8	0	8	8	0	8
LWS	LaiWu, ShanDong	Wild jujube	9/2010	19	0	19	19	0	19

Note: all specimens were larvae and were collected in October 2010.

phylogenetic tree, the haplotypes were involved in the monophyletic group of "*C. sasakii*" were retained and considered as *C. sasakii.*

Genetic Structure Analysis. A Median joining (MJ) network was calculated and drawn with the program NETWORK 4.5 (Bandelt et al. 1999) to investigate the possible relationships among haplotyes of *C. sasakii.* Genetic parameters for mitochondrial were estimated for the different collections from China using DnaSP 5.0 (Librado and Rozas 2009). The parameters included the number of polymorphic (segregating) sites (*S*), the total number of mutations (η), the average number of nucleotide differences (*K*), the number of haplotypes (*H*), haplotype diversity (*Hd*), nucleotide diversity (π), defined as the average number of pairwise nucleotide differences per site), and the nucleotide diversity with Jukes and Cantor correction [π (JC)] within each collection and region.

The ARLEQUIN version 3.1 (Excoffier et al. 2005) was used for pairwise F_{ST} estimation (a measure of genetic differentiation between populations) among populations, and for analysis of molecular variance (AMOVA), and pairwise haplotype distances (*P*-distance) were calculated by Mega 5.0 (Tamura et al. 2011). Gene flow within and among populations was approximated as *Nm* [analogous to $M = (1/F_{ST} - 1)/2$] (Slatkin 1993), where *Nm* is a measure of the extent of gene flow in an island model at equilibrium and was calculated by Dnasp 5.0 (Librado and Rozas 2009).

The correlation between F_{ST} and geographical distance of all populations was estimated using the Mantel test of IBD.1.5.2 (isolation-by-distance) (Bohonak 2002). The regression was made using F_{ST} against geographical distance. Pairwise geographic distances (straight-line distance) between collections were calculated by longitude and latitude.

Identification of Lineage-Specific Restriction Enzyme Sites and Determination of Lineages Based on Mitochondrial COI Gene PCR-RFLP. The nucleotide differences in the 37 mitochondrial *COI* gene haplotype sequences that were detected were analyzed to select a restriction endonuclease for distinguishing among sequences. A restriction endonuclease, BsaJI (NEB, MA) that cleaves DNA at "CCNNGG" sites was selected. All 180 individuals from the 16 collections were used to test the utility of the BsaJI-based PCR-RFLP. A 10-μl volume of PCR product was digested at 60°C for 2 h with 2 units of BsaJI. The BsaJI-digested PCR products were electrophoresed on 1.0% agarosegel and visualized by ethidium bromide staining. Based on the sizes of bands produced by BsaJI digestion, the lineage of each individual was determined.

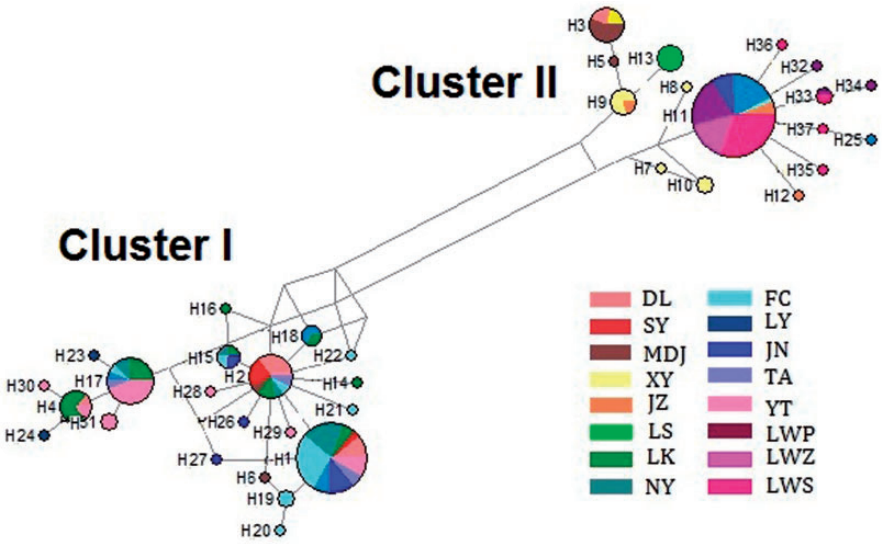

Fig. 2. The *C. sasakii* mitochondrial *COI* gene network constructed with the program Network 4.5. The area of each colored pie diagram reflects the number of individuals of each haplotype, and colored segments represent the proportion or the number of haplotypes that occur from each sampling collection. The code of the haplotype is "Hap" plus "number".

Table 2. Pairwise F_{ST} (below diagonal) and gene flow (above diagonal) of *C. sasakii* based on *COI* sequences

	DL	SY	MDJ	XY	JZ	LS	LK	NY	FC	LY	JN	TA	YT	LWP	LWZ	LWS
DL		9.70	0.65	0.40	0.31	0.17	2.74	4.53	3.79	1.25	2.31	10.52	1.80	0.13	0.14	0.11
SY	**0.00000**		1.27	0.55	0.33	0.16	2.31	1.50	1.90	1.98	2.35	2.23	1.80	0.15	0.13	0.14
MDJ	0.37681	0.16112		0.56	0.32	0.19	0.53	0.28	0.36	0.95	0.61	0.41	0.52	0.31	0.13	0.13
XY	**0.51438**	0.38829	0.39714		1.08	0.31	0.31	0.21	0.30	1.76	0.78	0.28	0.27	0.32	0.31	0.30
JZ	**0.62385**	**0.52766**	**0.56308**	**0.21385**		0.11	0.27	0.15	0.27	3.38	0.84	0.15	0.24	2.01	1.63	2.22
LS	**0.81488**	**0.72941**	**0.73810**	**0.63004**	**0.78125**		0.16	0.07	0.15	0.60	0.28	0.04	0.14	0.03	0.00	0.04
LK	**0.08991**	**0.08636**	**0.45371**	**0.60307**	**0.69130**	**0.86544**		1.45	1.03	0.85	1.06	3.65	8.71	0.11	0.13	0.09
NY	**0.01647**	0.11159	0.58228	0.66086	**0.74322**	**0.91560**	**0.21984**		10.34	0.85	1.49	24.26	1.03	0.06	0.06	0.06
FC	**0.05138**	0.12721	0.56450	0.61423	**0.69482**	**0.87948**	**0.28721**	-0.01760		0.84	2.09	9.56	0.79	0.11	0.13	0.09
LY	**0.25926**	**0.18882**	**0.35322**	0.20743	**0.12797**	0.63507	**0.33569**	**0.37825**	**0.33337**		5.36	1.73	0.78	1.17	1.52	1.00
JN	**0.09776**	0.07679	0.39931	**0.33578**	**0.35777**	**0.71313**	**0.25590**	**0.17610**	**0.12009**	**0.02274**		2.73	0.87	0.35	0.40	0.32
TA	**0.00000**	**0.00976**	**0.52194**	**0.62139**	**0.70955**	0.88889	**0.06685**	**0.00000**	**0.00000**	**0.32784**	**0.12829**		2.44	0.07	0.04	0.08
YT	**0.15457**	**0.12551**	**0.44640**	**0.62450**	**0.70690**	0.87677	**0.00000**	**0.29089**	**0.35222**	**0.36120**	**0.30312**	0.13410		0.10	0.11	0.08
LWP	**0.77385**	**0.69380**	**0.73601**	**0.52660**	**0.00751**	0.95965	**0.82595**	**0.87726**	**0.83275**	0.29233	**0.53040**	0.84784	0.83692		5.03	8.08
LWZ	**0.80036**	**0.71837**	0.76190	**0.56277**	**0.00000**	**1.00000**	**0.85242**	**0.90612**	**0.86111**	**0.30971**	0.55625	**0.87619**	**0.86353**	**0.04167**		15.19
LWS	0.77565	0.69467	0.73590	0.52902	**0.00000**	**0.96377**	**0.82748**	0.87987	0.83553	0.28872	0.53144	**0.85017**	**0.83858**	**0.00000**	0.00000	

The values in bold indicates $P < 0.05$.

Results

Genetic Differentiation and Gene Flow Between Collections. A total of 37 haplotypes of mitochondrial *COI* gene were detected and deposited in GenBank (JN974425–JN974452, KC815966–KC815974), and they were grouped into two clusters as showed by the haplotype network (Fig. 2). One cluster (code: cluster I) included 21 haplotypes (H1, H2, H4, H6, H14–H24, and H26–H31) that were distributed in 10 of the 16 host collections; cluster I haplotypes were detected in samples from MuDanJiang (MDJ), DaLian (DL), ShenYang (SY), LongKou (LK), NingYang (NY), FeiCheng (FC), LaiYang (LY), JiNan (JN), TaiAn (TA), and YanTai (YT). Another cluster (code: cluster II) included 16 haplotypes (H3, H5, H25, H7–H13, H32–H37) distributed in 12 of the 16 host collections; cluster II haplotypes were detected in samples from MuDanJiang (MDJ), DaLian (DL), ShenYang (SY), XianYang (XY), JinZhong (JZ), JiNan (JN), FeiCheng (FC), LaiYang (LY), LaiWu (LWP, LWS and LWZ), and LiangShan (LS).

The four collections labeled as LK, NY, TA, and YT contained only cluster I haplotypes, and these were designated as lineage I. The six collections labeled as XY, JZ, LS, LWP, LWZ, and LWS contained only cluster II haplotypes, and these were designated as lineage II. The other six collections (DL, SY, MDJ, FC, LY, and JN) contained a mixture of cluster I and II haplotypes. Both cluster I and II haplotypes were found in the collections from apple, jujube, and peach, only cluster II were included in wild jujube.

When F_{ST} ranged from 0 to 0.05, the genetic differentiation is small; F_{ST} ranged from 0.05 to 0.15, the genetic differentiation between populations is moderate; F_{ST} ranged from 0.15 to 0.25, the genetic differentiation is large; $F_{ST} > 0.25$, the genetic differentiation is very large (Weight 1978). Among the 120 pairwise F_{ST} (Table 2), 85 values were higher than 0.25 and 87 values were significant, indicating that most of populations were highly differentiated from other populations. Correlation between geographic distance and genetic distance was tested by isolation-by-distance (IBD) (Fig. 3). The test showed a significant positive relationship between pairwise F_{ST} values and geographic distance ($r = 0.3785$; $P = 0.0140$) among all of the collections.

Results of the estimates of gene flow (Table 2) revealed that TA and NY have the highest gene flow ($Nm = 24.26$), and LWS and LWZ have also the relatively high gene flow ($Nm = 15.19$). Although LS and LW

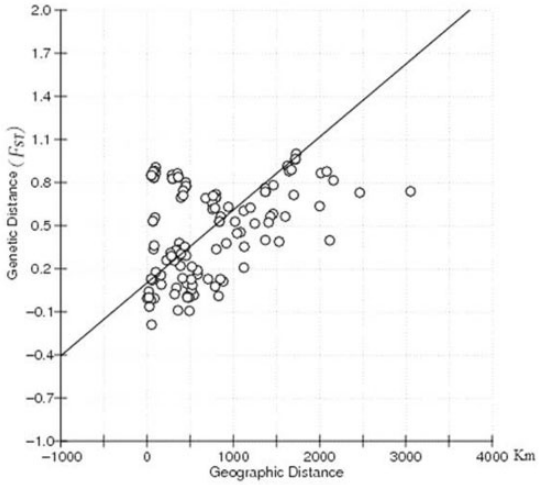

Fig. 3. Correlation between F_{ST} and distance (km) for pair-wise comparisons of 16 geographical *C. sasakii* populations.

Table 3. Diversity indexes of *C. sasakii* collections based on *COI* sequences

Collection (number of individuals)	*S*	η	*H*	*Hd*	π	*K*	π(JC)
DL(12)	10	10	4	0.74242	0.00577	3.39394	0.00582
SY(6)	9	9	3	0.73333	0.00789	4.60000	0.00782
MDJ(8)	10	10	4	0.64286	0.00668	3.92857	0.00674
XY(8)	7	7	4	0.75000	0.00613	3.60714	0.00167
JZ(5)	7	7	3	0.70000	0.00476	2.80000	0.00479
LS(5)	0	0	1	0.00000	0.00000	0.00000	0.00000
LK(17)	8	8	8	0.86786	0.00458	2.69118	0.00460
NY(11)	5	5	4	0.60000	0.00284	1.67273	0.00286
FC(19)	16	16	9	0.73099	0.00408	2.39766	0.00408
LY(18)	22	23	7	0.73856	0.01083	6.36601	0.01095
JN(11)	12	12	5	0.78182	0.00878	5.16364	0.00887
TA(4)	4	4	3	0.83333	0.00368	2.16667	0.00370
YT(16)	9	9	7	0.79167	0.00432	2.54167	0.00434
LWP(13)	3	3	4	0.42308	0.00100	0.58794	0.00010
LWZ(8)	0	0	1	0.00000	0.00000	0.00000	0.00000
LWS(9)	5	5	5	0.38596	0.38596	0.52632	0.00090

Note: *S*, number of polymorphic (segregating) sites; η, total number of mutations; *H*, number of haplotypes; *Hd*, haplotype diversity; π, nucleotide diversity; *K*, average number of nucleotide differences; π (JC), nucleotide diversity with Jukes and Cantor correction.

collections (WZ, LWP, LWS) have the low gene flow between them (the *Nm* between LS and LWZ, LWP, LWS was 0.00, 0.03 and 0.04, respectively).

Geographic Distribution of Haplotypes and Genetic Diversity. Ten of the 16 collections were found harboring unique haplotypes. In the MDJ, FC, LK, LY, and YT collections, 25.00, 15.79, 11.76, and 18.75% of the individuals, respectively, proved to be unique haplotypes, respectively. In the JZ, XY, JN, LWS, and LWP collections, 20.00, 25.00, 18.18, 15.79, and 15.38% of the individuals, belonged to the unique haplotypes, respectively.

The diversity indexes of LS collection and LW collections (LWZ, LWS, and LWP) were much lower than those in the other collections (Table 3). For instance, the *Hd* values of collections LWS, LWP, and LWZ were less than 0.42308, while the *Hd* values of the other seven collections were greater than 0.6000.

Results of AMOVA showed that there was significant genetic structure of *C. sasakii* among collections (Table 4), 55.96% ($P < 0.01$) of the variation was among collections and 44.04% was within the collections, which indicated that a considerable portion of the variation was observed among collections.

Table 4. Analysis of molecular variance (AMOVA) in collections of *C. sasakii*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among collections	14	301.989	1.74312Va	55.96*
Within collections	161	220.881	1.37193Vb	44.04
Total	175	522.869	3.11505	

*, $P < 0.01$.

Utility of BsaJI-Based Mitochondrial COI Gene PCR-RFLP. The restriction endonuclease BsaJI cleaves DNA at "CCNNGG", a pattern that was present in haplotypes from lineage II but not in haplotypes of lineage I (Fig. 4). Thus, BsaJI digested the 83 mitochondrial *COI* gene PCR products of individuals in lineage II but did not digest the 97 mitochondrial *COI* gene PCR products of individuals in lineage I (Table 1; Fig. 5). These results showed that the lineage of each individual could be determined based on the sizes of bands produced by BsaJI digestion.

Discussion

Understanding the genetic differentiation of pest insects is fundamental to understanding pest biology and management. Recent studies have shown that genetically differentiated lineages can differ in adaptation to environments or in invasive ability (Scheffer and Lewis 2005; Winkler et al. 2008). For example, two genetically differentiated lineages of the copepod *Eurytemora affinis* in the St. Lawrence River in North America have been identified. Although the lineage that primarily occurs in the central portion of the St. Lawrence River estuary has invaded freshwater lakes, the lineage in the upstream reaches of the estuary and downstream salt marshes has not (Winkler et al. 2008). The dipteran *Liriomyza sativae* has at least three genetic lineages including *sativae*-A, *sativae*-L, and *sativae*-W. However, only the *sativae*-W lineage was found to be invasive (Scheffer and Lewis 2005). Our analysis of mitochondrial *COI* gene revealed that the peach fruit moth, *C. sasakii*, has two cryptic mtDNA lineages in China, where it is native. Until now, the possible difference in biology (or ecology, or physiology) of the *C. sasakii* lineages was unknown.

Our results showed the genetic differentiation has significant correlation with the geographical distance (Fig. 3), but has no evidence for host plant associations. Host plant formation is an important process studied in many species that may explain their genetic structure (Inbar et al. 2004; Ros and Breeuwer 2007). Hua and Hua (1995) studied the esterase isozymes of three host biotypes from jujube, wide jujube, and apple, respectively. They found a great similarity exist between *C. sasakii* in hosts of jujube and wide jujube, while the two have a great differentiation with host of apple. The other patterns of random amplified polymorphic DNA (RAPDs) (Xu and Hua 2004) suggested the existence of two host biotypes, the *C. sasakii* in apricot was considered to be a new species with long genetic distance to the other hosts of *C. sasakii* (the *P*-distance between the two biotypes ranged from 0.453 to 0.673); they have suggested that genetic differentiation within *C. sasakii* was closely associated with host-plant identity. Although pairwise genetic distance between haplotypes (*P*-distance) in four host plant of apple, peach, jujube, and wild jujube ranged from 0.0016 to 0.0313 (data not shown) based on Tajima-Nei model, showed no biotypes were found in our study. Phylogenetics is one approach to assess the association between pest and host plants (Inbar et al. 2004; Ros and Breeuwer 2007). Although the genetic differentiation based on mitochondrial *COI* gene in the current study revealed the existence of two lineages, the lineages, however, were not closely associated with host identity. Our study revealed that the *C. sasakii* collections from apple, jujube, and peach contained haplotypes from both cluster I and II. The identical

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Hap1  AGATTATTAATTCGAGCTGAATTGGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap2  AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap4  AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap6  AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap14 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap15 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap16 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap17 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap18 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap19 AGATTATTAATTCGAGCTGAATTGGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap20 AGATTATTAATTCGAGCTGAATTGGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
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Hap23 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap24 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
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Hap31 AGATTATTAATTCGAGCTGAATTAGGAAATCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap3  AGATTATTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap5  AGATTATTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap7  AGATTACTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap8  AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap9  AGATTATTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap10 AGATTGCTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap11 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap12 AGATTGTTAATTCGAGCTGAATTAGGGAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap13 AGATTATTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGGAGATGATCAAATT 137
Hap25 AGAGTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGAGGATCAAATT 137
Hap32 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap33 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap34 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap35 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap36 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
Hap37 AGATTGTTAATTCGAGCTGAATTAGGAAACCCAGGATCATTAATTGAAGATGATCAAATT 137
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Fig. 4. Alignment of 37 haplotypes representing the *COI* sequences of 180 Chinese individuals of *C. sasakii.* Sequence alignment was generated with the Clustal W program. The BsaJI (CCNNGG) recognition site is boxed.

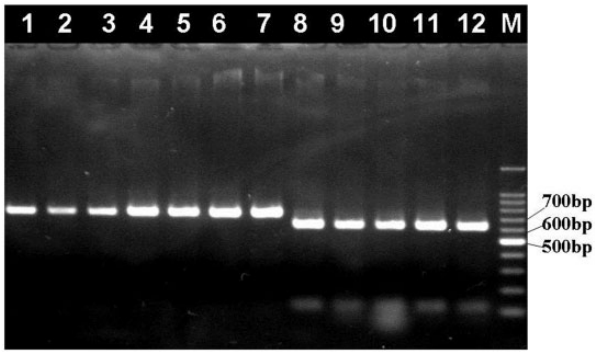

Fig. 5. Typical BsaJI-based mitochondrial *COI* gene PCR-RFLP gel profiles of *C. sasakii* individuals. Note: lanes 1–7 are from individuals belong to lineage I; lanes 8–12 are from individuals belong to lineage II; M, 100-bp ladder.

mitochondrial *COI* gene haplotypes can also be found on different host plant species, that means there were no closely correlation between mitochondrial divergence and host identity (Fig. 2).

The distribution of unique haplotypes within different collections showed that gene flow between collections was highly variable. The data suggested individuals naturally move between areas. For example, the locations of TA, NY, and FC are close to each other, and all of the haplotypes in the TA and NY collections were found in the FC collection. Similarly, all of the haplotypes in the LWZ collection were also found in the LWP and LWS collections. On the other hand, the movement of individuals between long-distance locations seems to be closely associated with human activities. For example, Hap11, a major haplotype in LW collections (representing 82.5% of all individual in the LW collections), could also be found in JZ (60.0%), FC (5.3%) and LY (50.0%) collections but not in JN, TA, and NY collections. As the map of Shandong indicates (Fig. 1), the distance among JN, TA, and NY are closer than that among JZ, FC, and LY. The movement of individuals with Hap11 among areas may be associated with human activities such as the transport of fruit.

The genetic diversity indexes of collections are variable greatly, which may be affected by the sample size. In the future, more individuals in each collection are needed to evaluate the genetic diversity. Determining whether the two cryptic mtDNA lineages differ in biological and ecological traits will require additional research, and the nuclear gene maybe important to verify this hypothesis. The information from that research should increase our understanding of *C. sasakii* evolution and management. The current paper demonstrates that the BsaJI-based PCR-RFLP can be used to differentiate the two cryptic mtDNA lineages within *C. sasakii.* The method should be useful for the reliable and rapid monitoring of the population dynamics of the two lineages in the field.

Acknowledgments

This work was funded by Public Service Sectors (agriculture) Special Funding for Research (No. 201103024) and the Taishan

Mountain Scholar Constructive Engineering Foundation of Shandong, China.

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Received 11 January 2015; accepted 22 May 2015.