Genetic Diversity and Inferences on Potential Source Areas of Adventive Frankliniella occidentalis (Thysanoptera: Thripidae) in Shandong, China Based on Mitochondrial and Microsatellite Markers

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GENETIC DIVERSITY AND INFERENCES ON POTENTIAL SOURCE AREAS OF ADVENTIVE *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE) IN SHANDONG, CHINA BASED ON MITOCHONDRIAL AND MICROSATELLITE MARKERS

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ABSTRACT

To reveal the genetic diversity and to infer potential source areas of adventive western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in Shandong, China, we used mitochondrial and microsatellite markers to analyze the genetic diversity of 15 populations from Shandong, as well as 3 populations from Yunnan and 2 populations from Beijing—these latter 2 sites having the earliest populations to establish in China—and 2 populations from California, which are part of the pest's native range in North America. Data involving the *mtCOI* gene and microsatellite markers showed that the Chinese populations were less diverse genetically than the native USA populations. The distribution of *mtCOI* haplotypes and percentage of shared alleles in this study suggested that the populations from Shandong may have arrived as a secondary incursion from Yunnan. We found that the diversity of mitochondrial alleles in some populations from Shandong had declined drastically, whereas the diversity of their nuclear alleles had remained high, i.e., the drastic loss of mitochondrial haplotype diversity in some populations was not accompanied by substantial reductions in nuclear allelic diversity. Therefore, further analyses of nuclear genetic diversity may demonstrate that it provides a better indication of the adaptability of an adventive species than mitochondrial genetic diversity. Also, the F_{ST} data and genetic diversity analysis suggest that the substantial gene flow among the Shandong populations might have minimized the bottleneck effects.

Key Words: western flower thrips; genetic diversity; *mtCOI*; microsatellites

RESUMEN

Para revelar la diversidad genética e inferir áreas de fuentes posibles del trips occidental de las flores, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) adventivos en Shandong, China, se utilizó marcadores mitocondriales y microsatélites para analizar la diversidad genética de 15 poblaciones (326 individuos) de Shandong, así como 3 poblaciones de Yunnan y 2 poblaciones de Beijing - estos 2 últimos sitios que tienen las primeras poblaciones establecidas en China - y 2 poblaciones de California, que forman parte del área de distribución nativa de esta plaga en América del Norte. Los datos relacionados con el gen *mtCOI* y marcadores microsatélites demostraron que las poblaciones chinas fueron menos diversas genéticamente que las poblaciones nativas de Estados Unidos. La distribución de los haplotipos *mtCOI* y porcentaje de alelos compartidos en este estudio sugiere que las poblaciones de Shandong pueden haber llegado de una incursión secundaria de Yunnan. Encontramos que la diversidad de alelos mitocondriales en las poblaciones de Shandong han disminuido drásticamente, mientras que la diversidad de los alelos nucleares han permanecido altos, es decir, la pérdida drástica de la diversidad de haplotipos no fue acompañada de una reducción sustancial de la diversidad alélica nuclear. Por lo tanto, nuevos análisis de la diversidad genética nuclear podrían demostrar que proporciona una mejor indicación de la capacidad de adaptación de una especie adventiva que la diversidad genética mitocondrial. Además, los datos de F_{ST} y el análisis de la diversidad genética sugieren que el flujo de genes sustancial entre las poblaciones de Shandong podría haber minimizado los efectos de cuello de botella.

Palabras Clave: trips occidental de la flor, la diversidad genética; *mtCOI*; microsatélites

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a very destructive invasive species. It feeds directly on plants, and also transmits many plant viruses (Brødsgaard 1994; de Kogel et al. 1997; Jones et al. 2005; Brunner & Frey 2010; Rugman-Jones et al. 2010). The pest is native to the western part of North America (Bailey 1940). Since the late 1970s, the western flower thrips has spread to more than 60 countries (Kirk & Terry 2003).

In China, the western flower thrips was first intercepted in Yunnan in 2000 (Jiang et al. 2001). An established population was found in Beijing in 2003 (Zhang et al. 2003) and this species was subsequently detected in other provinces including —in 2007—in Shandong (Zheng et al. 2007), one of the most important agricultural provinces in China, especially in vegetable production. Thus, the early-established populations from Yunnan and Beijing may be the potential source populations of the others in China. Our field survey resulted in collections of this thrips from 16 locations in Shandong Province in 2011 (Duan et al. 2013). Clearly, the genetic diversity and potential source areas from which this pest came to Shandong Province need to be systemically investigated. Such information will be helpful in revealing the pathways of invasion and spread and in managing this pest. We hypothesized that the genetic diversity (in both mitochondrial DNA and nuclear DNA) of the adventive *F. occidentalis* populations in Shandong Province would be much less than those of potential source populations because of the founder or bottleneck effects.

In the present study, we used mitochondrial and microsatellite markers to analyze the genetic diversity of 15 *F. occidentalis* populations from Shandong Province, as well as 3 populations from Yunnan and 2 populations from Beijing – these latter 2 sites having the earliest populations to establish in China, and 2 populations from California, which is part of the pest's native range in North America. Based on these analyses we sought to infer the potential source area(s) for the populations in Shandong Province.

Materials and Methods

Collection of *Frankliniella occidentalis* Samples

A total of 20 *F. occidentalis* populations were sampled from various host plants in Shandong, Beijing, Yunnan Provinces during 2010 and 2011. A total of 2 *F. occidentalis* populations were sampled from strawberry (*Fragaria ananassa* Duchesne; Rosales: Roaceae) in California in 2010. Only 2 or 3 thrips were collected from a single host plant separated by at least 1 m from the next sample, with only 1 thrips was collected from one leaf or flower. The adults were collected in a tube with 95% ethanol and stored at -20 °C. The thrips specimens were first identified by their morphological characteristics (Funderburk et al. 2007). The information on sampling locations, sampling dates, and hosts of each population is listed in Table 1.

Extraction of Genomic DNA, Amplification and Sequencing of *mtCOI* Gene

Genomic DNA was extracted from individual female adults as described in Duan et al. (2013). Briefly, one individual was put into a 0.2 mL centrifuge tube with 60 µL lysis buffer and was ground thoroughly. This preparation was incubated at 65 °C for 15 min and then at 95 °C for 10 min. This lysis was used as the DNA template in PCR amplication. A fragment of the mitochondrial cytochrome oxidase I (*mtCOI*) gene was amplified via standard PCR using the universal primers C1-J-1751 (5´GGATCACCTGATATAGCATTCCC3´) and C1-N-2329 (5´ACTGTAAATATATGATGAGCTCA3´) under the PCR conditions described in Simon et al. (1994). The PCR production was purified and then sequenced directly.

Microsatellite Genotyping

In addition to the 5 microsatellite loci that had been previously isolated by Brunner & Frey (2004), 3 more loci (GT310133, GT311293 and GT311492) were described and used (Duan et al. 2012). These 3 microsatellites were isolated from expressed sequence tags for *F. occidentalis* (Rotenberg et al. 2010). The characteristics of these microsatellites are shown in Table 2. These microsatellites were also used to amplify locus in 344 individuals in this study. The PCR reaction was performed as described in Brunner & Frey (2004) and the products were processed by an ABI 3730xl DNA analyzer.

Data Analysis

For *mtCOI* gene, a series of genetic parameters were estimated for 326 individuals from the Chinese populations and the American populations using DnaSP 5.0 (Librado & Rozas 2009). These parameters included the number of polymorphic (segregating) sites (*S*), the total number of mutations (η), the number of haplotypes (*H*), the haplotype diversity (*Hd*), the average number of nucleotide differences (*K*), and the nucleotide diversity with Jukes and Cantor correction [π(JC)] within each population and region.

For microsatellites, the alleles for each locus among various populations were calculated and the numbers of alleles shared among populations were estimated. The genetic diversity of each of the 20 populations was calculated using POP-

TABLE 1. THE 22 *FRANKLINIELLA OCCIDENTALIS* POPULATIONS USED IN THIS STUDY, THE LOCATIONS IN SHANDONG PROVINCE WHERE THEY WERE COLLECTED, THE PLANTS FROM WHICH THEY WERE COLLECTED AND THE DATES OF COLLECTION.

Population code	Sampling location	Collected from	Date
QD1	Qingsdao, Shandong	*Trifolium repens* L.	VI-2011
QD2	Qingsdao, Shandong	*Rosa chinensis* Jacq.	VI-2011
WH	Weihai, Shandong	*Rosa chinensis* Jacq.	VI-2011
RC	Rongcheng, Shandong	*Trifolium repens* L.	VI-2011
JNN	Jinan, Shandong	*Trifolium repens* L.	VII-2011
DZ	Dezhou, Shandong	*Trifolium repens* L.	VII-2011
ZB	Zibo, Shandong	*Trifolium repens* L.	VII-2011
BZ	Binzhou, Shandong	*Trifolium repens* L.	VII-2011
JNG	Jining, Shandong	*Trifolium repens* L.	VII-2011
QF	Qufu, Shandong	*Trifolium repens* L.	VII-2011
JX	Jinxiang, Shandong	*Trifolium repens* L.	VII-2011
DY	Dongying, Shandong	*Trifolium repens* L.	VII-2011
TAN	Taian, Shandong	*Trifolium repens* L.	VII-2011
DT	Dingtao, Shandong	*Trifolium repens* L.	VII-2011
SG	Shouguang, Shandong	*Capsicum annuum*	V-2011
BM	Mentougou, Beijing	*Phaseolus vulgaris* L.	XI-2010
BY	Yanqing, Beijing	*Phaseolus vulgaris* L.	XI-2010
YJ	Jinning, Yunnan	*Rosa chinensis* Jacq.	VI-2011
YC	Chenggong, Yunnan	*Rosa chinensis* Jacq.	VI-2011
YZ	ZhaoYang, Yunnan	*Trifolium repens* L.	VII-2011
USA1	California, USA	*Fragaria ananassa* Duchesne	VIII-2010
USA2	California, USA	*Fragaria ananassa* Duchesne	VIII-2010

GENE version 1.31 (Yeh et al. 1997). The average number of alleles per locus (*Na*), the effective number of alleles (*Ne*), the expected heterozygosity (*He*), the observed heterozygosity (*Ho*), and Nei's expected heterozygosity (*Nei*) were calculated based on microsatellite markers. The levels of genetic differentiation between pairs of populations were estimated using pairwise F_{ST} values computed with 10,000 permutations in Arlequin (Excoffier 2005). Estimates of gene flow were calculated as $Nm = 1/2[(1/F_{ST}) -1]$.

To identify bottleneck events, the possibility significantly excessive heterozygosity (signature of bottleneck) within any of the 20 Chinese populations was examined using BOTTLENECK software (Cornuet & Luikart 1996) under all 3 mutation models [Two Phase Mutation Model (TPM), Infinite Allele Model (IAM), and Stepwise Mutation Model (SMM)]. We used a TPM model with the default settings of 30% variation from the IAM model and 70% from the SMM model.

RESULTS

Genetic Diversity in Mitochondrial DNA

A total of 326 *mtCOI* sequences were obtained in this study (Table 3) and there were 7 *mtCOI* haplotypes (coded as Hap1-Hap6 and Hap23) in total. Hap1, Hap2, and Hap3 were found in the populations from Shandong (97.7%), Beijing (100.0%), Yunnan (100.0%), and California (80.0%). Hap4 and Hap23 were found only in the populations of Shandong. However, the percentages of these 2 haplotypes were very low (2.3%). Hap5 and Hap6 were found only in the native populations of the United States (20%). The genetic diversities of the 2 native populations from the United States were higher than those of the adventive populations in China (Table 4). For example, the *Hd* values of the 2 populations in the United States were 0.702 and 0.780, respectively, while the *Hd* values of the adventive populations in China ranged from 0.133 to 0.705. Among them, the *Hd* values of the 3 populations (YJ, YC, and YZ) from Yunnan were 0.629, 0.457, and 0.648, respectively; the *Hd* values of the 2 populations (BM and BY) from Beijing were 0.676 and 0.686, respectively; the *Hd* values of the 15 populations from Shandong ranged from 0.133 to 0.705 (Table 4).

Microsatellite-Based Genetic Diversity

Analysis of the 8 microsatellite loci revealed the presence of 74 alleles in the 15 populations from Shandong, 25 alleles in the populations from Beijing, 52 alleles in Yunnan populations and 75 alleles in the California, USA populations. The percentages of alleles shared between popu-

TABLE 2. CHARACTERISTICS OF THE MICROSATELLITE LOCI USED TO GENOTYPE THE 22 *FRANKLINIELLA OCCIDENTALIS* POPULATIONS FROM BEIJING, SHANDONG, YUNNAN AND CALIFORNIA *FRANKLINIELLA OCCIDENTALIS* POPULATIONS.

Locus name	Repeat motif	Primer sequence (5'-3')		Fragment size range (bp)	T_a(°C)	Reference
FOCC44	$(GT)_{49}$	h-TGTCACCAAGGCGGTGG	CGCTGGACCTTACCGAGAGA	90-105	60	Brunner & Frey (2004)
FOCC56	$(GA)_{42}$	t-TCAACCCCCATCACTCTTCC	CCTTGAGCTCCCCTCACCTC	193-209	60	
FOCC75	$(GA)_{23}$	f-GGATATTATTTTCCCGTCCCG	TGGTTCTTTTGTAAAGGCAGCG	184-244	60	
FOCC83	$(GT)_{46}$	t-GTCTGTCACCAAGGCGGTGG	CAGGTAACGCACAGTGCTGCTC	72-90	60	
FOCC125	$(GC)_6(GT)_{10}$	f-AACCCGCACCGTGCA	AGTTGGGCTGCCGTCC	138-156	60	
WD133	$(TG)_8$	f-TTCACCCACCTCCAGTCTATT	CCAGCACGGGACACAAATC	341-353	54	Duan et al. (2012)
WD293	$(TCC)_6$	h-CCAATGCGTGTTCAGTTAGC	AGACCTGATTCACCGTATGTTC	376-388	54	
WD492	$(TTG)_5$	h-TATGTTGACTATTGCCTTGC	TCTAACGGACTGAGCCCT	325-334	48	

Ta, PCR annealing temperature; letters (h, t, f) before the sequence of the forward primers indicate respectively fluorescent labels HEX, TAMRA and 6-FAM.

TABLE 3. *MTCOI* HAPLOTYPES OF EACH OF THE *FRANKLINIELLA OCCIDENTALIS* POPULATIONS USED TO ASSESS GENETIC DIVERSITY OF THE 22 *FRANKLINIELLA OCCIDENTALIS* POPULATIONS FROM BEIJING, SHANDONG, YUNNAN AND CALIFORNIA.

	China																					
	Shandong															Beijing		Yunnan			USA	
Haplotype	QD1	QD2	WH	RC	JNN	DZ	ZB	BZ	JNG	QF	JX	DY	TAN	DT	SG	BM	BY	YJ	YC	YZ	USA1	USA2
Hap1	8	7	9	8	8	11	5	12	13	13	9	2	9	10	15	3	7	8	11	8	5	13
Hap2	5	5	4		4		4	1			1	1	3	1		5	4	5	2	3	4	4
Hap3	2	2	1	6	3	4	6	2	1	2	3	8		2		7	4	2	2	4	2	3
Hap4											1	1										
Hap5																					3	5
Hap6																						1
Hap23		1	1	1																		

TABLE 4. DIVERSITY INDICES OF THE 22 *FRANKLINIELLA OCCIDENTALIS* POPULATIONS FROM BEIJING, SHANDONG, YUNNAN AND CALIFORNIA BASED ON *MTCOI* AND MICROSATELLITES.

Population code (Number of individuals tested, *mtCOI*/microsatellites)	*MtCOI*							Microsatellites				
	S	η	*H*	*Hd* (SD)	π (SD)	*K*	π (JC)	*Na*	*Ne*	*He*	*Ho*	*Nei*
QD1(15/15)	2	2	3	0.629(0.086)	0.00166(0.00032)	0.724	0.00166	4.625	3.223	0.635	0.264	0.555
QD2 (14/15	2	2	4	0.648(0.081)	0.00174(0.00033)	0.758	0.00174	4.875	2.838	0.596	0.349	0.576
WH (14/15)	2	2	4	0.538(0.115)	0.00134(0.00034)	0.582	0.00134	4.875	3.449	0.652	0.315	0.628
RC (14/15)	1	1	2	0.527(0.064)	0.00121(0.00015)	0.527	0.00121	4.000	2.667	0.554	0.328	0.534
JNN (15/15)	2	2	3	0.648(0.088)	0.00175(0.00034)	0.762	0.00175	4.875	2.831	0.549	0.331	0.530
DZ (15/15)	1	1	2	0.419(0.113)	0.00096(0.00026)	0.419	0.00096	4.500	3.232	0.652	0.372	0.628
ZB (15/15)	2	2	3	0.705(0.053)	0.00214(0.00026)	0.933	0.00215	4.625	3.087	0.629	0.400	0.608
BZ (15/15)	2	2	3	0.362(0.145)	0.00087(0.00037)	0.381	0.00088	4.625	2.767	0.606	0.344	0.585
JNG (14/15)	1	1	2	0.143(0.119)	0.00033(0.00027)	0.143	0.00033	4.625	2.799	0.584	0.335	0.564
QF (14/15)	1	1	2	0.264(0.136)	0.00060(0.00031)	0.264	0.00061	5.000	3.229	0.660	0.369	0.637
JX (14/15)	3	3	4	0.571(0.132)	0.00149(0.00043)	0.648	0.00149	4.250	3.173	0.634	0.350	0.612
DY (12/15)	3	3	4	0.561(0.154)	0.00188(0.00059)	0.818	0.00188	5.000	3.055	0.622	0.348	0.600
TAN (12/15)	1	1	2	0.409(0.133)	0.00094(0.00031)	0.409	0.00094	3.875	2.498	0.573	0.328	0.553
DT (13/15)	2	2	3	0.410(0.154)	0.00100(0.00041)	0.436	0.00100	3.875	2.349	0.533	0.327	0.514
SG (15/15)	1	1	2	0.133(0.112)	0.00031(0.00026)	0.133	0.00031	3.375	2.289	0.543	0.370	0.525
BM (15/15)	2	2	3	0.676(0.070)	0.00232(0.00024)	1.010	0.00232	2.500	1.842	0.404	0.330	0.390
BY (15/15)	2	2	3	0.686(0.068)	0.00192(0.00031)	0.838	0.00193	2.750	1.992	0.411	0.350	0.396
YJ(15/15)	2	2	3	0.629(0.086)	0.00166(0.00032)	0.724	0.00166	4.500	3.135	0.629	0.319	0.607
YC(15/15)	2	2	3	0.457(0.141)	0.00114(0.00039)	0.495	0.00114	5.000	2.831	0.594	0.330	0.573
YZ(15/15)	2	2	3	0.648(0.088)	0.00175(0.00034)	0.762	0.00175	4.625	3.258	0.617	0.343	0.594
USA1(14/15)	6	6	4	0.780(0.061)	0.00547(0.00128)	2.385	0.00551	6.000	4.326	0.724	0.375	0.692
USA2(26/29)	7	7	5	0.702(0.073)	0.00490(0.00099)	2.135	0.00493	8.125	4.858	0.694	0.352	0.681

Abbreviations: *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; *Na*, average number of alleles per locus; *Ne*, the effective number of alleles; *He*, the expected heterozygosity; *Ho*, the observed heterozygosity; and *Nei*, Nei's expected heterozygosity.

lations were as follows: Shandong and Beijing (42.9% to 66.7%), Shandong and Yunnan (86.8% to 97.4%), Shandong and California, USA (79.3% to 96.3%) (Fig. 1). Additionally, all of the alleles of the Beijing populations were found in Yunnan populations.

The Bottleneck test revealed that 14 of the 20 Chinese populations had a statistically significant excess of heterozygotes under the Infinite Allele Model (IAM), which suggests that these populations might recently have undergone a genetic bottleneck (Table 5). While there only 7 populations and 1 population had a statistically significant excess of heterozygotes under the Two Phase Mutation Model (TPM) and Stepwise Mutation Model (SMM), respectively (Table 5).

Microsatellite-Based Genetic Differentiation and Gene Flow

When considering each pair of populations, 61 of 231 values of pairwise fixation index F_{ST} (26%) were significant (Table 6). When considering each population pair among the populations from Shandong, 19 F_{ST} values out of 105 (18%) were associated with a significant exact test (Table 6). Our study revealed that 15 of the 19 significant values of F_{ST} between populations from Shandong were among those between QF or TAN and other populations. The F_{ST} values between the populations from Shandong and these from the California suggested that 4 F_{ST} values out of 30 (13%) were associated with a significant exact test. The pairwise F_{ST} values between the populations from Shandong and those from Beijing suggested that 22 F_{ST} values out of 30 (73%) were associated with a significant exact test. The pairwise F_{ST} values between the populations from Shandong and these from Yunnan suggested that 4 F_{ST} values out of 45 (9%) were associated with a significant exact test (Table 6).

The gene flow (*Nm*) between Shandong and Beijing populations ranged from 0.93-6.64, and 3 among 30 *Nm* values between the Shandong and Beijing populations were > 5.0. However, 38 of 45 values of the *Nm* values between the Shandong and Yunnan populations were > 5.0. Among the populations from Shandong, the gene flows between the TAN and other Shandong populations were relatively low (1.77-7.83). Similarly, the gene flows between the QF and other Shandong populations were also relatively low (1.58-5.06).

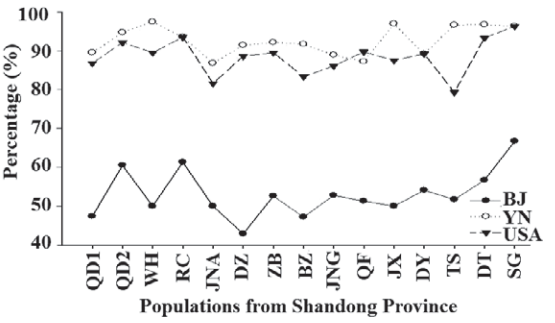

Fig. 1. The percentages of microsatellite alleles in common between the Shandong populations of *Frankliniella occidentalis* and the Beijing, Yunnan, and California populations.

Discussion

Molecular markers can be used to determine the pathway of spread of the invading organism (Gammon & Kesseli 2010; Dupont et al. 2010; Lombaert et al. 2010; Chu et al. 2011). For example, Chu et al. (2011) found the *mtCOI* haplotypes within the populations of *Bemisia tabaci* (Gennadius) biotype Q in Shandong, China can only be found in the western Mediterranean countries. In addition, the adventive Q-biotype populations have more shared alleles with the populations from the western Mediterranean countries than with the eastern Mediterranean countries. These results obtained by analyzing mitochondrial and nuclear (microsatellite) markers revealed that the populations of *B. tabaci* biotype Q in Shandong Province had arrived from western Mediterranean countries rather than from eastern Mediterranean countries. The *mtCOI* haplotypes in this study suggest that the populations from Beijing or Yunnan may be the potential source populations as the dominant haplotypes (Hap1-Hap3) in Shandong can be found in the 2 regions. The absence of the rare haplotypes (Hap4 and Hap23) in Shandong in Beijing or Yunnan may result from the limited number of samples from these regions. The percentage of the shared microsatellite alleles between Shandong populations and Yunnan populations (86.8% to 97.4%) was higher than those between Shandong and Beijing (42.9% to 66.7%), which further indicates that the populations in Shandong probably have come as secondary incursions from Yunnan. In addition, all of the alleles of the Beijing populations were found in Yunnan populations, which indicates that the populations in Beijing probably have also come as secondary incursions from Yunnan. This finding supports the conclusion of Yang et al. (2012) that the populations from southwest China (Yunnan province) may be the putative source populations of the other populations in China.

Based on *mtCOI* and microsatellite loci, Brunner & Frey (2010) demonstrated that there were 2 phylogenetic lineages within the western flower thrips in its native range in western North America. Among the 30 *mtCOI* haplotypes, 5 haplotypes adapted to hot-dry conditions and 25 haplotypes adapted to cool-moist conditions (Brunner & Frey 2010) belong to "Greenhouse strain" and "Lupin strain", respectively (Rugman-Jones et al. 2010),

TABLE 5. WITHIN POPULATION TESTS FOR OF HETEROZYGOSITY EXCESS *P*-VALUES.

Population code	County	Sampling location	Heterozygosity excess *P*-values		
			IAM	TPM	SMM
QD1	Qingdao	Chengyang	**0.01953**	0.09766	0.23047
QD2		Qingdao	0.27344	0.57813	0.84375
WH	Weihai	Weihai	**0.00586**	**0.01953**	0.27344
RC		Rongcheng	**0.00391**	**0.01953**	0.59375
JNN	Jinan	Jinan	0.47266	0.57813	0.72656
DZ	Dezhou	Dezhou	**0.00195**	**0.00391**	**0.02734**
ZB	Zibo	Zibo	**0.01953**	0.23047	0.47266
BZ	Binzhou	Binzhou	**0.03711**	0.57813	0.87500
JNG	Jining	Jining	0.05469	0.28906	0.59375
QF		Qufu	**0.00391**	0.15625	0.62891
JX		Jinxiang	**0.00580**	**0.01953**	0.09766
DY	Dongying	Dongying	**0.03711**	0.37109	0.96289
TAN	Taian	Taian	**0.02734**	0.27344	0.57813
DT	Heze	Dingtao	0.19141	0.62891	0.84375
SG	Weifang	Shouguang	**0.00586**	**0.01953**	0.37109
BM	Beijing	Mentougou	0.18750	0.23438	0.28906
BY		Yanqing	**0.21875**	0.28125	0.42188
YJ	Yunnan	Jinning	**0.01953**	**0.03711**	0.47266
YC		Chenggong	0.27344	0.67969	0.98633
YZ		Zhaoyang	**0.02734**	**0.03711**	0.42188

Bold indicates significant deviation from expected heterozygozity at $P < 0.05$

Abbreviations: TPM, Two Phase Mutation Model; IAM, Infinite Allele Model; and SMM, Stepwise Mutation Model. We used a TPM model with the default settings of 30% variation from the IAM model and 70% from the SMM model.

which have been referred to as WFT-G and WFT-L, respectively (Duan et al. 2013). Rugman-Jones et al. (2010) asserted that both strains have been found in their native California range. However, only WFT-L adapted to cool-moist conditions was found in California by Brunner & Frey (2010). The 2 populations from California in our study consisted of WFT-G. The present study showed that the haplotype in WFT-L (Hap23) was only detected in coastal regions of Shandong Province. In addition, the number of WFT-L individuals was very small. The distribution pattern of the 2 ecotypes may be associated with ecological factors, such as climate, or with their ratio within the initially introduced population (Duan et al. 2013). Data involving the *mtCOI* gene and microsatellite markers showed that the Chinese populations were less diverse genetically than the native USA populations considered in this study (WFT-G populations from California).

Our study revealed that 8 of 15 Shandong populations had higher *Hd* (mitochondrial haplotype diversities) than the lowest value of Yunnan population (YC), and 9 of 15 Shandong populations had higher *He* (expected heterozygosity at microsatellite loci) values than the lowest value of Yunnan population (YC). These findings are not consistent with our hypothesis that the genetic diversity (in both mitochondrial DNA and nuclear DNA) of the adventive *F. occidentalis* populations in Shandong Province would be much less than those of potential source populations. The higher genetic diversity of Shandong populations (in both mitochondrial DNA and nuclear DNA) may be associated with the multiple introductions of *F. occidentalis* populations from Yunnan and/or high subsequent gene flow between the introduced populations in Shandong during the past several yr. For instance, under the Two Phase Mutation and Stepwise Mutation models (Table 5), the number of populations that exhibited heterozygosity excess decreased, which indicated that the bottleneck effects within these populations is a transient feature, and can be expected to last only a few generations. In particular the populations of Shandong Province may have fairly substantial gene flows (Table 6), which may make the effects of local bottlenecks transient and difficult to detect.

To our surprise, we found much incongruence of nuclear and mitochondrial diversity in some populations from Shandong Province. The mitochondrial diversity index, *Hd*, of the various Shandong populations ranged from 0.133 to 0.705, while the nuclear (microsatellite) diversity index, *He*, of these same populations, ranged from 0.533 to 0.652. For example, the mitochondrial diversity index *Hd* value for the SG population

TABLE 6. THE PAIRWISE FIXATION INDEX (*FST*) VALUES (BELOW THE DIAGONAL) AND GENE FLOW (*NM*) (ABOVE THE DIAGONAL) BETWEEN THE 22 *FRANKLINIELLA OCCIDENTALIS* POPULATIONS. BOLD INDICATES SIGNIFICANT VALUES AFTER BONFERRONI CORRECTION ($P < 0.05$).

	QD1	QD2	WH	RC	JNN	DZ	ZB	BZ	JNG	QF	JX	DY	TAN	DT	SG	BM	BY	YJ	YC	YZ	USA1	USA2
QD1		Infinite	12.00	16.17	24.50	49.50	6.64	24.50	49.50	7.83	Infinite	16.17	5.06	12.00	16.17	2.63	2.63	16.17	Infinite	Infinite	49.50	16.17
QD2	-0.01		9.50	Infinite	24.50	16.17	5.06	16.17	Infinite	4.05	49.50	12.00	4.05	7.83	24.50	3.07	3.35	9.50	49.50	Infinite	7.83	7.83
WH	0.04	0.05		7.83	3.07	49.50	9.50	7.83	9.50	3.07	16.17	7.83	2.00	9.50	6.64	6.64	6.64	Infinite	7.83	6.64	7.83	6.64
RC	0.03	-0.01	0.06		12.00	12.00	3.35	9.50	24.50	2.63	16.17	12.00	2.28	7.83	16.17	2.44	2.44	6.64	24.50	16.17	5.06	6.64
JNN	0.02	0.02	0.14	0.04		5.75	2.63	7.83	12.00	4.05	9.50	7.83	4.05	5.75	4.50	1.50	1.42	3.35	Infinite	49.50	9.50	9.50
DZ	0.01	0.03	0.01	0.04	0.08		7.83	Infinite	24.50	12.00	49.50	24.50	3.67	49.50	6.64	5.06	4.50	24.50	16.17	16.17	49.50	16.17
ZB	0.07	0.09	0.05	0.13	**0.16**	0.06		9.50	6.64	4.05	6.64	2.83	3.07	4.50	2.13	2.28	2.83	24.50	4.50	5.75	5.06	3.07
BZ	0.02	0.03	0.06	0.05	0.06	0.00	0.05		Infinite	12.00	24.50	9.50	5.06	49.50	4.05	3.35	3.67	16.17	16.17	49.50	16.17	7.83
JNG	0.01	0.00	0.05	0.02	0.04	0.02	0.07	-0.01		4.50	Infinite	16.17	3.07	Infinite	6.64	4.05	4.50	24.50	Infinite	Infinite	9.50	9.50
QF	0.06	**0.11**	**0.14**	**0.16**	0.11	0.04	0.11	0.04	**0.10**		4.50	4.05	7.83	5.06	2.00	1.50	1.58	3.35	4.50	5.06	12.00	5.06
JX	-0.01	0.01	0.03	0.03	0.05	0.01	0.07	0.02	0.00	0.10		Infinite	2.44	Infinite	16.17	4.05	3.67	Infinite	Infinite	Infinite	16.17	16.17
DY	0.03	0.04	0.06	0.04	0.06	0.02	**0.15**	0.05	0.03	0.11	0.00		1.77	49.50	16.17	3.35	2.63	7.83	49.50	12.00	24.50	Infinite
TAN	0.09	0.11	**0.20**	**0.18**	0.11	**0.12**	**0.14**	0.09	**0.14**	0.06	**0.17**	**0.22**		1.88	1.58	0.93	1.06	1.88	3.07	4.50	3.35	2.13
DT	0.04	0.06	0.05	0.06	0.08	0.01	0.10	0.01	0.00	**0.09**	0.00	0.01	**0.21**		5.06	4.50	4.05	24.50	49.50	12.00	16.17	16.17
SG	0.03	0.02	0.07	0.03	0.10	0.07	**0.19**	**0.11**	0.07	**0.20**	0.03	0.03	**0.24**	0.09		2.83	2.44	5.75	7.83	9.50	4.05	6.64
BM	**0.16**	**0.14**	0.07	0.17	**0.25**	0.09	**0.18**	**0.13**	**0.11**	**0.25**	**0.11**	**0.13**	**0.35**	0.10	**0.15**		Infinite	5.06	2.83	2.44	2.83	3.35
BY	**0.16**	**0.13**	0.07	0.17	**0.26**	0.10	**0.15**	**0.12**	**0.10**	**0.24**	**0.12**	**0.16**	**0.32**	0.11	**0.17**	-0.02		5.75	2.44	2.44	2.63	2.83
YJ	0.03	0.05	0.00	0.07	**0.13**	0.02	0.02	0.03	0.02	**0.13**	0.00	0.06	**0.21**	0.02	0.08	0.09	0.08		9.50	12.00	6.64	5.75
YC	-0.01	0.01	0.06	0.02	0.00	0.03	0.10	0.03	0.00	0.10	0.00	0.01	**0.14**	0.01	0.06	**0.15**	**0.17**	0.05		Infinite	24.50	49.50
YZ	-0.02	-0.01	0.07	0.03	0.01	0.03	0.08	0.01	-0.01	0.09	0.00	0.04	0.10	0.04	0.05	**0.17**	**0.17**	0.04	-0.01		12.00	9.50
USA1	0.01	0.06	0.06	0.09	0.05	0.01	0.09	0.03	0.05	0.04	0.03	0.02	**0.13**	0.03	0.11	**0.15**	**0.16**	0.07	0.02	0.04		Infinite
USA2	0.03	0.06	0.07	0.07	0.05	0.03	**0.14**	0.06	0.05	**0.09**	0.03	0.00	**0.19**	0.03	0.07	0.13	**0.15**	**0.08**	0.01	0.05	0.00	

was the lowest (0.133), but the nuclear diversity *He* value was not the lowest (0.543) (Table 4). Our results agree with the view that the levels of diversity in mitochondrial DNA may not be a reliable guide to the levels of diversity in the nuclear DNA (Shao et al. 2004; DeHeer & Vargo 2008; Chu et al. 2011). These results support the view that bottleneck and founder events can lead to very rapid and drastic loss of mitochondrial diversity in some populations, however, this loss in haplotype diversity does not mean that nuclear allelic diversity must also decline to a similar extent (Chu et al. 2011). Another possibility of the incongruence is the small sample size used in our study may affect the accuracies in nuclear genetic diversity. Hale et al. (2012) showed that 25 to 30 individuals per population should be used for population genetic studies based on microsatellite allele frequencies. Thus larger sample sizes of *F. occidentalis* than in this study should be used in the future genetic studies with microsatellite loci to evaluate the potential effects of the sample size.

The values of F_{ST} between QF or TAN and other populations revealed that significant F_{ST} values are consistent with gene flow or bottleneck events. However, the Shandong populations that experienced bottleneck effects, as revealed by the Infinite Allele Model (IAM) (Table 5), do not necessarily have significant values of F_{ST}. For instance, the WH and RC populations experienced significant bottleneck effects as revealed by Two Phase Mutation Model (TPM) or Infinite Allele Model (IAM) (Table 5). However, few significant F_{ST} values were found between these 2 populations and other Shandong populations. These F_{ST} data suggest that the substantial gene flow among the Shandong populations might have minimized the bottleneck effects, which would be consistent with the genetic diversity analysis.

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