Sudden Widespread Distribution of Frankliniella occidentalis (Thysanoptera: Thripidae) in Shandong Province, China

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SUDDEN WIDESPREAD DISTRIBUTION OF *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE) IN SHANDONG PROVINCE, CHINA

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Abstract

In Shandong Province, China, the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), was first detected in Qingdao in 2007. The pest is composed of 2 genetic strains or types based on analyses of the mitochondrial DNA gene, *mtCOI*. These are known as "the Greenhouse strain" and "the Lupin strain", hereafter referred to as "WFT-G" and "WFT-L", respectively. To investigate the status and pathways of spread of this alien species in its new environment, we collected 78 samples of thrips from various plants in all of the 17 counties of Shandong Province during May-Jul, 2011. In total we made 16 collections of western flower thrips in 12 counties, and we analyzed the *mtCOI* gene of each of these samples. Most individuals (98.6%) in these 16 collections belonged to the WFT-G type and 3 (1.4%) belonged to WFT-L type. The results demonstrated that *F. occidentalis* has—without being noticed—become widespread in Shandong Province, and the main type is WFT-G. Field surveys coupled with genetic analyses proved to be helpful in revealing the invasion process including the invasion pathways or mechanisms, and such analyses may help in identifying approaches and options for prevention and management of the pest.

Key Words: western flower thrips, biological invasion, mitochondrial COI gene, sudden widespread distribution

Resumen

El trips occidental de las flores, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), fue detectado por primera vez en el 2007 en Qingdao en la provincia de Shandong, China. La plaga consiste de 2 cepas genéticas o tipos basados en el análisis del gen del ADN mitocondrial, mtCOI. Estos son conocidos como "la cepa de invernadero" y "la cepa Lupin", posteriormente referidas como "WFT-G" y "WFT-L", respectivamente. Para investigar el estatus y las vías de expansión de esta especie exótica en su nuevo ambiente, se recogieron 78 muestras de trips de diversas plantas en todos los 17 condados de la provincia de Shandong en mayo-julio del 2011. En total se hicieron 16 colecciones del trips occidental de las flores en 12 condados, y se analizó el gen mtCOI de cada una de estas muestras. La mayoría de los individuos (98.6%) en estas 16 colecciones pertenecían al tipo WFT-G y 3 (1.4%) de ellos pertenecían al tipo WFT-L. Los resultados demostraron que *F. occidentalis* tiene una amplia distribución en la provincia de Shandong y el tipo principal es el WFT-G. Los estudios de campo, junto con los análisis genéticos son útiles para revelar el proceso de invasión incluyendo las vías o mecanismos de invasión, y este tipo de análisis puede ayudar en la identificación de estrategias y opciones para la prevención y manejo de la plaga.

Palabras Clave: trips occidental de la flor, invasión biológica, gen mitocondrial COI, distribución generalizada

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The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), an important agricultural pest, severely damages ornamentals and vegetables directly by feeding and indirectly by transmitting viruses, and thus causes devastating losses of yield and/or market value (Jones et al. 2005). *Frankliniella occidentalis* is native to the western North America, where it has caused heavy damage to the ornamental industry as early as the 1940s (Bailey 1940). Since the late 1970s, international agricultural trade flows have increased very rapidly, and *F.*

occidentalis has arrived and established in many countries to become a cosmopolitan pest. Presently, this thrips is established in more than 60 countries including the United States, Canada, Australia, United Kingdom, and Japan (Kirk & Terry 2003).

Recent research (Brunner & Frey 2010; Rugman-Jones et al. 2010) revealed that *F. occidentalis* could be divided into 2 genetic entities or strains based on phylogenetic analyses using mitochondrial cytochrome oxidase I (*mtCOI*) gene sequences. These 2 genetic entities (also known as "Greenhouse strain" and "Lupin strain", thereafter referred to as "WFT-G" and "WFT-L" in this study, respectively) have been regarded as 2 ecotypes (Brunner et al. 2010) or cryptic species (Rugman-Jones et al. 2010). Prior studies showed that WFT-G or WFT-L type might differ in many biological traits including the fecundity, host adaptability, environmental adaptability, and insecticide resistance (de Kogel et al. 1997; Brødsgaard 1994; Brunner et al. 2010; Rugman-Jones et al. 2010). Unfortunately it seems that Rugman-Jones et al. (2010) gave the wrong primer sequences needed for the separation of the strains/haplotypes. Nevertheless many mitochondrial haplotypes of these 2 strains have been found in countries where the pest has become established (Brunner & Frey 2010; Rugman-Jones et al. 2010).

In China, *F. occidentalis* was first detected in Beijing in 2003 (Zhang et al. 2003) and in Shandong Province it was first detected in Qingdao in 2007 (Zheng et al. 2007). Indeed Rugman-Jones et al. (2010) showed that both the WFT-G and the WFT-L type of *F. occidentalis* have been detected in China. However, the spread status of the pest in Shandong Province during the past several years was unknown (Yang et al. 2012). In addition, we still do not know the type composition (WFT-G and WFT-L) of the pest in the region, which is closely associated with the management measures of the pest because of possible differences in their biological traits (de Kogel et al. 1997; Brødsgaard 1994; Brunner & Frey 2010; Rugman-Jones et al. 2010). We hypothesize that *F. occidentalis* might spread to a few of the counties neighboring Qingdao, because this pest has been established only for a few years.

In this study, the geographical distribution of *F. occidentalis* in Shandong Province was systematically established for the first time since it was first detected in the Province in 2007. We first collected the samples of thrips from various host plants from a total of 17 counties in Shandong Province during May-Jul 2011. Then the *mtCOI* gene of each of the samples was sequenced and analyzed to determine the distribution of this thrips and the type composition of the samples. We posited that the field survey would not only be helpful in revealing the invasion process and the invasion mechanism of this adventive alien species, but also in the prevention and management of the pest.

MATERIALS AND METHODS

Collection of *Frankliniella occidentalis* Samples

Seventy-eight thrips collections were sampled from various plant species in the field and greenhouse from the 17 counties of Shandong Province during May-Jul 2011. The adult thrips were collected in a tube with 95% ethanol and stored at -20 °C. The thrips specimens were first identified by dissecting microscope based on their morphological characteristics such as the number, size and location of the major setae on the head, prothorax and coloration characteristics (Funderburk et al. 2007). The information on sampling locations and sampling dates, and plants from which the thrips specimens were collected of each of the 16 populations are listed in Table 1.

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from individual female adults as described in Frohlich et al. (1999) with a little modification. Essentially the procedure was as follows. One individual was put into a 0.2 mL centrifuge tube with 60 µL lysis buffer (50 mmol·L^{-1}Tris-HCl (pH8.0), 20 mmol·L^{-1}NaCl, 1 mmol·L^{-1}EDTA, 1% SDS) 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 *mtCOI* gene was amplified via standard PCR using the primers C1-J-1751 (5'-GGATCACCTGATATAGCATTCCC-3') and C1-N-2329 (5'-ACTGTAAATATATGATGAGCTCA-3') under the PCR conditions described in Simon et al. (1994).

Haplotype Determination and Phylogenetic Analyses

Sequences were aligned with Clustal X (Thompson et al. 1997) and trimmed manually in MEGA5 (Tamura et al. 2011). All *mtCOI* sequences were checked for gaps, indels, numts, and pseudogenes by alignment using the multiple sequence editor Clustal X (Thompson et al. 1997). The *mtCOI* haplotypes were selected from all 225 *mtCOI* sequences in the present study and deposited in GenBank using DnaSP4.0 software (Librado et al. 2009). A phylogenetic tree was constructed using the neighbour-joining (NJ) or the minimum evolution (ME) method in MEGA5 with the *Scirtothrips dorsalis* (Hood) *mt-*

Table 1. The 16 *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.

County	Sampling locations	Longitude	Latitude	Collected from	Date
Qingsdao	Chengyang	120°23'41.39″	36°19′08.63″	*Trifolium repens* L.	VI-2011
	Qingsdao	120°23'35.13″	36°19′05.28″	*Rosa chinensis* Jacq.	VI-2011
Weihai	Weihai	122°09'02.86″	37°27′02.35″	*Rosa chinensis* Jacq.	VI-2011
	Rongcheng	122°26′06.33″	37°09′21.21″	*Trifolium repens* L.	VI-2011
Jinan	Jinan	116°59′45.87″	36°40′15.27″	*Trifolium repens* L.	VII-2011
Dezhou	Dezhou	116°20′48.18″	37°26′07.05″	*Trifolium repens* L.	VII-2011
Zibo	Zibo	118°02′59.41″	36°49′51.15″	*Trifolium repens* L.	VII-2011
Binzhou	Binzhou	118°01′50.29″	37°21′53.92″	*Trifolium repens* L.	VII-2011
Jining	Jining	116°34′56.09″	35°24′39.06″	*Trifolium repens* L.	VII-2011
	Qufu	117°00′34.56″	35°35′35.15″	*Trifolium repens* L.	VII-2011
	Jinxiang	116°18′41.13″	35°04′15.65″	*Trifolium repens* L.	VII-2011
Dongying	Dongying	118°38′20.28″	37°28′34.02″	*Trifolium repens* L.	VII-2011
Taian	Taian	116°59′38.13″	36°12′11.07″	*Trifolium repens* L.	VII-2011
Heze	Dingtao	115°34′01.66″	35°04′29.72″	*Trifolium repens* L.	VII-2011
Weifang	Shouguang	118°54′03.82″	36°50′20.28″	*Capsicum annuum*	V-2011
Yantai	Penglai	120°45′07.16″	37°49′22.66″	*Rosa chinensis* Jacq.	V-2011

COI sequence (GenBank No. GU570440) as an outgroup. The type of the haplotypes was determined based on the phylogenetic tree. The determination of the type (WFT-G or WFT-L) of each specimen was based on the diagnostic method of Rugman-Jones et al. (2010).

Results

Geographical Distribution of *Frankliniella occidentalis*

The 78 thrips collections in the 17 counties of Shandong Province (Fig. 1), included 16 *F. occidentalis* collections in 12 counties (Qingdao, Weihai, Jinan, Dezhou, Zibo, Binzhou, Jining, Dongying, Taian, Heze, Weifang, and Yantai) (Table 1). Among these 16 *F. occidentalis* collections, 15 were from ornamental plants and only 1 was collected from a vegetable crop.

Haplotype Composition of the Adventive *F. occidentalis* Populations

A total of 303 *mtCOI* sequences were retrieved from GenBank that had been deposited before 9-20-2011, and the information is listed in Table 2. A total of 46 haplotypes have been determined through the world. Among them, 22 haplotypes (coded as Hap1-Hap22) are found in WFT-G type and 24 haplotypes (coded as Hap23-Hap46) are found in WFT-L type. In this study, we sequenced 225 *mtCOI* in the collections from Shandong Province and only 5 haplotypes were found. Among the 5 haplotypes, 4 haplotypes (Hap1-Hap4) belongs to the WFT-G type and 1 haplotype (Hap5) belongs to WFT-L type. Overall 98.9% of the 225 individuals were WFT-G type and others (1.1%) were WFT-L type. Most individuals (98.6%) in the collections from Shandong belonged to WFT-G type and 3 individuals (1.4%), one each from Qingdao, Weihai, and Rongcheng belonged to the WFT-L type.

The most widespread haplotypes are Hap1, Hap2, and Hap3. Hap4 was found only in Dongying and Jining counties. Hap5 was found only in the coastal counties of Qingdao and Yantai.

Discussion

Contrary to our expectation, our study revealed that *F. occidentalis* suddenly and unnoticed become widespread in many regions of Shandong Province. Currently, we are not sure whether this species was already widespread in 2007. Nevertheless it seems possible that this thrips did spread rapidly within Shandong Province during the past 4 yr, and such rapid spread had been observed in Shandong Province with another invasive species, *Bemisia tabaci* (Gennadius) biotype Q (Hemiptera: Aleyrodidae) (Chu et al. 2007, 2010). *Frankliniella occidentalis* was found not only in the counties neighboring Qingdao, but also in counties far from Qingdao, which indicates that this thrips is spread mainly through human activities rather than naturally. Currently, *F. occidentalis* mainly damages the or-

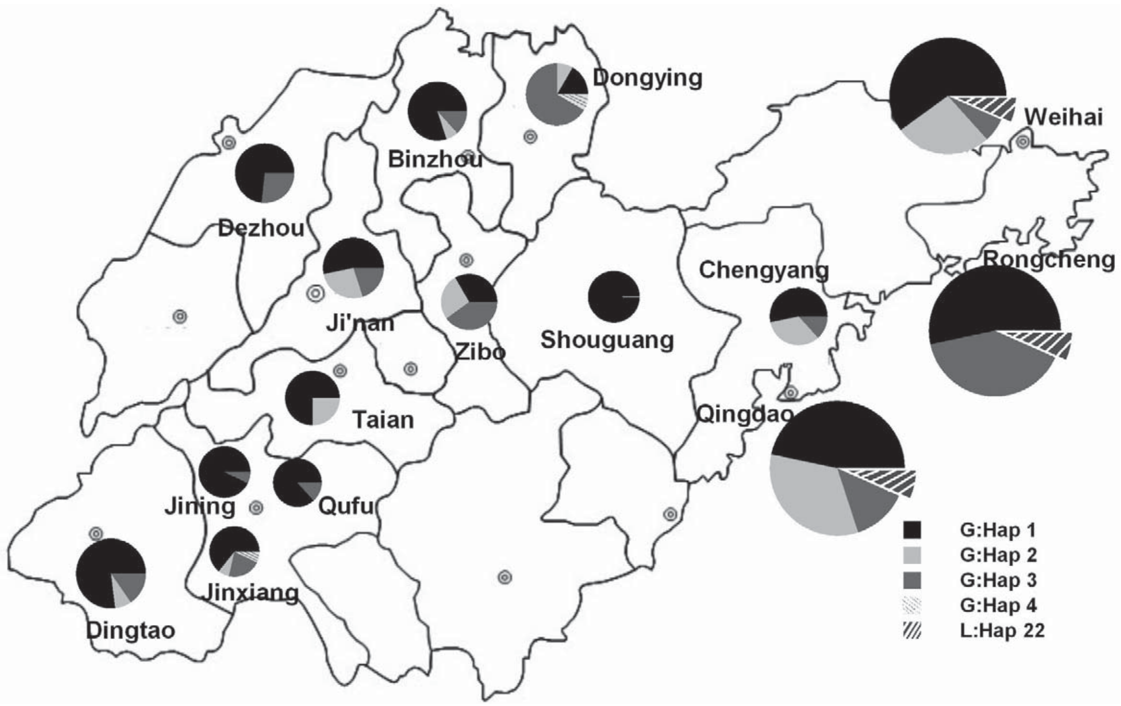

Fig. 1. Distribution of the western flower thrips in 12 of the 17 counties of Shandong Province, and the haplotype compositions of the 16 collections of this thrips in these counties. The sizes of the pie diagrams were chosen to fit into their respective counties, and they are not proportional to the number of samples taken.

namental plants in this Province. However, the infestation on the pepper crop in Weifang indicates that *F. occidentalis* may be gradually transferring to various vegetable crop species. Thus, it is essential to conduct additional field surveys of *F. occidentalis* on vegetable and fruit crops in the future.

In Shandong Province, both the WFT-G and WFT-L type of *F. occidentalis* have been detected in the field samples. However, the present study showed that the main type in this Province was the WFT-G type. There are 2 possibilities to explain the presence of both types or strains in the Province. The first possibility is that both types were represented in the first founder population to arrive. The haplotype composition among the collections indicated that there may have been multiple incursions into Shandong Province by *F. occidentalis*. Thus, if the initial founder population was mainly composed of WFT-G type, then the advantage of time may have enabled it spread widely and to become the most prevalent or dominant type. Another possibility is that WFT-G may be more invasive or more readily adapt to the environments of Shandong Province than WFT-L. Prior studies on their biological characteristics including fecundity (de Kogel et al. 1997), host adaptability (Rugman-Jones et al. 2010), environmental adaptability (Brunner et al. 2010) and insecticide resistance (Brødsgaard 1994) suggested that WFT-G has the biological advantages over WFT-L. Shandong has a warm-temperate monsoonal climate, but its coastal and inland regions experience a sharp differences in weather (http://english.peopledaily.com.cn/data/Province/shandong.html). WFT-L was only found in the eastern part of Shandong Province, which has cool-moist conditions. Thus, the WFT-G type might more readily adapt to the hoter and drier climatic conditions of most regions of Shandong Province. The distribution pattern of the 2 types of this thrips is consistent with the finding of Brunner et al. (2010) that WFT-G occurred mainly in the hot-dry conditions of its native range, while the WFT-L is better adapted to cool-moist conditions. The relationship between the geographic distribution pattern in this Province of the 2 types and temperature and humidity should also be explored in the future research.

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TABLE 2. COMPLETE LIST OF *FRANKLINIELLA OCCIDENTALIS MTCOI* HAPLOTYPES FROM THROUGHOUT THE WORLD THAT HAD BEEN DEPOSITED IN GENBANK BEFORE IX-20-2011.

Haplotype Code	GenBank Accession No.	Geographic Origin
Greenhouse strain		
Hap1	EF555841; EF555819; EF555810; EF555807	Beijing, China
	EF555845-48; EF555836; EF555806; EF555805; JF719597; EU363488; EU363487; EU363486; EU363484; EU363483	Yunnan, China
	HM246176	Liaoning, China
	EF591474-75; EF591477-78	Germany
	EF555801; EF555814; EF555820; EF555830; EF555832; EF555839; EF555840; EF555852	New Zealand
	EF555809; EF555815-16; EF555823-24; EF555826; HQ697596-98; EF555850-51	Netherlands
	EF555849; EF555844; EF555842; EF555835; EF555822; EF555813; EF555811; EF555808; EF555800	Australia
	EF555843; EF555837; EF555831; EF555827; EF555825; EF555821; EF555812; EF555803; EF555802; GU148112; GU14811; GU148025; GU148033; GU148035; GU148059; GU148061; GU148084; GU148085; GU148087; GU148094; GU372379-81; GU148126-30; GU148121-22; GU148115-17; GU148104-06; GU148039-42; GU148020-22	USA
	EF555838; EF555834; EF555833; EF555829; EF555828; EF555818; EF555817; EF555804; AB276374-76	Japan
	EU004556; EU004552	South Africa
	AM932027; AM932026	Sicily
	AM931992	Kenya
	AM932021; AM932016-18; AF378687; AF378686; EF469249	Unknown
Hap2	EF555870; EF555869; EF555866; EF555864; EF555863; EF555854	Beijing, China
	JF719596	Yunnan, China
	EF555862; EF555853; GU372382; GU148120; GU148118; GU148111; GU148110; GU148093; GU148091; GU148082; GU148032; GU148026; GU148023; GU148016-19; GU148075-77; JF429863-64; EF555865-68; EF555856-60;	USA
	EF555855	New Zealand
	EF555861	Netherlands
	EF591476	Germany
	EF469250	Unknown
Hap3	GU148114; GU148113; GU148086; GU148083; GU148064; GU148043; GU148037; GU148034; GU148024; GU148107-09; EF555887-89;	USA
	EF555876	Netherlands
	EF555885; EF555883; EF555871; EF555880; EF555875; EF555874; EF555881; EF213766	Beijing, China
	AM932005	Croatia
	JF719598; EF555886; EF555884; EF555882; EF555873; EF555877-79	Yunnan, China
	EF469245	Unknown

TABLE 2. (CONTINUED) COMPLETE LIST OF *FRANKLINIELLA OCCIDENTALIS MTCOI* HAPLOTYPES FROM THROUGHOUT THE WORLD THAT HAD BEEN DEPOSITED IN GENBANK BEFORE IX-20-2011.

Haplotype Code	GenBank Accession No.	Geographic Origin
Greenhouse strain		
Hap4	JF719595	Yunnan, China
Hap5	GU148089; GU148062; GU148058; GU148036	USA
	EU004554	Kenya
Hap6	JQ399902	USA
Hap7	AM932029	Unknown
Hap8	AM932025	Unknown
Hap9	EU363485	Yunnan, China
Hap10	AM932023; AM932022	South Africa
Hap11	EU363489	Heilongjiang, China
Hap12	EU363491	Heilongjiang, China
Hap13	EF213765	Beijing, China
	GU148123-25	USA
	EF555872	Australia
	EF469248	Unknown
Hap14	EU004553	Kenya
Hap15	GU372378	USA
Hap16	EU004555	Kenya
Hap17	FN545993	Kenya
Hap18	FN545992	Kenya
Hap19	FN545991	Kenya
Hap20	FN545981	UK
Hap21	AF378688	Unknown
Hap22	AF378685	Unknown
Lupin strain		
Hap23	EF555799	Beijing, China
	JF719599; EU363492	Yunnan, China
	GU372397; GU148097; GU148057; GU148052	USA
	EF469246	Unknown
Hap24	GU372404	USA
Hap25	GU372405-07	USA
Hap26	GU372403; GU148070	USA

TABLE 2. (CONTINUED) COMPLETE LIST OF *FRANKLINIELLA OCCIDENTALIS MTCOI* HAPLOTYPES FROM THROUGHOUT THE WORLD THAT HAD BEEN DEPOSITED IN GENBANK BEFORE IX-20-2011.

Haplotype Code	GenBank Accession No.	Geographic Origin
Lupin strain		
Hap27	GU372402	USA
Hap28	GU372398	USA
Hap29	GU372384	USA
Hap30	GU372383	USA
Hap31	GU148100; GU148056; GU148054; GU148051; GU148047-49; GU372385-37; GU148027-30; GU148078-80; GU148068-72	USA
Hap32	EF555794-98	New Zealand
	EU363490	Heilongjiang, China
	GU148102; GU148101	USA
	EF469247	Unknown
Hap33	GU372401; GU148096; GU148050	USA
Hap34	GU372399	USA
Hap35	GU372400	USA
Hap36	GU372396	USA
Hap37	GU372395	USA
Hap38	GU372394	USA
Hap39	GU372393	USA
Hap40	GU372392; GU372391; GU148099; GU148098; GU148095; GU148092; GU148090; GU148088; GU148081; GU148078; GU148063; GU148055; GU148053; GU148040; GU148038; GU148044-46; GU148065-67	USA
Hap41	GU372390	USA
Hap42	GU372389	USA
Hap43	GU372388	USA
Hap44	GU148079	USA
Hap45	GU148060	USA
Hap46	GU148031	USA

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