Phylogenetic analysis based on mitochondrial COI gene sequences in *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographic distribution

Satoshi TODA^{1,*} and Tamotsu MURAI²

¹ Grape and Persimmon Research Station, National Institute of Fruit Tree Science; Higashihiroshima, Hiroshima 739–2494, Japan

² Department of Bioproductive Science, Utsunomiya University; Utsunomiya 321–8505, Japan

(Received 26 June 2006; Accepted 28 December 2006)

Abstract

Thrips tabaci Lindeman is an extensively distributed pest insect that injures a wide range of crops. To investigate the intra-specific genetic diversity of this species, we analyzed an 810 bp region of the mitochondrial cytochrome oxidase gene subunit I (COI). Eight populations from six foreign countries and 18 Japanese populations were tested, and 17 different haplotypes were identified. Apparent differences were found between arrhenotokous and thelytokous strains in their COI sequences. A phylogenetic tree of the COI gene shows two distinct groups. We assume that these two groups correspond respectively to the arrhenotokous strain and the thelytokous strain. Japanese thelytokous types consisted of five haplotypes. Two haplotypes were identified from problematic populations in terms of the greater amount of damage they caused and the development of insecticide resistance. Both haplotypes were also found overseas, suggesting that some strains from overseas may have caused the above-mentioned problems in Japan.

Key words: *Thrips tabaci*; reproductive forms; phylogenetic analysis; COI; haplotype

INTRODUCTION

The onion thrips, *Thrips tabaci* Lindeman, is a major pest of alliaceous crops and is distributed widely throughout the world. This thrips has been known as a vector of *tomato spotted wilt virus* (TSWV) (Zawirska, 1976) and was recently recognized as a transmitter of a new tospovirus, *Iris yellow spot virus* (IYSV) (Doi et al., 2003). Although *T. tabaci* is a highly polyphagous species, it has been noted that there are several strains with different host preferences (Zawirska, 1976). Using RAPD-PCR analysis, Jenser et al. (2001) revealed a genetic difference between two populations collected from tobacco and onion, respectively. More recently, Brunner et al. (2004) conducted molecular phylogenetic analysis based on sequences of the mitochondrial cytochrome oxidase subunit I (COI) gene in some European populations and suggested that *T. tabaci* must be a complex of cryptic (sub) species in association with host preference.

Moreover, it is known that *T. tabaci* has two different reproductive forms, arrhenotoky and thelytoky, and shows geographic variation in the sex ratio (reviewed in Jenser and Szénási, 2004). Arrhenotoky is a form of parthenogenesis in which unfertilized eggs develop into haploid males, while thelytoky is complete parthenogenetic reproduction. Murai (1990) found that these two reproductive types are distinguishable by esterase zymogram. Nakao and Yabu (1998) suggested that thelytokous and arrhenotokous *Thrips nigropilosus* correspond to separate species, due to the existence of apparent genetic differences and reproductive isolation; however, the phylogenetic relationship between the two reproductive forms of *T. tabaci* is unclear, and their taxonomical relationship remains obscure.

Recently, *T. tabaci* has become a more serious pest in Japan. It has begun to cause serious damage to persimmon, which had not been recognized as a host plant of *T. tabaci* (Morishita and Oue, 2001).

* To whom correspondence should be addressed at: E-mail: toda@affrc.go.jp
DOI: 10.1303/aez.2007.309

Moreover, the damage to some crops, Satsuma mandarin (Tsuchiya, 2001; Fujikawa and Muta, 2003), asparagus (Matsumoto, 2004) and ornamental plants (Oida and Mihira, 2001), by *T. tabaci* has increased. In addition, reduced susceptibility of *T. tabaci* to insecticides has been observed in some areas of Japan (Morishita and Oue, 2001; Oida and Mihira, 2001; Nishimori et al., 2003; Murai, 2004). Recently, insecticide resistance in *T. tabaci* has been reported in the United States (Shelton et al., 2003) and Canada (Allen et al., 2005). These problems prompted us to consider that different strains have invaded Japan.

In this study, we analyzed and compared the nucleotide sequences of the COI gene from two reproductive forms of *T. tabaci* and inferred the degree of their genetic divergence. Furthermore, we conducted phylogenetic analysis to clarify the relationships between the 17 haplotypes found in this study. We then discuss the genetic background of

several problems occurring in Japanese *T. tabaci* populations.

MATERIALS AND METHODS

Thrips. Japanese *T. tabaci* samples were collected from a variety of host plants in 18 localities (Table 1). Species were identified by observation of morphological characteristics and a molecular diagnostic method (Toda and Komazaki, 2002). The reproductive form was identified in 13 populations which were offered in a viable condition. When no males appeared for several generations, the population was judged to be thelytokous. Arrhenotokous strains were established by individual rearing of females of populations in which males appeared. Thrips were reared on germinated broad bean (*Vicia faba* L.) under laboratory conditions (16L8D, 25°C) according to the method of Murai and Loomans (2001).

Table 1. Collection history and locality of *Thrips tabaci*

Country	Population name	Locality	Host plants	Year	Type ^a
Japan	HoNa	Naganuma, Hokkaido Pref.	<i>Allium cepa</i>	1999	
	AoRo	Rokunohe, Aomori Pref.	<i>Raphanus sativus</i>	2000	TH
	IwMo	Morioka, Iwate Pref.	Asteraceae spp.	1999	
	ChTa	Tateyama, Chiba Pref.	<i>Eustoma grandiflorum</i>	2003	
	ShHa	Hamamatsu, Shizuoka Pref.	<i>Allium cepa</i>	1999	TH
	NaCh	Chikumagawa, Nagano Pref.	<i>Allium cepa</i>	2005	AR
	OsHa	Habikino, Osaka Pref.	<i>Allium cepa</i>	2005	AR
	WaMo	Momoyama, Wakayama Pref.	<i>Diospyros kaki</i>	2001	TH
	WaKo	Kokawa, Wakayama Pref.	<i>Allium cepa</i>	2001	TH
	HiHi-1	Higashihiroshima, Hiroshima Pref.	<i>Solanum tuberosum</i>	2001	TH
	HiHi-2	Higashihiroshima, Hiroshima Pref.	<i>Allium cepa</i>	2005	AR
	ShIz	Izumo, Shimane Pref.	<i>Allium cepa</i>	1989	AR
	KaKa	Kan-onji, Kagawa Pref.	<i>Asparagus officinalis</i>	2002	TH
	SaKa	Karatsu, Saga Pref.	<i>Allium</i> × <i>wakegi</i>	2004	TH
	SaGe	Genkai, Saga Pref.	<i>Citrus unshiu</i>	2004	TH
	NaKu	Kuchinotsu, Nagasaki Pref.	<i>Trifolium repens</i>	2000	TH
	KaKu	Kushira, Kagoshima Pref.	<i>Allium fistulosum</i>	1999	
	OkNa	Nago, Okinawa Pref.	<i>Allium sativum</i>	1998	
Korea	KOR	Suwon	Unknown	1998	
Netherlands	NLD-1	Oudenbosch	<i>Allium porrum</i>	2000	M
	NLD-2	Meterik	<i>Allium porrum</i>	1999	M
France	FRA	Unknown (Imported)	<i>Allium porrum</i>	2000	
Israel	ISR-1	Unknown (Imported)	<i>Dianthus</i> spp.	2001	M
	ISR-2	Unknown (Imported)	<i>Liatris</i> spp.	2000	M
Kenya	KEN	Unknown (Imported)	<i>Dianthus</i> spp.	2000	
Peru	PER	Unknown (Imported)	<i>Asparagus officinalis</i>	2001	

^a AR: arrhenotoky, TH: thelytoky, M: including males.

Overseas samples were also used for phylogenetic analysis. Individuals of *T. tabaci* were collected from six countries: Korea, the Netherlands, France, Israel, Kenya and Peru (Table 1). Samples from the latter four countries were collected from crops imported to the Netherlands. They were offered as specimens in 100% ethanol.

DNA extraction and PCR amplification. Total genomic DNA was extracted from each single female and male adult thrips after Toda and Komazaki (2002). Thrips were homogenized in 100 μ l of lysis buffer (100 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), 1% sodium dodecyl sulfate (SDS), 0.1 mg/ml proteinase K) and incubated for 10 min at 65°C. The lysate was extracted once with one volume of TE-saturated phenol and once with chloroform/isoamyl alcohol (24 : 1). DNA was precipitated with ethanol and resuspended in 10 μ l of 0.1 $\times$ Tris-EDTA (TE) buffer (1 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (pH 8.0)) and stored at -20°C. To design a specific primer, the cytochrome oxidase subunit I (COI) region of mitochondrial DNA was amplified using the universal primers (sense) 5'-TATAGCATTCCCACGAATAAATAA-3' and (anti-sense) 5'-TACAGCTCCTATAGATAAAAC-3', designed by Zhang and Hewitt (1996) and by Navajas et al. (1996), respectively. A specific sense primer, 5'-TATAAGATTCTGACTTTTACCCCC-3', was designed on the basis of COI sequence data determined using these universal primers. This primer and the above-mentioned universal anti-sense primer were subsequently used for the analyses. The PCR thermal program in a DNA thermal cycler (DNA thermal cycler 9600, Applied Biosystems) was 95°C for 9 min for pre-heating, and then 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, and final extension at 72°C for 7 min as a final extension. PCR was performed in 40 μ l reaction volumes, containing 20 pmol of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.25 mM of each dNTP and 0.5 units of *Taq*-polymerase (AmpliTaq Gold®, Applied Biosystems).

Sequencing. PCR products were purified and dissolved in 0.1 $\times$ TE by ultrafiltration using Microcon® YM-100 (Millipore). They were sequenced directly using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI Prism® Model 377 automated DNA sequencer. Two specific primers, 5'-GTCTGATCAGTTATTTTA-

ACAGCC-3' and 5'-ATGAGAAATTAGTCCAA-ATCCTGG-3', were designed on the basis of sequence data determined beforehand and used for sequencing as internal primers. The DNA of each sample was sequenced twice for both strands.

Phylogenetic analysis. Six COI sequence data on *T. tabaci* submitted by Brunner et al. (2004) were obtained from the DNA database and used for phylogenetic analysis employing the sequence data obtained in this study. As an outgroup, we utilized the complete mitochondrion sequence data of *T. imaginis* (AF335993) (Shao and Barker, 2003). DNA sequences of *T. tabaci* and *T. imaginis* were aligned using Clustal W (Thompson et al., 1994). Phylogenetic analysis was performed using the PHYLIP 3.65 (Felsenstein, 2005) package. Divergence between sequences was estimated using the DNADIST program, with Kimura's two parameter distance option (Kimura, 1980). A tree was built using the neighbor-joining method employing the NEIGHBOR program. Bootstrap values were calculated using SEQBOOT and CONSENSE, packaged in PHYLIP. The phylogenetic tree was also estimated using the parsimony (DNAPARS) and maximum likelihood (DNAML) programs packed in PHYLIP.

RESULTS

The 856 bp region of the mitochondrial COI gene was successfully amplified in 118 specimens. The COI sequences used in this study were 810 bp excluding the primer region. Seventeen different haplotypes were identified. Their sequences have been deposited in the DDBJ nucleotide sequence database under accession numbers AB262428-AB262444. Variable nucleotide positions among the 17 COI haplotypes are shown in Table 2. The COI sequences showed polymorphism at 39 nucleotide positions (4.8%), including 37 transitions and two transversions. Although 38 of these were silent substitutions, one transversion caused an amino acid substitution in Haplotype 4 (Table 2).

Five and two haplotypes were identified from 10 thelytokous and four arrhenotokous populations, respectively (Tables 1 and 3). Table 3 shows the number of nucleotide substitutions in the COI gene among these seven haplotypes. A nucleotide difference between the two arrhenotokous haplotypes (Haplotypes 6 and 7) was found at only one site,

Table 2. Variable nucleotide positions in the 810 bp region of the 17 COI haplotypes of *T. tabaci*

Haplotype	Position																																							
	6	39	45	48	60	75	78	135	150	169	207	248	249	255	268	270	282	288	315	321	324	342	345	348	351	360	366	378	399	459	462	567	612	615	618	681	726	729	777	
1	G	A	A	A	G	A	A	A	G	A	A	C	T	C	C	A	A	T	C	C	T	G	G	A	A	C	C	C	A	T	C	C	T	A	C	C	A	A	T	
2	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	T	.	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	.	.	
3	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	C	T	T	.	.	.	.	.	.	T	T	G	.	.	.	.	.	T	.	.	.	C	
4	A	.	.	.	.	.	.	G	.	.	.	T	.	.	.	G	.	.	T	T	.	.	.	.	.	.	T	T	G	.	.	.	.	.	T	.	.	.	.	
5	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	.	.	.	T	T	G	.	T	.	.	.	.	T	T	.	.	
6	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	.	.	.	T	T	G	.	.	.	.	.	.	T	.	.	.	
7	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	.	.	.	T	T	G	.	.	.	.	.	.	T	.	.	.	
8	.	.	.	.	.	G	.	G	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	.	.	.	T	T	G	.	.	.	.	.	.	T	.	.	.	
9	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	T	T	.	.	.	G	.	.	T	T	G	.	.	.	.	.	.	T	.	.	G	.
10	.	.	.	G	A	.	G	G	A	.	.	.	C	T	T	G	G	.	T	C	A	A	.	G	T	T	T	.	.	.	.	.	C	.	T	.	G	.		
11	.	.	.	G	A	.	G	G	A	T	.	.	C	T	T	G	G	.	T	C	A	A	.	G	T	T	T	.	.	.	.	.	C	.	T	.	G	.		
12	.	.	.	G	A	.	G	G	A	.	.	.	C	T	T	G	G	.	T	C	A	A	.	G	T	T	T	.	.	.	T	C	.	T	.	G	.			
13	.	.	.	G	A	.	G	G	A	.	G	.	C	T	T	G	G	.	T	C	A	A	.	G	T	T	T	.	.	.	.	C	.	T	.	G	.			
14	.	.	.	G	A	.	G	G	A	.	.	.	C	T	T	G	G	.	T	C	A	.	.	G	T	T	T	.	.	.	.	C	.	T	.	G	.			
15	.	.	.	.	A	.	G	G	A	.	.	.	C	T	T	.	G	.	T	C	A	A	.	G	T	T	T	.	A	.	.	C	.	T	.	G	.			
16	.	G	.	G	A	.	G	G	A	.	.	.	C	T	T	.	G	.	T	C	A	A	.	G	T	T	T	.	A	.	.	C	.	T	.	G	.			
17	.	.	.	G	A	.	G	G	A	.	.	.	C	T	T	.	G	.	T	C	A	A	.	G	T	T	T	.	A	.	.	C	.	T	.	G	.			

Nucleotide in bold results in amino acid substitution.

Table 3. Number of nucleotide substitutions in COI sequences among seven haplotypes in which the reproductive form was confirmed

Haplotype No. (Population name)	10	12	14	15	16	6	7
10 (AoRo)	—						
12 (SaKa)	1	—					
14 (ShHa, AoRo, WaMo)	1	2	—				
15 (WaKo, KaKa, NaKu, SaGe)	3	4	4	—			
16 (WaMo, HiHi-1)	3	4	4	2	—		
6 (OsHa, NaCh, HiHi-2)	17	18	16	18	20	—	
7 (ShIz)	18	19	17	19	19	1	—

Haplotype numbers 10–16: thelytoky; 6, 7: arrhenotoky.

while the number of polymorphic sites among the four thelytokous haplotypes was 1–4 sites. In contrast, 16–20 substitution sites were found between the two reproductive forms.

The COI sequences used in phylogenetic analysis were 394 bp, which is common to all the sequences of *T. tabaci* and *T. imagines*. Although phylogenetic trees were estimated using the three methods of neighbor-joining (NJ), parsimony and maximum likelihood, their main stems showed the same pattern supported by high bootstrap values. We therefore show only the NJ tree in Fig. 1. The phylogenetic tree of the COI gene showed three distinct groups (Fig. 1). We call those groups

‘Group A’, ‘Group B’ and ‘Group C’. Groups A and B included Haplotypes 1–9 and 10–17, respectively. Group C consisted of two haplotypes, AY196845 and AY196846. All arrhenotokous strains and male specimens were in Group A, while all thelytokous strains were in Group B. High bootstrap values supported that Groups A and B are a monophyletic group, while Group C shows no monophyly with them (Fig. 1).

The frequency distribution of the 17 haplotypes in 18 Japanese and eight overseas populations is shown in Table 4. Arrhenotoky consisting of two haplotypes, Haplotypes 6 and 7, was found in four Japanese populations. Overseas, males were found

Fig. 1. Neighbor-joining tree based on 810 bases of the COI gene of *T. tabaci*. Bootstrap values (1,000 replicates) >80% are shown above the branches. *T. imaginis* (AF335993) served as an outgroup. The accession numbers in Groups A, B and C show the sequence data of *T. tabaci* obtained from DNA databases. TH, AR and M show that the thelytokous strain, arrhenotokous strain and males were contained in the samples, respectively.

in only two countries, the Netherlands and Israel. The geographic distribution of Haplotypes 1–9 in Group A did not overlap among three countries except for Haplotype 8. Haplotypes 1 and 2 formed a subgroup supported by a high bootstrap value (Subgroup 1 in Fig. 1). Five thelytokous haplotypes (Haplotypes 10, 12, 14, 15 and 16) were found in Japanese populations (Table 4). Haplotypes 10, 15 and 16 were found both overseas and in Japan. Haplotype 16 was found in three overseas populations, FRA, KEN and PER, and two Japanese populations, WaMo and HiHi-1. Haplotype 15, found in ChTa, WaMo, KaKa, SaGe and NaKu, was also found in ISR-1. In Japan, Haplotype 14 was detected over a geographically wide range.

DISCUSSION

A comparison of the nucleotide sequences of the mitochondrial COI gene revealed that Japanese arrhenotokous and thelytokous *T. tabaci* have at least two and five haplotypes, respectively. The nu-

cleotide sequence apparently differed between the two reproductive forms, while high homology was observed in each reproductive form. Moreover, phylogenetic analysis, including the overseas strain, showed that the samples used in this study were divided into two separate groups. Japanese arrhenotokous strains (ShIz, OsHa, NaCh, and HiHi-2) and all male samples, with a few females from the Netherlands (NDL-1 and -2) and Israel (ISR-1 and -2), formed an independent clade (Group A in Fig. 1). Since we can regard males as arrhenotokous, we assumed that this group was arrhenotokous. The two subgroups in Fig. 1 may indicate a difference in their geographic origin. In contrast, all thelytokous strains were included in Group B, suggesting that Group B is thelytokous. The divergence of Groups A and B was supported by a high bootstrap value (Fig. 1).

Brunner et al. (2004), after conducting molecular phylogenetic analysis, proposed three distinct major lineages (T, L1 and L2) in *T. tabaci*. They suggested that T is the tobacco-associated lineage,

Table 4. Frequency distribution of 17 COI haplotypes in 18 Japanese and eight overseas populations

Population name	Haplotypes																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
HoNa	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
AoRo	—	—	—	—	—	—	—	—	—	5	—	—	—	5	—	—	—
IwMo	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
ChTa	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—
ShHa	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
NaCh	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
OsHa	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
WaMo	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	—
WaKo	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—
HiHi-1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—
HiHi-2	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
ShIz	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
KaKa	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5	—	—
SaKa	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
SaGe	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
NaKu	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—
KaKu	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
OkNa	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	—
KOR	—	—	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—
NLD-1	—	—	—	—	1	—	—	1	1	—	—	—	1	—	—	—	—
NLD-2	—	—	1	2	3	—	—	3	1	—	—	—	—	—	—	—	—
FRA	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	5	4
ISR-1	5	—	—	—	—	—	—	3	—	—	—	—	—	—	1	—	—
ISR-2	1	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
KEN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—
PER	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—

and L1 and L2 are the leek-associated lineages. Two haplotypes, AY196845 and AY196846, belong to the tobacco type (T). Similarly, AY196842 and AY196843 are members of L1, and AY196831 and AY196832 are L2. These suggest that Groups A, B and C correspond to lineages L1, L2 and T, respectively. In this study, we found no samples belonging to Group C. This is attributable to the fact that no sample was collected from host plants of the tobacco type as described in Zawirska (1976). It has been pointed out that the tobacco type is arrhenotokous, and the leek type consists of both arrhenotoky and thelytoky (Zawirska, 1976). Thus, Brunner et al. (2004) and our study both suggest that an ancient arrhenotokous strain differentiated into two types, the tobacco (T) and leek (L) types, and then a thelytokous type (L2) was derived from the arrhenotokous leek type (L1). Chatzivassiliou et al. (2002) revealed that arrhenotokous populations collected from tobacco transmitted TSWV effi-

ciently (up to 48.5% transmission) and those from leek were poor transmitters (up to 3.1%), whereas no transmission was obtained with thelytokous populations from leeks. These facts show that the ability to transmit TSWV is closely linked to host preference and may also support the above-mentioned hypothesis in relation to the differentiation process of the three strains. Brunner et al. (2004) proposed that the relationship between T and the others corresponds to that of sibling species, and the two leek haplotypes (L1 and L2) correspond to typical host-race estimates according to their *Fst* value. In our study, the NJ tree with high bootstrap values suggested that Groups A and B are a monophyletic group, while Group C shows no monophyly with them. This result supports the phylogenetic relationship proposed by Brunner et al. (2004). Murai (data unpublished) observed that, although arrhenotokous males attempted to copulate with thelytokous females, the males were rejected,

suggesting the existence of reproductive isolation. This phenomenon was also observed between two reproductive forms of *T. nigropilosus* and suggested that they are a separate species (Nakao and Yabu, 1998). To discuss the taxonomical relationship between two reproductive forms of *T. tabaci*, it is necessary to investigate their reproductive isolation in detail.

Japanese thelytokous types consist of five haplotypes (Table 2). Haplotypes 12 and 14 were found only in Japan (Table 4). It was speculated that Haplotype 14 is the most common type distributed in Japan, since it was the most frequently found across Japan and has never been collected from problematic populations. Haplotypes 10, 15 and 16 were found in both Japanese and overseas populations (Table 3). Haplotype 10 was found also in Korea. Murai and Toda (2002) revealed that Haplotype 10 thrips collected in AoRo had a paler body color and smaller body size than that of Haplotype 14 collected in the same locality. Although there is a possibility that this haplotype originated overseas, we believe that this is not problematic type, since we have found no marked traits in insecticide resistance and host range in this haplotype. Haplotype 16 is also distributed in three overseas countries (France, Kenya and Peru), and Haplotype 15 was detected in Israeli samples. In this study, Haplotypes 15 and 16 were identified from persimmon, asparagus, Satsuma mandarin and florist crop populations in Wakayama, Kagawa, Saga and Chiba Prefectures, respectively (Table 1). They are problematic populations because of the extent of damage and/or decreased susceptibility to some insecticides (Morishita and Oue, 2001; Oida and Mihira, 2001; Matsumoto, 2004; Zen, personal communication). The expansion of damage on the above-mentioned crops and the decrease of insecticide susceptibility were all first reported in the late 1990s to early 2000s in Japan. Insecticide resistance in *T. tabaci* began to be reported in the late 1980s overseas (Cranshaw et al., 1989), and has recently become a serious problem (Shelton et al., 2003; Allen et al., 2005). These facts may suggest that different types of thelytokous strains invaded Japan during that period and caused agricultural problems; however, we need to analyze more overseas samples to clarify the relationship between Japanese and overseas populations.

In this study, we found a variety of mitochon-

drial COI haplotypes in *T. tabaci*. All populations which have been causing serious agricultural problems are thelytokous so far, suggesting that the problematic traits are maternally inherited. If there is genetic variation in such traits, we may develop a genetic marker which closely links to problematic traits using COI and/or other haplotypes. Thus, further genetic analysis may contribute to the development of an efficient control method of *T. tabaci*.

ACKNOWLEDGEMENTS

We are grateful to the following people for collecting Japanese thrips samples: T. Arai, H. Hayashi, S. Hayashikawa, O. Kanehira, S. Masui, E. Matsumoto, T. Mihira, M. Morishita, Y. Sakagami, M. Shibao and S. Zen. We are also grateful to Drs. G.-S. Lee (National Institute of Agricultural Science and Technology, Korea) and G. Vierbergen (Plantenziektenkundige Dienst, the Netherlands) for collecting and identifying the Korean and other five overseas samples, respectively. We also thank Dr. S. Komazaki of the National Institute of Fruit Tree Science for valuable advice on this study.

REFERENCES

- Allen, J. K. M., C. D. Scott-Dupree, J. H. Tolman and C. R. Harris (2005) Resistance of *Thrips tabaci* to pyrethroid and organophosphorus insecticides in Ontario, Canada. *Pest Manag. Sci.* 61: 809–815.
- Brunner, P. C., E. K. Chatzivassiliou, N. I. Katis and J. E. Frey (2004) Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. *Heredity* 93: 364–370.
- Chatzivassiliou, E. K., D. Peters and N. I. Katis (2002) The efficiency by which *Thrips tabaci* populations transmit *Tomato spotted wilt virus* depends on their host preference and reproductive strategy. *Phytopathology* 92: 603–609.
- Cranshaw, W. S. (1989) Control of organophosphate-resistant onion thrips. *Insecticide Acaricide Test* 14: 128.
- Doi, M., S. Zen, M. Okuda, H. Nakamura, K. Kato and K. Hanada (2003) Leaf necrosis disease of lisianthus (*Eustoma grandiflorum*) caused by *Iris yellow spot virus*. *Jpn. J. Phytopathol.* 69: 181–188 (in Japanese with English summary).
- Felsenstein, J. (2005) PHYLIP: Phylogeny inference package, version 3.6. Department of Genome Sciences, University of Washington, Seattle.
- Fujikawa, K. and T. Muta (2003) Occurrence and chemical susceptibility of onion thrips (*Thrips tabaci* Lindeman) in greenhouses of Satsuma mandarin in Kagoshima Prefecture. *Plant Prot.* 57: 61–64 (in Japanese).
- Jenser, G. and Á. Szénási (2004) Review of the biology and vector capability of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). *Acta Phytopathol. Entomol. Hung.* 39: 137–155.
- Jenser, G., Á. Szénási, O. Törjék, G. Gyulai, E. Kiss, L. Heszky and J. Fail (2001) Molecular polymorphism between population of *Thrips tabaci* Lindeman

- (Thysanoptera: Thripidae) propagating on tobacco and onion. *Acta Phytopathol. Entomol. Hung.* 36: 365–368.
- Kimura, M. (1980) A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16: 111–120.
- Matsumoto, E. (2004) Occurrence and management of *Thrips tabaci* on green asparagus. *Japan Agricultural Technology* 48(8): 34–38 (in Japanese).
- Morishita, M. and H. Oue (2001) Damage of persimmon fruit by onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), and its chemical control. *Proc. Kansai Plant Prot. Soc.* 43: 43–44 (in Japanese with English summary).
- Murai, T. (1990) Parthenogenetic reproduction in *Thrips tabaci* and *Frankliniella intonsa* (Insecta: Thysanoptera). In *Advances in Invertebrate Reproduction*. Vol. 5 (M. Hoshi and O. Yamashita eds.). Elsevier Science, Amsterdam, pp. 357–362.
- Murai, T. (2004) Current status of the onion thrips, *Thrips tabaci*, as a pest thrips in Japan. *Agrochemicals Japan* 84: 7–10.
- Murai, T. and A. J. M. Loomans (2001) Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. *Entomol. Exp. Appl.* 101: 281–289.
- Murai, T. and S. Toda (2002) Variation of *Thrips tabaci* in colour and size. In *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera* (R. Marullo and L. A. Mound eds.). Australian National Insect Collection, Canberra, pp. 377–378.
- Nakao, S. and S. Yabu (1998) Ethological and chemical discrimination between thelytokous and arrhenotokous *Thrips nigropilosus* Uzel, with discussion of taxonomy. *Jpn. J. Appl. Entomol. Zool.* 42: 77–83 (in Japanese with English summary).
- Navajas, M., D. Fournier, J. Lagnel, J. Gutierrez and P. Boursot (1996) Mitochondrial COI sequences in mites: evidence for variations in base composition. *Insect Mol. Biol.* 5: 281–285.
- Nishimori, T., K. Imai, M. Yamada, N. Miki and S. Kobayashi (2003) Insecticide susceptibility of onion thrips, *Thrips tabaci* Lindeman, collected in vegetable field and mass rearing method. *Plant Prot.* 57: 56–60 (in Japanese).
- Oida, H. and T. Mihira (2001) Damage from *Thrips tabaci* on florist crops and its management. *Agriculturist and Horticulturist* 56: 178–181 (in Japanese).
- Shao, R. and S. C. Barker (2003) The highly rearranged mitochondrial genome of the plague thrips, *Thrips imaginis* (Insecta: Thysanoptera): Convergence of two novel gene boundaries and an extraordinary arrangement of rRNA genes. *Mol. Biol. Evol.* 20: 362–370.
- Shelton, A. M., B. A. Nault, J. Plate and J.-Z. Zhao (2003) Regional and temporal variation in susceptibility to lambda-cyhalothrin in onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae), in onion fields in New York. *J. Econ. Entomol.* 96: 1843–1848.
- Thompson, J. D., D. G. Higgins and T. J. Gibson (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.
- Toda, S. and S. Komazaki (2002) Identification of thrips species (Thysanoptera: Thripidae) on Japanese fruit trees by polymerase chain reaction and restriction fragment length polymorphism of the ribosomal ITS2 region. *Bull. Entomol. Res.* 92: 359–363.
- Tsuchiya, M. (2001) Occurrence of blemished fruits caused by *Thrips tabaci* on greenhouse Satsuma mandarins in Shizuoka Prefecture. *Annu. Rep. Kanto-Tosan Plant Prot. Soc.* 48: 153–155 (in Japanese with English summary).
- Zawirska, I. (1976) Untersuchungen über zwei biologische Typen von *Thrips tabaci* Lind. (Thysanoptera, Thripidae) in der VR Polen. *Arch. Phytopathol. Pflanzenschutz* 12: 411–422.
- Zhang, D.-X. and G. M. Hewitt (1996) Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. *Insect Mol. Biol.* 6: 143–150.